NATIONAL NUCLEAR ENERGY SERIES
Manhattan Project Technical Section

Division VI - Volume 1

PHARMACOLOGY AND TOXICOLOGY

OF URANIUM COMPOUNDS

PHARMACOLOGY AND TOXICOLOGY OF URANIUM COMPOUNDS

With a Section on the Pharmacology and Toxicology of Fluorine and Hydrogen Fluoride

Edited by

CARL VOEGTLIN, Ph. D.

Formerly Chief of the Division of Pharmacology, National Institute of Health, U. S. Public Health Service; formerly Director of Cancer Research and Chief of the National Cancer Institute

and

HAROLD C. HODGE, Ph. D.

Professor of Pharmacology and Toxicology, School of Medicine and Dentistry, University of Rochester

First Edition

New York · Toronto · London

McGRAW-HILL BOOK COMPANY, INC.

1949

PHARMACOLOGY AND TOXICOLOGY
OF URANIUM COMPOUNDS

Printed in the United States of America

Lithoprinted

Edwards Brothers, Incorporated
Ann Arbor, Michigan

PART C. RESULTS OF SHORT-TERM EXPOSURES

This section summarizes the results of forty-six 30-day exposure studies of 12 uranium dusts at concentrations ranging from 20 to 0.05 mg U/cu m of air. The approximate concentration at which each of the dusts was studied is given in Table 10.30. In a few instances a

Table 10.30—Scope of 30-day Inhalation-exposure Studies

Approximate concentration level of dust, mg/cu m	Uranium dusts											
	UF_6	UO_2F_2	$UO_2(NO_3)_2 \cdot 6H_2O$	UCl_4	UF_4	UO_2	High-grade ore	UO_4	UO_3	$Na_2U_2O_7$	$(NH_4)_2U_2O_7$	U_3O_8
20	x	x	x*	x	x	x	x	x	x	x	x	x
10						x*						
5			x		x*		x					
3.3				x								
2	x	x	x	x		x						
1			x		x	x	x					
0.5		x	x	x	x							
0.2	x*	x	x	x*								
0.05	x			x								

Note: The x's indicate the levels at which inhalation-exposure studies were performed.

*Two studies of this level were made, one of which comprised groups of animals of a chronic study; these were accordingly not killed for examination at the end of 30 days.

study at a given level was repeated in the chronic experiments. The results of the first 30 days are included here. It is seen in Table 10.30 that the toxicity of certain compounds, namely, UF_6, UCl_4, $UO_2(NO_3)_2.6H_2O$, UF_4, UO_2, UO_2F_2, and high-grade ore, was explored in greater detail than was that of the higher oxides and the diuranates. Seven levels of the nitrate, six of the tetrachloride, and at least four levels of the dioxide and of each of the three fluorides were studied.

In addition, two 90-day and two 30-day control studies have been made, the results of which are summarized in the following section to furnish a base line for the five criteria employed in evaluating toxicity in the inhalation studies, namely, mortality, pathology, weight response, biochemistry, and hematology.

9. CONTROL STUDIES

By George F. Sprague, Jr.*

In forming an estimate of the condition or well-being of the animals used, the results of the histologic examination are probably the most revealing. The histological findings in two 30-day studies and two 90-day studies are summarized in Tables 10.31 and 10.32 and are discussed briefly. Weight response, mortality, and biochemical and hematologic data from one 30-day study are given in detail, along with a summary of such results from three other short-term studies, which summary supports or extends the conclusions derived. The study to be described comprised 95 animals: 5 dogs, 25 rabbits, 20 guinea pigs, 20 rats, and 25 mice, of which 10 rabbits and guinea pigs each were head exposed. The animals were exposed in the control chamber for a total of 216 hr: 6 hr a day for 36 exposure days. This time was more comparable to the combined period of conditioning and exposure used in the uranium-dust studies.

9.1 Mortality. No deaths occurred from any cause; one mouse was accidentally lost.

9.2 Histology. There were no tissue changes indicative of uranium damage. Moreover, there were no appreciable differences among the findings in the head-exposed and fully exposed rabbits and guinea pigs, and no significance could be attached to sex in the distribution of organ changes. There was, however, mild pathology in many of the animals examined. Mild chronic interstitial nephritis and small parasitic granulomata were the most common findings in dogs, each occurring in 5 of the 20 dogs studied. Chronic interstitial nephritis occurred in rabbits in approximately the same frequency as in dogs (15 of 70) but in every case was moderate in degree. Parasitic involvement of the bowel, mesenteric nodes, and/or liver was the most frequently occurring abnormality in rabbits (37 of 70). Abnormalities were less frequent in rats, and those observed were, for the most part, very mild. Slight vacuolization of the hepatic epithelium (presumably fat) and intestinal parasites were the most common findings in mice. Mild to moderate pulmonary inflammation (pneumonia, bronchitis, etc.) occurred in 16 of 40 guinea pigs. Table 10.31 shows the type, frequency, and severity of the tissue changes in these control animals.

* Work performed by G. F. Sprague, Jr., and W. M. Harrison.

Table 10.31 — Summary of Pathology of Two 30-day Control Studies

Species	Lung	Kidney	Other findings
30 rabbits FE*	1, moderate pneumonia 2, moderate hemorrhage and edema	7, moderate chronic interstitial nephritis 1, slight calcification in renal cortex 8, few interstitial round cells†	16, parasites in liver, omentum, and/or colon 5, essentially normal
6 rabbits HE* died	4, edema, hemorrhage, hyperemia; moderately severe	1, few interstitial round cells†	1, liver necrosis, mesenteric parasites 1, hemorrhage in thymus 1, mild fatty degeneration: liver 2, cause of death unknown
14 rabbits HE* sacrificed	2, mild bronchitis	2, chronic interstitial nephritis, moderate 7, few interstitial round cells†	9, parasites in liver, omentum, and/or bowel 1, fibrosis femoral marrow 2, essentially normal
50 rats FE*	2, mild bronchitis 5, very mild perivascular inflammation	6, mild peripelvic inflammation 1, parasites: renal pelvis 3, few interstitial round cells† 1, rather large embryonal tumor	2, parasites in large bowel 1, changes characteristic of Salmonellosis 30, essentially normal
10 dogs FE*	1, marked interstitial vacuolization; same in pulmonary nodes; possible fat	5, small parasitic granuloma 2, mild chronic interstitial nephritis 2, few interstitial round cells†	1, round worms in duodenum 1, testis, moderate degeneration 3, essentially normal
49 mice FE*	2, moderate bronchopneumonia 1, small adenoma	18, few interstitial round cells† 1, parasites in kidney	9, slight fatty degeneration in liver 1, benign tumor in ovary 10, parasites in bowel 1, parasites in skin 17, essentially normal
20 guinea pigs FE*	7, mild localized organizing pneumonia 2, mild interstitial pneumonia 1, mild bronchitis 3, mild pulmonary hemorrhage	1, mild chronic interstitial nephritis 9, few interstitial round cells†	7, essentially normal
3 guinea pigs HE* died	1, pulmonary embolism 1, interstitial pneumonia, moderately severe 1, mild hemorrhage and congestion	1, mild degeneration renal tubules 1, mild chronic interstitial nephritis	
17 guinea pigs HE* sacrificed	6, bronchopneumonia, mild to moderate	1, mild localized acute inflammation 3, few interstitial round cells†	1, mild focal necrosis in liver 1, abscess in lymph node 4, essentially normal

* FE = full exposure; HE = head exposure.

† This finding does not constitute an actual chronic nephritis but is important since round cell accumulations may be observed in the kidneys of animals after prolonged exposure to uranium.

Table 10.32 — Summary of Pathology of Two 90-day Control Studies

Species	Lung	Kidney	Other findings
20 rabbits	1, mild acute bronchitis 1, mild acute tracheitis 3, mild interstitial pneumonitis	6, moderate chronic interstitial nephritis	12, parasites in liver, omentum, and/or bowel 1, mild fatty degeneration 1, hemmorrhage and degeneration, testis 1, fibrosis of bone marrow (distal femur) 4, essentially normal
20 rats	2, mild perivascular inflammation 2, mild to moderate acute tracheitis	1, mild acute peripelvic inflammation 2, interstitial round cells: few *	1, parasites in liver 1, severe pancreatitis 12, essentially normal
10 dogs		3, mild chronic interstitial nephritis	4, worms in intestine 1, atrophy splenic follicles 1, aspermia and degeneration of testis 2, essentially normal

* This finding does not constitute an actual chronic nephritis but is important since round cell accumulations may be observed in the kidneys of animals after prolonged exposure to uranium.

9.3 Weight Response. The weight response of the five species of animals is considered typical of animals maintained under the conditions of handling and feeding of the short-term exposure studies. Each species, except the dog, which was mature at the start of the experiment, showed an appreciable and uniform weight increase from an inspection of the 50-day weight record (Fig. 10.31). Mean weight gains are recorded of from 10 per cent in the head-exposed rabbit to 87 per cent in the male rat. The gains were of such a magnitude as to indicate that the rather widespread pathology observed in the control animals was of a mild sort. A comparison of the weight gains of the head-exposed and the fully exposed animals shows that this form of restriction placed upon the animal during exposure retards growth to a limited extent. In the rapidly growing guinea pig the percentage weight gain of the head-exposed animal was on the average 80 per cent that of the unconfined guinea pig. In the more mature rabbit the weight response of the head-exposed animal was but 54 per cent of the animal not so restricted.

9.4 Biochemical Findings. Blood analyses, determined on 5 dogs and 10 rabbits at weekly intervals for 8 weeks, included nonprotein nitrogen, amino acid nitrogen, calcium, and phosphorus. Attempts were made three times each week for a period of 8 weeks to collect urine from 5 dogs and 10 rabbits to analyze for protein and reducing sugar. The rabbit urine was also analyzed for amino acid nitrogen, catalase, and phosphatase activity. The values of each determination all lay within the normal range given in Chap. 5, with the exception of

a single high nonprotein nitrogen value in one dog and of five high values in the rabbits. Of 102 samples for urinary protein in dogs, 15 showed positive values. No positive protein values were obtained in 214 samples from rabbits taken over the entire period. Eight positive sugar values were found in 101 samples from dogs and 4 of 214 samples in rabbits. Of 158 catalase-activity determinations in rabbits, only eight had values over 50 cu mm of oxygen, the arbitrarily chosen cutting point. Fifteen values of 183 determinations for urinary phosphatase activity in rabbits were above a cutting point of 3.0.*

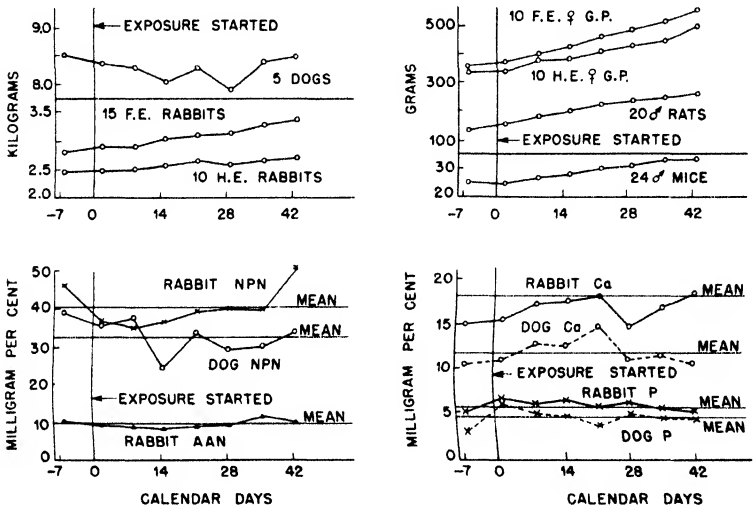


Fig. 10.31—Average toxicologic responses of control animals. Upper left and right, body-weight changes; HE and FE indicate head exposure and full exposure, respectively; lower left, NPN and amino acid nitrogen, AAN, of blood; lower right, calcium and phosphorus of blood.

9.5 Hematology. Twenty hematologic variables were determined weekly on 5 dogs, 10 rabbits, and 10 rats. The dogs and rabbits were those used for the biochemical studies. The first count was made 6 days before the animals were placed in the control chamber; the last count, 24 hr after the animals had been removed from the chamber. Table 10.33 shows the range of the mean values of several of the

* A cutting point is an arbitrary point in a set of values that is used to demarcate a boundary between one set and another. In its application here, cutting point separates normal control values from abnormal experimental values.

Table 10.33—Range of Mean Values of More Important Hematologic Constituents

Variables	Dogs*					Rabbits†					Rats†				
	Low	Count No.‡	High	Count No.‡	P value§	Low	Count No.‡	High	Count No.‡	P value§	Low	Count No.‡	High	Count No.‡	P value§
Red blood cells, $\times 10^6$	5.26	1	6.54	2	>0.05	4.95	8	5.67	4	>0.05	6.18	1	8.66	5	<0.01
Hemoglobin, g/100 ml	12.98	3	14.52	2	>0.05	11.44	2	12.42	5	>0.05	12.42	1	17.62	6	<0.01
Reticulocytes, %	0.34	1	0.72	4	>0.05	3.09	1	5.75	6	>0.01	3.81	5	9.27	2	<0.01
Platelets, $\times 10^5$	1.56	1	2.48	7	0.05>P>0.01	3.00	2	4.05	1	>0.05	3.05	4	7.31	7	<0.01
White blood count	9,170	6	11,830	3	>0.05	8,167	3	10,450	4	>0.05	15,045	3	24,855	5	<0.01
Absolute neutrophils	5,828	6	9,118	1	>0.05	2,500	7	4,217	4	>0.05	3,007	3	5,731	8	>0.05
Absolute lymphocytes	1,372	2	3,225	3	<0.01	4,092	3	5,558	4	>0.05	11,655	3	20,120	5	<0.01

*Five animals were used.

† Ten animals were used.

‡ Count number refers to the order or sequence in which the count was made. Counts were made approximately weekly; thus count 4 occurred in approximately the fourth week.

§ P value refers to statistical probability of the change of a variable to occur by chance (see Chap. 3).

more important hematologic constituents, the time at which the count was taken, and the corresponding P values for the test of significance of the findings.

9.6 Statistical Test of Hemogram. No consistent variation was observed in any of the seven variables for the dog and rabbit. The rats differed in this respect, although the variations that were observed were slight when compared with the values obtained in the initial count. The statistical treatment of seven variables shown in Table 10.33 revealed in only one instance, in that of the absolute lymphocytes (the product of the proportion of lymphocytes and the white blood count) of the dog, a P value smaller than 0.01, thereby indicating that this was the only constituent that did not show a chance variation. This was true similarly for the reticulocytes of the rabbits; for the rats, however, all variables but the absolute neutrophils showed trends not arising from chance throughout the course of the experiment. This would suggest that the dog and rabbit are better species on which to perform hematologic studies than the rat were it not for the fact that a statistical evaluation of the results of a similar 30-day control study showed trends in most of the seven variables in the rabbit and in four of the seven variables in the dog. This statistical treatment serves to emphasize the fact that exceptionally large changes must be obtained before any significance can be attached to hematologic results of experimental animals.

9.7 Discussion. A comparison of the results of the other three control studies with those of the study just detailed shows a low over-all mortality but otherwise shows results very similar to the other four criteria. The over-all mortality of the four control studies that embraced 300 animals was 16, or 5.3 per cent. Of these but 6 of the 300, or 2.0 per cent, died from natural causes; 10, or 3.3 per cent, died from accident. The chief causes of natural death were old age in three mice and pneumonia in three guinea pigs. The chief factor in the accidental deaths of eight rabbits was strangulation because of faulty construction of the head-exposure cages. One guinea pig died from faulty handling.

In view of the appreciable retardation in growth of the head-exposed animals, one may well question the wisdom of using this type of procedure. Although there is no direct proof forthcoming from these control studies favoring the use of this type of exposure, indirect evidence from other experimental studies reported herein indicates that the animals are in a better condition from a nervous standpoint than animals not so restricted. Apart from this also, the head-exposed animals ingest less dust, with consequent less likelihood of toxicity from sources other than inhalation, as compared with fully exposed animals, thus favorably disposing the head-exposed group.

Whereas the histologic results of the two 30-day control exposure studies (Table 10.31) present a fairly representative picture of the normal conditions of the animals, a review of additional histologic results made on animals in two 90-day control studies shows the extent of pathology in other animal groups not subjected to exposure-chamber conditions but maintained continuously in living quarters. Table 10.37, listing the frequency of occurrence in three species of the histologic conditions of the lung and kidney (and occasionally other organs) important in uranium-inhalation studies, shows that, in general, one may expect a certain amount of mild pathology in so-called "normal animals." A greater number of rats than of dogs or rabbits were histologically normal.

10. URANIUM HEXAFLUORIDE

By Charles J. Spiegl*

10.1 Introduction. The large-scale production and utilization of the hexafluoride has required a critical evaluation of the material as a potential industrial health hazard.

The hexafluoride is a volatile compound and is extremely reactive toward most substances. On contact with the moisture of the atmosphere, UF_6 instantaneously hydrolyzes, liberating dense white fumes of uranyl fluoride, UO_2F_2, and hydrogen fluoride, HF, both potentially toxic materials. Accordingly no undecomposed UF_6 was detected† at or below 20 mg UF_6/cu m, the concentration range used in the 30-day studies. Hereafter by UF_6 will be meant the products of hydrolysis of UF_6, i.e., UO_2F_2 and HF and not the unhydrolyzed material introduced into the atmosphere. It is obvious therefore that the toxicologic responses noted during the 30-day studies represent the combined action of these two hydrolysis products, at least at the higher levels of exposure. As the concentration is reduced, the effects of hydrogen fluoride become negligible.

A feature of considerable toxicologic importance of the UF_6 exposures, making this material unique among uranium-dust exposures, was the extreme fineness of the particle size of the uranium available for inhalation. Developed from the gas in essentially molecular form, a fine-particle-size smoke with little tendency to aggregate was produced, with the bulk of the particles less than 0.5 μ in diameter.

* Toxicologic procedures by C. J. Spiegl, A. I. Schepartz, J. Minor, J. Marx, M. Schlamowitz, and U. C. Pozzani.

† Tests made with diphenylamine-impregnated paper according to directions given in a communication from J. Greenspan, Columbia University, indicated the absence of UF_6.

10.2 Special Features of Exposure Equipment. The only important detail of procedure that has not been described under the section on Materials and Methods is that of the specific type of exposure chamber and feed systems employed for UF_6. The exposure chamber embodied only certain of the features described therein. The chief points of difference were in (1) the size, (2) material of construction, and (3) types and arrangement of cages. Because of the highly corrosive nature of HF, the unit was made entirely of Monel metal and because of expense was limited to a 4-ft cube, which was set 3 ft above the floor. Small portable cages of a size suitable for the individual species were placed in two layers within the chamber.

The methods of introducing the toxic material are described in Sec. 6.2d. The vaporization method was used for experiments at 20, 3.0, and 0.3 mg UF_6/cu m, whereas the blending method was used for a second experiment at the 0.3-mg level and also at the 0.075-mg level.

Thirty-day inhalation studies were performed at levels of 20, 3, 0.3, and 0.075 mg UF_6/cu m (equivalent to 13.3, 2.0, 0.2, and 0.05 mg of uranium, respectively) in order to delineate the range of toxicologic response. In one instance, 0.3 mg UF_6/cu m, two studies were performed at the same level, making a total of five 30-day experiments for which the chief toxicological findings are presented. In addition several acute studies with extremely high flood concentrations, of from 1,000 to 2,300 mg UF_6/cu m, were performed in order to estimate the type and degree of damage that might be expected from accidental exposures when huge amounts of material are suddenly released into the atmosphere.

10.3 Concentration of Test Substance in Exposure Atmospheres. The control of concentration within the exposure unit was given particular attention because of the strict dependence of toxicological response on this factor. Hourly sampling of from ⅓ to 1 cu m of air was used to monitor concentration. Data showing the degree of deviation from the desired concentration are presented for the highest and for the lowest, 19.8 and 0.075 mg/cu m, of the five levels studied (Table 10.34).

The table shows that the control of the concentration at the highest level was superior to that at the lowest level, approximately 80 per cent of the samples being within ±10 per cent of the highest concentration, whereas the same percentage of the samples was within ±50 per cent at the lowest level. Intermediate levels showed proportional deviations within these limits. At low concentrations of UF_6 in the air, precise regulation was difficult because of the extremely minute quantities of the material used. Thus at any given instant only 0.46 mg of the compound was present in the entire volume of the 216-cu ft

chamber during exposure of animals to 0.075 mg UF_6/cu m. An increase of only 0.1 mg of UF_6 above the desired amount in the entire exposure unit produced therefore, roughly a 22 per cent elevation in concentration.

Table 10.34 — Dust Concentration

Weighted-mean concentration, mg UF_6/cu m	Deviation, mg UF_6/cu m	Samples within given deviation, %
19.8	±1.5	60
19.8	±2.5	81
19.8	±3.5	94
19.8	±4.5	97

Note: desired concentration, 20 mg UF_6/cu m; standard deviation, 1.7 mg UF_6/cu m; number of samples, 156.

Weighted-mean concentration, mg UF_6/cu m	Deviation, mg UF_6/cu m	Samples within given deviation, %
0.075	±0.023	60
0.075	±0.038	81
0.075	±0.053	92
0.075	±0.068	99

Note: desired concentration, 0.075 mg UF_6/cu m; standard deviation, 0.0225 mg UF_6/cu m; number of samples, 114.

UF_6 was more exactly metered than the other uranium compounds, because of its ease of volatilization. On the other hand, the material created a number of difficulties involved in its introduction into the exposure unit because of its highly corrosive nature, solidification below 62°C, and ready hydrolysis with consequent formation of plugs in tubes and lines.

10.4 30-Day Studies. (a) Signs of Toxicity. The outward toxic signs in the animals ranged from severe irritation of the mucous membranes to nonspecific general indications of ill health. The conjunctivitis made its appearance within from 4 to 5 days after the start of exposure, whereas the generalized effects occurred during the second and third weeks of exposure. In severe poisoning from exposure to the 20-mg level, dogs exhibited complete lack of appetite for a period of a week or more. Accompanying refusal to eat was severe dehydration of the animal characterized by shrinkage of the muscle and an inelastic condition of the skin. Animals succumbing from exposure showed mucous discharge and encrustation about the eyes,

conjunctivitis, severe inflammation of the gums, and weakness and apathy just prior to death. Rats and mice at this level also showed some irritation of the eyes and nose and usually exhibited an unkempt appearance as evidence of ill health. Rabbits and guinea pigs often appeared normal even shortly before death. At lower concentrations, 3 mg or less, few or no symptoms were observed in any species.

(b) Mortality. Uranium hexafluoride was highly lethal at a concentration of 20 mg to all five species. At this level the mortality ranged from 100 per cent in the mouse and rabbits to 40 per cent in the dog. According to mortality the susceptibility of different species may be grouped into three categories: (1) high susceptibility, the mouse and rabbit; (2) medium susceptibility, the rat; and (3) low susceptibility, the guinea pig and the dog (Table 10.35).

The 3-mg level was lethal only to the two most susceptible species, the mouse and rabbit, but represented a sublethal but definitely toxic level for the other species. Thus, roughly a seven-fold decrease in the concentration of UF_6 in the air from 20 to 3 mg reduced the mortality in rats from 75 to 0 per cent, in the guinea pigs from 45 to 5 per cent, and in dogs from 40 to 20 per cent. Because of the high susceptibility of the mouse and rabbit to uranium, the mortality of these species was not appreciably altered by the same decrease in concentration, changing only from 100 to 92 per cent in the mouse and only from 100 to 80 per cent in the rabbit.

At the 0.3-mg level few deaths were occasionally noted. In the most susceptible species the mortality was reduced to 5 per cent in the mouse and to 14 per cent in the rabbit. At the lowest concentration, 0.075 mg, mortality was lower than that found in a similar group of control animals not exposed to uranium dust (Sec. 9).

(c) Weight Response. The growth curves of animals exposed to various concentrations of UF_6 provided an approximate index of toxicity except for the dog. At the higher levels the degree of weight loss was commensurate with the concentration and with species susceptibility. At the 20-mg level all species lost weight, the most pronounced decrease being that in rabbits, which lost 21 per cent during the 30-day period. Guinea pigs showed a decrease of 13 per cent; rats, 6 per cent; and dogs, 3 per cent. At the 3-mg level, rabbits, rats, and dogs showed slight decreases in weight, whereas guinea pigs remained at a stationary level. The higher susceptibility of the rabbit was again in evidence at this concentration. Concentrations at 0.3 mg or lower resulted in only slightly impaired growth in all species. The growth rate at the 0.075-mg level was normal (Table 10.36).

(d) Biochemical Findings. Blood. Determinations of certain chemical constituents of blood showed that nonprotein nitrogen (NPN) and urea nitrogen values were dependable criteria of toxicity, relatively

Table 10.35 — Mortality of Animals Exposed 6 Hr Daily for 30 Days to Atmospheres Containing UF_6

Species	Concentration level of compound, mg UF_6/cu m	No. of deaths per no. exposed	Mortality, %
Mouse	20	20/20	100
	3	23/25	92
	0.3*	1/19	5
Rabbit	20	10/10	100
	3	8/10	80
	0.3	0/10	0
	0.3*	3/12	25
Rat	20	15/20	75
	3	0/20	0
	0.3	1/19	5
	0.3*	1/175	0.5
	0.075*	0/165	0
Guinea pig	20	9/20	45
	3	1/20	5
	0.3	0/20	0
	0.075*	1/30	3
Dog	20	2/5	40
	3	1/5	20
	0.3*	0/5	0
	0.3*,†	0/19	0
	0.075*	0/15	0
Total	20	56/75	75
	3	34/98	35
	0.3	2/91	2
	0.3*,†	4/187	2
	0.075†	1/210	0.5

*Two separate experiments at this level were performed.
† First 30 days of a chronic study.

Table 10.36 — Body-weight Change in Various Species Following Inhalation of UF_6

Concentration level of UF_6, mg/cu m	Weight change,* %			
	Rabbit	Rat	Guinea pig	Dog
20	−21	−6	−13	−3
3	−12	−8	+1	−12
0.3	0	+3	+11	+9
0.3†	+3	+75‡		+10
0.075†		+70‡	+11	−2

*For initial weights, see Tables 10.41 to 10.45.
†Very young, growing animals were used in these experiments.
‡The first 30 days of a chronic study.

specific for renal dysfunction, and readily made and widely accepted. The pattern of increases in NPN and urea nitrogen was uniform for both the rabbit and the dog. Maximal values were obtained from the seventh to ninth days of exposure, followed by a decrease toward normal in surviving animals. In animals that died, peaks were uniformly observed prior to death (Fig. 10.32).

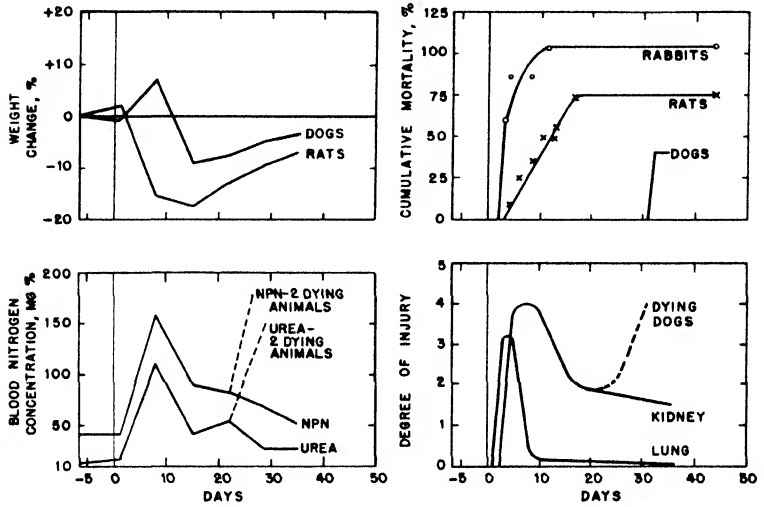


Fig. 10.32 — Typical course of toxicologic responses to UF_6 (20-mg level). Upper left, weight changes of representative species; upper right, cumulative mortality for species of high, intermediate, and low susceptibility; lower left, blood nonprotein and urea nitrogen; lower right, schematic representation illustrating sequence of renal and pulmonary injury.

The maximal values obtained, either individual or mean, decreased with decreasing concentrations of UF_6 in the atmosphere. At the 20-mg level all rabbits and dogs showed azotemia. The maximal value observed in the dog was 316 mg % NPN and 166 mg % urea nitrogen. Rabbits showed values of 372 mg % NPN and 270 mg % urea nitrogen. Decreasing the concentration of UF_6 to 3 mg produced an elevation in the blood constituents of only 1 of 5 dogs, a species less sensitive than the rabbit, and a moderate increase in 5 of 10 rabbits. Maximal values at this level were 157 mg % NPN and 134 mg % urea nitrogen in dogs, 180 mg % NPN, and 119 mg % urea nitrogen in rabbits. Levels of 0.3 mg UF_6 or lower induced only infrequent rises of doubtful significance. It was thus apparent that exposure of dogs and rabbits to atmospheric concentrations of UF_6 ranging from 20 to 3 mg

produced severe azotemia in these species. Maximal effects occurred at the higher level of exposure during the seventh to ninth days (Table 10.36).

Urine. Analysis of urinary constituents showed that rabbits exposed to the 20-mg level suffered from marked proteinuria. Rabbits exposed to 3 mg showed a slight and infrequent appearance of protein, and those exposed to either the 0.3- or 0.075-mg level exhibited only insignificant changes. Dogs exposed to 0.3- and 0.075-mg levels, respectively, showed only traces of protein in the urine (Table 10.37).

Table 10.37 — Biochemical Results for Animals Exposed 6 Hr Daily for 30 Days to Atmospheres Containing UF_6

Concentration level of compound, mg UF_6/cu m	Species	Blood			Urine protein
		NPN, mg %	Urea nitrogen, mg %	Remarks	
20	Dog	Max. value 316	Max. value 166	All animals showed azotemia	Not determined
20	Rabbit	Max. value 372	Max. value 270	All animals showed azotemia	Marked proteinuria
3	Dog	Max. value 157	Max. value 134	Elevation in blood NPN and urea N in 1 of 5 animals	Not determined
3	Rabbit	Max. value 180	Max. value 119	Moderate increases in blood NPN and urea N in 5 of 10 animals	Not determined
0.3	Rabbit	Not determined	Not determined		Slight, infrequent proteinuria
0.3*	Dog	Insignificant rises	Insignificant rises		Insignificant rises
0.3*	Rabbit	Insignificant changes	Insignificant rises		Insignificant changes
0.075*	Dog	Insignificant changes	Insignificant rises		Insignificant changes

*Refers to the first 30 days of a chronic study.

(e) Pathology. The most characteristic pathologic findings in animals exposed for a 30-day period to UF_6 were represented by renal-tubular degeneration followed by regeneration. At high levels of UF_6 concentration in the atmosphere, pulmonary hemorrhage and edema were also observed. No other tissues consistently showed damage.

All species exposed to 20 mg of UF_6 showed severe renal-tubular injury characterized by degeneration, necrosis, regeneration, and the presence of casts in the tubules. At this relatively high level of exposure, dying animals showed pulmonary pathology typical of acute HF poisoning. The lungs showed edema, hemorrhage, and emphysema in some cases. Varying degrees of acute inflammation were found in

bronchi, alveoli, and alveolar interstices. Animals sacrificed after 30 days showed much milder pulmonary injury than those dying 4 to 9 days after exposure.

Table 10.38 -- Pathology of Animals Exposed for 30 Days to Atmosphere of UF_6

Concentration level of compound, mg UF_6/cu m	Dog	Rat	Rabbit
20	Severe pulmonary edema and hemorrhage; extensive renal tubular damage	Early severe pulmonary irritation; renal tubular necrosis followed by regeneration*	Pulmonary edema and hemorrhage; severe renal tubular damage
3	Slight pneumonia; moderate renal tubular damage	Moderate to severe renal injury*	Severe pulmonary edema; moderate to severe renal tubular damage
0.3	Mild renal changes	Mild renal damage at 6 to 9 days, no significant damage at 30 days*	Mild to moderate renal damage
0.3†	Slight indications of renal tubular damage in sections taken at 10-day biopsy	Mild renal damage in rats sacrificed at 5 to 7 days; none at later periods*	Not observed
0.075†	No tissue changes in sections taken at 10-day biopsy	No changes related to exposure*	Not observed

*Rats studied by serial sacrifice.
†First 30 days of a chronic study.

At the 3-mg level, moderate to severe renal injury was noted in dogs, rabbits, and rats. Only one dog of five, which was sacrificed when moribund after approximately 3 weeks of exposure, showed extensive tubular necrosis and regeneration in the kidney cortex. The other four dogs sacrificed after 30 days showed renal pathology of a more chronic nature—typical and atypical regeneration of kidney tubules limited mostly to the corticomedullary region. Renal injury of moderate to moderately severe degree as well as evidence of acute pulmonary irritation was found in dying rabbits but only regenerative renal changes in surviving animals. Serial sacrifice of rats revealed moderate renal tubular degeneration and necrosis that was followed by tubular regeneration. There was not, however, complete repair by the end of the 30-day period (see Chap. 4).

At the 0.3-mg level, mild but consistent changes characterized by tubular regeneration in the corticomedullary zone were present in the kidneys of dogs. Rats and rabbits showed a mild renal tubular damage of a degenerative nature. The incidence of detectable alteration in

rats was low, but the animals exhibiting the changes were those sacrificed after 6 and 9 days of exposure. This fact is considered of significance, since, in a second study at 0.3 mg/cu m, slight tubular necrosis was noted in rats sacrificed at from 5 to 7 days but none in those sacrificed at 1, 2, 3, 14, or 28 days (Table 10.38). At the 0.075-mg level, no injury was found in rats sacrificed after 1, 2, 3, 4, 5, 7, 14, and 28 days (see Chap. 4).

(f) Summary of Histologic Findings. Histologic studies confirmed and amplified the toxic indications from the mortality rate among three species.

1. The most characteristic histologic changes were renal, characterized by tubular degeneration and necrosis followed by regeneration. The damage diminished in intensity with the decreasing concentration of the compound in the air until at 0.075 mg/cu m no injury was evident.

2. At relatively high levels of UF_6 concentration in the atmosphere (20 mg/cu m), early pulmonary hemorrhage and edema similar to that found in HF poisoning occurred. Resolution of the pulmonary condition was apparently effected quickly thereafter if the animal survived.

3. The critical period of maximal damage was found to be almost invariably from 7 to 9 days after the start of exposure.

4. Of three species studied histologically (the rabbit, rat, and dog) the rabbit was found to be the most susceptible to pulmonary damage.

(g) Hematology. Hematologic examination revealed a remarkable constancy in 20 variables, primarily cytologic, of the blood following exposure to uranium hexafluoride. No significant changes were noted in numerical values of hemoglobin, red blood count (RBC), white blood count (WBC), absolute number of neutrophils and lymphocytes, or differential WBC in dogs, rabbits, or rats exposed to any of the levels of UF_6. An apparent decrease in eosinophils was observed in dogs at the 20-mg/cu m level. At this level also rats showed a slight drop, according to a statistical test of probability, in RBC and hemoglobin. Both of these changes, however, were on the borderline of significance and were complicated by wide fluctuations of values even during the control period. Accordingly, concentrations of UF_6 up to 20 mg/cu m, which were sufficient to produce over-all mortality of 79 per cent, did not produce notable changes in 20 hematologic constituents.

(h) Distribution of Uranium in Tissues. The content of uranium in tissues was determined spectrochemically, and, although this method was neither so sensitive (the lower limit of quantitative estimation for soft tissue was 0.2 μg U/g; for bone, 5 μg U/g) nor so accurate as might have been desired, nevertheless it was capable of giving valuable preliminary information.

Animals exposed for 30 days showed little evidence of deposition or storage of uranium. The highest frequency of occurrence, as well as the largest concentration of uranium, has been found in the kidney with lesser amounts in the lung. No very high concentrations were found in the lung because of the ready solubility of UO_2F_2 in body fluids. Only small, relatively infrequently occurring amounts have ever been found in the liver and in the bones studied, namely, the pelvis, femur, the femoral epiphysis and diaphysis, and the spinal column. These data were obtained by spectrochemical analysis, however, and somewhat different results were obtained by the more sensitive fluorophotometric method (see Chap. 2).

Among individuals of a given species, the deposition of uranium varies considerably. Thus, at the 20-mg level, concentrations range from less than 0.2 to 7.3 μg/g in rat kidneys. Occasionally higher values were found in the lung than in the kidney, but with a larger variability among different animals. The liver of rats exposed at this level contained amounts on the order of 0.4 μg. Few samples of bone contained more than 5 μg/g. Results cited for rats were typical of dogs and rabbits. The apparent lack of storage of the element following the inhalation of UF_6 is further substantiated by the finding that no significant increase in concentration was observed upon increased exposure of the animals.

At decreasing concentrations of UF_6, although uranium was found less frequently, the amounts present were not widely different from those found at higher concentrations. At 0.075 mg UF_6/cu m in the atmosphere, only 2 of 62 tissues analyzed contained detectable amounts of uranium (Table 10.39).

Table 10.39 — Frequency of Occurrence of Detectable Amounts of Uranium in Tissues of Exposed Dogs, Rats, and Rabbits

Concentration level of compound, mg/cu m	Kidney		Lung		Liver		Bone*	
	No. positive per no. exposed†	Freq., %	No. positive per no. exposed†	Freq., %	No. positive per no. exposed†	Freq., %	No. positive per no. exposed†	Freq., %
20	38/45	84	30/49	61	11/49	22	24/151	16
3	18/52	35	12/52	23	3/50	6	1/48	2
0.3	12/66	18	6/64	9	6/66	9	0/46	0
0.075	0/21	0	2/20	10	0/21	0		

* Bones studied included epiphysis, diaphysis, pelvis, and whole femur.

† A value was considered positive if greater than 0.2 μg U/g of soft tissue or 5.0 μg U/g of bone as analyzed by the spectrochemical method.

A summary of the results of the criteria of toxicity are given separately for each of the five studies in Tables 10.41 to 10.45.

10.5 Study of Animals Exposed to Flood Concentrations. In order to determine the type of toxic response arising from flood exposures

such as might on occasion arise in accidents, groups of laboratory animals were exposed for 10 min to concentrations of UF_6 within the range of 942 to 2,284 mg/cu m. This produced a smoke that was optically impenetrable through a 3-ft layer. At these high concentrations it is possible that considerable amounts of UF_6 may have existed unhydrolyzed in the exposure chamber for short periods owing to the lack of sufficient moisture in the air for complete hydrolysis. Accordingly a pathology peculiar to molecular UF_6 is a possibility. On the other hand, at these concentrations a potential atmosphere of from 215 to 515 mg/cu m of HF could arise from complete hydrolysis, an overwhelmingly lethal dose of fluoride (see Chap. 17, section on HF).

The UF_6 was introduced into the exposure chamber in a continuous stream. Concentrations were measured as described in Sec. 8 with six 1-min samples taken during the 10-min period.

(a) Results. Flood concentrations of UF_6 for 10 min were highly irritating to rats, mice, and guinea pigs. Otherwise the responses were the same as those produced by exposure to 20 mg/cu m.

(b) Signs of Toxicity. Toxic signs were most pronounced in mice, less so in rats, and least in guinea pigs. Upon removal from the chamber following the 10-min exposure, all animals were gasping for breath, conjunctivitis was present in most animals, and the eyes of mice were encrusted and sometimes closed. All species showed severe irritation of the nasal passages typical of chemical irritation. The acute irritation persisted in surviving animals for a period of several hours.

(c) Mortality. Exposures of animals for 10 min produced mortality in all three species. In mice the percentage ranged from 20 at 942 mg UF_6/cu m to 95 at 2,284 mg UF_6/cu m. In rats and guinea pigs the

Table 10.40 -- Percentage Mortality at Flood Concentration

Concentration level of material	Mouse*		Rat†		Guinea pig‡	
	Mortality during exposure, %	Mortality during 30 days after exposure, %	Mortality during exposure, %	Mortality during 30 days after exposure, %	Mortality during exposure, %	Mortality during 30 days after exposure, %
2,284 mg UF_6/cu m (1,540 mg U/cu m)	30	95	0	75	27	40
1,083 mg UF_6/cu m (730 mg U/cu m)	0	70	0	30	0	20
942 mg UF_6/cu m (636 mg U/cu m)	0	20	0	10	0	13

* Twenty mice were used at each concentration level in this experiment.
† Twenty rats were used at each concentration level in this experiment.
‡ Fifteen guinea pigs were used at each concentration level in this experiment.

ranges were from 10 to 75 per cent and from 13 to 40 per cent, respectively, for the same concentrations. The only animals dying during the exposure period were six mice and four guinea pigs, all at the 2,284-mg UF_6/cu m level (Table 10.40). The period of maximal incidence of mortality lasted from the fifth through the eighth day of observation after exposure, with only a few animals succumbing after the tenth day.

(d) Pathology. Rats sacrificed in a daily serial study after the termination of exposure showed severe damage to the renal cortical tubules. This type of injury was at its maximum in animals sacrificed between 5 and 8 days after exposure. Processes of regeneration followed renal tubular damage (pulmonary damage in a large number of animals was observed grossly by the staff of the Inhalation Section). Pulmonary changes in animals sacrificed 1 to 4 days after inhalation of the UF_6 were mild and of an inflammatory nature. One rat dying on the fifth day showed rather severe acute lung injury.

(e) Discussion. The above results show that UF_6 was highly toxic. A dust concentration of 3 mg/cu m and above caused a high mortality in almost all the species exposed, pronounced tissue injury, depressed growth rate, and changes in biochemical constituents of blood and urine. No changes were observed in the hematologic constituents.

A sequence of two effects was characteristic of exposure to this compound at the higher levels. The primary effect was pulmonary irritation, first noted after 1 day of exposure but disappearing after the fifth day. At this time the secondary effect, renal injury, became dominant.

It is believed that the two hydrolysis products of UF_6 are responsible for the separate effects. Hydrogen fluoride is known to be a severe pulmonary irritant, but uranyl fluoride probably contributes to this effect because of its fluorine content. On the other hand, only the uranyl fluoride gives rise to the observed renal damage.

Except under the very unusual circumstances of flood concentration, after which animals died within 2 hr presumably of pulmonary damage from the combined toxic agents, uranyl fluoride alone is undoubtedly the chief toxic agent. This is especially true at lower levels where renal injury is the principal cause of death. In addition to its high concentration of uranium, 77 per cent, uranyl fluoride is extremely soluble in body fluids and is rapidly absorbed by the lungs. Although the lung is the principal site of entry of the compound into the body, uranium is found more frequently in the kidney as shown by spectrochemical analysis. The presence of uranium in the kidney interferes with the normal functioning of this organ. As a consequence, blood urea nitrogen and nonprotein nitrogen concentrations

Table 10.41 — Animals Exposed for 30 Days to 20 Mg UF_6/cu m (Summary of Results)

1. Mortality. Uranium hexafluoride was one of the most toxic of uranium compounds.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	20/20	100
Rabbit	10/10	100
Rat	15/20	75
Guinea pig	9/20	45
Dog	2/5	40

2. Weight Changes. Uranium hexafluoride caused weight loss in all species.

Species	Mean initial wt., g	Wt. change, %
Rabbit	3,700	−21
Rat	250	−6
Guinea pig	690	−13
Dog	6,600	−3

3. Biochemistry.

Blood, Dogs and Rabbits. High values of blood urea N and NPN during the first 8 days of exposure were followed by normal values at the end, indicating recovery by surviving animals. Early deaths among rabbits prevented accumulation of extensive data.

		Calendar days							
Species	Determination	−13	−6	+1	+8	+15	+22	+29	+36
Dog	Urea N, mg %*	14	11	14	119	40	57	76	25
Rabbit	Urea N, mg %*	18	16	15	270				
Dog	NPN, mg %*	35	40	39	160	89	85	157	55
Rabbit	NPN, mg %*	57	51	50	372				

* Mean values.

Urine, Rabbits. High protein values were observed before death. Anuria was noted in 50 per cent of animals for several days.

4. Pathology.

Dog. Severe pulmonary edema and hemorrhage. Extensive renal tubular damage.

Rat. Early severe pulmonary injury. Tubular necrosis followed by regeneration in the kidney.

Rabbit. Pulmonary edema and hemorrhage. Severe renal tubular damage.

5. Hematology. No significant changes.

become elevated, protein appears in the urine, and, upon autopsy, renal tubular necrosis is evident.

The simultaneous presence of uranium and fluoride in the hydrolysis products of uranium hexafluoride, UO_2F_2 and HF, appears to produce an additive toxicologic response. At levels of 20 mg of UF_6,

Table 10.42 — Animals Exposed for 30 Days to 3 Mg UF_6/cu m (Summary of Results)

1. Mortality. The most susceptible species (mouse and rabbit) showed high mortality. For all other species this 3 mg/cu m represented a borderline level.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	23/25	92
Rabbit	8/10	80
Rat	0/20	0
Guinea pig	1/20	5
Dog	1/5	20

2. Weight Changes. Weight losses were noted in all species except the guinea pig.

Species	Mean initial wt., g	Wt. change, %
Rabbit	3,000	−12
Rat	230	−8
Guinea pig	500	+1
Dog	11,700	−12

3. Biochemistry. High values of blood urea N and NPN during the first 8 days of the exposure were followed by low (recovery) values at the end.

			Calendar days				
Species	Determination	Control period	+1	+8	+15	+22	+29
Dog	Urea N, mg %*	10	35	42	32	41	11
Rabbit	Urea N, mg %*	13	13	115	79	44	11
Dog	NPN, mg %*	30	56	89	54	82	36
Rabbit	NPN, mg %*	38	55	180	112	90	37

* Mean values.

4. Pathology.
   Dog. Mild pneumonia. Moderate renal tubular damage.
   Rat. Moderate to severe renal injury.
   Rabbit. Severe pulmonary edema. Moderate to severe renal tubular damage.
5. Hematology. No significant changes.

or above, the influence of one element on the degree of injury caused by the other may be important. Thus, at the level mentioned, pulmonary pathology similar to that caused by HF was found even though the concentration of HF (3.5 mg/cu m) was, judging from other experiments (Chap. 17), insufficient of itself to produce acute pulmonary injury. Likewise, the UO_2F_2 formed in the presence of HF by the hydrolysis of UF_6 caused a higher mortality from the type of renal

Table 10.43 — Animals Exposed for 30 Days to 0.3 Mg UF_6/cu m (Summary of Results)

1. Mortality. The low mortality indicated that this level was essentially nontoxic.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	1/19	5
Rabbit	0/10	0
Rat	1/19	5
Guinea pig	0/20	0
Dog	0/5	0

2. Weight Changes. No weight losses were recorded.

Species	Mean initial wt., g	Wt. change, %
Rabbit	4,000	0
Rat	225	+3
Guinea pig	700	+11
Dog	8,800	+9

3. Biochemistry. Slight, infrequent proteinuria occurred in rabbits.
4. Pathology.
   Dog. Mild renal changes.
   Rat. Mild renal damage at 6 to 7 days. Very little damage at 30 days.

involvement found in uranium toxicity than did a corresponding level of UO_2F_2 used alone.

At levels of 3 mg of UF_6 or lower, the amount of HF present is too small to give an effect of its own or to influence appreciably the toxicity of uranium. Consequently at these lower levels the response is essentially that of UO_2F_2.

In contrast to the severe renal and pulmonary injury, no changes in 20 blood cytologic constituents were found. It must be concluded from the 30-day studies, therefore, that the commercially available uranium hexafluoride is not a hemocytotoxic agent. One must further conclude that under the conditions of these experiments insufficient radiation is emitted from the amounts of uranium retained by the body to affect the hemogram.

The toxicity picture typical of uranium hexafluoride is portrayed best by concentration levels permitting partial survival of the animal group. Maximal effects are noted from the fifth to eighth days. During this time a high rate of mortality, elevation of blood urea nitrogen and nonprotein nitrogen, weight loss, and renal pathology are seen. Animals surviving approximately ten days of exposure show a gradual return toward normal and an improvement in their general condition

Table 10.44 — Animals Exposed for 30 Days to 0.3 Mg UF_6/cu m (Summary of Results)
(First 30 days of the chronic run)

1. Mortality. The low mortality indicated that this level was essentially nontoxic except to rabbits.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	3/12	25
Rat	1/175	1
Dog	0/19	0

2. Weight Changes. No weight losses were recorded.

Species	Mean initial wt., g	Wt. change, %
Rabbit	2,700	+3
Rat	155	+75
Dog	7,600	+10

3. Biochemistry. No significant changes occurred in the blood or urine of dogs and rabbits.
4. Pathology.
   Dog. Indications of slight tubular damage in biopsy sections taken at 10 days.
   Rat. Mild renal damage in rats sacrificed at 5 to 7 days. None at later periods.
5. Hematology. No significant changes were found.

Table 10.45 — Animals Exposed for 30 Days to 0.075 Mg UF_6/cu m (Summary of Results)
(First 30 days of a chronic study)

1. Mortality. Very low mortality indicated that this level was nontoxic.

Species	No. of deaths per no. exposed	Mortality, %
Rat	0/165	0
Guinea pig	1/30	3
Dog	0/15	0

2. Weight Changes. An insignificant decrease in weight was noted in dogs. Other species grew normally.

Species	Mean initial wt., g	Wt. change, %
Rat	160	+70
Guinea pig	875	+11
Dog	7,600	+2

3. Biochemistry. No significant changes occurred in the blood or urine of dogs.
4. Pathology. No significant damage was found in dogs or rats.
5. Hematology. No significant changes were found.

despite continued daily exposure. Thus, exposure to UF_6 may result in early pulmonary injury followed by severe renal damage and a return to apparently normal functional state.

(f) Summary. The following are the toxicological effects found upon exposure of laboratory animals to UF_6.

1. At high concentrations (942 mg UF_6/cu m and above) the compound is severely irritating to the eyes, nose, and mucous membranes observed clinically.

2. Mortality among rabbits, mice, guinea pigs, and dogs is high at 20 mg UF_6/cu m. At 3 mg UF_6/cu m only the rabbits and mice are affected, and at 0.3 mg UF_6/cu m only few deaths occur in any species. A level of 0.075 mg UF_6/cu m is nontoxic. The most sensitive species are the rabbit and the mouse.

3. Weight changes are pronounced at 20 mg UF_6/cu m, decreasing to minimal at 0.3. No abnormal effects are encountered during exposure to 0.075 mg.

4. Marked elevations in blood urea nitrogen and nonprotein nitrogen concentrations are found at the higher levels of exposure. Below 3 mg UF_6/cu m, no azotemia is apparent. The appearance of protein in the urine parallels azotemia.

5. At 20 mg UF_6/cu m, severe edema and hemorrhage of the lungs is found at from 2 to 4 days.

6. Severe renal tubular necrosis and degeneration are found in all species exposed at 20 to 3 mg UF_6/cu m. Damage is minimal at 0.3 and absent at 0.075 mg/cu m.

7. Resolution of the lungs apparently takes place following the second week of exposure to 20 mg/cu m in surviving animals.

8. No significant hematological changes occur.

9. Only small amounts of uranium are found in tissues. In order of decreasing frequency, the element is found in kidney, lung, liver, and bone.

10. Animals surviving approximately 2 weeks of exposure tend to return toward normal in all criteria studied despite their continued exposure to UF_6 for a total of 30 days.

11. URANYL FLUORIDE

By Aser Rothstein*

11.1 Introduction. It has been pointed out in the previous section dealing with uranium hexafluoride that a close relationship exists between this compound and uranyl fluoride. On exposure of uranium

* Work done by A. Rothstein and P. Dygert, toxicologists; assisted by D. Dittman, H. Berke, S. Laskin, and H. Oberg. Renal clearances were by H. Wills, physiologist.

hexafluoride to the atmosphere, the uranium appears wholly in the form of uranyl fluoride, along with another product, hydrogen fluoride. Accordingly, uranyl fluoride is one of the chief toxic agents when the hexafluoride is liberated into the atmosphere. In order to define the toxicity of the uranyl fluoride separately from that of either the hexafluoride or hydrogen fluoride, a series of four 30-day studies were undertaken to determine the toxicological responses of several species of laboratory animals exposed to inhalation of UO_2F_2 dust at concentrations of 12, 2.5, 0.6, and 0.2 mg/cu m of air (equivalent to 9.2, 1.9, 0.46, and 0.15 mg of uranium, respectively).

Although uranyl fluoride contains two potentially toxic elements, uranium in amount of 77 per cent and fluorine in amounts of 12 per cent, it was found that uranium was by far the most injurious, at least during short-term experiments. Therefore all references to toxicological responses in this section are concerned with the effects of uranium alone.

As used in these experiments, uranyl fluoride was a light-yellow, soluble, crystalline anhydrous powder, acquiring, when introduced into the atmosphere, 2 molecules of water of crystallization. In this form it is somewhat hygroscopic but is sufficiently stable to permit relative ease of introduction into the exposure unit as a dust. Other than its high water solubility, uranyl fluoride has no other physicochemical properties of interest from a toxicologic standpoint.

11.2 Exposure Unit. The animals were exposed in a typical head-exposure unit as described in Sec. 6.1f for three of the four levels studied. Only the rabbits, cats, and guinea pigs were head exposed; the dogs, rats, and mice were bodily exposed. One of the studies, that at the highest level, was performed in a full-exposure unit, a 4-ft cube in which all animals were bodily exposed.

The dust feed used in three of the four experiments was of the pressure-feed type described in Sec. 6.2. That used in conjunction with the experiment performed in the full-exposure unit (12-mg level) was a crude elutriator on which no provision was made for a continuous, controlled rate of dust flow.

11.3 Exposure Conditions. The dust concentrations with standard deviations for the four experiments were 12.2 ± 5.7, 2.8 ± 1.4, 0.65 ± 0.23, and 0.19 ± 0.08 mg of UO_2F_2/cu m of air (9.2, 2.2, 0.50, and 0.15 mg U/cu m of air). Table 10.46 indicates the variation encountered in the chamber-dust concentration during each experiment. It is readily seen that in no experiment was the concentration really constant but was maintained within a relatively narrow range about the desired mean. However, there was certainly good differentiation between various experiments, with little overlapping of concentration ranges. Only in the 12.2-mg level experiment were the deviations

Table 10.46 — Dust Concentration

Experiment 1

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
12.2	±4.0	45
12.2	±6.0	62
12.2	±8.0	79
12.2	±10.0	90

Note: desired concentration, 20.0 mg UO_2F_2/cu m; standard deviation, ±5.73 mg/cu m; number of samples, 141.

Experiment 2

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
2.8	±1.0	50
2.8	±1.5	70
2.8	±2.0	81
2.8	±2.5	91

Note: desired concentration, 2.5 mg UO_2F_2/cu m; standard deviation, ±1.4 mg/cu m; number of samples, 150.

Experiment 3

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
0.65	±0.1	29
0.65	±0.2	56
0.65	±0.3	73
0.65	±0.4	88

Note: desired concentration, 0.50 mg UO_2F_2/cu m; standard deviation, ±0.23 mg/cu m; number of samples, 144.

Experiment 4

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
0.186	±0.05	44
0.186	±0.10	69
0.186	±0.15	94
0.186	±0.20	98

Note: desired concentration, 0.20 mg UO_2F_2/cu m; standard deviation, ±0.08 mg/cu m; number of samples, 218.

sufficiently large to place some question on the validity of the mean concentration as a measure of toxicological events, since at some intervals the concentrations were as high as 25 mg/cu m.

The temperature of the air within the chamber in three of the four experiments was maintained thermostatically at 70 to 72°F, whereas no attempt was made to control the air temperature in the other (12.2-mg level) experiment. There seems to be no doubt that the air temperature in the latter experiment was at times undesirably high (possibly 85 to 90°) and might have influenced toxicologic response.

11.4 Exposure Schedule. A similar pattern of procedure was used in all four experiments. Animals were exposed to UO_2F_2 dust for 6 hr a day, 6 days a week for 5 weeks. Preceding the exposure period there was a 2-week conditioning period during which the animals were acclimatized to the exposure conditions (except in the 12.2-mg level). During the exposure period, animals were sacrificed at intervals and tissues were taken for histological examination. At the end of the exposure period, large groups of animals were sacrificed both for histological study and also for analysis of tissues for uranium. Each week animals were weighed, and blood samples were taken for hematological and biochemical studies. Urine samples were taken daily for analysis, and renal-function tests were performed at intervals. These tests were performed in a manner similar to that described in Sec. 8.3.

11.5 Signs of Toxicity. Acute signs were apparent at only the highest (12.2-mg) level in dogs and cats. These animals exhibited anorexia after a few days of exposure followed by rhinitis and polydypsia. Vomiting that later became bloody was a terminal symptom, as were severe muscular weakness and general lassitude.

11.6 Mortality. At the 12.2-mg level over 50 per cent of all species except the rat died as a result of exposure; however, at the 2.8-mg level there was only 13 and 26 per cent mortality in rabbits and mice, respectively, but none in dogs, cats, and rats, and essentially none in guinea pigs. At the 0.65-mg level, only mice showed a significant mortality, and at the 0.19-mg level there was no significant mortality in any of the species (Table 10.47).

There was certainly a marked species difference in susceptibility. Mice were the most susceptible, followed by rabbits, then dogs, cats, and guinea pigs and finally rats, with no deaths even at the highest level. The levels for which no significant mortality resulted among the six species were as follows:

Rat, 12.2 mg/cu m and lower; dog, 2.8 mg/cu m and lower; cat, 2.8 mg/cu m and lower; guinea pig, 2.8 mg/cu m and lower; rabbit, 0.65 mg/cu m and lower; mouse, 0.19 mg/cu m and lower.

Table 10.47 — Mortality of Animals Exposed Daily to UO_2F_2 Dust

Species	Concentration level of UO_2F_2, mg/cu m	No. of deaths per no. exposed	Mortality, %
Mouse	12.2	20/20	100
	2.8	11/42	26
	0.65	7/51	14
	0.19	2/41	5
Rabbit	12.2	5/6	83
	2.8	3/24	13
	0.65	1/24	4
	0.19	1/21*	5
Dog	12.2	2/2	100
	2.8	0/6	0
	0.19	0/6	0
Cat	12.2	2/2	100
	2.8	0/4	0
Guinea pig	12.2	11/20	55
	2.8	1/30	3
Rat	12.2	0/20	0
	2.8	0/30	0
	0.65	0/10	0
	0.19	0/10	0

* Two additional rabbits died, one of peritonitis and one with severe liver necrosis, associated possibly with the presence of "snuffles" (Pasteurella lepiseptica) in the colony.

11.7 Weight Response. At the 12.2-mg level, dogs, rabbits, and guinea pigs showed an appreciable weight loss, compared to a slight dip in weight at the 2.8-mg level for rabbits and guinea pigs, but not for dogs or rats. At the 0.65- and 0.19-mg levels there were no significant changes in body weights of any of the species (Table 10.48). Thus guinea pigs and rabbits tended to show the greatest weight losses, followed by dogs. Rats showed the least effect.

Table 10.48 — Body-weight Changes in Animals Exposed to Uranyl Fluoride

Concentration level of UO_2F_2, mg/cu m	Weight, change			
	Dog	Rabbit	Guinea pig	Rat
12.2	Severe loss	Severe loss	Severe loss	Moderate loss
2.8	None	Moderate loss	Moderate loss	None
0.65	No data	None	No data	None
0.19	None	None	No data	None

11.8 Hematology. Hematological studies were made on dogs and rats at the 12.2- and 2.8-mg levels. No significant changes were observed.

11.9 Biochemical Findings. A number of biochemical tests have been useful as sensitive indicators of renal damage. The urinary protein, catalase, and amino acid nitrogen have proved to be the most sensitive and earliest indicators; changes appeared within 3 to 5 days. The blood nonprotein nitrogen was elevated above normal after 1 week and was a sign of more severe damage. All the variables except urinary amino acid nitrogen, after a marked initial rise, tended to return toward normal during the latter part of the exposure period. In Table 10.49 the biochemical data for rabbits are summarized in terms of the number of animals showing a particular abnormality, the frequency of abnormal values during the exposure period for each variable, and the extent of the abnormality as measured by the mean increase in the concentration of the variable during the exposure period.

At the 12.2-mg level, the blood NPN rose in every animal compared to 6 of 10 animals at the 2.8-mg level, 2 of 10 at the 0.65-mg level, and none of 15 animals at the 0.19-mg level. Similarly the frequency of occurrence of abnormal values decreased from 56 per cent at the 12.2-mg level to 2 per cent at the 0.19-mg level, with intermediate values for the 2.8- and 0.65-mg levels. Other variables also showed a progressive decrease in the number of abnormal values as the dust concentration was lowered. In so far as the biochemical results are concerned, the levels might be rated for toxic response as follows: extremely toxic, 12.2 mg/cu m; very toxic, 2.8 mg/cu m; moderately toxic, 0.65 mg/cu m; very slightly toxic, 0.19 mg/cu m.

A comparison of the four variables studied in this experiment indicates that the urinary amino acid nitrogen was most sensitive in this particular experiment, followed by catalase, protein, and blood nonprotein nitrogen. For example, at the 0.65-mg level, 8 of 10 animals showed abnormal urinary amino acid nitrogen compared to 6 of 10 for catalase, 4 of 10 for protein, and 2 of 10 for nonprotein nitrogen. The frequency of abnormal values was 28 per cent for amino acid nitrogen, 19 per cent for catalase, 16 per cent for protein, and 9 per cent for NPN.

Fewer studies were made on dogs. At the 12.2-mg level, two out of two dogs showed a marked rise in blood NPN (over 200 mg %). At the 0.19-mg level, none out of three dogs showed an abnormal blood NPN or urinary amino acid, and one out of three dogs showed a rise in urinary protein.

11.10 Uranium Content of Tissues. After the end of the 5 weeks of exposure, dogs, rabbits, and rats were killed, and various tissues were analyzed for uranium content by the spectrochemical method.

Table 10.49 — Biochemical Findings

Concentration level of dust, mg/cu m	Blood nonprotein nitrogen			Urine								
				Protein			Catalase			Amino acid nitrogen		
	No. of abnormal animals per no. animals studied	Freq., %	Δ E, * mg %	No. of abnormal animals per no. animals studied	Freq., %	Δ E, * mg %	No. of abnormal animals per no. animals studied	Freq., %	Δ E, * mg %	No. of abnormal animals per no. animals studied	Freq., %	Δ E, * mg %
12.2	5/5	56	63									
2.8	6/10	24	12	10/10	52	41				10/10	69	35
0.65	2/10	9	6	4/10	16	8	6/10	19	11	8/10	28	10
0.19	0/15	2	0	1/15	6	0	1/9	2	6	1/7	8	1

*Mean increase during exposure.

Table 10.50 — Frequency of Occurrence of Detectable Amounts of Uranium in Tissues

Species	Concentration level of exposure, mg/cu m	Lung		Liver		Kidney		Femur		Spinal column		Pelvis	
		No. with U per no. examined	Freq., %	No. with U per no. examined	Freq., %	No. with U per no. examined	Freq., %	No. with U per no. examined	Freq., %	No. with U per no. examined	Freq., %	No. with U per no. examined	Freq., %
Rabbit	12.2	6/7	86	1/7	16	4/7	57	0/3	0				
	2.8	5/10	50	8/10	80	9/10	90	8/9	89	6/9	67	8/10	80
	0.65	0/10	0	3/10	30	1/10	10	0/10	0	2/10	20	1/10	10
	0.19	0/10	0	1/9	11	1/10	10	0/10	0			0/10	0
Rat	12.2	5/10	50	1/10	10	9/11	82						
	2.8	8/10	80	0/10	0	6/10	60	9/1	100	8/9	89	7/9	78
	0.65	1/10	10	1/10	10	1/10	10			2/10	20	2/9	22
	0.19	2/10	20	0/10	0	2/10	20	0/10	0			0/10	0
Dog	12.2	2/2	100	2/2	100	2/2	100	0/2	0				
	2.8	3/4	75	1/4	25	5/5	100	3/3	100	3/5	60	4/5	80
	0.19	0/2	0	0/3	0	1/3	33	0/3	0			0/3	0

Unfortunately the sensitivity of the method was changed during the course of the experiments so that the results are not strictly comparable.

Table 10.50 indicates that in general uranium appeared with greater frequency when the dust concentration was increased, appearing with varying frequency in the lung, liver, kidney, femur, spinal column, and pelvis. No particular species differences were observed except in the rat liver, which showed lower frequencies than rabbit and dog livers.

11.11 Renal-function Tests. Renal-function tests were performed weekly on rabbits exposed to the 2.8-, 0.65-, and 0.19-mg levels. Clearances of chloride, diodrast, and inulin were used as measures of tubular reabsorption, tubular excretion, and glomerular filtration, respectively. Table 10.51 indicates that severe disturbances of renal function occurred only in the highest level studied (2.5-mg experiment), with marked changes in all three functions. The most severe changes occurred within the first 2 weeks with a striking increase in chloride clearance and a decrease in diodrast and inulin clearance. Thereafter the latter clearances tended to return to normal, whereas the chloride remained high. At the 0.65-mg level, only the animal at 14 days showed a rise in chloride clearance and a drop in diodrast. At the 0.19-mg level the diodrast and inulin were even higher than normal, particularly in the animal at 21 days.

Table 10.51 — Renal-function Tests on Animals Exposed to Uranyl Fluoride

Days of exposure	Clearances								
	Chloride, % of normal			Diodrast, % of normal			Inulin, % of normal		
	2.8-mg level	0.65-mg level	0.19-mg level	2.8-mg level	0.65-mg level	0.19-mg level	2.8-mg level	0.65-mg level	0.19-mg level
1	351	138		49	109		77	100	
4	185	103		1	142		4	113	
7	318	99	105	4	78	155	12	81	108
14	295	321	96	9	55	87	29	98	106
21	310	103	100	61	142	322	79	110	308
28	244	98	107	63	64	114	75	71	70
35	164	115	94	83	71	134	80	66	179
Mean	267	140	100	39	94	162	50	94	154

11.12 Histopathology. The only organ in which histologic changes were consistently found was the kidney; this discussion will be limited to that organ. Serial study of rats and rabbits exposed to inhalation of UO_2F_2 dust, in concentrations of 12.2, 2.8, and 0.65 mg/cu m, showed a fairly definite characteristic progression of histological

changes typical of that usually seen during exposure to uranium compounds. A detailed description of the pattern of tubular degeneration and regeneration is given in Chap. 4. In general the pattern was similar in rabbits and rats, although less severe in rats. Thus at the 2.8-mg level both rats and rabbits showed renal injury during the exposure period, but only the rabbits showed evidence of damage at the end of the exposure period. Rats at that time were essentially normal. No serial studies were made on other species.

At the 12.2-mg level severe renal damage was observed in dogs, guinea pigs, rabbits, mice, and cats. Less severe damage was found in rats. At the 2.8-mg level there was moderate damage in dogs and rabbits but less in rats. At the 0.65-mg level there were slight changes in most of the rabbits but no damage in rats. At the 0.19-mg level there were very slight but definite changes in about 50 per cent of the dogs and rabbits but none in the rats.

Thus from a histological point of view the various experiments could be rated, in regard to toxicity, as shown in the accompanying table.

Level, mg/cu m	Response
12.2	Very toxic to dogs and rabbits, moderately toxic to rats*
2.8	Moderately toxic to dogs and rabbits,* slightly less toxic to rats*
0.65	Slightly toxic to dogs and rabbits,* not toxic to rats
0.19	Very slightly toxic to dogs and rabbits,* not toxic to rats

*Studied by serial sacrifice as well as by terminal sacrifice.

The only distinct species difference was the marked resistance of rats compared to rabbits and dogs.

11.13 Discussion. It has been pointed out in the introduction that the toxicity of uranium hexafluoride is in reality the sum of the toxicities of uranyl fluoride and hydrogen fluoride. A major difference in particle size exists between the uranyl fluoride produced by hydrolysis of uranium hexafluoride and that produced by grinding bulk uranyl fluoride preparatory to these studies. The hydrolysis product is a fume with a very small particle size (0.056μ in diameter), whereas the ground material has a considerably larger size (ranging up to 10μ, with a mean of approximately 1μ). Although there is at present no evaluation of the effect of such dissimilarity of particle size on toxicity, there seems no doubt that differences do exist because of differential retention of different sizes of particles.

Apart from the particle-size factor, other differences in toxicity observed between uranium hexafluoride and uranyl fluoride can be attributed to the presence of hydrogen fluoride as a hydrolysis product of the hexafluoride. This is particularly noticeable at the higher exposure levels in which considerable pulmonary damage was found in the case of the hexafluoride but not in that of the uranyl fluoride. At lower exposure levels, where the hydrogen fluoride did not cause pulmonary damage, the toxicity for both the uranium hexafluoride and uranyl fluoride was confined to the renal effect of uranium and was of the same general pattern and extent. The two compounds are of approximately equal effect at low concentrations, both showing minimal damage at approximately 0.2 to 0.3 mg. At high and flood concentrations the uranium hexafluoride is the more toxic because of the additional effect of hydrogen fluoride on the respiratory system.

A summary of all the measures of toxicity are given separately for each of the four levels in separate tables (Tables 10.52, 10.53, 10.54, and 10.55).

Table 10.52 — Animals Exposed for 30 Days to 12.2 Mg UO_2F_2/cu m
(Summary of Results)

1. Mortality. Uranyl fluoride is one of the more toxic of uranium compounds.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	20/20	100
Rabbit	5/6	83
Dog	2/2	100
Cat	2/2	100
Guinea pig	11/20	55
Rat	0/20	0

2. Weight Changes. Weight losses were observed in all species.

Species	Wt. change
Rabbit	Severe loss
Guinea pig	Severe loss
Dog	Severe loss
Rat	Moderate loss

3. Biochemistry. Dogs and rabbits all showed a marked rise in blood NPN.
4. Pathology. Extensive renal tubular degeneration and regeneration in dog and rabbit. Less severe damage in rat.
5. Hematology. Changes were of doubtful significance.
6. Summary. This level is extremely toxic to all species except the rat, causing severe damage and high mortality.

Table 10.53 — Animals Exposed for 30 Days to 2.8 Mg of UO_2F_2/cu m (Summary of Results)

1. Mortality. Only mice and rabbits show significant mortality.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	11/42	26
Rabbit	3/24	13
Dog	0/6	0
Cat	0/4	0
Guinea pig	1/30	3
Rat	0/30	0

2. Weight Changes. Only rabbits and guinea pigs show a weight loss.

Species	Wt. change
Rabbit	Moderate loss
Guinea pig	Moderate loss
Dog	None
Rat	None

3. Biochemistry, Rabbits.

Determination	No. of animals showing abnormality per no. exposed	Frequency of abnormal values, %	Mean increase in concentration, mg %
Blood NPN	6/10	74	12
Urinary protein	10/10	51	41
Urinary amino acid	10/10	69	35

4. Pathology. Moderate renal damage in dogs and rabbits. Slightly less renal damage in rats.
5. Hematology. No significant changes.
6. Renal Function, Rabbits. Increased chloride and decreased diodrast and inulin clearances indicate severe upset of renal function. After 3 weeks of exposure, diodrast and renal clearances tend to return toward normal.

Exposure, days	Chloride, % of normal	Clearance Diodrast, % of normal	Inulin, % of normal
1	351	49	77
4	185	1	4
7	318	4	12
14	295	9	29
21	310	61	79
28	244	63	75
35	164	83	80
Mean	267	39	50

7. Conclusion. This level is only slightly toxic to rats, moderately toxic to dogs, guinea pigs, and rabbits, and more toxic to mice. It produces marked functional changes in rabbits despite the low mortality.

Table 10.54 — Animals Exposed for 30 Days to 0.65 Mg of UO_2F_2/cu m (Summary of Results)

1. Mortality. Only mice showed a significant mortality.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	7/51	14
Rabbit	1/24	4
Rat	0/10	0

2. Weight Changes. Weights were normal in rabbits and rats.
3. Biochemistry, Rabbits.

Determination	No. of animals showing abnormality per no. exposed	Frequency of abnormal values, %	Mean increase in concentration
Blood NPN	2/10	9	6 mg %
Urinary protein	4/10	16	8 mg %
Urinary catalase	6/10	19	11 cu mm
Urinary amino acid	8/10	28	10 mg %

4. Pathology. Slight renal tubular damage in most rabbits. No renal damage in rats.
5. Renal Function, Rabbits. Only the animal at 14 days showed an abnormal function.

Exposure, days	Chloride, % of normal	Diodrast, % of normal	Inulin, % of normal
1	138	109	100
4	103	142	113
7	99	78	81
14	321	55	98
21	103	142	110
28	98	64	71
35	115	71	66
Mean	140	94	94

6. Conclusion. This level is nontoxic for rats and rabbits and toxic for mice. There were moderate functional and histological renal disturbances in most of the rabbits.

11.14 Summary. 1. Mortality among mice, rabbits, dogs, cats, and guinea pigs (but not rats) was high at 12.2 mg/cu m. At 2.8 mg/cu m there was moderate mortality in mice and rabbits, but not in dogs, cats, guinea pigs, and rats. At 0.65 mg/cu m only mice showed a significant mortality. At 0.19 mg/cu m there was no significant mortality in any species.

2. Weight changes were pronounced at 12.2 mg/cu m, except in rats; were moderate in rabbits and guinea pigs at 2.8 mg/cu m; and were insignificant at 0.65 and 0.19 mg/cu m.

Table 10.55—Animals Exposed for 30 Days to 0.19 Mg of UO_2F_2/cu m (Summary of Results)

1. Mortality. There was no significant mortality in any species.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	2/41	5
Rabbit	1/21	5
Dog	0/6	0
Rat	0/10	0

2. Weight Changes. Weights were normal in rabbits, dogs, and rats.
3. Biochemistry.
   Rabbits. There were borderline changes in only one or two animals.
   Dogs. Three showed normal blood NPN and urinary amino acid. One showed proteinuria.

Determination	No. of animals showing abnormality per no. exposed	Frequency of abnormal values, %	Mean increase in concentration
Blood NPN	0/15	2	0 mg %
Urinary protein	1/15	6	0 mg %
Urinary catalase	1/8	2	6 cu mm
Urinary amino acid	1/7	8	1 mg %

4. Pathology. Very slight renal tubular changes in about half the dogs and rabbits. No renal changes in rats.
5. Renal Function, Rabbits. No decrease in function was found as measured by chloride, diodrast, and inulin clearance.
6. Conclusion. This level is nontoxic for rats and very slightly toxic to dogs and rabbits. No unequivocal functional changes were observed, but slight histological changes were found in the kidneys of about half the dogs and rabbits.

3. There was a marked azotemia in dogs and rabbits at 12.2 mg/cu m, a moderate azotemia in 6 of 10 rabbits at 2.8 mg/cu m, a slight azotemia in 2 of 10 rabbits at 0.65 mg/cu m, and no azotemia in dogs or rabbits at 0.19 mg/cu m.

4. Urinary protein, catalase, and amino acid nitrogen showed a parallel decrease with the azotemia in both frequency and severity of effect at the lower dust-concentration levels. These variables were more sensitive indicators, however, and they showed positive in more animals. Positive values were frequent in all experiments except the 0.19-mg level, which was borderline.

5. At the 12.2-mg level, severe renal tubular degeneration and regeneration were found in dogs and rabbits. More moderate changes were found in the rats. At 2.8 mg/cu m, moderate changes occurred in dogs and rabbits. Slight changes occurred in rats. At the 0.65-mg level slight changes were observed in rabbits and no changes in rats.

At the 0.19-mg level, minimal damage was found in rabbits and dogs and none in rats.

6. No significant hematological changes were observed.

7. Uranium was found in lungs, kidneys, livers (except rats), and bones of dogs, rabbits, and rats. The amount and frequency of occurrence was high at the 12.2-mg level and approached the detectable limit at the 0.19-mg level.

8. Renal-clearance studies indicated marked functional disturbances at the 2.8-mg level. The chloride clearance was elevated, and diodrast and inulin clearances were depressed, indicating decreased tubular reabsorption and excretion and glomerular filtration, respectively. At the 0.65-mg level, only slight dysfunction was observed, and at the 0.19-mg level function was normal.

9. Animals surviving a 2-week period of exposure tended to return toward normal during the latter part of the experiment despite continued exposure to UO_2F_2.

10. Marked species differences in susceptibility were found. The rat was particularly resistant compared to the other species.

12. URANYL NITRATE

By Eugene Roberts*

12.1 Introduction. The recent widespread industrial use of uranyl nitrate and its use in the past for the development of experimental uranium nephritis has made doubly desirable the determination of the inhalation toxicity of this compound as a standard basis of comparison with that of other uranium dusts. Selection of the nitrate for past work has been made because it is the most soluble of all uranium compounds and in addition allows the observation of the effects of uranyl ion uncomplicated by those of anions such as fluoride which, in contrast to the nitrate, enhance the toxicity of the uranium. For the above reasons an intensive study of inhalation toxicity of uranyl nitrate was made in six experiments at five different levels of exposure with the concentrations in the atmosphere ranging from approximately 0.3 to 20 mg/cu m (equivalent to 0.14 to 9.4 mg of uranium per cubic meter). These studies were exceptional in that, in addition to the usual criteria for detecting uranium poisoning, special studies of certain aspects of carbohydrate metabolism, liver function, renal clearance, and the influence of added salts to the diet were made.

* Toxicologic procedures performed by C. W. Bishop, F. W. Wichser, R. H. Amdur, A. G. Pettengill, G. J. Mogridge, R. C. Baxter, J. A. Tornaben, R. Gerson, E. Roberts, C. J. Spiegl, and D. C. Brodie.

Considerable emphasis was also placed on the catalase activity in the urine and its relationship to urinary protein, blood nonprotein nitrogen, and urea nitrogen. The sequence of increase and quantitative relationship of urinary catalase and protein, sensitive indicators of uranium poisoning, to the other measures of toxicity were established. Serial renal biopsies in dogs produced interesting information of a histological and histochemical nature.

12.2 Exposure Unit. The animals were exposed in a typical full-exposure unit, the details of which are described in Sec. 6.1e.

The dust was introduced into the chamber by means of an agitated-elutriator type of dust feed described in Sec. 6.2c. Prior to its use in the feed the nitrate was ground once to a dust in the Bantam Mikro-pulverizer, care being taken to maintain the temperature below the melting point of 62°C. The dust was dried over calcium chloride at a vacuum of approximately 15 mm for at least 4 days before grinding and just before use in the exposure unit. At this stage the compound corresponded approximately to the formula $UO_2(NO_3).4H_2O$. The purpose of the desiccation was to produce a dust whose entry into the chamber could be more suitably controlled. After its introduction into the chamber the dust acquired additional water to form the stable hexahydrate.

12.3 Concentration of Uranium in Exposure Atmospheres. The dust concentration in the atmospheres produced for the six uranyl nitrate studies was maintained approximately at the desired levels, although there was a rather large standard deviation of the mean in almost every case. Results of the dust concentrations expressed in terms of $UO_2(NO_3)_2.6H_2O$ obtained in two representative 30-day studies are given in Table 10.56.

The distribution of the dust throughout the chamber in which the exposures were performed is described in Sec. 8.

12.4 Signs of Toxicity. Frank clinical symptoms of uranium poisoning were observed only in the animals exposed at the 20-mg level. In the dog there were anorexia, loss in weight, tenderness in the region of the kidneys, a dryness of the fur, vomiting, respiratory râles, and severe dehydration. Anorexia and loss in weight were also observed in the rabbit. In the guinea pig, rat, and mouse these findings were not manifest.

At the lower levels of exposure, only the dying rabbits showed a marked loss in weight.

12.5 Mortality. The mortality of the animals in all the studies is summarized in Table 10.57 and Fig. 10.33. Mortality was highest in the rabbits among those species exposed at more than one level, attaining 80 per cent at the 20-mg/cu m level. At the 4.5-mg level the mortality was still 80 per cent, and at the 0.3-mg level, the lowest

Table 10.56—Exposure to Dust Concentrations of $UO_2(NO_3)_2.6H_2O$

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
4.54	±1.71	66.7
4.54	±3.42	95.0
4.54	±5.13	99.0

Note: desired concentration, 5 mg $UO_2(NO_3)_2.6H_2O$/cu m; standard deviation, ±1.71 mg/cu m; number of samples, 193.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
0.53	±0.32	66.7
0.53	±0.64	95.0
0.53	±0.96	99.0

Note: desired concentration, 0.5 mg $UO_2(NO_3)_2.6H_2O$/cu m; standard deviation, ±0.32 mg $UO_2(NO_3)_2.6H_2O$/cu m; number of samples, 271.

concentration of uranyl nitrate hexahydrate at which rabbits were exposed, it was 19 per cent. All four of the cats exposed at the 4.5-mg level, the only level at which this species was exposed, succumbed. There was no mortality in dogs at levels below 20 mg. At the 20-mg level, however, three of four dogs were killed when moribund; in the

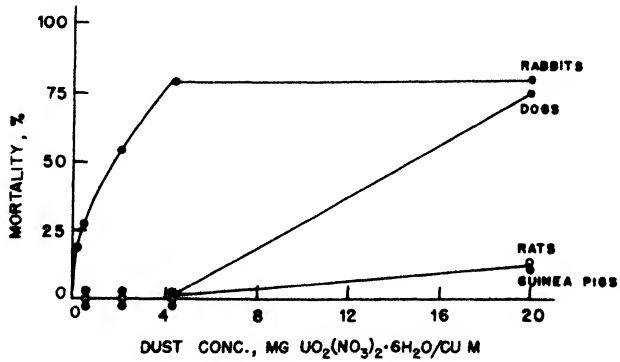


Fig. 10.33—Mortality of four species of animals exposed to uranyl nitrate hexahydrate dust at levels of from 0.3 to 20 mg/cu m.

Table 10.57 — Mortality of Animals Exposed Daily to Uranyl Nitrate Hexahydrate Dust

Species	Concentration level of $UO_2(NO_3)_2.6H_2O$, mg/cu m	No. of deaths per no. exposed	Mortality, %
Dog	20	3/4*	75
	4.5	0/5	0
	1.94†	0/20	0
	0.27	0/20	0
Guinea pig	20	2/20	10
	4.5	0/20	0
	1.94†	0/30	0
	0.27	0/29	0
Mouse	20	0/39	0
	4.5	3/50	6
	0.53	2/50	4
Rabbit	20	8/10	80
	4.5	16/20	80
	1.94†	8/15	53
	0.53	8/30	27
	0.27	3/16	19
Rat	20	2/20	10
	20	3/30	10
	4.5	0/30	0
	1.94†	0/150	0
	0.53	0/46	0
	0.27	0/150	0
Cat	4.5	4/4	100
Total	20‡	5/24	21
	20‡	13/99	13
	4.5	23/129	18
	1.94†	8/195	4
	0.53	10/126	8
	0.27	3/195	2

* The three animals that died were sacrified when moribund. Two had had renal biopsies.

† Refers to the first 30 days of a chronic study.

‡ Two separate experiments at this level were performed.

case of two of three of the dying animals, it is likely that the morbidity was enhanced by renal biopsies performed at close intervals. The mortality in both the guinea pigs and the rats was only 10 per cent at the highest level of exposure tested, 20 mg, indicating exceptional resistance of these two species to the effects of exposure to uranyl nitrate dust. At lower levels there were no deaths in either of these two species attributable to the exposure. Although none of 39 mice

died following a 30-day exposure to 20 mg of uranyl nitrate hexahydrate, 3 out of 50 and 2 out of 50 succumbed at the 4.5- and 0.5-mg levels, respectively. The significance of these last two mortality figures is doubtful, and it is believed that the mouse may have the same order of resistance as the rat and the guinea pig to uranyl nitrate.

12.6 Body Weight. (a) 20-mg Level. Changes in weight have proved to be relatively unreliable guides to toxicity in short-term studies of uranyl nitrate hexahydrate. At the 20-mg level, three of the four dogs that were killed before the termination of the experiment had lost approximately one-quarter of their original body weight at the time of sacrifice. The one surviving dog showed a decrease of 6 per cent from its original weight 30 days after the start of exposure. Other exposed animals, which included rats, rabbits, and guinea pigs, lost weight during the experimental period. Mice, on the other hand, gained weight (Table 10.58). The female mice showed a gain in weight

Table 10.58 — Mean Weight Change of Animals Exposed to Uranyl Nitrate Hexahydrate Dust at 20 Mg/cu m

Species	No. of animals	Fate	Total period of observation, days	Mean weight		Mean weight change, %
				Initial, g	Final, g	
Rat	27	Survived	29	268	253	−5.6
Rat	3*	Died	12–14	279	244	−12.6
Rabbit	2	Survived	29	3,700	3,200	−13.5
Rabbit	8	Died	8–17	4,000	3,513	−12.2
Guinea pig	18	Survived	34	678	658	−2.9
Guinea pig	2	Died	21–22	751	541	−27.9
Mouse, female	19	Survived	34	15	20	+33.3
	0	Died				
Mouse, male	20	Survived	34	18	22	+22.2
	0	Died				

* One death accidental.

of 33 per cent, and the male mice a mean gain of 22 per cent for the experimental period. In general, those animals which died exhibited a loss in weight greater than or equal to that exhibited by the surviving animals for the entire exposure period.

(b) 4.5-mg Level. In this study the animals with the highest mortality, the rabbits and cats, exhibited the greatest weight loss (Table 10.59). The rabbits that died lost weight uniformly before dying,

Table 10.59—Mean Weights of Animals Exposed to 4.5 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter at Observation Period Shown

Species	0 days*		6 days		13 days		20 days		21 days		27 days		36 days	
	Mean wt., g	No.	Mean wt., g	No.	Mean wt., g	No.	Mean wt., g	No.	Mean wt., g	No.	Mean wt., g	No.	Mean wt., g	No.
Rabbit:														
Died	3,450	16	3,420	16	2,830	12	2,970	1			2,680	1		
Survived	3,700	4	3,590	4	3,510	4	3,510	4			3,590	4	3,610	4
Dog	6,570	5	6,330	5	6,350	5	6,220	5			6,200	5	6,230	5
Rat	272	30	271	30	277	30					284	24	278	24
Cat	2,400	4	2,380	4	2,350	4			2,170	1	1,970	1	1,700	1
Guinea pig	550	20	545	20	568	20	586	20			597	20	606	20

*Zero day indicates the day prior to the first exposure.

whereas those that survived showed little change. The three cats that died early in the experiment showed no marked loss in weight prior to death, whereas the cat that died at the end of the experiment showed a progressive decrease in weight throughout the experimental period. The dogs showed only a slight loss in mean weight at this level, whereas the rats and guinea pigs evidenced slight to moderate gains.

(c) 1.9-mg Level. There appeared to be a fluctuation in the weight of the dogs at this level, the means for both sexes ranging from 8.37 to 9.06 kg during the control period and from 7.95 to 8.92 kg during the experimental period. The lowest mean value of 7.90 kg that occurred during the second week of exposure may possibly be significantly lower than the other experimental values. Neither the male nor the female rats showed any unusual trends, gaining weight at a rate comparable to the litter-mate controls (Table 10.60). The rabbits at this level showed a loss in mean weight from 3.2 kg at 5 weeks before the beginning of exposure to 2.5 kg 6 weeks after the beginning of exposure, but again the greater losses were exhibited by the dying animals. The guinea pigs gained weight uniformly from the start of the exposure until the end of the period of observation.

(d) 0.5-mg Level. Individual animal weights were observed only in the case of the rabbits in the group of animals exposed to 0.5 mg. The mean weights of the rabbits that survived the entire experimental period showed an increase of from 3.11 kg at the start of exposure to 3.47 kg at the end of the experiment. Five of the seven animals that died or were sacrificed before the end of the experiment showed a marked decrease from the original weight, while two showed a gain.

(e) 0.3-mg Level. There were no significant changes in the weights of the dogs at this level of exposure, the mean weight for both sexes ranging between 8.43 and 8.8 kg during the 4 weeks of the control

period and between 8.35 and 8.8 kg for the experimental period. The rats of both sexes showed gains in weight for the period that were comparable to those exhibited by litter-mate controls (Table 10.60). No significant trend was observed in the weight of the rabbits, but the guinea pigs gained uniformly at this level.

Table 10.60 — Changes in Weight of Experimental and Control Rats during the Period of Exposure to 1.9 and 0.3 Mg of $UO_2(NO_3)_2.6H_2O$/cu m

Week No.	Exposure period, hr	Mean weights of rats, g					
		Male			Female		
		Control	0.3-mg level	1.9-mg level	Control	0.3-mg level	1.9-mg level
-4*	0		133.7	137.4		105.7	111.5
-3	0		159.6	159.7		120.4	124.6
-2	0	170.3	175.1	187.1	124.9	134.1	140.2
-1	0	198.7	199.4	205.1	142.8	145.5	149.2
1	18.0	220.0	214.9	224.2	157.2	154.9	157.9
2	44.8	238.9	228.8	232.2	163.5	159.1	162.6
3	77.0	252.4	238.8	244.6	172.0	165.0	168.6
4	112.0	261.7	255.1	258.5	181.3	171.9	174.5
5	139.8	273.3	260.6	266.4	185.1	173.7	178.7

* Minus sign indicates intervals prior to exposure.

12.7 Hematology. The 20-mg exposure was the only level that produced hematologic changes of possible significance. The changes in blood cellular constituents of 10 rabbits and 15 rats were studied. In the rats a count 24 hr after the exposure and weekly counts thereafter revealed a fall in the number of red blood cells, hemoglobin, and absolute lymphocytes. The white count and absolute neutrophils exhibited an initial rise followed by a fall. Reticulocytes first fell and then rose slightly. The rabbits exhibited a rise in platelets, white count, and absolute neutrophils. There were no other changes. The results are tabulated below:

Constituent*	Animal	
	Rabbit	Rat
RBC		Falls
Hemoglobin		Falls
WBC	Rises	Rises then falls
Absolute neutrophils	Rises	Rises then falls
Absolute lymphocytes		Falls
Platelets	Rises	
Reticulocytes		Rises then falls

* There were no blood cellular changes except those indicated above.

12.8 Pathology. (a) 20-mg Level. Injury to the kidney was the primary finding from exposures to uranyl nitrate hexahydrate, a finding common to that in other experiments with uranium dusts. In the case of three of four dogs, however, pulmonary changes were also observed. This finding, together with the clinical evidence, indicates that exposures to 20 mg may produce irritation of the lungs. Histologically, severe renal tubular damage was observed in all four dogs, and a progressive decrease in the alkaline phosphatase activity was noted in staining the kidney sections made from two serially biopsied dogs. Although there was much renal epithelium undergoing repair at this time, the enzymic activity appeared to be progressively decreasing, which emphasizes the importance of making simultaneous physiological and morphological observations of injured tissue.

(b) 4.5-mg Level. Renal injury occurred in the rabbits, dogs, and rats, the animals examined following exposure at this level. The rabbits dying during the period from 14 to 22 days of exposure exhibited tubular necrosis accompanied by reparative processes. Rabbits surviving the exposure but killed on the thirty-fifth day likewise showed tubular changes, although of a lesser degree. Four dogs showed evidences of renal injury as manifested by reparative processes when killed at the end of the experiment. The fifth animal had granulomata of the kidney of unknown etiology.

A serial picture of renal injury was afforded by a histologic examination of sections of the kidneys of 32 rats killed at the rate of a rat per day throughout the exposure. Renal injury first became apparent on or about the seventh day of exposure at this level and remained moderate until either the twenty-fifth or twenty-sixth day, when it became pronounced. On the other hand, this sequence was not apparent in all animals sacrificed during exposure. Certain of the rats killed after 16 days of exposure possessed normal kidneys, whereas only 5 of 10 rats sacrificed after 35 days showed even mild changes typical of uranium poisoning in the renal tubules.

(c) 1.9-mg Level. Significant renal changes in rats at this level were observed only in the tubules. No changes were seen in the glomeruli. It is difficult to deduce the sequence of events with which the injury occurred because of the wide variation among animals. The earliest detectable morphological changes were observed on the second day of exposure. These changes, which were of a degenerative nature, were extremely slight. Degeneration and necrosis of tubular epithelium were readily observed in the kidneys of the animals sacrificed during the first week. However, these were of no more than moderate degree. The animals studied after the first 2 weeks showed only slight evidence of active epithelial damage. Active epithelial regeneration was observed on the third day, occurring only shortly

after the first detectable cellular breakdown. In cases where only slight changes were observed, the affected tubules were those in the region of the arcuate vessels. In cases with more severe damage, some tubules in the region just parallel to the subarcuate zone and the medullary rays were involved. In general, the changes in the rats with the greatest amount of renal injury at each time interval showed a sequence of events typical of that usually occurring during exposures to moderately damaging levels of uranium compounds by inhalation (see Chap. 4).

The findings in the kidneys of the two dogs that were biopsied on the tenth day and in the one sacrificed at the same time were similar in nature and degree. These changes were limited to the inner cortex and medullary rays and were characterized by mild degeneration and necrosis with moderate amounts of regenerative epithelium, some of which was quite active.

(d) 0.5-mg Level. An extensive study of the pathological findings in serially and terminally sacrificed rats and in dying and terminally sacrificed rabbits was performed by three pathologists independently of one another and without prior knowledge of the animals' histories, thus enabling an unbiased evaluation of the effect of uranyl nitrate on the lung, liver, and kidney. Inhalation of the nitrate at this level produced relatively severe damage to rabbit kidney but extremely slight damage to the rat kidney. However, no pathological changes attributable to uranium were observed in the lung and liver of either the rabbits or the rats.

(e) 0.3-mg Level. Slight renal injury typical of uranium poisoning occurred in one of three rats sacrificed at the end of 28 days of exposure. The kidneys of five rats sacrificed after 1 week were all normal, as were those of all rats sacrificed weekly in groups of three through the 4 weeks of exposure. No other significant changes were observed in these animals. In two dogs upon which renal biopsies were performed after 10 days of exposure, there were very mild changes in the inner kidney cortex. Unfortunately in the dog sacrificed after 10 days a severe chronic nephritis masked any damage from uranium. The above slight and infrequently observed changes, although doubtless resulting from exposure, do not seem to be of sufficient severity to cause concern.

12.9 Biochemistry. (a) Dogs. 20-mg Level. An extensive biochemical study was made on four dogs exposed at this level. The changes in blood nonprotein and urea nitrogen and in potassium were followed as a measure of retention of the products of metabolism resulting from renal damage, and total blood carbon dioxide was followed as a reflection of the disturbances in acid-base equilibrium. Measurements of serum protein were made to ascertain whether the

excretion of urinary protein affected the amount of circulating serum protein. Glucose tolerance of those animals was also measured at intervals during the exposure.

The two biopsied dogs showed a steady rise in NPN and a parallel rise in urea from the beginning of exposure until killed 12 days later, at which time the values for NPN were 252 and 356 mg %, respectively (Fig. 10.34). One of the two unoperated dogs had an NPN of 156 mg % when killed on the nineteenth day of the experiment, and the remaining animal, which survived for the entire experimental period of 35 days, attained on the third day of exposure a maximal value for NPN of 73 mg % which returned to normal at the end of the first week and remained so until the conclusion of the experiment.

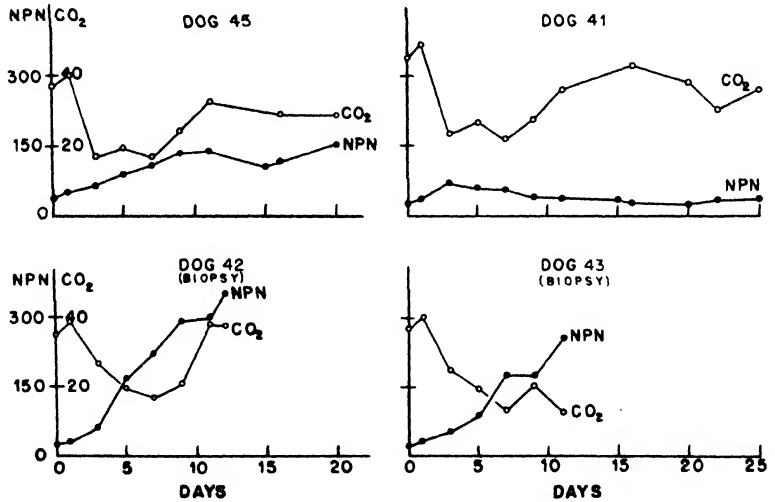


Fig. 10.34—The NPN and carbon dioxide, CO_2, in the blood of dogs following daily exposure to uranyl nitrate hexahydrate dust (20-mg level). The NPN is expressed in milligram per cent; CO_2, in volume per cent.

All the dogs exhibited a marked drop in total blood CO_2 from the normal value of 40 vol. % after the initiation of the exposure, attaining minimal value between the first and seventh day, with a trend to return toward normal values thereafter except in the case of one of the dogs which had an abnormally low value of 13.5 vol. % at sacrifice. The minimal values were in general less than one-half of the control values, indicating a relatively severe acidosis in all cases.

Glucose Tolerance in Dogs. (C. W. Bishop and E. Roberts.) The blood-glucose levels were determined in dogs fasted for 18 hr before

the test was made and at the following intervals after the injection of 1 g of glucose per kilogram of body weight (50 per cent solution) into the external jugular vein: 15 min, 30 min, 1 hr, 1 ½ hr, and 2 hr.

The first determinations were performed prior to exposure. The second determinations were made after 4 days of exposure in the case of dogs 41, 43, and 45 and after 7 days in the case of dog 42. Additional experiments were performed on dogs 41 and 45 during the exposure. It is clear from data of a typical experiment (Fig. 10.35)

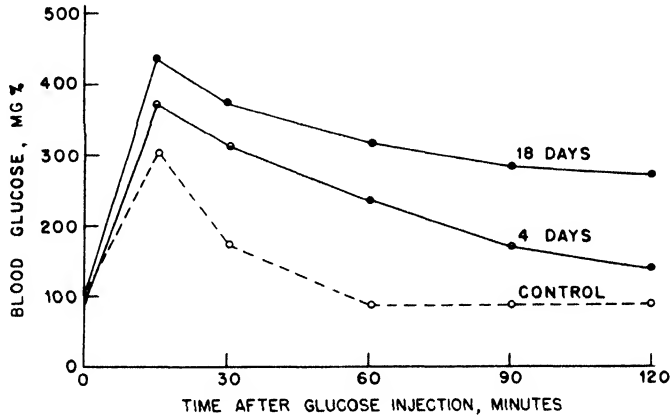


Fig. 10.35—Glucose-tolerance curves of a dog, before exposure (control) and after 4 and 18 days of exposure to uranyl nitrate hexahydrate dust (20-mg level).

that the glucose tolerance was significantly decreased after a brief exposure. There was a progressive decrease in glucose tolerance throughout the experimental period in this animal. The dog (No. 41) showing the mildest clinical symptoms of toxicity and the smallest alterations in weight and blood NPN exhibited also the least decrease in glucose tolerance. In fact, in an experiment performed on this animal 25 days after the beginning of the exposure, the glucose tolerance was found to be normal. That the altered carbohydrate metabolism in these animals was not caused by the production of diabetes was indicated by the fact that the fasting blood-sugar values were essentially normal and that the symptoms associated with this condition were not manifest.

The most marked urinary findings in dogs showed excretion of relatively large quantities of protein (maximal values 400 to 800 mg %) and sugar by the animals, the greatest excretion occurring on the first 6 days of exposure. Thereafter both the excretion of protein and

sugar decreased. There was also a decrease of urinary creatinine excretion during the exposure.

(b) Rats. Blood-NPN and total blood-CO_2 values were determined at 14 and 30 days after the start of exposure in blood obtained by heart puncture in from two to five rats in each of the following six dietary groups: unexposed controls receiving the stock diet, and five groups of exposed rats receiving, respectively, (1) the unsupplemented diet, (2) $NaHCO_3$, (3) sodium citrate, (4) $KHCO_3$, and (5) potassium citrate, each in quantities equivalent to 1 per cent $NaHCO_3$ added to the stock diet on a cation-equivalent basis (Table 10.61). The mean

Table 10.61 — Mean Total Blood CO_2 and NPN of Rats* Exposed to $UO_2(NO_3)_2.6H_2O$ Dust at a Mean Concentration of 20 Mg/cu m

Group	Dietary supplement	14 days						30 days					
		Blood CO_2, vol. %			NPN, mg %			Blood CO_2, vol. %			NPN, mg %		
		Mean	Range	No.†	Mean	Range	No.†	Mean	Range	No.†	Mean	Range	No.†
Untreated controls	None	51	47–55	5	46	34–64	5						
1	None	64	60–69	4	83	64–108	5	52	44–56	4	111	74–140	5
2	$NAHCO_3$	60	59–60	4	52	40–58	5	47	39–56	5	70	58–90	5
3	Na citrate	67	62–73	2	52	40–72	4	53	49–57	5	62	56–68	4
4	$KHCO_3$	55	47–59	3	41	36–44	5	46	38–53	4	57	46–74	5
5	K citrate	53	45–61	5	45	44–46	5	48	39–62	5	59	54–68	5

* Determinations were performed on rats selected at random from each group.
† Number of determinations.

total blood-CO_2 values showed no marked alterations at the 14-day period. In fact, the mean values of all the experimental groups were higher than that of the control group, showing, perhaps, a tendency toward alkalosis rather than acidosis. The group receiving no dietary supplement was the only group exhibiting an abnormally high mean value for NPN (83 mg %) at this time. Thirty days after the beginning of the exposure all the experimental groups showed a decrease in total blood-CO_2 and an increase in NPN over the 14-day values. However, in none of the groups was the value for CO_2 significantly different from the value of 51 vol. % found previously in the unexposed controls. The highest mean value for NPN at this time (111 mg %) was exhibited by the group receiving the unsupplemented diet, the next highest (70 mg %) by the rats receiving the $NaHCO_3$. The elevations were smaller in the case of the other three groups.

The mortality of four of the dietary groups ranged between 5 and 20 per cent for the entire experimental period, and that in the group receiving 1 per cent of dietary $NaHCO_3$ attained 50 per cent. The results are opposed to those obtained in numerous instances in which

the dietary administration of alkali has been demonstrated to decrease the mortality of animals poisoned by a single injection of a soluble uranium compound. The animals receiving $KHCO_3$ in the diet exhibited the same mortality as did the exposed controls (10 per cent).

Glucose Tolerance and Liver- and Muscle-glycogen Formation in Rats. (C. W. Bishop and E. Roberts.) These aspects of carbohydrate metabolism were studied in rats exposed for approximately 40 hr over a period of a week to an atmosphere containing 20 mg of uranyl nitrate per cubic meter. In both the controls and the uranyl nitrate-exposed animals the blood-glucose as well as liver and gastrocnemius muscle-glycogen levels were determined in groups of five rats before fasting, after a 24-hr fast, and at the following eight time intervals after the intraperitoneal injection of 350 mg of glucose per 100 g of body weight: 15 min; 30 min; and 1, 2, 3, 4, and 8 hr. The results are shown in Fig. 10.36.

Blood Glucose. The nonfasting and fasting blood-sugar levels of the treated animals were the same as for the controls; however, a marked deviation from the normal glucose-tolerance curve was exhibited by the uranium-treated animals. The mean value attained 15 min after the sugar injection was 486 mg % as compared to 243 mg % for the controls. The blood-sugar level of the controls returned to a normal mean value of 105 mg % within 2 hr after the injection, from which level the values decreased regularly to about 87 mg % at the 8-hr period. The results from the uranium treated animals showed no such regularity. Mean values higher than those observed at 15 min after the injection of the glucose in the controls were obtained at the 30-min and 4-hr periods in the group treated with uranyl nitrate. From these data it is concluded that a disturbance occurred in the ability of the exposed rats to return the blood sugar to pre-injection levels.

Liver Glycogen. Rats exposed to the nitrate showed no significant decreases in nonfasting liver-glycogen values or in their ability to form liver glycogen from injected glucose (Fig. 10.36). For the periods following 1 hr after the injection the mean values for the uranyl nitrate rats and the controls were virtually identical. The rats exposed to uranyl nitrate appeared, however, to have slightly more liver glycogen after a 24-hr fast, at the 15-min, 30-min, and 1-hr intervals than did the controls.

Muscle Glycogen. No large differences were observed in the mean values for muscle glycogen of the experimental and control animals through the 1-hr period. However, the control animals thereafter possessed greater quantities of muscle glycogen than did the animals exposed to uranyl nitrate. The quantitative effect of this decrease in

muscle glycogen on the carbohydrate economy is probably much greater than that of the elevated blood-sugar levels.

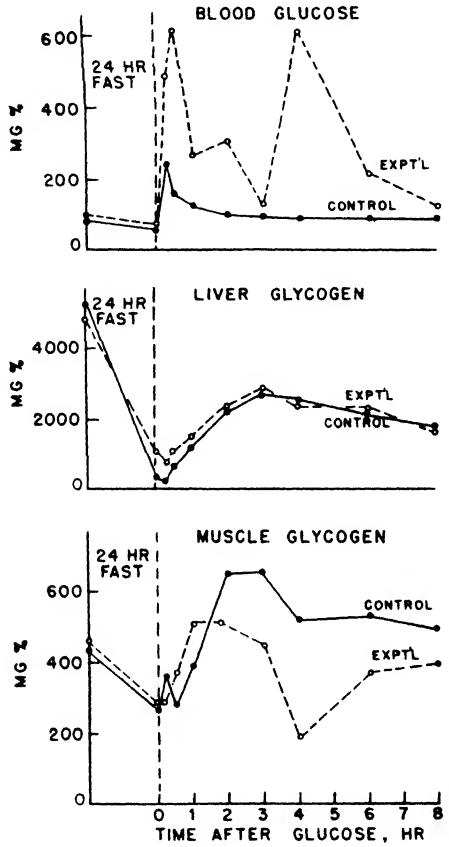


Fig. 10.36 — Curves of glucose tolerance and of formation of liver and muscle glycogen in rats, before exposure (control) and after 40 hr of exposure (experimental) to uranyl nitrate hexahydrate (20-mg level).

(c) Rabbit and Dog. A rise was noted in the nonprotein and urea nitrogen in the blood of rabbits within 4 days after the initiation of exposure to the 20-mg level. On the eighth day of exposure the mean nonprotein nitrogen value reached 250 mg %. Subsequently the values returned to normal in those animals that survived.

4.5-mg Level. Blood nonprotein nitrogen and urea nitrogen determinations were performed on 10 rabbits and 5 dogs in this study. The

rabbits showed greater values for NPN and urea nitrogen than did the dogs. On the thirteenth day after the start of the experiment seven of the eight surviving rabbits had elevated NPN and urea values, and three showed NPN values greater than 200 mg %. The three rabbits that survived never attained values as high as did those that died, and at the end of the exposure the survivors had normal values. In contrast, the dogs did not show such high values at any time during the experiment. On the thirteenth day three of the dogs showed elevated values of blood NPN and urea, but at the end of the experiment only one had a slight elevation in blood NPN. The highest value for NPN exhibited by any of the dogs throughout the experiment was 86 mg %.

1.9-mg Level. At no time was there observed a significant elevation in the blood NPN of the 10 dogs studied during the month of exposure. Values for total blood CO_2 were also never strikingly altered, although a suggestion of a lowering in the CO_2 content occurred on the twelfth and nineteenth days after the start of exposure. The only urinary variable that was studied in the dogs was that of protein. There was a significant rise in the urinary protein excretion of dogs on the fifth day after the beginning of exposure and extending through the twenty-fourth, whereupon the values returned to normal.

0.5-mg Level. The biochemical studies both with respect to urinary findings and blood NPN in 12 rabbits studied at this level are discussed in considerable detail as illustrative of the type of procedure employed and of the results obtained in this and other dust-exposure studies. In addition, a study of the correlation of urinary-catalase and alkaline-phosphatase activities and of protein and blood NPN and urea N in these rabbits is recorded, with estimations of renal-tubular-damage ratings from histological readings.

Urinary-catalase and Alkaline-phosphatase Activity and Protein Excretion. Increases in one or two, and infrequently in all three, of these urinary constituents occurred during the exposure period. During the experimental period there were 91 of 264 urine samples with a trace or more of protein, and 33 of 256 with 4 or more units of alkaline-phosphatase activity. In contrast, only 1 of the 24 control urine samples had a catalase-activity value greater than 60 cu mm of O_2, and none showed any protein or more than 3 units of phosphatase activity. The frequency of occurrence of values of catalase above 60 and protein in detectable amounts (Fig. 10.37) increased sharply up to the fifth day after the initial exposure, at which time 92 per cent of the catalase values were at or above 60 cu mm, and 75 per cent of the samples showed a trace or more of protein. The frequencies of occurrence for catalase and protein values above those found in the control period reached a peak on the fifth day. The protein declined

from the eighteenth day, attaining essentially normal values on the thirty-first and thirty-second days, whereas the catalase was more irregular. The frequency of occurrence of elevated phosphatase values was considerably lower than that for the other two constituents.

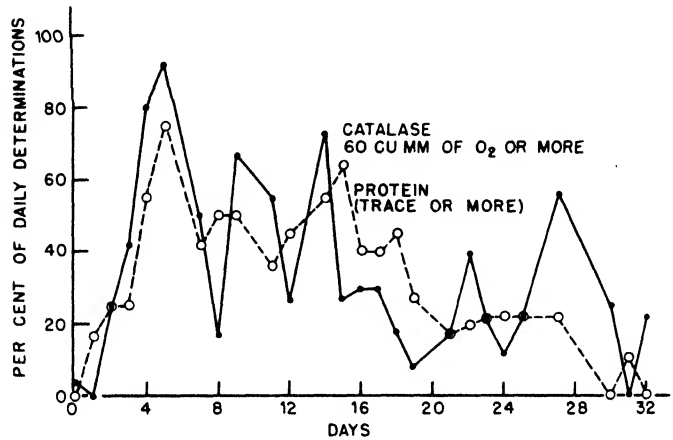


Fig. 10.37 — Frequency of occurrence of urinary catalase activity and protein of rabbits exposed to the 0.5-mg level of uranyl nitrate hexahydrate.

The occurrence of values of the three constituents studied above the arbitrary limits appeared to be related, since in 48 per cent of the instances in which such quantities of catalase and protein appeared they appeared together, and in about 50 per cent of the instances in which phosphatase appeared it was accompanied by either catalase or protein or both. Each of the 12 rabbits studied exhibited at some time during the experiment values of one or more of the constituents which were higher than those exhibited during the control period. The quantitative relationship between catalase and protein was analyzed statistically, and the correlation was found to be .92.

Blood Chemistry. Blood NPN and urea N determinations were performed on six rabbits prior to the first exposure and on 12 rabbits at weekly intervals thereafter. On the eighth day after the initial exposure, 5 of the 12 exposed rabbits had values of NPN greater than 50 mg %. The highest value shown by the six rabbits whose values were determined prior to exposure was 42 mg %. Two of 11 rabbits had values over 50 mg % after 15 days of exposure, likewise 5 of 11 after 22 days of exposure, and 3 of 9 after 29 days. Three rabbits in which values of NPN exceeded 200 mg % died shortly thereafter. Two

rabbits that had NPN values of 134 and 106 mg %, respectively, on the eighth day of the experiment fell to below 50 mg % on the twenty-ninth day. Only two of the rabbits did not show any NPN values above 50 mg % during the experiment. Three of the high values found in the moribund animals were similar to those commonly observed in rabbits in acute uranium poisoning.

On the basis of these results it is concluded that the 0.5-mg level of uranyl nitrate hexahydrate employed in this experiment is injurious to the kidney of the rabbit.

Correlations of Urinary Catalase, Protein, and Blood Nonprotein Nitrogen and Urea Nitrogen with the Estimations of Renal-tubular-damage Ratings from Histological Sections in Rabbits at the 0.5-mg Level. (Statistical analyses by D. V. Tiedeman and M. J. Wantman. Evaluation of tubular damage by R. G. Metcalf.) It was the purpose of this study to analyze the biochemical data and the estimates of renal-tubular damage obtained from 11 of the above rabbits from the point of view of comparing the validity of the biochemical indicators, employing the estimate of typical histological renal-tubular damage as the criterion. The time of the first appearance of the biochemical changes was also noted. The kidney sections were examined at the end of the 30-day experimental period.

If a variable is to be a valuable predictor or indicator of renal injury produced by uranium salts, then animals showing the highest values of that variable during the experimental period should also show the greatest amount of damage as estimated by a criterion acceptable to the average clinician. Therefore the criterion employed in this study was the estimation of tubular damage on sections stained by hematoxylin and eosin, a procedure routinely employed in most medical laboratories. Since it has been suggested by Dounce, Roberts, Wills, and Tien Ho Lan that an elevated value of urinary catalase in an animal showing normal values before treatment is a specific indicator of renal-tubular disturbance and that uranium damage to the kidney is of primarily tubular origin, only the histological changes observed in the tubules of the kidney were evaluated. The evaluation of the damage was made on a nine-point scale in which zero stood for no damage and in which eight stood for severe damage.

Statistical Procedures. (See Chap. 3.) The method employed for determining catalase activity did not distinguish between values above 200 cu mm of O_2. Consequently an attempt was made to estimate statistically what the value of all determinations of catalase over 200 cu mm would have been had the scale not been cut off at 200. To do this a distribution was made of 471 experimental determinations of catalase for the group of rabbits employed in this experiment and a group

of rabbits in another experiment. This distribution was normalized, and a table of "standard values" was prepared. The conversion permitted an expression of the catalase values as a linear scale. For convenience the mean of the standard values was arbitrarily set at 500 and the standard deviation was set at 100. The protein values were changed to standard values by a similar procedure, since the method employed did not distinguish values above 100 mg %.

The general method employed in this analysis was to establish the relationship between ratings of histologically observed renal-tubular damage and the various predictors as determined by the product-moment correlation coefficient.

The following correlations with the kidney-damage ratings were found for the biochemical variables studied: catalase, .55; protein, .56; NPN, .72; and urea N, .62. The difference between the highest correlation of .72 and the lowest value of .55 is not significant. Thus it is entirely possible that in another similar study the order of the correlations might be reversed. The correlations between the biochemical variables and the damage estimates are probably real, since increases in urinary catalase and protein, blood NPN, and urea N, as well as renal histological damage, can be produced at will by single injections of uranium salts, and the intensity of the changes, within limits, is greater with larger doses. The significance of the correlation in the case of urinary protein and catalase is further supported by the fact that the correlation between these two variables is .92, although the urinary protein is believed to come mainly from the serum proteins, whereas it is thought that urinary catalase is derived from affected tubular epithelium. Since the correlations of these biochemical variables with tubular injury are not significantly different from each other, it is concluded that in the case of rabbits under the conditions of this experiment all the biochemical variables studied were of comparable value in predicting or indicating the extent of renal injury. At lower levels of exposure the validity of all the indicators of renal damage decreases.

The reliability of estimating the degree of tubular necrosis and regeneration in rabbits, i.e., the relationship of one judge's rating with that of another, ranged from .50 to .73, with an average of about .6.* Therefore it could not be expected that the correlation between these readings and the chemical variables would be any higher. It should be pointed out that the sections were studied after the phase of maximal histological damage, which occurs 6 to 10 days after the beginning of exposure.

* It should be noted that this degree of agreement among judges when subjective judgment is involved is of the order of magnitude commonly reported.

A further study was made of the relationship between urinary catalase and protein and tubular-damage rating by determining whether or not the animals that showed the largest variations in their predictor values would also show the greatest amount of tubular injury. The correlations between the variances (square of the standard deviation) and the damage ratings were −0.15 and 0.67 for catalase and protein, respectively. Although the relationship for catalase is within the realm of chance, that for the protein may be significant.

Of the 12 rabbits, 9 showed elevated catalase prior to elevated protein values, and 3 showed elevated values for both constituents on the same day. High values of catalase appeared in rabbit urine as much as 96 hr before high protein values. From all the injections and inhalation experiments performed on rabbits to date, in which these variables were studied, it can be concluded that, in general, catalase appears before protein or simultaneously with it in rabbits but that there are individual cases where protein appears first.

0.3-mg Level. There was an elevation in the protein excretion of the 10 dogs between the ninth and twelfth days, with a subsequent return to normal. No alterations in blood NPN or total blood CO_2 were observed. The rabbits exhibited marked elevation in urinary catalase that appeared between the fourth and sixteenth days after the beginning of exposure. No significant alterations were noted in the blood NPN, and no regular trend was observed in urinary protein. This finding corresponds to the previous observations in rabbits that had been studied after receiving 0.005 mg of uranium as uranyl nitrate per kilogram by a single intravenous injection. The results are consistent with the interpretation that, in rabbits exposed at low concentrations, catalase is the most sensitive of the three variables studied.

Summary of Biochemical Findings. A period of maximal renal damage, as judged by biochemical and physiological tests, occurred at some time during the first 3 weeks after the start of exposure. The intensity of the changes in the biochemical variables decreased from the highest exposure level of 20 mg to the lowest level of 0.3 mg, as did the other criteria of toxicity employed. Of the three species studied, the rabbit was the most sensitive. The dog and rat were next in order. Significant alterations in urinary catalase and protein excretion and blood nonprotein nitrogen were observed in the rabbit at the 0.5-mg level, but no marked changes in blood NPN were noted in the dog at a level 10 times higher. The rat showed only a minor elevation in blood NPN even at the 20-mg level. The urinary catalase was the most sensitive indicator of renal alterations in the rabbit, appearing in abnormal quantities in the urine at the 0.3-mg level at a time when no other signs of abnormality were noted.

12.10 Distribution of Uranium in Tissues. (a) 20-mg Level. Uranium, determined spectrographically, was found in two out of four samples of lung tissue and in three out of five samples of kidney but was not detected in the specimens of liver and spleen that were analyzed from the four dogs. None of the 14 tissue samples exhibited a uranium content that indicated a marked accumulation of uranium in the soft tissues for this period of time even at this high level of exposure.

Of the 369 spectrographic analyses of tissues of rats, rabbits, and guinea pigs, which included samples of the kidney, lung, liver, and assorted skeletal tissues, 65 gave positive values for uranium content. The majority of the positive values were in the kidney and lung. The rats exhibited a detectable uranium content twice as often in the kidney as in the lung; the rabbits showed a reversed picture. In the case of the guinea pig both tissues showed the presence of uranium in about an equal number of cases. The tissues of the rabbit had a higher concentration in general than those of the other species.

(b) 4.5-mg Level. Spectrographic analyses performed on 565 samples, which included specimens of the lung, liver, kidney, spleen, and selected osseous tissues of the experimental animals, showed uranium most consistently in the kidney — in 32 out of 97 samples (33 per cent). Next in order of occurrence were the skeletal tissues (36 out of 186 samples, or 19.4 per cent) and the liver (18 out of 94 samples, or 19.2 per cent). The lung and spleen showed uranium less frequently than did the other tissues.

(c) 1.94- and 0.3-mg Levels. Uranium concentrations were determined by the more sensitive fluorophotometric method in the lung, liver, kidney, and femur of rats sacrificed at varying periods during the exposure (Table 10.62). There appeared to be no marked accumulation of uranium in any of the tissues studied for this period of time. The highest values were found most consistently in the kidney, uranium being detected in every specimen examined at the 1.9-mg level and in all but one at the 0.3-mg level. In general, a greater amount of uranium was found in the kidneys of the animals at the higher of the two levels. Uranium was also detected in amounts as high as 1.1 μg U/g of lung in rats at the 1.9-mg level, and smaller amounts were observed at the lower level. The findings in the liver, bone, and especially the femur, although at times showing significantly high values, were inconsistent in that zero values were also encountered.

12.11 Prothrombin Time, Fibrinogen Level, and Bromsulfalein Retention. Table 10.63 contains the results of these tests of four dogs and eight rats exposed to 0.3 mg/cu m.

Table 10.62 — Distribution of Uranium in Tissues* of Serially Sacrificed Rats Exposed to Atmospheres Containing $UO_2(NO_3)_2.6H_2O$

Calendar days	Exposure, hr		Lung, μg U/g of tissue		Liver, μg U/g of tissue		Kidney, μg U/g of tissue		Femur, μg U/g of tissue	
	0.3-mg level	1.9-mg level	0.3-mg level	1.9-mg level	0.3-mg level	1.9-mg level	0.3-mg level	1.9-mg level	0.3-mg level	1.9-mg level
2	6.0	6.0	0.0	0.2	0.0	0.3	0.4	0.5	0.0	0.0
3	12.0	12.0	0.0	0.2			0.1	5.0	0.0	0.0
				0.1				1.3		0.0
4	18.0	18.0	0.0	0.4	0.5	0.0	0.1	5.9	0.8	0.8
5	24.0	24.0	0.1	0.4			0.1	1.0	0.0	0.1
				0.2				1.0		0.0
6	30.0	30.0	0.1	0.3	0.0	2.3	0.3	1.9	2.4	0.0
				0.3		0.2		0.8		0.0
8	26.5	29.0	0.2	0.5	0.0	0.1	0.3	0.5	0.0	0.0
9		42.0		0.1		0.0		0.7		0.2
10		48.0		0.3				1.3		0.6
11		54.0		0.1		0.0		0.7		0.5
12		60.0		0.1				1.9		1.4
				0.3						0.2
15	59.8	58.5	0.0	0.0	0.0	0.2	0.0	0.8	0.0	0.0
29	121.7	124.0	0.1	1.1	0.1	0.0	1.7	1.6	0.0	0.0

*Determined by the fluorophotometric method. "0.0" means below the limits of sensitivity of the method for tissue analyses.

Table 10.63 — Prothrombin Time, Fibrinogen Level, and Bromsulfalein Retention (Mean Values) of Dogs and Rats Exposed to 0.3 Mg of $UO_2(NO_3)_2.6H_2O$/cu m

Species	Week No.	Exposure period Days	Exposure period Total hr	Prothrombin time, sec	Fibrinogen level, mg %	Bromsulfalein retention, % 5 min	Bromsulfalein retention, % 30 min
Dog*	−3	−17	0.0	25.4	307	10.5	0
Rat†	−3	−17	0.0	39.2	311		
Dog‡	2	13	55.5	26.9	275	18.3	0
Rat§	2	13	55.5	39.9	292		
Dog‡	4	25	111.5	27.1	290	23.7	0
Rat**	4	25	111.5	40.7	234		

* Four animals.
† Eight animals.
‡ Five animals.
§ Ten animals.
** Seven animals.

The bromsulfalein retention of the dogs was somewhat increased during the second and fourth weeks of exposure while the fibrinogen levels dropped slightly. No significant changes were observed in the prothrombin time. In the rats there appeared to be a depression of

the fibrinogen during the fourth week of exposure. None of these changes were striking, nor were they indicative of severe hepatic injury. They may have possibly indicated a slight direct or indirect action of the exposure on the liver functions measured. Of the four rabbits that were studied, one exhibited a markedly increased prothrombin and fibrinogen content of plasma on the tenth day of exposure, just before death. Another one of the rabbits also exhibited a very high plasma-prothrombin level after the tenth day. This indicates that in these two rabbits there was considerably more change with respect to these two variables than in the rats and dogs.

12.12 Diodrast, Inulin, and Chloride Clearance of Dogs. (R. C. Baxter, J. A. Tornaben, and E. Roberts; chemical determinations were performed by E. R. Main; J. H. Wills served as technical adviser in these studies.) Clearance studies of inulin for glomerular filtration, diodrast for tubular excretion, and chloride for tubular reabsorption were performed as described in Sec. 8. In addition, the blood NPN and urinary catalase and protein were studied concomitantly with clearances. The blood nonprotein nitrogen was employed as an index of retention of the nitrogenous products of metabolism, the catalase and protein as sensitive spot tests of active renal-tubular injury.

(a) 1.9-mg Level. (See Figs. 10.38 and 10.39.) In two dogs studied at this level there was a fall in inulin clearance during the third week of exposure with a return toward normal values on the fifth week. No significant difference was observed seven and nine days, respectively, after the start of the experiment. Both dogs showed a decreased diodrast clearance throughout the period of observation. After a week of exposure one dog showed a fall in diodrast clearance of approximately 21 per cent, whereas the other had a decrease of 69 per cent, indicating a rather severe derangement of the excretory capacity for diodrast. By the thirty-fifth and thirty-seventh days there was a return toward normality in both dogs, with the diodrast clearances attaining values approximately 85 per cent of the control values. There was no change of the chloride clearance in one of the dogs, but in the other one there was a fall nine days after the start of exposure, an increase over the control values at 23 days, and an essentially normal value 37 days after the beginning of the exposure. In both dogs the values for blood NPN were normal at all times. There were significant rises in catalase and protein excretion, with normal values occurring at the end of the exposure period. The peak in the catalase excretion occurred during the first 3 weeks, whereas that in the protein excretion occurred in the middle 2-week period. The maximal catalase excretion coincided with the greatest fall in diodrast clearance.

(b) 0.3-mg Level. In contrast to the findings at the higher level, there appeared to be no significant decreases in the diodrast clearances of the three dogs, and, peculiarly enough, each animal showed

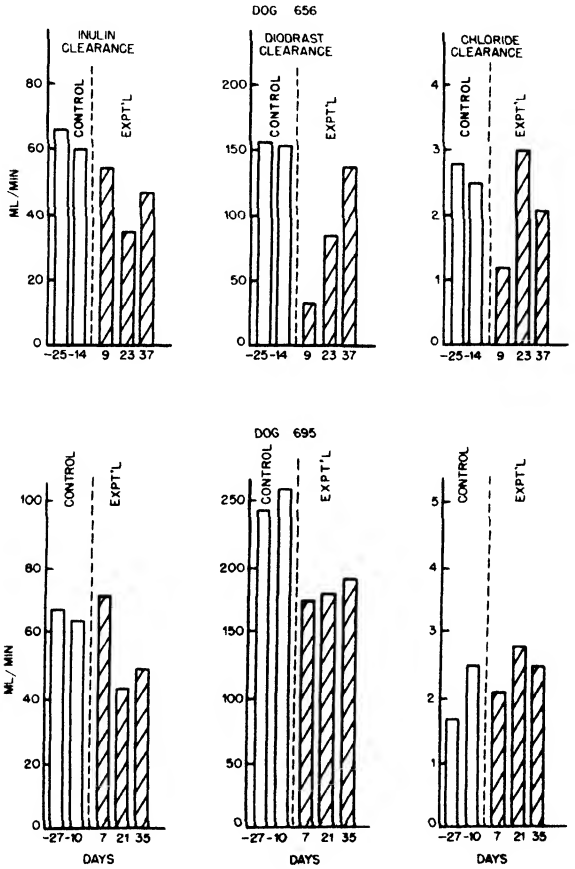


Fig. 10.38—Renal clearance of two dogs exposed to the 1.9-mg level of uranyl nitrate hexahydrate.

at some time during the exposure period a considerably higher mean value for diodrast clearance than was found in the control period. There were no marked alterations in the inulin clearance, but there was a tendency toward an increased chloride clearance, a phenomenon not observed in the two animals exposed at the higher level. There were no significant changes in blood NPN. Although there was an increased excretion of catalase, with one or two abnormal values for

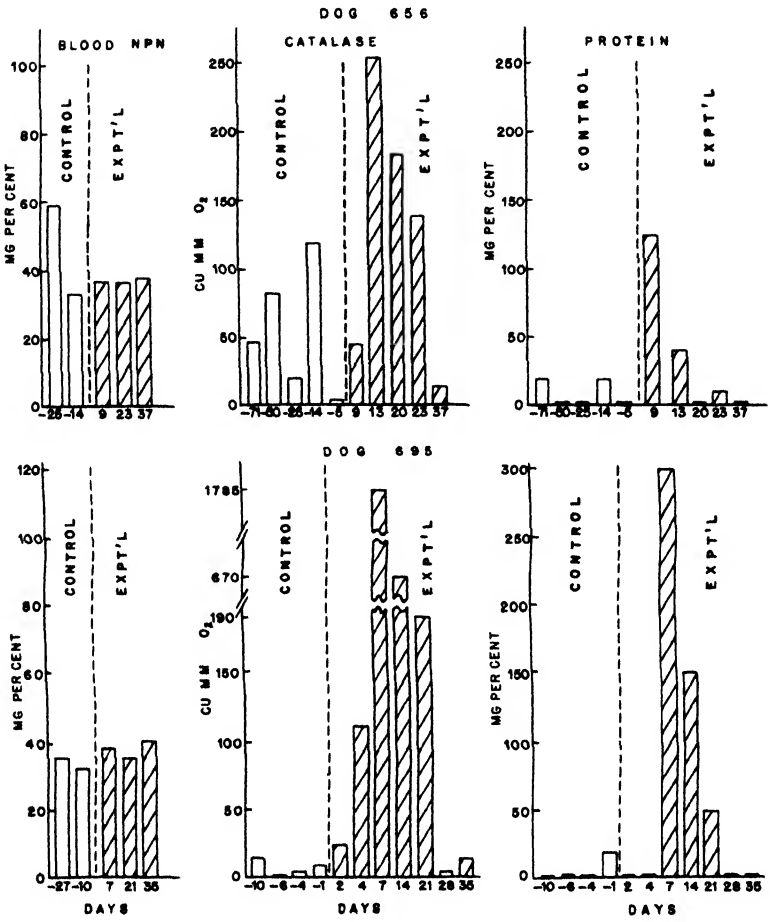


Fig. 10.39 — Blood NPN and urinary catalase and protein values of two dogs exposed to the 1.9-mg level of uranyl nitrate hexahydrate.

urinary protein in each of the animals, the intensity and duration of the response were less than in the case of the two dogs at the 1.9-mg level. (See Figs. 10.40 and 10.41.)

These data indicate that at the 1.9-mg level, a level approximately seven times the present maximal allowable concentration for this compound, there was definite functional change in the kidney of the exposed animals as expressed in a lowered diodrast clearance, a change indicative of decreased tubular excretory function. Although this is probably accompanied by some histological changes, since

such changes were found in other similarly exposed animals, there is every reason to believe that the damage can be compensated for successfully in otherwise healthy animals. A further indication that no serious consequences can ensue from a 1-month exposure of the dog at this level is the normality of the blood NPN. The functional changes in the three dogs at the 0.3-mg level were minimal as reflected in the criteria employed. This is in agreement with the histological and clinical observations in 20 other dogs that were exposed to this concentration of uranyl nitrate hexahydrate.

Results of further studies on these dogs extended to a period of approximately 130 days will be reported in Division VI, Volume 4, of the National Nuclear Energy Series.

12.13 Summary and Conclusions. 1. Uranyl nitrate hexahydrate administered by inhalation to five species daily for 30 days at concentrations ranging from 0.3 to 20 mg/cu m exerted primarily a nephrotoxic action with possible secondary effects on tissues such as lung and liver only at the highest concentrations.

2. In general there was higher mortality among the rabbits, less among the dogs. The rat, mouse, and guinea pig were least affected by the exposure. The dog, but not the remaining species, was about as susceptible as the rabbit to renal injury.

3. Some of the criteria employed in the evaluation of the state of the kidney were positive at all the concentrations employed; however, only minimal changes were observed at the 0.3-mg level, and these were detected by biochemical and histologic techniques.

4. The intensity of the changes in the biochemical constituents decreased from the highest exposure level of 20 mg to the lowest level of 0.3 mg, as did the other criteria. Blood NPN frequently attained values as high as 200 mg % in rabbits at 4.5 mg and above; at 0.5 mg only an occasional animal reached this value, and at 0.3 mg no marked elevation occurred in any animal. The determination of blood NPN was the least sensitive of the biochemical and physiological tests employed, however. Values of NPN remained normal even when renal injury was noted both histologically and by decreased diodrast clearance, and when high urinary-catalase activity and large amounts of protein were found in the urine.

5. From these responses it was concluded that the uranyl nitrate hexahydrate at 0.3 mg is the lowest level that uniformly produces uranium injury in dogs. The slight injury that occurred during the first 2 to 3 weeks of exposure was followed by regeneration despite continuation of the exposure. The 0.3-mg level likewise produced some renal injury in the rabbit but not in the rat, as far as could be judged from histologic examination in this species.

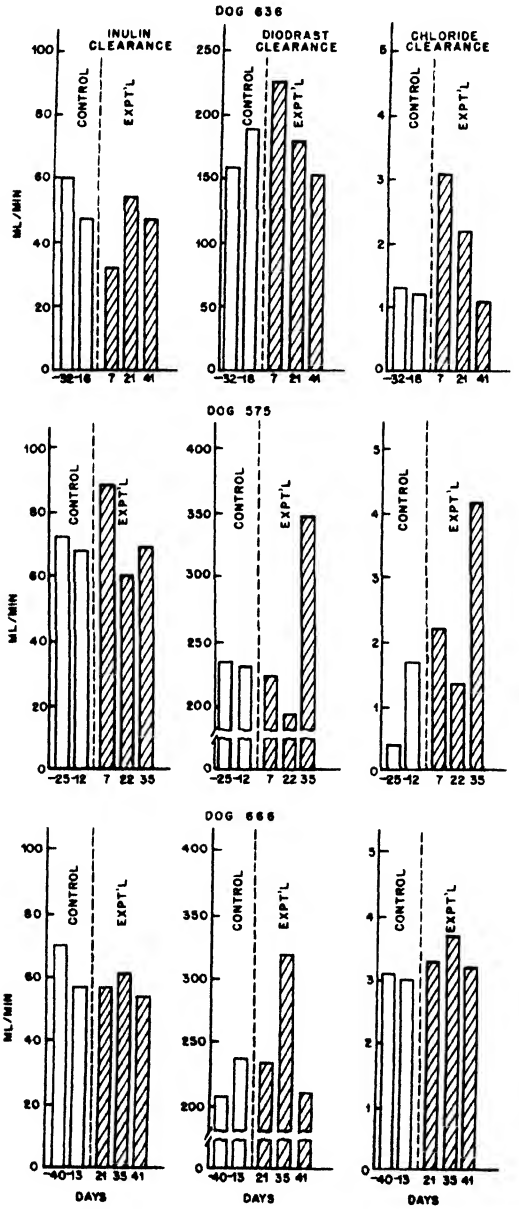


Fig. 10.40—Renal clearances of three dogs exposed to the 0.3-mg level of uranyl nitrate hexahydrate.

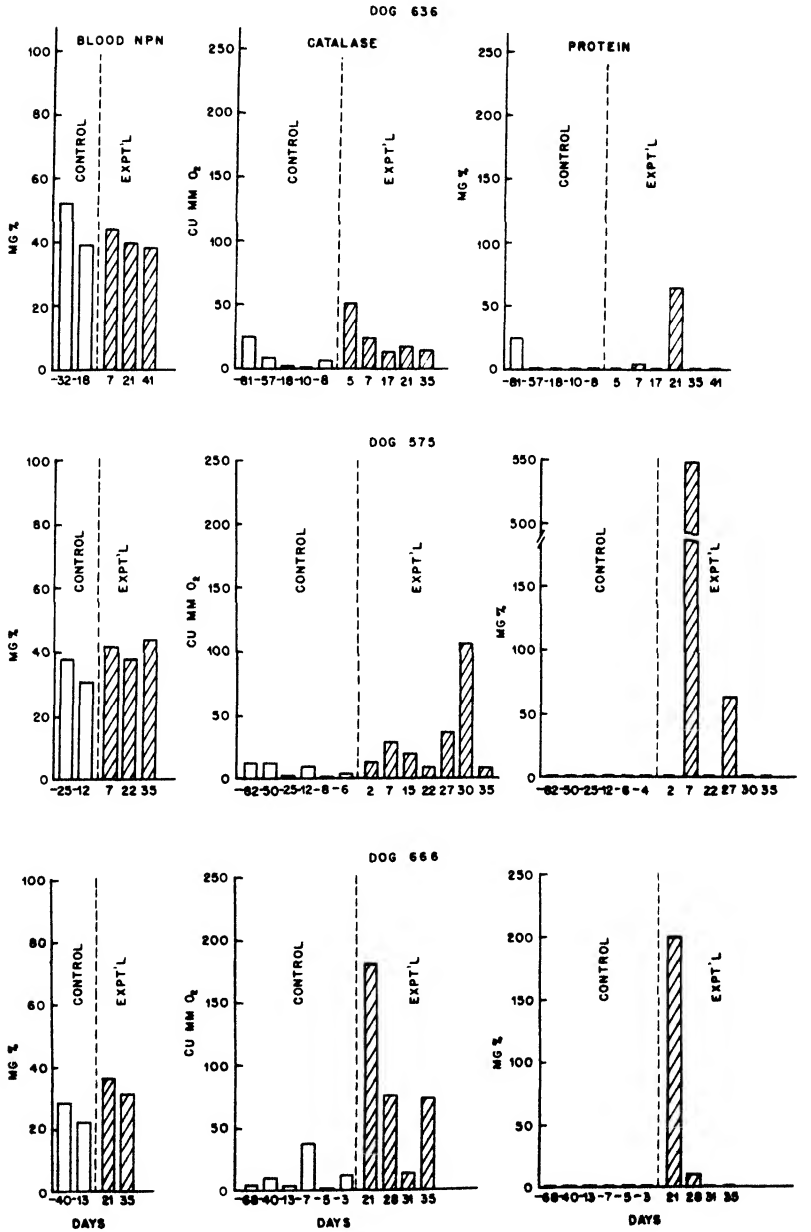


Fig. 10.41 — Blood NPN and urinary catalase and protein values of three dogs exposed to the 0.3-mg level of uranyl nitrate hexahydrate.

6. The ability to return glucose to normal blood levels after its administration under standard conditions was decreased in both the rat and dog, and the formation and retention of muscle glycogen was impaired in the rat as a result of exposure at the 20-mg level. The formation of liver glycogen in the rat proceeded normally.

7. The studies detailed in this section give a wide range of tests and procedures useful in evaluating uranium toxicity at different levels of exposure.

13. URANIUM TETRACHLORIDE

By Joseph J. Rothermel*

13.1 Introduction. Uranium tetrachloride, UCl_4, because of its peculiar physicochemical properties proved to be the most difficult of the several dusts tested for inhalation toxicity. Uranium tetrachloride, a bright-green crystalline material, is extremely hygroscopic, fuming immediately on exposure to the atmosphere, producing a number of hydrolysis and oxidation products, among which are the oxychloride, $UOCl_2$; uranyl chloride, UO_2Cl_2; the dioxide, UO_2; and hydrogen chloride, HCl. One of the chlorides is a tetravalent, the other a hexavalent uranium substance, both being soluble in the body fluids. The characteristics and toxicologic properties of UO_2 are described elsewhere in this chapter.

Important also from the toxicologic standpoint is the production of the gaseous hydrolysis product hydrogen chloride, which on a molar basis is formed in considerable proportion. In this respect uranium tetrachloride resembles uranium hexafluoride in that the tetrachloride contains the nephrotoxic constituent uranium, and also the pulmonary irritant hydrogen chloride. On a molecular basis, however, hydrogen chloride is approximately one-fourth as toxic as hydrogen fluoride.

Hereafter, in this section, by UCl_4 will be meant the products of hydrolysis, oxidation, and decomposition, $UOCl_2$, UO_2Cl_2, UO_2, and HCl, as well as the unhydrolyzed tetrachloride, as they occurred in the experimental chamber. Thus the toxicologic responses noted during the 180-hr studies represent the combined action of four uranium-containing compounds and the gaseous hydrogen chloride. The toxicologic importance of the latter cannot be disregarded at the higher levels; at the lower levels the effects of hydrogen chloride become negligible.

* Toxicologic procedures performed by Nils Eriksen, J. B. Field, J. J. Rothermel, J. Cobler, J. Brinkman, A. Shannon, J. Tornaben, and R. Maiers.

13.2 Exposure Equipment. Two types of exposure chambers were employed for the six levels. The one used for the 18-, 3.3-, 1.8-, and 0.2-mg levels was a rectangular enclosure with a capacity of approximately 38 cu ft, constructed of sheet steel and lined with sheet lead to resist chemical attack by the hydrolysis products of UCl_4. When in operation the unit contained small portable wire-meshed cages with compartments of different sizes suitable for the accommodation of five species of animals. The other chamber, which was used for the 0.3- and 0.1-mg levels, was constructed of wood and had a capacity of 324 cu ft. Its essential features are described in Sec. 6.1e.

A ball-mill feed was used to introduce the toxic material into the exposure unit (Sec. 6.2b). The dust from the ball-mill feed entered at mid-height through a port in one end of the chamber, and the dust-air mixture was withdrawn at a slightly higher level at the other end so as to aid in the suspension of the dust about the animal-exposure cages. The exhaust air was passed through a water scrubber that removed the soluble products from the chamber air before discharge into the outside atmosphere. A slight modification of the method of introducing the dust was resorted to at the lower concentrations by passing the dust through an elutriator before it entered the chamber. This gave a better control of the dust concentration through a reduction in the amount of dust from the feed.

In order to delineate the toxic responses to uranium tetrachloride, six 180-hr (approximately 30-day) inhalation studies were performed at the following levels: 18, 3.3, 1.8, 0.3, 0.2, and 0.1 mg UCl_4/cu m of air, equivalent to 11, 2.1, 1.1, 0.2, 0.1, and 0.06 mg U/cu m, respectively. Two of these studies, the 0.3- and 0.1-mg levels, represented the first 30 days of two year-long, chronic studies.

In all six studies the exposures were performed 3 to 7 hr each day, 6 days per week, for a total of 180 hr, apportioned over 35 to 40 calendar days. In the course of this work two different lots of uranium tetrachloride were used because of the depletion of the original stock set aside for the studies. The first lot that was used at the 18- and 3.3-mg levels was a dark-green and relatively coarse crystalline powder of widely varying particle size. The other lot, used for the remainder of the levels, was a very finely ground powder with a median particle size of approximately 3.5 μ. It is believed that the animals exposed to the coarser material were actually subjected to a higher concentration than was measured, because of the difficulty in maintaining a uniform suspension of the heavier particles in the chamber atmosphere. The sampling was performed 8 to 10 in. above the animal cages where only the lighter, more readily suspended particles predominated. The method of sampling dust concentration employed the Greenburg-Smith impinger.

With the second lot of uranium tetrachloride more assurance was placed on the values of dust-concentration samples because of the greater uniformity and fineness of particle size. The filter-paper dust sampler[9] was used for the four levels (1.8, 0.3, 0.2, and 0.1 mg) employing the finely ground dust.

13.3 Dust Concentration in Exposure Atmosphere. The control of the concentration within the exposure unit required close attention because of the physical nature of the dust and because the high toxicity of the compound demanded the use of low ranges of concentration. Table 10.64 shows the percentage of samples at given deviations from each of the levels as calculated from the weighted mean of individual samples taken hourly throughout each exposure.

Table 10.64 — Exposure to Dust Concentrations of UCl_4

Weighted-mean concentration, mg UCl_4/cu m	Deviation, mg/cu m	Samples within given deviation, %	Standard deviation, mg/cu m	No. of samples
18*	±2.0	15	11	143
	±6.0	46		
	±10.0	71		
	±14.0	82		
3.3*	±0.7	47	1.2	139
	±1.3	69		
	±2.2	88		
1.8	±0.2	33	0.61	181
	±0.4	53		
	±0.6	69		
	±0.8	80		
0.31†	±0.05	38	0.13	180
	±0.10	65		
	±0.15	75		
0.18	±0.02	19	0.08	164
	±0.06	43		
	±0.10	84		
	±0.14	93		
0.10†	±0.02	47	0.04	116
	±0.04	79		
	±0.06	90		

* Coarsely ground material used in these experiments.
† Refers to first 30 days of a 1-year study.

Table 10.64 shows that the deviation from the actual mean concentration was less at levels in which finely ground material was used (1.8, 0.3, 0.2, and 0.1), thus providing narrower ranges of dust concentration at levels where this was a primary requirement. Eighty

per cent of the samples were within ±67 and ±78 per cent of the weighted-mean concentration of the 18- and 3.3-mg levels, respectively, in which the coarser material was used. By comparison the same percentage of samples at the lower levels was well within 50 per cent of their respective weighted-mean concentrations.

13.4 Signs of Toxicity. At the higher levels of exposure the extremely poor condition of the animals was strikingly apparent. At the conclusion of each day's exposure, heavy green deposits of tetrachloride adhered to the fur of the animals, giving the animals an unkempt appearance. Although attempts were made to remove the deposit, they were not completely successful, and as the experiment progressed the fur retained a matted green appearance. There was also apparent on the teeth a heavy greenish-black deposit, although no gross dental changes resulted. Likewise, heavy deposits of the material were found on the anterior dorsal half of the tongue, buccal mucosa, and oral vestibule. The most common findings were marked dehydration, erythema and irritation of the epithelium of the tongue, and buccal mucosa. In the rats the deposits resulted not only from the inhalation of the compound but apparently also from licking of the uranium products from the fur.

Loss of appetite and of weight was general in animals exposed at the highest levels. There were, however, no external indications of pulmonary embarrassment attributable to the presence of hydrogen chloride.

13.5 Mortality. Uranium tetrachloride at the 18-mg level was highly lethal to the rabbit and mouse, moderately lethal to the rat, but only occasionally lethal to the guinea pig (Table 10.65). This order of species susceptibility is identical with that of the UF_6 (Sec. 10), the similarity of which to UCl_4 has already been pointed out. Characteristic of these materials was the speed with which the mortality occurred; all the 51 mice died within the first 6 days of exposure, and all six of the rabbits between the sixth and nineteenth day. The peak of mortality in the rat did not occur until the third and fourth week, only two rats dying before this time (Fig. 10.42, upper left).

At the 3.3-mg level, mortality in the mouse and the rat was considerably decreased but that of the rabbit remained relatively high. No deaths occurred among any of the animal species, which included the dog, at any level below 3.3 mg.

13.6 Weight Response. The weight response of animals furnished a dependable criterion of the degree of toxicity. In general a more favorable weight response occurred in all species with a decrease in dust concentration (Table 10.66). The exceptions noted were attributable to a higher mean initial weight of the group indicating maturity

Table 10.65 — Mortality of Animals Exposed 6 Hr Daily for Total of 180 Hr to UCl_4 Dust

Species	Concentration level* of dust, mg UCl_4/cu m	No. of deaths per no. exposed	Mortality, %
Rabbit	18	6/6	100
	3.3	4/6	67
	1.8	0/6	0
	0.31	0/20	0
	0.18	0/10	0
Mouse	18	51/51	100
	3.3	12/70	17
	0.18	0/49	0
Rat	18	13/20	65
	3.3	1/20	5
	1.8	0/20	0
	0.31	0/125	0
	0.18	0/19	0
	0.10	0/125	0
Guinea pig	18	1/20	5
	3.3	0/20	0
	0.31	0/30	0
Dog	1.8	0/4	0
	0.31	0/19	0
	0.10	0/19	0

* For levels of 18 and 3.3 mg UCl_4/cu m, a relatively coarse crystalline powder was used in this study, rendering uncertain the values of the dust concentration. For levels of 1.8, 0.31, 0.18, and 0.10 mg UCl_4/cu m, a very finely ground powder was used.

in the animals and consequent slower rate of growth. A typical weight response of two of the species, rabbit and rat, exposed at the 18-mg level is represented graphically in Fig. 10.42, upper right. A very similar growth pattern was observed in the animals exposed to the 3.3- and 1.8-mg levels, but the changes were not so marked. At these concentrations the higher susceptibility of the rabbit was again in evidence. At the three lowest levels gradual weight gains were observed in all species except the dog, which showed a slight weight loss, a trend noticed in a group of control dogs.

13.7 Biochemical Findings. (a) Blood. Determinations of blood nonprotein nitrogen and urea in rabbits (blood samples taken on second and ninth calendar days of the experiment) showed a pattern of changes similar to that for UF_6 in that maximal values were observed after approximately 8 days of exposure to both the 18- and 3.3-mg

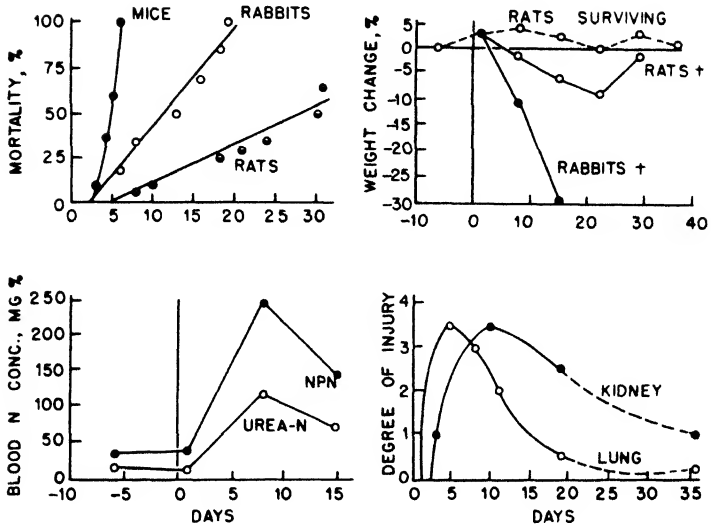


Fig. 10.42 — Average toxicologic responses to UCl_4-dust exposures at 18 mg/cu m. Upper left, mortality of three animal species; upper right, changes in body weight from preexposure values; lower left, changes in NPN and urea nitrogen of blood of rabbits; lower right, schematic representation of the course of pulmonary and renal injury. Crosses indicate deaths.

Table 10.66 — Body-weight Change of Animals Exposed to UCl_4*

Concentration level of dust, mg UCl_4/cu m	Weight change, %				
	Rabbit	Rat	Guinea pig	Mouse	Dog
18	−30	+0.6	−0.5	†	
3.3	+9	−4	+20	+10	
1.8	+24	+31			+0.3
0.31‡	+14	+84	12		−6
0.18	+15	−2§		+15	
0.10‡		+84			−10

* Weight changes for rabbits at the 18-mg level include those of animals that died as well as those that survived; all other weight changes are for survivors only.

† No weight change was recorded because death occurred before the scheduled time of weighing.

‡ Refers to the first 30 days of a chronic study.

§ The mean initial weight of this group of adult rats was 343 g in comparison with an approximate mean weight of 200 g for the groups on the other levels.

levels, followed by a decrease to normal values in surviving animals. In animals that died, the NPN and urea nitrogen rose to levels incompatible with life (Table 10.67).

At the 18-mg level all the rabbits were azotemic after 1 week of exposure. At this level the highest NPN and urea nitrogen values recorded were 458 mg % and 246 mg %, respectively. Averaged values of six rabbits showed the highest value at any time to be 250 mg % for blood NPN, 115 mg % for urea nitrogen (Fig. 10.42, lower left).

The maximal values for these blood constituents at the 3.3-mg level were noticeably lower than those at the higher level, even among rabbits that died from the exposure, although the surviving rabbits still showed well-elevated values of NPN that later decreased to within normal range. Neither dogs nor rabbits showed azotemia at the 1.8-mg level. Concentrations of 0.3 mg and lower induced no abnormal changes in these constituents during the 180 hr of exposure.

(b) Urine. Rabbits exposed to the 1.8-mg level showed a slight and infrequent proteinuria but no glycosuria. At levels of 0.3 mg and below, in which both dogs and rabbits were tested, neither protein nor reducing sugar appeared in concentrations above those occurring normally.

In rabbits exposed to the 18-mg level the urinary amino acid nitrogen rose gradually to three times the preexposure value. At the 0.2-mg level no significant changes were observed.

13.8 Pathology. Like all the uranium compounds studied, uranium tetrachloride produced renal-tubular necrosis in animals exposed to atmospheres containing it. In addition, pulmonary hemorrhage and edema were observed in rabbits exposed to high concentrations of UCl_4 (18 mg/cu m), a finding also observed in UF_6 exposures. Evidence of mild pulmonary irritation was noted in serially studied mice at this level and in rabbits at the 3.3-mg level.

The rabbits and mice exposed to the 18-mg level showed moderately severe degeneration and necrosis of the renal-cortical tubular epithelium in early-dying animals and degeneration as well as associated processes of regeneration in animals dying later during the exposure. Progressive changes in serially sacrificed mice were typical of those usually observed during exposure to moderately high concentrations of uranium compounds (see Chap. 4). In addition to renal damage, pulmonary and respiratory changes were also observed in rabbits. The toxic action of uranium tetrachloride on mucous membranes was indicated by acute inflammation of the trachea and by ulceration of the tongue. Damage to the lung itself was of varying intensity ranging from mild acute interstitial inflammation to moderately severe hemorrhage and edema (Fig. 10.42, lower right).

Table 10.67 — Biochemical Studies on Animals Exposed to UCl_4

Concentration level of dust, mg UCl_4/cu m	Animal species	Blood		Urine		
		Nonprotein nitrogen	Urea nitrogen	Protein	Sugar	Amino acid nitrogen
18	Rabbit	Max. value* 458 mg %	Max. value* 246 mg %			Values started rising during first week and continued until death. Mean value increased 3-fold over preexposure value
3.3	Rabbit	The 4 animals that died showed high values before death; max. value 348 mg %; the 2 animals that survived had elevated values that then returned to normal				
1.8	Rabbit	No changes		Slight, infrequent proteinuria	Did not appear	
1.8	Dog	No changes				
0.31	Rabbit	No changes		No values above normal		
0.31	Dog	No changes		No values above normal	Did not appear	
0.18	Rabbit	No changes	No changes	Did not appear	Did not appear	No significant changes
0.10	Dog	No changes		No values above normal	Did not appear	

*All animals showed azotemia.

At the 3.3-mg level, rabbits showed pulmonary changes that were much less severe than at the higher level and renal changes that consisted of moderate degeneration and necrosis of the cortical tubular epithelium with associated processes of regeneration. Rats exposed to the 3.3-mg level showed renal pathology similar to that seen in the rabbits but of less severity.

Dogs exposed to the 1.8-mg level showed renal-tubular-epithelial changes limited for the most part to the corticomedullary region but showed no pulmonary damage. This level also produced mild to moderate damage in the kidneys of rabbits but produced in rats no renal changes that were detectable after 180 hr exposure apportioned over 39 days. The fact that the kidneys in rats were essentially normal at the end of 180 hr of exposure does not preclude the possibility that changes, which were later repaired, occurred during the exposure.

Except for extremely mild renal-tubular changes in kidney sections obtained from dogs after 10 days of exposure to 0.3 mg, and extremely slight alteration in renal tubular epithelium in mice after 1 and 2 weeks of exposure to 0.2 mg, no morphological changes related to exposure to UCl_4 were observed in dogs, rats, and rabbits at the 0.3-, 0.2-, and 0.1-mg levels (Table 10.68).

13.9 Hematology. Hematology performed on several animal species at all levels except that at 0.2 mg showed no changes in the numbers of blood-cellular constituents that were sufficiently consistent to allow the use of this measure as an index of uranium poisoning in animals exposed to uranium tetrachloride. The 18-mg level seemed to show more definite changes than other levels, but the changes could not be correlated with the more definite signs of toxicity, i.e., mortality, changes in biochemical constituents, and histological changes. For example, there was a continuous fall in the hemoglobin of the rat in which there was considerable mortality, but, on the other hand, there was a marked rise in the hemoglobin of the rabbit for which the same level was uniformly lethal. Likewise, there were identical trends in the two species with respect to the red-blood-cell counts but also a reverse and opposite response in the reticulocytes.

13.10 Distribution of Uranium in Tissues. Spectrochemical analysis for the content of uranium in the kidney, lung, liver, bones, and teeth of the rabbits, rats, and guinea pigs showed a high incidence of uranium in both the hard and soft tissues at the 18-mg level, a noticeable decrease in incidence at the 3.3-mg level, and only a rare occurrence in the tissues of any of the species exposed at the lower levels (Table 10.69). An improvement in the preparation of the samples occurred at levels below 3.3 mg. Whereas uranium was found uniformly in the osseous tissue and in the majority of the soft tissues

Table 10.68 — Pathological Observations in Animals Exposed to UCl_4

Concentration level of dust, mg UCl_4/cu m	Dog	Rat	Rabbit	Mouse
18			Inflammation of mucosa of the trachea; pulmonary damage ranged from mild to severe; moderately severe renal damage with peak at 8 to 11 days followed by regeneration	Extensive renal-tubular necrosis within 3rd day followed by regeneration on 4th to 6th day
3.3		Mild pulmonary changes; mild degeneration and necrosis of renal-cortical tubular epithelium with associated processes of regeneration	Moderate to mild pulmonary hyperemia and hemorrhage; moderate renal-tubular degeneration and necrosis	
1.8	Moderate damage to renal tubular epithelium	No renal damage detectable after 39 days	Mild to moderate kidney damage	
0.31	Extremely mild renal-tubular changes in sections obtained after 10 days of exposure	No morphological changes related to treatment in rats serially sacrificed		
0.18		No changes related to treatment	No changes related to treatment	Extremely slight alteration in renal tubular epithelium observed at only 1 and 2 weeks of exposure
0.10	No abnormalities observed in sections of kidney obtained after 10 days of exposure	No morphological changes related to treatment in rats serially sacrificed		

Table 10.69 — Frequency of Occurrence* of Uranium in Tissues of Dogs, Rats, Rabbits, and Guinea Pigs Exposed to UCl_4

Tissue	Concentration level of dust, mg UCl_4/cu m	No. of occurrences per no. exposed	Occurrences, %
Kidney	18	27/31	87
	3.3	17/32	56
	1.8	0/21	0
	0.31	0/22	0
	0.18	0/14	0
	0.10	0/21	0
Lung	18	24/33	76
	3.3	16/32	50
	1.8	0/23	0
	0.31	2/22	9
	0.18	0/14	0
	0.10	1/21	5
Liver	18	20/32	65
	3.3	3/32	9
	1.8	0/20	0
	0.31	2/22	9
	0.18	0/15	0
	0.10	1/21	5
Bones	18	101/101	100
	3.3	64/210	30
	1.8	0/133	0
	0.31	0/21	0
	0.18	1/107	1
	0.10	1/21	5
Teeth	18	89/89	100
	3.3	33/105	31
	1.8	0/78	0
	0.18	2/66	3

*Occurrence of uranium was recorded for soft tissues if the amount was more than 0.2 μg/g of wet tissue and for hard tissues if the amount was more than 5 μg/g of ash.

analyzed at the 18-mg level, a somewhat higher frequency of occurrence of uranium was found in the kidney and lung at the 3.3-mg level than in the bone. In the liver at this level only occasional samples showed the presence of detectable amounts of uranium.

Summaries of four of the six levels studied showing the responses for each criterion of toxicity are given in Tables 10.70 to 10.73.

13.11 Discussion. The determination of the toxicity of uranium tetrachloride was a difficult study. The distribution of the material

Table 10.70—Animals Exposed for 180 Hr to 18 Mg UCl_4/cu m (Summary of Results)

1. Mortality. Uranium tetrachloride is one of the more toxic of the uranium compounds.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	6/6	100
Mouse	51/51	100
Rat	13/20	65
Guinea pig	1/20	5

2. Weight Changes. Appreciable weight loss was recorded in only the rabbit. All mice died within first 6 days.

Species	Mean initial wt., g	Wt. change, %
Rabbit	2,920	−30
Rat	164	+0.6
Guinea pig	471	−0.5

3. Biochemistry.
   Blood, Rabbit. Both blood NPN and urea N rose to high values during the first 8 days of exposure. Because of the early deaths of the rabbits, it was not possible to observe a return to normal values.

	Calendar days			
Determination	−6	+1	+8	+15
NPN, mg %*	34	37	249	146
Urea N, mg %*	17	12	116	69

* Mean values.

   Urine, Rabbit. Urinary amino acid nitrogen started rising during the first week and continued rising until death of the rabbits.
4. Pathology.
   Rabbit. Irritation of mucous membranes leading to ulceration and acute inflammation of the tongue and trachea. Pulmonary irritation and moderately severe renal damage.
   Mouse. Early extensive renal-tubular necrosis followed by regeneration.
5. Hematology. No significant changes were observed in rabbits and rats.

within the chamber was far from uniform, especially at the two highest concentrations, because of the impossibility of uniformly distributing dust particles that are constantly acquiring increasing amounts of water during the time of suspension in the atmosphere. Furthermore, with hygroscopic particles aggregation to larger particle sizes is common, and the high density of the material further complicates the problem. At the lower level, although certain of the disturbing

Table 10.71 — Animals Exposed for 180 Hr to 3.3 Mg UCl_4/cu m (Summary of Results)

1. Mortality. At this concentration uranium tetrachloride was highly toxic to rabbits, moderately toxic to mice, and only mildly toxic to rats.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	4/6	67
Mouse	12/70	17
Rat	1/20	5
Guinea pig	0/20	0

2. Weight Changes. All species showed a weight gain except the rat.

Species	Mean initial wt., g	Wt. change, %
Rat	196	−4
Rabbit	3,060	+9
Mouse	20.8	+10
Guinea pig	398	+20

3. Biochemistry.
   Blood, Rabbit. Blood NPN rose to a high value during the first 8 days of exposure. Two rabbits that died on the fourth and fifth days had an NPN value of about 200 on the first day after exposure.

	Calendar days							
Determination	−13	−6	+1	+8	+15	+22	+29	+36
NPN, mg %*	38	39	100	164	56	48	38	49

* Mean values.

   Urine, Rabbit. During the third week of exposure urinary ammonia nitrogen excretion rose sharply to five times the normal value.
4. Pathology.
   Rabbit. Moderate to mild pulmonary hyperemia and hemorrhage. Moderate renal-tubular degeneration and necrosis along with regenerative processes.
   Rat. Mild degeneration and necrosis of renal-cortical tubular epithelium with associated processes of regeneration.
5. Hematology. No abnormal changes occurred in rabbits or rats.

factors prevailed, they occurred to a lesser degree, and the distribution conditions were considerably improved. This resulted from the use of a new lot of material of smaller particle sizes and of dust more highly attenuated. Both of these factors tended to produce more favorable conditions for uniform distribution of dust.

Uranium tetrachloride inhaled as dust at or above the 3.3-mg level was highly toxic to the rabbit and mouse, moderately toxic to the rat, but only mildly toxic to the guinea pig. The same order of species susceptibility was observed in inhalation exposure studies of uranium

Table 10.72 — Animals Exposed for 180 Hr to 1.8 Mg UCl_4/cu m (Summary of Results)

1. Mortality. No deaths occurred among 4 dogs, 6 rabbits, and 20 rats.
2. Weight Changes. All species showed a slight to moderate gain in weight.

Species	Mean initial wt., g	Wt., change, %
Dog	7,700	+0.3
Rabbit	2,530	+12
Rat	180.3	+31

3. Biochemistry:
   Blood, Dog and Rabbit. Blood NPN remained within the normal range throughout the experiment.
   Urine, Rabbit. Slight infrequent proteinuria.
4. Pathology.
   Dog. Moderate damage to renal tubular epithelium after 39 days of exposure.
   Rabbit. Mild to moderate kidney damage.
   Rat. No renal damage detectable after 39 days.
5. Hematology. No abnormal changes occurred in dogs or rabbits.

Table 10.73 — Animals Exposed for 180 Hr to 0.1 Mg UCl_4/cu m (Summary of Results)

1. Mortality. No deaths attributable to exposure resulted among 19 dogs and 125 rats.
2. Weight Changes. The dogs showed a slight loss in weight.

Species	Mean initial wt., g	Wt. change, %
Dog	8,300	−10
Rat	131.4	+84

3. Biochemistry.
   Blood, Dog. Blood NPN remained within the normal range throughout the experiment.
   Urine, Dog. Urinary-protein values were all within normal limits.
4. Pathology.
   Dog. No abnormalities observed in sections of kidney obtained after 10 days of exposure.
   Rat. No morphological changes related to treatment.
5. Hematology. No abnormal changes occurred in dogs and rats.

hexafluoride but resembled only in part the order of susceptibility to uranyl fluoride, further emphasizing the difference between uranium compounds capable or incapable of liberating pulmonary irritants. The peculiar susceptibility of the rat and the mouse to pulmonary irritants, of which halogen acids are examples, is well known. On the other hand, the maximal theoretical concentration of the hydrogen chloride even at the highest level of exposure could never have reached a level sufficient to produce of itself a mortality in any of the

species exposed. The upper limit of safety of atmospheres containing hydrogen chloride is 50 mg/cu m as shown in experiments by Machle *et al.* [57] with rabbits and guinea pigs continued over a period of 120 hr. Assuming complete hydrolysis of the uranium compound, the concentration of hydrogen chloride could never have exceeded 7 mg/cu m at the highest level. Although, unfortunately, no rats and mice were included in the study of hydrogen chloride exposures, it is known that hydrogen fluoride produces no mortality in rats and mice exposed to 7 mg/cu m of hydrogen fluoride (see Chap. 17). It must be conceded, therefore, that hydrogen chloride acts in a capacity secondary to that of uranium.

It should be noted that in addition to the potential toxicity of the hydrogen chloride, the uranium toxicity observed is the resultant of four uranium materials. Of these, uranyl chloride undoubtedly is the main contributor to the toxicity in so far as it bears the uranyl ion, the most toxic of the ions of uranium. The toxicity of uranium oxychloride has never been determined. On the other hand, the toxicity of uranium dioxide has been established (see Sec. 15) and at the 20-mg level has been found to be moderately toxic to rabbits only. The contribution to toxicity by the undecomposed tetrachloride is unknown.

Suggestive evidence of the multiplicity of uranium products in the exposure chamber is provided by the observation of five types of particles distinct as regards color in samples taken for particle-size measurement. Regardless of what specific compounds were responsible for the observed toxicity, the fact remains that most of these were very highly soluble materials, which condition, in the case of uranium, results in high toxicity.

13.12 Summary. Uranium tetrachloride, when inhaled by laboratory animals exposed to various concentrations, showed the following:

1. At the 18-mg level, mortality was high in the rabbits and mice, moderate in the rats, and low in the guinea pigs. At the 3.3-mg level, mortality in the mice and the rats was considerably decreased but in the rabbits remained relatively high. No mortality was observed in the rabbits, rats, mice, guinea pigs, and dogs at and below concentrations of 1.8 mg/cu m.

2. At the 18-mg level the rabbits showed a pronounced loss in body weight, whereas the rats and guinea pigs showed weight losses during the first 3 or 4 weeks of exposure, followed by a return to their preexposure weight. At the 3.3-mg level a very similar but less pronounced picture was observed in the weight of the rabbits and rats, but the guinea pigs showed a continuous weight gain throughout the experiment. No abnormal weight changes were observed in any of the species at or below the 1.8-mg levels.

3. Rabbits showed a marked azotemia at the 18-mg level and a moderate azotemia at the 3.3-mg level; however, neither rabbits nor dogs were azotemic at or below the 1.8-mg level.

4. Marked proteinuria and glycosuria occurred in rabbits exposed to the higher concentrations, but only slight and infrequent proteinuria occurred in rabbits at the 1.8-mg level. Neither rabbits nor dogs developed proteinuria at concentrations below 1.8 mg/cu m. In rabbits significant increases in urinary amino acid nitrogen were observed at the 18-mg level but not at the 0.2-mg level.

5. At the 18-mg level rabbits and mice showed moderately severe degeneration and necrosis of the renal-cortical tubular epithelium in early-dying animals and degeneration as well as regeneration in animals dying later during the exposure. In addition pulmonary changes were observed in the dying rabbits. At the 3.3-mg level mild to moderate changes were found in the kidneys of rabbits and rats and lungs of rabbits. At the 1.8-mg level both dogs and rabbits showed moderate renal-tubular damage, but no changes were found in the rats. At concentrations of 0.3 mg/cu m and lower only very slight renal damage was observed in the dogs and mice and no morphological changes in rabbits and rats.

6. No significant changes were observed in the hemogram of the dog, rabbit, or rat.

7. At the 18- and 3.3-mg levels, uranium was found in the lung, liver, kidney, bones, and teeth of rabbits, rats, and guinea pigs. At the lower levels practically no uranium was found in either the soft or hard tissues.

14. URANIUM TETRAFLUORIDE

By H. P. Dygert*

14.1 Introduction. The determination of the inhalation toxicity of uranium tetrafluoride, the "green salt" of industry, represents a study of one of the more widely used intermediates in the uranium chemical industry. In a number of operations it is handled in a dry state in a finely pulverized form, forming a potential exposure hazard for personnel and a consequent industrial-hygiene problem. Because of the importance of the problem, tests of the green salt have been made at five levels of exposure ranging from 24 to 0.5 mg/cu m.

* Toxicologic procedures performed by A. Rothstein, H. P. Dygert, H. A. Oberg, D. Dittman, H. L. Berke, R. Sanford, G. Laush, J. P. Zambuto, and E. Fisher.

The dust was nonhygroscopic and presented no special difficulties in the preparation of dusty atmospheres for study. Its density of 6, however, required special attention for the maintenance of a uniform distribution of the exposure atmosphere. The dust had a moderate solubility in water, greater than that of one of the other tetravalent dusts (UO_2) studied but far less than uranium tetrachloride. Contrary to other uranium compounds, UF_4 was the only one of twelve studied that showed a solubility in blood serum not appreciably different from that in water. Another property of some toxicologic concern is the oxidizability of the tetrafluoride as indicated by the presence in commercial samples of from 2 to 3 per cent of highly toxic uranyl fluoride (Sec. 11). This oxidative process may also be expected to contribute to the toxicity of the tetrafluoride locally in the lungs. Another impurity, the dioxide, also occurs in the tetrafluoride, but this oxide has a low order of toxicity (Sec. 15).

The study of UF_4 presents toxicological information on a nonoxygenated tetravalent uranium compound that contains, in addition to uranium, an ion of demonstrated toxicity, namely, fluoride. The results obtained with this dust afford an interesting comparison with that of hexavalent uranium compounds containing either oxygen or fluorine.

14.2 Exposure Conditions. The five studies were performed at average exposure levels of 24, 5, 4, 0.7, and 0.5 mg UF_4/cu m, equivalent, respectively, to 18, 3.8, 3, 0.6, and 0.38 mg U/cu m. The experiments were performed in units of the full exposure type described in Sec. 6.1e. At the highest level a crude dust feed was used, which consisted of an elutriating bottle containing the dust. The introduction of the dust into the chamber was accomplished by means of a hand-operated intermittent jet of compressed air and occurred at 15-min intervals of 1-min duration. This feed led to a less well-controlled dust concentration than if a pressure feed had been used. During the first 9 days of the exposure, the mean initial concentration was 40 mg UF_4/cu m. After this time a better control of the dust concentration gave a mean concentration of 18 mg of dust per cubic meter for the remainder of the experimental period, a value more closely approximating the desired mean of 20 mg/cu m. The over-all mean for the 30-day study at this highest level was 24 mg UF_4/cu m. The mean concentration and the percentage of samples deviating from the mean at each exposure level are given in Table 10.74. Dust concentrations produced by the pressure feed were uniformly good for the four lower levels. A good example of an exceptionally well-controlled dust concentration is illustrated by the results at the 0.5-mg level; two-thirds of the samples deviated no more than ± 20 per cent of the mean concentration.

Table 10.74 — Dust Concentration of UF_4 in Milligrams per Cubic Meter

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
24*	± 2	14
24*	± 4	33
24*	± 6	61
24*	± 8	79

Note: desired concentration, 20.0 mg UF_4/cu m; standard deviation, ± 15.6 mg/cu m; number of samples, 211.

*Average of 18 to 40.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
4.8	± 0.5	27
4.8	± 1.0	49
4.8	± 1.5	72
4.8	± 2.0	86

Note: desired concentration, 5 mg UF_4/cu m; standard deviation, ± 1.1 mg/cu m; number of samples, 102.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
3.8	± 0.5	41
3.8	± 1.0	68
3.8	± 1.5	81

Note: desired concentration, 4.0 mg UF_4/cu m; standard deviation, ± 1.2 mg/cu m; number of samples, 177.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
0.7	± 0.1	17
0.7	± 0.2	38
0.7	± 0.3	60
0.7	± 0.4	78

Note: desired concentration, 0.7 mg UF_4/cu m; standard deviation, ± 0.29 mg/cu m; number of samples, 189.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
0.5	± 0.1	67
0.5	± 0.2	88
0.5	± 0.3	88

Note: desired concentration, 0.5 mg UF_4/cu m; standard deviation, ± 0.1 mg/cu m; number of samples, 84.

The particle size of the bulk UF_4 dust had a median value of 0.8 μ with a standard geometric deviation of 2.5 determined by the microprojector method (Sec. 8.2).

14.3 Signs of Toxicity. Clinical signs of poisoning were most obvious in the cat and dog at the highest level. These animals showed anuria and polydipsia by the 7th day of exposure. On the 8th day rhinitis and conjunctivitis were first noted, and vomiting occurred immediately after drinking. The vomitus, at first copious and extremely viscous, later decreased in volume and became bloody. The cat and the dog on the 10th and 13th day, respectively, exhibited muscular weakness. Within a few days thereafter these animals evidenced instability of gait. Shortly thereafter they became moribund, and death occurred 48 hr later.

No outward signs of toxicity occurred in any animals exposed at the four lower levels.

14.4 Mortality. Only the highest of the five levels resulted in appreciable mortality (Table 10.75), as 21 per cent of the 66 animals exposed at this level died. In general, death occurred during the first 9 days when the average concentration was 40 mg/cu m. In the subsequent period, while the average concentration was approximately 18 mg/cu m, deaths occurred in animals of the same species after a longer exposure period. Mortality was most pronounced in the cat and dog but less in the rat, rabbit, and guinea pig. No deaths occurred among the mice. Deaths occurred in the majority of the cases between the first and third weeks of exposure. There was only one death in a total of 669 animals exposed at the remaining four levels. Accordingly, mortality from exposures to uranium tetrafluoride dust was noticeable only at a range of approximately 18 to 40 mg/cu m.

14.5 Weight Changes. Weight losses among all species were prominent at the highest level of exposure, and at this level the greatest weight loss occurred among the dying animals. Losses amounted to between 18 and 28 per cent. Weight changes among the surviving animals ranged from a maintenance of body weight to gains of 48 per cent in a single surviving rabbit. Consistent with the results of mortality, at the four lower levels animals either showed gains similar to those of healthy, growing animals or maintained their weight characteristic of adult animals (Table 10.76).

14.6 Biochemical Findings. The biochemical determinations used consisted of (1) less sensitive but reliable indices of uranium poisoning, such as blood NPN and urea N at the highest level and (2) more sensitive indicators of toxicity, such as urinary protein, amino acid nitrogen-creatinine ratios, and catalase and phosphatase activity at the three lowest levels, in addition to blood NPN determinations. At the 5-mg level no biochemistry was performed. Marked biochemical

Table 10.75 — Mortality of Animals Exposed Daily to UF_4

Species	Concentration level of dust, mg UF_4/cu m	No. of deaths per no. exposed	Mortality, %
Rabbit	24*	1/3	33
	4	0/19	0
	0.5	0/10	0
Cat	24*	3/3	100
Dog	24*	1/1	100
	5	0/4	0
	4	0/18	0
	0.7	0/18	0
Rat	24*	6/19	32
	5	1/30	3
	4	0/200	0
	0.7	0/200	0
	0.5	0/30	0
Guinea pig	24*	3/20	15
	5	0/20	0
	4	0/20	0
	0.5	0/20	0
Mouse	24*	0/20	0
	5	0/40	0
	0.5	0/40	0

* The value 24 represents the average of the range 18 to 40.

Table 10.76 — Weight Changes in Animals Exposed to UF_4

Concentration level of dust, mg UF_4/cu m	Wt. change, %					
	Rabbit	Cat	Dog	Rat	Guinea pig	Mouse
24[a]	−24[b]			−26[c]	−28[d]	
	+48[e]	−18	−26	+28[f]	0[g]	
5			−2	0	+32	+9.8
4	+12		+3	+52[h]	0	
0.7			+6	+36[h]		
0.5	+37.2			−0.4	−3.0	+9.6

a Average of 18 to 40.
b Two died.
c Six died.
d Three died.
e One survived.
f Thirteen survived.
g Seventeen survived.
h These were immature rats; those at the 5- and 0.5-mg levels were adult animals.

changes were found in three species exposed at the highest level, and slight or no changes were noted in two other species (Table 10.77a,b). The rise of NPN and urea N concentration in the blood of dog, cat, and

Table 10.77a — Biochemistry

Concentration level of dust, mg UF_4/cu m	Species	No. of animals	Max. value blood NPN, mg %	Max. value blood urea N, mg %	Remarks
24*	Rabbit	3	306	88	Azotemia in 2 animals
24*	Cat	3	396	100	Azotemia in 2 animals
24*	Dog	1	398	80	Azotemia in 1 animal
24*	Rat	14	82	31	Slight azotemia in 4 animals

* Average of 18 to 40 mg UF_4/cu m.

Table 10.77b — Biochemistry

Concentration level of dust, mg UF_4/cu m	Species	No. of animals	Blood NPN	Protein	Catalase	Phosphatase	AAN-creatinine ratio
4	Rabbit	10	No azotemia	Normal			Slight elevation in 2d week; max. value 1.0
4	Dog	10	No azotemia	Normal			No excessive values
0.7	Dog	10	No azotemia	Normal			No excessive values
0.5	Rabbit	10	No azotemia	Normal	Elevation in 9; max. value > 200 cu mm of O_2	Slight elevation in 9; max. value 19.6 K-A units	

rabbit reached values as high as 398 and 100 mg %, respectively; that of the rat and guinea pig attained only slight (82 mg % NPN) or no elevation. Animals examined at the three lower levels showed neither azotemia nor proteinuria but did show biochemical evidence of toxicity at the 4-mg level by more sensitive tests, such as urinary amino acid, catalase, and phosphatase activities. Urinary amino acid nitrogen-creatinine ratios increased from 0.3 to a value of 1.0 in the rabbit, but no increase was noted in the urine of dogs exposed at this level. Additional evidence of toxicity was given by an increase in urinary catalase and phosphatase activity in rabbits exposed at the 0.5-mg level. Elevation in the catalase values did not occur until the 16th day, and that of phosphatase, not until the 32nd day of the exposure period.

14.7 Hematology. Hematologic studies performed on animals at all levels of exposure except the 0.5-mg level showed no changes in

any species that differed significantly from that of the controls. The species studied included the dog, rat, and the guinea pig.

14.8 Pathology. Histologic examination of critical tissues of animals exposed at each of the five levels showed marked renal injury only at the highest. This was uniformly found in all five species. Progressively less renal injury was seen at the 5- and 4-mg levels, and no demonstrable tissue changes related to the exposure were seen at the two lowest levels. Although renal pathology appeared in approximately the same intensity in all the species at the highest level of exposure, differences in pathologic response became apparent at lower levels of exposure. At the 5-mg level no pathology typical of uranium poisoning was found in the rat, guinea pig, or mouse, whereas there was moderate renal involvement in the dog. This species difference in renal cellular response was again noted at the 4-mg level, but below this level no further injury in this species was observed. No pulmonary damage of any sort was seen in exposure to uranium tetrafluoride.

The type of renal changes resulting from exposures to this dust at the highest level consisted of moderate to severe necrosis of the corticomedullary tubular epithelium in animals dying throughout the exposure period, whereas in the surviving animals—rabbit, rat, and guinea pig—necrosis was less widely apparent and was associated with numerous regenerative changes at end of the 30-day period. At the 5- and 4-mg levels* evidence of slight to moderate renal injury was suggested by the generalized nature of the regenerative process of the tubular epithelium in dogs. It should be reemphasized that no such evidence of renal injury was seen in the rat, guinea pig, and mouse exposed to the same conditions (Table 10.78).

14.9 Uranium in Tissues. The qualitative aspects of distribution of uranium were determined by the spectrochemical method in the animals exposed at the 24-, 5-, and 0.5-mg levels (Table 10.79). The frequency with which the element occurred in seven tissue samples in each of five species was determined on the basis of its occurrence in amounts greater than 1 μg. At the highest level, uranium was commonly found in all tissues analyzed except the spleen. At the two lower levels, 5 and 0.5 mg, a notable decrease in frequency occurred in the soft tissues, and no uranium was detectable in bones.

A separate study of the digestive tract and integument was made to determine the relative amounts of uranium in extrarespiratory sites.

*At the 5-mg level all animals were studied after 30 days, and at the 4-mg level dogs and rats were studied within the first 10 days of exposure.

Table 10.78 — Pathology of Animals Exposed to UF_4

Concentration level of dust, mg UF_4/cu m	Rabbit	Rat	Dog	Cat	Guinea pig	Mouse
24 (18 – 40)	Moderate typical renal injury in 2 of 3 dying or sacrificed animals; moderate regenerative changes, thickened basement membranes of the tubules, and tubular casts	Acute typical renal injury in all 6 dying; very mild changes with typical regeneration and nearly complete repair in all 11 sacrificed	Typical renal injury evidenced as necrosis of the corticomedullary tubular epithelium; only 1 dog studied	Severe injury in 1 dying cat; moderate typical renal injury in a second dying cat; autolysis in third cat	Of 3 dying guinea pigs, 1 showed subacute typical renal injury; 1 showed acute renal injury; 1 showed chronic renal injury; 2 of above had moderate pulmonary infection; 17 sacrificed guinea pigs showed moderate regenerative changes	
5		No injury was seen in kidneys of 27 sacrificed rats; salmonellosis* present in a few of rats examined	Four sacrificed dogs showed typical renal injury with slight to moderate amounts of regenerated epithelium present		No injury in 5 sacrificed guinea pigs	No injury in 5 sacrificed mice
4		No injury in rats sacrificed serially about every third experimental day	Very slight early tubular changes in inner cortex of kidney, these were typical changes found in 1 sacrificed and 1 biopsied dog after 10 days			
0.7		No injury in rats sacrificed serially about every third experimental day	No renal injury in 1 sacrificed nor in 1 biopsied dog after 10 days			
0.5	No injury in 10 sacrificed rabbits	No injury was seen in 40 sacrificed rats; salmonellosis syndrome present in a few rats examined			No injury in 5 sacrificed guinea pigs	No injury in 5 sacrificed mice

* Salmonellosis syndrome was characterized by edematous cecal lymph nodes, ulceration of cecum, and focal necrosis of liver and spleen.

Table 10.79 — Uranium Distribution in Five Species of Animals as Determined by the Spectrochemical Method

Concentration level of dust, mg UF_4/cu m	Soft tissues								Bone					
	Lung		Kidney		Liver		Spleen		Pelvis		Spinal column		Femoral epiphysis	
	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%
24*	45/46	98	17/43	40	12/44	27	0/3	0	18/30	60	23/41	56	16/35	46
5.0	11/25	44	3/22	14	2/22	10	1/24	4	0/25	0	0/22	0	0/24	0
0.5	5/29	17	0/25	0	1/28	3	1/28	5	0/29	0	0/28	0	0/29	0

*The figure 24 represents an average of values ranging from 18 to 40.

The analyses were performed by the spectrochemical method on animals exposed at the highest concentration. The uranium content of the stomach and intestine was of the same order of magnitude as that of the lung in five species save the rat; in this species relatively enormous amounts of uranium were found in the stomach and intestine of two animals examined. This was likewise true of the integument. The amount of uranium in the tongue was slightly less than that in the lung but of approximately the same order of magnitude as in the trachea.

The concentration of uranium was quantitatively estimated by the fluorophotometric method (Chap. 11) in four tissues of rats exposed at the 4- and 0.7-mg levels. Eight to 10 samples of each tissue were analyzed at each level. By this method, values of 0.1 μg or greater represent an actual increase of uranium in the tissues. Table 10.80 shows the range and mean of the values. The lung contained the greatest amounts of uranium, and the remaining tissues contained amounts just indicative of retention.

Table 10.80 — Concentration of Uranium in Rats Determined by the Fluorophotometric Method

Concentration level of dust, mg UF_4/cu m	Lung, μg U/g		Kidney, μg U/g		Liver, μg U/g		Femoral epiphysis, μg U/g	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
4	0.7–6.1	3.1	0.0–0.4	0.1	0.0–0.4	0.1	0.0–0.7	0.1
0.7	0.2–1.1	0.5	0.0–2.1	0.3	0.0–0.1	0.01	0.0–0.3	0.1

14.10 Discussion. The low order of uranium toxicity in animals shown by the inhalation-exposure studies of the tetrafluoride is typical of the response of tetravalent uranium dusts that have in common (1) a toxicity that is moderate at comparatively high levels of dust exposure and (2) a sharp demarcation in response at lower levels. This is in striking contrast to the toxicity of uranium hexafluoride and uranyl fluoride, both of which contain uranium and fluorine, but, unlike the tetrafluoride, contain hexavalent uranium. The toxicity at the highest levels of these two hexavalent compounds is invariably severe and, moreover, persists at relatively low levels of exposure (Secs. 10 and 11).

Relative to other tetravalent uranium compounds, tetrafluoride is somewhat more toxic than the corresponding dioxide (Sec. 15). This in all probability results from a combination of two factors, namely, the greater solubility of the tetrafluoride in comparison with that of

the dioxide and the presence of a small percentage of the toxic hydrolysis product of UF_6, which is UO_2F_2. Evidence that this impurity contributed to the observed toxicity is derived from the similarity in clinical response of animals exposed at the highest level of UF_4 and UO_2F_2, as regards irritation of the mucous membranes, eye and nose, and gastric mucosa. Ample evidence is presented throughout these studies that the toxicity of the uranium dusts is dependent upon their solubility in body fluids. Concerning the contribution to toxicity by the fluoride ion, no evidence is available from either the studies reported here or from the continuation of these studies to be reported later if the contribution from the uranyl fluoride contaminant is neglected.

14.11 Summary. Toxicity resulting from daily intermittent exposures of animals at levels of 24, 5, 4, 0.7, and 0.5 mg UF_4/cu m inclusive was prominent only at the highest level. At this level mortality was pronounced, histologic changes were severe, weight losses were in evidence, and biochemical changes were marked. A summary of the changes in toxicity with progressively decreasing exposure concentrations follows:

1. Symptoms of uranium poisoning were noted only in animals exposed to the highest level (18 to 40 mg/cu m) and consisted of irritation of mucous membranes, vomiting, polydipsia and anuria, loss in weight, typical renal tubular necrosis, and ultimately death in some animals.

2. Twenty-one per cent of the 66 animals exposed at the highest level succumbed. In marked contrast, only 1 rat died among a total of 669 animals of four species exposed among the four lower levels, illustrating the sharply demarcated mortality response typical of tetravalent uranium compounds.

3. Biochemical measures capable of estimating with three different degrees of sensitivity the severity of uranium poisoning showed (1) sharply elevated nonprotein and urea nitrogen at the highest level of exposure, (2) moderately elevated amino acid nitrogen-creatinine ratios at the 3-mg level of exposure, and (3) distinctly elevated catalase and phosphatase activities in the urine of animals exposed at the 0.5-mg level. Nonprotein nitrogen of the blood and protein of the urine were followed at the three lowest levels, but no changes were noted. The rabbit and the dog were the species from which the biochemical determinations were principally derived.

4. Severe renal tubular changes were observed in all five species exposed at the highest level, but in animals surviving exposures even at the highest level, regenerative changes were characteristic. The typical pathology of uranium poisoning appeared only in the dog at the 5- and 4-mg levels and to a lesser degree than observed at the highest

level. No indication whatsoever of uranium injury was noted in animals exposed at the 0.7- and 0.5-mg levels.

5. Qualitative estimation of the frequency of uranium, by the spectrochemical method in tissues of animals exposed at three of the higher levels, showed uranium to be distributed in 6 out of 7 cortical tissues. At the 5- and 0.5-mg levels retention in the bone had decreased to such an extent as to be not detectable by the spectrochemical method, although uranium was detected in a significant number of instances in the lungs of these animals. Uranium was much less commonly found in the kidney, liver, and spleen.

A quantitative estimation of the amounts of uranium by the fluorophotometric method, in the tissues of rats exposed at the 4- and 0.7-mg levels, showed that the lung was the only tissue in which uranium attained a significant concentration.

15. URANIUM DIOXIDE

By Aser Rothstein*

15.1 Introduction. Uranium dioxide, a dark-brown material possessing the highest density (10.5) of the uranium dusts studied, has a very low solubility in both water and blood plasma. At present its widespread use in industry results in widespread human exposure. It was therefore given a priority rating of 9, despite its anticipatedly low toxicity. This compound was chosen as typical of insoluble uranium compounds uncomplicated by the presence of constituents of potentially toxic nature. A rather comprehensive program of studies of inhalation toxicity was accordingly undertaken. This comprised five 30-day experiments between dust-concentration levels of 22 and 1.3 mg/cu m. Two of these studies constitute the first 30 days of the 1-year chronic experiments. Particular notice was given in these experiments to relative accumulation of uranium in lungs and kidneys and the relative damage to these two organs.

15.2 Exposure Conditions. (a) Exposure Chamber. Two of the five studies, those at the 9.3- and 2-mg levels, were performed in a typical head-exposure unit (Sec. 6.1f) equipped with a pressure-type dust feed (Sec. 6.2a). The other three experiments were performed in a full exposure unit of 64 cu ft capacity similar to that described in Sec. 6.1e. A very crude elutriator-type dust feed was used for the

* Toxicological studies by H. P. Dygert, H. Berke, A. Rothstein, J. Field, D. Dittman, N. Kaplan, H. Oberg, G. Laush, and R. Sanford; work on urinary ketone bodies by I. Slotnik; phenol-red tests by D. Dittman and H. Berke.

22-mg level. A pressure-type feed was used for the 10.4-mg level and a ball-mill feed for the 1.3-mg level (Sec. 6.2b).

(b) Air Temperature and Relative Humidity. The chamber air temperature was maintained between 70 and 80°F in all except the highest exposure level (22 mg). The latter experiment was one of the first undertaken, and no temperature controlling mechanism was available at that time. During this experiment air temperatures were at times undesirably high (about 90°F) and may have contributed to observed toxicity.

No attempt was made to control the relative humidity which fluctuated between 35 and 40 per cent.

(c) Chamber-dust Concentration. There was no more than the usual variation in chamber-dust concentration. The actual mean concentrations obtained of 22.0, 10.4, 9.3, 2.0, and 1.3 mg/cu m, equivalent to 19, 9.2, 8.2, 1.76, and 1.06 mg U/cu m, respectively, approximated closely the desired concentrations of 20, 10, 10, 2, and 1 mg of UO_2/cu m.

Samples of chamber air were taken hourly by the filter-paper method (Sec. 8.1a). The quantities of uranium dust were determined both gravimetrically and colorimetrically by the ferrocyanide method. The coefficients of variance (ratio of standard deviation to mean) averaged about 25 per cent. Fifty per cent of all dust concentration samples were within 10 to 20 per cent of the mean, and 90 per cent were within 30 to 50 per cent of the mean (Table 10.81).

(d) Particle Size. The median particle size of UO_2 dust in chamber air was 0.4 μ with a standard geometric deviation of approximately 2, according to the approximate methods described in Sec. 8.2.

(e) Exposure Schedule. Five species — dog, rabbit, guinea pig, rat, and mouse — were used in one or another of the experiments. At the 1.3-mg level, only dogs and rats were exposed; at the 2.0- and 22.0-mg levels, all species except dogs; at the 10.4-mg level, all species except mice; and at the 9.3-mg level, all five species.

The experiments consisted of a 2-week control period during which the animals were conditioned in the chamber to the experimental procedures, followed by a 5-week period during which animals were exposed to UO_2 dust, 6 days a week. At the 22-mg level there was no conditioning period. The usual tests of toxicity were used, including mortality, body weight, blood and urine chemistry, pathology, hematology (except at the 9.3- and 2.0-mg levels), and uranium deposition in tissues. Special studies included phenol-red test of kidney function at the 9.3- and 2.0-mg levels and urinary ketone excretion at the 22-mg level.

Table 10.81—Dust Concentration of UO_2

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
22.0	± 3.0	41
22.0	± 6.0	66
22.0	± 9.0	82
22.0	± 12.0	92

Note: desired concentration, 20 mg UO_2/cu m; standard deviation, ± 5.90 mg/cu m; number of samples, 164.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
10.4	± 1.0	40
10.4	± 2.0	69
10.4	± 3.0	87
10.4	± 4.0	93

Note: desired concentration, 10.0 mg UO_2/cu m; standard deviation, ± 2.5 mg/cu m; number of samples, 163.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
9.3	± 1.0	44
9.3	± 2.0	75
9.3	± 3.0	90
9.3	± 4.0	96

Note: desired concentration, 10.0 mg UO_2/cu m; standard deviation, ± 1.57 mg/cu m; number of samples, 160.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
2.0	± 0.3	27
2.0	± 0.6	59
2.0	± 0.9	75
2.0	± 1.2	88

Note: desired concentration, 2.0 mg UO_2/cu m; standard deviation, ± 0.69 mg/cu m; number of samples, 168.

Weighted-mean concentration mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
1.3	± 0.1	43
1.3	± 0.2	65
1.3	± 0.3	75
1.3	± 0.4	84

Note: desired concentration, 1.0 mg UO_2/cu m; standard deviation, ± 0.87 mg/cu m; number of samples, 174.

15.3 Mortality. Only at the 22-mg level was any significant mortality observed. This amounted to 60 per cent and was confined to only one species, the rabbit. At other levels one or two animals died, and in each case infection rather than uranium poisoning was the apparent cause of death (Table 10.82).

Table 10.82 — Mortality of Animals Exposed to UO_2

Concentration level, mg/cu m	Rabbit		Guinea pig		Mouse		Dog		Rat	
	No. of deaths per no. exposed	Deaths, %	No. of deaths per no. exposed	Deaths, %	No. of deaths per no. exposed	Deaths, %	No. of deaths per no. exposed	Deaths, %	No. of deaths per no. exposed	Deaths, %
22.0	6/10	60	1/20	5	0/40	0			0/15	0
10.4	2/18	10	0/30	0			0/19	0	2/123	2
9.3	1/24	4	1/18	5	1/45	2	0/6	0	0/44	0
2.0	0/23	0	0/18	0	0/48	0			0/30	0
1.3*							0/19	0	4/122	4

*Refers to first 30 days of a 1-year study.

15.4 Body Weight. Severe weight losses were observed in the rabbits at the 22-mg level, especially in those animals that died. The surviving rabbits showed some recovery of body weight during the latter part of the exposure period. Rats exposed at this level lost no weight but failed to gain at the normal rate. Guinea pigs showed normal growth. In all levels below 22 mg/cu m, no significant weight losses were found in any of the species (Table 10.83).

Table 10.83 — Body Weight of Animals Exposed to UO_2

Concentration level of compound, mg UO_2/cu m	Weight change			
	Dog	Rabbit	Guinea pig	Rat
22.0		Marked loss	Normal	Slight loss
10.4	Normal	Slight loss	Normal	Normal
9.3	Normal	Normal	Normal	Normal
2.0		Normal	Normal	Normal
1.3	Normal	Normal		Normal

15.5 Hematology. No significant changes attributable to uranium poisoning were observed at the 22-, 10.4-, or 1.3-mg levels.

15.6 Biochemical Constituents. (a) Blood. There were no significant increases in blood nonprotein or urea nitrogen of dogs at the 1.3-, 9.3-, and 10.4-mg levels, or in rabbits at the 2.0-, 9.3-, and 10.4-mg levels. At the 22-mg level there was a marked rise in both

constituents in rabbits. In those animals which died, these constituents continued to rise until death, when values higher than 300 mg % for NPN and 150 mg % for urea nitrogen were attained, but in the survivors there was a return to normal values during the latter part of the exposure.

(b) Urine. The following urinary constituents were analyzed: protein of rabbits at the 2.0-, 9.3-, and 10.4-mg levels, protein of dogs at the 1.3- and 10.4-mg levels, amino acids, catalase, and phosphatase of rabbits at the 2.0- and 9.3-mg levels, and ketones of rabbits at the 22-mg level. Only in the case of ketone excretion at the 22-mg level were there any changes that could be attributed to uranium poisoning, and these changes were not sufficiently high to be of diagnostic value. Changes were confined to the β hydroxybutyric acid fraction.

15.7 Pathology. Severe renal injury was found only in rabbits at the 22-mg level. The observed injury in rabbits was typical of uranium poisoning, with marked tubular necrosis in the dying animals and considerable regeneration in the survivors examined at the end of the exposure period. Rats showed no tissue injury at this level. At the 10.4- and 9.3-mg levels, no change was found in rats (examined at serial intervals), guinea pigs, or rabbits. Two of six dogs showed very slight renal change after 30 days exposure to the 9.3-mg level. No changes were observed in biopsy sections from two dogs after 10 days exposure at the 10.4-mg level. At the 1.3- and 2.0-mg levels, no tissue injury was found in any species.

No tissue injury related to uranium poisoning was seen in the lungs.

15.8 Deposition of Uranium in Tissues. At the 22-mg level the uranium deposited in the tissues of exposed animals was determined by the spectrochemical method (Chap. 2). This method was not particularly well adapted to quantitative tissue analyses. Consequently the data showed considerable variation, especially in bone samples. Uranium was found in the lungs of nearly all the animals, in the kidneys of about half the animals, in one-quarter of the bone samples, and in only a few liver samples.

In the studies performed at the 1.3-, 2.0-, 9.3-, and 10.4-mg levels, the uranium content of tissues was determined by the fluorophotometric method (Chap. 2), which proved far more sensitive and reliable. After 30 days of exposure, uranium was found rarely in any tissues except the lungs at any of the levels. There was a consistent accumulation in the lungs of rats, amounting to 8 or 9 μg/g of tissue at the 1.3- and 2.0-mg levels and 38 to 40 μg/g of tissue at the 9.3- and 10.4-mg levels. These data illustrate the relative insolubility of this compound, which accumulated in the lungs much faster than it was

Table 10.84 — Animals Exposed for 30 Days to 22 Mg UO_2/cu m (Summary of Results)

1. Mortality. Only rabbits showed significant mortality.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	6/10	60
Guinea pig	1/20	5
Mouse	0/40	0
Rat	0/15	0

2. Body Weight.

Species	Wt. change
Rabbit	Severe loss
Rat	Slight loss
Guinea pig	Normal

3. Biochemistry. Blood nonprotein and urea nitrogen of rabbits were markedly elevated, especially in those animals that died.
4. Pathology. Severe typical renal changes found in rabbits but not in rats. No damage to lungs.
5. Hematology. No significant changes.
6. Uranium Deposition in Tissues. Uranium found in lungs of all animals, in kidneys of half the animals, in one-quarter of the bone samples, and rarely in the liver.
7. Conclusion. The compound UO_2 at 22 mg/cu m is very toxic to rabbits, causing renal damage and high mortality. It is relatively nontoxic to rats, guinea pigs, and mice.

removed. Its nonoccurrence in the kidneys and in the bones indicates that very little leaves the lungs and accounts for the low toxicity observed.

15.9 Phenol-red Tests of Kidney Function. Phenol-red tests performed weekly on rabbits at the 2- and 9.3-mg levels indicated no diminution of renal function resulting from exposure to uranium dioxide.

15.10 Summary and Conclusions. 1. Uranium dioxide is a relatively nontoxic compound when inhaled as a dust in intermittent daily exposures. Only at the 22-mg level was there notable toxicity, and this occurred only in rabbits.

2. The toxicity resulting from inhalation of high concentrations of uranium dioxide is typical of uranium poisoning, consisting largely of renal tubular necrosis, uremia, loss of weight, and ultimate death.

3. The rabbit was the most sensitive species, showing high mortality at the 22-mg level but no toxic effects at the 10.4-mg level.

Table 10.85 — Animals Exposed for 30 days to 10.4 Mg UO_2/cu m (Summary of Results)

1. Mortality.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	2/18	10
Rat	2/123	2
Dog	0/19	0
Guinea pig	0/30	0

2. Body Weight.

Species	Wt. change
Rabbit	Slight loss
Rat	None
Dog	None
Guinea pig	None

3. Biochemistry. (a) No changes in blood NPN and urea nitrogen or urinary protein of dogs. (b) No change in urinary protein of rabbits.
4. Pathology. No histopathology.
5. Hematology. No significant changes.
6. Uranium Deposition in Tissues. Consistent accumulation of uranium in lungs of rats to 40 μg/g of tissue. Little uranium in other tissues.
7. Conclusion. Despite accumulation of uranium in lungs, little appears in other tissues, and little or no toxicity is present.

Table 10.86 — Animals Exposed for 30 Days to 9.3 Mg UO_2/cu m (Summary of Results)

1. Mortality.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	1/24	4
Guinea pig	1/18	5
Mouse	1/45	2
Dog	0/6	0
Rat	0/44	0

2. Body Weight. Normal.
3. Biochemistry. Urinary protein, catalase, amino acid nitrogen, and phosphatase of rabbits are normal.
4. Pathology. No histopathology.
5. Phenol-red Test. Normal.
6. Uranium Deposition in Tissues. Up to 40 μg U/g of lung, but little in other tissues.
7. Conclusion. No toxic symptoms observed.

Table 10.87 — Animals Exposed for 30 Days to 2.0 Mg UO_2/cu m (Summary of Results)

1. Mortality.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	0/23	0
Guinea pig	0/18	0
Rat	0/30	0
Mouse	0/48	0

2. Body Weight. Normal.
3. Biochemistry. Blood and urine constituents normal.
4. Pathology. No histopathology.
5. Phenol-red Tests. Normal.
6. Uranium Deposition in Tissues. Up to 9 μg U/g of lung, but little in other tissues.
7. Conclusion. No toxic effects.

Table 10.88 — Animals Exposed for 30 Days to 1.3 Mg UO_2/cu m (Summary of Results)

1. Mortality.

Species	No. of deaths per no. exposed	Mortality, %
Dog	0/19	0
Rat	4/122	4

2. Body Weight. Normal.
3. Biochemistry. Blood and urine constituents normal.
4. Pathology. No histopathology.
5. Hematology. No changes.
6. Uranium Deposition in Tissues. Up to 8 μg U/g of lungs, but little in other tissues.
7. Conclusion. No toxic effects.

Rats showed no renal histological changes in any of the experiments but failed to gain weight at the normal rate at the 22-mg level. At the 9.3- and 10.4-mg levels, the highest to which this species was exposed two of eight dogs showed minor histological changes but no other symptoms. Guinea pigs showed no signs of toxicity at any level.

4. The relative insolubility of uranium dioxide accounts for the appearance of very little uranium in kidneys and bone, and relatively little renal damage. Despite a marked accumulation of the inhaled dust in the lungs, there was no sign of any local respiratory irritation, at least during the 5-week period of this experiment.

16. HIGH-GRADE ORE

By U. C. Pozzani*

16.1 Introduction. High-grade ore is the starting point of the uranium chemical industry. From the time ore is mined until it enters chemical purification processes, large quantities are handled repeatedly. Because of the number of personnel involved in the processing of the ore, it becomes important to determine whether the handling of the ore constitutes an industrial hazard. To this end, three inhalation-toxicity studies were undertaken.

The ore differs from other uranium dusts in that it contains a number of constituents that are potentially toxic in addition to its chief component, uranium. Two uranium oxides, UO_2 and U_3O_8, compose the bulk (68.5 per cent) of the potentially toxic material. Other metallic oxides are present in considerable proportions, i.e., lead oxide 6 per cent, nickel oxide approximately 1 per cent, and cobalt oxide 0.4 per cent. Analysis of the ore is given in Chap. 1. Of all these minor constituents, lead should definitely be reckoned with as a possible toxic agent.

The ore was composed of extremely insoluble uranium oxides and somewhat more soluble oxides of elements of lower atomic weight. The ore was nonhygroscopic and had a density of approximately 7. Even though the atmospheric stability of the dust allowed comparative ease in the preparation of dusty atmospheres, its high density made difficult the maintenance of uniform dust concentrations.

The three inhalation studies were designed to define the permissible level of exposure of animals to the ore and to determine (1) the physiological, biochemical, and histological changes occurring in the experimental animals; (2) the relative effects of the chief toxic agents, i.e., uranium and lead; and (3) to form an estimate of the relative distribution and storage of these constituents in animal tissues. The highest of the three levels was set at approximately 36 mg/cu m (equivalent to approximately 20 mg U/cu m) of air. From the results of this study, two other levels were selected; one, at approximately 5 mg/cu m, to give border-line responses; the other, at approximately 1 mg/cu m, to produce little or no toxic response. Biochemical tests included blood nonprotein and urea nitrogen, and urinary catalase and protein. In order to estimate the relative contribution to toxicity of lead and uranium, respectively, the relative distribution and storage of these two elements in the tissues was determined. Measurements

* Toxicologic procedures performed by C. S. Weil, C. Horton, H. P. Dygert, H. A. Oberg, U. C. Pozzani, H. Wilson, and R. Sanford.

of the distribution of these elements in the chamber atmosphere were also made to ascertain whether stratification of any dust component occurred during circulation of the ore dust in the exposure unit. The usual observations of weight response, mortality, gross and microscopic examinations of tissues, hematologic changes, and clinical signs were made. Because of the important relation of particle size of dust to toxicity, a determination of this factor was also made.

16.2 Exposure Conditions. The levels at which the inhalation studies were performed were at 36, 4.8, and 1.4 mg of ore per cubic meter of air, equivalent to 22, 2.9, and 0.84 mg of uranium, respectively. The highest level was performed in a unit smaller than that for the lower two and with a dust feed of a cruder design. Both units were the full-exposure type described in Sec. 6.1e. That which was used at the highest level was a 4-ft transite-lined wooden cube, and that used at the two lower levels was approximately a cube of 252 cu ft capacity. The ore dust at the highest level was introduced into the smaller chamber by an intermittent stream of cotton-filtered compressed air that passed through an elutriating bottle containing the ore. The introduction of the dust occurred at 15-min intervals of 1-min duration. The dust feed used at the two lower levels was the pressure type described in Sec. 6.2a.

(a) Exposure Schedule. Animals were exposed at the highest level for 30 days on the average of 4.4 hr per day for a total of 132 hr. At the two lower levels, animals were exposed for approximately 6 hr per day for 30 days for a total of 171 hr.

(b) Concentration of Ore Dust in Exposure Atmospheres. The mean concentration of the highest exposure level for the entire exposure period of 30 days was 36.1 mg/cu m, as monitored by an hourly sample taken with the filter-paper dust sampler. Ninety-five per cent of the samples were within ± 64 per cent of the mean concentration, and over half of the sample concentrations fell within 33 per cent of this mean figure. The control of the dust concentration at the two lower levels was superior to that at the highest level, a greater number of samples falling closer to the mean. At the 4.8- and 1.4-mg levels, 95 per cent of the samples fell within ± 32 and 44 per cent, respectively, of the mean concentration (Table 10.89).

The median particle size of the ore dust measured by the Bausch & Lomb counter was 0.56 μ with a standard deviation of 1.7 μ. This determination was made at the highest level of exposure only.

(c) Distribution of Lead and Uranium in the Exposure Atmospheres. Because the lead component of the ore may have some toxicological effects, tests were made to ascertain whether the concentration of this constituent relative to that of uranium was the same in the chamber air as in the bulk dust. To this end, filter-paper dust samples

Table 10.89 — Dust Concentration of High-grade Ore

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
36.1	± 5	22.5
36.1	± 10	54.0
36.1	± 20	84.0
36.1	± 23	95.0

Note: desired concentration, 33.3 mg of high-grade ore per cubic meter; standard deviation, ± 11.3 mg/cu m; number of samples, 149.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
4.8	± 0.37	38.3
4.8	± 0.74	66.7
4.8	± 1.48	95.0
4.8	± 2.22	99.0

Note: desired concentration, 5.0 mg of high-grade ore per cubic meter; standard deviation, ± 0.74 mg/cu m; number of samples, 150.

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
1.4	± 0.1	17.5
1.4	± 0.2	39.0
1.4	± 0.3	58.5
1.4	± 0.61	95.0

Note: desired concentration, 1.0 mg of high-grade ore per cubic meter; standard deviation, ± 0.305 mg/cu m; number of samples, 185.

were taken at four different positions during the course of exposure at the 1.4- and 4.8-mg levels and the lead-uranium ratio was determined by spectrochemical and ferrocyanide colorimetric methods, respectively. The results of these tests showed that the ratio of lead to uranium in the exposure atmosphere did not vary greatly from that found in the previously analyzed sample of ore before its distribution in the chamber (Table 10.90) and indicated that no appreciable stratification of either component occurred.

16.3 Signs of Toxicity. Outward manifestations of toxicity were negligible with the exception of the rabbits exposed at the highest

Table 10.90 — Relation of the Amounts of Lead and Uranium in the Circulating High-grade-ore Dust at Different Concentrations in the Exposure Unit*

Position of samples	No. of samples	Pb:U ratio Mean	Pb:U ratio Range
At 4.8 mg/cu m			
Directly inside rear wall of chamber	5	0.071	0.045–0.120
Inside of chamber, center	7	0.072	0.039–0.135
Inside of chamber, top	1	0.152	
Immediately inside door of chamber, bottom	1	0.057	
At 1.4 mg/cu m			
Directly inside rear wall of chamber	10	0.088	0.050–0.180
Inside of chamber, center	1	0.050	
Inside of chamber, top	5	0.064	0.027–0.080
Immediately inside door of chamber, bottom	5	0.092	0.056–0.146

*Ratio of Pb to U in pulverized ore is 0.093 to 1.

level, which had a coccidial infection. Animals that died during the exposure showed signs of ill health for only a very brief period prior to death.

16.4 Mortality. Mortality at any of the three levels of exposure was not a prominent feature. At the 36-mg level, only 7 of 80 animals or 8.8 per cent* died during exposure. Although 1 of 20 rats and 3 of 40 mice died, this number approximates the mortality in control animals. Moreover, of 3 guinea pigs that died (out of 20) the organism streptoccus septicemia was isolated in two of these animals. Deaths were not confined to a definite period but were scattered throughout the 30 days of the study (Table 10.91).

At the two lower levels, mice showed the only significant mortality of the five species exposed (mouse, rat, dog, rabbit, and guinea pig). Twenty-one per cent succumbed at the 4.8-mg level and 12 per cent at the 1.4-mg level. The observation that the lowest mortality of this species occurred at the highest level cannot be explained. The occasional deaths distributed among the other species at these levels are insignificant.

* This number excludes the rabbits that were eliminated from this calculation because of coccidial infection. Six of 10 rabbits died after periods ranging from 6 to 20 calendar days of exposure (25 to 80 exposure hours). Coccidia, isolated from both the bile and feces, indicated that coccidiosis played an important part in the death of these animals.

Table 10.91 — Mortality of Animals Exposed to High-grade-ore Dust

Concentration level of high-grade ore, mg/cu m	Mouse		Rat,	Dog,	Rabbit,	Guinea pig,
	No. of deaths per no. exposed	Mortality, %	no. of deaths per no. exposed	no. of deaths per no. exposed	no. of deaths per no. exposed	no. of deaths per no. exposed
36	3/40	8	1/20		6/10*	3/20†
4.8	9/43	21	0/45	0/5	1/15	1/20
1.4	6/50	12	0/45	0/5	0/15	1/20

*Coccidial infection occurred in this group.
†Streptococcal septicemia was diagnosed for two of these guinea pigs.

16.5 Body-weight Response. Striking weight losses were not a characteristic feature of the response of the animals to exposures at any level with the exception of the comparatively small groups of animals that died. Although an over-all gain in weight was observed in most surviving animals, many sporadic changes in weight occurred — changes that were not consistent for the same species and sex at various exposure levels (Table 10.92). For example, the weight

Table 10.92 — Weight Change* in Animals Exposed to High-grade-ore Dust

Concentration level of high-grade ore, mg/cu m	Weight change, %				
	Rabbit	Rat	Dog	Mouse	Guinea pig
36	−11† +7‡	−3			−14† +2.0†
4.8	+12	+50	+10	+0.7	+45
1.4	+17	+23	+14	+13	+6

*The percentage weight change represents the average weight gain of both sexes of the same species or of the dying and surviving animals of one species. For initial weight, see Tables 10.96, 10.97, and 10.98.
†Dying animals.
‡Surviving animals.

of the female guinea pigs, but not that of the males of this group, fluctuated during exposure at the lowest level, in contrast to the finding of normal growth in both sexes in this species from exposure at the intermediate level. A similarly discrepant weight response was noted in the comparison of the weight changes in male and female mice. Female mice gained more than the males at the intermediate level, whereas the males showed the greater weight gain at the lowest level of exposure.

Only two animal groups weighed less at the end of the exposure than at the start. These were both sexes of rats at the 36-mg level and male mice at the 4.8-mg level. A graphical representation of the weight response of animals exposed at the intermediate level is given in Fig. 10.43.

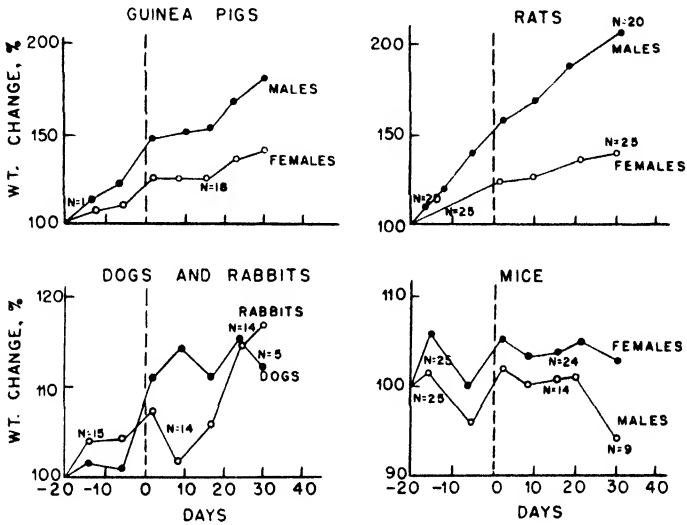


Fig. 10.43 — Change in body weight of animals exposed to high-grade-ore dust at 4.8 mg/cu m.

16.6 Biochemical Findings. The biochemical studies performed on dogs and rabbits comprised the quantitative determination of non-protein nitrogen and urea of blood and protein and catalase activity of urine (Table 10.93). Only one of these tests, blood NPN, was used uniformly on the rabbits at each of the three levels.

The maximal blood NPN level showed a progressive decrease with decreasing exposure levels. From a mean maximal value of 177 mg % (highest individual value, 398 mg %) at the highest exposure level, the NPN level fell to a mean maximal value of 82 mg % at the intermediate level and finally at the 1.4-mg level to 48 mg %, a value definitely within normal limits. (Coccidiosis was present in certain of the rabbits at the highest exposure level, but this condition of itself does not give rise to elevated blood NPN values.) At the lowest level, no animal showed an abnormal NPN value. Blood urea nitrogen estimations at the highest level paralleled those of the blood NPN in the rabbits. Blood NPN values of dogs exposed at the two lower levels

Table 10.93 — Biochemical Findings on Animals Exposed to High-grade-ore Dust

Concentration level of high-grade ore, mg/cu m	Species	Blood nonprotein nitrogen	Blood urea N	Urinary protein	Urinary catalase
36	Rabbit	8 of 10 rabbits showed azotemia; max. 398 mg %	Max. 350 mg %; all rabbits showed azotemia		
4.8	Dog	No change			
4.8	Rabbit	4 of 10 rabbits showed temporary azotemia; 1 died after a max. value of 324 mg %			
1.4	Dog	Changes within normal range; max. individual value, 59 mg %			
1.4	Rabbit	No changes		No changes	Inconclusive changes

yielded only normal values. Likewise, urinary protein concentrations or rabbits exposed at the lowest level were normal, and catalase activity values obtained on rabbits at this level varied so little from normal as to indicate no positive sign of uranium injury.

16.7 Pathology. Histological examination of tissues from groups of animals of all three exposure levels revealed definite tissue damage, the intensity of which decreased progressively and consistently from the highest to the lowest exposure level (Table 10.94). Of the three tissues — kidney, lung, and liver — regularly examined from animals exposed at the three exposure levels, only the lung and kidney were noted to have been affected by the treatment, and, of the five animal species used, only the rabbit and the rat were studied at all three levels. The rabbits showed moderate kidney and lung damage at the highest exposure level, decreased kidney and no lung damage at the intermediate level, and no lesions definitely attributable to exposure at the lowest level (Table 10.94). Serial sacrifice of rats during exposure to the highest level revealed moderate renal tubular injury followed by tubular regeneration. Following exposure to the intermediate level, 2 of 10 rats showed mild renal tubular injury. Rats studied at serial intervals during exposure to the lowest level

Table 10.94 — Pathology

Concentration level of dust, mg/cu m	Dog	Rat	Rabbit	Mouse	Guinea pig
36		Moderate changes in kidneys	More extensive kidney damage than in rats; moderate to moderately severe pulmonary damage		
4.8	Typical active renal damage in 5 of 5; hemorrhagic lung in 1 dog	Traces of epithelial degeneration in tubules of kidney in 2 of 10; no changes in lung and liver	Kidney damage of moderate degree in 6 of 14	Mild renal changes in 4 of 5	Typical renal changes in 1 of 5
1.4	Mild typical renal injury; almost complete repair in 3 of 5; definite mild acute injury in one 7-day biopsied dog	No tissue changes related to treatment	Chronic nephritis in experimental and control rabbits	Slight renal injury in 2 of 5	No typical renal changes

showed no significant changes. At the two lower levels the tissues of dogs, mice, and guinea pigs were also examined. Typical renal damage was noted in all the five dogs at the intermediate exposure level. Of the five dogs at the lowest exposure level, only three exhibited renal injury, which was of a very mild intensity and showed almost complete repair. Renal biopsy of one dog after 7 days of exposure showed definite though mild acute renal injury. Of five mice studied after exposure to each of the two low levels, four at the intermediate level and three at the low level showed evidence of very mild renal tubular injury. Only one of five guinea pigs showed any typical renal injury at the intermediate level, and at the lowest level no renal changes were noted. All changes in the kidneys of experimental animals, with the exception of chronic nephritis, are typical of those usually associated with uranium poisoning.

16.8 Hematology. Hematologic examination of over twenty cellular constituents of the exposed animals revealed a pronounced uniformity in these constituents throughout the exposures. The rats and rabbits at the 36-mg level and the dogs, rats, and rabbits at the 1.4-mg level had counts weekly throughout the exposure period. It is to be noted that no stippling of cells characteristic of lead intoxication was observed at any level.

16.9 Distribution and Retention of Uranium and Lead in Tissues. The determination of uranium by the spectrochemical method in lung,

liver, kidney, and bone of five animal groups exposed to high-grade ore revealed a retention of uranium in all tissues except the liver but inappreciable retention of lead in any tissues except the femur (Table 10.95). In the lungs of the 10 rats, decreasing concentrations of uranium were noted with progressively decreasing dust concentrations to which the animals were exposed. The same trend was noted also in the kidneys of this species, although to a less marked degree. As would be expected, the bone of the rat exposed at the highest level contained appreciably more uranium than at lower levels, but certain difficulties in the spectrographic method of analysis makes further comparison difficult. Analysis of the uranium content of the liver yielded results that were not appreciably different from those of the controls.

The above animals were sacrificed 2 days after the termination of the exposure. In order to determine the storage of uranium in these animals, analyses were also made 28 days after exposure on similar groups of animals. It is seen in Table 10.95 that the uranium content

Table 10.95--Mean Amounts of Uranium and Lead Found in Tissues* of Animals Exposed to High-grade Ore

Concentration level of high-grade ore, mg/cu m	Animals		Days after end of exposure	Uranium, μg/g				Lead, μg/g			
	Species	No.		Lung	Kidney	Liver	Bone	Lung	Kidney	Liver	Femur
36	Rat	10	2	272.0	4.3	0.09	46.5†				
			28	962.0‡	0.2	0.2	4.2†				
36	G. pig	8	27	91.0	9.9	4.8	26.1†				
36	Rabbit	6	1	39.5	1.7	0.4	22.0§				
4.8	Rat	10	2	15.5	3.9	1.1	<5.0**	1.1	0.4	0.2	43.1
1.4	Rat	9	2	9.1	3.8	0.9	<10.0	0.2	0.4	0.2	35.1
0 (control)	Rat	10		<0.2	<0.2	<0.2	<10.0	0.6	0.2	0.2	18.0

* The unit weight of tissue represents moist weight for the soft tissue, dry-ash weight for bone.
† Skeleton.
‡ This value was confined chiefly to 3 of the 10 tissue samples tested.
§ Epiphysis.
** Entire femur.

of the skeleton and the kidney was considerably lower in the latter group. The fact that the uranium content of the lungs of rats sacrificed 28 days after exposure was four times that of rats sacrificed immediately after exposure is explained on the basis of three exceptionally high values of a total of 10 analyses.

A similar distribution of uranium was found in the guinea pigs and rabbits exposed at the highest level.

The lead content of the liver of the 10 rats exposed at each of the two lower levels did not differ from that of tissues from five control rats. On the other hand, the lead content of the femur at the two levels was from 2 to 2 ½ times greater than normal.

A summary of results of each test used in evaluating the toxic response at each of the three levels is given in Tables 10.96 through 10.98.

16.10 Discussion. The primary problem in evaluating the toxicological response to the high-grade-ore dust is the question whether a true uranium poisoning is involved or whether the response is a combined effect of several toxic agents. Among the possible contributors to toxicity other than uranium, lead is most probably concerned. At the highest (36-mg) level, the dusty atmosphere contained more than 2 mg Pb/cu m. Even at the intermediate level of 4.8 mg, the amount of circulating lead was 0.293 mg/cu m, a concentration approximately twice the maximal allowable concentration of this element in factory air. This "allowable" concentration, although representing a "safe" limit, occasionally produces a mildly toxic response among lead workers. This fact makes the toxicity of the ore, still observable at the 4.8-mg level, more readily explicable. Moreover the slight and transient renal changes seen in dogs at the lowest level are more easily interpreted than they would be on the basis of uranium toxicity alone. The uranium oxides U_3O_8 and UO_2, which compose 68 per cent of ore, are known from short-term exposure studies to have a very low toxicity (Secs. 15 and 21). At the 10-mg level of UO_2 as well as at two levels tested below, no deaths and only slight and infrequent pathological changes were observed in animals exposed for 30 days. On the other hand, in the case of the ore, death and definite tissue injury was observed not only at the 4.8-mg level but also at the 1.4-mg level. The peculiar susceptibility of the mouse to the high-grade ore is not characteristic of uranium poisoning. Of the 343 animals exposed at all levels, the mouse mortality accounted for 75 per cent of all deaths (18 of 25). The only other instances of particularly high susceptibility in the mouse were found in uranium compounds containing other potentially toxic constituents, notably fluoride (UF_6 and UO_2F_2). These pieces of evidence suggest the conclusion that some constituent other than uranium, presumably lead, contributed to the toxicity of the ore. On the other hand, no definite specific evidence is forthcoming from any of the three studies that lead played a part in the toxicity. No characteristic stippling of the red cells or liver damage were observed. Therefore it might be concluded that, although there is no specific evidence that directly points to lead as a toxic

Table 10.96 — Animals Exposed for 30 Days (132 Hr) at 36.1 Mg/cu m Level to High-grade Ore (Summary of Results)

1. Mortality. Some mortality was produced.

Species	No. of deaths per no. exposed	Mortality, %
Guinea pig	3/20*	15
Rabbit	6/10†	60
Mouse	3/40	8
Rat	1/20	5

*Streptococcus septicemia was diagnosed in two animals.
†Coccidiosis was present in rabbit colony.

2. Weight Changes. Weight changes were for the most part slight and sporadic.

Species	Wt. change, %	Original wt., g
Rat	-3	244
Rabbit	-11*	2,300*
Rabbit	+7†	2,700†
Guinea pig	13.9*	266*
Guinea pig	+1.9	313†

* Dying animals.
† Surviving animals.

3. Biochemistry. Most of the rabbits showed azotemia.

Determination	Calendar days +1	+8	+15	+22	+29
Urea N, mg %*	19	121†	33‡	20§	34
NPN, mg %	44	177	119	53	61

* Mean values.
† One died before this value was reached.
‡ Three died, and one was sacrificed before this date.
§ Two died before this date.

4. Pathology. This concentration was toxic to rabbits and rats. No histopathological observations were made on guinea pigs and mice.
Rat. Moderate renal damage.
Rabbit. More extensive kidney damage than in rats, and moderate to moderately severe pulmonary damage.
5. Hematology. No significant changes appeared in hemograms of the rat and rabbit.
6. Distribution and Retention of Uranium in Tissue. Considerable amounts of uranium were found in the lung, skeleton, and kidney of exposed animals.

Species	Days after end of exposure	Lung*	Kidney*	Liver*	Bone†
Rat	2	272	4.3	0.09	46.5§
Rat	28	962.0‡	0.2	0.2	4.2§
Guinea pig	27	91.0	9.9	4.8	26.1§
Rabbit	1	39.5	1.7	0.4	22.0**

*Amounts are expressed in terms of micrograms of uranium per gram of wet tissue; mean values.
†Amounts are expressed in terms of micrograms of uranium per gram of tissue ash; mean values.
‡Three of ten samples contained very high concentrations of uranium.
§Skeleton.
**Epiphysis.

Table 10.97—Animals Exposed for 30 Days (171 Hr) at 4.8-Mg/cu m Level to High-grade Ore (Summary of Results)

1. Mortality. This produced only slightly higher mortality than the 1.4-mg level.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	9/43	21
Rabbit	1/15	7
Guinea pig	1/20	5
Dog	0/5	0
Rat	0/45	0

2. Weight Changes. Weight gains were recorded in all species.

Species	Wt. change, %	Original wt., g
Rat	+50	158
Guinea pig	+45	495
Rabbit	+12	3,140
Dog	+10	8,300
Mouse	+0.7	24

3. Biochemistry. Dogs showed no conclusive changes, but some of rabbits showed azotemia.

		Calendar days							
Species	Determination	−20	−13	−6	+1	+8	+15	+22	+29
Dog	NPN, mg %*	40.8	44.0	33.8	25.8	33.0	35.0	42.6	31.8
Rabbit	NPN, mg %*	42.3	41.1	32.6	40.0	81.8	68.5	69.1	41.6

* Mean values.

4. Pathology. This concentration was toxic to dogs, rabbits, and mice and questionably toxic to rats and guinea pigs.
   Dog. Typical active renal damage in all five.
   Rat. Two of ten rats showed traces of epithelial degeneration in tubules of kidney.
   Rabbit. Six of fourteen rabbits showed kidney damage to moderate degree.
   Mouse. Four of five mice showed renal changes of an acute nature.
   Guinea Pig. One of five guinea pigs showed typical renal changes.
5. Uranium and Lead Content of Tissues of Exposed Rats. The highest concentration of uranium was found in the lungs; the highest concentration of lead was found in the femur.

Element	Lung*	Kidney*	Liver*	Femur†
Uranium	15.5	3.9	1.1	5.0
Lead	1.1	0.4	0.2	43.1

*Amounts (mean values) are expressed in terms of micrograms of element per gram of wet tissue.

†Amounts (mean values) are in terms of micrograms of element per gram of tissue ash.

Table 10.98 - Animals Exposed for 30 Days (171 Hr) at 1.4 Mg/cu m Level to High-grade Ore (Summary of Results)

1 Mortality. This concentration produced low mortality in five species of laboratory animals.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	6/50	12
Guinea pig	1/20	5
Rat	0/45	0
Dog	0/5	0
Rabbit	0/15	0

2. Weight Changes. Weight gains were recorded in all species.

Species	Wt. change, %	Original wt., g
Guinea pig	+6	626
Mouse	+13	23
Dog	+14	7,000
Rabbit	+17	2,900
Rat	+23	162

3. Biochemistry. Neither dogs nor rabbits showed any conclusive changes in blood NPN.

		Calendar days							
Species	Determination	-20	13	-6	+1	+8	+15	+22	+29
Dog	Blood, mg %*	36.5	33.5	28.5	25.6	31.3	31.5	47.5	38.0
Rabbit	NPN, mg %*	37.9	47.7	37.9	35.4	39.2	35.9	46.6	35.8

*Mean values.

Urinary Protein and Catalase. Rabbits showed no significant changes.

		Calendar days							
Species	Determination	-12	-5	+2	+8	+14	+21	+28	+35
Rabbit	Catalase, mg %*				37.1	5.1	16.7	29.2	16.4
Rabbit	Protein, mg %*	3.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0

*Mean values.

4. Pathology. This concentration is not toxic to rats, guinea pigs, or rabbits but possibly is slightly injurious to dogs and guinea pigs.
Dog. Mild, typical uranium renal injury followed by almost complete repair.
Rat. No tissue damage traceable to uranium treatment.
Rabbit. Renal changes not attributable to treatment.
Mouse. Slight, active typical renal damage in three of five.
Guinea Pig. No renal injury.
5. Hematology.
Dogs and Rabbits. No significant change in hemogram.
Rats. Showed inconclusive changes in hemoglobin, platelets, absolute neutrophils, and absolute lymphocytes.
6. Uranium and Lead Content of Tissues of Exposed Rats. Small amounts of uranium were found in the lung and kidney. Lead content of the femur was twice that of normal.

Element	Lung*	Kidney*	Liver*	Femur†
Uranium	9.1	3.8	0.9	<10.0
Lead	0.2	0.4	0.2	35.1

*Amounts are expressed in terms of micrograms of element per gram of wet tissue.
†Amounts are expressed in terms of micrograms of element per gram of tissue ash.

factor in high-grade-uranium-ore poisoning, the evidence in general and the increased lead content of the femurs in particular tend to incriminate this element.

16.11 Summary. The following are the toxicological effects of high-grade uranium ore on laboratory animals:

1. Mortality at any of the three levels was not a prominent feature. At the 36-mg level, 7 of 80 animals died; at the 4.8-mg level, 11 of 128 succumbed; and at the 1.4-mg level, 7 of 135 died. Deaths were confined for the most part to the mice.

2. Weight losses among the exposed animals were confined to two groups, the rats at the 36-mg level and the male mice at the 4.8-mg level. Other species showed an over-all gain in weight.

3. Markedly elevated blood NPN and urea values were found in the rabbits at the 36-mg level. Elevation to a lesser degree of the NPN value was observed in the rabbits at the 4.8-mg level, and no elevation of the NPN value was found in the rabbits at the 1.4-mg level.

4. All species showed at least moderate to mild renal damage at the 36- and 4.8-mg levels, but moderate to severe pulmonary damage was observed only in the rabbits at the 36-mg level. At the 1.4-mg level, dogs, rabbits, and mice showed very mild to questionable renal damage.

5. Hematologic examination revealed no significant changes in the cellular constituents of the blood.

6. Considerable amounts of uranium were found in the lung and to a lesser degree in the kidney of exposed animals. Lead was not found to any appreciable extent in the tissues, except in the bones where concentration of this element was found to be more than twice that found in control animals.

17. URANIUM TRIOXIDE

By Aser Rothstein*

17.1 Introduction. A determination of the inhalation toxicity of the trioxide of uranium was made at a single level of concentration at approximately 20 mg of dust per cubic meter of air. Because a priority rating of 5 was assigned to this compound, the information derived from a single study was considered sufficient for the purpose. This short-term study of uranium toxicity, one of the first made but more comprehensive and detailed than most, was designed to furnish information on three features basic to the understanding of uranium

* Toxicologic procedures performed by A. Rothstein, D. Dittman, and E. Same. Renal function tests performed by H. Wills.

toxicity: (1) the relative usefulness of various correlatives of toxicity; (2) the sequence of events of uranium intoxication by the inhalative route; and (3) the differences in species susceptibility. Relative to the first of these subjects, considerable attention was paid in this study to the biochemical and physiological changes that occurred in animals poisoned with uranium trioxide. The biochemical constituents examined were urinary protein, creatinine, and reducing sugar; the physiological changes comprised the study of the effects attendant on renal damage and included clearances of creatinine, urea, chloride, and phenol red following increasing periods of exposure of rabbits. The value of these measures of toxicity were compared critically with more commonly used criteria of uranium poisoning, namely, weight response, mortality and blood nonprotein nitrogen, urine volume, and histologic changes in the tissues. The second feature of this study, the establishment of the chronology of toxicologic response, concerned determination of the order of histologic, physiologic, and biochemical changes in relation to the deposition of uranium in tissues. Conclusions relative to the differences in species susceptibility were possible because the number of animal species was sufficiently diverse and the group size sufficiently large to show variations in species susceptibility, an important consideration if results of these animal studies are to have any carry-over value to man.

One of four oxides of uranium, UO_3 is characterized by its high density, 7.7; high content of uranium, 83 per cent; and low water solubility, 1 mg %. Most striking from the standpoint of toxicity, however, is the fortyfold increase in solubility of the trioxide in plasma. In this respect it resembles the peroxide but differs markedly from the dioxide and the tritaoctoxide. Its plasma solubility is thought to be caused by formation with the bicarbonate of the plasma of a soluble complex with the formula $Na_4UO_2(CO_3)_3$. This allows for rapid distribution throughout the body of what is an apparently insoluble and consequently nontoxic uranium compound with the effects of a highly toxic compound. This solubility characteristic of uranium trioxide is undoubtedly a major cause of the toxicity reported herein.

A total of 152 animals of six species (dog, cat, rabbit, guinea pig, rat, and mouse) were exposed for approximately 6 hr a day, 6 days a week, for 4 weeks—a total of 131 hr.

17.2 Exposure Unit. The animals were exposed in a typical head-exposure unit described in Sec. 6.1f. Only rabbits, cats, and guinea pigs were head-exposed; dogs, rats, and mice were totally exposed.

The method of introducing the dust utilized a type of dust feed in use prior to the adoption of the finally accepted types described in Sec. 6.2. It consisted of a dust reservoir, the floor of which was a

toothed cylinder. Upon rotation of the cylinder the dust was carried out of the reservoir by a jet of air that forced the dust into the air stream entering the input side of the Rotoclone. The known variables in the operation of this dust feed included the pressure of the air jet, the speed of the tooth cylinder, and the stirring of the dust in the reservoir. Despite careful control of these variables, only moderate success was achieved in maintaining the desired concentration of 20 mg of dust per cubic meter of chamber air.

Table 10.99 shows the degree of departure of the concentrations in the dust samples measured approximately every hour during each exposure period from the desired level. Although the actual weighted-mean concentration of 19.04 mg/cu m approximated the desired concentration of 20 mg/cu m, the standard deviation was rather large with this type of feed, amounting to 7.7. The concentration approached values up to 30 to 40 mg/cu m for short periods of time.

The mean particle size, measured by the approximate method of the Bausch & Lomb counter, was 0.7 μ.

Table 10.99—Dust Concentration

Weighted-mean concentration, mg UO_3/cu m	Deviation, mg/cu m	Samples within given deviation, %
19.04	±4.0	27
19.04	±8.0	55
19.04	±12.0	72
19.04	±16.0	89

Note: desired concentration, 20 mg UO_3/cu m; standard deviation, ±7.72 mg UO_3/cu m; number of samples, 238.

17.3 Signs of Toxicity. Loss of appetite and the temporary loss in weight, diuresis in dogs and cats, and anuria in the rabbits prior to death were the only external signs of uranium poisoning.

17.4 Mortality. A relatively high mortality was observed in all species as a result of the 4-week exposure. Twenty-six animals, or 20 per cent of the total of 130 used in the mortality study, died (Table 10.100). The cat and rabbit showed the highest mortality rates, 100 and 67 per cent, respectively, whereas the dog, guinea pig, rat, and mouse all showed a mortality lower than 20 per cent, indicating a marked difference in species susceptibility.

The first animal death was that of a mouse on the fifth day; the other deaths were distributed throughout the period between the fifth

and twenty-fifth days with a definite peak occurring in the mortality curve during the third week of exposure. Of 26 deaths, 5 occurred during the first week, 6 during the second week, 13 during the third week, but only 2, that of a cat and mouse, during the fourth week. The mortality peak was especially pronounced in rabbits. One of 18 died the first week, 3 the second week, but 8, or 57 per cent of the sur-

Table 10.100 — Mortality of Animals Exposed Daily for 28 Days to 19 Mg of UO_3 Dust per Cubic Meter

Species	No. died per no. exposed	Mortality, %
Cat	4/4	100
Rabbit	12/18	67
Dog	1/6	17
Guinea pig	2/21	10
Rat	3/31	10
Mouse	4/50	8

vivors, died the third week. None of the remaining 6 died during the fourth week (Fig. 10.44, upper left).

17.5 Body-weight Response. During the exposure period each species lost weight for the first 2 to 3 weeks but thereafter either gained or maintained its body weight without further loss. Prior to the beginning of exposure, the rabbits, guinea pigs, and rats, which were young animals, showed a normal growth response, whereas the dogs, which were mature, showed no change in weight. The greatest decrease in weight occurred during the second week of exposure in rats and guinea pigs and during the third week in rabbits and dogs.

Figure 10.44, upper right, shows the weight response for the five species. For the rabbits, in which there was considerable mortality, a plot of the weight response of those which died is also given.

Although no attempt was made in this experiment to determine the effect of the head restrictions upon the growth rate of the animal, it has been pointed out in Sec. 9 (Control Studies) that animals so restricted had a slower rate of growth than those not so restrained. In the control studies no sharp declines in growth rate were observed comparable to those found in this experiment. Therefore it can be concluded that the loss in weight in the animals head-exposed to UO_3 dust resulted from the toxic effects of uranium and not as a result of the experimental procedure.

17.6 Physiological Findings. Effects of uranium trioxide exposure on physiological responses of the body were evaluated (a) from measurement of urine volume and (b) from renal clearances.

(a) Urine Volume. Urine was collected from the time the animals were taken from the chamber late in the afternoon until they were replaced in the chamber the next morning. In the case of cats and rabbits, urine volume represented a 24-hr sample, because these animals retained their urine while in the chamber. In the case of the dogs, not all the urine was retained, so that the experimental values represent a minimal value of the 24-hr urine volume. Figure 10.44, lower left, shows that a definite diuresis occurred in dogs and cats following exposure, whereas in rabbits a tendency toward anuria was present especially just prior to death. Differences between the control, the last, and the next to the last urine sample before death are significantly different as indicated by the P values 0.03 and 0.04.

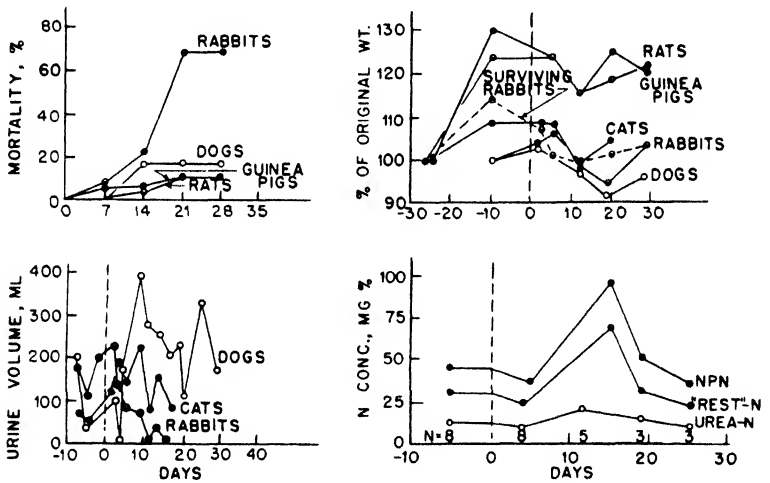


Fig. 10.44—Average toxicologic responses to uranium trioxide dust exposures at 19 mg/cu m. Upper left, mortality of four animal species; upper right, changes in body weight from preexposure values; lower left, changes in volume of urinary excretion; lower right, changes in NPN, urea nitrogen, and residual nitrogen ("rest" N) of blood of rabbits.

(b) Renal Clearance. Renal clearances of chloride, creatinine, diodrast, phenol red, and urea were determined on five rabbits at 10, 19, and 28 days during exposure. The clearances, expressed in terms of percentages of normal values of stock rabbits, are given in Table 10.101. Creatinine, phenol-red, and urea clearances were markedly low except for two values for rabbit 80 that were questionable. Diodrast clearances were very low in one animal of three. Chloride

clearances were elevated in all five animals. Although a strict interpretation of these physiological data is difficult, the values indicate a general disturbance in kidney function.

Table 10.101 — Renal Clearances* in Rabbits Exposed to UO_3 Dust

Animal No.	Days from start of exposure	Chloride clearance	Creatinine clearance	Diodrast clearance	Phenol-red clearance	Urea clearance
78	10	228	9.7		3.3	29.1
96	19	228	20.0		7.3	33.7
80	28	205	168.0†	31.2	194.0†	
97	28	172	47.5	81.5	18.6	52.8
98	28	205	8.6	117.0	38.6	75.6

*The values are expressed in percentage of average values for stock rabbits.

†These values are questionable. The reported urinary concentrations of the substances concerned were even higher than those of stock rabbits with the same rate of urine flow.

17.7 Changes in Chemical Constituents of Blood and Urine. Urine samples collected on alternate days thrice weekly and blood samples collected weekly were obtained from 3 dogs, 2 cats, and 10 rabbits. Control samples were collected during the week preceding the start of the exposure. Determinations of urinary protein, reducing sugar and creatinine, and nonprotein nitrogen and urea in the blood were made.

(a) Urinary Protein and Reducing Sugar. Each rabbit, dog, and cat in the biochemical study showed a marked increase in protein excretion with a peak value 5 to 10 days following the start of the exposure. After this period the protein level decreased, but it never returned completely to normal. Elevated values first appeared in some animals after 4 days of exposure, and all animals showed a marked proteinuria after 1 week (Fig. 10.45, lower). Statistical analysis indicated that these changes were highly significant.

The determinations of urinary sugar excretion did not present such clear-cut results as those of urinary protein. This was primarily because, in contrast to normal urinary protein values, the normal urinary sugar values varied considerably from animal to animal and among samples of a given animal. Statistical analysis of the results indicated a significant difference between the sixth exposure day and the control period. However, an analysis of the variance during the control and exposure period indicated that only 5 of the 10 animals

showed significant changes. To some extent the peak of sugar excretion paralleled that of protein, especially in the dogs (Fig. 10.45, upper).

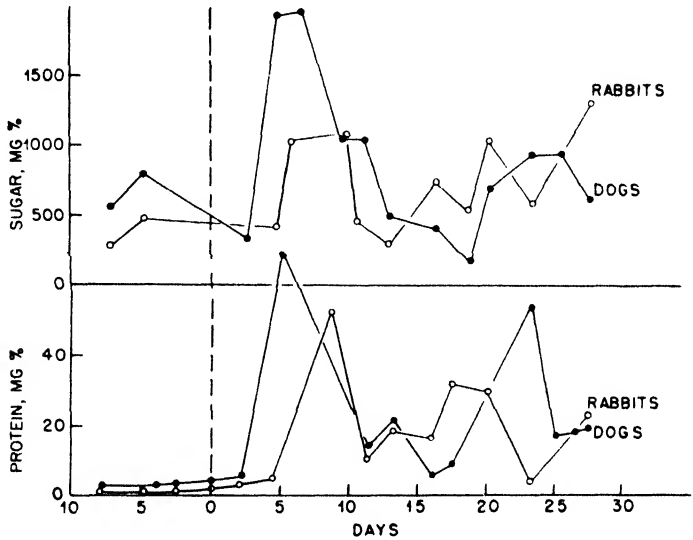


Fig. 10.45—Average change in excretion of urinary constituents of two animal species exposed to UO_3 dust.

(b) Creatinine Excretion. No significant changes were observed in the 24-hr creatinine excretion in 10 rabbits.

(c) Nonprotein and Urea Nitrogen of Blood. There was a marked rise in NPN in each of three species tested after 1 to 2 weeks of exposure as observed from weekly determinations. Most of this rise was accounted for by an increase in "rest" nitrogen (nonurea NPN). This was especially true at the peak of the rise (Fig. 10.44, lower right). The highest NPN value observed during the experiment was in the rabbit, 212 mg %; highest value in the cat was 138 mg %; and highest in the dog was 90 mg %. Of the three rabbits that survived, two showed a peak at the end of 2 weeks, but one showed no abnormal values whatsoever.

17.8 Pathology. Gross and microscopic examinations were made of the tissues of 10 rabbits, 10 rats, and 5 dogs. Of this group, 1 dog and 8 rabbits died during the exposure period, and the remaining animals were sacrificed at the end of the experiment. Tissues studied included lung, liver, kidney, and vertebral and femoral marrow.

(a) Gross Examination. Gross changes seen in the kidneys were characterized by swelling and gray discoloration at the corticomedullary junction. In the lungs of rabbits that died during the exposure there was gross evidence of hemorrhage and consolidation. The liver did not show damage consistently, although in a number of rabbits fatty livers were observed.

(b) Microscopic Examination. Kidney. The tubules were the site of most severe damage histologically. Of the animals studied, the rabbits were most affected. The tubules of the dogs and the rats were affected to a lesser extent, possibly because the rabbits died and were examined within 20 days. Injury ranged from mild to severe necrosis of tubular epithelium. Various stages of degeneration as well as regeneration were commonly observed. (See Chap. 4 for details of renal changes.) Glomerular changes were observed in dogs.

Lung. Histologic examination indicated that rabbits that died within the first 20 days of exposure showed most severe damage, whereas those that survived showed no damage at the twenty-eighth day. Pulmonary changes in dogs and rats were very slight.

Liver. Fatty livers were found in 5 of the 8 rabbits that died and in 3 of 10 rats but in none of the dogs. The damage was moderate.

Serial Sections of Brain and Splanchnic Nerve. A detailed histological study of the brain and the splanchnic and sciatic nerves of one dog exposed for 28 days indicated no demonstrable damage to these tissues.

Bone Marrow. Differential counts made of the cellular elements of bone marrow of nine experimental rats at the end of the exposure and a group of 109 control rats showed significant differences between the two groups in the number of myeloblasts and lymphoid cells. Although the bone-marrow changes were statistically significant, the absence of similarly clear-cut changes in the hemogram raises some question as to the meaning of the bone-marrow changes, and accordingly no interpretation of these data in terms of causal relationship to uranium exposure is made. (See Chap. 4 for a discussion of bone-marrow changes in animals exposed to uranium.)

17.9 Hematology. A statistical analysis of the hematological results obtained weekly from 5 dogs, 10 rabbits, and 10 rats indicates that the changes observed during exposure were of doubtful significance and were of little diagnostic value in uranium toxicity.

17.10 Uranium Deposition in Tissues. Uranium analyses were performed spectrochemically on selected tissues of 22 guinea pigs, 12 rats, 9 rabbits, 4 dogs, and 3 cats. The lung, liver, kidney, and epiphyses of the long bones of the leg, pelvis, and spinal column were

studied. The embryos of some species were also analyzed. In addition, analyses of the tissues of groups of three rats and three guinea pigs sacrificed weekly during the exposure were made. Another group was killed 4 weeks after the termination of the exposure to determine the retention of uranium in the tissues after cessation of exposure.

As a result of these analyses it was found that the accumulation of uranium in the tissues was much more rapid during the first 2 weeks of exposure than subsequently. Moreover very little uranium appeared to be lost from the tissues within 4 weeks after the cessation of exposure.

The lungs showed the highest frequency of the occurrence of uranium, the liver the lowest, and other tissues an intermediate frequency. No marked species differences were observed.

Six cat and four guinea pig embryos from exposed mothers were analyzed. Uranium was absent from all samples. The embryos varied in their state of development from one-third to almost full term.

17.11 Discussion. (a) Relative Value of Correlatives of Uranium Poisoning. Numerous criteria were employed to obtain a precise description of the symptomatology, the sequence and extent of events, and the mechanism of uranium toxicity. In this study of UO_3 dust inhaled at a comparatively high level, 20 per cent of all exposed animals died, all lost weight, and most showed severe pathology in either the lung or kidney or both, along with functional changes in the kidney as measured by renal clearance studies and by changes in the concentrations of various biochemical constituents of blood and urine. At lower levels of exposure, the increasing value of more sensitive indicators, such as renal-function tests, is obvious, but at highly toxic levels, such as obtained in this study, mortality is one of the most useful of the various correlatives as a measure of the toxicity, but even here it is not a completely trustworthy guide. In inhalation studies, such species as the guinea pig and rabbit are prone to infections, conditions that are frequently aggravated by the excitement and change in environment of the exposure unit. Moreover, group size must be large, and significant mortality must occur; the death of one or two animals has little meaning.

The significant histologic changes observed usually were indicative of severe to moderate uranium damage, though changes may not have been sufficient to cause death. With extreme care, histologic changes of low degree may be differentiated from histologic aberrations commonly present in untreated animals. In so far as growth rate is concerned, loss of weight found in all the animals, although indicating a general debility and loss of appetite unmistakably suggesting the

toxicity of uranium, in no way indicated which organs or which biological mechanisms were affected.

Renal clearances of both inorganic and organic substances proved an excellent test for specific renal damage. Unfortunately, in the rabbit this test required the sacrifice of an animal for each determination, prohibiting large-scale work. With dogs, however, this difficulty might be avoided.

Biochemical changes in the blood and urine proved valuable correlatives of uranium poisoning both because of their ease in obtaining information and the definitiveness of the data. Excretion of protein in the urine proved to be the first sign of toxicity. Excretion of reducing sugar, although showing changes in the early stages of uranium poisoning, proved too inconstant a correlative to be of value. Excretion of creatinine, on the other hand, showed no significant change.

Blood nonprotein nitrogen was not so early an indicator as was urinary protein but was a reliable index of severe uranium poisoning. Death was usually preceded by very high values. On the other hand, blood urea nitrogen showed less elevation than nonprotein nitrogen.

(b) Sequence of Events in Uranium Intoxication by Inhalation. The sequence of events following the inhalation of the water-insoluble UO_3 dust is dependent first upon the solution of the oxide by the body fluids passing through the lung. Although UO_3 is insoluble in water, it dissolves rapidly in bicarbonate to form $Na_4UO_2(CO_3)_3$, a soluble complex. There seems no doubt that the formation of this carbonate complex plays an important role in transportation of uranium across the alveolar membrane. The appearance of high concentrations of uranium in the lungs within a few days after the start of exposure was confirmed by direct analysis. Although the percentage of retention of the inhaled UO_3 has not yet been determined, it may be estimated that, if the air turnover is approximately 25 liters/hr in the rabbit and the retention is assumed to be 50 per cent, then at a UO_3 concentration of 20 mg/cu m the daily intake for a 6-hr exposure day is approximately 1.5 mg. Although a number of other desirable points of information are lacking, such as the absorption from the lungs, the amount entering the body by way of the gastrointestinal tract, and the amount excreted, the analyses of the lung for uranium indicate no marked accumulation of uranium in this organ after the first week. Therefore uranium was absorbed as rapidly as it entered the respiratory tract. Uranium was also found in the kidney and bone after 1 week of exposure.

A marked diuresis and proteinuria appeared within 2 to 3 days from the start of exposure. This was followed by marked elevations

in nonprotein nitrogen and urea of the blood on the third to fifth day. No change in weight response was observable before the fifth day, but changes became most marked during the second week. Likewise the first animal death occurred on the fifth day, but the majority of deaths occured much later during the third week of exposure. Histologic changes were most prominent in the kidneys, with some lung involvement, especially during the first part of the exposure period. Unfortunately the sequence of histological changes cannot be stated as definitely as desired in this experiment because of the infrequency with which the tissues were examined. No surviving animals were examined before the eighth day of exposure. The pathology noted terminally was one of repair, indicating that, in so far as the lung and kidney damage was concerned, it was the initial exposure that gave rise to most of the damage. Once the process of repair was initiated, further exposure caused little insult. These findings, in common with those of other inhalation studies, indicate a tolerance to uranium-dust exposures at the end of 3 weeks that is not present during the early period of exposure. The growth, mortality, and biochemical data are in conformity with this conclusion.

(c) Differences in Species Susceptibility. Despite the similarity in the intensity in certain of the toxic symptoms, there were marked differences in species susceptibility as measured by mortality. Because of the severity of the toxicity, it was the only variable that was capable of showing differences. Cats and rabbits had a high mortality, whereas the other species had a low mortality. The dog, a relatively resistant species on this basis, showed less uremia than did the cat and rabbit. On the other hand, the dog demonstrated as high a proteinuria and glycosuria as did the rabbit and cat, both susceptible species. The histologic changes in dogs and rats were less severe than those in rabbits, although these observed changes may possibly have been apparent only. Rabbits may have died before repair processes were substantial, whereas, in the case of dogs that survived, time allowed repair of this damage.

If the results of these animal experiments are applicable to man, the use of as wide a variety of species as possible is a necessity in order to obtain as complete a spectrum of susceptibility and resistance as possible. If no toxicity is observed in the most sensitive species under given exposure conditions, it is reasonable to assume that the concentration is probably safe for man.

17.12 Summary. 1. Uranium trioxide dust at a concentration of 19 mg/cu m and a standard deviation of 7.7 and a mean particle size of 0.7 μ was found to be toxic for dogs, cats, rabbits, guinea pigs, rats, and mice.

2. There was a large difference in species susceptibility. On the basis of mortality the most susceptible species were the cat and the rabbit, and, in decreasing order, the dog, rat, guinea pig, and mouse.

Table 10.102 — Animals Exposed for 24 Days to 19 Mg UO_3/cu m (Summary of Results)

1. Mortality. Cats and rabbits were most susceptible.

Species	No. of deaths per no. exposed	Mortality, %
Cat	4/4	100
Rabbit	12/18	67
Dog	1/6	17
Guinea pig	2/21	10
Rat	3/31	10
Mouse	4/50	8

2. Weight Changes. All species lost weight during the first 2 to 3 weeks. The surviving animals gained weight thereafter.
3. Biochemistry.

Determination	Animals showing abnormal values, %
Urinary protein	100
Blood NPN	64
Blood urea	55
Urinary sugar	50
Urinary creatinine	7

4. Pathology. Severe renal tubular damage in dogs, rats, and rabbits. Lung damage in rabbits during first 2 to 3 weeks.
5. Hematology. No clear-cut changes.
6. Kidney Function. Rabbits showed elevated renal clearance of chloride and diminished clearances of creatinine, diodrast, phenol red, and urea.
7. Uranium Deposition in Tissues. Uranium appeared in lungs, kidneys, and bones.
8. Conclusion. UO_3 at 20 mg/cu m is very toxic to all species and especially to cats and rabbits. Although UO_3 is relatively insoluble in water, it is very soluble in bicarbonate, forming the complex $Na_4UO_2(CO_3)_3$. This probably accounts for the relatively high toxicity of the compound.

3. Histological examination of various organs indicated that the kidney was most severely damaged. Some lung damage was evident in rabbits during the first 3 weeks of exposure.

4. The most sensitive criterion of toxicity was urinary protein, which reached abnormal values in all the animals studied after a few days of exposure. Urinary sugar excretion increased during exposure

but not to such an extent that this variable could serve as a good indicator of toxicity.

5. The animals that survived the first 3 weeks of exposure showed a marked improvement in general health during the fourth week. All animals lost weight during the second and third weeks, but surviving animals gained thereafter.

There was a definite anuria associated with approaching death. Blood NPN, although not so sensitive a criterion as urinary protein, was valuable as an indicator of extensive damage. The rise in blood NPN was largely occasioned by nonurea nitrogen. Renal-clearance studies indicated that the functioning of the kidney was markedly disturbed.

Uranium deposition occurred to a large extent in kidneys and lungs but to a lesser extent in livers. Uranium was also found in various bones (vertebral column, pelvis, and epiphysis of long bones) and teeth. No uranium was found in guinea pig or cat embryos.

18. URANIUM PEROXIDE

By H. Paul Dygert*

18.1 Introduction. The toxicologic response resulting from the inhalation of uranium peroxide dust in animals was studied as part of a program to determine the toxicity of a number of intermediates to which there was limited human exposure.

Because of the low priority rating of this material, less extensive information was demanded than for those materials with higher ratings. Accordingly, the toxicity of UO_4 was determined at one level only. The peroxide employed was a yellow hydrated compound of acicular form, with remarkably low density after grinding in comparison with that of other uranium compounds. The latter property allowed easy dispersal of the material as a dust into the inhalation-chamber atmosphere. The only other property of interest was the increase in solubility in serum over that in water.

Exposures were conducted at an approximate level of 20 mg/cu m of air for a period of 23 exposure days with an average exposure of approximately 5 hr a day, 5 days a week, and 3 hr on the sixth day. One hundred and twenty animals, including six species, were exposed: 1 dog, 4 cats, 5 rabbits, 20 rats, 20 guinea pigs, and 70 mice. Exposure was performed in a full-exposure unit of 64 cu ft size with

* Toxicologic procedures performed by H. A. Oberg and H. P. Dygert.

features similar to those described in Sec. 6.1e. The dust was introduced intermittently into the chamber by using a dust elutriator activated by a hand-operated jet of air. The dust concentration obtained by this method of introduction yielded a mean of 19.5 mg UO_4/cu m (13.1 mg U) as determined from the average of the weighted means of individual daily samples. Samples taken at hourly intervals showed a standard deviation from the mean of 5.8. Table 10.103 shows the range of concentrations of 114 dust samples taken throughout the exposure period and the percentage of samples at given deviations from the mean. The dust samples taken from the chamber during exposure of the animals for measurement of particle size were distinct from other dusts in their needlelike form, with long axis two or three times that of the short. The size of the particles measured by the approximate method of the Bausch & Lomb counter (Sec. 8.2) possessed a mean of 0.86 μ with a standard geometric mean of 1.69.

Table 10.103 -- Dust Concentration

Weighted-mean concentration, mg UO_4/cu m	Deviation, mg/cu m	Samples within given deviation, %
19.5	± 2	26
19.5	± 4	57
19.5	± 6	70
19.5	± 8	80
19.5	± 10	88

Note: desired concentration, 20 mg UO_4/cu m; standard deviation, ± 5.8 mg UO_4/cu m; number of samples.

18.2 Signs of Toxicity. No outward manifestations of toxicity prior to death were observed other than loss of appetite and weight.

18.3 Mortality. Uranium peroxide was lethal for all species exposed except the dog. The one dog exposed was killed, for reasons unrelated to the study, 6 days after the start of exposure without having shown any signs of uranium poisoning. Exposures at the 19.5-mg level were almost uniformly fatal for both the cat and the rabbit and were lethal to the majority of the heavier of two groups of mice. The mice of lower weight, however, showed a considerably lower mortality. The peroxide was only occasionally lethal to rats (Table 10.104).

18.4 Weight Response. All animals exposed, except one surviving rabbit, lost weight. Weight losses are recorded separately for the animals that died and those that survived (Table 10.105). Dying animals showed a greater weight loss than those that survived. This

amounted to approximately 25 per cent in the rat and guinea pig, somewhat less in the rabbit and cat. Weight losses became evident in the different animal species between the second and sixth day. The

Table 10.104 — Mortality of Animals Exposed 5 Hr Daily for 23 Days to Atmospheres Containing 19.5 Mg of UO_4

Species	No. of deaths per no. exposed	Mortality, %
Cat	4/4	100
Rabbit	4/5	80
Mouse*	25/40	63
Guinea pig	8/20	40
Mouse†	7/30	23
Rat	2/20	10
Dog	0/1	0‡

* Average weight 21 – 29 g.
† Average weight 16 – 19 g.
‡ Dog killed on sixth day, after 4 days of exposure, because of viciousness.

Table 10.105 — Weight Change in Animals Exposed to UO_4

Species	No. died	No. survived	Wt. change, %
Rat	2		-24.7
		18	-6.3
Guinea pig	8		-22.8
		12	-7.1
Rabbit	4		-19.3
		1	+3.8
Cat	4	0	-11.3

four cats and two of the rabbits lost weight continually until death, whereas the three remaining rabbits either exhibited gains just prior to death or, in the case of the one surviving rabbit, recovered the loss and continued showing normal weight response. The rats regained a portion of the lost weight with a peak at the fifteenth day, after which loss in weight was again observed. This loss continued to the end of exposure. Guinea pigs did not recover from the initial weight loss during the exposure period.

18.5 Hematology. The hematologic studies made in 20 rats and 5 rabbits revealed no significant changes.

18.6 Biochemical Findings. Nonprotein and urea nitrogen in the blood of two cats and five rabbits showed increases following exposure. In all animals, except one rabbit that was killed at the end of the experiment, the rise was continuous until death. The maximal values obtained are shown in Table 10.106. Blood creatinine showed a definite rise in three of the rabbits succumbing from exposure, and only slight increases in the two remaining rabbits.

Table 10.106 — Biochemical Findings for Blood of Animals Exposed to UO_4

Species	Nonprotein nitrogen, mg %	Urea nitrogen, mg %	Creatinine	Remarks
Rabbit	Max. value 256	Max. value 173	Elevation in 3 of 5; max. value, 19.5 mg %	All animals showed azotemia
Cat	Max. value 511	Max. value 186	Elevation in 1 of 2; max. value, 15.4 mg %	All animals showed azotemia
Dog	48	29	No elevation	Slight elevation of NPN and urea N on fourth exposure day

18.7 Pathology. The tissues of 4 rabbits and 18 rats were examined grossly and microscopically. Three of 4 rabbits and 2 of the 18 rats died before the conclusion of the 23-day exposure period; the remainder, however, were examined at the termination of the exposure. In the rabbit moderate corticomedullary tubular necrosis was observed, accompanied by some typical and atypical regeneration of the tubular cells. In a few instances there were slight degenerative glomerular changes as well. The lungs of the rabbit exhibited edematous alveoli, alveolar hemorrhage, hyperemia, and atelectasis.

In the rats, most of which were observed at the end of the experiment, typical regeneration of corticomedullary tubular epithelium was seen. In certain sections there was evidence of hyaline thickening of the basement membrane of regenerated tubules. Mild vacuolization of the glomerular tuft was occasionally noted.

18.8 Distribution of Uranium in Tissues. Uranium, analyzed by the spectrochemical method, was found in all tissues examined — kidney, lung, pelvic bone, spinal column, femoral epiphysis, and liver. Detectable concentrations were observed most frequently in the kidney (75 per cent of the cases) and progressively less frequently in the tissues in the order mentioned (Table 10.107).

Table 10.107 — Frequency of Occurrence of Uranium in Tissues of Animals Exposed to 19.5 Mg UO_4/cu m, Determined by the Spectrochemical Method*†

	No. with uranium per no. exposed	Per cent
Kidney	15/20	75
Lung	15/22	68
Pelvic bone	13/22	59
Spinal column	11/21	53
Femoral epiphysis	11/22	50
Liver	5/21	24

*A value was considered positive if equal to or greater than 0.2 μg/g of soft tissue or if equal to or greater than 0.5 μg/g for bone.

†Although the tissue of animals that died throughout the exposure period are included here, little variation in frequency distribution of uranium in tissues was observed among animals whose tissues were examined at these periods and those analyzed at the termination of the experiment.

18.9 Bone-marrow-differential Counts. Examination of cytological constituents of bone marrow of 16 rats showed a significantly higher percentage of lymphoid cells than did the bone-marrow differentials of more than 100 control rats. Percentages of other cellular constituents were not significantly different from those of controls, with the exception of myeloblasts that were also present in higher percentage than in the controls. The latter change is less significant than that observed in lymphoid cells (see Chap. 4).

18.10 Discussion. Uranium peroxide dust was definitely toxic when inhaled for repeated intermittent daily periods at a concentration approximating 20 mg/cu m of air. The comparatively high solubility of this oxide of uranium in serum at body temperatures is undoubtedly a major factor in producing this toxicity. The acicular crystalline form of the inhaled dust may have produced mechanical irritation in the lung, thus adding to the chemical toxicity. Further evidence of the toxic nature of the peroxide was the general weight loss among all the five species exposed; occasional individual exceptions were noted in one surviving rabbit and in the dog exposed for but 4 days. Observations of weight response showed that all cats and rabbits had begun to lose weight by the sixth day of exposure. At this time also deaths began to occur among the cats, rabbits, and mice. The guinea pigs were somewhat more resistant, no deaths occurring within this group until after more than a week of exposure. Variation in species susceptibility in response to exposure to peroxide is therefore apparent.

Examination of histologic sections found renal and pulmonary changes of similar nature in each species, but the degree of renal damage was far greater than that of pulmonary damage. Consistent with these findings, all cats and rabbits upon which blood NPN determinations were made showed elevated levels compatible with the degree of renal pathology with one exception. The frequency of occurrence of uranium in the six tissues examined was highest in the kidney. Noteworthy also was the relatively high frequency of occurrence of uranium in the lung and in the three osseous tissues, femoral epiphysis, pelvis, and spinal column.

18.11 Summary. The toxic effects of inhaling uranium peroxide dust at a concentration approximating 20 mg/cu m of air (13.1 mg of uranium) over a period of 26 calendar days revealed the following:

1. Forty-two per cent of 120 animals comprising six species died, mortality occurring in the following species: cats, 4 of 4; rabbits, 4 of 5; and guinea pigs, 8 of 20. A 62 per cent mortality occurred in a group of 40 mice with a weight range of 21 to 29 g, whereas only 23 per cent mortality occurred in a group of 30 mice with a weight range of 16 to 19 g. The one dog exposed was killed before showing signs of toxicity.

2. Only one animal, a surviving rabbit, gained weight. All other species showed an average weight decrease amounting to as much as 25 per cent of the weight at the start of exposure.

3. Nonprotein nitrogen and urea nitrogen of blood of four rabbits and two cats showed progressively increasing values that reached a maximum at the time of death.

4. Kidneys of 3 dying rabbits and 18 rats killed at the end of the 26-day-exposure period showed mild to moderate corticomedullary tubular necrosis, mild typical and sometimes atypical regeneration, and the presence of tubular casts. A mild glomerular vacuolization was occasionally noted. The lungs of the three dying rabbits showed markedly edematous alveoli. The liver and spleen showed no injury of note.

5. Uranium was most frequently detectable in the kidney and lung, with a somewhat higher percentage frequency in renal tissue. The bones studied (pelvic bone, femoral epiphysis, and spinal column) showed approximately equal percentage frequency findings that were in turn somewhat lower than that determined for the lung. The liver contained detectable uranium concentrations in only a few instances.

6. Although the solubility of UO_4 in water was of similar magnitude to that of two less toxic uranium oxides, UO_2 and U_3O_8, its 25-fold greater solubility in human serum is consistent with its greater toxicity.

19. SODIUM DIURANATE

By Aser Rothstein*

19.1 Introduction. On sodium diuranate, $Na_2U_2O_7.1\ ½\ H_2O$, a relatively insoluble orange substance, only a single inhalation study was made. This was performed at a level of 20 mg/cu m. In the course of this study emphasis was laid on three aspects of uranium poisoning: (1) the chronological sequence of events during exposure, especially from a histologic point of view, (2) the changes in biochemical constituents of blood and urine, and (3) the extent of recovery during 12 weeks following the exposure. Although animals of six species were used in the experiment, most of the measurements were made on rabbits, a species suited for the biochemical work. In order to obtain information regarding the histological events, a series of 30 rabbits was sacrificed at intervals throughout the exposure and postexposure periods. Biochemical determinations were made on the same animals so that correlations were possible between chemistry and pathology. In addition, mortality, hematology, growth rate, renal function, and uranium deposition in tissues were studied.

19.2 Conditions of Exposure. (a) Exposure Chamber. A typical head-exposure unit (Sec. 6.1f) equipped with a pressure type of dust feed (Sec. 6.2a) was used. Only rabbits, cats, and guinea pigs were head exposed, whereas dogs, rats, and mice were placed bodily in the chamber in wire-mesh cages. Air at a temperature of 70 to 80°F was exchanged in the chamber about once every minute.

(b) Dust Concentration. Samples of dust-laden air were taken hourly during the exposure period by the filter-paper method (Sec 8.1a). The actual mean concentration was 20.3 mg/cu m of the diuranate (15 mg U/cu m), a value close to the desired concentration of 20 mg. There was no more than the usual variation in the chamber dust concentration. The standard deviation was 3.2 mg/cu m, and 80 per cent of the determinations lay between ± 6 mg or within ± 30 per cent of the mean (Table 10.108).

The bulk dust had a median particle size of 0.77 μ with a standard deviation of 2.3.

(c) Exposure Schedule. A total of 199 animals representing six species (6 dogs, 4 cats, 42 rabbits, 30 guinea pigs, 67 rats, and 50 mice) was used. The animals were first placed in the chamber without dust for a 2-week period, during which they were acclimatized to

* Work performed by A. Rothstein, D. Dittman, H. Berke, and J. Cobler; renal clearance studies by H. Wills.

exposure conditions. This was followed by a 5-week exposure to sodium diuranate dust. The animals were within the chamber 6 hr a day, 5½ days a week (3 hr on Saturdays).

Eighteen rabbits were sacrificed during the exposure period, and numbers of the other species were sacrificed at the end of the period. The surviving rabbits were studied over a 12-week period following completion of the exposure.

Table 10.108 — Dust Concentration of $Na_2U_2O_7$

Weighted-mean concentration, mg/cu m	Deviation, mg/cu m	Samples within given deviation, %
20.26	± 2.0	42
20.26	± 4.0	68
20.26	± 6.0	80
20.26	± 8.0	90

Note: desired concentration, 20.0 mg/cu m; standard deviation, 3.24 mg/cu m; number of samples, 208.

19.3 Signs of Toxicity. No gross signs of toxicity were noted other than the usual signs of loss of appetite and weight accompanying uranium poisoning.

19.4 Mortality. Of the total of 150 animals in the mortality group, 32, or 21 per cent, died during the exposure period. The highest mortality was found in rabbits (39 per cent) and mice (38 per cent), compared to intermediate mortality in guinea pigs (13 per cent), and no mortality in dogs, cats, and rats (Table 10.109). Most of the animals died during the second and third weeks.

Table 10.109 — Mortality from Exposure to $Na_2U_2O_7$

Concentration level of compound, mg/cu m	Species	No. of deaths per no. exposed	Mortality, %
20.26	Rabbit	9/23	39
20.26	Mouse	19/50	38
20.26	Guinea pig	4/30	13
20.26	Dog	0/6	0
20.26	Cat	0/4	0
20.26	Rat	0/36	0

19.5 Body Weight. (Fig. 10.46). The rabbits, guinea pigs, and rats were young, growing animals. They gained weight during the control

period but failed to gain or lose weight during the first 2 or 3 weeks of the exposure period. The cats and dogs were full grown. They lost weight during exposure. During the last 2 weeks of exposure all surviving animals, except the cats, gained weight, indicating a recovery of body function despite continued exposure.

The surviving rabbits showed a very marked acceleration of growth during the month following the end of the exposure period.

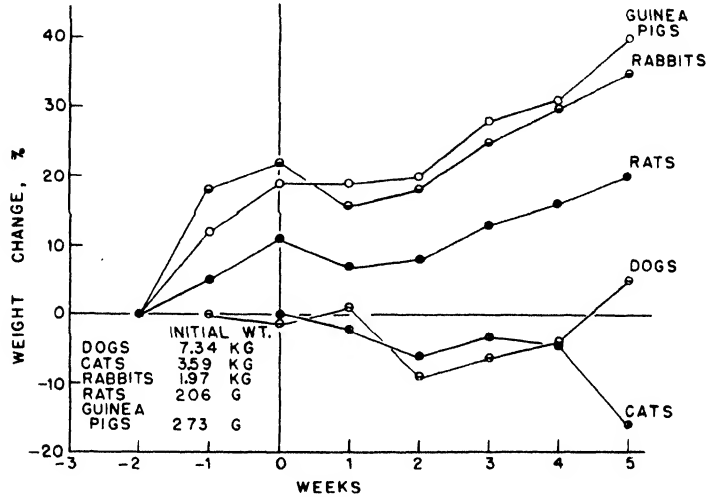


Fig. 10.46—Changes in body weight of five animal species exposed to $Na_2U_2O_7$ dust at 20 mg/cu m.

19.6 Pathology. No lesions other than renal that could be attributed to uranium treatment were noted in any animals. Development of renal changes in rabbits followed the usual pattern for animals exposed to inhalation of toxic levels of uranium dust. Very slight degeneration was first observed 2 days after the beginning of exposure and was followed on the 3rd day by a definite tubular necrosis. These changes of an active degenerative type were seen at maximal severity on the 7th, 12th, and 16th days. Although two animals studied during the second week (9th and 14th days) showed less damage of this type, it seems reasonable to assume from this and other experiments that with moderately high concentrations of uranium the peak of active damage is reached during the second week. Sloughed necrotic cells in the renal cortical tubules were seen throughout the exposure period but were extremely few in animals studied after the 21st day. Further

evidence of tubular destruction was present in the form of hyaline and finely granular casts in the medullary tubules. These were especially numerous between the 7th and 23rd days.

Tubular regeneration was first observed on the fifth day and in this case was slight but definite and quite active. Tubular regeneration persisted throughout the exposure period and was most extensive between the 16th and 35th days. It was predominantly typical, although atypical cells were present in all cases. There were also tubules that stained normally but were small, constricted, and thin-walled. This type of tubular alteration was accompanied by an increase in surrounding stroma. No changes were noted in the glomerular and capillary tufts, although there was seen a pink-staining granular material in the capsular spaces of the outer cortex in one case and slight hyaline thickening and epithelial proliferation in some Bowman's capsules in animals sacrificed between the 25th and 31st days.

The latter part of the exposure period was marked by a return toward normal that was suggested more by a change in the type of renal alteration than by a decrease in the total renal abnormality.

During the first 5 weeks following the end of the exposure period, renal abnormalities were definite but much less extensive than those seen in the latter part of the exposure period. The changes were characterized by slight typical and atypical regeneration that in some cases was accompanied by tubular constriction. Following the fifth postexposure week, renal pathology was negligible except for an irregularity of cortical tubules, especially in the corticomedullary zone. These tubules showed irregularity in arrangement as well as irregularity of the epithelium forming the tubules.

If we assume that the rabbits studied in the postexposure series had already undergone the same changes seen in animals sacrificed in the second week of exposure, then we can conclude that the renal epithelium is capable of returning to almost complete structural normalcy.

Rabbits that died on the 14th and 17th days after the beginning of exposure showed renal changes similar to those seen in animals sacrificed at about the same time, although somewhat more severe. In these cases death can be assumed to have resulted from renal damage, since no other significant pathological findings were noted.

Dogs and rats examined after 35 days of exposure showed typical renal changes, largely regenerative in nature, although evidence of active necrosis was seen in the form of sloughed dead cells in the lumina of regenerating tubules. In dogs there was an increase in interstitial stroma and some thickening in hyperplasia of Bowman's capsules.

Renal damage was much more severe in dogs than in rats. Since there was no group of rabbits studied that was comparable in exposure time to the rats and dogs, it is impossible to make comparisons of renal response. (See Chap. 4 for definitions and illustrations of terms used here.)

19.7 Hematology. There were no marked changes in any of the counts, nor were there changes common to the three species examined. In dogs there were no consistent changes except in percentage of eosinophils. In rabbits there was a slight but steady rise in red count and hemoglobin, white count, percentage of neutrophils, and absolute neutrophils and a decrease in lymphocytes. In rats there was a slight rise in white count and percentage of neutrophils and eosinophils, especially during the first 2 weeks of exposure. It seems doubtful that any of the changes reported in this experiment are of significance.

19.8 Blood and Urine Chemistry. A large number of biochemical constituents of rabbits were studied, including urinary protein, creatinine, creatine, ammonia, and lactate and blood nonprotein nitrogen, urea nitrogen, carbon dioxide, and lactate. Urinary output was also measured. In dogs only blood nonprotein nitrogen and urea nitrogen were determined. The biochemical work was undertaken to clarify the mechanism of uranium intoxication and also to find out whether any of these blood or urine determinations could be used as a sensitive test of uranium toxicity.

(a) Blood Constituents (Fig. 10.47). Nonprotein and Urea Nitrogen. In rabbits there was a marked uremia as indicated by an increase in the blood nonprotein and urea nitrogen, starting on the fourth to fifth day and continuing until death in those animals that died but returning toward normal after 3 weeks in the survivors. Of the 15 animals from which blood samples were obtained after the third day of exposure, 12 showed a marked elevation of urea nitrogen. Three rabbits that died showed NPN values of 215, 250, and 280 mg %, confirming other experimental work that indicated that values of 200 mg % or over are a sign of approaching death.

The data for blood urea nitrogen parallel those for blood nonprotein nitrogen, except in the case of one rabbit that had at the time of its death an NPN of 215 mg % and a urea nitrogen of only 50 mg %. During the control period the mean NPN was 38 mg %, of which 17 mg % was urea nitrogen and 21 mg % was nonurea nitrogen. During the exposure period the mean NPN of all determinations was 70 mg %, an increase of 32 mg % over the control mean. The experimental mean for urea was 42 mg %, an increase of 25 mg %. Thus, on the average, urea accounted for 78 per cent of the increase in nonprotein nitrogen.

In dogs there was a small but definite increase in blood nonprotein nitrogen. The mean control value was 32 mg %, compared to a mean experimental value of 50 mg %. After 2 weeks of exposure, the mean was 60 mg % and the highest individual value 81 mg %.

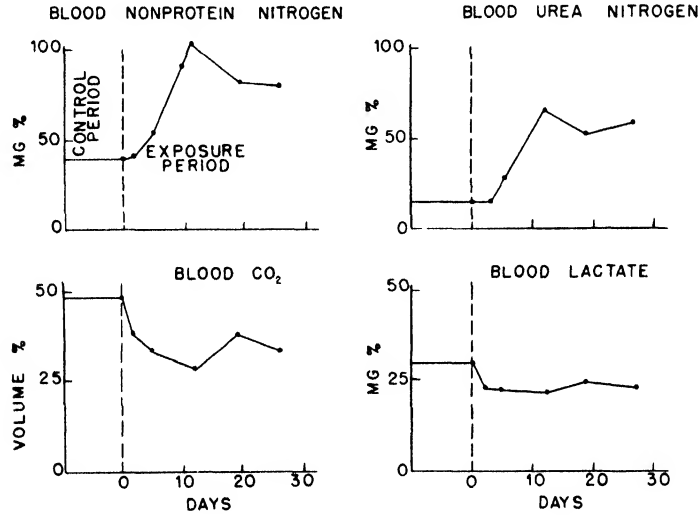


Fig. 10.47 — Average change in chemical constituents of blood of rabbits exposed to $Na_2U_2O_7$ dust.

Carbon Dioxide. There was a definite acidosis in rabbits during the exposure period as measured by a decrease in blood carbon dioxide. This decrease was most marked during the second week and returned to a higher but still subnormal value for the remainder of the exposure period. The control mean blood carbon dioxide was 49 vol. %, with a range of 40 to 60. During the second week of exposure there was a drop to 30 vol. %, followed by a rise to 39 vol. % during the remainder of the experiment. The most marked acidosis was observed in three animals that died, with values of 10, 22, and 23 vol. % just prior to death. Although the majority of animals showed a fairly marked decrease in blood carbon dioxide, a few did not.

Lactate. Blood lactate was studied in order to establish its relationship, if any, to the marked acidosis that occurs in uranium-poisoned animals. Although there was a slight decrease in blood lactate of most of the animals during the exposure period, there was certainly no obvious relationship to the blood carbon dioxide. Neither were the

changes extensive enough to be of any clinical significance, although they were of statistical significance.

(b) Urinary Constituents (Figs. 10.48 and 10.49). An examination of the urinary data of rabbits in this and in other experiments has indicated that a good deal of variance in the data is associated with variations in fluid turnover of the animals and with urinary dilution due to spillage of drinking water. A convenient method has been found to eliminate a great deal of this variance by using the creatinine concentration as an indicator of urinary dilution. The ratio of the concentration of any urinary constituent to the concentration of creatinine is used as a measure of the rate of excretion of that constituent. The advantage of the ratio lies in the fact that a spot sample of urine can be used, thus eliminating the necessity of obtaining accurate 24-hr samples, a difficult procedure in rabbits.

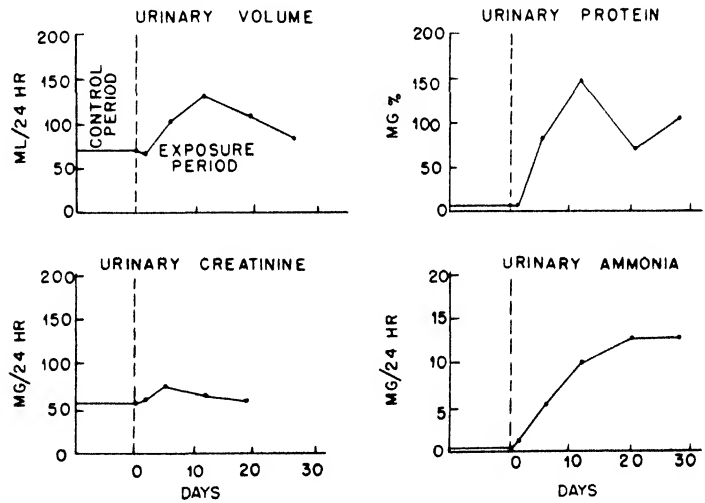


Fig. 10.48 — Average change in urinary excretion of rabbits exposed to $Na_2U_2O_7$ dust.

In normal animals the rate of creatinine excretion is fairly constant and is almost independent of fluid turnover. In uranium-poisoned animals the rate of creatinine excretion is not affected unless damage is so acute that anuria results. Therefore creatinine concentration can be used as a reference point. In both animals and human beings the ratio of a given urinary constituent to creatinine shows far less variance than does the concentration of that constituent. The ratio is so

much more consistent that its use adds considerably more significance to urinary data in the case of constituents which are normally present and which, it is suspected, will change as a result of uranium poisoning. The ratio is of little use in the case of abnormal urinary constituents such as protein.

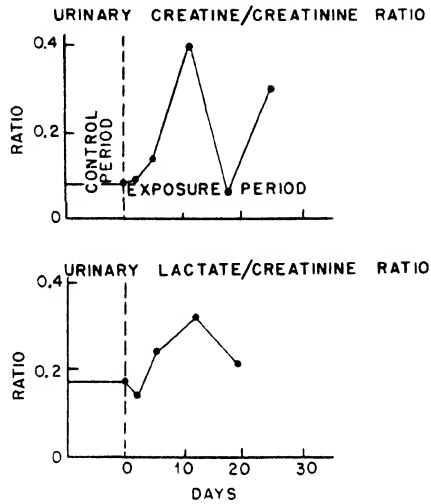


Fig. 10.49 — Average change in excretion of urinary constituents of rabbits exposed to $Na_2U_2O_7$ dust.

Protein (Fig. 10.48). There was a very marked rise in urinary protein after 3 to 5 days of exposure, the rise continuing until the end of the second week, followed by a drop to lower values and a second peak in some of the animals in the fifth week. After the third day of exposure every animal from which a urine sample was taken showed proteinuria, but there seemed to be no definite quantitative relationship between the extent of uranium damage and the extent of proteinuria. Three animals that died did not show particularly high protein values, nor did the animals with the most extensive uremia show the highest values. In fact, some of the animals that showed no change in nonprotein nitrogen had very high urinary protein.

Creatinine. There were no significant changes in the urinary creatinine excretions during the exposure period.

Creatine. It was found that the ratio of creatine to creatinine gave more consistent data than did the creatine concentration, although the result was not qualitatively different. Between the fifth and fifteenth

days of exposure there was a significant rise in the rate of creatine excretion that seemed to be related to the uremia. Of 12 animals on which data were available, 9 showed a rise in both urinary creatine and blood nonprotein nitrogen, 2 showed no rise in either variable, and only 1 showed a rise in nonprotein nitrogen and not in creatine. Three animals that died all had very high creatine and nonprotein nitrogen values. The correlation between urinary creatine and blood nonprotein nitrogen was .68, a highly significant figure.

Lactate. There was a significant rise in urinary lactate in the second week of exposure in most of the animals, followed by a return to almost normal during the latter part of the exposure period. Changes were not so great as in the case of some of the other constituents and were therefore of less significance with respect to their use as a diagnostic test for uranium poisoning. There seemed to be no definite relationship between the increased lactate excretion and the acidosis or uremia.

Ammonia. There was a very marked increase in the ammonia content of urine of all rabbits, starting on the third to fifth days and continuing throughout the exposure period. The ammonia excretion was highest during the second week but never returned to normal during the experiment. The animals that died did not necessarily show the highest values, nor was there any marked parallelism between the blood NPN and urinary ammonia.

Although there was some parallelism between urinary ammonia and protein, it seems unlikely that the two are causally related. It is more probable that the increased ammonia excretion is related to the acidosis that accompanies uranium poisoning. The correlation between the urinary ammonia and blood CO_2 was .29, which is more than twice the standard error, indicating that the two variables may be inversely related.

(c) Biochemical Variables during the Postexposure Period. Thirteen rabbits that survived the exposure period were studied for a few weeks after exposure. Blood nonprotein and urea nitrogen remained normal for 4 weeks in the five animals studied, but blood CO_2 was subnormal in the first week in all five animals. Thereafter three of the animals were normal and two remained low. Urinary protein, creatine, creatinine, and lactate were close to normal in all 13 animals, except for a few scattered high values. Unfortunately no control data were available to permit a strict evaluation.

19.9 Uranium Deposition in Tissues. The tissues of all sacrificed animals were analyzed for uranium content by the spectrochemical method (Chap. 2). The tissues analyzed were the lung, liver, kidney, vertebral column, pelvis, femoral epiphysis, and teeth. A total of 449 tissues from 65 animals (43 rats, 17 rabbits, and 5 dogs) were studied.

Uranium was found to occur with varying frequencies and amounts in different tissues. Unfortunately there was a great deal of variance in the data that revealed many inconsistencies and made interpretation difficult.

The lungs of nearly all exposed animals contained uranium. At the end of the exposure period the mean concentration in rabbits and rats was about 35 μg/g of tissue and in dogs about 14 μg/g. Uranium was found in the liver of only a few animals, but the frequency was relatively high in the kidney, with a mean concentration of about 4 μg/g. The results for bone and teeth were variable with one-quarter of the tissues showing positive results. There were no obvious differences between different bones. Some showed relatively large quantities of uranium, whereas some taken after the same interval of exposure showed none. There were no marked species differences.

19.10 Renal-function Tests on Rabbits. Renal-function tests were performed on the surviving rabbits during 13 weeks following the end of the exposure period. Clearances of chloride, diodrast, and inulin were measured on one rabbit each week. The results shown in Table 10.110 indicate that diodrast and inulin clearances were normal, but

Table 10.110—Renal Clearances of Surviving Rabbits during the Postexposure Period

Animal No.	Time from end of exposure, weeks	Chloride, % of control	Diodrast, % of control	Inulin, % of control
263	1		74	65
264	2		116	76
266	3		119	124
267	4	321	135	98
268	5	197	130	153
270	6	172	112	90
271	7	205	99	126
272	8	236	145	133
295	10		137	124
296	11	202	81	72
298	12	141	141	155
Mean		211	117	111

that chloride clearance was twice normal, indicating a reduced tubular reabsorption of chloride. The abnormal chloride clearance persisted for the duration of the 13-week study despite the fact that the animals had returned to normal in all other respects.

19.11 Discussion. Within 2 days after the start of exposure, degenerative changes were observed in the kidney tubules, accompanied

by a slight proteinuria and ammonuria. The active renal degeneration reached its maximal severity by the sixteenth day, accompanied by a very marked proteinuria, ammonuria, acidosis, uremia, creatinuria, and polyuria. Blood lactate showed little change, although urinary lactate was elevated. There was also an appreciable loss of weight during this period.

Slight tubular regeneration was first observed on the fifth day. From the sixteenth day until the end of the exposure period, regeneration was very extensive, mostly typical, accompanied by diminishing toxicologic symptoms. Uremia and creatinuria disappeared. Urinary protein, ammonia, and lactate and blood CO_2 approached more normal values. The animals gained weight.

After the end of the exposure period, the kidneys approached the normal, and damage became negligible after the fifth week except for some irregularity in tubular arrangement. Likewise, except for a slightly lower blood CO_2 in some of the animals, the biochemical variables were normal. The animals gained weight at the normal rate. A study of renal function indicated that glomerular filtration and tubular excretion had returned completely to normal, as measured by inulin and diodrast clearances, but tubular reabsorption of chloride, as measured by chloride clearance, remained lower than normal even 12 weeks after the end of the exposure period and showed no tendency to return to normal.

It may be concluded that rabbits tend to recover from moderately severe uranium poisoning if they survived the first 3 weeks of exposure. This recovery occurred despite continued exposure and continued at a rapid rate for several weeks after exposure. From the point of view of weight measurements, histological examination of the kidney, and biochemical tests, recovery was almost complete. But from a renal-function point of view, the kidney did not return to its original state even 12 weeks after the end of the exposure. That the recovered kidney was perfectly adequate in function was witnessed by the good health of the animals, though the kidney had a lower tubular chloride reabsorption than normal.

19.12 Summary. 1. Inhalation of 20 mg of sodium diuranate per cubic meter of air proved toxic to all species studied (dog, cat, rabbit, guinea pig, rat, and mouse). There were marked species differences. The rabbit and mouse showed a high mortality (39 and 38 per cent, respectively); the guinea pig showed a low mortality (13 per cent); the dog, cat, and rat showed no mortality.

2. All species at first failed to maintain their normal growth rate. After the third week, however, there was a tendency toward recovery and normal weight gain.

Table 10.111 — Animals Exposed for 35 Days to 20 Mg of $Na_2U_2O_7$/cu m (Summary of Results)

1. Dust Concentration.

Concentration, mg/cu m	Deviation	Samples within given deviation, %
20	± 2.0	42
20	± 4.0	68
20	± 6.0	80
20	± 8.0	90

2. Mortality.

Species	Mortality, %
Rabbit	39
Mouse	38
Guinea pig	13
Dog	0.0
Cat	0.0
Rat	0.0

3. Body Weight. All animals lost weight first 2 to 3 weeks but gained thereafter.
4. Hematology. No changes.
5. Pathology. Renal degeneration and necrosis reached a peak after 2 weeks. Regeneration starts in 1 week and continues during exposure and for 5 weeks after the end of exposure. There is complete recovery of the kidney in the postexposure period, except for a slight irregularity in tubular arrangement. Dogs showed more severe renal damage than rats. Dogs showed glomerular and interstitial changes.
6. Biochemistry.
   Blood. NPN and urea rose to a peak and returned to normal. Lactate decreased slightly. CO_2 decreased and remained low throughout. Dying animals showed highest NPN and lowest CO_2.
   Urine. Protein and NH_3 rose rapidly to a peak and remained abnormal. Creatinine showed no change. Creatinine increased, correlated with blood NPN. Lactate increased, perhaps related to acidosis.
7. Uranium Deposition in Tissues. Uranium appears in lungs of most animals, in most kidneys, in about one-fourth of bones but not in liver.
8. Renal Clearances. In postexposure period, diodast and inulin clearances were normal, but chloride clearance remained high.
9. Conclusions. $Na_2U_2O_7$, 20 mg/cu m, is quite toxic, especially to rabbits and mice. The damage, largely renal, is most severe during the 2nd and 3rd weeks, and thereafter recovery takes place despite continued exposure. Recovery during the postexposure period is almost complete from a histological and functional point of view except for a slight tubular disarrangement and a decreased chloride reabsorption.

3. Ten rats sacrificed after the end of the exposure showed moderate renal tubular changes. Five dogs also sacrificed at this time showed more severe tubular changes as well as an interstitial and a glomerular reaction. Twenty-seven rabbits sacrificed at intervals

throughout exposure showed progressive degeneration and necrosis followed by regeneration of tubular epithelium. By the end of the exposure period there was a definite sign of a return toward normal that was complete by the fifth week after exposure had ceased, except for some irregularities in the arrangement of cortical tubules. Three rabbits that died showed renal damage similar to, but more severe than, that of rabbits sacrificed after the same exposure time.

4. There were no consistent hematological changes.

5. Biochemical studies on rabbits indicate that functional damage was largely renal. There was a marked uremia as measured by the rise in blood nonprotein nitrogen and urea nitrogen to a peak during the second week of exposure with a return toward normal at the end of the exposure period. This was accompanied by an acidosis (measured as reduced blood CO_2) that continued throughout the exposure period and in some animals for several weeks thereafter. The acidosis could not be accounted for by any rise in blood lactate. Acidosis and uremia were most severe in three animals that died during exposure.

6. Urinary protein was markedly increased after 3 days of exposure, reaching a peak during the first 2 weeks with a tendency for a partial return to normal thereafter. Urinary ammonia also showed a marked increase after from 4 to 5 days, reaching a peak after 2 weeks and remaining at this high level for the rest of the exposure period. Urinary creatinine displayed no significant change, but there was a marked rise in creatine during the second exposure week. This creatinuria was related to the uremia, for there was a correlation of .68 between blood NPN and urinary creatine. Urinary lactate also rose during the second week of exposure and was normal thereafter. There seemed to be no significant correlation between urinary lactate excretion and any of the other biochemical variables.

7. Dogs showed a moderate rise in blood nonprotein nitrogen and urea nitrogen.

8. Uranium was found deposited in the lungs and kidneys but not in the livers of exposed dogs, rats, and rabbits. The bones and teeth showed variable amounts.

9. Renal-function tests on rabbits during the postexposure period indicated normal diodrast and inulin clearances, but a high chloride clearance indicated that a decreased renal reabsorption of chloride had persisted for 12 weeks after exposure had ceased.

10. Despite the occurrence of severe renal damage during the exposure, the kidneys showed a remarkable power of regeneration. Several weeks after the end of the exposure period they were almost normal from a histological and functional standpoint.

20. AMMONIUM DIURANATE

By H. P. Dygert*

20.1 Introduction. The assessment of the toxicity of ammonium diuranate by the inhalative route represents a study of one of the minor intermediates in the ramified chemistry of uranium. During processing the salt is handled wet, and human exposures are accordingly of low grade.

The salt, unusual for an inorganic ammonium salt, is highly insoluble in water but is moderately soluble in the plasma. From this fact alone a moderate toxicity might be expected. The compound has, however, a potential source of toxicity aside from that contributed by uranium. A review of the literature indicates that, although there is little question of the individuality of the toxicity of the ammonium ion, the reported effects of its salts are not unmixed. Difficulty is experienced in determining precisely the effects attributable to the ammonium ion itself and those of the acidosis produced by hydrolysis of its salts. In the body, more than one site appears to be involved. The liver is probably the chief site, although the number of red blood cells are markedly reduced, and at high concentrations the involvement of the nervous system also occurs.

It is not unreasonable, therefore, that similar responses to greater or lesser degree may form part of the toxicity picture resulting from exposure to the ammonium diuranate dust. Although the liver is not the primary organ affected by uranium, it is believed to be involved at least secondarily. A greater effect on this organ may possibly be anticipated with this dust than with other uranium dusts studied. Because of the possible acidosis contributed by the ammonium salt, it might also not be surprising that enhanced acidotic response and its concomitant effects would result from exposures to this salt. For similar reasons, more importance is placed on the hematologic findings in this study than in other uranium-dust studies.

20.2 Exposure Conditions. Exposures were performed at a level approximating 20 mg/cu m for a period of 30 exposure days (34 calendar days or 152 exposure hours). One hundred animals, comprising 10 rabbits, 30 rats, 20 guinea pigs, and 40 mice, were exposed. Daily exposures were conducted in a full-exposure unit of 64 cu ft capacity, the construction of which was similar to that described in Sec. 6.1e. The dust was introduced into the chamber by means of a gear feed. Dust fell from a reservoir upon a rotating ridged drum that allowed

* Toxicologic procedures performed by H. P. Dygert and H. A. Oberg.

controlled amounts of dust to fall through a port in the center of the exposure unit, whence it was immediately distributed throughout the unit by the air turbulence created by a fan.

The control of the dust concentration with this type of feed device was only moderately satisfactory. Although the weighted mean dust concentration of 17.9 mg/cu m (12.2 mg of uranium/cu m) approximated the desired 20 mg/cu m of dust; the standard deviation was 9.7 (Table 10.112). Somewhat more than half of the dust-concentration samples varied from the weighted mean by ±8 mg/cu m, or by more than ±40 per cent.

The particle-size distribution of the dust circulating within the chamber possessed a mean of 0.5μ, with a standard deviation of 1.85 as determined by the microprojector method following collection in the Thermal Precipitator (Sec. 8.2).

Table 10.112 — Ammonium Diuranate Dust Concentration

Concentration level of compound, mg/cu m	Deviation, mg/cu m	Samples within given deviation %
17.9	±2	15
17.9	±4	26
17.9	±6	39
17.9	±8	52
17.9	±10	69

Note: desired concentration, 20 mg of dust per cubic meter; standard deviation, ±9.7 mg/cu m; number of samples, 197.

Because the analytical value of uranium in the commercially obtained ammonium salt was sufficiently below the theoretical to make further investigation of the composition of the salt desirable, an analysis was made of the salt for the presence of other constituents that might seriously affect the toxicologic response. Table 10.113 shows the results of such analyses. It was concluded, on the basis of the above analyses, that the commercial sample contained 86 per cent of the theoretical amount of uranium and no appreciable quantities of soluble ammonium salts as impurities.

20.3 Signs of Toxicity. Five of 10 rabbits showed mild but persistent nasal bleeding shortly before death. Many of the rats showed similar signs of nasal irritation after the eleventh exposure day. No other signs of toxicity were manifest in any of the exposed animals prior to death.

Table 10.113—Analysis of Ammonium Salt

	Data supplied, %*	Found, %†	Theoretical, %
U	61.65	59.6	68.5
NH_3	3.6	3.9	4.5
H_2O	3.8		10.3
Na	2.8		
SO_4	12.8		

* From an industrial source.
† Results submitted by the analytical group of this section.

20.4 Mortality. The ammonium salt was uniformly lethal for the rabbit, and it was commonly lethal for the mouse, but it produced no mortality in the rat (Table 10.114). The 20 per cent mortality in the guinea pig is believed to be attributable in part at least to an intercurrent infection in the group. The time at which deaths occurred varied with the species. All the rabbits had succumbed by the tenth calendar day of exposure; certain of the deaths in mice occurred as late as the thirty-sixth day.

Table 10.114—Mortality of Animals Exposed 6 Hr Daily for 30 Days to $(NH_4)_2U_2O_7.4H_2O$ Dust at 17.9 Mg/cu m

	No. of deaths per no. exposed	Mortality, %
Rabbit*	10/10	100
Mouse	21/40	53
Guinea pig†	4/20	20
Rat	0/30	0

* One animal was killed when moribund.
† These animals died from causes other than uranium poisoning.

20.5 Weight Response. Of the three species for which weight-response data were recorded, the rat was the only species that showed an over-all weight gain at the end of exposure (Table 10.115). But even in this species a loss in weight was observed following the first week of exposure. After the second week of exposure the rate in the gain of weight was the same as that prior to exposure. A similar type of weight response was noted in the surviving guinea pigs during the first three weeks of exposure (Fig. 10.50). Subsequently an over-all

weight loss was noted that was apparently occasioned by an infection. All the rabbits died before significant weight change occurred.

Table 10.115 — Body-weight change in Animals Exposed to Ammonium Diuranate

Animal	Body-weight change, %
Guinea pig:*	
Died	−10
Surviving	−3
Rabbit †	0
Rat ‡	+13

* Intercurrent infection in this group.
† All died.
‡ None died.

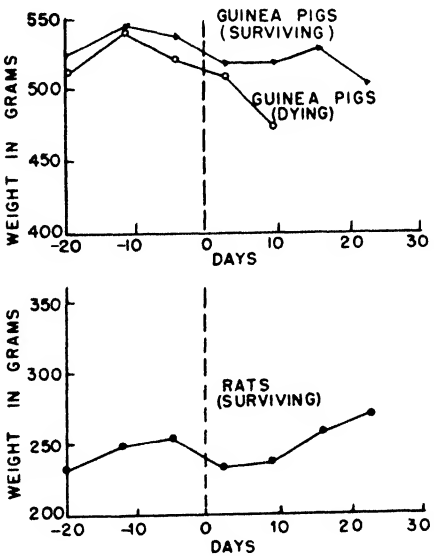


Fig. 10.50 — Change in body weight from preexposure values of guinea pigs and rats exposed to $(NH_4)_2U_2O_7$ dust.

20.6 Pathology. Tissue injury in the rabbits, all of which died, was strikingly uniform in nature and was characterized by rather severe necrosis of the tubular epithelium of the nephrons and by extensive pulmonary irritation including edema, hemorrhage, and necrosis.

As a result of a serial histologic study, in which one control and two experimental rats were sacrificed at frequent intervals throughout each week of exposure, renal damage, characterized by necrosis of the tubular epithelium, was first noted on the third exposure day. Maximal damage was attained on the sixth day, after which time reparative processes became increasingly evident, and by the twenty-eighth day the kidneys appeared more nearly normal than at any time after the initial damage. An incidental bronchopneumonia of the interstitial type was observed in 25 per cent of the animals examined in this group (Table 10.116). This is a rather frequent occurrence in control guinea pigs (see Table 10.31 in control studies, Sec. 9).

Table 10.116 — Pathology of Animals Exposed to Ammonium Diuranate

Organ	Rabbit	Rat
Kidney	Severe typical damage of corticomedullary tubular epithelium	Progressive damage described as most severe on the 6th day; from this day on, regenerative tissue became progressively more important, and by the 28th day kidneys appeared almost normal
Lung	Considerable evidence of irritation, including edema, hemorrhage, and necrosis	Twenty-five per cent exhibited interstitial bronchopneumonia

20.7 Hematology. The hematologic study of both rabbits and rats showed changes unusual for uranium-dust exposures. Both species showed similar changes in a number of the constituents measured. Each species showed a rise in the neutrophils and in the lymphocytes. Consistent with the most severe toxicity noted in the rabbits was the greater change in the cellular constituents of this species than in the rats. The rats also exhibited a fall of 1 million in the red blood count and loss of 4 g of hemoglobin per 100 ml of blood, a moderate fall in the white blood count, and a rise in the eosinophils. These additional changes noted in the rats, but not noted in the rabbits, undoubtedly resulted from the fact that the rabbits died before such changes could become manifest (Table 10.117).

20.8 Uranium Content of Tissues. Uranium, determined by the spectrochemical method at the termination of exposure, was found distributed in all the six tissues examined. Uranium was most often found in the kidney and lung of the guinea pig, rabbit, and rat, less frequently in the osseous tissues, and least frequently in the liver

(Table 10.118). Presented also in Table 10.118 are the results on rats grouped according to increasing lengths of exposure to ammonium salt. The results show, in general, a progressive increase in the frequency of uranium in the tissues analyzed upon continued exposure of the animals.

Table 10.117 — Hematology

Blood constituent	Rabbit (2 counts)	Rat (7 counts)
Neutrophils, %	Rise, 11	Slight rise
Lymphocytes, %	Fall, 8	Slight fall
Neutrophils, absolute	Slight rise	Rise, then normal
Lymphocytes, absolute	Slight fall	Fall
RBC		Fall, 1 million
Hemoglobin		Fall, 4 g/100 ml
WBC		Moderate fall
Eosinophils, %		Rise

Table 10.118 — Frequency Distribution of Uranium in Tissues of Animals Exposed to Ammonium Diuranate

Species	Kidney		Lung		Liver		Femoral epiphysis		Pelvis		Spinal column	
	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%	No. with U per no. exposed	%
Rat*	2/12	17	1/11	9	2/14	14	2/14	14	1/13	8	3/13	23
Rat†	7/20	35	8/17	47	3/19	16	3/13	23	5/19	26	5/19	26
Rat	13/30	43	27/29	93	6/29	21	13/25	52	14/24	58	15/25	60
Guinea pig	10/14	72	13/14	93	6/15	40	7/13	54	8/14	57	8/13	61
Rabbit	10/10	100	6/9	67	1/9	11	2/10	20	3/9	33	2/9	22

* Rats sacrificed before the 10th day of exposure.
† Rats sacrificed after the 10th day of exposure.

20.9 Discussion. The toxic response of animals following exposures to ammonium diuranate dust represents another instance of parallelism between solubility of uranium salt in plasma and toxic response. The observed toxicity could not have been predicted upon the basis of water solubility, as this is negligible, whereas in serum it is moderate. This situation is comparable to that found in the study

of uranium peroxide (Sec. 18). Similar solubilities of these two salts and similar mortality rates are noted among the species exposed to each of these two compounds in tests carried out at the same level of exposure.

Consistent with the indications in the literature on the effects of the ammonium ion, considerable change was noted in the cellular constituents of the blood. The reported decrease in erythrocytes was observed in the rats exposed to the ammonium salt during the fourth week. On the other hand, no pathology was found in the liver of the exposed animals, although this might have been anticipated from literature reports.

20.10 Summary. Ammonium diuranate dust inhaled by laboratory animals at atmospheric levels approximating 18 mg/cu m produced the following responses:

1. The over-all mortality of rabbits, mice, guinea pigs, and rats was 31 per cent. The compound was invariably fatal for the rabbit, commonly fatal for the mouse, questionably fatal for the guinea pig, but nonfatal for the rat.

2. Consistent with mortality results, the rat was the only species that showed a weight gain at the end of exposure, but even this species sustained a moderate weight loss during exposure. Similar responses were noted in the guinea pig.

3. Pathology was marked by uniformly severe necrosis of the renal tubular epithelium and by extensive pulmonary irritation in the rabbit. A serial histologic study in the rat revealed renal damage by the 3rd day and maximal damage by the 6th, followed by reparative processes that resulted in the kidney being more nearly normal by the 28th day than at any time after the initial injury. It should be noted that these findings were made on surviving rats; the preceding findings were made on dying rabbits.

4. Definite and consistent changes were observed in the cytologic constituents of the blood of the rabbit and the rat. More elements were involved in the rat, the surviving species, and included a fall in erythrocytes and white blood cells and a rise in the eosinophils, in addition to changes in the neutrophils and lymphocytes noted in both rabbits and rats.

5. A serial spectrochemical analysis of the tissues showed uranium with constantly increasing frequency in the tissues of rats upon continued exposure. Uranium was distributed in all of six tissues analyzed, which included the lung, kidney, liver, and three bony structures, but was found most frequently in the lung and kidney. A similar finding was made in the tissues of the guinea pig and rabbit.

21. URANIUM TRITAOCTOXIDE

By H. Paul Dygert*

21.1 Introduction. The information on animal inhalation toxicity of U_3O_8 was derived from a single 1-month study. This study represented a beginning of the entire series of inhalation studies and was undertaken before refined methods of preparation of uranium-dust atmospheres had been attained and before all departments had been fully organized. Being the first study of its kind, it was less extensive than the others, and its data were approximate.

This oxide possessed the following properties of toxicological interest: Its extreme stability under ordinary atmospheric conditions makes the dust-feed problem a relatively simple one. On the other hand, its high density of nearly 8 considerably complicates the problem of maintaining a uniform dust concentration within the exposure unit. Its very low solubility in water and in body fluids makes for a predictably low toxicity.

The toxicity was assayed from a consideration of weight response, mortality, and histologic change. Some aid in the interpretation of the toxic response was also gained from a limited amount of blood chemical and hematologic work. In an effort to interpret the effects of exposure, spectrochemical analyses for the uranium content of certain critical tissues of the exposed animals were made. Comparisons have been drawn between solubility of the oxide in body fluids and its toxicity and between uranium concentration in the tissue and histologic damage.

21.2 Materials and Methods. The chamber in which the animals were exposed to the dust was a small unit of 64 cu ft capacity of the full-exposure type described in Sec. 6.1e. The dust was introduced into the unit in intermittent jets from a dust elutriator operated by hand. The mean dust concentration for the exposure period was 17 mg/cu m of air (14.5 mg U/cu m) with an extreme range of from 3.3 to 57 mg/cu m (2.8 to 49 mg U/cu m). Only two high values were obtained throughout the exposure period, and these occurred on the twelfth day. All other values were far closer to the mean. The dust concentration in these experiments was determined from one to three times daily by means of either the electrostatic precipitator or the Greenburg-Smith impinger. In the latter, water was used as a collecting medium. Particle size of the dust determined by the microprojector method was 2.0μ with a standard geometric mean of 3.2. The

* Work done by H. E. Stokinger and H. P. Dygert.

range of particle counts within the exposure unit was from 10 million to 103 million particles per cubic foot of air.

Exposures were continued for a period of 26 days from 4 to 6 hr daily on 16 guinea pigs, 9 rats, and 12 mice; 8 rabbits were exposed for 9 days only because of the occurrence of an infection in the group.

21.3 Signs of Toxicity. No animals of the four species exhibited any certain signs of toxicity either during exposure or subsequent to it up to the time of sacrifice, which in the case of certain animal groups constituted a period of 95 days. A possible exception was a lessened growth rate in the rats noted below. A loss in weight observed in both the guinea pig and the rabbit shortly after the start of exposure was thought to be unrelated to the exposure.

21.4 Weight Response. The rabbit and mouse showed an over-all gain in weight at the end of the experimental 26-day period of 31 and 27 per cent, respectively, whereas weight gains in the guinea pig and rat were subnormal (approximately 4 per cent). The weight response was apparently complicated by a latent infection in the guinea pigs, for a loss in weight occurred in the controls similar to that of the exposed group. In the rabbits, likewise, interpretetion of the weight curves was made difficult by an infection in certain members of the group. In the case of the rabbit, five of the eight animals died from an infection of undetermined cause, and the three survivors showed a continuous weight gain after a loss sustained during exposure. The extent of the effect of the infection on weight is unknown. The mouse also showed a continuous increase in weight that was greater than that of the controls.

21.5 Mortality. Although eight deaths occurred in the 45 animals exposed, it was felt that infection was a contributing factor in the observed mortality. Of these eight, one guinea pig died during, and two after, the exposure period. Five rabbits died, either during or after exposure, definitely as a result of a latent infection. The causative organism of neither of these infections was isolated.

21.6 Blood Chemistry. Occasional analyses of blood for nonprotein nitrogen showed slightly elevated values in 2 of 10 samples taken at random among the guinea pigs, but the elevated values appeared only in those samples taken 4 weeks after the termination of exposure and may be presumed to be unrelated to exposure. Five samples of rat blood taken at this time showed no elevated nonprotein nitrogen values. No significant change was observed in blood sugar values of a few samples taken from guinea pigs and rats 4 weeks after termination of exposure.

21.7 Histology. Sections of kidney, lung, liver, spleen, and heart of 31 animals were examined without controls. Three showed evidences of renal injury as indicated by a moderate degree of regenerative tubular changes. These findings were confined to two guinea pigs and one rat.

Pulmonary changes in a small number of animals were considered to be incidental findings. The renal changes were those usually observed during the stage of epithelial regeneration that follows injury by uranium compounds. The pulmonary changes are probably not significant, although they pointed toward some pulmonary irritation.

21.8 Hematology. No conclusive findings were observed from a hematologic study of the four species. Wide variations in the blood picture were in evidence, but these were not appreciably different from those of control animals.

21.9 Uranium Content of Tissues. Eighty spectrochemical analyses were made for uranium content of the lung, kidney, and liver of the four species sacrificed at the end of the 26 days of exposure and at intervals thereafter up to periods of 3 months. These analyses showed highest concentration in the lung, considerably less in the kidney, and, with but two exceptions, less than the minimal detectable amount in the liver. The liver of animals sacrificed four weeks after the termination of exposure showed no detectable amounts of uranium with the exception of two samples from the guinea pig. No essential difference in the uranium concentration was noted between the lungs of animals killed 28 days after the termination of exposure and those analyzed at the end of exposure. Only small amounts of uranium were observed in the tissues of rabbits killed 3 months after cessation of the 9-day exposure. Although uranium was undetectable in 8 of 10 tissue specimens analyzed, it is of interest to note that the lungs of two rabbits killed at this time still showed measurable amounts of uranium. The ribs of one of these animals also contained small amounts of uranium, but none was found in the ribs of the other rabbit thus analyzed.

21.10 Discussion and Summary. The compound U_3O_8, when inhaled as a dust in daily repeated exposures at concentrations approximating 17 mg/cu m, and with a median particle size of 2.0μ, showed only occasional indications of mild toxic reaction in the guinea pig, rat, and mouse in a period of 26 days. In rabbits similarly exposed for 9 days, no toxic symptoms were observed, although retention of uranium in the lung and in the bone was shown for a period as long as 3 months after the cessation of exposure. These observations of extremely mild toxicity are based on the absence of definite mortality

attributable to uranium poisoning, occasional findings of mild renal tubular injury and of pulmonary irritation, and decidedly questionable elevation of nonprotein nitrogen of the blood in isolated instances. This paucity of definitely toxic manifestations was found despite uniformly high concentrations of uranium in the lungs of all the animals and considerable quantities in the kidney as long as 1 month after the end of exposure.

The low order of toxicity of this oxide is directly predictable from the in vitro findings of its extremely low solubility in body fluids. Other more soluble uranium compounds exhibited far more marked toxicity under similar conditions. With this dust, however, the greater portion of the material was retained in the lung, whereas soluble dusts rapidly disappear from this site. The concentration of the octoxide in the lung was 100 times that in the kidney; soluble uranium dusts are concentrated in the kidney, the organ primarily damaged by uranium. The extreme insolubility of this oxide in the lung was further evidenced by an almost imperceptible decrease in concentration during a period of at least 30 days after the exposure had ceased.

PART D. TESTS OF RESPIRATORY PROTECTIVE DEVICES IN ATMOSPHERES CONTAINING URANIUM DUST

By H. Paul Dygert*

The purpose of the tests was to determine from among the protective devices currently available those acceptable for use in atmospheres containing uranium dust. Because the toxicity by inhalation of uranium compounds was unknown at the initiation of these tests, the concentration of dust, as uranium, permitted to penetrate the filtering device was arbitrarily set at 150 μg/cu m of air. This was the same concentration as the interim choice for the permissible exposure of factory personnel.

22. TEST PROCEDURES

22.1 Test Chamber. The chamber in which the tests were made was a 4-ft cube lined with transite and set with two lateral glass windows in opposite walls. One side was hinged and served as a door. Uranium dust was fed into the chamber (diagrammatically represented in Fig. 10.51) by means of a dust feed at A, through the duct B into

*Work done by H. E. Stokinger, H. P. Dygert, H. Oberg, and R. Sanford.

an opening C in the center of the roof of the unit. Here a fan D circulated the dust uniformly through the chamber. A Rotoclone removed the test atmosphere from the chamber at a constant rate of 180 cfm through the outlet E in the rear wall. The test atmosphere was sampled for concentration and particle size at F. The respiratory device G, mounted on the glass electrostatic precipitator H or, as was later used, a filter-paper mask sampler[58] was inserted into the chamber. The test atmosphere was drawn through the respirator filter and thence through the dust-collecting unit at the rate of 32 liters/min, which was approximately double the resting inspiratory rate of man. The amount of dust that penetrated the filter and was retained by the collecting unit was analyzed, and the amount of penetration calculated. Only those protective devices allowing no more than the penetration limit indicated above were designated as acceptable.

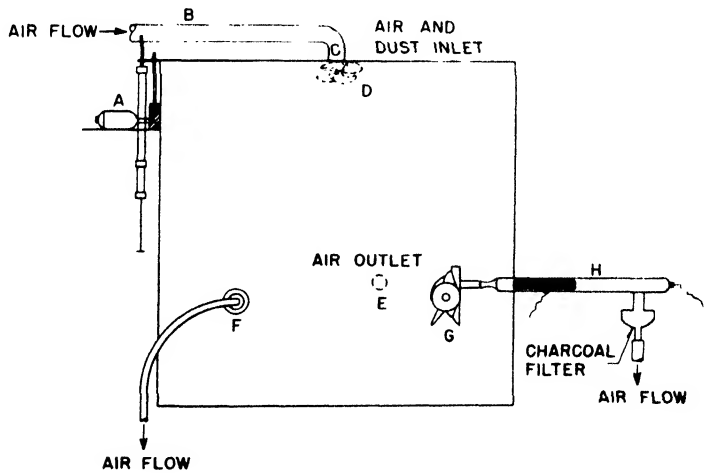


Fig. 10.51—Dust chamber for tests of respiratory protective devices (schematic). A, dust feed; B, air-dust inlet duct; C, inlet port; D, fan; E, air outlet; F, dust-sampling port; G, respirator and mount; H, electrostatic precipitator.

22.2 Preparation of Dusty Atmospheres. The dust feeds employed were selected according to the individual requirements of the dust as indicated in Sec. 6.2.

During respirator tests animal experiments were also performed in the same chamber. This necessitated a constant air change, which was maintained by the Rotoclone. The temperature of the air in the chamber ranged from 70 to 90°F; relative humidity ranged from 40 to 75 per cent; air turnover in the chamber was 180 cfm.

Respiratory protective devices were tested in atmospheres containing 10 of the dusts listed in Chap. 1; no tests were made of uranium trioxide and sodium diuranate dusts.

The preparation of the dust, determination of particle size, and dust concentration were those described under Sec. 8.2, except that the thermal precipitator was used in sampling the test atmosphere for particle size.

22.3 Tests of Respirator Efficiency. Protection against the industrially used uranium dusts required the testing of two types of protective devices: (1) respirators and (2) gas masks. In the first type, the efficiency of the whole facepiece as well as the filters was tested in order to detect possible flaws of design and workmanship. In the second type, canisters only were tested. Canisters are required in work with certain of the uranium dusts because respirator filters do not afford protection against the toxic gaseous products of hydrolysis liberated on contact with the moisture in ordinary atmosphere (HCl and HF from UCl_4 and UF_6, respectively). The selection of the devices was made on the basis of high efficiency in tests made with similar substances by the U. S. Bureau of Mines,[18] or because comfort and fit indicated suitability for industrial use. The latter factor is important because workers are negligent in the use of an uncomfortable respirator irrespective of its efficiency.

The method of testing, on which project Dr. H. H. Schrenk served as consultant, was essentially that used by the U. S. Bureau of Mines[7] for the approval of respiratory protective devices. Preparatory to testing, the facepiece of each respirator was affixed to a metal shield with Duxseal (a nonhardening plastic obtained from Johns-Manville Corporation) in such a manner as to assure an airtight mount, and the shield and respirator were connected to the end of the dust-collecting unit (G in Fig. 10.51).

During the test the atmosphere containing uranium dust was drawn through the apparatus at a rate of 32 liters/min, and the dust penetrating the filter was retained by the dust-collecting unit. The collected dust was analyzed both gravimetrically and colorimetrically by the ferrocyanide method, and the amount in milligrams of uranium element penetrating the filter per cubic meter of air sampled was calculated.

The initial and final resistance of the respirator was determined at an air flow of 85 liters/min. The average initial resistance of respirator filters approximated 35 mm of water. Each set of filters was tested until the resistance reached a value of 50 mm of water. Beyond this resistance it is difficult for man to breathe easily while working.

As work progressed it was found that maximal penetration of the non-hydrolyzing compounds occurred during the first 6 hr of the test, and, therefore, even though the resistance had not reached 50 mm of water, the test was considered completed. The hydrolyzing compounds either wetted the filters, resulting in a decreased filtering efficiency, or elements instrumental in removing gaseous hydrolysis products became saturated and efficiency proportionally decreased.

The initial resistance of gas-mask canisters was approximately 85 mm of water when tested at the rate of 85 liters/min. This exceeded the 50-mm limit mentioned above, but because this type of protective device is intended for use for only short periods of time the high resistance was not considered critical. Canisters were run until a resistance of 100 mm of water was reached.

In most instances respirators were tested in triplicate, whereas only one canister was tested against each compound because the attainment of the 100-mm resistance for canisters required long periods of testing. Approximately 60 respirator and 12 canister tests were made, or a total of 180 respirator and 12 canister tests were performed. The same respiratory protective devices were not tested for acceptability against each compound because variation in physical and chemical characteristics of the substances, such as hygroscopicity, products of hydrolysis, and particle size and form, made this inadvisable. Test atmospheres approximating 20 mg of dust per cubic meter were produced. The average concentration of dust, as uranium, penetrating the filter was compared to the permissible limit of penetration.

In addition to rating the respirators with respect to their filtering efficiency, an attempt was made to evaluate the relative comfort of each device by making comparative tests of fit and ease in wearing.

22.4 Results. The results of the total number of tests of respirator efficiency are given in Table 10.119 and show the ratings of each respirator according to comfort and acceptability for each of the 10 dusts tested. The results may be considered either from the standpoint of the type of respirators or from the standpoint of dust against which they were tested, depending upon the interest.

According to type of respirators, the Mine Safety Appliances Respirator BM-2133 showed the greatest efficiency in all tests. On the other hand, Dustfoe Respirator BM-2148 had only slightly less efficiency but had a higher comfort rating. Pulmosan Respirator BM-2112, although possessing a relatively high efficiency, had such a low comfort rating that it was tested in only a few instances. Respirators BM-2128 and 2130 showed variable efficiencies on testing with the

Table 10.119 — Tests of Acceptability of Respiratory Protective Devices for Dusts of 10 Uranium Compounds

		Respirators															Gas-mask canisters						
		Pulmosan	Dupor	Willson 750 L	Willson Fume	M.S.A. Dust and Mist	M.S.A. All Dust	Am. Op. R-9100	Am. Op. R-1000 T	M.S.A. Comfo Metal Fume	CESCO Healthguard	M.S.A. Dustfoe	Willson Dust and Fume	C.W.S. Aerosol	Navy N.C. Mask	M.S.A. Chemical Cartridge	M.S.A. All Service	Willson Universal	M.S.A. All Service	Acme All Purpose	For acid gases, smoke, dust; M.S.A. GMB-1	For acid gases, organic vapors, smoke, dust; M.S.A. GMC-1	For acid gases, ammonia, organic vapors, smoke, dust; M.S.A. GME-1
U.S. Bur. of Mines approval No.		2112	2124	2125	2128	2130	2133	2137	2138	2139	2142	2148	2149	None	None	None	1405	1433	1434	1435	None	None	None
Comfort rating*		4	2	3	4	2	2	1	1	2	2	1	3	4	4	3	4	4	4	4	4	4	4
Permissible limit of penetration, mg U/cu m	Dust																						
0.10	UF_6 UO_2F_2 UCl_4				Yes No	No	Yes No			No		No	No	Yes No		No	No	Yes No	Yes		No	Yes	
0.15	$UO_2(NO_3)_2$ $(NH_4)_2U_2O_7$ UO_4 High-grade ore	Yes Yes	Yes	Yes Yes Yes	No Yes Yes	Yes	Yes Yes Yes Yes	Yes	Yes Yes No	Yes Yes Yes Yes	Yes Yes Yes Yes	Yes Yes Yes Yes	Yes Yes Yes	Yes Yes	Yes								
0.50	UF_4 UO_3 U_3O_8		Yes		No Yes	Yes Yes Yes	Yes Yes Yes	No	No	Yes Yes	Yes	Yes Yes Yes	No Yes	Yes									No

*Comfort ratings are designated 1, 2, 3, or 4; 1 is the most comfortable, 4 the least.

different compounds. Inspection of the acceptability ratings of canisters shows a number of instances in which a device was not acceptable. These were among a group that either were not approved by the U. S. Bureau of Mines or were designated of lower efficiency than the canisters BM-1433, 1434, and 1435 and the Chemical Warfare Service aerosol mask. No canister or respirator, even among the best available, was acceptable for use in atmospheres of UCl_4. The hydrolysis products of this compound attacked the paper filtering medium and greatly decreased its effectiveness. It must be pointed out, however, that, although the most effective canisters were generally superior to respirator filters in filtering dusts from the air, their comfort rating was low because, in every case, use of such a device necessitated the wearing of a gas mask.

From the point of view of the compounds against which the devices were tested, UF_6, UO_2F_2, and UCl_4 were the most difficult to remove from the atmosphere. As stated above, no protective device tested, either respirator or canister, effectively removed UCl_4 and its products from the inspired air. No respirator and only one canister, all-service Model S, BM-1434 of Mine Safety Appliances, effectively removed UO_2F_2 from the atmosphere, and one respirator and three canisters were designated acceptable for use in atmospheres of UF_6. It must be noted, however, that the demands placed on the filtering devices by these three compounds were particularly exacting because of the extremely low limit for allowable penetration. Those respirators tested against atmospheres containing dusts of moderate toxicity, namely, ammonium diuranate, the peroxide, high-grade ore, or materials having large particle size such as uranyl nitrate, were in most instances acceptable. The greater number of devices found acceptable for use in atmospheres containing these four compounds, as compared to the previous group of three substances, UF_6, UO_2F_2, and UCl_4, is understandable when it is realized that each was either a dry amorphous dust or a dust of large particle size or peculiar particle form, all of which enabled the respirator filter to remove the particulate matter more easily. The slightly higher penetration limit set for these compounds was another factor contributing to the greater effectiveness of filtering media in the devices tested. The third group of compounds, including UO_2, U_3O_8, and UF_4, although having lower toxicity reflected by the highest permissible penetration limit of 0.5 mg/cu m, was somewhat difficult to filter from the test atmosphere because a considerable portion of each of these was of relatively small particle size and showed little tendency to aggregate.

The respiratory protective devices are produced by the following companies: Pulmosan, Pulmosan Safety Equipment Corp.; Dupor,

H. S. Cover; Willson, Willson Products, Inc.; M.S.A., Mine Safety Appliances Co.; Am. Op., American Optical Co.; CESCO, Chicago Eyeshield Co.

PART E. DISCUSSION AND INTERPRETATION OF RESULTS

The investigation of the toxicology of uranium, a venture dictated by the wartime emergency, has resulted in an exhaustive and comprehensive survey of the subject. The project had as its ultimate goal the safeguarding of workers engaged in the production of uranium from the dangers of acute or recurrent exposures. Therefore, the project's chief purpose was to determine the toxicity following inhalation of all uranium dusts used in industry on any significant scale.

Because the methods of inhalation require considerable time to obtain even approximate information, resort was made to other routes of exposure to supply much-needed toxicity data. To this end the ingestion studies (Chap. 7) were of great value in supplying rapidly the approximate order of toxicity of the various materials and later permitting an estimate of the relative amount of toxicity contributed by the ingestion. Likewise, toxicity following application of these uranium materials to the skin (Chap. 8) and to the eye (Chap. 9), although of a more qualitative nature, served to fix the limitations of the inhalation work. The determination of toxicity following parenteral administration of the uranium compounds (Chap. 6), in which the dosage was accurately known, furnished an absolute relation between dosage and toxicity from which the approximate exposure by inhalation might be estimated. The mechanism studies (Chap. 14) have aided in the design and the interpretation of the inhalation studies.

The discussion here is concerned only with the results of short-term studies and centers about three general subjects: (1) engineering know-how in the construction and design of inhalation-exposure equipment, (2) dust inhalation, and (3) the toxicology of uranium.

23. CONTRIBUTIONS OF ENGINEERING TO INHALATION-EXPOSURE EQUIPMENT

Engineering development in the construction of animal exposure units for the large-scale study of uranium dusts has favored the choice of cubic units of at least 250 cu ft capacity of wooden construction with plywood lining and transite floor. Windows comprised more than half of the wall space to permit full observation of the animals, and a horizontally split door, the lower portion of which served as a ramp to allow convenient transport of cages, comprised the major part of one side.

Although the head-exposure chamber (Sec. 6.1f) was originally designed as an experimental unit to minimize the amount of uranium material ingested by the animals, numerous tests of the value of the head-exposure unit in this regard have failed to produce striking evidence of its advantage in uranium-dust exposures. Accordingly, in view of its more complex and expensive construction, the work entailed in loading the unit, and the relatively small contributions to inhalation toxicity by the oral, cutaneous, and ocular routes, it has been concluded that the head-exposure unit offers insufficient advantages to advocate its use in uranium-dust exposures.

The highly corrosive action of a number of the uranium compounds to materials usually employed in the construction of exposure units necessitated special resistant linings or coatings of the interior of the units, especially at the higher concentrations. Special lining of the chambers with either lead or copper sheeting to fit the individual requirements was found suitable when concentrations were maintained at a high level. For all ordinary use, however, corrosion-resistant paints were found highly satisfactory. Such paints can be applied as frequently as needed and leave a continuous, smooth hard surface to which dust does not adhere, thereby maintaining a spotless interior.

The exposure of animals in cages with individual compartments goes far to ensure uniform dust intake by eliminating animal crowding. Cage units, either on casters or small trucks, speed considerably the transfer of animals to and from the unit.

The problem of delivering controlled concentrations of uranium dusts of from 20 to 0.1 mg/cu m was solved by the development of three types of mechanical feeds. Differences in physical state, hygroscopicity, particle size, and density of the uranium materials necessitated basically different systems. All the dusts, except the nitrate and the tetrachloride, are relatively nonhygroscopic and were handled conveniently by the pressure type of feed (Sec. 6.2a). The tetrachloride and nitrate, hygroscopic materials, were handled respectively by a rotating ball-mill feed (Sec. 6.2b) and an agitated elutriator (Sec. 6.2c).

The delivery of metered amounts of gaseous uranium hexafluoride was accomplished by a nitrogen blending unit (Sec. 6.2f).

An estimate of the precision of which these instruments were capable is given by the fact that, at the lowest levels tested, less than 1 mg was in circulation in a 250 cu ft unit at any one instant. As the dusts have a density of from 2.8 to nearly 11, such a value constitutes an extremely minute mass of material for accurate control and distribution.

Experience has shown that, where highly dense particulate matter is concerned, the use of inlet lines for conduction of the dusts into

exposure units is unsatisfactory; introduction of the dust from the feed directly into the interior of the chamber is the most practical method. For the regulation of air circulation and the control of temperature and humidity, air-conditioning units are advocated. By this means the temperature during exposure is conveniently maintained at approximately 72°F. Temperatures should never be allowed to exceed 80°F because of the known increase in toxicity with rise in temperature,[59] as has been demonstrated for arsenic. For air turnover in the unit, a Rotoclone has proved highly satsifactory for continuous use in providing approximately one to one-half air exchanges per minute. In addition to this, propeller-type fans were required in strategically located sites throughout the unit in order to provide generalized turbulence to ensure a reasonably uniform distribution of the heavy uranium dusts. Fans in each corner directed to the center of the unit, or a single fan directly under the central air-dust inlet, proved to be the most satsifactory arrangement.

The simplest and most efficient means for estimating the concentration of circulating uranium dusts during exposure was the modification of the filter-paper sampler of Fairhall.[13] This method was especially advantageous for those dusts that were nonhygroscopic. A simple weighing, directly following the taking of the sample, allowed immediate control of the chamber-dust concentration. For these dusts the correlation between the gravimetric and colorimetric analyses was sufficiently satisfactory so that, at levels of 3 mg and above, colorimetric analysis was only infrequently required as a check. The ferrocyanide method, as modified here from the original method of Benard and Tessier, proved eminently satisfactory for routine analyses of uranium dust-air mixtures.

24. DUST INHALATION

24.1 Relation of Dust Characteristics to Toxicity. The toxic effects resulting from inhalation of uranium dusts are a function of the physical characteristics of the dusts as well as of the chemical nature or uranium. The particle size, density, and solubility are most important because they affect the amount of inhaled uranium that is retained in the respiratory tract and the rate at which uranium is transported from the respiratory tract to the kidneys, where it has its primary toxic action.

Some of the inhaled dust is deposited on the nasal mucosa, some on the walls of the trachea, bronchi, and bronchioles, and some in the alveoli; however, a certain fraction is not retained at all and is present in the exhaled air. The relative quantities of material retained in

the various parts of the respiratory tract are largely a function of the particle size and particle density, although other factors may also be important. Of the retained dust, only that which can be dissolved into the circulation will reach the kidney and result in typical uranium toxicity. In the case of soluble materials, the particles deposited either on the nasal mucosa or in the alveoli may be absorbed. In the case of the insoluble dusts, probably the smaller particles, which reach the alveoli, are important.

Particles of insoluble dust smaller than 1 μ were found to be inherently more toxic than particles of larger size. This finding has been clearly demonstrated in rats in which uranium dioxide of carefully graded particle size, from 10 to 0.2 μ, had been intratracheally injected. Weight losses, elevation of blood nonprotein nitrogen, and retention of phenolsulfonphthalein dye were all markedly increased at the lowest particle-size ranges. The differences in response appeared to be associated with the movement of the material in the body after its introduction into the lung. Although larger particles were removed from the lung more rapidly, smaller particles were responsible for greater deposition of uranium in the kidney and femur (Sec. 8.3).

A development of practical value incidental to the foregoing work was the method of preparation of graded particle sizes in quantity. The method was a modification of that of Cummings[56] and consisted of suspending and settling of dusts in a process of recyclization in 21 operations. By this process the size of successive fractions did not overlap by more than 10 per cent.

24.2 Measurement of Particle Size. Because of the importance of particle size to inhalation toxicity, considerable effort has been expended in establishment of standard methods for sampling and measuring uranium-dust particles for the purpose of its ultimate adoption by industry for routine use as well as for experimental work. This has involved (1) development of a standard dust-sampling apparatus, (2) extension of the practical limits of the light microscope, and (3) use of the electron microscope.

1. The British Cascade Impactor, selected as the instrument of greatest promise for sampling dusts for size determination, was redesigned for standardized production, and a fifth stage was added for the collection of finest size particles in order to suit the range encountered industrially. Extremely fine machining was dictated by the requirement of curtailing losses within the instrument so that, in the model used, losses were reduced to less than 10 per cent. Fairly good separation of particle sizes on each stage was obtained with the new design, and, following calibration of the instrument, a simple

chemical analysis of each stage constitutes all that is required to determine quantitatively the amount of dust within a definite size range in the air sample. With the aid of a portable vacuum pump, this instrument allowed samples to be taken in the field and the chemical analysis made later. The instrument was given thorough testing with four uranium dusts during operation of the animal-exposure units.

2. Past work on measurement of small particle sizes has been handicapped by the limitations of the light microscope. Extension of the limit of resolution to a certain degree has been provided by viewing the particles through a liquid medium of a refractive index different from that of the particle. This procedure has offered only partial solution to the problem, however. As a result, number and size of particles below 1.0 μ measured in the past by the light microscope have been in serious error.

A technique that has resulted in an increase of the visible range of dust particles utilizes solid film coatings of materials of high refractive index. Selenium, with a refractive index of 2.8, has proved to be most suitable in this respect, producing thin, transparent, and relatively stable films by the simple procedure of vacuum evaporation of selenium directly onto the dust particles on slides taken from the Cascade impactor. The selenium-coating procedure has resulted in the detection of large numbers of particles either unseen or misrepresented in size by previous procedures. By this technique the limits of resolution were extended with a resultant smoother frequency distribution of the particle size shifted toward lower limits. Thus a sample of UF_4 dust obtained during the operation of an animal-exposure unit had a median particle size of 0.89 μ when measured by usual procedures; by measurement involving the selenium-coating technique, a median size of 0.25 μ was found for the same sample. These results demonstrate the value of the high-refractive-index technique in problems of counting and sizing of particles whose distributions are close to the resolution limits of the optical microscope. It is anticipated that this technique will find wide application in industry.

3. The electron microscope has been used for the determination of particle sizes of dusts derived from atmospheric hydrolysis of gaseous uranium derivatives. Particles derived by such means are of sizes classified as fumes. The uranyl fluoride derived from the atmospheric hydrolysis of uranium hexafluoride was such a fume. Measurement of its particle size from pictures taken in the electron microscope have indicated a median of 0.056 μ with considerable mass of material much below this range. For accurate size measurement of such particles the electron microscope is the only instrument at present available (Sec. 8.2).

24.3 Local Effects on the Lung. One other factor involved in inhalation studies consists of the possibility of local irritation in the lungs due to the presence of foreign material. In the short-term experiments some local effects on the lungs have been found, especially at high exposure levels. In the case of materials such as UF_6, there is a particularly marked lung irritation by HF, a hydrolysis product. However, in general the lung involvement is not nearly so damaging as the more specific action of uranium on the kidney.

25. CONTRIBUTIONS OF INHALATION PROCEDURES TO URANIUM TOXICITY

Chief among the contributions were:

1. A description was given of the acute toxic responses of 12 uranium dusts and the delineation of the nontoxic from the toxic levels following inhalation of seven of the more important dusts.
2. A number of practical criteria of uranium toxicity were established, certain of which have been described for the first time.
3. The pattern of acute toxic response was clearly defined.
4. Information on relative susceptibility of five animal species was adduced.
5. From a consideration of four primary criteria, arrangement of the dusts on a graded scale of relative toxicities was possible.
6. A comparison of the relative dosages that produce equivalent toxicities furnished the basis for an estimate of the contribution of ingested dust to the apparent toxicity by inhalation. In a more qualitative manner the contributions by the routes of percutaneous and ocular absorption have been estimated.
7. Finally, a calculation of the percentage dust retention of an animal exposed to a dusty atmosphere of known concentration was made based on the toxicity of an injected dose.

25.1 Acute Toxicity of Uranium Dusts. From consideration of the results of the numerous exposure studies it is now possible to designate (1) definitely toxic levels, (2) levels producing mild transitory changes, and (3) levels incurring no detectable histologic damage in moderately susceptible species. Such results, which represent the heart of the inhalation program, are summarized in Tables 10.120 and 10.121 for seven of the more important dusts. It is seen that the hexafluoride, uranyl fluoride, the tetrachloride, and the nitrate that constitute the more soluble uranium dusts are not only fatal to certain species but occasion considerable renal involvement and some pulmonary damage at all levels above and including 2.5 mg/cu m. Even the 0.2-mg level is occasionally lethal to certain of the more susceptible animals and produces slight renal damage that is generally transitory. On the other hand, the less soluble dusts, the tetrafluoride,

Table 10.120 — Summary of Acute Toxic Response of Animals Following Repeated Daily Inhalation of Uranium Dusts

Approximate concentration level of dust, mg/cu m	Response to	
	UF_6, UO_2F_2, UCl_4, $UO_2(NO_3)_2.6H_2O$	UF_4, high-grade ore, UO_2
20	Generally fatal to most species; severe renal and frequently pulmonary damage	Occasionally fatal to some species; mostly moderate renal damage; severe renal damage in certain species only
2.5	Fatal to certain species; severe to moderate renal damage; occasional pulmonary damage	Nonfatal or rarely fatal; mild or no renal damage
0.2	Fatal to an occasional animal; slight renal damage in certain species	
0.05	Nonfatal; no histologic damage	

Table 10.121 — Uranium-dust Levels Producing Minimal or No Histologic Changes in the Kidney of the Dog

Dust	Lowest level showing mild, transitory response, mg U/cu m	Highest level showing no detectable response, mg U/cu m
UF_6, UO_2F_2, UCl_4, $UO_2(NO_3)_2.6H_2O$	0.2	0.05
High-grade ore	1.0	
UF_4	3.0	0.8
UO_2	10.0	2.0

the dioxide, and high-grade ore, are rarely fatal and produce little or no renal pathology at the 2.5-mg level. Complete freedom from response upon repeated exposure to the soluble dusts is not attained until the level of 0.075 mg of uranium is reached. By contrast, the insoluble dusts produce no injurious effects at levels of from approximately tenfold to thirtyfold higher concentration.

Compounds (not shown in the tables) of lesser importance from the point of view of industrial health that were tested at a single level of

approximately 20 mg/cu m were only moderately lethal to certain species and produced, in general, a moderate degree of renal pathology. The most toxic of these was the trioxide, which produced fairly extensive renal damage and mortality. The peroxide and the diuranates of ammonium and sodium were intermediate in effect, with tritaoctoxide being so slightly toxic as to be outside the usual range of findings of these dusts.

25.2 Criteria of Uranium Poisoning. In the course of the experimental work, numerous tests both old and new have been employed in an effort to discover sensitive, quantitative, and if possible, specific indicators of uranium toxicity. When attempts to find a specific indicator for uranium toxicity were unsuccessful, efforts directed toward changes in biochemical constituents and in physiological function that were associated with disturbances in renal function were highly fruitful. As a result of this approach three biochemical tests have been shown to be early sensitive indicators of uranium poisoning. Biochemical constituents of the urine have proved to give more distinct changes than those of blood in low-grade exposures. Similarly, in the physiological field, renal-function tests have proved good indicators of moderate damage.

Favored in the rank of first place among early sensitive biochemical indicators are urinary catalase, protein, and the amino acid nitrogen-creatinine ratios. Two of these tests, the determination of urinary catalase activity and amino acid nitrogen-creatinine ratios, have been shown for the first time to be applicable to uranium poisoning. Quantitative changes are observable on the second and third day in either of these constituents following a 6-hr exposure to dust levels ultimately producing just detectable histopathology. Of the three tests, urinary protein is the simplest to perform, but, in view of the more frequent appearance of this constituent from causes other than uranium poisoning, the use of more than one index is advocated. Multiple indices are desirable for the detection of toxic effects, since independent or overlapping responses in the animal organism are thus examined. Evidence on this point is the demonstration that, whereas protein is equally as good as catalase in the dog, this is not so for the rabbit exposed at 0.3 mg/cu m. In the latter species increased catalase activity in the urine is detectable sooner than is protein. The use of amino acid nitrogen (AAN) as a sensitive index is made possible through the use of the simple device of comparing this constituent with creatinine, a metabolite appearing in reasonably constant rate of excretion in the urine. This ratio is equivalent to the ratio of the clearances of amino acid nitrogen and creatinine. Urinary amino acid, moreover, continues elevated after protein and catalase and NPN have returned to normal in the rabbit. Measurements of AAN are

most satisfactory in rabbits but not in dogs. Clinical application of the above tests to workers employed in the uranium industry is feasible. Changes in urinary phosphatase activity, after thorough tests, have been adjudged less sensitive than the above constituents as an early sign of uranium poisoning.

Renal-function tests of value as indices have consisted in the determination of the change in the ability of the kidney to clear the blood of diodrast, inulin, chloride, and phenolsulfonphthalein. Diodrast clearance, which is a measure of tubular excretion, was the only one of three (diodrast, inulin, and chloride) that showed a marked change after nine days in dogs exposed at the 2-mg level of uranyl nitrate. By comparison, unquestionable changes occurred in urinary catalase and protein in the same animals. As an indication of the lesser degree of sensitivity of the renal-function tests, only questionable changes in the renal clearances occurred in the dogs exposed at a tenfold lower concentration of the nitrate, although elevations in urinary catalase and protein were evident (Sec. 13). On the other hand, the fact that chloride clearances performed on rabbits weekly for 13 weeks following exposure to 20 mg $Na_2U_2O_7$/cu m remained elevated while in all other tests the animals were normal would indicate a special usefulness of this renal clearance test in uranium poisoning. The ability of the kidney to reabsorb chloride is reduced for protracted periods once initial damage has been incurred (Sec. 19). The extent of the effect may be such as not to cause the animal any distress as indicated by a generally normal condition, including absence of histologic abnormalities. Phenolsulfonphthalein (PSP) retention, an empirical test, although yielding no information in addition to that of diodrast or PSP clearance, offers the technical advantage that several successive measurements may be made without sacrifice of the animal.

In a broad sense, functional tests, e.g., renal clearances, give a kinetic picture of the disturbances within an organ over a period of hours. Biochemical determinations give a more static picture. For a correlation with changes in morphology, assay of the functional state is more important. On the other hand, it should be noted that renal-function tests, although offering certain advantages not gained by biochemical tests, are rather involved procedures that demand considerable technical experience, which limits the application to relatively small numbers of animals. Accordingly the choice of a biochemical or physiological test in uranium poisoning depends in part on the type of information desired and in part on expense at which it is to be gained.

Histologic changes are also among the most sensitive guides to uranium poisoning. Although less quantitative than the above-mentioned tests and usually requiring the sacrifice of an animal, the method is

capable of detecting subtle renal alterations in animals shortly after the most delicate biochemical indications of injury have made their appearance.

Reliable indicators of uranium toxicity are provided by elevations in nonprotein nitrogen and urea of blood, changes in body weight, physical signs, and mortality. Usually these only become manifest in uranium poisoning of considerable severity and are of greatest service in the determination of the degree of severity and in prognosis.

Hematology, a frequent diagnostic aid in heavy-metal toxicity, is of no value as an index of uranium poisoning.

25.3 Characteristics of Uranium Intoxication. Perhaps most characteristic of all in the complex pattern of response in uranium intoxication is the time lag between initial exposure and grossly manifest symptoms. In uranium-dust exposures uncomplicated by the presence of other toxic substances, HF or HCl, no deaths have been recorded in any animal species before the fifth day subsequent to the first exposure.* The period of the lag phase varies with the solubility and concentration of the uranium dust. Deaths from exposures to soluble uranium compounds, such as uranyl fluoride and uranyl nitrate, may occur within the first week at a level of 20 mg/cu m. With more insoluble compounds, such as uranium tetrafluoride and sodium diuranate, the time of peak mortality is prolonged into the second and third weeks. The time lag between exposure and final outcome has also obvious dependence on variation among species (see following section).

Moreover, once the animal has received a lethal exposure dose, cessation or continuance of the exposure has little effect on prolonging or foreshortening the final outcome. Either the animal dies eventually (usually within 2 to 3 weeks from the initial exposure) or it recovers with complete or nearly complete return of normal body function. As far as has been determined from 30-day studies there is no indication that animals tend to persist in a state of chronic uranium toxicity. The fact that recovery from severe uranium injury is possible in the face of continued exposure to dust concentrations that decimate certain of the species has lead to the concept of tolerance. The entire pattern of response in all the numerous dust-exposure studies indicates repeatedly and consistently recovery of weight losses, return to normal blood levels, and regeneration of injured tissues in animals surviving initial depression of these functions. Indications of tolerance are not incompatible with histologic observations of dust-exposed

*Animals with peculiar susceptibility to pulmonary irritation, such as the mouse, have succumbed from exposures to UF_6 (UO_2F_2 + HF) within a matter of minutes at concentrations approximating 1 g/cu m and within 2 to 3 days following a 2-hr exposure to UO_2F_2 at 20 mg/cu m.

animals where characteristics of the regenerated renal cells differ from the original nonpoisoned cells (Chap. 4, Sec. 4.1b). Tests of acquired tolerance to uranium and a description of its nature are to be found in Chap. 12.

The sequence of events in uranium poisoning following inhalation have been adequately described in this chapter (Sec. 19), and that of renal histologic changes in Chap. 4, Sec. 4.5.

25.4 Differences in Species Susceptibility to Uranium-dust Exposures. Although the relative degree of susceptibility of laboratory animal species can be categorically given for a single uranium dust, there is no satisfactory method for determining the order of susceptibility of species for uranium dusts generally. This difficulty arises because few of the test materials were uncomplicated by the presence of potentially toxic anions (of elements other than uranium, previously discussed) to which different species have peculiar susceptibilities. For example, the mouse is highly susceptible to UF_6 and UCl_4, and the rat is highly resistant to UO_2F_2. Furthermore, susceptibility based on different criteria such as mortality and pathology may result in a different choice of order. As a case in point, the dog is about equally as susceptible as the rabbit to renal injury, but under the same conditions of exposure the dog survives whereas the rabbit succumbs. Recognizing these differences, the order of susceptibility to uranium-dust exposures for six species of laboratory animals is: most susceptible — rabbit, cat; intermediately susceptible — dog, mouse; least susceptible — guinea pig, rat.

25.5 Relative Toxicity of 12 Uranium Dusts. From a consideration of the criteria discussed in the foregoing sections, it is possible to relate the over-all toxicity to laboratory animals of one uranium dust with that of another. The criteria chosen were mortality, weight response, and biochemical and histological changes. Scores of from 1 to 4 for each criteria were used to grade the severity of the response. In order that the results would be based on similar types of evidence, the dog, rat, and rabbit were the species chosen for estimating the score. For each level of exposure, the lowest possible score for all four criteria, representing the greatest toxicity was 4; the highest possible score, 16, representing the least toxicity. For the assignment of the relative order of toxicity of each of the dusts, the scores of each level (in the majority of instances three levels were used) were weighted in respect to relative change in the scores with decreasing concentration. As a result of this procedure, the relative order of the toxicity of 12 uranium dusts derived from inhalation-toxicity studies is given in Table 10.122.

Table 10.122—Relative Toxicity by Inhalation of 12 Uranium Dusts

(Greatest toxicity, or lowest possible score, 4; lowest toxicity, or highest possible score, 16)

Toxic response score	Dust	Level, mg U/cu m
4	UF_6	13
5	UO_2F_2, UCl_4	>9, 11
5.5	$UO_2(NO_3)_2.6H_2O$	11
6.5	UO_4	13
7	UO_3	16
8	High-grade ore	20
9	$Na_2U_2O_7$, UF_4	15, 18
9.5	$(NH_4)_2U_2O_7$	12
11	UO_2	19
15	U_3O_8*	15

* The position of U_3O_8 is fixed with less certainty than others because of less detailed study.

As has been already indicated in former sections, the most soluble compounds were relatively the most toxic. These stand at the top of the list. Only small differences are seen to exist among the four most toxic compounds: UF_6, UO_2F_2, UCl_4, and $UO_2(NO_3)_2.6H_2O$. Although each compound exhibited special features, nevertheless the over-all toxic response of each of the four compounds is about the same. The relatively high position of high-grade ore on the scale would be surprising in view of its large percentage composition of the insoluble, relatively nontoxic oxides, UO_2 and U_3O_8, were it not for the fact that a considerable portion of lead and some copper, nickel, and other heavy metals accompany the uranium oxides. The position of UO_2 and U_3O_8 on the scale is relatively far below the usual range of toxicity of uranium compounds.

The above representation discloses a different order than would have resulted had the arrangement been based on mortality alone, the usual criterion of toxicity. On this basis, uranyl nitrate would

occupy a median position on the scale. This placement would be in error because, although mortality is not a striking feature of this compound, other toxic manifestations such as renal damage are continually observed at levels far below those of most uranium dusts tested. Other exceptions are UO_2 and UF_4 that exhibited a sharp cutoff in toxicity below the 20-mg level. On the whole, however, a linear response with change in concentration is observed. Again, the mortality from UO_2F_2 dust at 2.5 mg is approximately equivalent to that produced by $Na_2U_2O_7$ at 20 mg, a tenfold difference in response. Such a difference does not appear on the figure. In these respects the representation is limited.

25.6 Contribution to Toxicity by Routes Other than Inhalation. A factor that has given considerable concern has been the part played by the chance ingestion of uranium dust in the toxicity by inhalation. As pointed out in the introduction to this chapter, all technical provisions possible had been made to minimize oral dust intake during exposure, but the nature of large-scale exposures is such that accumulation of dust by settling in the cages and on the fur is unavoidable. There is a real possibility that the occasionally ingested large particle, which in mass constitutes a significant exposure dose, would complicate the inhalation-toxicity picture.

An estimate of the potential effect that any ingested dust might have on inhalation toxicity was possible only after sufficient data had been accumulated from studies involving both routes of administration. This has been done for several uranium compounds (Chaps. 8 and 9). The ratio of the oral dose producing a toxicity equivalent to a given inhalation dose is a means of expressing the relative toxicity by each route. The ratio was arrived at from a comparison of the known daily oral dose with the calculated inhalation dose. The latter calculation, which utilizes the ventilation rates of Benedict and assumes a 50 per cent dust retention for a given exposure atmosphere, gave for a 10-kg dog, a 0.3-kg rat, and a 2-kg rabbit a total daily dust-retention value of 50, 5, and 18 per cent, respectively, of the exposure concentration. The final ratio of oral to inhalation dosages based on equivalent mortality for the dog, rat, and rabbit, and for the equivalent weight loss for the rat, are given in Table 10.123.

It is seen that a given uranium compound is many times less toxic by ingestion than by inhalation, varying from approximately 65 for UO_2F_2 to 10,000 for UF_4. In the few instances for which data were available to determine the ratio for more than one species, the values were in reasonable agreement. Less satisfactory agreement was found among the ratios calculated on the basis of equivalent weight losses in the rat. A reasonable explanation of the discrepancy would

appear to be the unpalatability of the diet for the rat. This would result in lowered food intake, which would in turn produce an apparently greater weight loss for a given oral dose causing the ratio between the oral and inhalation dose to decrease, the finding actually observed.

Table 10.123—Relation of Oral Dose to Inhalation Dose at Equivalent Toxicities
(Based on data in Chaps. 7 and 10)

Uranium compound	Oral dose÷inhalation dose*			
	Dog†	Rat†	Rabbit†	Rat‡
UO_2F_2	65	75		50
$Na_2U_2O_7$	<100			
UO_4		125		30
$UO_2(NO_3)_2.6H_2O$	200	300	220§ –300	75
UCl_4		250		250
High-grade ore	2,500			
UF_4	10,000			3,330
UO_2				3,330

*Calculation of average daily inhalation dose based on:
1. The following ventilation rates (Benedict, "Basal Metabolism"):

Species	Weight, kg	Ventilation rate, liters/min
Dog	10	3.0
Rat	0.3	0.3
Rabbit	2	1.0

2. Fifty per cent retention of exposure-dust concentration.

†Based on equivalent mortality.
‡Based on equivalent weight loss.
§Based on renal pathology and weight loss.

Before a conclusion can be reached regarding the effect of ingestion on inhalation, it is necessary to consider whether the animals under conditions of exposure could possibly ingest regularly, each day, part of, all, or more than 65 to 10,000 times the inhaled dose, realizing that 65 to 10,000 represents doubling the inhalation toxicity. For a rat exposed to 20 mg/cu m and retaining 5 per cent of the concentration, or 1 mg, this would require that 65 mg of UO_2F_2, or 10,000 mg of UF_4, be ingested daily. Such ingestion would then have the effect of doubling the apparent inhalation toxicity. From all the analytical data

available such intakes appear to be excessive. The maximal amount of uranium analytically determined in the gastrointestinal tract (with contents) was 10 mg. More commonly amounts of from 0.2 to 0.6 mg of uranium in the intestinal tract were found. Assuming these values to be the result of ingestion for a period of 1 day, the maximal contribution to inhalation toxicity by orally ingested dust for UF_4 would be 10/10,000 or 0.1 per cent. For a dust of lower dosage ratio such as $UO_2(NO_3)_2.6H_2O$, the contribution would be 4 per cent; for UO_2F_2, 15 per cent.

With the information afforded on dosage ratios and from the considerations just discussed, two conclusions appear justified regarding the effects of oral intake of uranium compounds on the toxicity by inhalation: (1) The amount of uranium compound ingested in the course of inhalation may contribute to the apparent inhalation toxicity, more especially in the rat and mouse. (2) The enhancement of inhalation toxicity by ingested dust may, under the most unfavorable circumstances, amount to 15 per cent with the more soluble dusts but becomes negligible with the more insoluble dusts at inhalation exposure concentrations of 20 mg/cu m.

(a) Percutaneous Absorption. From calculations of the relative dosages that produce equivalent mortality by percutaneous absorption (Table 8.1) and by inhalation, it was found that uranyl nitrate applied to the shaved back of the rabbit was about 130 times less lethal by the cutaneous route than by inhalation. For other species, the rat and guinea pig, differences between the cutaneous and inhalative doses were far greater. In terms of the contribution by cutaneous toxicity these ratios represent the maximum. Under normal conditions of exposure, dust penetrates the heavily furred skin of animals only with exceptional difficulty, and in most species normal habits of cleanliness remove a considerable portion of any adherent dust. Accordingly, from these admittedly scant data it is reasonable to assume that the possibilities of enhancement of toxicity by the percutaneous route during dust exposures are exceedingly small.

(b) Ocular Absorption. Owing to the qualitative nature of the work it is possible to make only an extremely rough calculation of the ratio of ocular (Chap. 9) to pulmonary dosage for equivalent mortality response, but within this restriction the uranium compounds seem to be from 1¼ to 2 times more toxic than by the cutaneous route. Unfortunately, direct measurement of the amount of uranium dust deposited in the palpebral region is extremely difficult to make. None has been attempted. Merely clinical observations of severe ocular irritation have been noted with the uranyl fluoride and uranium tetrafluoride at the highest levels studied. As has been noted in the above sections,

at high levels the addition to inhalation toxicity by ocular absorption may, therefore, possibly be significant with certain of the uranium compounds.

25.7 Calculation of Dust Retention from Parenterally Injected Doses. In a single study in which sufficiently detailed data were available on the relation between dosage and toxicity by inhalation and by injection, it has been calculated that the retention of uranyl nitrate hexahydrate dust at an exposure level of 0.5 mg/cu m is from 20 to 50 per cent for the rabbit. The median particle size of this extremely soluble dust is approximately 10 μ and is far larger than other dusts on which exposure studies are made. More information on this extremely important subject is expected to be available soon in a separate study especially designed for this purpose.

26. RECOMMENDATIONS OF RESPIRATORY PROTECTIVE EQUIPMENT

Most respirators that had been previously certified by the U. S. Bureau of Mines to offer protection against specific dusts of heavy metals and their compounds have been retested by methods approved by the Bureau to determine their serviceability for the worker in atmospheres containing uranium dusts. The additional tests were made necessary because of the low permissibility limit (0.15 mg U/cu m) and because of the fineness and the wide variation in the physical and chemical characteristics of the industrial uranium dusts. For this reason a number of respirators claiming to offer protection against fumes were tested as well. In the case of two uranium materials, UF_6 and UCl_4, against which simple paper or felt respirator filters did not offer sufficient protection, a number of different canisters, most of which were also approved by the Bureau, were similarly retested.

In the case of UF_6, gas-mask canisters were found that would protect against both the uranium fume and the halogen vapor, and respirators were found that would give protection against the uranium components of this material. On the other hand, no respirator or canister of a number tested was found satisfactory for continued use in UCl_4 atmospheres. A few offered partial protection for short periods from UCl_4; longer use resulted in destruction of the filter.

Because the low permissibility limit and the fineness of the particle size of the uranium dusts placed rather rigid restrictions for acceptability on respirators, not all were found satisfactory. Moreover, the number of satisfactory respirators was further reduced by the important consideration of comfort during use. This latter consideration is of such practical importance that frequently respirators

showing border-line efficiency were favored by plant workers over others more efficient but less comfortable. Respirators with greater filtering area give greater comfort by offering less resistance to breathing under the high ventilation rates of heavy work, but a limit is soon reached at which the increase in bulkiness cancels the advantage. The various respirators and canisters rated according to both comfort and filtering efficiency are tabulated in Part D.

The chief difficulty in respiratory protection is that of convincing the worker of its continuous necessity during operations in dusty atmospheres. Education and constant supervision of the worker in this regard is essential. Individual respirators should be assigned to the personnel, and filters changed and facepieces cleaned on a schedule suited to the conditions of use. Regular inspection or tests of respirators should be made to ensure uniformity of the product.

BIBLIOGRAPHY

1. L. U. Gardner, D. E. Cummings, and G. R. Dowd, Experimental Inhalation of Bituminous Coal Dust and Its Effects Upon Primary Tuberculosis Infection in Guinea Pigs, J. Ind. Hyg., 15: 456 (1933).
2. L. U. Gardner, Studies on the Relation of Mineral Dusts to Tuberculosis. I. The Relatively Early Lesions in Experimental Pneumokoniosis Produced by Granite Inhalation and Their Influence on Pulmonary Tuberculosis, Am. Rev. Tuberc., 4: 734 (1920-1921).
3. L. U. Gardner and M. Dworski, Studies on the Relation of Mineral Dusts to Tuberculosis. II. The Relatively Early Lesions Produced by the Inhalation of Marble Dust and Their Influence on Pulmonary Tuberculosis, Am. Rev. Tuberc., 6: 782 (1922-1923).
4. M. J. Stewart and A. C. Haddow, Demonstration of the Peculiar Bodies of Pulmonary Asbestosis ("Asbestosis Bodies") in Material Obtained by Lung Puncture and in the Sputum, J. Path. Bact., 32: 172 (1929).
5. L. U. Gardner, Studies on the Relation of Mineral Dusts to Tuberculosis. III. The Relatively Early Lesions in Experimental Pneumokoniosis Produced by Carborundum Inhalation and Their Influence on Pulmonary Tuberculosis, Am. Rev. Tuberc., 7: 344 (1923).
6. K. R. Drinker and P. Drinker, Metal Fume Fever. Results of Inhalation by Animals of Zinc and Magnesium Oxide Fumes, J. Ind. Hyg., 10: 56 (1928).
7. H. H. Schrenk, Testing and Design of Respirator Protective Devices, U.S. Bur. of Mines Inform. Circ. 7086, 1939; Procedure for Testing Gas Masks, U.S. Bur. of Mines Schedule 14E (December, 1941).
8. J. Arnold, Untersuchungen ueber Staubinhalation und Staubmetastase, F. C. W. Vogel, Leipzig, 1885.
9. A. von Jns, Experimentelle Untersuchungen ueber Kieselstaubinhalation, Arch. exper. Path. Pharm., 5: 169 (1876).
10. Y. Saito, Experimentelle Untersuchungen ueber die quantitative Absorption von Staub durch Tiere bei genau bekannten Staubgehalt der Luft, Arch. Hyg. u. Bakt., 75: 134 (1912).

11. A. Mavrogordato, Experiments on Effect of Dust Inhalation, J. Ind. Hyg., 17: 439 (1918).
12. L. U. Gardner, Relation of Mineral Dusts to Tuberculosis, Am. Rev. Tuberc., 4: 734 (1920).
13. L. T. Fairhall and R. R. Sayers, The Relative Toxicity of Lead and Some of Its Common Compounds, U.S. Pub. Health Bull. 253 (1940).
14. C. E. Brown, Studies in Dust Retention. III. J. Ind. Hyg., 13: 293 (1931).
15. A. M. Van Wijh and H. S. Patterson, Per Cent of Particles of Different Sizes Removed from Air by Breathing, J. Ind. Hyg., 23: 31 (1940).
16. T. Bedford and G. G. Warner, Measurement of Concentrations of Air-borne Dust, J. Ind. Hyg., 24: 315 (1942).
17. J. H. Yoe, Some Observations on Reactions between Certain Metallic Ions and the Ammonium Salt of Aurintricarboxylic Acid, J. Am. Chem. Soc., 54: 1022 (1932).
18. H. H. Schrenk, List of Respiratory Protective Devices Approved by the U.S. Bureau of Mines, Inform. Circ. 7237 (March, 1943).
19. W. E. Gibbs, Clouds and Smokes, The Blakiston Company, Philadelphia, 1924.
20. S. B. Drinker and T. H. Hatch, "Industrial Dust," McGraw-Hill Book Company, Inc., New York, 1936.
21. J. M. Dallavalle, "Micromeritics," Pitman Publishing Corp., New York, 1943.
22. G. Lehmann, Die Funktion der menschlichen Nase als Staubfilter, Arbeitsphysiol., 7: 167 (1933). See also J. Ind. Hyg., 17: 37 (1935).
23. F. J. Tourangeau and P. Drinker, The Dust Filtering Efficiency of the Human Nose, J. Ind. Hyg. Toxicol., 19: 53 (1937).
24. T. F. Hatch, Behavior of Microscopic Particles in the Air and in the Respiratory System, in "Aerobiology," ed. by F. R. Moulton, Pub. Am. Ass. Advance. Sci., 17: 102 (1942).
25. C. E. Brown, Quantitative Measurements of the Inhalation, Retention, and Exhalation of Dusts and Fumes by Man. II. Concentrations Below 50 Mg per Cubic Meter, J. Ind. Hyg., 13: 285 (1931).
26. H. L. Blumgart, Absorption of Lead by the Upper Respiratory Passages, J. Ind. Hyg., 5: 153 (1923).
27. A. M. Van Wijh and H. S. Patterson, The Percentage of Particles of Different Sizes Removed from Dust-laden Air by Breathing, J. Ind. Hyg., 23: 31 (1940).
28. G. Martin *et al.* Researches on the Theory of Fine Grinding. I. Law Governing the Connection Between the Number of Particles and Their Diameters in Grinding Grushed Sand, Trans. Brit. Ceram. Soc. 23: 61 (1923).
29. T. Hatch and S. P. Choate, Statistical Description of the Size Properties of Non-uniform Particulate Substances, J. Franklin Inst., 210: 793 (1929).
30. A. Hazen, Storage to Be Provided in Impounding Reservoirs, Trans. Am. Soc. Civil Engrs., 77: 1539 (1914).
31. C. E. Brown *et al.* Microprojection Method for Counting Impinger Dust Samples, U.S. Bureau of Mines, Rept. Invest. 3373 (1938).
32. E. M. Chamot and C. W. Mason, "Handbook of Chemical Microscopy," Vol. II, John Wiley & Sons, Inc., New York, 1931.
33. C. P. Shillaber, "Photomicrography," John Wiley & Sons, Inc., New York, 1944.
34. W. D. Foster and H. H. Schrenk, Petrographic Identification of Atmospheric Dust Particles, U.S. Bureau of Mines, Rept. Invest. 3368 (1938).
35. C. C. Twort *et al.* Disinfection of Closed Atmospheres with Germicidal Aerosols, J. Hyg., 40: 253 (1940).
36. V. Bryson, E. Sansome, and S. Laskin, Aerosolization of Penicillin Solutions, Science, 100: 33 (1944).
37. K. R. May, The Cascade Impactor: An Instrument for Sampling Coarse Aerosols, J. Sci. Instr. 22: 187 (1945).

38. J. J. Bloomfield and J. M. Dallavalle, The Determination and Control of Industrial Dust, U.S. Pub. Health Bull. 217 (1935).
39. G. D. Hanna and W. M. Grant, Preliminary Note on a Technique for Mounting Diatoms in Realgar and Other Substances, J. Roy. Microscop. Soc., Series III, 59: 174 (1939); Apparatus for Mounting Diatoms in Realgar and Other Substances, J. Roy. Microscop. Soc., Series III, 60: 152 (1940).
40. U. Strong *et al.* Procedures in Experimental Physics, Prentice-Hall, Inc., New York, 1939.
41. D. G. Brubaker, Light and Electron Microscopy of Pigments, Anal. Chem., 17: 184 (1945).
42. V. K. Zworkin *et al.* "Electron Optics and the Electron Microscope," John Wiley & Sons, Inc., New York, 1945.
43. W. Findeisen, Ueber das Absetzen kleiner, in der Luft suspendierter Teilchen in der menschlichen Lunge bei der Atmung, Arch. ges. Physiol. (Pfluegers), 236: 367 (1935).
44. K. Slavjansky, Experimentelle Beitraege zur Pneumonkoniosislehre, Virchow's Arch. path. Anat. 48: 326 (1869).
45. N. Tchistovitch, Des phenomenes de phagocytose dans les poumons, Ann. inst. Pasteur, 3: 337 (1889).
46. W. T. Sewell, The Phagocytic Properties of the Alveolar Cells of the Lung, J. Path. Bact., 22: 40 (1918).
47. N. C. Foot, Studies on Endothelial Reactions. IV. The Endothelium in Experimental General Miliary Tuberculosis in Rabbits, J. Exptl. Med., 33: 271 (1921).
48. F. A. McJunkin, The Origin of the Phagocytic Nuclear Cells of the Peripheral Blood, Am. J. Anat., 25: 27 (1919).
49. H. H. Permar, An Experimental Study of the Mononuclear Phagocytes of the Lung, J. Med. Res., 42: 9 (1920).
50. K. B. Lehmann, Studien ueber technische und hygienische wichtige Gase und Daempfe. XIV. Das Giess oder Zinkfieber, Arch. Hyg. u. Bakt., 72: 358 (1910).
51. M. C. Winternitz and G. H. Smith, "Preliminary Studies in Intratracheal Technique; Pathology of War Gas Poisoning," Yale University Press, New Haven, 1920.
52. A. S. Minot, Lead Studies. V. The Distribution of Lead in the Organism after Absorption by the Lungs and Subcutaneous Tissues, J. Ind. Hyg., 6: 137 (1924).
53. H. L. Blumgart, Lead Studies. VI. Absorption of Lead by the Upper Respiratory Passages, J. Ind. Hyg., 5: 153 (1923).
54. A. Axelsson and R. Bringel, Zur Entstehung und Verbreitung der Lungentuberkulose des Kaninchens bei vorher mobilisiertem Reticuloendothel, Beitr. Klin. Tuberk., 76: 493 (1931).
55. E. H. Kettle and R. Hilton, The Technique of Experimental Pneumoconiosis, Lancet, 222: 1190 (1932).
56. D. E. Cummings, Studies on Experimental Pneumonokoniosis. IV. The Separation of Particulate Matter Smaller Than Screen Size into Graded Fractions, J. Ind. Hyg., 11: 245 (1929).
57. W. Machle, K. V. Kitzmiller, E. W. Scott, and J. F. Treon, The Effect of the Inhalation of Hydrogen Chloride, J. Ind. Hyg. Toxicol., 24: 8 (1942).
58. H. P. Dygert, R. Sanford, and H. Oberg, Relative Efficiencies of Dust Sampling Devices as Applied to the Collection of U-Dusts, Univ. Rochester, Atomic Energy Project Rept. M-1582 (1944).
59. H. E. Stokinger, F. L. Dorn, R. A. Boak, and C. M. Carpenter, The Effect of Fever on the Distribution of Arsenic in the Tissues of Rabbits Injected Intravenously with Mapharsen, Am. J. Syphilis, Gonorrhea, Venereal Diseases, 28: 465 (1944).

Chapter 11

THE DISTRIBUTION AND EXCRETION OF URANIUM

By William F. Neuman*

1. INTRODUCTION

A brief survey of the literature revealed a number of papers on the distribution and excretion of injected uranium (see Bibliography, references 1 to 9), wherein various methods were employed and various conclusions were reached. For example, some authors[6,7] considered the liver as an important site of uranium deposition, whereas others[3,4] reported the liver to be uranium-free. Some[1,3] found uranium to be excreted in the bile, a fact denied by others.[7]

All these investigators agreed that uranium accumulates in the kidneys after administration. Most of the recent publications indicated that large quantities of injected uranium (as much as 85 per cent of the dose) are excreted in the urine.

None of these investigators examined bone as a possible site of storage in spite of the fact that Hoffmann[10] found uranium in the bones of normal untreated animals.

The purpose of the experiments to be described was (1) to obtain more information about deposition in the kidney especially as related to urinary excretion, (2) to examine the skeletal system for possible storage, and (3) to settle, if possible, the dispute regarding liver deposition.

The chronological order of the experiments on the fate of absorbed uranium was as follows: (1) the development of a reliable method of analysis for uranium in biological specimens, (2) the examination of representative tissues of rats at various time intervals after uranium injections, in each case checking the data obtained by an analysis of

*Work done by W. F. Neuman, R. W. Fleming, A. B. Carlson, J. F. O'Leary, P. E. O'Connell, B. J. Mulryan, J. H. Wills, M. W. Neuman, M. Anders, and P. Hutchens.

the entire animal and all excreta, (3) confirmatory studies on other species of animals, and (4) exploratory studies on the factors that influence the distribution and excretion of uranium.

The development of the protein-isolation method for uranium in biological material and its subsequent determination by fluorophotometry is described in Chap. 2. All analyses were made by this method.

2. EXPERIMENTAL WORK

It was impracticable to analyze a large experimental animal, such as a dog, in toto, employing platinum ware (see Chap. 2) for the collection of samples. Accordingly the rat was chosen as the experimental animal in the "balance" studies, where the entire animal was analyzed. An additional reason for this choice was that the results of a great deal of research on the toxicity of uranium in the rat were available.

Rats given uranium intravenously were sacrificed for study at periods varying from 45 min to 44 days after the injection. In this way, information was obtained on the sequence of changes that took place in the distribution and excretion of uranium after administration.

2.1 Cages and Animals. The first problem was to obtain some means of collecting urine and feces quantitatively and separately. An

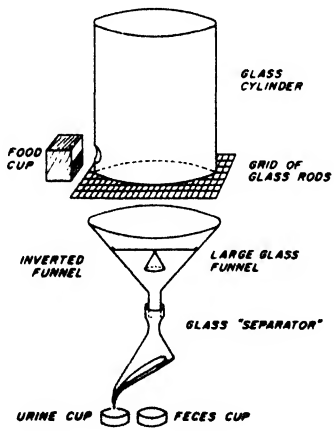


Fig. 11.1—All-glass metabolism cage for rats.

all-glass cage was constructed which permitted a satisfactory separation of urine and feces. Further, the all-glass construction made it possible to remove uranium quantitatively from the cage with an acid solution before and after excreta collections. Kimball made sugges-

tions and gave advice on cage construction. A diagrammatic sketch of the cage is presented in Fig. 11.1.

In testing these cages, it was found necessary to use young adult rats in order to obtain successful separation of urine and feces. Therefore all the distribution experiments were carried out on male (approximately 9 weeks old) and female (approximately 16 weeks old) rats weighing approximately 200 g.

An experiment was designed to test whether complete recovery of uranium could be obtained from the cage itself. A rat was placed in a clean glass cage for 24 hr. The rat was then removed, and 10 ml of human urine containing 0.1 mg of uranium was sprinkled around the sides and bottom of the cage. Twenty-four hours later the cage was washed thoroughly with 2N hydrochloric acid containing 0.1 per cent detergent (Igepal CTA Extra, General Dyestuff Corporation). Urine, feces, and washings were collected together, evaporated to dryness, ashed, and analyzed. The recoveries from three such experiments were 86.3, 88.0, and 95.7 per cent. The average, 90.0 per cent, compares well with 92.5 per cent recovery from pure solutions.

In all subsequent experiments, the cages were washed thoroughly with the HCl-detergent solution before and after excreta collections. The washings were added to the urine sample.

2.2 Diet. It was recognized that the diet might have a considerable influence on the relative distribution and excretion of uranium. Accordingly a synthetic ration was chosen which could be defined and reproduced. It was necessary to add indigestible roughage (celluflour) to obtain feces of the proper consistency for successful operation of the urine-feces separator. Rats that were kept on this ration for months grew well and evidenced no deficiency symptoms. The diet finally selected and used in all experiments had the following composition (parts by weight):

Casein	25	Lard	7.5
Starch	40	Cod-liver oil	2.5
Sugar	10	Salt mixture[11]	4.0
Yeast	5	$CaCO_3$	1.0
Alfalfa	4	Natola*	0.1
Celluflour	20	Choline chloride	0.2
		Vitamin mixture†	0.21

*Fish-liver-oil concentrate, commercially available.
†The vitamin mixture had the following composition:

	Mg		Mg
Thiamin	80	Calcium pantothenate	250
Riboflavin	160	*p*-Amino benzoic acid	250
Pyridoxin	120	Inositol	500
Nicotinic acid	250	$CuSO_4$ (not present in salt mixture[11])	500

With the high celluflour content (approximately 20 per cent) of the diet, rats usually suffered from diarrhea for a few days after being placed on the synthetic ration. Because of this and other considerations, all experimental animals were given a minimal "equilibrating period" of 1 week on the ration before the administration of uranium.

2.3 Method of Administration. To ensure complete absorption of the uranium, the full dose was injected directly into the blood. The rat's tail was immersed in lukewarm water to obtain dilation of the blood vessels. The rat was then wrapped in a clean towel and placed in a brass cylinder. The rat was usually quiet. The tail was held firmly, allowing slack in case of twitching, and the needle inserted in a convenient vein. In any case in which it was doubtful that the entire dose entered the vein properly, the animal was sacrificed without further study. As a further check, the section of the tail in which the dose was injected was analyzed to make sure that the uranium had not "localized."

The hexavalent uranium was injected as a solution of uranyl nitrate dissolved in water (0.5 mg U/ml) in doses of 1.8 or 2.5 mg U/kg of body weight.

To avoid oxidation the following scheme was adopted for the preparation and administration of tetravalent uranium: 50 to 80 mg of UCl_4 was weighed by difference in a covered weighing bottle to minimize hydration. The salt was immediately transferred to a brown bottle containing 100 mg of sodium hydrosulfite and diluted to 100 ml with 0.2M sodium acetate buffer at pH 4.6. The whole process was done as quickly as possible, and the animals were injected immediately. An aliquot was taken for analysis to determine accurately the dose administered. The dose varied between 1.5 and 3.0 mg U/kg of body weight.

2.4 Dissection. The rat to be sacrificed was anesthetized lightly with ether, and blood was collected by aspiration from an axillary pouch made according to Kuhn.[12] The rat was dissected with clean instruments that were kept under a 2 per cent bicarbonate solution when not in use. The following samples were taken: kidney; urinary bladder; spleen; genitals; heart; lung; liver; stomach and contents; intestines; leg muscle; skin and hair; femurs and humeri; tibias, fibulas, radii, and ulnas; pelvic girdle; three or four vertebras; ribs; skull; tail; and what were termed "soft carcass" and "bone carcass." All soft tissue scraps and trimmings were placed in the soft-carcass sample. All bones not sampled as above were cleaned superficially of flesh and placed in the bone-carcass sample.

Later, as results clearly indicated that there was practically no deposition of uranium in soft tissues other than kidney, a smaller number of samples was taken. The same bone samples were taken as

before, but the bladder, genitals, heart, lung, stomach, and muscle samples were pooled with the soft carcass.

2.5 Analytical Recoveries. As mentioned previously, various reports in the literature are in disagreement regarding the distribution and excretion of uranium (see Bibliography, references 1 to 10). It is likely that much of the disagreement resulted from inaccuracies in analytical technique. To prevent such inaccuracies from giving misleading results in the present experiments, a balance study was conducted on each rat examined; each animal together with its excreta was analyzed in toto. The summarized balance results are presented in Table 11.1. Included in Table 11.1 are the results of "control" isolations [protein isolations from pure $UO_2(NO_3)_2$ solutions] carried out concurrently with the animal studies. It should be pointed out that

Table 11.1—Over-all Recoveries in the Balance Experiments

	Number	Mean recovery, %	Standard deviation, %
Control isolations	38	85	8
Short-term animals	16	90	7
Long-term animals	7	108	13

in these experiments most errors were "positive" errors. For example, of 30 analyses of normal urine specimens that are supposedly uranium-free, some will give slightly positive results, some true blanks, and some slightly negative results. However, negative results are reported as zero, giving an average that is positive. Therefore, as expected, in the metabolism studies the highest recoveries were observed in those animals from which the greatest number of samples were taken (greatest accumulation of positive errors). In practice, it was the long-term animals from which the greatest number of specimens were taken (urine and feces collections over a period of 10 to 40 days). Accordingly, the results obtained from long-term animals have been tabulated separately in Table 11.1.

It appeared from these data that all the dose could be accounted for quantitatively by the experimental techniques and methods. Actually the recoveries were slightly high; from 90 to 108 per cent recovery was obtained with animals as compared with 85 to 92 per cent (see Chap. 2) obtained with pure solutions of $UO_2(NO_3)_2$.

2.6 Uranium Content of Normal Tissues. In order to evaluate the results obtained after the administration of uranium, it was necessary first to establish the quantities of uranium present in tissues of normal untreated animals.

Two rats taken from the stock Wistar colony were placed on the synthetic ration for 1 week, sacrificed, and dissected as described above. Each tissue was analyzed for uranium content. The results are recorded in Table 11.2.

Table 11.2 — Uranium Content of Untreated Animals

Tissue	Rat 1			Rat 2		
	Wt., g	Total uranium,* μg	μg U/g	Wt., g	Total uranium,* μg	μg U/g
Kidneys	1.63	0.03	0.02	1.66	0.00	0.00
Bladder	0.08	0.02	0.25	0.10	0.00	0.00
Spleen	1.16	0.03	0.03	1.35	0.01	0.01
Genitals	2.38	0.00	0.00	2.54		
Heart	0.81	0.06	0.07	0.82	0.06	0.07
Lungs	1.15	0.03	0.03	1.03	0.00	0.00
Liver	8.72	0.22	0.04	9.65	0.00	0.00
Stomach	4.82	0.04	0.01	1.55	0.02	0.01
Intestines	17.66	0.12	0.01	20.32	0.13	0.01
Muscle	42.00	0.38	0.01	27.21	0.00	0.00
Skin and hair	33.09	0.45	0.01	31.15	0.39	0.01
Carcass	47.55			71.40	0.00	0.00
Femur and humerus	0.79	0.00	0.00	1.01	0.00	0.00
Tibia, fibula, radius, and ulna	1.26	0.00	0.00	0.85	0.00	0.00
Pelvic girdle	0.67	0.00	0.00	0.75	0.00	0.00
Vertebras	1.06	0.11	0.10	1.21	0.00	0.00
Ribs	0.41	0.06	0.15	0.35	0.00	0.00
Skull	2.71	0.00	0.00	2.97	0.00	0.00
Blood	7.03	0.14	0.02	7.77	0.08	0.01

*Here 0.00 means "less than 0.005." Actually analyses totaling less than 0.1 μg are of doubtful significance.

It is clear from these data, which are in agreement with those of Hoffmann,[10] that the quantities of uranium occurring in normal animals are extremely small. Only 3 of 36 tissues analyzed evidenced a concentration greater than 0.1 μg U/g (1×10^{-7} g). The concentrations ranged from 2×10^{-7} to 1×10^{-8} g U/g of tissue, or less. The largest amount of uranium found in any tissue was 0.45 μg. Therefore in subsequent experiments any tissue having a uranium content in excess of 0.6 μg was arbitrarily considered significant. Since the dose varied from 300 to 500 μg, this quantity represented 0.1 to 0.2 per cent of the dose.

3. THE DISTRIBUTION AND EXCRETION OF HEXAVALENT URANIUM

From the toxicological standpoint, hexavalent uranium salts (UO_2X_2) are of prime importance, first, because of their solubility (readily absorbed) and second, because of the frequency with which they are

met in industrial processes. Accordingly, the experiments with uranyl compounds were more extensive than with other uranium compounds. Two representative salts, $UO_2(NO_3)_2$ and $UO_2(CH_3COO)_2$, were studied.

Within the limits of biological variation, a reproducible pattern of distribution and excretion was observed. Hexavalent uranium was found to be deposited in bone and kidney and excreted in the urine.

3.1 Disappearance of Uranium from the Circulation. Uranium leaves the blood stream very rapidly. Data have been obtained from two species: rabbits and rats; the literature contains one report[8] on dogs. The rabbits and rats were injected intravenously; the dogs,

Table 11.3—Variation of Blood-uranium Level with Time (Rats)

Time after intravenous administration, hr	Blood-uranium level	
	Females, μg/ml	Males, μg/ml
0	36*	36*
0.75	1.5	
2.5	0.2	0.1
8		0.1
12	0.1	0.1
24	0.0	0.0
48	0.1	0.1
960	0.0	0.0

*Initial value was calculated on the assumption that the whole injected amount was immediately distributed uniformly in the blood volume and that blood volume in the rat is 7 per cent of its body weight.

intraperitoneally. All three species evidenced a very rapid disappearance of blood uranium.

The data obtained on rats are summarized in Table 11.3. Figure 11.2 presents graphically the results of the rabbit experiments and Holman and Douglas's dog experiments.[8] Included in Fig. 11.2 are the results obtained on a nephrectomized rabbit.

The data support the following conclusions: (1) that the distribution of absorbed uranium takes place rapidly, (2) that, once distributed, there is little or no mobilization of the deposited uranium, since the blood stream is practically uranium-free from 2½ hr to 40 days after administration, (3) that some tissue or tissues other than kidney are capable of storing uranium, for the disappearance of uranium from the blood of a nephrectomized animal is nearly as rapid as from the blood of a normal animal, and (4) that the presence of uranium in the blood is so transient that the blood-uranium level could not be used to estimate either the degree of exposure or the storage of uranium in the body.

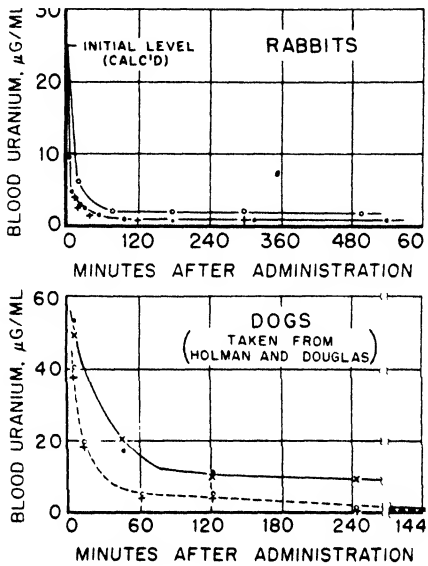


Fig. 11.2—Relationship between time after administration and blood-uranium level. All rabbits were given uranium intravenously. In the upper graph, values represented by dots and plus signs were from normal rabbits; those represented by circles were obtained from a nephrectomized rabbit. The dogs were given uranium intraperitoneally. In the lower graph the dashed line represents data from dogs receiving sodium citrate. The points represented by dots, circles, crosses, and plus signs represent data obtained from different individual dogs.

3.2 Urinary Excretion. Since the blood-uranium level falls very rapidly to an insignificant concentration, it is obvious that uranium, if it is to be excreted in any quantity, must appear in the excreta within the first few hours after administration.

Data obtained from three species (rats, cats, and rabbits) indicate that this is the case. Most of the total excretion took place in the first 24 hr after administration; thereafter only small, ever-decreasing quantities were excreted. Practically all the excreted uranium was found in the urine. Holman and Douglas[8] obtained similar results from the study of dogs. The average urinary excretion of rats is presented in Fig. 11.3. Table 11.4 contains the summarized results of studies on cats and rabbits. These animals were anesthetized and infused with 0.85 per cent saline. The bladders of the animals were cannulated, and, after intravenous injection of various doses of uranyl acetate, urine was collected over varying periods. Dounce prepared these animals.

In the rabbits and cats most of the excretion took place during the first few hours. For example, cat 1 excreted 17.5 per cent of the dose in the first 4 hr; in the next 4-hr period, only 2 per cent more was

Table 11.4 — Urinary Excretion of Uranium by Cats and Rabbits during the First 4 Hr after Intravenous Administration

Cats		Rabbits	
Dose, mg U/kg	Percentage of dose in urine	Dose, mg U/kg	Percentage of dose in urine
3.37	17.5	1.67	17.8
3.37	19.0	1.67	8.9
6.22	27.4	1.67	14.2
12.1	18.5	5.61	23.2
	Av. 20.6		Av. 16.0

found in the urine. It is interesting too that there seemed to be little or no correlation between magnitude of the dose and the percentage found in the urine.

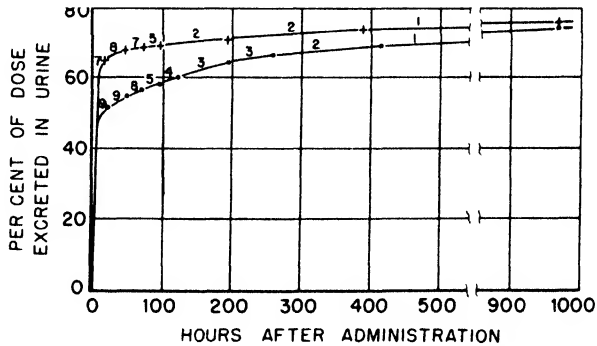


Fig. 11.3 — Averaged urinary excretion of uranium by rats; data for males represented by dots, for females by plus signs. The numbers above the curves indicate the number of animals used in obtaining the average.

In rats, also, most of the excretion took place soon after the injection. There was a significant difference between the early urinary excretion of the male and female rats. Apparently this observed difference was more closely associated with the difference in the age of the animals than with their sex per se. In these experiments all animals weighed 200 ±10 g; consequently the males were approximately 9 weeks, the females approximately 16 weeks of age. The relation between excretion and age will be discussed later in more detail.

Effect of Acid-Base Balance. Early work in this[13] and other laboratories[14] indicated that alkali administration decreased the toxicity of uranium as evidenced by mortality. It was soon evident that alkali administration exerted a marked effect on the urinary excretion of uranium.

Specifically, simultaneous administration of uranium and alkali resulted in an increase in the amount of uranium excreted and a decrease in the amount retained by the kidney. For a detailed discussion of the mechanism by which alkali administration exerts this effect

Table 11.5 — Urinary Excretion Data Showing the Effect of Acid-Base Balance on the 4-hr Output of Uranium by Cats and Rabbits

No. of animals	Diet	Infusion fluid	Systemic acid-base balance	Dose, mg U/kg	Av. percentage of uranium dose excreted
		Cat			
1	Normal	0.85% $NaHCO_3$	Alkaline	3.37	65
5	Normal	0.85% NaCl	Normal	3.37 – 12.1	24
2	Meat + NH_4Cl	0.85% NaCl	Acid	3.37	10
		Rabbit			
1	Normal	0.85% $NaHCO_3$	Alkaline	5.61	71
4	Normal	0.85% NaCl	Normal	1.67 – 5.61	16
3	Oat	0.85% NaCl	Acid	1.67 – 5.61	3.6

the reader is referred to Chap. 14. To illustrate the effect of alkali, representative data have been assembled in Table 11.5. The animals were prepared by Dounce as described above and injected intravenously with various doses of uranyl acetate. It is readily apparent that acidifying substances exert an effect opposed to that of alkaline substances. Rabbits and cats that were given acid-producing diets excreted a much smaller portion of the dose.

3.3 Fecal Excretion. Although the amounts of uranium found in the feces of treated animals were significant, they appear unimportant when compared in a quantitative sense with amounts found in urine. The average total amount found in the feces of 23 rats was only 3.9 per cent of the dose. This average includes total fecal collections for periods ranging from ¾ to 960 hr after administration. There was no correlation between the total amount of fecal uranium and the time after administration.

There is good reason to believe that the small amounts found in feces did not represent true excretion but rather contamination. The intestines and contents removed from the carcass never contained

more than 0.5 per cent of the dose. Forty-eight hours or more after administration the quantities found in the intestines and contents were insignificant. Two possible sources of contamination were:

1. Direct contamination. The feces rolled down the sides of the cage funnel, which were wet with previously excreted uranium-rich urine.

2. Indirect contamination by ingestion. A few drops of urine (on the first day) clinging to the fur provided means by which the rat could obtain uranium orally (by licking) to the extent of several per cent of the dose.

3.4 Deposition in Kidney. Concurrent with the rapid fall of the uranium concentration of the blood during the first few hours after administration was the deposition of uranium in the kidney. The data obtained from rats are presented in Fig. 11.4. The highest kidney

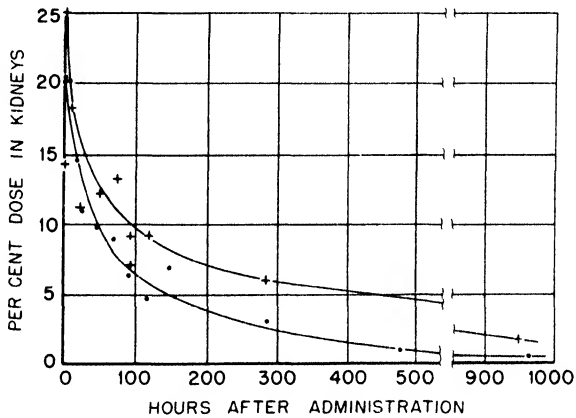


Fig. 11.4—Relationship between time after administration and the uranium content of the rat kidney; data for males are represented by dots, for females by plus signs.

values were noted between ¾ and 2½ hr after uranium was injected and amounted to about one-third of the injected dose. After 2½ hr, the uranium was gradually mobilized from the kidney and was present in almost insignificant quantities at the end of 40 days (960 hr). The uranium that appeared in the urine after the first 24 hr (from the second to the fortieth day) probably represented that which had been fixed initially in the kidney. During this period the blood concentration was insignificant and could not have been the source of the uranium that appeared in the urine. The decrease in the uranium content of the kidneys was from an initial 20 per cent to 1 or 2 per cent of the injected dose, an amount about equal to that which appeared in the urine.

As is shown later, the relative proportion of the dose that is deposited in the kidney appears to vary inversely with the storage in bone and the alkaline reserve of the animal after investigation. Bone

Table 11.6 — Effect of Acid-Base Relations on Uranium Deposition in Cat Kidney*

Diet	Infusion fluid	Percentage of dose found in kidneys	Percentage of dose found in urine
Normal	0.85% bicarbonate	0.8 at 5 hr	66
Normal	0.85% saline	3.3 at 8 hr	19.5
Acid (NH_4Cl)	0.85% saline	48.0 at 4 hr	14
Acid (NH_4Cl)	0.85% saline	72 at 8 hr	7.9

*These data were obtained from individual animals, each injected with 3.37 mg U/kg as uranyl acetate (anhydrous).

storage will be discussed in a later section. Data illustrating the effect of acid-base relations on uranium deposition in the kidney are presented in Table 11.6. A detailed discussion of the relationship between alkali reserve and uranium storage in the kidney is found in Chap. 14.

Location of Uranium within Kidney. Two studies were conducted on the histological location of the uranium that deposits in the kidney. Anders and Hutchens studied the problem by means of tissue-staining techniques. W. F. Neuman and Wills employed a radioactive isotope of uranium to obtain autoradiographs of the uranium distribution in the kidney tissue.

The two methods of study, uranium stain and autoradiograph, gave complementary results. The autoradiograph technique proved to be a sensitive indicator of the uranium deposited in the kidney. Definition, however, was poor; exact histological detail was lacking. With the staining technique, only areas having a high concentration of uranium could be studied, but histological detail was present.

For the autoradiograph studies, rats were given a fatal dose of uranyl nitrate hexahydrate (5 mg/kg), containing U 232 equivalent to a dose of 2 microcuries/kg, and sacrificed at intervals of from 1 to 6 hr after the injection. Immediately after sacrifice of the animals, kidney slices were taken, placed in alcohol or alcohol-chloroform mixtures, and, after 1 day of fixation, transferred to xylol and embedded in paraffin. The method of fixation and embedding was shown by fluorometric analysis to remove insignificant quantities of uranium from the slices. Sections of from 5 to 10 μ were made and fixed to glass slides. The dried slides were placed section side down on the emulsion of a Wratten metallographic plate. After an exposure of

from 10 days to 3 weeks the plates were developed fully. The sections on the slides were stained with hematoxylin and eosin for maximum color contrast.

Figure 11.5 illustrates the results obtained, showing separate prints of the stained section, the autoradiograph, and the picture obtained by

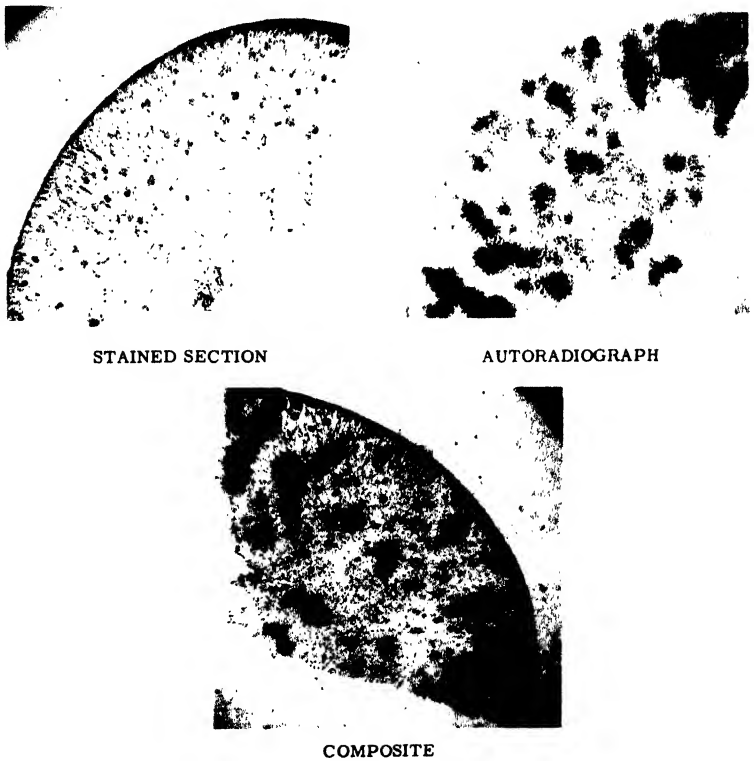


Fig. 11.5 — Autoradiograph and stained section showing the distribution of uranium in the kidney.

superimposition. Examination of the group of composite pictures obtained from the kidneys of four rats treated as described above has led to the conclusion that, although some of the uranium is distributed quite generally throughout the kidney, most of it tends to be concentrated in localized areas. There seems to be no definite correlation between the loci of the metal and structural elements of the kidney, although the metal rarely was deposited in glomerular regions.

Two methods of staining were employed: direct color development of the uranium with ferrocyanide[15] and the indirect use of uranium as

a mordant for hematoxylin.[16] A comparison of results of the studies of kidneys stained for uranium with the results of fluorophotometric analyses indicated that consistent staining occurred only if the uranium concentration in the kidney was greater than 48 μg/g. Since these methods were rather insensitive, good results were obtained only when large doses were administered and then only for short periods after administration.

Following intraperitoneal injection of uranyl nitrate hexahydrate in rats in doses of the order of 50 mg/kg, a positive stain for uranium in the kidney may be obtained as early as 20 min after the injection. The uranium first appeared at the lumen edge of the epithelial cells of the (scattered) proximal convoluted tubules in the outer and midzones of the cortex. Most of the material remained in this location for several hours, although in a few cases it appeared in debris in the tubular lumina as early as 40 min after the injection. The results of examination of animals 1 to 3 days after the injection were somewhat varied; in some, stainable uranium occurred almost entirely in the lumen edge of tubular epithelium, and in others it was entirely in casts in the collecting tubules.

Doses of this order (50 mg/kg) caused degenerative changes detectable with hematoxylin and eosin stain as early as 1½ to 2 hr after injection. These degenerative changes were in the same zone of the kidney as that in which the stainable uranium was first observed. Not all tubules showing a positive uranium stain showed degeneration of the epithelial cells, nor did all degenerating tubules show a positive stain for uranium. In many cases, however, the two occurred in the same tubule. After the first few hours following injection, the damage to the tubules became very extensive, involving much greater areas of the cortex than that which showed a positive uranium stain. By the time tubular destruction reached a maximum, the uranium content began to decline as shown by staining.

3.5 Deposition in Liver. Uranium was never found to accumulate in the livers of animals injected with uranyl compounds. There was practically no deposition in the liver whether the dose was administered orally, intraperitoneally, intravenously, or by inhalation. As an illustration, the data from intravenously injected rats are presented in Table 11.7. The total uranium present varied from 0.1 to 0.9 per cent of the dose.

Other animal species gave similar results. The liver of a dog contained less than 0.2 per cent of the 22.5 mg of uranium administered intravenously 3 hr previously. Rabbits were also examined with similarly negative results.

It was found later that tetravalent uranium, in contrast to the hexavalent form, was deposited in the liver and spleen when injected into an animal. It is possible that the divergence of reports in the literature (see Bibliography, references 3, 4, 6, and 7) regarding the deposition of uranium in the liver can be attributed to a lack of control of the valence state of the uranium compounds injected, as well as to inaccuracies in analytical technique.

Table 11.7 — Uranium Content of Livers of Rats Given Uranyl Nitrate Hexahydrate Intravenously

Time after administration, hr	Uranium concentration in liver	
	Males, μg U/g	Females, μg U/g
0.75	0.5	0.6
2.5	0.5	0.3
8	0.3	
12	0.3	0.6
24	0.3	
48	0.4	0.5
72	0.6	0.6
96	0.3	0.5
120	0.3	0.4
288	0.6	0.1
480	0.1	
960	0.1	0.1

3.6 Deposition in Soft Tissues. In this category all soft tissues except kidney are considered. Within 45 min after administration large quantities of uranium (as much as 19 per cent of the amount given)

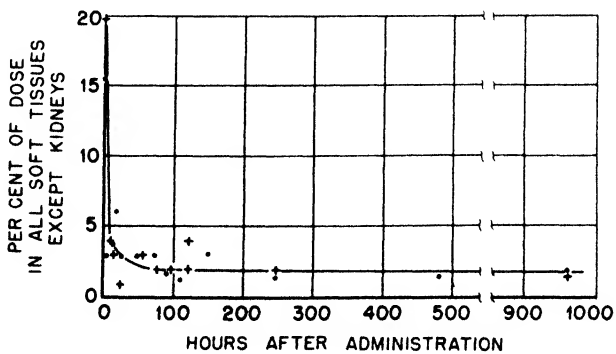


Fig. 11.6 — Relative absence of uranium in soft tissues of rats; values obtained from males are represented by dots, from females by plus signs.

were found in the soft tissues of rats. At this time there were considerable quantities of uranium in the blood. Later, concurrent with the fall in blood uranium, the soft-tissue content fell to a very low concentration (1 to 2 per cent of the dose) and remained low as long as 40 days after administration. Results are presented graphically in Fig. 11.6.

It is probable that the large quantities of uranium found in soft tissues immediately after injection represented that which was in the extracellular fluid, and, as the uranium was rapidly removed from the circulation by bone and kidney, the concentration in soft tissues fell rapidly. No significant fixation of uranium was noted in any of the soft tissues examined. The tissues studied were urinary bladder, spleen, gonads, heart, lungs, stomach, intestines, skin and hair, and muscle.

3.7 Deposition in Bone. None of the previously reported studies (see Bibliography, references 1 to 9) of uranium distribution dealt with deposition in bone. Tannenbaum reported at a Manhattan Project meeting in 1944 that the bones of mice fed uranium salts contained

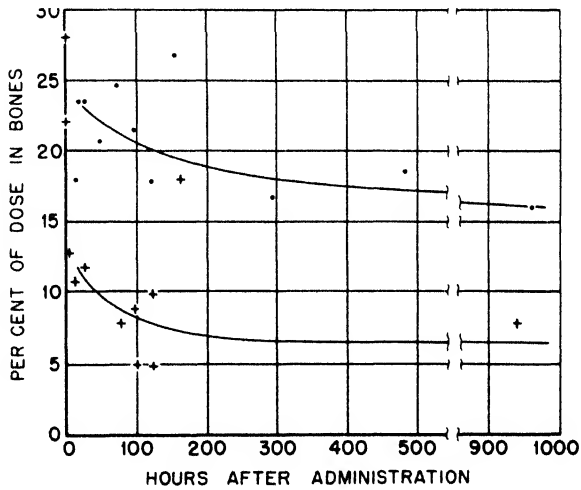


Fig. 11.7—Relationship between total bone storage of uranium and time after administration; values obtained from male rats are represented by dots, from female rats by plus signs.

some of the metal. It was found in the present experiments that, from the long-term point of view, nearly all the uranium retained by rats was stored in the bones. These data are presented in Fig. 11.7.

Forty days after administration, 80 to 90 per cent of the uranium still in the rat was located in the calcified structures. The greatest concentration was noted shortly (2½ hr) after the dose was administered. There was only a slight decrease in the quantity of uranium retained by the bones during the 40 subsequent days.

One of the striking results was the discovery that a consistently greater amount was stored in bones of males than of females. This suggested that some physiological factor was involved which warranted further study. The rats used weighed 200 g each. At this weight the average male rat is 9 weeks of age, the average female 16 weeks. It was therefore questionable whether the physiological factor was related to sex or age. M. W. Neuman conducted a series of experiments designed to elucidate this physiological factor and to learn something of the mechanism by which uranium is deposited in bone.

(a) The Effect of Age on Bone Deposition. The bones of females at this weight (200 g) have reached a more advanced stage of ossification and presumably have a less extensive circulation than those of males. This suggested that the difference in uranium deposition might depend on the availability of uranium to the bone substance, i.e., less uranium was available to bone in the female because of the decreased blood supply. This hypothesis was borne out by the differences in uranium content that were found in different parts of the skeleton. The vertebras, which have a high proportion of cancellous bone and presumably a greater area of bone in contact with the circulation, showed the greatest affinity for uranium, whereas the skull, which has a high proportion of dense bone, showed the least affinity for uranium. The relative uranium concentrations of various parts of the skeleton are tabulated in Table 11.8.

Table 11.8 -- Comparison of Uranium Deposition in Different Parts of the Skeleton

Sex	No. of animals	Uranium in bone ash, μg/g					
		Skull	Tibia, fibula, radius, and ulna	Femur and humerus	Rib	Pelvic girdle	Vertebra
Male	13	14.4	21.0	22.4	23.9	24.5	28.3
Female	11	9.4	8.6	9.7	10.3	9.4	13.1

If the circulation hypothesis is correct, young male and female animals of the same weight that are similar in age and whose bones are equivalent with respect to ossification should show no sex difference in the uranium deposition in bones. Furthermore, since the process of ossification is very active and the circulation extensive in the bones

of young animals, there should be a greater uptake of uranium in their bones than in those of adults. The bones of rachitic animals should also have an increased circulation and a greater uranium deposition. These hypotheses were tested.

Young litter-mate male and female rats from 3 to 6 weeks old were placed on the synthetic diet for 4 or 5 days and then injected intraperitoneally with 2.5 mg of uranium as $UO_2(NO_3)_2.6H_2O$ per kilogram (same dose as given to adults). From 2½ to 48 hr after injection they were killed and their femurs and humeri and occasionally skulls and

Table 11.9–Uranium Content of Combined Femur and Humerus of Rats of Various Ages

Group*	Uranium content of ash, μg/g					
	Analysis 1	Analysis 2	Analysis 3	Analysis 4	Analysis 5	Average
Weanlings:						
Males, 46 g	20	57	51†	63†		52.3
Females, 38 g	62	46	37†	62†		50.6
Sexually mature:						
Males, 114 g	35†	35†	30†			33.1
Females, 103 g	40†	38‡	26†	28†		32.6
Adult:						
Males, 200 g	35	22	12	25	26	23.8
Females, 200 g	5.9	10	15	11		10.4

*Weights given are average body weights.
†Three animals.
‡Two animals.

Table 11.10—Uranium Content of the Skull and Vertebras of Young and Adult Rats

Group*	Uranium content of ash, μg/g†					
	Analysis 1	Analysis 2	Analysis 3	Analysis 4	Analysis 5	Average
Weanlings, skulls:						
Males		29	20*			22.4
Females	33	32	27*			29.1
Adults, skulls:						
Males	13	13	16	14	18	15.8
Females	12	8	10	10		10
Weanlings, vertebras:						
Males	22	58	74*			60.4
Females	78	52	59*			61.4
Adults, vertebras:						
Males	36	23	36	28	25	29.5
Females	15	16	17			15.8

*Three animals.
†Figures are from single analysis of material pooled from three animals.

vertebras were removed and analyzed. In some cases the bones of three animals were pooled before analysis.

The results of the analyses of the bones of normal animals are listed in Tables 11.9 and 11.10.

It is clearly evident, even with this small group of animals, that the bones of weanlings had a significantly higher uranium concentration and therefore retained a greater percentage of the dose than the bones of adults. Perhaps this explains the fact observed by MacNider[17] that, to obtain a given kidney injury, young animals must be given a larger dose (on a body-weight basis) than adults. Furthermore no sex difference in uranium deposition was observed in the weanling animals.

The immature endocrine balance of the weanlings might have influenced the above-mentioned results. Therefore the long bones of rats that had just reached sexual maturity were also studied. The bones of these sexually mature animals (Table 11.9) had a uranium concentration intermediate to those of weanlings and adults. Again no sex difference was apparent.

Two 11-week-old female rats that had been fed a rachitogenic[18] diet for 2 months were injected with the same dose (2.5 mg U/kg) and sacrificed 2½ hr later. The results are recorded in Table 11.11.

Table 11.11 — Uranium Content of the Bones of Rachitic Rats

Animal wt., g	Bone	Uranium concentration	
		μg/g of ash wt.	μg/g of wet bone wt.
93	Femur and humerus	48.5	6.8
	Vertebras	71.3	5.5
	Skull	23.8	5.5
76	Femur and humerus	40.8	4.6
	Vertebras	100	6.2
	Skull	15.7	3.6

These rats were extremely rachitic. The ash content of their long bones was 11 to 15 per cent as compared with 40 to 50 per cent in the long bones of normal females of the same age. The uranium content was greater in the rachitic rats than in normal animals of the same age and about the same as that in weanlings.

These results were confirmatory evidence for the hypothesis that variation in the uranium content of different bones is due to the variation in the extent of the circulation. This is based on the assumption that there is a more effective circulation in young bones than in old, in vertebras than in long bones, in long bones than in skull. No data on the relative circulation of different bones could be found in the literature. Therefore these assumptions could not be tested.

In any case, other explanations do not adequately explain the observed results. For example:

1. Large amounts of uranium found in bones having a good blood supply cannot be attributed to the blood content per se, because the blood is practically uranium-free as early as 2½ hr after intravenous administration.

2. Differences in uranium deposition in various bones cannot be attributed to differences in the rate of bone growth because the deposition takes place in about 45 min, a period of time in which the actual accretion of bone substance is negligible.

3. It might be assumed that much of the uranium deposited in bone is taken up by the organic matrix, and, since there is a greater proportion of organic material in the bones of young than of adult rats, more uranium would be found per gram of ash. However, this assumption would not explain the differences observed in the deposition of uranium in bone of adult male and female rats, since there was little if any difference in the proportion of organic to inorganic material found in the bones of these animals.

(b) Effect of Acid-Base Balance on Bone Storage. As mentioned above, the administration of alkali to uranium-injected animals resulted in an increased urinary excretion and a decreased kidney deposition. In some of the animals given alkali and uranium simultaneously, as much as 88 per cent of the dose could be recovered in the urine. It is obvious in such cases that only a very small portion of the dose could have been stored in the bones. It remained to be determined experimentally whether alkali administration prevented deposition of uranium in the skeletal system.

M. W. Neuman conducted the following experiment to test the effect of acid-base balance on the deposition of uranium in bone. Littermate rats (120 to 160 g of body weight) were divided into four groups of nine each. Animals in Group 1 were given fox chow and water ad lib. throughout the experimental period. Animals in Group 2 were given the same treatment as those in the control group except that they received sodium bicarbonate by stomach tube (0.5 g/kg twice a day) on the second, third, and fourth days after the uranium injections. Group 3 was fed fox chow containing 0.5 per cent sodium bicarbonate continuously from 1 week before to 1 day after the uranium injection. Thereafter, animals were given the regular fox chow ration. Group 4 was fed fox chow containing 0.3 per cent ammonium chloride from 1 week before to 1 day after the uranium injection. Thereafter, these animals too received unsupplemented fox chow. Animals in all four groups were given 2.5 mg U/kg as $UO_2(NO_3)_2.6H_2O$ intraperitoneally

and sacrificed on the fifth day after the injection. The femurs were taken for analysis. Specimens from three animals were pooled. The analytical results are presented in Table 11.12.

Table 11.12 — Effect of Acid-Base Relations on Storage of Uranium in Bone

Group No.	Preinjection supplement	Postinjection supplement	μg U/g of bone ash	Av. μg U/g of bone ash
1a	None	None	10	20
1b	None	None	24	
1c	None	None	26	
2a	None	$NaHCO_3$ by stomach tube	21	14
2b	None	$NaHCO_3$ by stomach tube	9	
2c	None	$NaHCO_3$ by stomach tube	12	
3a	$NaHCO_3$ in diet	None	13	18
3b	$NaHCO_3$ in diet	None	20	
3c	$NaHCO_3$ in diet	None	19	
4a	NH_4Cl in diet	None	17	16
4b	NH_4Cl in diet	None	13	
4c	NH_4Cl in diet	None	18	

The data do not indicate a clear-cut relationship between acid-base relations and storage of uranium in bone. Although the uranium concentration in the bones of animals in all three experimental groups averaged lower than the concentration found in the bones of the control animals, none of the differences was significant.

3.8 General Summary. Within the limits of the data at hand, a provisional general synthesis of the fate of hexavalent uranium in the animal body may be made. A simplified diagram of the events that presumably take place has been constructed in Fig. 11.8.

1. Uranium enters the blood via all routes of administration.

2. In the blood stream, uranium exists mainly in two forms: a non-diffusible complex with the plasma proteins and a diffusible complex with bicarbonate. Both forms are reversibly in equilibrium with each other (see Chap. 14).

3. The bicarbonate-uranium complex enters the extracellular fluid bathing the soft tissues (a), but it either does not penetrate the cells or, if it does, is not "fixed." It therefore reenters the circulation (b).

4. The bicarbonate-uranium complex is filtered (c) by the kidney glomerulus. If the blood level of bicarbonate is increased there is a relatively greater proportion of the uranium present as the diffusible bicarbonate complex; therefore the rate of filtration of uranium is

increased. If the alkaline reserve is high and a significant concentration of bicarbonate remains in the urine leaving the kidney, little uranium will combine with the protein surfaces (f) of the tubules; most will be eliminated (d) or reabsorbed (e). Conversely, if all the

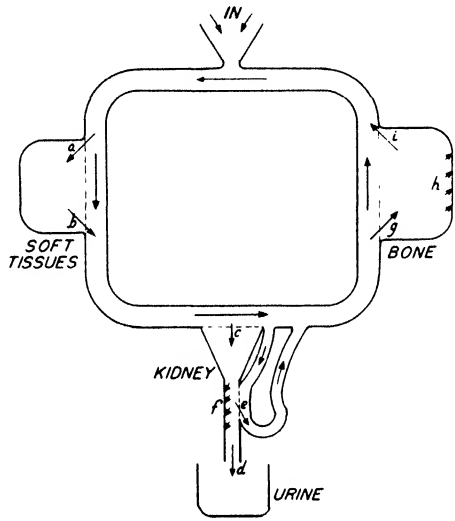


Fig. 11.8 — Schematic diagram showing the pathways taken by uranium as it leaves the circulation.

bicarbonate is reabsorbed by the renal tubules, more of the filtered uranium will "stick" (f) to the tubules, thereby damaging cell surfaces; less will be excreted and less reabsorbed.

5. At the same time these events are taking place in the kidney, the bicarbonate-uranium complex filters (g) into the fluid bathing the bone. The mechanism whereby uranium is deposited in the bone (h) is not clear. That which is not deposited reenters the circulation (i).

6. In alkalosis the distribution is shifted; less uranium is deposited in the kidney, more is excreted in the urine. In acidosis more uranium is deposited in the kidney, less is excreted in the urine.

7. Young animals, because of the extensive circulation throughout the skeleton, deposit more of the dose in the bone system than do adults.

4. THE DISTRIBUTION AND EXCRETION OF TETRAVALENT URANIUM

4.1 Introduction. From the toxicological standpoint, the pharmacology of tetravalent uranium is less important than the pharmacology

of hexavalent uranium. Primarily this difference in pharmacological importance is due to the difference in chemical properties of the two valence forms. Because of its protein affinity (discussed in Chap. 13), U_4 cannot pass a semipermeable membrane. It is believed, therefore, that little uranium in the tetravalent state can enter the animal body except by direct injection. Accordingly, for purposes of comparison, only a preliminary investigation was made of the distribution and excretion of uranium in rats following the injection of the tetravalent form.

In the experiments with uranyl salts, data from an animal receiving a faulty injection or giving poor recovery were discarded, and the experiment repeated. In the experiments with the reduced tetravalent ion, no such selection of data was made. These results, therefore, cannot be considered accurate in a quantitative sense, although they illustrate well the qualitative differences between the two common valence forms of uranium in regard to distribution and excretion. UCl_4 was administered intravenously with bisulfite in an acetate buffer solution, as described above at approximately the same dose level as in the U_6 experiments (1.5 to 3.0 mg U/kg).

4.2 Disappearance of Uranium from the Blood. Tetravalent uranium leaves the blood stream rapidly but not so fast as does the hexavalent form. Blood values obtained at varying time intervals after

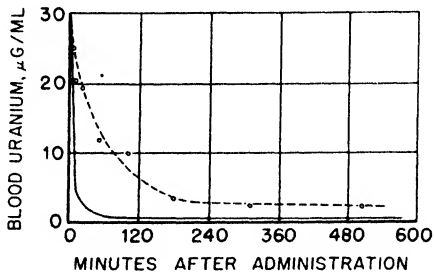


Fig. 11.9—Rate of disappearance of blood uranium, U_4 vs. U_6. The broken line indicates the results obtained after the administration of U_4.

administration of U_4 to a rabbit are presented in Fig. 11.9. Rats also showed a rapid removal of blood uranium as the data in Table 11.13 indicate. The blood level of U_4 (1.2 μg/ml) 7 hr after administration was 10 times that of U_6 (0.1 μg/ml). Even though significant quantities of tetravalent uranium were left in the blood at this time, more than 90 per cent of the dose had been removed.

4.3 Excretion. In contrast to the rapid urinary excretion of hexavalent uranium, only a small part of the dose (about one-tenth) of U_4

appears in the urine in the first 24 hr after injection. Also in contrast to the behavior of hexavalent uranium, considerable amounts (as much as 20 per cent) were found in the feces.

The data on the U_4 excretion of rats are listed in Table 11.14. About 9 per cent of the dose was found in the urine during the first 24 hr

Table 11.13 — Variation of Blood Tetravalent Uranium Level with Time (Rats)

Time after administration, days	Blood-uranium level, μg/ml	
	Males	Females
0	36*	36*
0.1	14.7	4.2
1.0	0.1	0.1
5	0.1	0.0
10	0.0	0.1
20	0.0	0.0
44		

*Initial value was calculated on the assumption that the whole injected amount was immediately distributed uniformly in the blood volume and that blood volume in the rat is 7 per cent of its body weight.

Table 11.14 — Excretion of Tetravalent Uranium by Rats

Time of sacrifice, days after injection	Percentage of injected uranium		
	In urine		In feces, total
	First 24 hr	Total	
Male			
0.1		16	
1.0	14.5	14.5	1.5
5.0	4.1	7.1	15
10	10.8	26.6	11.7
20	7.0	25.6	21.5
44	8.0	27.0	19.3
Female			
0.1		15.6	1.5
1.0	7.2	7.2	1.8
5.0	12	18	5.3
10	8.8	20	8.7
20	5.8	21	16
44	8.4	27	

after administration; thereafter, significant quantities continued to be excreted for the 40-day period. Fecal excretion was almost negligible (1.5 to 1.8 per cent of the dose) during the first 24 hr. On the second, third, and succeeding days, the amounts of uranium found in feces increased considerably. In fact, 20 to 40 days after the injection the proportion of the dose that was excreted via the intestine was almost as great as that excreted via the urinary tract.

4.4 Deposition in Kidney. Tetravalent uranium deposits rapidly in the kidney. The data obtained on rat kidney are presented in Fig. 11.10. The data are too variable to show conclusively that a smaller proportion of the dose of U_4 than U_6 is deposited, but this is indicated. Following the first day after injection, the concentration of uranium in the kidney decreased markedly and reached a very low level by the end of 44 days.

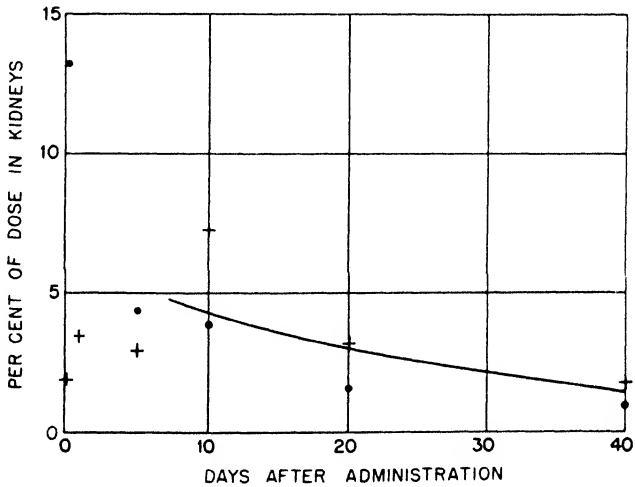


Fig. 11.10—The deposition of U_4 in rat kidney; values obtained from male rats are represented by dots, from female rats by plus signs.

Experiments described in Chap. 14 suggest that only small quantities of U_4 can be filtered by the kidney glomerulus. It is therefore not clear why such a large portion of the injected dose should be fixed by the kidney. Possibly some of the uranium injected in the U_4 form is oxidized in the circulation. The oxidized portion would be filtered by the glomerulus and fixed in part by the kidney tubules.

4.5 Deposition in Tissues Other Than Kidney. The distribution of U_4 was found to be quite similar to that of U_6 in some respects; there was practically no deposition in soft tissues, and a large proportion

of the dose was found in the skeletal system. However, one marked difference was noted in the behavior of the two valence forms. Unlike U_6, the tetravalent uranium accumulated in the livers of the injected animals. The data obtained on rats are summarized in Table 11.15.

Table 11.15—Deposition of U_4 in Rat Tissues Other Than Kidney

Time after administration, days	Percentage of injected uranium		
	Liver and spleen	Soft tissues	Skeleton
	Males		
0.1	33	25	13
1.0	19	5.8	11
5	31	7.9	13
10	27	1.2	16
20	9.5	1.6	10
44	4.5	0.5	8.6
	Females		
0.1	18.4	7.8	13
1	51	1.3	6.4
5	36	2.0	11
10	22	2.8	23
20	12	0.8	8.6
	9.1	1.5	7.1

It is evident that a large portion of the dose (as much as one-half) was found in the liver and spleen shortly after the injection. From the fifth to the forty-fourth day after administration the uranium content of the liver diminished quite markedly. It is likely that a large part of the dose initially deposited in liver and spleen was excreted in the feces. Increased fecal excretion of uranium began at about the same time the liver content began to diminish. During the period that uranium was mobilized from the liver, urinary excretion amounted to only 5 to 10 per cent of the dose.

Tetravalent uranium was deposited rapidly in bone. As in the case of U_6 injections, there was a slight, if significant, decrease in the uranium content of bones with increasing time after administration.

There appeared to be no appreciable deposition of the tetravalent uranium in soft tissues other than liver, spleen, and kidney.

4.6 Comparison of the Distribution and Excretion of U_4 and U_6. A general comparison of the distribution and excretion of U_4 and U_6 is presented in Table 11.16.

Table 11.16 — Comparison of the Distribution and Excretion of U_4 and U_6

Tissue	Hexavalent uranium	Tetravalent uranium
Kidney	Approximately 20% of the dose deposited immediately followed by a mobilization to urine	Approximately 10% of the dose deposited immediately, followed by mobilization, possibly to urine
Liver	No significant deposition	Significant deposition (as much as 50%) followed by mobilization, possibly to feces
Bone	Approximately 10 to 30% of the dose deposited immediately followed by a slow if significant mobilization	Approximately 10 to 20% of the dose deposited immediately followed by a slow if significant mobilization
Urine	Rapid (24-hr) excretion of large proportion of dose (50%) followed by continued excretion of small amounts	Rapid excretion of small proportion (10%) of dose followed by continued excretion of small amounts
Feces	No significant excretion at any period following injection	Significant excretion on the 2d, 3d, and and 4th days after injection. Continued excretion of small amounts

BIBLIOGRAPHY

1. A. Rabuteau, "Eléments de toxicologie et de medicine legale," 2d ed., p. 849, Paris, 1867.
2. R. H. Chittenden and A. Lambert, Trans. Conn. Acad. Arts and Sci., I: viii (1888-1892).
3. H. T. Karsner, S. P. Reimann, and S. C. Brooks, J. Med. Research, 39: 169 (1918).
4. H. Eitel, Arch. exptl. Path. Pharmakol., 135: 188 (1928).
5. W. C. Hunter, Ann. Internal Med., 1: 747 (1928).
6. W. Straub, Arch. exptl. Path. Pharmakol., 138: 134 (1928).
7. H. D. Jones and R. Goslin, Am. J. Physiol., 105: 693 (1933).
8. R. L. Holman and W. A. Douglas, Proc. Soc. Exptl. Biol. Med., 57: 72 (1944).
9. G. E. Gustafson and S. Koletsky, Arch. Internal Med., 74: 416 (1944).
10. J. Hoffmann, Biochem. Z., 311: 247 (1942).
11. P. B. Hawk and B. L. Oser, Science, 74: 369 (1919).
12. L. R. Kuhn, Science, 93: 504 (1941).
13. W. F. Neuman and B. Mulryan, Univ. Rochester, Atomic Energy Project Rept. M-1691 (1944).
14. W. MacNider, J. Exptl. Med., 23: 171 (1916).
    G. L. Donnelly and R. L. Holman, J. Pharmacol. Exptl. Therap., 75: 11 (1942).
15. W. Gerard and C. Cordier, Arch. biol., 43: 367 (1932).
16. R. Krause, Enzyklopaedie der mikroskopischen Technik, Vol. II, p. 994, 1926-1927.
17. W. MacNider, Physiol. Revs., 4: 599 (1924).
18. Pharmacopoeia of U.S., 12th revision, p. 636, Mack Printing Co., Easton, Pa., (1942).

Chapter 12

TOLERANCE TO URANIUM COMPOUNDS

By Frances L. Haven*

Tolerance may be defined as the ability to endure or resist the toxic action of a chemical. Mortality constitutes an adequate measure of tolerance to uranium. Tolerance may be either natural or acquired. The term "resistance" is sometimes used instead of tolerance, and "susceptibility" for a lack of natural tolerance. Natural tolerance should not be referred to as "immunity," since this term implies a special kind of resistance to infectious diseases. Tolerance acquired by repeated sublethal doses of uranium may be measured by the survival of the animals following the administration of a dose that is fatal to normal control animals. Within this chapter, such an acute dose of uranium will be referred to as the "test dose." Although other criteria of tolerance to uranium might be used — e.g., changes in body weight, catalase excretion, retention of urea nitrogen, nonprotein nitrogen, and creatinine — mortality response is the only criterion employed in this chapter.

1. SURVEY OF THE LITERATURE

1.1 Introduction. Although the early literature dealing with experimental nephritis produced by uranium contains no specific studies of tolerance, the phenomenon of tolerance is suggested or mentioned in several reports. During the frequent prescription of uranyl nitrate as a supposed remedy for diabetes, physicians observed that larger doses were necessary after temporary cessation of therapy. In an investigation of renal secretion Suzuki[1] in 1912 increased the dosage of uranium in guinea pigs and was the first to point out the decrease

*Work done by Ruth Crossland, Frances L. Haven, Challiss Randall, Virginia Edwards, and Joyce O'Leary.

in sensitivity of regenerated tubular epithelium to repeated uranium poisoning.

1.2 First Study of Tolerance. The first investigator to study tolerance as such was Gil y Gil[2] in 1923-1924. As the result of Suzuki's observation that regenerated tubular epithelium became less sensitive to uranium upon repeated injections, Gil y Gil thought that repeated administration of sufficiently small quantities of uranium might cause the renal epithelium to tolerate doses larger than the lethal. Tolerance in repeatedly poisoned animals might mean, according to Gil y Gil, that uranium no longer passed through the kidney but was excreted by other channels. By employing the ferrocyanide method of analysis and by observing typical uranium kidneys in mice injected with urine of uranium-treated rabbits, Gil y Gil was led to discard his hypothesis because his data showed that a given dose of uranium was excreted rather rapidly in the urine of an animal that had received previous smaller doses of uranium.

1.3 Study of Tolerance Based on Kidney Histology. In a comprehensive study, Hunter[3] sought to prove that the rabbit's renal epithelium acquired resistance to uranyl nitrate. The distal and proximal segments of the convoluted tubule of the kidney were established as the site of injury by uranium. The smallest doses of uranyl nitrate that would kill the original renal tubular cells but not the animal were determined, and the time necessary for complete regeneration of epithelium was established as 14 days. Hunter then started with the doses that killed renal cells and doubled these doses every 14 days until from 16 to 40 times the lethal dose had been given subcutaneously, and in other rabbits, from 9 to 40 times the lethal dose intravenously. The kidney tubules of these animals contained uninjured regenerated epithelium or tolerant cells. By employing the biological method used by Gil y Gil, Hunter found that the peak of excretion of uranium in an acutely poisoned animal was on the third day, whereas in a tolerant animal given the same dose it was on the first and second days. He suggested that in acute poisoning the uranium combined with the protein of the kidney tubule to delay excretion, whereas in a tolerant animal the uranium did not combine with protein of the regenerated epithelium and therefore passed more rapidly into the urine.

MacNider[4] maintained that repair of tubular epithelium after injury by uranium occurred by two processes: (1) a regeneration of convoluted tubule cells that had not been too severely injured by uranium and (2) an ingrowth of cells in the terminal portion of the proximal convoluted tubule or from the upper end of the descending limb of

Henle's loop. The first type of epithelium had no resistance to subsequent injections of uranium; the second type was cytologically different from normal convoluted tubular epithelium in that it was atypical and flattened and was resistant to a second injury from uranium, even in double the amount of the first. Somewhat later MacNider[5] also presented evidence for a resistant type of epithelium in the liver of tolerant animals.

1.4 Biochemical Studies of Tolerance. According to MacNider[4] the kidney that had regenerated with the atypical flattened epithelium exhibited resistance to a second injection of uranium. There was less depletion of the alkali reserve of the blood, better phenolsulphonphthalein elimination and less retention of urea nitrogen, nonprotein nitrogen, and creatinine than in the case of the kidney which had not regenerated with this type of epithelium. Garnier and Marek[6] noted less retention of urea in rabbits following each new injection of uranium while albuminuria and glycosuria became more marked. According to these investigators, death from massive doses of uranium occurred from hepatic lesions and hypoglycemia rather than from nephritic retention of urea.

2. NATURAL TOLERANCE

Since the determination of the natural tolerance of an animal for uranium is in this work the determination of the toxicity based on mortality response, detailed data on mortality have not been included in this section but will be found in the chapters on toxicity (Chaps. 6, 7, 8, and 10). The degree of toxicity of a compound for an animal varies inversely as the natural tolerance of the animal. Comparison of the mortality response to a given dose of uranium in animals of different species, sexes, and ages indicates the relative degree of natural tolerance to this substance.

2.1 Species Differences. Doses of uranium salts known to be lethal to one species of animal may not be lethal to a second species. Such differences depend on many factors, most of which are unknown. Change of the route of administration of uranium may change the order of species susceptibility to a given compound.

(a) Parenteral Route. Uranyl nitrate has been administered both intraperitoneally and intravenously to several species of animals, and the mortality response has been obtained.

Intraperitoneal. The approximate LD_{50}'s expressed as milligrams of uranium per kilogram and based on the mortalities obtained within 3 weeks after doses of 5 to 8 mg of uranyl nitrate hexahydrate per

kilogram in adult male rats and 5 mg of uranyl nitrate hexahydrate per kilogram in adult female rats were 2.5 and 1, respectively (work of Haven, Table 6.3). Mortalities obtained on white mice given graded doses of uranyl nitrate indicated an approximate LD_{50} of 6 to 8 mg U/kg (work of Haven, Table 6.7). Thus the natural tolerance of the white mouse was found to be from three to four times that of the Wistar rat.

Additional evidence for the existence of a species difference came from consideration of the mortalities for C_3H mice given graded doses of uranyl nitrate. In work done by Haven (Tables 6.8 and 6.9) an approximate LD_{50} of 25 mg U/kg was indicated for male and female mice of this strain. Hence, the natural tolerance of the C_3H mouse was at least ten times greater than that of the Wistar rat. In addition to this species difference in natural tolerance, a strain difference also was found, since the C_3H mouse had from three to four times the natural tolerance of the white mouse.

Intravenous. Species differences in natural tolerance have been demonstrated by Orcutt (Table 6.10) after intravenous injections of uranyl nitrate. Rabbits, guinea pigs, Wistar rats, and white mice were given doses as low as 0.01 mg/kg and increasing serially by the factor of 1.67. The dose above which all animals of a species were killed and the dose below which no animals were killed were taken as indices of the relative susceptibility among the species. By this method the following order of increasing natural tolerance to intravenously injected uranyl nitrate was established: rabbits, guinea pigs, rats, and mice.

(b) Percutaneous Route. In work done by Orcutt (Table 8.2) animals given uranyl nitrate by the percutaneous route possessed various degrees of natural tolerance in increasing order according to species, as shown by the following ratios of the LD_{50}'s: rabbits, 1; rats, 8; guinea pigs, 36; and mice (white), 128. By this route as well as by the parenteral route, mice had far more natural tolerance to uranyl nitrate than rats.

(c) By Ingestion. When animals given 2 per cent uranyl nitrate hexahydrate in the diet (Maynard, Chap. 7, Sec. 8.4, Fig. 7.30) are arranged according to the highest levels tolerated for 30 days, the following increasing order of natural tolerance among species is obtained: rabbits, dogs, rats, and hamsters.

2.2 Age Differences. Age differences in natural tolerance have been established by mortality studies of acute and subacute toxicity. Determinations of the mortalities during the first 24 hr following the intraperitoneal administration of uranyl nitrate hexahydrate to rats indicate that toxicity is related to age rather than to body weight

(Hodge, Chap. 6, Sec. 2.1a). For example, the LD_{50}'s for 24 hr were 204 mg/kg for 200- to 300-g males and 135 mg/kg for females of the same weight. Males weighing 300 to 400 g, of the same age as the 200- to 300-g females, gave a comparable LD_{50}, namely, 128 mg/kg. An increase in toxicity with increasing age was established for uranyl nitrate, uranyl fluoride, and uranium tetrachloride. Thus, by the study of 24-hr mortality, a decrease in natural tolerance of the rat with increasing age was demonstrated.

When a dose of 5 mg of uranyl nitrate hexahydrate per kilogram was given to rats and the 2- and 3-week mortalities were determined, no difference between young and adult animals was apparent (Chap. 6, Sec. 2.6, Fig. 7.6).

As shown by Maynard (Chap. 7, Sec. 2.2a), there was a markedly higher mortality in adult rats (70 ± per cent) placed on a diet containing 2 per cent uranyl nitrate hexahydrate than in weanling rats (15 ± per cent) treated in the same manner.

2.3 Sex Differences. As pointed out in the preceding section, apparent differences in the 24-hr mortality following intraperitoneal administration of acute doses of uranyl nitrate to male and female rats are more correctly attributable to age than to sex (Chap. 6, Sec. 2.1a). With uranium tetrachloride buffered by sodium acetate the toxicity seemed to be less for females than for males of the same age.

Although sex differences in toxicity were not clearly indicated when acute doses of uranium salts were given, one might expect to find such differences following smaller doses. When such doses of uranyl nitrate were given to adult rats of both sexes, 2- and 3-week mortalities at the same dose level were higher among the females than the males. That age was not a factor was shown by the use of brother-sister litter-mate rats. Approximate LD_{50}'s in milligrams of uranium per kilogram were 2.5 for male rats and 1 for female rats. The same sex difference was obtained when young rats were used. Approximate LD_{50}'s in milligrams of uranium per kilogram were 2 for male rats and 1 for female rats.

When uranyl fluoride was administered to adult rats in doses ranging from 1.25 to 4.0 mg/kg, the females again were less resistant than the males. The approximate LD_{50}'s, in milligrams of uranium per kilogram, were 2.5 for males and 1 for females. Although the data are insufficient for definite conclusions, no sex difference was indicated for young rats given this compound.

In general, no sex difference in natural tolerance to uranium has been established in rats given acute doses. After smaller doses, however, male rats showed a greater natural tolerance than did female rats, as shown in the accompanying summary table.

Summary Table for Natural Tolerance

Age and species	Compound	Approximate LD_{50}, mg U/kg	
		Male	Female
Rats:			
Young	Uranyl nitrate	2	1
Adult	Uranyl nitrate	2.5	1
Adult	UO_2F_2	2.5	1
Mice:			
Albino	Uranyl nitrate	6–8*	
C_3H	Uranyl nitrate	25	20–24

*Mice of both sexes.

3. ACQUIRED TOLERANCE

Acquired tolerance to uranium may be produced by sublethal exposure by different methods of administration: parenteral, percutaneous, ingestion, or inhalation. Previous investigators have used parenteral administration, which has the obvious advantage that definitely known amounts of uranium are introduced into the animal.

3.1 Parenteral Route. Throughout this investigation tolerance has been produced by two methods; in both of these a soluble uranium salt was administered intraperitoneally. By the first method, doses that were approximately one-fifteenth the lethal dose were administered at short time intervals over a period of 3 weeks and were followed within 2 days by the test dose. By the second method, an initial dose amounting to about one-tenth the lethal dose was given, and this was followed at regular intervals, varying from 10 to 20 days, by doses each double the preceding dose. The first method was also used on rabbits by Garnier,[6] and the second was used by Hunter.[3]

(a) Method of Repeated Small Doses. Mortality Responses. Young rats of both sexes, either litter mates or matched by weight, were given 11 intraperitoneal injections of 0.33 mg of uranyl nitrate hexahydrate per kilogram every other day. The second day after injection of the last small dose, the test dose of 5 mg of this compound per kilogram was given intraperitoneally. Body weights were recorded at the time of each injection and daily thereafter until death or gain in weight occurred. The experiment was terminated 2 weeks after injection of the test dose.

From the mortality results shown in Table 12.1, it is apparent that, in female animals at least, the method of repeated doses produced tolerance to the test dose. The low mortalities for all the male rats

Table 12.1 — Mortality in Young Wistar Rats Following Intraperitoneal Injection of a Test Dose with and without Previous Administration of Small Doses of Uranyl Nitrate, $UO_2(NO_3)_2.6H_2O$

Group	Dose, mg/kg		No. of deaths per no. of rats	Mortality, %	Treatment
	Repeated	Test			
			Male		
1	0.33	5	0/10	0	
4		5	3/10	30	
9*	0.33	5	1/10	10	0.5% $NaHCO_3$ diet
10*		5	2/10	20	0.5% $NaHCO_3$ diet
8	0.33	5	1/10	10	
			Female		
1	0.33	5	4/10	40	
4		5	7/10	70	
9*	0.33	5	4/10	40	0.5% $NaHCO_3$ diet
10*		5	9/10	90	0.5% $NaHCO_3$ diet
8	0.33	5	2/10	20	

* Litter mates.

are explained by the fact, discovered later, that the dose of 5 mg of uranyl nitrate hexahydrate per kilogram was too low to constitute a true test dose; a dose of 6 mg/kg should have been used. It is interesting to note that rats on the 0.5 per cent sodium bicarbonate diet acquired tolerance.

Essentially the same technique was used to produce tolerance to uranium tetrachloride in young rats of both sexes (Table 12.2). The size of the repeated dose was 1.65 mg/kg and that of the test dose was 25.0 mg/kg. The mortalities following the test dose were 10 and 0 per cent for male and female rats, respectively, that received repeated doses, as compared with 90 per cent for both the groups that were not pretreated. Thus a marked tolerance to uranium tetrachloride had been produced.

Uranyl nitrate was given to adult litter-mate rats by the method of repeated doses (Table 12.3). The size of the small dose and the interval and the duration of dosage were the same as in young rats that received this compound. However, three of the groups of male rats received a test dose of 6 mg of uranyl nitrate hexahydrate per kilogram instead of 5 mg/kg. The experiment was terminated 3 weeks after administration of the test dose. When the mortalities following the test dose are compared for the groups that had received repeated doses and for those that had not had this treatment, it is evident that tolerance was produced in adult rats by this method.

Table 12.2—Mortality in Young Wistar Rats Following Intraperitoneal Injection of a Test Dose with and without Previous Administration of Small Doses of Uranium Tetrachloride

Group	Dose, mg/kg Repeated	Dose, mg/kg Test	No. of deaths per no. of rats	Mortality, %
		Male		
3	1.65	25	1/10	10
6		25	9/10	90
		Female		
3	1.65	25	0/8*	0
6		25	9/10	90

* Two rats died prior to injection of the large dose.

Table 12.3—Mortality in Adult Wistar Rats Following Intraperitoneal Injection of a Test Dose with and without Previous Administration of Small Doses of Uranyl Nitrate, $UO_2(NO_3)_2.6H_2O$

Group	Dose, mg/kg Repeated	Dose, mg/kg Test	No. of deaths per no. of rats	Mortality, %	Treatment
			Male		
11*	0.33	5	0/10	0	
13*		5	4/10	40	
12*	0.33	5	1/10	10	3-week interval between injection of last small dose and test dose; control, Group 13
36†	0.33	6	0/20	0	
38†		6	14/20	70	Underfed
40†		6	12/20	60	
43‡	0.33	5	0/13	0	
			Female		
11*	0.33	5	0/10	0	
13*		5	8/10	80	
12*	0.33	5	4/10	40	3-week interval between injection of last small dose and test dose; control, Group 13
37†	0.33	5	1/20	5	
39†		5	20/20	100	Underfed
41†		5	19/20	95	
44‡	0.33	5	2/15	13	

* Groups marked with asterisk were all from one litter.
† Groups marked with dagger were all from a second litter.
‡ Groups marked with double dagger were from a third litter.

Effect of Underfeeding. The administration of uranium salts, whether by ingestion or by injection, causes considerable loss of appetite and consequent loss of weight or failure to gain weight normally. The effect of underfeeding on tolerance was therefore studied. Accordingly, Groups 38 and 39 (Table 12.3) were underfed to the extent that the average body weights were comparable with those of the experimental Groups 36 and 37 during repeated dosage and at the time of administration of the large dose. Such underfeeding, instead of decreasing the mortality from the test dose, increased it slightly. In fact, deaths occurred among the male rats in the underfed group from 24 to 48 hr before those in the control group. Hence lowered food intake during repeated dosage was not a factor in producing tolerance.

Duration of Tolerance. In order to obtain information regarding the duration of tolerance the rats of Group 12 (Table 12.3) were not given the test dose (5 mg of uranyl nitrate hexahydrate per kilogram) until 21 days after termination of the repeated doses. Mortalities were 10 and 40 per cent for male and female animals, respectively, as compared with 0 per cent for both sexes (Group 11) when administration of the test dose was not delayed. Hence about one-half of the female animals had lost their acquired tolerance by 21 days after cessation of repeated dosage.

Eight of the twenty rats in Group 36 (Table 12.3) that survived the test dose of 6 mg of uranyl nitrate hexahydrate per kilogram as the result of preceding repeated small doses were given a second dose of 6 mg/kg 43 days later. One-half of the rats died 8 to 10 days after injection.

The results of these two experiments indicate that in the rat tolerance was not lost immediately after the termination of the period of repeated dosage. Also, male animals may retain their acquired tolerance longer than female animals. However, the possibility exists that the sex difference was due to the greater natural tolerance of the male animals.

Size of Small Dose Required for Production of Tolerance. After establishing the fact that rats acquired tolerance to uranyl nitrate hexahydrate by repeated injections of 0.33 mg/kg, an attempt was made to find the lower range of a repeated dose that would fail to induce tolerance. With this aim in mind, an experiment was carried out in which 20 adult female rats received repeated injections of 0.1 mg/kg followed by a test dose of 5 mg/kg (Group 68). The mortality within this group was 100 per cent as compared with 90 per cent for the litter-mate control group (Group 69). Thus the dose of 0.1 mg/kg was too small to cause the rats to acquire tolerance to the large dose.

A second experiment was performed in which 0.2 mg/kg was injected repeatedly into 20 adult female rats. The mortality following

the test dose of 5 mg/kg was 55 per cent as compared with 100 per cent for the litter-mate control group. Thus only part of the group had acquired tolerance.

The relationship between the mortality following an intraperitoneal test dose (5 mg/kg) of uranyl nitrate hexahydrate and the size of the preceding repeated intraperitoneal dose in adult female rats is shown

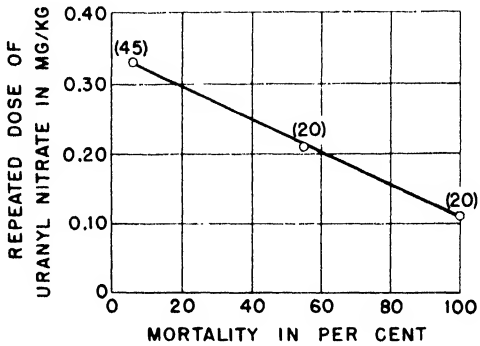


Fig. 12.1 — Relationship between the mortality following an intraperitoneal test dose (5 mg/kg) of uranyl nitrate hexahydrate and the size of the preceding repeated intraperitoneal dose in adult female rats. Solutions used for the two lower doses were analyzed for uranium by the fluorophotometric method. The number of animals represented by each point is given in parentheses.

in Figure 12.1. Data for mortality after the test dose following repeated doses of 0.33 mg/kg were taken from Table 12.3, Groups 11, 37, and 44. Figure 12.1 shows that the production of tolerance to an acute dose of uranyl nitrate hexahydrate is inversely proportional to the size of the preceding repeated small dose.

(b) Method of Increased Doses. Ten-day Interval between Doses. Adult litter-mate rats of both sexes were given an initial intraperitoneal dose of 0.5 mg of uranyl nitrate hexahydrate per kilogram. This dose was followed at 10-day intervals by intraperitoneal doses, each double the amount of the preceding dose. Body weights were recorded at the time of each injection and daily thereafter until death or gain in weight occurred. After a definite gain in weight was apparent, the animals were weighed approximately every other day until the next injection. Doses and mortality results are shown in Table 12.4.

No mortalities occurred in rats of either sex until administration of the dose of 8 mg/kg, which caused a mortality of 10.5 per cent for the males and 5 per cent for the females. The mortality following a

single dose of 8 mg/kg (Chap. 6) was 100 per cent for male rats and most assuredly would have been the same for the female rats, since doses of only 5 mg/kg gave mortalities varying from 70 to 100 per cent. Hence the method of increased doses at 10-day intervals produced tolerance in rats of both sexes.

Table 12.4 — Tolerance of Adult Rats to $UO_2(NO_3)_2.6H_2O$ Administered Intraperitoneally Every 10 Days in Increasing Doses

Dose		Males			Females		
			Mortality, %			Mortality, %	
$UO_2(NO_3)_2$.$6H_2O$, mg/kg	Uranium equiv.	No. of deaths per no. of rats	Original group	Survivors	No. of deaths per no. of rats	Original group	Survivors
0.5	0.24	0/20	0	0	0/20	0	0
1.0	0.47	0/19*	0	0	0/20	0	0
2.0	0.95	0/19	0	0	0/20	0	0
4.0	1.90	0/19	0	0	0/20	0	0
8.0	3.79	2/19	10.5	10.5	1/20	5	5
16.0	7.58	2/17	10.5	11.8	8/19	40	42.1
32.0	15.17	15/15	79.0	100	11/11	55	100

*One rat killed because of acute labyrinthitis.

After the dose of 16 mg/kg, the mortality from this and all preceding doses totaled 21 per cent for male rats as compared with 45 per cent for female rats. Thus the sex difference in toxicity of uranyl nitrate shown after a single dose (Chap. 6) and in the production of tolerance by the method of repeated doses (Table 12.1) was apparent when increased doses were given at 10-day intervals.

Twenty-day Interval between Doses. Adult litter-mate rats of both sexes (litter mates of the rats in the preceding section), were given an initial intraperitoneal dose of 0.5 mg of uranyl nitrate hexahydrate per kilogram. This dose was followed at intervals of 20 days by further intraperitoneal doses, each double the amount of the preceding dose. Body weights were recorded as described above. Doses and mortality results are shown in Table 12.5. By the time the dose of 8 mg/kg was given, 55 per cent of the male animals and 40 per cent of the female animals had died. Thus the 10-day interval between doses was better than the 20-day interval for production of tolerance to uranyl nitrate.

Fourteen-day Interval between Doses. The method of increased doses was used on adult rats to produce tolerance to uranyl fluoride. Adult male rats were given an initial dose of 0.25 mg/kg of this compound; adult female animals received an initial dose of 0.125 mg/kg.

Table 12.5 — Tolerance of Adult Rats to $UO_2(NO_3)_2 \cdot 6H_2O$ Administered Intraperitoneally Every 20 Days in Increasing Doses

Dose		Males			Females		
			Mortality, %			Mortality, %	
$UO_2(NO_3)_2 \cdot 6H_2O$ mg/kg	Uranium equiv.	No. of deaths per no. of rats	Original group	Survivors	No. of deaths per no. of rats	Original group	Survivors
0.5	0.24	0/20	0	0	1/20	5	5
1.0	0.47	0/20	0	0	0/19	0	0
2.0	0.95	2/20	10	10	0/19	0	0
4.0	1.90	9/18	45	50	7/19	35	36.8
8.0	3.79	3/9	15	33.3	4/12	20	27.3
16.0	7.58	5/6	25	66.7	6/7*	30	85.7

*One rat killed because of acute labyrinthitis.

Table 12.6 — Tolerance of Adult Male Rats to Uranyl Fluoride Administered Intraperitoneally

Increased-dose experiment						Single-dose experiment*	
Dose		Interval from last dose, days	No. of deaths per no. of rats	Mortality, %		No. of deaths per no. of rats	Mortality, %
				Original group	Survivors		
UO_2F_2, mg/kg	Mg U/kg						
0.25	0.19		0/20	0	0		
0.5	0.39	14	0/20	0	0		
1.0	0.77	14	0/20	0	0		
2.0	1.55	14	1/20	5	5.0		
4.0	3.09	14	2/19	10	10.5	19/20	95
6.0	4.64	14	2/17	10	11.8		
8.0	6.18	21	3/15	15	20.0		
10.0	7.73	14	2/12	10	16.7		
12.0	9.28	14	1/10	5	20.0		
14.0	10.82	15	1/9	5	11.1		
16.0	12.37	14	3/8	15	37.5		
18.0	13.91	14	1/5	5	20.0		
20.0	15.46	15	3/4	15	75.0		

*Each animal in this experiment was a litter mate of an animal in the increased-dose experiment.

Table 12.7—Tolerance of Adult Female Rats to Uranyl Fluoride Administered Intraperitoneally

Increased-dose experiment						Single-dose experiment*	
Dose		Interval from last dose, days	No. of deaths per no. of rats	Mortality, %		No. of deaths per no. of rats	Mortality, %
				Original group	Survivors		
UO_2F_2, mg/kg	Mg U/kg						
0.125	0.10		0/20	0	0		
0.25	0.19	14	0/20	0	0		
0.50	0.39	14	0/20	0	0		
1.0	0.77	14	0/20	0	0		
2.0	1.55	14	0/20	0	0		
2.5	1.93					20/20	100
3.0	2.32	14	0/20	0	0		
4.0	3.09	14	5/20	25	25		
5.0	3.87	14	2/15	10	13.3		
6.0	4.64	15	1/13	5	7.7		
8.0	6.18	14	3/12	15	25		
10.0	7.73	14	0/9	0	0		

* Each animal in this experiment was a litter mate of an animal in the increased-dose experiment.

These doses were increased at first by doubling each preceding dose every 14 days (Tables 12.6 and 12.7). At the time the initial doses were given, two groups of litter-mate animals received acute doses of 4.0 mg/kg for the male animals and 2.5 mg/kg for the females; mortalities were 95 and 100 per cent, respectively. Only 10 per cent of the male animals that had received the increased doses died after the 4 mg/kg dose; none of the females died after the dose of 3 mg/kg. The doses that followed these were always less than double the preceding dose, a procedure recommended by Hunter.[3] Tolerance to uranyl fluoride was produced in adult rats of both sexes by the method of increased doses administered every 14 days.

3.2 Percutaneous Route. The repeated percutaneous administration of small doses of uranyl nitrate in ethereal solution has been shown by Orcutt (Chap. 8) to cause a different sort of tolerance in guinea pigs. The mortalities from repeated exposure (no large or test dose was given) were compared with the mortalities calculated to occur from a single dose equal to the cumulative amount of the small doses. The guinea pigs tolerated a given dose of uranyl nitrate applied to the skin much better when administered in repeated small fractions than in a single application.

3.3 By Ingestion. Rats given 2 per cent uranyl nitrate hexahydrate by ingestion appeared to have acquired tolerance. Weanling rats were put on this diet for 6 ½ months, at which time they were returned to the uranium-free diet. Eight and one-half months later they were put back on the diet containing 2 per cent uranyl nitrate hexahydrate. The mortality after 30 days on the diet was 15 per cent. Control rats of the same age, when placed on the 2 per cent diet, suffered an 85 per cent mortality in the same time interval.

In a second experiment, rats were put on the 2 per cent uranyl nitrate hexahydrate diet for the first time at the age of 10 months. Five of the six male rats and all nine female rats died. Rats that had been on a 0.1 per cent uranyl nitrate hexahydrate diet for 9 months were put on the 2 per cent diet. Three of the five male and four of the seven female animals died. Finally, rats that had been on 0.5 per cent uranyl nitrate hexahydrate diet for 9 months were put on the 2 per cent diet. None of the six male animals and only one of the seven female animals died. From these experiments it is evident that inclusion of small amounts of uranyl nitrate in the diet made rats tolerate large amounts, which in nontolerant rats of this age produced a mortality of 70 to 80 per cent (see Sec. 2.3).

3.4 By Inhalation. Evidence that acquired tolerance to uranium was produced by inhalation is given by Spiegl. Twenty rats that had been exposed 7 weeks previously to a "flood" concentration of uranium hexafluoride for 10 min and 20 normal rats were exposed for 26 days to 20 mg UF_6/cu m. The mortalities for the two groups were 15 and 75 per cent, respectively. Hence tolerance to this uranium compound was acquired as the result of previous exposure by inhalation.

4. MANIFESTATIONS AND MECHANISM OF TOLERANCE

With further study it may eventually be possible to establish criteria of tolerance other than mortality response to a test dose. With this purpose in mind and in order to gain a better understanding of the phenomenon of tolerance, additional data have been collected in the mortality experiments reported above and will be presented in the following sections.

4.1 Changes in Body Weight. Since the inclusion of body-weight data for individual animals of a group or for all groups is impossible, average body weights will be presented for representative groups only.

(a) Method of Repeated Small Doses. Figure 12.2 shows the average body weight for the young male rats of Group 1, Table 12.1, throughout the repeated dose period and for control rats of the same

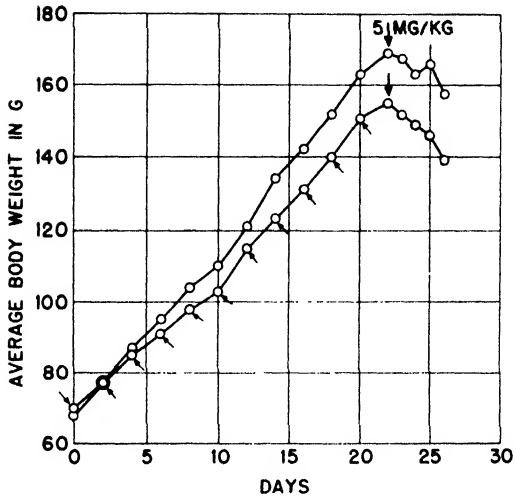


Fig. 12.2—Relationship between the average body weight and time for young male rats. Animals of Group 1 (Table 12.1), which is represented by the lower curve, were each injected with 0.33 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg at each point on the curve prior to injection of the test dose (5 mg/kg). Animals of Group 4 (Table 12.1), which is represented by the upper curve, received only the test dose (5 mg/kg).

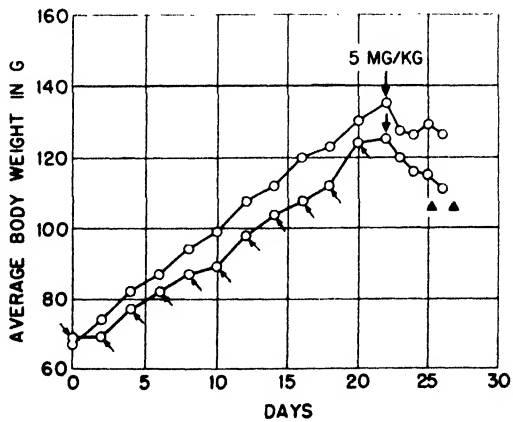


Fig. 12.3—Relationship between the average body weight and time for young female rats. Animals of Group 1 (Table 12.1), which is represented by the lower curve, were each injected with 0.33 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg at each point on the curve prior to injection of the test dose (5 mg/kg). Animals of Group 4 (Table 12.1), which is represented by the upper curve, received only the test dose (5 mg/kg). Deaths are indicated by ▲.

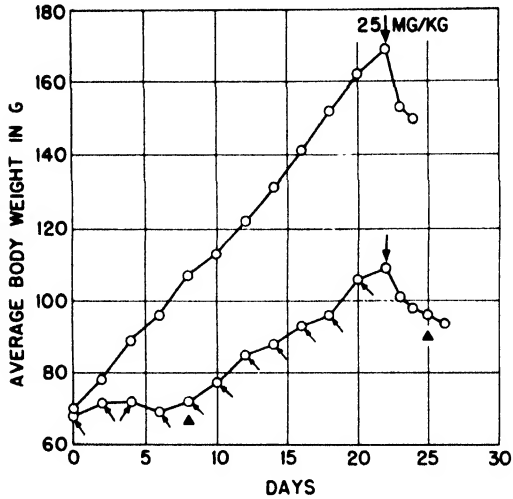


Fig. 12.4 — Relationship between the average body weight and time for young male rats. Animals of Group 3 (Table 12.2), which is represented by the lower curve, were each injected with 1.65 mg UCl_4/kg at each point on the curve prior to injection of the test dose (25 mg/kg). Animals of Group 6 (Table 12.2), which is represented by the upper curve, received only the test dose (25 mg/kg). Deaths are indicated by ▲.

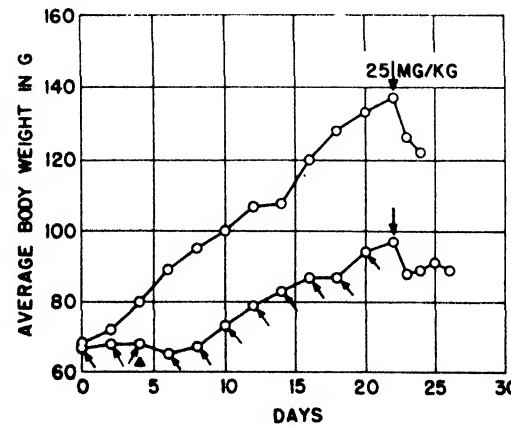


Fig. 12.5 — Relationship between the average body weight and time for young female rats. Animals of Group 3 (Table 12.2), which is represented by the lower curve, were each injected with 1.65 mg UCl_4/kg at each point on the curve prior to injection of the test dose (25 mg/kg). Animals of Group 6 (Table 12.2), which is represented by the upper curve, received only the test dose (25 mg/kg). Deaths are indicated by ▲.

weight and sex (Group 4). After injection of the test dose, the body-weight curve is continued for only 4 days, since deaths of animals within the control group make the curve meaningless beyond this point. Figure 12.3 is the body-weight curve for the female rats of Groups 1 and 4. Figures 12.4 and 12.5 show the body-weight curves for the young male and female rats, respectively, that received repeated doses of uranium tetrachloride (Table 12.2) and for control groups of the same age and sex.

As shown in Figs. 12.2 to 12.5, young rats of both sexes continued to grow throughout the period of repeated injection of small doses of uranium salts, but at a somewhat slower rate than normal rats of the same age. At the time of injection of the test dose of nitrate (Fig. 12.2 and 12.3) the average body weights of the repeated-dose groups were for males 8.3 per cent and for females 7.4 per cent below the weights of the corresponding control groups. For the groups given repeated doses of the tetrachloride these figures were 35.5 and 29.2 per cent for males and females, respectively. Hence retardation of growth was more marked during repeated dosage with the tetrachloride than with the nitrate. This difference was probably not due solely to the difference in uranium content of the small dose (0.156 mg U_6/kg and 1.04 mg U_4/kg) or the state of valence of the element. A greater amount of strong acid is injected with the chloride than with the nitrate, which might account for the two deaths, one at the third injection (Fig. 12.5) and one at the fifth injection (Fig. 12.4) of uranium tetrachloride. In spite of the pronounced effect of the small doses of the tetrachloride on growth rate, the animals were markedly tolerant to the large dose (Table 12.2).

The growth curves for adult male rats given repeated doses of 0.33 mg/kg (Group 36, Table 12.3) and for normal litter mates (Group 40) are shown in Fig. 12.6. Similar curves for adult female rats (Groups 37 and 41, Table 12.3) are shown in Fig. 12.7. The curves for adult rats during repeated dosage differ from the corresponding curves for young rats (Figures 12.2 and 12.3) in that the adult rats suffered an initial loss in body weight amounting to 5.6 and 4.6 per cent for males and females, respectively. Attainment and maintenance of the initial body weight in these groups were achieved at the time of the tenth injection on the eighteenth day after the first injection. At the time of injection of the test dose, namely, 22 days after the initial injection, the average body weights for the male rats were 16.4 per cent and for the female rats 21.3 per cent below those of the corresponding control groups. Hence retardation of growth during repeated dosage was greater in adult rats than in young rats of both sexes.

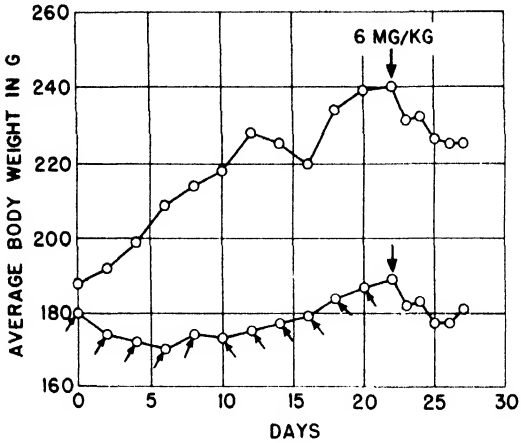


Fig. 12.6 — Relationship between the average body weight and time for adult male litter-mate rats. Animals of Group 36 (Table 12.3), which is represented by the lower curve, were each injected intraperitoneally with 0.33 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg at each point on the curve prior to injection of the test dose (6 mg/kg). Animals of Group 40 (Table 12.3), which is represented by the upper curve, received only the test dose (6 mg/kg).

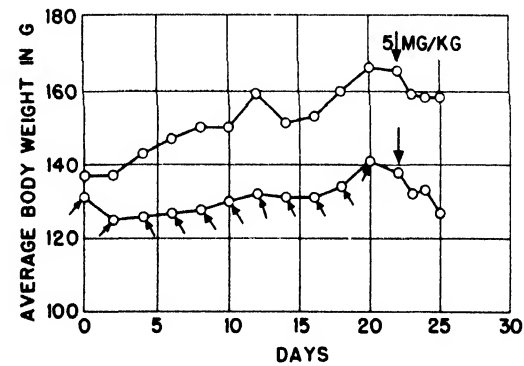


Fig. 12.7 — Relationship between the average body weight and time for adult female litter-mate rats. Animals of Group 37 (Table 12.3), which is represented by the lower curve, were each injected intraperitoneally with 0.33 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg at each point on the curve prior to injection of the test dose (5 mg/kg). Animals of Group 41 (Table 12.3), which is represented by the upper curve, received only the test dose (5 mg/kg).

The growth curves for adult female rats given repeated doses of 0.1 mg/kg and for normal litter mates are shown in Fig. 12.8. From Fig. 12.1 it is evident that this repeated dose was too small to cause the rats to acquire tolerance to the large dose. The growth curve for

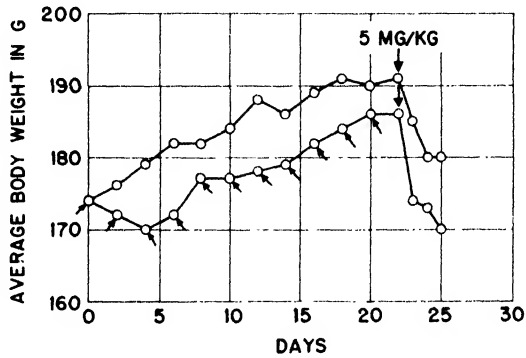


Fig. 12.8 — Relationship between the average body weight and time for adult female litter-mate rats. Animals of Group 68 (Sec. 3.1a), which is represented by the lower curve, were each injected intraperitoneally with 0.1 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg at each point on the curve prior to injection of the test dose (5 mg/kg). Animals of Group 69 (Sec. 3.1a), which is represented by the upper curve, received only the test dose (5 mg/kg).

these rats differs in three respects from the curves for female rats that received 0.33 mg/kg (Fig. 12.7). In the first place, the initial loss in weight for this group was only 2.4 per cent. In the second place the attainment and maintenance of the initial body weight occurred in only 8 days at the time of the fourth injection, in contrast to 18 days at the time of the tenth injection for the group that received 0.33 mg/kg. Finally the average body weight for the group was only 2.6 per cent below that of the litter-mate control group at the time of injection of the test dose.

(b) Method of Increased Doses. The growth curves for rats made tolerant by repeated injections of increased doses are shown in Figs. 12.9 and 12.10, in which the interval between doses was 10 days, and in Figs. 12.11 and 12.12, in which the corresponding interval was 20 days (Tables 12.4 and 12.5).

All four of the curves are similar in two respects: (1) a loss in weight followed the injection of each dose, and (2) a gain in weight preceded injection of the next dose. The curves for the rats with the 10-day dosage interval differ in two respects from the curves for the

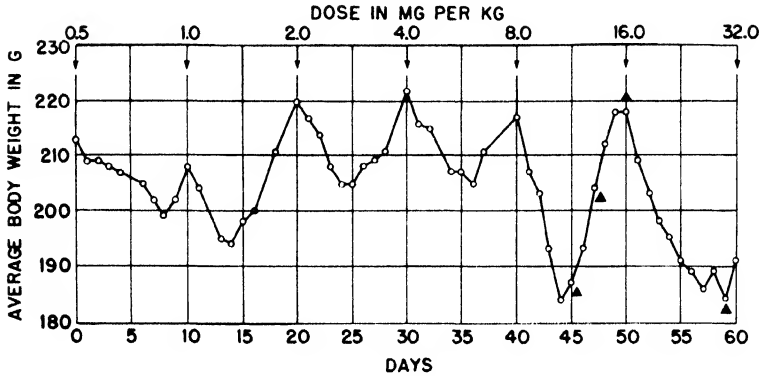


Fig. 12.9 — Average body weight of adult male rats given increased intraperitoneal doses of $UO_2(NO_3)_2 \cdot 6H_2O$ at 10-day intervals (Table 12.4). Deaths are indicated by ▲.

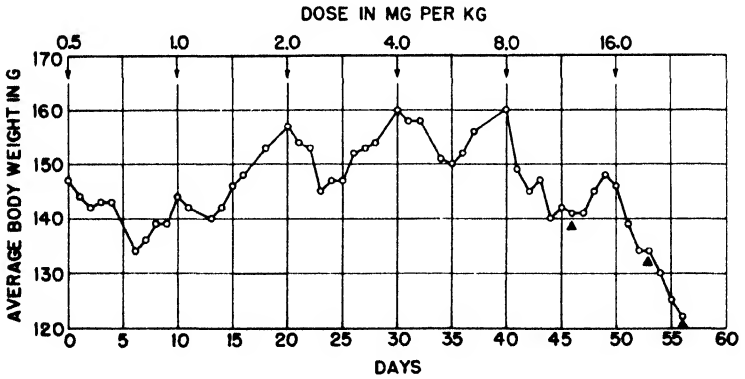


Fig. 12.10 — Average body weight of adult female rats given increased intraperitoneal doses of $UO_2(NO_3)_2 \cdot 6H_2O$ at 10-day intervals (Table 12.4). Deaths are indicated by ▲.

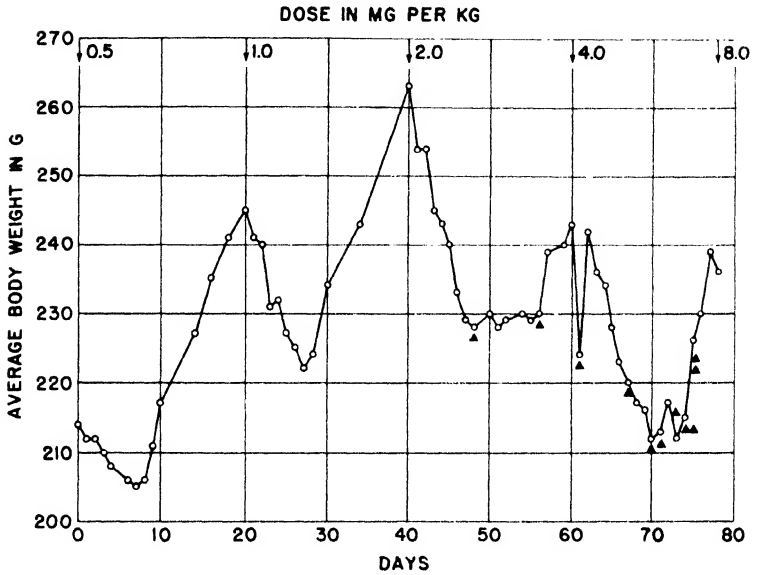


Fig. 12.11 — Average body weight of adult male rats given increased intraperitoneal doses of $UO_2(NO_3)_2 \cdot 6H_2O$ at 20-day intervals (Table 12.5). Deaths are indicated by ▲.

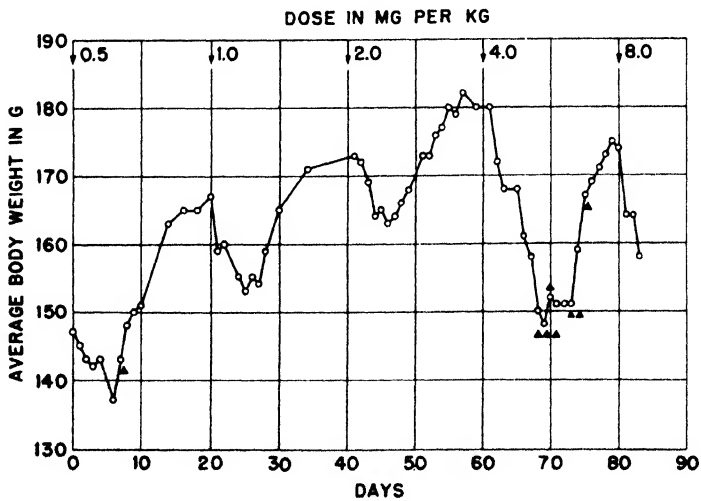


Fig. 12.12 — Average body weight of adult female rats given increased intraperitoneal doses of $UO_2(NO_3)_2 \cdot 6H_2O$ at 20-day intervals (Table 12.5). Deaths are indicated by ▲.

animals with the 20-day dosage interval: (1) the maximum weights attained by the rats with the 10-day interval are only 4.2 and 8.1 per cent higher than the initial weights for males and females, respectively, as contrasted with 22.9 and 23.8 per cent higher for males and females, respectively, where the interval between doses was 20 days; and (2) except for the first injection, the time at which the maximum weight loss occurred after each dose was shorter for animals with the 10-day interval between doses than for those of the same sex with the 20-day interval, as shown in the accompanying table.

Time (Days) of Occurrence of Maximum Weight Loss after Injection

Dose, mg/kg	10-day interval		20-day interval	
	Male	Female	Male	Female
0.5	8	6	7	6
1.0	4	3	7	5
2.0	5	3	8 - 10	5
4.0	6	5	10 13	7
8.0	4	4		

In general, the female rats began to gain sooner after each injection than did the male rats.

As pointed out in Sec. 3.1b, the 10-day interval was preferable to the 20-day interval for producing tolerance. The longer interval between doses, although seemingly offering the advantage of greater weight gain, must actually have been deleterious, perhaps by affording time for the loss of some response of the animal to the preceding dose. This hypothesis is supported by the fact that underfeeding per se did not reduce but rather increased the mortality after an acute dose (Table 12.3).

4.2 Excretion of Uranium. In the literature the suggestion was made that tolerant animals excreted uranium more rapidly than normal animals. At present we have very little data on the excretion of uranium in tolerant rats. In an experiment designed to test the urinary excretion of citric acid of two rats during increased dosage with uranyl nitrate at approximately 12-day intervals, data were obtained by Randall[12] on the excretion of uranium in the first 24-hr urine sample (Fig. 12.13). William F. Neuman (Chap. 11, Sec. 3.2) found that rats given a single injection of 5 mg of uranyl nitrate hexahydrate per kilogram excreted from 40 to 70 per cent of the dose in the urine in the first few hours. The values depicted in Figure 12.13, through

the 4 mg/kg dose only, are similar to those found by Neuman, although somewhat lower, owing perhaps to a difference in diet of the rats. In view of the wide range in Neuman's values, or, more specifically, the variation from animal to animal, the possibility of showing increased urinary excretion of uranium by tolerant as compared with nontolerant animals becomes remote. The demonstration would require the use of large numbers of animals.

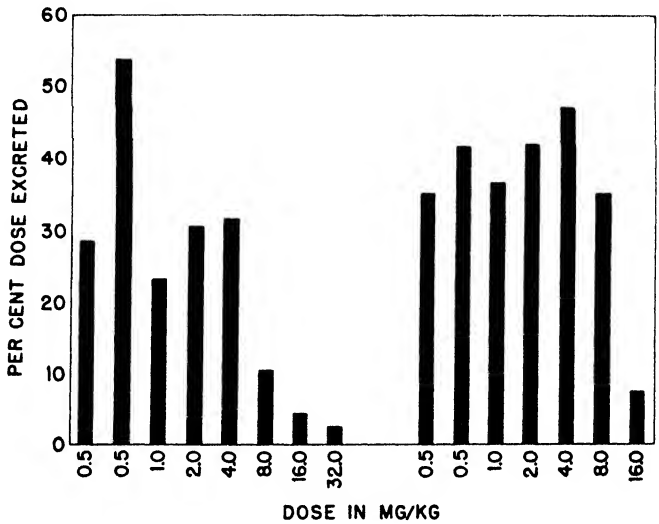


Fig. 12.13 — Excretion of uranium in the first 24-hr urine sample of two rats following increased doses of $UO_2(NO_3)_2 \cdot 6H_2O$.

4.3 Retention of Uranium by the Kidneys. Although differences in urinary excretion of uranium would be difficult to demonstrate, differences in uranium content of the kidneys of tolerant animals and of animals that received a single dose might become evident.

Nineteen male rats and twelve female rats, all young adults, were killed 20 days after having received intraperitoneally 5 mg of uranyl nitrate hexahydrate per kilogram. The kidneys were analyzed for uranium by the fluorophotometric method of Bloor (Chap. 2, Sec. 2.1). The kidneys of the tolerant rats of Groups 1 and 8 (Table 12.1) were analyzed for uranium 23 days after the test dose of 5 mg/kg. The average values for uranium content expressed as percentage of the 5-mg/kg dose in the total kidney tissue and the number of animals (in parentheses) are shown in the accompanying table.

Uranium Content of Kidney, as Percentage of Administered Test Dose

Sex	After single dose of 5 mg/kg	After repeated small doses plus single dose of 5 mg/kg
Males	3.39 (19)	2.08 (10) 1.59 (9)
Females	4.78 (12)	2.70 (6) 2.01 (8)

The differences in uranium content of the kidneys between the animals that received the repeated doses followed by the 5-mg/kg dose and those that received only the 5-mg/kg dose are statistically significant. The standard deviations were much greater for the single dose than for the repeated doses, which indicates that the repeated dosage may have made the kidneys more uniform in some respect. At any rate, the data show that the kidneys of tolerant animals retained less uranium than the kidneys of nontolerant animals.

4.4 Excretion of Citric Acid. The excretion of citric acid in adult rats before and after a single intraperitoneal dose of uranyl nitrate has been studied by Randall. Figure 12.14 shows the typical excretion pattern for citric acid in 24-hr urine samples and the body weight of a male rat following a single dose of 2.5 mg of uranyl nitrate hexahydrate per kilogram. Within 2 to 3 days after injection the urinary citric acid increased to a value three or four times the normal, after which it fell again to the normal level. At about 10 days after injection the urinary citric acid began to rise again, reached a value three to four times the normal, and remained above normal for approximately 10 days. This second increase in excretion of citric acid occurred when the animal began to gain weight.

Inspection of the citrate-excretion pattern shown in Fig. 12.4 shows that the excretion of citrate was above normal between about the tenth and twentieth days after injection. Thus it seems likely that animals made tolerant by the administration of increased doses at 10- and 20-day intervals (Tables 12.4 and 12.5) would be excreting citrate in amounts above normal each time an increased dose was given. In 1931 Hakomori[7] claimed that citrate forms a complex with uranium. Fanta (reported by Dounce in Chap. 1) and William F. Neuman (Chap. 8, Sec. 4) have established the complexing ability of citrate. These facts led to the hypothesis that the increased excretion of citric acid

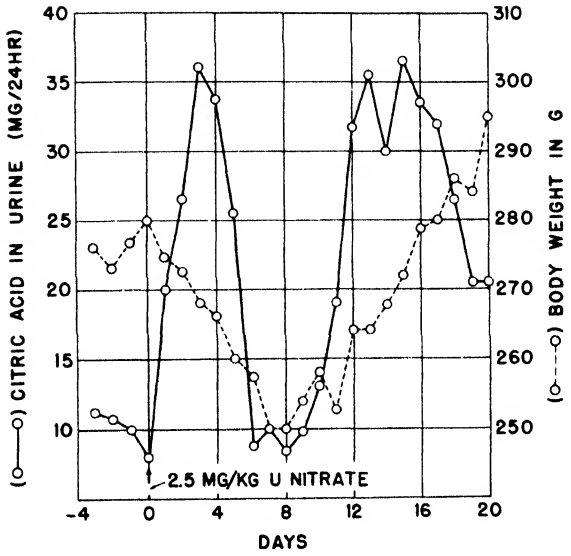


Fig. 12.14 — Urinary excretion of citric acid and the body weight of an adult male rat following the intraperitoneal administration of 2.5 mg $UO_2(NO_3)_2 \cdot 6H_2O$/kg. The solid line indicates excretion of citric acid. The dashed line indicates body weight.

might explain the mechanism of tolerance, perhaps by aiding the excretion of uranium as the citrate complex, thus preventing combination with protein and damage to the kidney tubule. This hypothesis would be strengthened by demonstrating that the urinary excretion of citric acid was above normal at the time that the test dose of uranyl nitrate was given to a rat made tolerant by either method used to produce tolerance. Figure 12.15 shows the urinary excretion of citric acid determined by Randall[6] in an adult male rat during the development of tolerance to uranyl nitrate hexahydrate by the method of repeated small doses (0.33 mg/kg). The excretion of citric acid was approximately twice normal at the time the test dose of 5 mg/kg was given and remained above normal for at least 20 days thereafter. On the other hand, in two rats that received repeated doses of 0.1 mg/kg, a dose too small to cause tolerance to the test dose as shown in Sec. 3.1a and in Fig. 12.1, the urinary excretion of citric acid was normal or below normal when the test (fatal) dose was given. The urinary excretion of citric acid was determined by Randall in an adult male rat during the administration of increased doses of uranyl nitrate and is shown in Fig. 12.16. The level of excretion was high each time a larger dose was given.

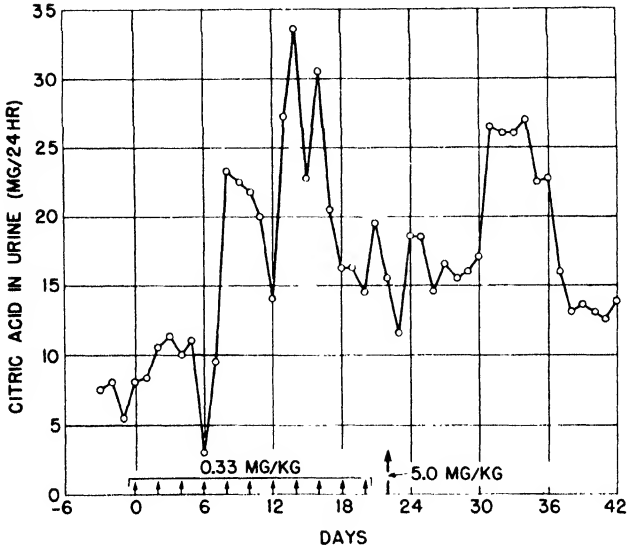


Fig. 12.15—Urinary excretion of citric acid of an adult male rat during the intraperitoneal administration of small doses of uranyl nitrate hexahydrate (0.33 mg/kg) and following the test dose (5 mg/kg).

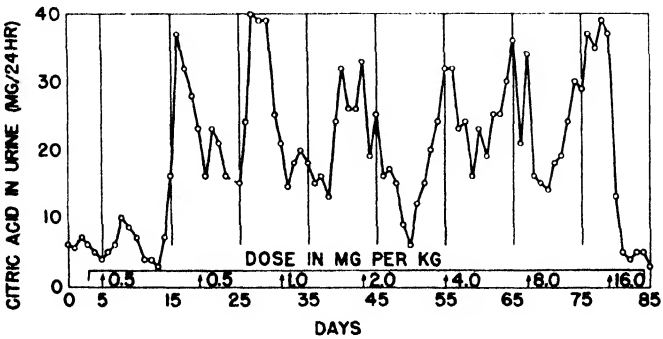


Fig. 12.16—Urinary excretion of citric acid of an adult male rat during the intraperitoneal administration of increased doses of $UO_2(NO_3)_2 \cdot 6H_2O$.

The excretion of citric acid was studied by Randall in a male rat already made tolerant by 11 injections of 0.33 mg of uranyl nitrate hexahydrate per kilogram followed by a single injection of 5 mg/kg, then injections of 6 mg/kg and 7 mg/kg at 3-week intervals. The level of citric acid excretion was found to be about twice normal 14 days after the 7-mg/kg dose. Five days after a further dose of 5 mg/kg the citric acid excretion rose to 1 ½ times the level at injection. When the excretion of citric acid was determined 6 weeks later, the level was again found to be about four times the normal. Hence in a tolerant rat the urinary excretion of citric acid seems to remain high for some time after dosage is discontinued.

4.5 Other Urinary Changes. Additional urinary changes have been found in tolerant animals. Average values for urinary volume were high and pH low in two rats made tolerant by injection of increased doses of uranyl nitrate hexahydrate every 20 days. The same was true for a rat made tolerant by the method of repeated small doses followed at 3-week intervals by doses of 5, 6, and 7 mg/kg.

According to Orcutt (Chap. 8), rabbits that received repeated dermal exposure to uranyl nitrate hexahydrate, averaging 0.016 g/kg in ethereal solution, showed a fluctuating rise in urinary protein. By the twenty-fifth day after the first exposure, protein fell to less than 100 mg % and showed no further rise, although exposure was continued. Animals that received a single application had a peak on the fifth day; the values fell to less than 100 by the fourteenth day and eventually returned to 0.

4.6 Biochemical Changes in the Kidney and Liver. The livers of tolerant rats showed several changes from normal. The ratio of liver weight to body weight decreased from normal in all groups that received repeated small doses of uranium salts (Table 12.8). A highly significant decrease in the percentage of dry residue of the liver (increase in water content) occurred in the rats tolerant to uranyl nitrate. Fatty livers were present in the group that received uranium tetrachloride.

Kidneys of tolerant rats were significantly enlarged and showed a decrease in the percentage of dry residue. The ratio of kidney weight to body weight decreased from normal in rats made tolerant to uranium tetrachloride.

In control rats studied by Crossland,[9] the degree of unsaturation (iodine number) of the phospholipid fatty acids of the kidney decreased about 25 per cent following a single 5-mg/kg dose, whereas the same value for liver increased about 15 per cent. Iodine numbers on kidneys and livers of tolerant rats (Group 8, Table 12.1) were normal.

Table 12.8 — Relationship between Organ Weight, Body Weight, and Certain Organ Constituents of Normal and Tolerant Rats

Group	No. of rats		Time after test dose, days	Body weight, %		Dry residue, %		Total lipid of liver, %	
	Male	Female		Liver	Kidney	Liver	Kidney	Wet	Dry
Normal	12	8		4.7	0.92	29.6		4.2	14.2
	8	8			0.86		21.8		
Tolerant to uranyl nitrate *	19	14	23	3.8†–4.2†	1.02†–1.05†	28.7†	19.4†	4.2	
Tolerant to UCl_4‡	9	8	40	4.0†	0.80†	29.9	21.0†	4.4†	14.8†

* See Table 12.1, Groups 1 and 8.
† Significant difference from normal.
‡ See Table 12.2, Group 3.

A decrease in phosphatase activity of kidney brei was found by Lan in rats that received an acute dose of uranyl acetate. Phosphatase values were likewise very low or absent in kidneys of tolerant rats. Probably no regeneration of phosphatase occurs, since kidney brei of tolerant rats was still devoid of activity 65 days after the last acute dose.

5. DISCUSSION

Species differences in mortality following the administration of uranyl nitrate by three different routes clearly indicate the existence of a striking natural tolerance to this compound. The rat possessed less natural tolerance than the mouse, and the white mouse far less than the C_3H mouse.

On the basis of mortality following a test dose, male rats have been shown to possess more natural tolerance and to develop greater acquired tolerance to uranyl nitrate than do female rats, which may be related to the fact recognized earlier[10] that females retained a greater percentage of the dose in the kidney than males of the same age. William F. Neuman's work (Chap. 11, Sec. 3.7) on distribution and excretion of uranium in the rat has confirmed this finding and has added the fact that male rats of the same weight store more uranium in bones than female rats, thus sparing the kidney.

The work on acquired tolerance shows that either the method of repeated doses or the method of increased doses may be used to produce tolerance in rats. The small dose used in the method of repeated doses should probably be large enough to cause considerable functional injury to the kidney in order to produce tolerance. The slight

changes in body weight following repeated doses too small to produce tolerance and the greater changes following doses that produced tolerance would indicate that kidney damage is a prerequisite to tolerance. The markedly increased ratio of kidney weight to body weight and the concomitant decrease in percentage of dry residue, the absence of kidney phosphatase, the high urinary volumes, and the low variable pH's are all indicative of kidney damage in tolerant rats.

The average body-weight data for adult rats made tolerant by the repeated-dose method (Figs. 12.6 and 12.7) show that the initial body weight is exceeded by about the time of the ninth injection. Concurrently the urinary excretion of citric acid during repeated dosage is at its height (Fig. 12.15). The excretion of citric acid remained normal after the repeated administration of a dose of uranyl nitrate hexahydrate (0.1 mg/kg) too small to cause tolerance to the test dose. By the method of increased dosage the 10-day interval was better than the 20-day interval for producing tolerance. At 10 days after a single injection (Fig. 12.14) the excretion of citric acid had begun to rise, whereas at 20 days the excretion was falling. Each time an increased dose was given at 12-day intervals the excretion of citric acid was high (Fig. 12.16).

These facts are in agreement with the hypothesis that the enhanced urinary excretion of citric acid following administration of uranium is concerned with tolerance. As has been pointed out, no increase in urinary excretion of uranium by tolerant rats was found, which may be accounted for by the wide variation in excretion from animal to animal following a single dose. The kidneys of tolerant rats retain less uranium than those of nontolerant animals, a fact that may be explained by assuming that uranium, having filtered through the glomerulus as bicarbonate complex, combines with citric acid present in the tubule and is excreted. In the absence of citric acid, uranium would combine with protein of the tubule and cause kidney damage.

Evidence in support of this assumption is presented by Randall,[11] who carried out an experiment designed to imitate the flow of urine through the kidney tubule. Two solutions were prepared, both of which contained 200 mg of dialyzed albumin, 0.87 g of sodium chloride, and 600 μg of uranium. To the experimental solution 198.0 mg of citric acid was added. The pH was adjusted to 5.0, and the volume to 100 ml. Both solutions were filtered through a collodion membrane, and citric acid and uranium determinations were made on the protein-free filtrates. No uranium was found in the control filtrate, whereas in the experimental filtrate 81 per cent came through the membrane. The concentration of citric acid before and after filtration was exactly the same, showing that none was held back by the protein. In a similar

experiment in which twice the amount of citric acid was used, 86 per cent of the uranium filtered through the membrane. Again none was found in the control filtrate.

In conclusion, the available evidence indicates that the presence of citric acid in the kidney tubule in concentrations above normal may account for acquired tolerance to the toxic action of uranium.

BIBLIOGRAPHY

1. T. Suzuki, "Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen," Carl Fischer, Jena, 1912.
2. C. Gil y Gil, Beitr. path. Anat. u. allgem. Path., 72: 621 (1923-24).
3. W. C. Hunter, Ann. Internal Med., 1: 747 (1928).
4. W. deB. MacNider, J. Exptl. Med., 49: 411 (1929).
5. W. deB. MacNider, Southern Med. J., 29: 1189 (1936).
6. M. Garnier and J. Marek, Presse méd., 40: No. 1, 829 (1932).
7. S. Hakomori, Science Repts. Tôhoku Imp. Univ., First Ser., 20: 736 (1931); see Chem. Abstracts, 26: 40 (1932).
8. C. Randall, Univ. Rochester, Atomic Energy Project Rept. M-1860 (March, 1946).
9. R. S. Crossland, Univ. Rochester, Atomic Energy Project Rept. M-1877 (January, 1946).
10. F. L. Haven, Univ. Rochester, Atomic Energy Project Rept. M-1687 (April, 1945).
11. C. Randall, Univ. Rochester, Atomic Energy Project Rept. M-1868 (April, 1946).
12. C. Randall, "Citric Acid of Bones and Urine of Uranium-poisoned Rats," Univ. Rochester Master's Thesis, 1946.

Chapter 13

THE ACTION OF URANIUM ON ENZYMES AND PROTEINS

By Alexander L. Dounce and Tien Ho Lan*

1. GENERAL INTRODUCTION

Uranium compounds fall under the general classification of heavy-metal compounds, many of which are known to act as poisons when administered in relatively small doses. Such compounds are immediately suspected of interfering with the catalytic machinery of cells, both because of the smallness of the toxic doses and because in vitro experiments have shown that many of these compounds act as protein precipitant and denaturants as well as enzyme poisons. From these considerations it was thought desirable at the outset of the work with uranium to find out how it behaved toward various isolated enzymes and proteins and to compare its behavior in these respects to the corresponding behavior of other heavy metals. This was particularly desirable at the time when it was not quite clear that the primary action of low doses of uranium is to damage the kidney tubules. It was felt that if, for example, a particular enzyme or enzyme system that was especially sensitive to uranium compounds could be found, it might be possible to make some prediction about the chronic action of uranium and perhaps to find therapeutic agents for uranium poisoning.

A further consideration that encouraged the continuance of enzyme and protein work was the thought that plasma proteins must be important in the transport of uranium in the blood stream.

As a result of the work on the action of uranium compounds on enzymes and proteins it can be stated that hexavalent uranium (the valence form that is of chief concern in the animal body) probably is

*Work done by Alexander L. Dounce and Tien Ho Lan, with the assistance of William Connors, Marian Kaley, Betty Robinson, and Daphne Rothermel. Thanks are due the Department of Medicine of the University of Rochester for furnishing the partially purified thrombin and fibrionogen that were used in the experiments in this chapter.

not a specific enzyme poison under physiological conditions in the sense that arsenic or mercury are specific poisons for sulfhydryl enzymes. Hexavalent uranium shows a strong tendency to form very slightly dissociated complexes under physiological conditions with, for instance, organic acids and bicarbonate. Since these complexes apparently do not affect enzymes, uranium probably is not a particularly powerful enzyme poison in the animal body except in special locations, such as the kidney tubules, where the reabsorption of bicarbonate and drop in pH allow sufficient UO_2^{++} to accumulate to permit a combination of U_6 with enzymes and cell proteins.

It is apparently the UO_2^{++} ions that combine with proteins and inhibit enzymes. It has been found that in all cases so far studied the inhibitory action of hexavalent uranium on isolated enzymes or enzyme systems increases very sharply as the pH decreases, apparently because of liberation of UO_2^{++} from complexes. This point will be illustrated in the following experiments. The reader is referred to Chap. 1 for a detailed discussion of the chemistry of complexes of U_6.

In regard to combination of hexavalent uranium with proteins, it can be stated definitely that it combines rather firmly with acid groups of proteins and that it probably can combine only in loose ionic or salt-type linkages with basic groups. Sulfhydryl groups do not combine with U_6. The U_6 has a very slight or unmeasurable effect in denaturing proteins at a pH range from 6 to 8.

The combination of U_6 with proteins is sufficiently firm so that some strong complexer such as bicarbonate, citrate, or malate must be used to reverse the combination. The presence of even a small amount of combined U_6 destroys the crystallizability of egg albumin, but crystallizability can be restored by prolonged dialysis with repeated additions of a strong complexer. The presence of a small amount of U_6 also affects the mobility of plasma proteins in the Tiselius electrophoresis cell.

In regard to the problem of transport in the blood stream, it can be stated that U_6 is chiefly combined with bicarbonate but also partly with plasma proteins. The lower the plasma bicarbonate content, the more U_6 is carried by protein.

There is less quantitative work on U_4 than on U_6, since it has been felt that in case of lung exposure to U_4 the material entering the plasma would be U_6 rather than U_4. Work on the oxidation-reduction problem has made it fairly clear that the chemistry of uranium in the animal body is chiefly the chemistry of U_6 for all modes of entry of uranium except by direct injection. By the latter method U_4 can of course be introduced into the body. For a detailed account of work to support this statement, the reader is referred to Chap. 1.

It has been found that in general U_4 appears to have a somewhat greater affinity for protein than has U_6 and that it is usually a somewhat more severe enzyme poison than is U_6. The latter point is difficult to prove, however, since the U_4 might contain small amounts of impurity in the form of other heavy metals that are absent in U_6 compounds. The use of U_4 in enzyme poisoning experiments is hampered by the insolubility of U_4 at pH values in the neighborhood of 7.0 where most enzyme reactions are carried out.

The U_4 has somewhat less tendency to form complexes with bicarbonate than does U_6, and because of this and its apparently greater affinity for protein it must be carried in the blood stream to a considerable extent in the form of complexes with blood protein, after being injected. However, it is also possible that some U_4 is carried as colloidal oxide stabilized by plasma protein. These statements are borne out by excretion studies (see Chaps. 11 and 15).

The detailed material that follows shortly will explain the general reactions of U_6 and to some extent the reactions of U_4 with proteins and also will show the behavior of a number of enzymes in the presence of U_6 and sometimes U_4. Much of the work with proteins is of a qualitative nature, since it was necessary to cover much territory in a short time, and since it was found that decisive information was obtainable from qualitative work in this field. On the other hand, much of the enzyme work is quantitative, since few conclusions can be drawn from qualitative experiments in this field. As a rule, few striking differences among enzymes in regard to sensitivity to U_6 or to U_4 have been found, but measurable differences do occur. That these differences in sensitivity are important physiologically is somewhat doubtful, as will be seen from the work on the enzymes of organs of animals poisoned by uranium.

The details of the experiments now will be given. Section 2 of this chapter deals with proteins, and Sec. 3 with enzymes.

2. ACTION OF URANIUM COMPOUNDS ON PROTEINS OF EXPERIMENTAL MATERIAL IN VITRO

The general aim of this part of the chapter is to give experimental evidence supporting deductions concerning the combination of uranium (chiefly in the hexavalent form) with proteins. The results of experiments on the precipitating action of uranyl compounds (U_6) on proteins also will be stated. The action of U_4 will be discussed separately.

2.1 Nonremoval of Uranium from Protein by Washing or Dialysis.

1. It has been found that crystallized egg albumin which has been completely precipitated by uranyl acetate contains combined uranium which is not readily removed by washing with water.

Approximately 1.0 g of once-recrystallized, thoroughly dialyzed egg albumin in 30 ml of distilled water was treated with 5 ml of 5 per cent uranyl acetate dihydrate solution (≈250 mg of uranyl acetate dihydrate, or 119 mg of uranium metal). The precipitated protein was washed four times with about 50 ml of distilled water in each washing. A rough test for uranium carried out by Bloor using the ferrocyanide method after wet-ashing the protein with H_2SO_4 showed that approximately 40 mg of uranyl acetate remained combined with the protein after the fourth washing. That considerable uranium had remained combined could be seen by the rather strong yellow color of the protein precipitate.

The first and second washings, which evidently contained considerable uranium, judging by the yellow color, were not analyzed. The third washing removed about 16 mg of uranyl acetate from the precipitated protein, whereas the fourth washing removed only 2 mg. From these results it is apparent that uranyl acetate combined with the egg albumin in some manner when it was added to the egg-albumin solution, and that the protein-uranyl complex then precipitated.

2. In a different type of experiment, thrice-recrystallized egg albumin was treated with just sufficient uranyl acetate to cause complete precipitation. The precipitate after centrifugation was dissolved by the addition of water and 2 or 3 drops of 1M citrate buffer of pH 6.0, yielding a rather concentrated yellow solution. Following this, the solution was dialyzed for 2 days against repeated changes of distilled water. The solution inside the dialysis sac lost most of but not all its yellow color, indicating that some of the U_6 remained inside the sac. At the end of the dialysis no U_6 could be detected outside the dialysis sac using the ferrocyanide method.

An attempt was made to crystallize the dialyzed albumin, using the buffered ammonium sulfate procedure. But crystallization could not be brought about, in spite of the fact that the dialyzed albumin was soluble and apparently not denatured. A control experiment on the original albumin resulted in easy crystallization. Repeating the whole experiment twice gave exactly the same results. It is now known that the reason for lack of crystallization was failure to remove all the uranium from the protein even by prolonged dialysis. This indicates that some type of fairly firm chemical combination between the U_6 and albumin has taken place. Dialysis against distilled water with repeated additions of a strong complexing reagent for U_6 (such as malate) will readily restore crystallizability as will be shown later on, so that the failure to recrystallize was not caused by denaturation of the protein molecule. Part of the difficulty of removing U_6 from proteins by dialysis may lie in the inability of all the uranium to diffuse through the dialyzing membrane at high pH values.

2.2 Effect of pH on the Precipitability of Proteins by U_6. It has been found that pH has an important influence on the precipitation of proteins by uranyl compounds. In this and the following section the evidence has been gathered from rough test-tube experiments in which protein (usually egg albumin) in concentrations from 0.1 to 1.0 per cent was treated with small amounts of 1 per cent $UO_2(Ac)_2.2H_2O$ solution. With uranyl acetate used as precipitating agent, in all cases studied the effect of lowering the pH from a value in the neighborhood of 7.0 to values in the neighborhood of 5.0 was to increase the precipitating action of the uranyl acetate on the protein. Precipitability increases as pH falls and appears to be maximal in the pH range of 4.0 to 5.5 as a general rule. If the pH is raised to 8.0 by the cautious addition of dilute NaOH, the protein precipitate generally dissolves. Also, if the pH is lowered to a value in the neighborhood of 2 to 3, the precipitate usually dissolves. This variation with pH in the precipitating action of uranyl acetate on proteins places it in a class that is distinct from the heavy metals like Pb and Hg and also from the alkaloidal reagents. The heavy metals mentioned usually precipitate proteins as well or better in neutral or slightly alkaline solution than in slightly acid solution, whereas the precipitating action of the alkaloidal reagents is increased as the pH decreases and does not suddenly vanish at pH values in the neighborhood of 2 to 3.

Extensive work has not been done on the variation of the ability of uranyl nitrate to precipitate proteins as the pH is changed, but it can be stated that in general the same sort of phenomenon occurs.

It is now realized that the effect of pH on the precipitability of proteins by uranyl compounds is at least in part explained by its effect on the amount of uncomplexed uranyl ion present in solution, since it is apparent that uranyl ion complexed with buffer anion (such as acetate) has no precipitating action on protein in cases so far studied. There appears to be a competition for the uranyl ion between the protein and any organic anion that may be present. With anions such as acetate, lowering of the pH means formation of more uncomplexed UO_2^{++}, which can react with the protein. Strong complexers like citrate or malate more or less inhibit the effect of pH on the precipitability of protein by uranyl compounds, since they prevent any precipitation even at the lower pH ranges of 4 to 5.

2.3 Salt Effect on Precipitation of Protein by Uranyl Acetate. It has been found that some salt-free proteins will yield little or no precipitate in the presence of excess uranyl acetate until some inert salt is added to the solution. For example, beef-serum albumin, chicken and dog hemoglobin, histone prepared from isolated nuclei of hepatic cells, and cytochrome C do not form precipitates when treated with uranyl acetate at pH values from 5 to 7, but, if a little

sodium chloride is added, precipitates form immediately. A concentration of 0.9 per cent NaCl or somewhat less is sufficient to cause this precipitation. Other salts, such as KCl, $(NH_4)_2SO_4$, and Na_2SO_4, have the same action as NaCl. On the other hand, many salt-free proteins form immediate precipitates when treated with sufficient uranyl acetate. Examples of such proteins are egg albumin, urease, and beef-serum globulin. A possible explanation of this effect of salt on the precipitation of proteins by uranyl acetate will be given under Sec. 2.10.

It appears to be possible to separate egg albumin and beef-serum albumin partially by precipitation of the well-dialyzed mixture with uranyl acetate. A simple experiment showing this is as follows:

One milliliter of approximately 1 per cent dialyzed egg albumin was mixed with 1 ml of approximately 1 per cent dialyzed beef-serum albumin. To the mixture was added approximately 2.5 ml of 1 per cent uranyl acetate. The precipitate that formed was centrifuged down at about 16,000 rpm in the centrifuge with high-speed attachment. The clear supernatant remained clear upon the further addition of about 0.5 ml of uranyl acetate. Subsequent addition of a pinch of NaCl to this clear solution caused a heavy precipitate to form. This second precipitate was undoubtedly chiefly serum albumin, whereas the first precipitate obtained by adding excess uranyl acetate without NaCl probably consisted chiefly of egg albumin.

Both the precipitates obtained with and that without the addition of salt were instantly soluble when a drop of trisodium citrate was added to the solutions, and both could thereafter be readily precipitated by heat denaturation, indicating that the precipitation by uranyl acetate had not caused denaturation. A positive identification of the components separated by the uranyl acetate precipitation was not made. This could be done by diffusion studies, ultracentrifugation, or immunological studies after removal of the uranyl acetate.

An attempt was made to apply the salt effect to separate plasma albumin from plasma globulins. Conditions were established such that, on adding uranyl acetate to thoroughly dialyzed plasma, a precipitate was obtained which corresponded in dry weight roughly to the amount of globulin present. However, after dialysis of the precipitate with citrate to remove the uranium, it was found by electrophoresis that only a very slight separation of globulins from albumin had been obtained. The electrophoresis was carried out by Alling.

It is probable that some type of framework precipitate is formed when proteins are precipitated by uranyl acetate, and that in mixtures much cross combination occurs. This point will be discussed in more detail later.

2.4 Effect of Buffer Anions, Especially Strong Complexers Like Bicarbonate, Citrate, and Malate, in Dissolving Protein Precipitated by Uranyl Compounds. It has been found that, in all cases except one so far investigated, the use of citrate, bicarbonate, or malate causes protein precipitated by uranyl acetate to go into solution very rapidly. Malate and citrate work well in the pH range of 5 to 8, and bicarbonate works well at any pH where it is stable. Relatively low concentrations of these buffers will act in this manner. For instance, sodium bicarbonate functions above pH 7.0 when in 0.025M concentration, and citrate functions when in concentrations of 0.01 to 0.05M at pH 5 to 8. Malate is similar to citrate.

An apparent exception to this general type of behavior was encountered when an attempt was made to dissolve histone (from isolated nuclei of rat liver) in dilute sodium citrate. The histone had been precipitated by adding dilute uranyl acetate and dilute sodium chloride. No solution occurred. In fact it was found that sodium citrate could be used in place of NaCl in effecting a precipitation of histone by uranyl acetate. There is at present no explanation for this phenomenon, although the high isoelectric point of the histone may be involved.

If acetate is substituted for citrate, malate, or bicarbonate, it will effect a solution of most proteins precipitated by uranyl acetate, but larger amounts are required, so that 0.1 to 0.5M acetate may be needed, depending upon the pH. The reason for this is clear, from work on the physical chemistry of uranium compounds in solution. There is a competition between the buffer anion and the protein for the uranyl ion. Since citrate, malate, and bicarbonate all complex the uranyl ion very strongly, not much of any of these anions is needed to remove the bulk of the uranium from the protein and so permit solution of the latter. But acetate complexes the uranyl ion less strongly than citrate, malate, or bicarbonate, and hence more of it is required to remove the uranyl ion from the protein (by the mass-law effect). There is no evidence that the uranyl complexes of any of the buffer anions discussed above combine with protein except possibly in the sense of forming very easily dissociable salts.

Phosphate buffer is similar to acetate buffer in its action on dissolving proteins precipitated by uranyl acetate, except that it does not work well below pH 6 to 7, and, once the precipitated protein has dissolved, a precipitate of uranyl phosphate is likely to form gradually. Bicarbonate and citrate can dissolve this precipitated uranyl phosphate.

2.5 Proteins Investigated for Precipitability by Uranium Compounds. A number of proteins were tested in obtaining the information thus far covered. They include egg and serum albumin; dog,

chicken, guinea pig, and cow hemoglobin; beef-plasma euglobulin; urease; catalase; cytochrome C; histone (from liver-cell nuclei); and some other enzymes, both purified and partially purified. Table 13.1 shows whether or not salt is required to precipitate the proteins by uranyl acetate.

Table 13.1 — Classification of Various Proteins According to Whether Salt (NaCl) Must Be Present for Them to Be Precipitated by Uranyl Acetate

Protein	Salt requirement for precipitation with U_6
Egg albumin	No salt required for precipitation with uranyl acetate
Serum globulin	
Human erythrocyte catalase	
Urease	
Serum albumin (beef, horse)	Salt is required for precipitation with uranyl acetate
Hemoglobin (cow, dog, horse, guinea pig, chicken)	
Cytochrome C (beef heart)	
Histone (from rat-liver-cell nuclei)	

2.6 Existence of the Complexed Uranyl Ion in Solution as an Anion. From the experimental work thus far covered it was surmised that the uranyl ion was behaving as an anion at high pH values and as a cation at low pH values. This surmise was made because of the partial resemblance of the precipitating action of uranyl compounds on proteins to the corresponding action of alkaloidal reagents, which are known to be anions. It was thought that the precipitant was the complex uranyl anion rather than the uranyl cation.

From what is now known of the behavior of the uranyl ion in forming complexes with buffer anions like acetate, citrate, malate, and bicarbonate, the original idea that the uranyl complex anion was the precipitating agent is untenable. It evidently is the uranyl cation that is the precipitating agent, and there is a competition for this cation between the protein (which complexes it) and the buffer anion that happens to be present. But the erroneous interpretation served its purpose, since it led to a detailed investigation of the reaction of the uranyl ion with various buffer anions to yield anionic or neutral complexes. The results of this investigation were of value in explaining

the behavior of uranyl compounds under physiological conditions. For a detailed investigation of these complexes, reference is made to Chap. 1.

2.7 Does the Uranyl Ion Alter, Damage, or Denature Protein? It has been shown thus far that the uranyl ion combines rather firmly with proteins at pH values ranging from 3 to 8, that this combination under favorable conditions effects precipitation of the protein, and finally that protein precipitated in this way can be dissolved by the addition of small amounts of various buffer anions that complex the uranyl ion. The question now arises as to whether this combination in any way changes the protein irreversibly so that on complete removal of the uranyl ion the protein would be left in an altered state.

The answer to this question would have to be determined separately for each protein in the body to obtain a complete picture of the situation, but the evidence strongly points to a nondamaging influence of the uranyl ion on most proteins, provided the pH is kept within limits that are safe for the protein in question.

One point contributing to this evidence is the solubility of various proteins precipitated by uranyl acetate in solutions containing buffer anions in low concentrations, which strongly complex the uranyl ion. If the proteins were denatured by the uranyl ion, their solubility should be lost, except in solutions containing strong acids, strong bases, or high concentrations of substances like urea or guanidine hydrochloride. But the solubility is not lost, and therefore it can be surmised at once that the protein has not been completely denatured.

However, mere solubility at pH values near the isoelectric point of the protein is not an especially delicate test for slight alterations in the protein molecule. Denaturation apparently can vary in degree and conceivably could be very subtle in certain instances.[1]

In order to test the absence of denaturation in a more delicate manner, the effect of the uranyl ion on the recrystallizability of crystallized egg albumin has been investigated.

Here the experiment was carried out as in Sec. 2.1, except that the egg albumin precipitated with uranyl acetate was suspended in about 5 ml of water to form a strong solution and then was dissolved by the addition of a few drops of 1M malate buffer of pH 6.0. From time to time during the dialysis, malate buffer was added to the protein a few drops at a time, and the distilled water outside the dialysis sac was changed frequently. After about 48 hr, U_6 could not be detected inside or outside the dialysis sac by means of the ferrocyanide test. Use of the usual method of recrystallization with buffered ammonium sulfate resulted in easy recrystallization of the egg albumin as needles. Although the thrice-recrystallized albumin originally

employed in this experiment was in the form of plates, and the material recrystallized after the removal of U_6 was in the form of needles, this change in crystal form probably should not be considered as significant, since crystallization as plates as a rule simply requires slow crystallization with optimal concentration of egg albumin. The use of malate should not be considered different in any way from the use of citrate or bicarbonate, since these substances all are strong complexers of the uranyl ion and since the success in recrystallization after dialysis with malate was attributable to the repeated additions of malate buffer to the protein during the dialysis.

The results of this experiment show that judging by the test of recrystallizability, which is a reasonably delicate indicator of absence of denaturation, egg albumin is not injured by precipitation with uranyl acetate at pH 5.0 to 5.5 (the pH range at which precipitation was carried out). The experiment also indicates that practically all the U_6 must be removed from egg albumin before it can be crystallized. Why this is so is not known. It might be of interest to determine exactly the quantity of uranium necessary to prevent crystallization by using the microfluorometric method for analysis or by adding carefully measured microportions to egg-albumin samples and subsequently attempting crystallization.

Another qualitative experiment that illustrates the relative mildness of the action of uranyl compounds on proteins is one in which partially purified liver arginase was shown to retain much of its activity after being precipitated by uranyl acetate. In this experiment 1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ was added to 1 ml of rather strong arginase solution, and the precipitate was filtered off. The original suspension was tested for arginase activity by a procedure used by Dounce[2] in testing the arginase activity of liver nuclei and liver suspensions. The filtrate was tested in the same manner and was found to have no activity; nor did it contain any precipitable protein. The activity of the suspension of precipitated enzyme plus uranyl acetate was practically the same as that of the original enzyme solution, when allowance was made for the dilution. In testing for activity, excess arginine carbonate was used as substrate. Because of the complexing action of carbonate on U_6, this substrate dissolved the precipitated enzyme and probably removed all the uranium from it.

This experiment shows that arginase, a rather labile enzyme, can be precipitated by uranyl acetate without apparent loss of activity, and hence without denaturation. The arginase solution employed was a solution of beef-liver arginase purified about thirty times. Such an excess of uranyl acetate was added that, even considering the impurity of the enzyme, it could hardly have escaped reacting with the uranyl acetate.

Similar results also were obtained using crystalline urease. The urease, after being allowed to stand in the refrigerator over night and following precipitation with uranyl acetate, was found to be perfectly soluble in citrate buffer and was found by qualitative tests to possess a high degree of activity.

2.8 The Effect of the Addition of Uranyl Compounds on the Electrophoretic Behavior of Plasma Proteins. (Work done by E. Alling.) Blood was drawn from healthy subjects. Coagulation was prevented by adding 25 mg of potassium oxalate per 10 ml of blood. To 9 ml of plasma was added 2 ml of 2 per cent uranyl acetate dihydrate solution drop by drop with constant stirring (≎ 40 mg of uranyl acetate dihydrate or 21 mg of uranium metal). A small precipitate (possibly of uranyl phosphate) was filtered off. The filtrate was diluted with 7 ml of veronal buffer of ionic strength 0.1, pH 8.5. After 16 hr of rocking dialysis in a cellophane membrane against 2 liters of similar veronal buffer at 20°C, the plasma was subjected to electrophoresis in the standard 11-ml single-section Tiselius cell for 3 hr at a potential gradient of 6.8 volts/cm. Schlieren patterns were obtained by means of Longsworth's moving-plate technique.

The patterns were obviously abnormal. All the peaks had moved further than in normal plasma, and the fibrinogen peak had either disappeared or more probably merged in the beta globulin peak, which was much displaced toward the albumin peak. The mobilities of the various peaks were calculated. The mobilities of the albumin and alpha and gamma globulin peaks were 6 per cent above normal, whereas that of the beta peak was 10 per cent above normal. The $alpha_2$ peak was so distorted by the beta peak that it was impossible to measure the distance traveled.

This experiment was twice repeated in identical fashion except that an attempt was made to separate the uranyl acetate, which was presumably combined with the proteins, by a 36-hr dialysis against many changes of veronal buffer. Electrophoresis of these samples showed similar abnormally high mobilities.

In another experiment 9 ml of plasma with similar additions of uranyl acetate and veronal buffer was treated by the addition of 1 ml of a 0.9M solution of neutral sodium malate. The plasma was then dialyzed for 2 hr against 200 ml of veronal buffer. The addition of sodium malate with subsequent dialysis was repeated twice more. Electrophoresis of this plasma showed entirely normal patterns, with normal mobilities. The fibrinogen peak had reappeared. This experiment was repeated with identical results.

The most probable explanation of the increases in the mobilities of the various proteins is that uranium-containing ions have increased

the total net negative charge of the protein ions. It is obviously impossible, from these experiments, to state the mechanism of this change.

2.9 Combination of Uranium Compounds with Denatured Protein. It was possible for W. F. Neuman's group to make use of some of the information obtained in the previously summarized test-tube experiments on the action of uranyl acetate on protein to devise a new quantitative method for the precipitation of U_6. The U_6 is allowed to combine with protein (egg albumin), which then is precipitated by denaturation of the albumin. Such a method was particularly useful in separating the uranium from bone salts. The details will be found in W. F. Neuman's reports (see Chap. 11). The work is included in this report because it shows that the dissociation of the U_6-protein complex is sufficiently low (in the presence of excess protein) so that microgram quantities of U_6 can be quantitatively precipitated in this manner. It also shows that combination of U_6 with a denatured protein as well as with a native protein is relatively firm. The combination of uranium with denatured protein at the isoelectric point of the latter is so firm that washing with 0.1M acetate buffer of pH 5 will not remove the U_6, although 0.1M bicarbonate will remove it readily, and 0.025M bicarbonate will remove it to the extent of about 60 to 70 per cent in two washings. The U_4 also combines firmly with denatured egg albumin and can be removed by 0.1M bicarbonate but not with 0.025M bicarbonate.

2.10 Specific Groups of Protein Combining with U_6 and U_4. (a) Carboxyl Groups of Proteins Combine with U_6. The reasons for this statement are the following:

1. Ionized COOH groups of all organic acids so far have been found to complex with U_6 (see Chap. 1).

2. The titration curves of proteins with uranyl nitrate added show less acid-combining power of the U_6-protein system below the isoelectric point than is given by the sums of the acid-combining powers of the constituents. The titration of proteins in the presence of U_6 will now be presented in detail.

Experimental Procedure. (Written by P. Fanta.) The titrations were performed in a 50-ml beaker with a mechanical stirrer. A Beckman pH meter fitted with external electrodes was used to determine the pH. In each case sufficient distilled water was added to bring the volume at the beginning of the titration to 20 to 25 ml. Approximately 0.1N carbonate-free sodium hydroxide solution was stored in a bottle fitted with a siphon and a soda-lime tube.

The titration data are presented graphically in Figs. 13.1 to 13.7. In each case four curves are presented: A, the titration of the protein; B, the titration of uranyl nitrate; C, the titration of protein plus

uranyl nitrate; and D, a throretical curve obtained by adding A and B. The difference between curves C and D may be regarded as related to the degree of interaction between the uranyl nitrate and the proteins.

Figure 13.1. The protein used in this experiment was a sample of once-recrystallized, dialyzed hen ovalbumin.

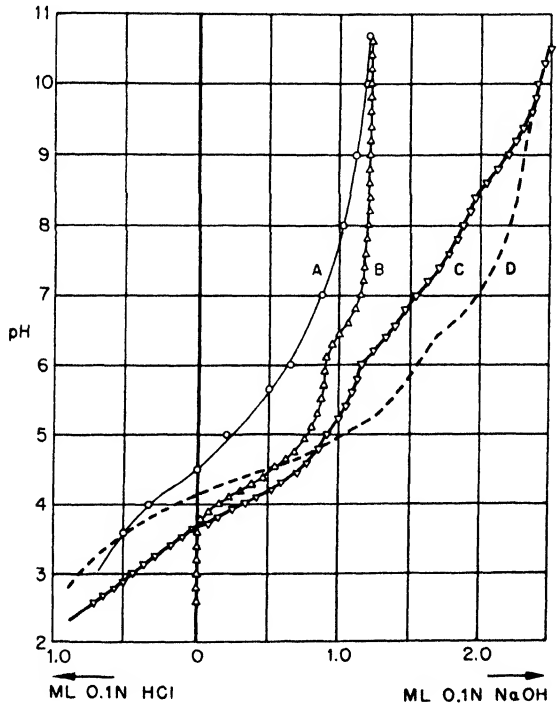


Fig. 13.1 — Effect of U_6 on the titration of egg albumin. Curve A, 5 ml of 6 per cent once-recrystallized egg albumin; curve B, 1 ml of 0.05M uranyl nitrate hexahydrate; curve C, 5 ml of 6 per cent once-recrystallized egg albumin + 1 ml of 0.05M uranyl nitrate hexahydrate; curve D = A + B.

Figure 13.2. The protein used in this experiment was a sample of crystallized lyophiled beef-serum albumin purchased from the Armour Research Laboratories of Chicago. At the beginning of the titrations represented by curves A and C a 2-ml portion of 0.1N hydrochloric acid was added to lower the pH. This may have resulted in more or less denaturation of the protein in each case.

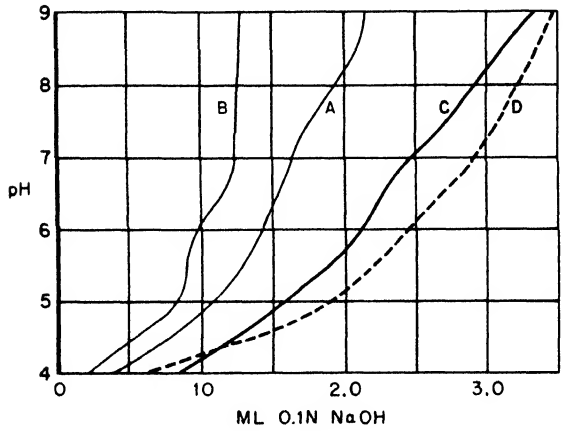


Fig. 13.2 — Effect of U_6 on the titration curve of crystallized beef-serum albumin, probably acid-denatured. Curve A, 5 ml of 5 per cent beef-serum albumin; curve B, 1 ml of 0.05M uranyl nitrate hexahydrate; curve C, 5 ml of 5 per cent beef-serum albumin + 1 ml of 0.05M uranyl nitrate hexahydrate; curve D = A + B.

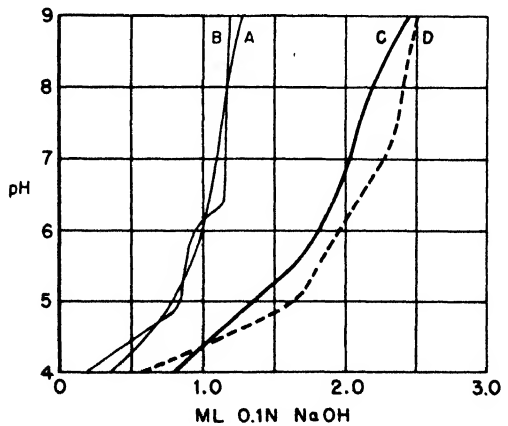


Fig. 13.3 — Effect of U_6 on the titration curve of twice-crystallized dialyzed horse-serum albumin, probably acid-denatured. Curve A, 10 ml of 2.5 per cent horse-serum albumin; curve B, 1 ml of 0.05M uranyl nitrate hexahydrate; curve C, 1 ml of 0.05M uranyl nitrate hexahydrate + 10 ml of 2.5 per cent horse-serum albumin; curve D = A + B.

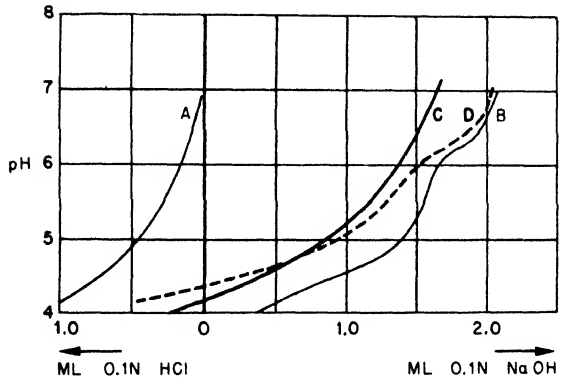


Fig. 13.4 — Effect of U_6 on the titration curve of crystallized horse-serum albumin. Curve A, 10 ml of 2.5 per cent horse-serum albumin; curve B, 1 ml of 0.01M uranyl nitrate hexahydrate; curve C, 10 ml of 2.5 per cent horse-serum albumin + 1 ml of 0.1M uranyl nitrate hexahydrate; curve D = A + B.

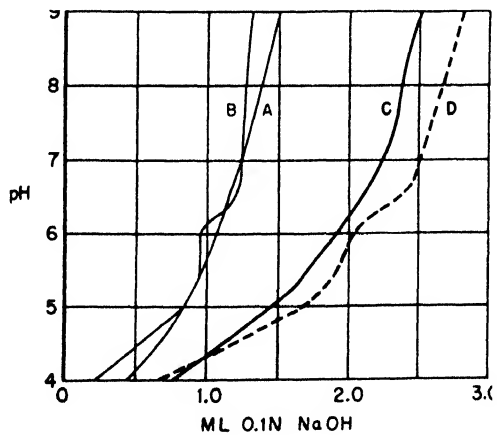


Fig. 13.5 — Effect of U_6 on the titration curve of acid-denatured beef hemoglobin. Curve A, 5 ml of 5 per cent acid-denatured beef hemoglobin; curve B, 1 ml of 0.05M uranyl nitrate hexahydrate; curve C, 1 ml of 0.05M uranyl nitrate hexahydrate + 5 ml of 5 per cent acid-denatured beef hemoglobin; curve D = A + B.

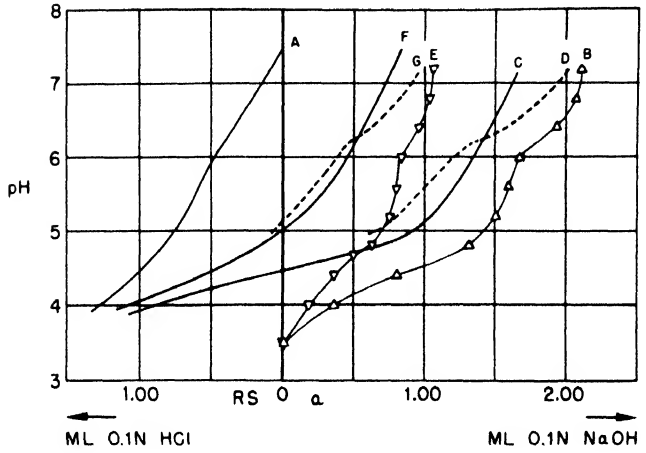


Fig. 13.6 — Effect of U_6 on the titration curve of beef hemoglobin. Curve A, 5 ml of 5 per cent once-recrystallized beef hemoglobin; curve B, 1 ml of 0.1M uranyl nitrate hexahydrate; curve C, 1 ml of 0.1M uranyl nitrate hexahydrate + 5 ml of 5 per cent once-recrystallized beef hemoglobin; curve D = A + B; curve E, 0.5 ml of 0.1M uranyl nitrate hexahydrate; curve F, 0.5 ml of 0.1M uranyl nitrate hexahydrate + 5 ml of 5 per cent beef hemoglobin; curve G = A + E.

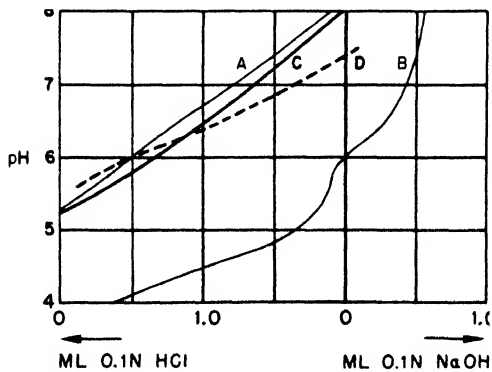


Fig. 13.7 — Effect of U_6 on the titration curve of once-recrystallized horse hemoglobin. Curve A, 5 ml of 5 per cent horse hemoglobin; curve B, 1 ml of 0.1M uranyl nitrate hexahydrate; curve C, 5 ml of 5 per cent horse hemoglobin + 1 ml of 0.1M uranyl nitrate hexahydrate; curve D = A + B.

Figure 13.3. The protein used in this experiment was a sample of once-recrystallized, dialyzed horse-serum albumin prepared according to the procedure of Hewitt[3] and preserved by lyophiling. The crystallizability of the protein was not destroyed by the lyophiling. At the beginning of the titrations represented by curves A and C, 1-ml portions of 0.1N hydrochloric acid were added to lower the pH to a convenient value (3.2 to 3.6). This may have resulted in more or less denaturation of the protein.

Figure 13.4. This experiment with horse-serum albumin was similar to the preceding one except that precautions were taken to avoid denaturation. At the beginning of titrations A and C, measured volumes of sodium hydroxide were added to bring the initial pH values to near 7. The titration was carried out with hydrochloric acid, and the data were recalculated to allow for the added base.

Figure 13.5. Denaturation probably resulted in this experiment with once-recrystallized beef hemoglobin when the protein was brought to an initial pH of about 3.0.

Figure 13.6. Denaturation was avoided in this experiment with once-recrystallized beef hemoglobin by the use of an initial pH of about 7.0.

Figure 13.7. The protein used in this experiment was a sample of once-recrystallized horse hemoglobin prepared by a modification of the method of Gubler and Schmidt.[4] In our procedure washed horse cells were hemolyzed with saponin in water. Furthermore 0.1N acetic acid instead of 0.1N hydrochloric acid was employed in the acidification steps. Washing of the crystals with 25 per cent alcohol was omitted. For use in the titration experiments the dialyzed hemoglobin was brought to pH 8.1 by the addition of carbonate-free sodium hydroxide.

Discussion of Results. An examination of the curves discloses that in each case the calculated curve crosses the protein curve at a well-defined pH value that can be designated as the "crossing point." The crossing always occurs in the same sense; i.e., compared to the acid- and base-combining power of pure protein, the protein in the presence of uranyl ion has diminished acid-combining power below the crossing point. Above the crossing point the uranyl nitrate shows a diminished base-combining capacity, whereas the base-combining capacity of the protein probably is not changed. Both of these effects no doubt can be ascribed to combination of U_6 with acid groups of the protein as explained in Chap. 1.

In the case of the proteins that were not subjected to extremes of pH, and thus presumably retained their native states, the crossing points are reasonably close to the isoelectric points of the native

proteins reported in the literature. This is shown by the data in Table 13.2.

Table 13.2—Comparison of Isolation Points with Crossing Points for Various Proteins

Protein	Crossing point	Isoelectric point*
Native proteins:		
Egg albumin	4.7	4.6
Horse-serum albumin	4.7	4.7
Beef hemoglobin	6.35	6.8
Horse hemoglobin	6.25	6.8
Denatured proteins:		
Beef-serum albumin	4.3	?
Horse-serum albumin	4.3	?
Beef hemoglobin	4.2	?

*P. B. Hawk and O. Bergeim, "Practical Physiological Chemistry," 11th ed., The Blakiston Company, Philadelphia, 1944.

At the crossing point there is no effect on the titration curve caused by the interaction of the protein and U_6, but it must not be assumed that there is no interaction between the protein and the U_6 at this point. On the contrary there is a strong interaction. The titration curves obviously must cross at some point if the signs of the algebraic differences between the two curves are opposite in alkaline and acid solutions.

(b) Combination of U_6 with Basic Groups of Protein. There is no evidence to show that U_6 combines with basic groups of proteins except in the sense of forming loose ionic linkages.

This statement that U_6 probably does not combine with basic groups of proteins except in an ionic sense is a reversal of earlier ideas expressed by this laboratory. The latter were based for the most part on a misinterpretation of the results of titration experiments with proteins.

Since at acid pH values (2 to 3) proteins precipitated by U_6 dissolve, and since at these pH values the chief form of U_6 is probably the UO_2^{++} ion that could hardly combine with ionized basic groups, it can be concluded that U_6 will not combine with basic groups of proteins even in very acid solution (see Chap. 1).

(c) Does U_6 Combine with the SH Groups of Proteins? U_6 has no affinity whatsoever for SH groups of proteins or amino acids in so far as can be determined. The evidence is as follows:

1. Urease, an SH enzyme that is poisoned by the merest traces of silver, lead, or mercury, is relatively insensitive to U_6 (see Sec. 4 of this chapter, under Urease).

2. Excess of SH compounds such as cysteine and glutathione have no effect on the reversal of enzyme poisoning by U_6. This is particularly marked in the case of phosphorylase, which is more sensitive to U_6 than are most other enzymes. Cysteine inhibits the poisoning of phosphorylase by Hg but not by U_6.

3. Cysteine appears to have no effect in vivo on the poisoning of animals by U_6 (see Chap. 15).

4. Uranyl acetate does not prevent the decolorization of porphyrindene by cysteine.*

(d) Does U_6 Combine with Alcoholic or Phenolic Groups? There is no evidence to show that U_6 has any marked affinity for single aliphatic hydroxyl groups or single phenolic groups in the absence of adjacent acid groups. Using the technique of testing the power of the compound in question to dissolve albumin precipitated by uranyl acetate at pH 4.5 to 5.0, it had been found that methyl and ethyl alcohol, isoamyl alcohol, glycerol, and phenol do not dissolve the precipitated albumin in any concentration, and hence the functional groups in question (alcoholic or phenolic OH groups) are not complexers of U_6. Catechol by this test turns out to be only a relatively weak complexer of U_6 in spite of the fact that it does yield a complex as evidenced by the formation of a red color in the presence of U_6.

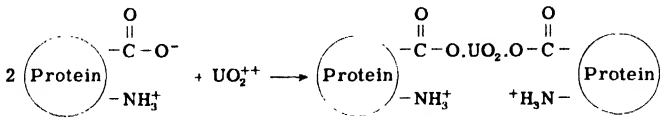
It must be noted, however, that compounds like citric acid, with at least two acid groups separated by not more than two carbon atoms and on one a hydroxyl group, are especially strong complexers for U_6 (Barron and coworkers, Chicago Report CH-2429), and hence under these special conditions it appears probable that the OH group does complex with U_6. It is also to be recalled that glyceryl phosphate is a strong complexer for U_6, and it is possible, although not demonstrated, that here also one of the OH groups complexes with the U_6 as well as the phosphate radical.

2.11 A Theory to Explain the Precipitating Action of U_6 on Protein. It has been shown that U_6 combines rather firmly with acid groups of proteins, that proteins appear to be precipitated by U_6 to the greatest extent in the neighborhood of their isoelectric points, and that in

*The porphyrindene sample was kindly furnished by Dr. J. P. Greenstein of the National Cancer Institute at Bethesda, Md.

certain cases relatively small amounts of salt as well as U_6 are required in order for the protein to precipitate.

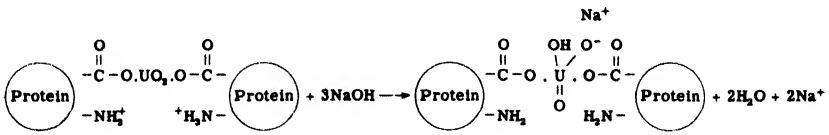
The following type of reaction appears likely between uranyl acetate and protein, near the isoelectric point of the latter:



Such a reaction could give rise to the formation of a framework type of precipitate if the ionization of basic groups were insufficient to keep the aggregated protein molecules in solution and if the degree of aggregation were sufficiently high. On the other hand, if sufficient ionization of basic groups occurred, the aggregates, particularly if not very large, might easily remain soluble unless a small amount of electrolyte were added to neutralize charges on the ionized basic groups locally.

If this theory of protein precipitation is correct, it should be possible to detect an aggregation of certain proteins treated with uranyl acetate near the isoelectric point in the absence of salt. Any appropriate physical method for measuring protein molecular weights could be employed, and even viscosity measurements might be used. The protein tested would of course be one that does require the presence of a small amount of salt in order to be precipitated by U_6.

It is not clear why proteins can form soluble complexes with U_6 when the pH is raised sufficiently above the isoelectric point. A possibility is that the complexed U_6 becomes ionized according to the following hypothetical reaction:



The chief distinction between precipitation of proteins by U_6 and by other heavy metals such as mercury appears to be that there is less tendency for the protein to form soluble complexes with the usual heavy metals than with U_6 as the pH is raised. In addition, the ordinary heavy metals appear to have less precipitating action than has U_6 at or slightly below the isoelectric point of the protein in question.

It is likely that the precipitation of protein by any type of heavy metal involves formation of some sort of framework precipitate.

2.12 Study of the Effect of Salt on the Precipitation of Protein by U_6 at Different pH Values. It may be instructive at this point to cite some simple test-tube experiments with crystallized horse hemoglobin to show the effect of pH on the salt effect already described, since these experiments furnish some supporting evidence for the general theory just outlined.

1. To 2 ml of 5 per cent horse hemoglobin free from toluene that has been previously neutralized to pH 8.0 with dilute NaOH, 2.0 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ is added. No precipitation takes place. In fact the constituents can be mixed in all proportions without the occurrence of any precipitation.

Next, 0.2 ml of saturated sodium chloride or a few crystals of solid NaCl are added, followed by mixing. A heavy gelatinous precipitate of hemoglobin forms at once. The pH of the solution is about 4.5. That the hemoglobin is not denatured can readily be demonstrated by adding at once 0.2 ml of 0.5M trisodium citrate. The hemoglobin will quickly dissolve, leaving a perfectly clear solution. If toluene is present, some denaturation will occur.

2. The preceding experiment is repeated, except that 1 per cent $UO_2(Ac)_2.2H_2O$ that has been previously neutralized to about pH 6.8 with NaOH is used. To prepare the latter solution, 1 volume of 0.1N NaOH is added to 1 volume of 2 per cent $UO_2(Ac)_2.2H_2O$, and the pH is measured with the glass electrode. NaOH is then added, drop by drop, until the pH is 6.8. In this experiment the addition of the NaCl causes only a slight precipitation of the albumin. If the pH is now adjusted to 5.5, the amount of precipitate is increased; if the pH is lowered still further to 4.5, precipitation is practically complete. Denaturation can be avoided by working rapidly. Although the U_6 appears to have no denaturing action on proteins per se, leaving the hemoglobin too long at a pH as low as 4.5 might cause denaturation, especially at room temperature.

3. If Experiment 1 is repeated up to the addition of NaCl, and then the pH is carefully adjusted to 6.5 to 6.8 with 0.1N NaOH without adding any NaCl, a certain amount of hemoglobin is precipitated.

4. If one repeats Experiment 2 and then adjusts the pH to 8.0 with 0.1N NaOH, the small amount of precipitate disappears.

These experiments can be interpreted as follows. It is assumed as already outlined in detail in Sec. 2.9 that protein precipitation by U_6 may occur as the result of aggregate formation brought about by a complexing reaction of UO_2^{++} with the protein molecules, followed by neutralization of charges on the molecules. In the case of hemoglobin, U_6 itself is unable to produce neutralization of charges; if complete precipitation of the protein is to occur, some inorganic salt such as NaCl must be added. The reason precipitation of the hemo-

globin is best at pH 4.5 is that here UO_2^{++} cations are liberated from the uranyl acetate complex; at the same time there are still a number of ionized carboxyl groups present on the hemoglobin molecule. At pH 7.0, even though more acid groups of the protein are ionized, less UO_2^{++} ions are present, since a large part of the uranium is present as uranyl acetate complex which does not combine with the protein, except possibly in an ionic sense. However, even at pH 7.0 apparently enough UO_2^{++} ions can be formed to give a measurable amount of precipitation in the presence of NaCl. If the pH is lowered to 4.5, precipitation occurs at once.

2.13 Removal of Uranium from Proteins by Malate, Citrate, and Bicarbonate; Physiological Applications; Distribution of U_6 in Plasma between Bicarbonate and Protein. It has been shown that anions which are strong complexers for U_6, such as malate, citrate, and bicarbonate, will dissolve protein precipitated by U_6 and in so doing remove the U_6 from its combination with protein. Weaker complexers like acetate are less effective.

In plasma the two materials that are capable of complexing U_6 strongly and that are present in highest concentration are bicarbonate and protein. Therefore as a first approximation the partition of U_6 between bicarbonate and protein in plasma should be studied as a means of ascertaining how U_6 is transported in plasma. The only possibility of a serious competitor for U_6 besides protein and bicarbonate appears to be lactate in high concentration, as might occur temporarily under conditions of heavy exercise.

The partition of U_6 at concentrations to be expected initially in the blood of heavily poisoned animals has been studied quantitatively by Fanta, using the ultrafiltration technique. The results of this study are reported in Chap. 1. His studies have shown that the U_6 in plasma appears to be transported chiefly by bicarbonate and protein, as predicted.

2.14 Distinction between U_6 and U_4 in Regard to Combination with Protein. Little can be said here of a quantitative nature, but something can be stated of a qualitative nature. The main points of interest are as follows:

1. The U_4 is apparently at least as strong a protein precipitant as U_6 for the proteins investigated (egg and serum albumin, chicken and dog hemoglobin, urease, etc.). The U_4 can be tested by dissolving UCl_4 in 0.1M acetate buffer and raising the pH to about 5.0 by the cautious addition of NaOH. Above pH 5.5 the U_4 precipitates. The U_4-acetate solution is then added to the protein in question.

2. The U_4-protein precipitates can be dissolved by citrate buffer in low concentration, and usually by 0.025M bicarbonate, but the latter will not dissolve U_4 oxide that has been precipitated by the addition

of dilute NaOH to UCl_4 solution. One-tenth molar bicarbonate will remove microgram quantities of U_6 quantitatively from denatured egg albumin (W. F. Neuman *et al.*). Although U_4 precipitated as hydrous oxide is not soluble in 0.025M bicarbonate, U_6 precipitated as hydrous oxide (or hydroxide) is readily soluble in 0.025M bicarbonate. Dilute NaOH will dissolve protein precipitated by UCl_4, in the presence of a small amount of sodium acetate.

Thus bicarbonate complexes U_4 somewhat less strongly than it complexes U_6, whereas protein, at least at pH values between 4 and 6, may complex U_4 even more strongly than it complexes U_6. For these reasons it can be expected that U_4 will be carried largely by protein in the plasma or that it will exist there as a colloidal oxide or hydroxide and therefore not be able to pass through the glomerular barrier readily. That the U_4 does not pass the glomerular barrier readily is indicated by experiments on U_4 excretion in the urine (see Chap. 15).

3. The U_4 is quantitatively precipitated by denatured egg albumin in microgram quantities (W. F. Neuman *et al.*).

4. The U_4 appears to combine with SH groups and hence would be expected to have a more marked inhibiting action on SH enzymes than has U_6. In the case of urease and phosphorylase this appears to be true. Reference is made to Sec. 4 in this chapter, under Urease and under Phosphorylase.

5. Apparently U_4 can combine with at least some proteins without denaturing them. In the case of oxyhemoglobin, there is no immediate spectral change caused by adding relatively small amounts of U_4 at pH 5.5, indicating that denaturation has not occurred. But gradually the oxyhemoglobin is changed to hemoglobin. Protein such as egg albumin that has been precipitated by U_4 can be redissolved readily by adding a small amount of citrate buffer or a larger amount of bicarbonate buffer, indicating that denaturation has not occurred. Urease can be precipitated by U_4, allowed to stand overnight in the refrigerator, and then can be redissolved by the addition of a small amount of pH 6 citrate buffer apparently without any serious loss in activity.

2.15 Nucleic Acid and Nucleoprotein. The U_6 will precipitate thymus nucleic acid (prepared according to Hammarsten), and citrate will dissolve this precipitate. This is also true of yeast nucleic acid prepared according to Johnson.[5] Phosphoric acid groups must be involved in the complexing of nucleic acid with U_6 since it is known that phosphate combines with U_6 and that glyceryl phosphate is a strong complexer for U_6. The general theory of protein precipitation by U_6 based on the idea of aggregation and neutralization of charge probably could be applied to the precipitation of nucleic acid by U_6.

globin is best at pH 4.5 is that here UO_2^{++} cations are liberated from the uranyl acetate complex; at the same time there are still a number of ionized carboxyl groups present on the hemoglobin molecule. At pH 7.0, even though more acid groups of the protein are ionized, less UO_2^{++} ions are present, since a large part of the uranium is present as uranyl acetate complex which does not combine with the protein, except possibly in an ionic sense. However, even at pH 7.0 apparently enough UO_2^{++} ions can be formed to give a measurable amount of precipitation in the presence of NaCl. If the pH is lowered to 4.5, precipitation occurs at once.

2.13 Removal of Uranium from Proteins by Malate, Citrate, and Bicarbonate; Physiological Applications; Distribution of U_6 in Plasma between Bicarbonate and Protein. It has been shown that anions which are strong complexers for U_6, such as malate, citrate, and bicarbonate, will dissolve protein precipitated by U_6 and in so doing remove the U_6 from its combination with protein. Weaker complexers like acetate are less effective.

In plasma the two materials that are capable of complexing U_6 strongly and that are present in highest concentration are bicarbonate and protein. Therefore as a first approximation the partition of U_6 between bicarbonate and protein in plasma should be studied as a means of ascertaining how U_6 is transported in plasma. The only possibility of a serious competitor for U_6 besides protein and bicarbonate appears to be lactate in high concentration, as might occur temporarily under conditions of heavy exercise.

The partition of U_6 at concentrations to be expected initially in the blood of heavily poisoned animals has been studied quantitatively by Fanta, using the ultrafiltration technique. The results of this study are reported in Chap. 1. His studies have shown that the U_6 in plasma appears to be transported chiefly by bicarbonate and protein, as predicted.

2.14 Distinction between U_6 and U_4 in Regard to Combination with Protein. Little can be said here of a quantitative nature, but something can be stated of a qualitative nature. The main points of interest are as follows:

1. The U_4 is apparently at least as strong a protein precipitant as U_6 for the proteins investigated (egg and serum albumin, chicken and dog hemoglobin, urease, etc.). The U_4 can be tested by dissolving UCl_4 in 0.1M acetate buffer and raising the pH to about 5.0 by the cautious addition of NaOH. Above pH 5.5 the U_4 precipitates. The U_4-acetate solution is then added to the protein in question.

2. The U_4-protein precipitates can be dissolved by citrate buffer in low concentration, and usually by 0.025M bicarbonate, but the latter will not dissolve U_4 oxide that has been precipitated by the addition

of dilute NaOH to UCl_4 solution. One-tenth molar bicarbonate will remove microgram quantities of U_6 quantitatively from denatured egg albumin (W. F. Neuman *et al.*). Although U_4 precipitated as hydrous oxide is not soluble in 0.025M bicarbonate, U_6 precipitated as hydrous oxide (or hydroxide) is readily soluble in 0.025M bicarbonate. Dilute NaOH will dissolve protein precipitated by UCl_4, in the presence of a small amount of sodium acetate.

Thus bicarbonate complexes U_4 somewhat less strongly than it complexes U_6, whereas protein, at least at pH values between 4 and 6, may complex U_4 even more strongly than it complexes U_6. For these reasons it can be expected that U_4 will be carried largely by protein in the plasma or that it will exist there as a colloidal oxide or hydroxide and therefore not be able to pass through the glomerular barrier readily. That the U_4 does not pass the glomerular barrier readily is indicated by experiments on U_4 excretion in the urine (see Chap. 15).

3. The U_4 is quantitatively precipitated by denatured egg albumin in microgram quantities (W. F. Neuman *et al.*).

4. The U_4 appears to combine with SH groups and hence would be expected to have a more marked inhibiting action on SH enzymes than has U_6. In the case of urease and phosphorylase this appears to be true. Reference is made to Sec. 4 in this chapter, under Urease and under Phosphorylase.

5. Apparently U_4 can combine with at least some proteins without denaturing them. In the case of oxyhemoglobin, there is no immediate spectral change caused by adding relatively small amounts of U_4 at pH 5.5, indicating that denaturation has not occurred. But gradually the oxyhemoglobin is changed to hemoglobin. Protein such as egg albumin that has been precipitated by U_4 can be redissolved readily by adding a small amount of citrate buffer or a larger amount of bicarbonate buffer, indicating that denaturation has not occurred. Urease can be precipitated by U_4, allowed to stand overnight in the refrigerator, and then can be redissolved by the addition of a small amount of pH 6 citrate buffer apparently without any serious loss in activity.

2.15 Nucleic Acid and Nucleoprotein. The U_6 will precipitate thymus nucleic acid (prepared according to Hammarsten), and citrate will dissolve this precipitate. This is also true of yeast nucleic acid prepared according to Johnson.[5] Phosphoric acid groups must be involved in the complexing of nucleic acid with U_6 since it is known that phosphate combines with U_6 and that glyceryl phosphate is a strong complexer for U_6. The general theory of protein precipitation by U_6 based on the idea of aggregation and neutralization of charge probably could be applied to the precipitation of nucleic acid by U_6.

Since nucleoprotein is made up of nucleic acid and protein, both of which combine with U_6, nucleoprotein must combine with U_6 and U_4 and must be precipitated by U_6 or U_4 under proper conditions, but experiments have not been conducted with this type of material.

3. ACTION OF URANIUM COMPOUNDS ON VARIOUS ENZYMES AND ENZYME SYSTEMS IN VITRO AND IN VIVO

3.1 Introduction. The general aim of the work concerned with the effect of uranium compounds on enzymes was first to ascertain whether the general inhibitory effects are severe or light, and second to attempt to determine whether a particular enzyme or enzyme system could be preferentially inhibited. If uranium were a powerful enzyme poison, and particularly if it were known that uranium affected some particular enzyme of enzyme system considerably more than others, it should be possible to explain its toxic action on this basis and perhaps to prevent or reverse the poisonous action.

Various poisons are known to act rather specifically on certain enzymes or groups of enzymes. For instance, cyanide acts on some iron porphyrin enzymes such as cytochrome oxidase and catalase and at least partly for this reason is a powerful poison to the respiratory system of cells, although other enzymes also are no doubt inhibited. Certain metals such as arsenic, lead, and mercury have a strong affinity for SH groups and hence are extremely poisonous to SH enzymes such as urease, in vitro. The in vivo action may be somewhat less marked because these metals can combine with other groups of inert proteins and hence have less chance to single out SH groups with which to combine.

In addition to reversibly inhibiting SH enzymes, heavy metals of the type listed above can also cause an irreversible denaturation of the enzyme in vitro. For a general discussion of the action of heavy metals on enzymes and cells, see Clark.[6]

In regard to the action of U_6 on enzymes in vitro, its action may be mild or moderately severe, depending upon the conditions used in the experiment and upon the particular enzyme in question. In most of the work done in this laboratory the uranium compound (usually uranyl acetate) has been added to the substrate followed by addition of the enzyme. Since most substrates and also buffers have the ability to complex U_6, there is a competition for the U_6 by the enzyme and the substrate as well as by the buffer employed, with the result that part of the added U_6 is not accessible to the enzyme.

It is also possible to add partially neutralized uranyl nitrate to the enzyme before addition of the substrate, as has been done by investigators at the University of Chicago. Since there appears to be a

fairly rapid equilibration between U_6, protein, and the low-molecular-weight complexing agent for U_6, the two types of experiments may not be so widely divergent as was at first thought. One point that should be kept clearly in mind, however, is the fact that high concentrations of substrate (such as are commonly used in the determination of enzymes) often tend to lower the effect of the uranium compound on the enzyme, because many substrates tend to form complexes with uranium. There is evidence that U_6 complexed with low-molecular-weight substances such as organic acids or bicarbonate has no effect on enzymes or proteins. In the cell the single substrate concentrations are much lower than the concentrations usually employed in vitro, but, on the other hand, there are so many different substrates present that the sum total of all substrates capable of complexing with U_6 may be comparable to the concentrations of the single substrates used for experiments in vitro. In addition, there are a great number of proteins in the cell, all of which undoubtedly can complex U_6 (or U_4).

Finally it must be realized that the bicarbonate and inert protein of the plasma are excellent protective agents against cellular poisoning by uranium compounds because of their strong tendency to complex U_6. How this effect is overcome to allow kidney poisoning by U_6 will be discussed in Chap. 15 on mechanism.

It has been found in general that U_6 reversibly inhibits many enzymes to a slight or moderate degree when the experiments have been carried out as indicated above and when the pH is below 6.5. Some enzymes, however, are hardly inhibited at all. On the other hand, at least two — phosphorylase and thrombin — are rather severely inhibited. For additional enzymes not investigated in the Rochester laboratory, the work of Barron *et al.* at the University of Chicago should be consulted.

One general influence on the inhibition of enzymes by U_6 compounds that has been found thus far always to prevail is the effect of pH. With all the enzymes investigated in this laboratory, the degree of inhibition by U_6 increases as the pH decreases. This phenomenon can be explained at least partly by the fact that the precipitating action and hence probably the general aggregating tendency of U_6 on proteins generally increases as the pH approaches the isoelectric point of the protein, for proteins with the isoelectric point in the pH range 4 to 6. This phenomenon is also partly attributable to the fact that lowering of the pH often acts upon the buffer system (and sometimes the substrate) so as to increase the amount of uncomplexed uranyl ion in solution. It is evidently the uncomplexed uranyl ion that combines with the enzyme to effect an inhibition of its action. The pH effect on the poisoning of enzymes by U_6 will be brought up again in the

discussion on mechanism, in order partially to explain the localized action of U_6 on the kidney tubule.

The effect of pH on the inhibition of enzymes by U_4 has not been extensively studied, owing to the insolubility of U_4 at pH values above 5.0 to 5.5. If U_4 were neutralized to pH 7.4 before being added to an enzyme preparation, it would be present chiefly as colloidal or precipitated oxide and therefore would not be expected to poison the enzyme. Changing the pH has been found to influence the inhibition of urease by U_4 in exactly the same manner as it influences the inhibition of this enzyme by U_6.

The effect of U_6 on enzymes appears to be rather mild compared to the effect of a metal like Hg, which apparently cannot only reversibly inactivate an enzyme but can in time irreversibly inactivate it, presumably by denaturation.[7]

From a consideration of the results of experiments on the limited number of enzymes studied in this laboratory, it appears likely that U_6 would not affect enzymes seriously at body pH in cells at the concentrations that might be expected from the administration of toxic but nonlethal doses, and perhaps even of lethal doses, unless some special mechanism existed for concentrating the U_6 in or on the surface of certain specialized cells and for enhancing its action there by the removal of complexing agents. That such special mechanisms do occur in the kidney has been demonstrated (see Chap. 15).

At the present time it is doubtful that the poisoning of specific enzymes in the kidney accounts for the action of U_6 on this organ, but instead it seems probable that there is a sufficiently general action of U_6 on enzymes and proteins of the surfaces of kidney tubular cells to account for the damage. This will be discussed in more detail in Chap. 15.

An additional reason for assuming a relatively nontoxic effect of U_6 on the enzymes of cells other than those of the kidney is the likelihood that U_6 administered in comparatively low doses does not enter cells other than kidney cells in appreciable amounts, partly because of its particular mode of transportation in plasma and partly because its concentration in blood falls rapidly after administration, owing to deposition in bone and kidney. In vivo studies have not contradicted this assumption.

It should be noticed that the general information about the action of U_6 (and to a more limited extent of U_4) on enzymes, together with the known ability of the kidney to concentrate administered U_6, leads to the conclusion that the effect of U_6 on the body in moderately low doses must be primarily an effect on the kidney, and that effects on other organs or tissues are likely to be secondary to the initial kidney damage. This argument does not hold for massive doses of U_6, in

which case general enzyme inhibition no doubt can occur in organs other than the kidney.

The work with enzymes has contributed to an understanding of the effect of alkali in uranium poisoning (see Chap. 15) and, together with other work (physiological, toxicological, and physicochemical), has made it appear somewhat doubtful that an antidote for uranium poisoning that is more effective than bicarbonate will be found. Bicarbonate can reverse enzyme inhibition caused by U_6 and in general complexes so strongly with U_6 that in its presence an inhibiting action of U_6 in relatively low concentrations on enzymes is not likely to be found. The inhibition of enzymes by U_6 can be reversed by citrate as well as by bicarbonate (and presumably also by malate or any other strong complexing agent), but complexing agents other than bicarbonate thus far employed either are toxic to the animal or else are too rapidly metabolized by the body to be effective as antidotes. In any case they do not appear to have a much greater complexing affinity for U_6 than does bicarbonate. Moreover, bicarbonate is an alkalinizing agent that probably is effective in aiding kidney repair as well as in removing U_6.

Some ideas about the general mechanism of the effect of uranium compounds on various types of enzyme systems now follow, and after this will be given in detail individual experiments with a number of enzymes.

3.2 General Mechanism of the Effect of Uranium Compounds on Enzymes and Enzyme Systems.* (a) Reaction of U_6 with Apoenzyme Protein. All enzymes thus far highly purified have been found to be simple proteins or to consist of a protein apoenzyme plus a rather firmly bound nonprotein prosthetic group such as hematin and flavine-adenine dinucleotide. Examples of the first class are urease, pepsin, trypsin, and chymotrypsin, and examples of the second type are catalase and *d*-amino oxidase. A third type of enzyme consists of protein apoenzyme that requires the presence of a nonprotein coenzyme. The coenzyme may be thought of as a highly dissociable prosthetic group. Examples of this third class are lactic dehydrogenase, which requires coenzyme I (codehydrogenase I or diphosphopyridine nucleotide), and Robinson ester dehydrogenase, which requires coenzyme II (codehydrogenase II, or triphosphopyridine nucleotide).

Since all enzymes are undoubtedly proteins or have a component that is a protein, it can be expected at the outset that the same general effects observed in the test-tube experiments with inert protein such as egg or serum albumin also will be observed with the enzymes. Indeed some of the proteins employed in the general protein experi-

*The reader unfamiliar with the details of modern enzymology is advised to refer to "Chemistry and Methods of Enzymes."[8]

ments were crystallized enzymes. In particular it would be expected that U_6 would combine with carboxyl groups of the enzyme. Such combination with presumably nonfunctional groups might be without effect on enzymatic activity until many carboxyl groups were involved or considerable aggregation or precipitation of the enzyme had taken place. An analogous case is the acetylation of pepsin. Here activity is not lost until a certain number of acetyl groups have been added.[9]

It seems likely that in many cases the action of U_6 on enzymes can be satisfactorily explained by this nonspecific action. It must be conceded that in the case of enzymes like phosphorylase and thrombin a more specific combination of U_6 with a necessary functional group of the enzyme might be involved. But even in such cases it is possible that one or more key acid groups of ordinary amino acids are required for combination of enzyme with substrate and that these key acid groups combine with the uranium and so are no longer accessible to the substrate.

(b) Possibility of Interaction of U_6 with Prosthetic Groups or Coenzymes, Including SH Groups. 1. There is no evidence to show that U_6 can combine with SH groups. This has been mentioned already, and details will be found in Sec. 4 of this chapter under experiments with urease and phosphorylase. On the other hand, U_4 apparently can combine with SH groups. The evidence appears under work on phosphorylase in Sec. 4 of this chapter.

2. There is no evidence from studies on cytochrome oxidase and catalase that reasonably small amounts of U_6 can combine with a hematin prosthetic group in such a way as to hinder seriously the action of a hematin enzyme at body pH. The details of the work will be found under the experiments on catalase and cytochrome oxidase.

3. It has been found that *d*-amino oxidase is not seriously affected by U_6. It might be concluded from this that U_6 is not able to combine with flavine-adenine dinucleotide (the prosthetic group of *d*-amino oxidase) in such a way as to block enzyme activity. But the *d*-amino oxidase employed was not purified. Hence it can be concluded only that under the conditions of the experiment in question, in which substances that could complex U_6 no doubt were introduced, no serious blocking of the flavine-adenine dinucleotide prosthetic group occurred.

4. Work with lactic dehydrogenase has indicated that U_6 does not combine with coenzyme I (in low concentrations) in such a way as to block the catalytic action of this prosthetic group in the presence of excess substrate (lactic acid). But coenzyme I prepared by the method of Williamson and Green[10] does appear to complex U_6, so that the whole matter may be one of competition for U_6 by the lactic acid, coenzyme I, and apoenzyme.

The apparent ability of coenzyme I to complex U_6 was investigated by determining the ability of this material to dissolve egg albumin precipitated by U_6 (see Sec. 2 of this chapter). The test can be carried out as follows: To 2.0 ml of a 1 per cent crystallized egg albumin that has been dissolved in water and well dialyzed add 0.1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ solution. Shake. Add the substance to be tested as a complexer in a known amount (as solution or solid). Shake. Determine how much is required just to dissolve the egg-albumin precipitate completely. In the case of coenzyme I prepared according to Williamson and Green, a few milligrams is sufficient. The purity of this coenzyme preparation was not investigated, but the authors stated that their preparation was about 85 per cent pure. Coenzyme I might complex U_6 because of its pyrophosphate group.

5. Pyridoxine complexes U_6 but not so strongly as does bicarbonate. Pyridoxal phosphate (the coenzyme of tyrosine decarboxylase and some other amino acid decarboxylases and of transaminase) has not been available, so it cannot be stated whether this prosthetic group combines strongly with U_6 or not. This prosthetic group should be investigated, however.

6. Investigators at the University of Chicago have reported that yeast carboxylase, a thiamin-pyrophosphate enzyme, is not very sensitive to the action of U_6.

7. Any coenzyme or phosthetic group containing a primary phosphate group may be suspected of complexing U_6, especially if this group is adjacent to an OH group. Such complexing of U_6 might or might not cause inhibition of the reaction normally catalyzed by the coenzyme or phosthetic group in question. Thus far a fairly high order of sensitivity of only two enzymes to U_6 has been demonstrated in this laboratory. These enzymes are phosphorylase and thrombin. In the case of phosphorylase it is known that the probable prosthetic group (adenylic acid) contains a phosphate group. It is not known whether or not phosphate groups are present in thrombin.

In the case of an enzyme like lactic dehydrogenase, which requires coenzyme I for activity, it is possible that lack of marked sensitivity was caused by a successful competition of the substrate (lactate) for the U_6. Also a fairly large amount of coenzyme I was employed in the experiments with lactic dehydrogenase. However, it must also be pointed out that the phosphate groups in coenzyme I are probably secondary phosphates, in contrast to the primary phosphate of adenylic acid, and this may mean less affinity for U_6. Furthermore there is no evidence that these secondary phosphate groups take part in the catalytic reaction involving coenzyme I.

It has been found that alkaline phosphatase is not sensitive to U_6. This is not surprising in view of the fact that excess substrate was used. In this reaction it is the substrate rather than the enzyme that contains the phosphate group. Moreover, the pH employed (9.0 to 9.2) was so high that the U_6 may have been unable to complex even with the primary phosphate group of the substrate. There is no information on this point. In the case of acid phosphatase, some inhibition was observed in spite of excess substrate, but here the pH is so low (5.5) that partial precipitation of the apoenzyme by the U_6 can be expected.

Adenosine triphosphatase from muscle and phosphoglucomutase from muscle were not affected markedly by U_6, but here again excess substrate was employed, and the substrate rather than the enzyme contains the phosphate groups.

All known enzymes with phosphate-containing coenzymes or prosthetic groups, especially if the phosphate is primary and adjacent to an OH group, should be carefully investigated for sensitivity to U_6, and it is regrettable that this approach was not thought of earlier so that more information might have been acquired.

In summary, it seems probable that as a rule the ability of U_6 to inhibit certain enzymes can be explained by combination of U_6 with the protein component of the enzyme rather than with the prosthetic group or coenzyme, although U_6 may inhibit certain enzymes by combining with prosthetic groups or coenzymes that have primary phosphate groups.

3.3 Possible Replacement of a Natural Metallic Activator by U_6. Another way in which a metal might affect an enzyme or enzyme system would be to replace a natural metallic activator, yielding an inactive uranoenzyme. That U_6 does not act in this manner with arginase is already apparent from the work on arginase discussed under proteins. Arginase requires the presence of a metal such as Mn^{++} or Co^{++} for activation.[11] No other cases are known that would throw more light on this possible action of uranium on enzymes.

3.4 Possible Denaturation of Enzymes by U_6. Still another way in which a metal might block enzyme action would be by outright denaturation of the apoenzyme. From what has been said in Sec. 1 on proteins, it is quite certain that uranium compounds do not in general act in this manner in vitro and probably not in vivo.

3.5 Possible Interference of U_6 with Protein Synthesis. A further possibility to be considered in regard to the inhibitory action of uranium compounds on enzymes in vivo is that protein synthesis (and hence enzyme synthesis) in the cell might be directly blocked. Such an explanation of the toxic action of uranium in the body seems unlikely in view of the relative rapidity with which toxic effects can be demonstrated.

3.6 Possible Activation of Proteolytic Enzymes by Uranium. A final possibility to be considered in connection with the interaction of uranium compounds with enzymes is that proteolytic enzymes (or other enzymes of degradation) might be activated by the action of the uranium compounds. This possibility has not been investigated.

3.7 Order of Magnitude of the In Vitro Inhibition of Enzymes by Uranium Compounds. Some idea of the general effectiveness of uranium as an enzyme poison can be gained by comparing its action with the action of metals such as silver and mercury on enzyme urease, which is extremely sensitive to the action of many heavy metals. For instance, 4 μg of crystallized urease at pH 6.5 is inhibited to the extent of about 95 per cent by 0.025 μg of silver nitrate. This gives by calculation, using 483,000 as the molecular weight of urease,[12] a ratio of approximately 15 atoms of silver to 1 molecule of urease. At pH 6.5, 0.1 μg of $HgCl_2$ inhibits 4 μg of urease to the extent of about 90 per cent. The above weights of $HgCl_2$ and urease yield by calculation a ratio of about 50 atoms of Hg to 1 molecule of urease.

In the case of U_6, however, it is found that 2 mg of $UO_2(Ac)_2.2H_2O$ ($\simeq$1.1 mg of uranium metal) in maleate buffer inhibits 2 μg of urease only to the extent of 40 per cent at pH 6.0. These weights yield by calculation a ratio of approximately 20,000 moles of uranyl acetate to 1 mole of urease. By dividing the sensitivity of urease to uranyl acetate by the sensitivities of urease to silver and mercury, it is found that urease is more than 13,000 times more sensitive to silver than to uranyl acetate, and more than 400 times more sensitive to mercury than to uranyl acetate.

The latter figures represent minimal values for two reasons: The first is that absorption of silver and mercury on glass may remove an appreciable portion of these metals from solution at such low concentrations as were used in the experiments. The second is that, even with the high amount of uranyl acetate employed, the urease was inhibited only to the extent of 40 per cent rather than 95 per cent as in the case of silver at the concentration used, or 90 per cent in the case of mercury at the concentration used.

Urease is not especially sensitive to U_6 in comparison with enzymes such as phosphorylase and thrombin, but it is more sensitive than some enzymes such as catalase. Phosphorylase is inhibited at least as strongly by U_6 as by silver and mercury. At pH 6.7, 0.5 mg of this enzyme is inhibited to the extent of 90 per cent by 1 mg of $AgNO_3$, and to the extent of 99 per cent by 1 mg of $HgCl_2$. The sensitivity of phosphorylase to U_6 was about the same as its sensitivity to mercury when the inhibition by these substances was measured at levels of 1 mg of $UO_2(Ac)_2.2H_2O$ and 1 mg of $HgCl_2$. But when levels of uranyl acetate

and $HgCl_2$ were reduced to 0.01 mg there still was 20 per cent inhibition by the $UO_2(Ac)_2.2H_2O$ and zero inhibition by mercury. At 0.1 mg of $HgCl_2$ inhibition was nearly complete, so that in the case of mercury complete inhibition of phosphorylase sets in suddenly at a critical level, whereas in the case of uranyl acetate the inhibition increases more gradually as the amount of U_6 is increased. The important point to bear in mind is that at levels of U_6 and mercuric chloride where serious inhibition occurs (i.e., inhibition greater than 50 per cent), phosphorylase has roughly the same order of sensitivity to uranyl acetate on a dry-weight basis as to mercuric chloride or to silver nitrate. On a molecular basis this would mean that phosphorylase is about twice as sensitive to the U_6 as to mercury. In all the preceding studies, from 0.3 to 0.6 mg of crystallized phosphorylase were used.

It can be calculated (see Sec. 4) that urease is about eight times more sensitive to mercury than phosphorylase is to mercury, silver, or $UO_2(Ac)_2.2H_2O$ on a dry-weight basis. On a molecular basis the sensitivity of urease to mercuric chloride is in the neighborhood of four times the sensitivity of phosphorylase to U_6. (The molecular weight of phosphorylase is 340,000 to 400,000, whereas that of urease is 483,000; and the molecular weight of uranyl acetate is 424.2, whereas that of mercuric chloride is 271.5.)

3.8 Action of Complex Ions in Preventing Enzyme Inhibition by U_6. In the experiments for determining the order of sensitivity of phosphorylase and urease to U_6, special efforts were made to prevent reduction of sensitivities of these enzymes. For instance, maleate buffer was used at pH values low enough so that at least some dissociation of the U_6-buffer complex could occur. If, instead of maleate buffer, 0.025M bicarbonate buffer is employed and the pH is raised to 7.4, then 1 mg of $UO_2(Ac)_2.2H_2O$ no longer has an appreciable inhibiting effect on 0.5 mg of phosphorylase. This is due to complexing of the U_6 by the bicarbonate. The complex, as stated before, dissociates only to a very slight extent, and apparently it is only the dissociated UO_2^{++} that can affect the phosphorylase.

Although the effect of bicarbonate on the poisoning of urease by U_6 was not investigated, it was found that citrate, another strong complexer for U_6, prevented the action of U_6 on urease at the concentrations employed in the experiments already mentioned.

It is true that complexing of silver and mercury by the buffer anions present also can be expected, but apparently such complexing action for some reason does not prevent poisoning of urease by mercury or silver as effectively as the complexing of U_6 with bicarbonate prevents poisoning of phosphorylase by U_6.

3.9 Action of Citrate and Bicarbonate in Reversing the Inhibitory Effect of U_6. It has been found that 0.025M bicarbonate as well as 0.1M citrate can reverse the poisoning of enzymes by U_6. Presumably more dilute citrate would also be effective (see Sec. 4 under the individual experiments). This shows that U_6 does not appear to exert an irreversible damaging action on enzymes, at least in short-time experiments. This finding is in agreement with the work on the general action of U_6 on proteins. The reader is referred to the work of Barron and his collaborators at the University of Chicago for a rather detailed investigation of the effect of citrate in reversing enzyme poisoning by U_6.

3.10 In Vivo Action of U_6 on Enzymes. The in vitro experiments in the absence of strong complexers show a potentiality of U_6 to poison an enzyme (phosphorylase) severely, provided that conditions are just right. But, in the body, conditions in most cases are not favorable for the inhibitions of enzyme activity by U_6, since, on the one hand, plasma contains bicarbonate in appreciable concentration (about 0.024M) as well as high concentrations of protective protein (albumin, for instance) and, on the other hand, cells contain many substrates that can complex U_6, in addition to protein and bicarbonate. Moreover, U_6 may not penetrate cells in the body other than possibly kidney cells. Finally it has been found that kidney phosphorylase is not inhibited for a period of 1 to 2 days by poisoning animals (rats) with relatively large doses of uranyl acetate [5 mg of $UO_2(Ac)_2.2H_2O$, $\simeq$2.6 mg of uranium metal per kilogram of body weight], in spite of the in vitro findings. Thus it is necessary to be very cautious about interpreting the in vivo action of U_6 as being directly attributable to the poisoning of enzymes found to be sensitive to U_6 in vitro.

One enzyme has been found that has a high order of sensitivity to U_6 in vitro, and possibly it is affected by U_6 in vivo. This enzyme is thrombin (see Sec. 4.18 of this chapter under Thrombin).

It has been found that massive doses of U_6 disturb the blood-clotting process, and therefore thrombin may be inhibited. However, blood clotting could also be disturbed in many other ways than by a direct inhibition of thrombin—for instance, by an indirect action of damaged kidneys in disturbing liver function.

3.11 Comparison between U_6 and U_4 as Regards Enzyme Poisoning. In regard to the in vitro effect of U_4 on enzymes, there is relatively little information, chiefly because of technical difficulties occurring because of the great insolubility of U_4 at pH values much above 5.0. However, U_4 seems to inhibit urease and phosphorylase under certain conditions (see Sec. 4 of this chapter) more than does U_6. This statement must be accepted with some caution, since it seems possible that

the U_4 sample used (UCl_4) might possibly have contained other heavy metals not present in the U_6 compounds used (nitrate and acetate).

4. ACTION OF U_6 AND U_4 ON ENZYMES IN VITRO; EXPERIMENTAL MATERIAL

4.1 Adenosine Triphosphatase. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Adenosine triphosphatase, an enzyme that catalyzes the liberation of acid-labile phosphate from adenosine triphosphate (A.T.P.), was prepared from rabbit muscle by Greenstein's method.[13] This preparation consists of a purified myosin having adenosine triphosphatase activity. Although it was formerly thought that adenosine triphosphatase and myosin were identical, this assumption now appears to be erroneous.[14]

This preparation was tested for presence of alkaline phosphatase and phosphorylase, with negative results. The substrate used was the sodium salt of A.T.P. prepared from the barium salt by precipitating the barium with anhydrous Na_2SO_4 and washing well with acidulated water.

The activity of the adenosine triphosphatase (Fig. 13.8) was measured by the amount of inorganic phosphate liberated from adenosine

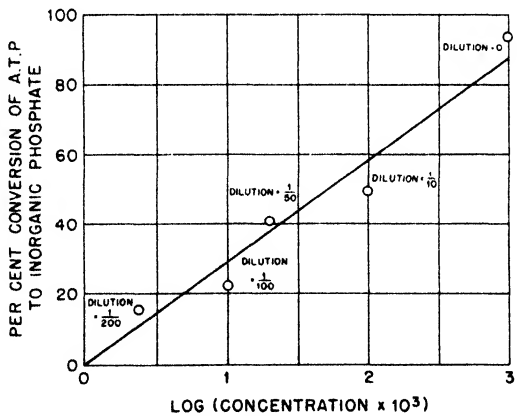


Fig. 13.8 — Variation of adenosine triphosphatase activity with concentration.

triphosphate in maleate buffer at 25°C and was expressed as percentage conversion of substrate to free phosphate. The experiments were carried out as follows: 1 ml of adenosine triphosphatase preparation (0.046 g diluted in maleate buffer) plus 0.05 ml of 0.18N $CaCl_2$ (as an

activator) were added to 1.0 ml of substrate. The substrate present corresponded to 5 mg of the barium salt of A.T.P. and on complete hydrolysis yielded 0.07 to 0.08 mg of phosphate corresponding to 64 per cent phosphorus. This mixture was incubated for different periods of time. At the end of the incubation period, 1.5 ml of 10 per cent trichloracetic acid was added, and the precipitate was filtered off through glass wool and then was washed several times with dilute acetic acid. The inorganic phosphate present in the combined filtrates was measured by the method of Fiske and Subbarow.[15]

The inhibitory effects of U_6 and Hg^{++} on adenosine triphosphatase were studied in parallel. Each experiment was carried out as stated previously by adding 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\simeq$560 μg of uranium metal) or 1,000 μg of $HgCl_2$ to the substrate solution, which had been adjusted to the desired pH and was maintained at 25°C throughout the experiment. The enzyme was then added to the poisoned substrate. Three determinations of enzyme activity were made during a period of 45 min.

It is clearly shown in Table 13.3 that U_6 caused no significant inhibition of adenosine triphosphatase activity at pH 7 and 7.9 (see Fig. 13.9) but caused a slight inhibition at pH 5.5 (Fig. 13.10). Mercury showed just the reverse effect with respect to pH, as is seen in Table 13.3.

Table 13.3 — Effect of pH on the Inhibition of Adenosine Triphosphatase by U_6 and Mercury

	Inhibition, %	
pH	U_6	Hg
5.5	25.8	36.2
7.0	6.9	78.5
7.9	0.0	86.9

These findings agree with the statements concerning the increasing ability of U_6 to inhibit enzymes as pH is lowered because of the fact that more uncomplexed UO_2^{++} ions are present in the solution.

Adenosine triphosphatase is an enzyme containing active SH groups that can easily be blocked by Hg^{++}. The addition of 2 mg of glutathione to the mercury-poisoned enzyme-substrate mixture gave about 20 per cent protection in one determination, as is shown in Fig. 13.11.

4.2 Adrenaline Oxidase. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Crude adrenaline oxidase was prepared

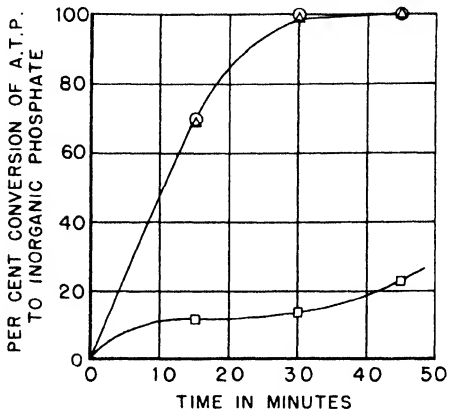


Fig. 13.9 — Inhibition of adenosine triphosphatase by uranium and mercury in maleate buffer of pH 7.9. ○, adenosine triphosphatase alone; △, adenosine triphosphatase + 1,000 μg of $UO_2(Ac)_2.2H_2O$; □, adenosine triphosphatase + 1,000 μg of mercuric chloride.

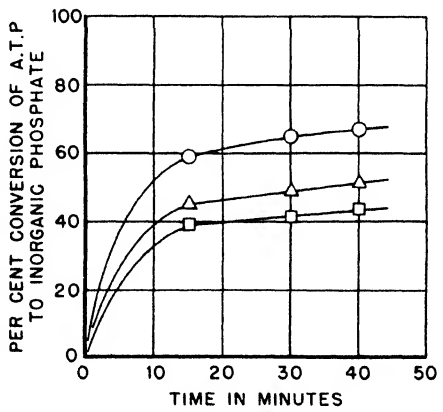


Fig. 13.10 — Inhibition of adenosine triphosphatase by uranium and mercury in maleate buffer of pH 5.5. ○, adenosine triphosphatase alone; △, adenosine triphosphatase + 1,000 μg of $UO_2(Ac)_2.2H_2O$; □, adenosine triphosphatase + 1,000 μg of mercuric chloride.

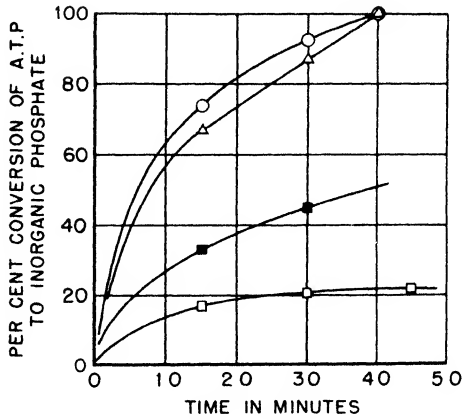


Fig. 13.11 — Effect of glutathione on inhibition of adenosine triphosphatase by uranium and mercury in maleate buffer of pH 7.0. ○, adenosine triphosphatase alone; △, adenosine triphosphatase + 1,000 μg of $UO_2(Ac)_2.2H_2O$; □, adenosine triphosphatase + 1,000 μg of mercuric chloride; ■, adenosine triphosphatase + 1,000 μg of mercuric chloride + 2 μg of glutathione.

by the method of Blaschko.[16] The activity of the enzyme in 1 ml of solution was determined by measuring the QO_2* in the presence of epinephrine (2.3 mg) in phosphate or maleate buffers of pH 7.3, using the Warburg apparatus. The autoxidation of the epinephrine was prevented by the addition of 0.001M KCN and 4 mg of glutathione to the reaction mixture. One thousand micrograms of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal) was added to the enzyme-substrate mixture. This mixture was prepared as follows: 2.28 mg of adrenaline in 0.2 ml of 0.1M phosphate buffer was placed in one side arm of a Warburg flask. Eight-tenths milliliter of 0.1M phosphate buffer and 1 ml of enzyme solution were placed in the principal chamber of the vessel when uranium was not to be added, and after temperature equilibration the adrenaline was mixed with the buffer substrate. When uranium was to be added, 0.1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ neutralized to pH 6.5 was added to the second side arm of the Warburg flask and was later mixed with the enzyme-substrate mixture immediately after adding the substrate. No inhibition was observed when phosphate buffer was used. Probably an insoluble U_6 phosphate was formed. An insignificant degree of inhibition was also observed when 0.1M maleate buffer was used in place of 0.1M phosphate buffer.

*The QO_2 is defined as the cubic millimeters of O_2 consumed per hour per milligram of tissue on a dry-weight basis.

When epinephrine solution was kept at room temperature at pH 7.3, autoxidation occurred slowly, and a brownish-red oxidation product was formed. A brown color developed rapidly when U_6 acetate was added to the epinephrine solution, but this probably was caused by complexing of the U_6 with the epinephrine rather than by the oxidation product, although there is no direct evidence to support this statement other than the immediate development of the color.

4.3 *d*-Amino Oxidase. (Work done by Tien Ho Lan.) The activity of *d*-amino acid oxidase of tissue brei was determined, using the Warburg apparatus, by measuring the oxygen consumption with *dl*-alanine as substrate according to Klein's method.[17] Rat liver or kidney brei was prepared in 0.1M maleate buffer of pH 8.3 in a glass homogenizer. One milliliter of the brei, containing about 50 mg of tissue (by dry-weight determination), was put into the main vessel of the Warburg flask. Twenty-five milligrams of *dl*-alanine dissolved in 0.2 ml of 0.1M maleate buffer of pH 8.2 was put into one side arm, and 0.1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ solution ($\simeq$560 μg of uranium metal) previously neutralized to pH 6.0 was put into the other side arm. Five-hundredths milliliter of 3N KOH solution was put into the central well of the flask. Maleate buffer, 0.1M, was added to the main compartment of the flask as needed to make the total volume of solution 2 ml. After temperature equilibration was established at 25°C, the substrate and the uranyl acetate were mixed with the tissue brei, and the oxygen consumption was measured every 15 min for a period of 60 min. Under such conditions, U_6 did not cause any significant inhibition of the activity of *d*-amino acid oxidase in the kidney brei, judging from control experiment without uranium, but it caused a slight inhibition in the liver brei. The results are shown in Fig. 13.12.

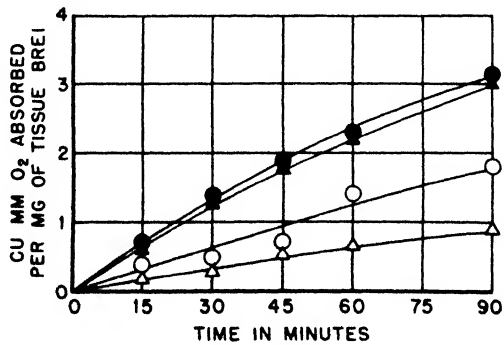


Fig. 13.12—*d*-Amino acid oxidase activity in rat-kidney and liver brei with and without uranyl acetate. ○, rat-liver brei + alanine; △, rat-liver brei + alanine + 1,000 μg of $UO_2(Ac)_2.2H_2O$; ●, rat-kidney brei + alanine; ▲, rat-kidney brei + alanine + 1,000 μg of $UO_2(Ac)_2.2H_2O$.

4.4 Salivary Amylase. (Work done by Tien Ho Lan.) Human saliva was collected after rinsing the mouth. The saliva was diluted 10 times with veronal-sodium acetate buffer of pH 6.8 (the optimal pH for salivary amylase), and the precipitate of mucin was removed. One per cent starch solution was also prepared in veronal-sodium acetate buffer of pH 6.8. The activity of salivary amylase was determined by measuring the time required to decolorize the blue color of the I_2-starch complex. To 3 ml of 1 per cent starch solution was added 0.1 ml of 0.01N iodine solution and 0.1 or 0.2 ml of 1 per cent uranyl acetate dihydrate ($\simeq$ 560 or 1,120 μg, respectively, of uranium metal), previously neutralized to pH 6.0. Finally, to this mixture 1 ml of the diluted saliva was added, and the time required for the disappearance of blue color was recorded. The results are shown in Table 13.4. It is obvious that under the conditions of the experiment no inactivation of salivary amylase by the added uranyl acetate was observed.

Table 13.4 — Effect of U_e on the Amylase Activity of Human Saliva

Test solution	Time required for decolorizing the blue color of I_2-starch, min
3 ml of starch solution + 1 ml of saliva + 0.1 ml of I_2	44
3 ml of starch sol. + 0.1 ml of uranyl acetate [1,000 μg of $UO_2(Ac)_2.2H_2O$]	43
3 ml of starch sol. + 0.2 ml of uranyl acetate [2,000 μg of $UO_2(Ac)_2.2H_2O$]	44

If a solution was made from commercial pancreatin as a source of amylase, with the same buffer but at a pH of 7.2, it was found that the quantities of uranyl acetate used above caused no inhibition of enzyme activity.

If malt was used as a source of amylase, it was found that at pH 4.8 with the same buffer there was also no inhibition of amylase activity by the amounts of uranyl acetate employed above. It is even possible that the uranyl acetate caused a slight activation of the enzyme.

4.5 Arginase. (Work done by Alexander L. Dounce with assistance of Daphne Rothermel.) In the first experiments with arginase, 1 per cent arginine carbonate *l* form) was used as substrate at pH 9.0. Later when the strong complexing action of carbonate for U_e was appreciated, the experiments were repeated using arginine monohydrochloride neutralized to pH 9.0 with NaOH. In neither case was any

additional buffer used (other than the arginine carbonate or the arginine itself.)

The arginase solution was one that was prepared from beef liver by fractional precipitation with cold dioxane, followed by fractional precipitation with cold acetone. The solution was a light-yellow color, transparent, and practically free from hemoglobin and catalase. The activity per gram of dry weight expressed as monomolecular reaction velocity K [from $K = (1/t) \log (A/A-X)$] was about 100. The K per gram of rat liver is about 3.5 per gram.[18]

Arginase activity was measured as described by Dounce.[18] The solution of the monohydrochloride contained an amount of arginine equivalent to that used in the arginine carbonate solution.

Five-hundredths milliliter of arginase solution was used in carrying out the arginase determination, and this amount was sufficient to cause decomposition of about 18 per cent of the substrate in 1 min.

In all the experiments the enzyme was allowed to act on the substrate for 1 min.

It was found that 1 mg of $UO_2(Ac)_2 \cdot 2H_2O$ ($\eqsim$ 560 μg of uranium metal) failed to cause appreciable inhibition of arginase activity when added to the substrate in the experiments with arginine carbonate, and in the experiments with arginine monohydrochloride the same amount of uranyl acetate produced only 17 per cent inhibition. This result is in agreement with the results of other enzyme experiments in which inhibition of enzymes by U_6 at pH values of 7 or higher was not observed when the U_6 was added to the substrate.

In regard to the action of massive amounts of uranyl acetate on arginase, reference is made to Sec. 1 of this chapter, where an experiment is described that shows that arginase can be precipitated by uranyl acetate and redissolved without apparent loss in activity.

The experiment was also carried out by adding 1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 10 mg, or 5.6 mg of uranium metal) to 1 ml of arginase solution ($\eqsim$ 17 mg of protein), and testing an aliquot of the mixture without removal of the uranium, using arginine carbonate as substrate. No marked diminution in enzyme activity occurred, so that the enzyme at least was not irreversibly inhibited.

The above experiments indicate that arginase is not sensitive to the action of U_6. Contrasting the action of mercuric chloride with that of uranyl acetate, it was found that, using arginine carbonate for substrate as outlined above, 1,000 μg of $HgCl_2$ produced 99 per cent inhibition of arginase activity. A curve showing percentage inhibition of arginase plotted against the amount of $HgCl_2$ added is shown in Fig. 13.13. The same amount of enzyme was used as in the experiments with uranyl acetate.

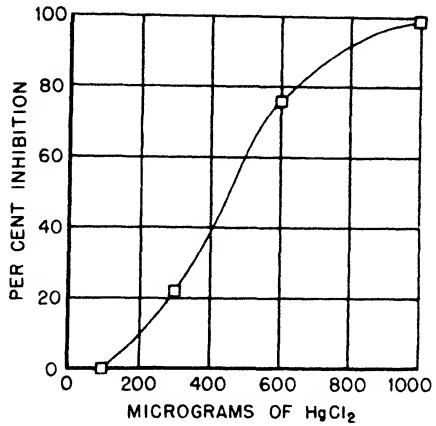


Fig. 13.13—Inhibition of arginase by $HgCl_2$ at pH 9.0.

4.6 Carbonic Anhydrase. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Carbonic anhydrase, which is abundant in red cells, is the enzyme that catalyzes the following reversible reactions:

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

$$HCO_3^- \rightleftharpoons CO_2 + OH^-$$

The enzyme is responsible for the rapid excretion of CO_2 from the blood in the lungs.

The activity of carbonic anhydrase can be determined either manometrically by measuring the rate of evolution of CO_2 from H_2CO_3 or by measuring the rate of formation of H_2CO_3 in the presence of excess of CO_2. In the present work the activity of carbonic anhydrase was determined according to the method of Philpot and Philpot.[19] By use of this method, the rate of formation of H_2CO_3 in the presence of excess of CO_2 was determined. The substrate solution for each determination is prepared by saturating with CO_2 a mixture consisting of 10 ml of 0.00263M $NaHCO_3$ and 1 ml of a solution approximately 0.3M with respect to Na_2CO_3 and 0.2M with respect to $NaHCO_3$. To this substrate solution 10 drops of an 0.08 per cent solution of bromthymol blue are added as in indicator. Carbon dioxide current is passed through the solution throughout the experiment. The substrate solution is kept at 0°C by keeping it in an ice-water bath.

Under the conditions just described, it would take about 70 sec to form enough H_2CO_3 to change the blue color of the bromthymol blue

into the yellow color that is present at or below pH 6.0, without the addition of any enzyme. For each series of experiments, a control determination without enzyme was carried out. Throughout the in vitro experiments, oxalated laked blood or a crude preparation of carbonic anhydrase made according to Meldrum and Roughton[20] was used. The rate of formation of H_2CO_3 was increased by the addition of enzyme solution according to a linear function.

Method of Expressing Carbonic Anhydrase Activity. It has been found by use of the method of Philpot and Philpot[20a] that for purified or partially purified carbonic anhydrase solution the activity of the enzyme in units can be obtained by the following equation:

$$\text{Units} = \frac{E}{E_u} = \frac{\frac{1}{T_e} - \frac{1}{T_c}}{\frac{1}{t_c}} = \frac{T_c - T_e}{T_e}$$

In this equation E represents the amount of enzyme present in the solution being analyzed, which gives observed time of T_e seconds elapsing before the color change has occurred; T_c is the time elapsing before the color change occurs in the control solution (without enzyme); and E_u is one unit of enzyme. The equation given above can be derived by the use of simple algebra if it is assumed that the amount of enzyme present is, in general, proportional to the quantity $\left(\frac{1}{T_e} - \frac{1}{T_c}\right)$ and define the unit as the amount of enzyme which will make T_e equal to $T_c/2$.

Thus the amount of enzyme present can be expressed as units, and in the case of tissue the number of units per milligram of tissue can be determined.

It is unfortunately true that the amount of enzyme present is not strictly a linear function of $(T_c - T_e)/T_e$ in the case of whole blood and possibly in the case of tissue, but in this work so little change was encountered in the activities of carbonic anhydrase in different samples of blood or different samples of tissue that, over the narrow range of values occurring, linearity between the amount of enzyme and $(T_c - T_e)/T_e$ still can be assumed. Therefore the above formula can be applied without serious error.

Effects of Uranium on Carbonic Anhydrase. In the literature many inorganic salts have been reported to inhibit the activity of carbonic anhydrase. It has been found in this laboratory that uranium forms a

complex salt with the bicarbonate ion. Because of these reports a series of in vitro experiments have been carried out to study the effects of uranium salts on carbonic anhydrase. In order to compare the effect of U_6 with the effect of another heavy metal that is known to inhibit the action of carbonic anhydrase, $HgCl_2$ has been tested in parallel with U_6.

Both whole blood and crude carbonic anhydrase preparations from rat or beef blood were tested with and without the addition of uranyl acetate or $HgCl_2$.

The effect of uranyl acetate on carbonic anhydrase was studied by two methods: In the first method various concentrations of uranyl acetate were added to the complete enzyme-substrate system. In the second method, uranyl acetate of different concentrations was directly added either to diluted whole blood or to the crude carbonic anhydrase preparation, and the mixture was allowed to stand from 5 to 100 min before adding to the substrate mixture. The activity of enzyme was measured as described above.

In the first method, when 0.5 ml of rat blood diluted 1 : 200 with water was added to 11 ml of substrate mixture, the rate of formation of H_2CO_3 was almost double that of the control, as indicated by the decrease in time required for the color change of bromthymol blue from 70 to 34 sec. The same amount of rabbit blood diluted 1 : 100 with water decreased the time required for the color change of bromthymol blue from 70 to 20 sec. This indicated that rate of formation of H_2CO_3 was increased to more than three times that of the control. Under the same condition the addition of 0.01 ml of the crude enzyme preparation to the substrate mixture almost tripled the rate of formation of H_2CO_3, since the time required for the color change of bromthymol blue was decreased from 70 to about 25 sec. The addition of 1,000 or 2,000 μg of $UO_2(Ac)_2.2H_2O$ ($\hat{=}$ 560 or 1,120 μg of uranium metal, respectively) to the enzyme-substrate system did not cause any inhibition of carbonic anhydrase either when whole blood or the crude enzyme preparation was used. This was shown by the fact that the time required for the color change of bromthymol blue in the enzyme-substrate system was about the same with or without the addition of uranyl acetate. The experiments were repeated several times with rat blood, rabbit blood, and crude carbonic anhydrase prepared from rat blood and beef blood. The results are shown in Table 13.5.

In order to study the effect of uranyl acetate on carbonic anhydrase by the second method described above, two series of experiments were carried out. In the first series, diluted blood or crude carbonic anhydrase preparation was added to the substrate mixture, and the enzyme activity was determined as a control. In the second series,

Table 13.5 — Effect of Uranyl Acetate in Presence of Substrate on Carbonic Anhydrase

Reaction mixture*	Time required for pH change, sec	Enzyme activity, units	Inhibition, %
Control (substrate alone)	70		
S + 0.5 ml of d. rb. bl.	20	2.50	
S + 0.5 ml of d. rb. bl. + 1,000 μg of uranyl acetate dihydrate	20	2.50	0
S + 0.5 ml of d. r. bl.	34	1.06	
S + 0.5 ml of d. r. bl. + 1,000 μg of uranyl acetate dihydrate	34	1.06	0
S + 0.05 ml of crude C.A. from rat blood	25	1.80	
S + 0.05 ml of crude C.A. from rat blood + 1,000 μg of uranyl acetate dihydrate	25	1.80	0
S + 0.01 ml of crude C.A. from beef blood	25	1.80	
S + 0.01 ml of crude C.A. from beef blood + 1,000 μg of uranyl acetate dihydrate	24	1.92	0

*Where S is substrate; d. rb. bl. is diluted rabbit blood; d. r. bl. is diluted rat blood; and C.A. is carbonic anhydrase.

the same amount of diluted blood or crude carbonic anhydrase was mixed with different amounts of uranyl acetate, and the mixture after standing for a certain period of time was added to the substrate mixture. The time required for the color change of bromthymol blue was about the same in both series of experiments, so that no inhibition was observed. The results are shown in Table 13.6.

Comparison of the Effect of $HgCl_2$ with the Effect of Uranyl Acetate on Carbonic Anhydrase. In order to compare the effect of Hg^{++} with the effect of U_6 on carbonic anhydrase, 1,000 μg of $HgCl_2$ was added to the substrate immediately after the addition of the crude carbonic anhydrase. The crude carbonic anhydrase was prepared from beef blood. In the presence of 0.01 ml of this preparation, 27 sec was required for the color of bromthymol blue in the substrate mixture to change to yellow. The time required for the same color change of bromthymol blue was increased to 48 sec when 1,000 μg of $HgCl_2$ was added to the substrate before the enzyme was added, so that the added $HgCl_2$ caused a 71 per cent inhibition of the carbonic anhydrase activity. When the same amount of $UO_2(Ac)_2.2H_2O$ (1,000 μg, ≙560 μg of uranium metal) was added in the same way, the time required for the color change of bromthymol blue was 24 sec. Therefore the added

Table 13.6—Effect of Allowing Carbonic Anhydrase to Stand in the Presence of Uranyl Acetate without Added Substrate

Reaction mixture*	Time required for pH change, sec	Enzyme activity, units	Inhibition, %
Control (substrate alone)	70		
S + 0.5 ml of d. rb. bl.	21	2.33	
S + 0.5 ml of d. rb. bl. + 1,000 μg of uranyl acetate dihydrate; enzyme allowed to stand with U_6 before testing:			
5 min	20	2.50	0
10 min	20	2.50	0
15 min	20	2.50	0
S + 0.5 ml of d. r. bl.	16	3.38	
S + 0.5 ml of d. r. bl. + 1,240 μg of uranyl acetate dihydrate; enzyme allowed to stand with U_6 before testing:			
15 min	16	3.38	0
30 min	17	3.12	7.7†
100 min	17	3.12	7.7†
S + 0.01 ml of crude C.A. from beef blood	25	1.80	
S + 0.01 ml of crude C.A. from beef blood + 2,000 μg of uranyl acetate dihydrate; enzyme allowed to stand with U_6 before testing:			
20 min	23	2.04	0

* Where S is substrate; d. rb. bl. is diluted rabbit blood; d. r. bl. is diluted rat blood; and C.A. is carbonic anhydrase.

† Probably not a significant amount.

uranyl acetate caused no inhibition of the carbonic anhydrase activity. The results are shown in Table 13.7.

4.7 Choline Oxidase. (Work done by Tien Ho Lan.) The activity of choline oxidase in liver and kidney brei with and without added uranyl acetate was determined by measuring oxygen consumption in the Warburg apparatus according to the method of Mann and Quastel,[21] except that 0.1M maleate buffer of pH 7.0 was used instead of the phosphate buffer of the same pH value. In experiments involving uranyl acetate, 0.1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ solution previously neutralized to pH 6.0 [1,000 μg of $UO_2(Ac)_2.2H_2O$, ≙ 560 μg of uranium metal], was put into one side arm and 1 mg of choline hydrochloride

Table 13.7 — Comparison of the Effect of $HgCl_2$ with the Effect of Uranyl Acetate on Carbonic Anhydrase

Reaction mixture*	Time required for pH change, sec	Enzyme activity, units	Inhibition, %
Control (substrate alone)	70		
S + 0.01 ml of C.A.	27	1.59	
S + 0.01 ml of C.A. + 1,000 μg of $HgCl_2$	48	0.46	71
S + 0.01 ml of C.A. + 1,000 μg of uranyl acetate dihydrate	24	1.92	0

* Where S is substrate and C.A. is carbonic anhydrase.

dissolved in 0.2 ml of maleate buffer of pH 7.0 was put into the other side arm of the flask. Five-hundredths milliliter of 3N KOH solution was put into the central well of the flask, and 0.1M maleate buffer of pH 7.0 was added to the main compartment of the flask as needed to make the final volume of the solution 2 ml. After temperature equilibration had been established at 25°C the substrate and the uranyl acetate were mixed with the tissue brei, and the oxygen consumption caused by the oxidation of the choline hydrochloride was measured at 15-min intervals for a period of 60 min. Addition of uranyl acetate did not cause any significant inhibition, as is shown in Fig. 13.14.

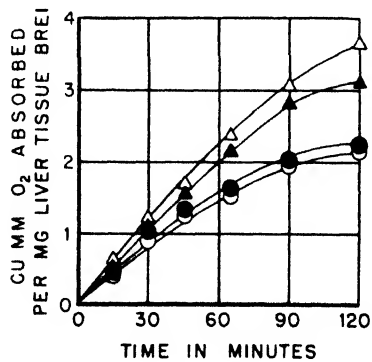


Fig. 13.14 — Choline oxidase activity of rat-liver tissue brei with and without uranyl acetate. ○, rat-liver brei alone; △, rat-liver-tissue brei + 1,000 μg of $UO_2(Ac)_2.2H_2O$; ●, rat-liver-tissue brei + 1 mg of choline hydrochloride; ▲, rat-liver-tissue brei + 1 mg of choline hydrochloride + 1,000 mg of $UO_2(Ac)_2.2H_2O$.

4.8 Catalase. (Work done by Alexander L. Dounce with the assistance of William Connors.) Crystallized beef-liver catalase of Kat. f. = 30,000 was used as enzyme.[22]

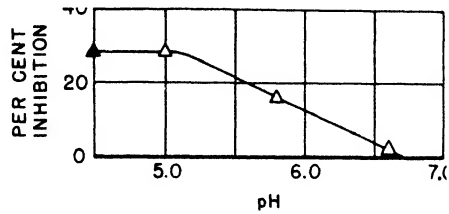


Fig. 13.15—Inhibition of catalase by uranyl acetate in the presence of 0.05M acetate buffer, plotted against pH. △, 5 mg of $UO_2(Ac)_2.2H_2O$ + 0.0037 mg (1.5×10^{-8} millimoles) of catalase; ▲, 15 mg of $UO_2(Ac)_2.2H_2O$ + 0.011 mg (4.4×10^{-8} millimoles) of catalase.

This enzyme is rather insensitive to U_6. Catalase precipitated by uranyl acetate is still partially active. The curve showing the percentage inactivation of catalase by uranyl acetate in the presence of acetate buffer plotted against pH is shown in Fig. 13.15. A similar curve using maleate buffer is shown in Fig. 13.16.

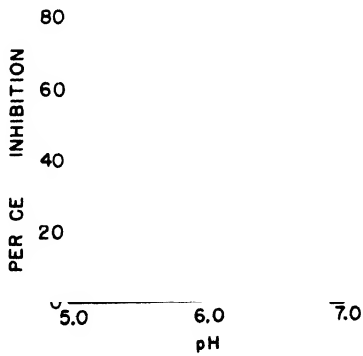


Fig. 13.16—Inhibition of catalase by uranyl acetate in the presence of 0.05M maleate buffer, plotted against pH. ▲, 15 mg of $UO_2(Ac)_2.2H_2O$ + 0.011 mg (4.4×10^{-8} millimoles) of catalase; △, 5 mg of $UO_2(Ac)_2.2H_2O$ + 0.0037 mg (1.5×10^{-8} millimoles) of catalase.

A corresponding curve showing percentage inactivation of catalase by mercuric chloride in the presence of maleate buffer plotted against pH is shown in Fig. 13.17.

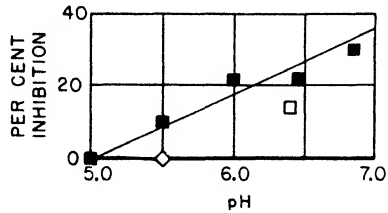


Fig. 13.17—Inhibition of catalase by $HgCl_2$ in 0.05M maleate buffer. ■, 200 μg of $HgCl_2$ + 0.0037 mg (1.5×10^{-8} millimoles) of catalase; ◇, 50 μg of $HgCl_2$ + 0.0037 mg (1.5×10^{-8} millimoles) of catalase; □, 100 μg of $HgCl_2$ + 0.0037 mg (1.5×10^{-8} millimoles) of catalase.

It can be seen that catalase is more sensitive to mercuric chloride than to uranyl acetate. Moreover, the effect of pH on inactivation is exactly opposite in the case of mercuric chloride and uranyl acetate.

In the case of inhibition by uranyl acetate, any enzyme ought to show a higher degree of inactivation at pH 5.0 than at 7.0, since at the lower pH value there is more U_6 not complexed with buffer anion and therefore free to react with protein. This situation would probably hold no matter whether the U_6 acted by blocking a single specific group of the enzyme, or whether it acted by blocking a relatively large number of acid groups. In the case of catalase it is evident that the active center (which is the hematin group) is not blocked by U_6. The action of U_6 in relatively high concentration in inactivating catalase is probably best explained by its tendency to aggregate and precipitate the enzyme. Mercury probably acts in a similar way. The tendency of both U_6 and Hg^{++} to react with COO^- groups of the enzyme is increased as the ionization of the acid groups of the protein increases. In the case of U_6, however, this effect is more than offset by the increased complexing action of the buffer anion at high pH values. Though Hg^{++} can form complexes with certain buffer anions including bicarbonate, it appears that the affinity of the Hg^{++} for protein is so much higher than its affinity for the nonprotein complexing agents that the latter do not exert the same effect they are able to exert in the case of U_6, so that the precipitating and inactivating action of $HgCl_2$ on catalase increases as the pH increases.

4.9 Cytochrome Oxidase. (Work done by Alexander L. Dounce.) This enzyme was prepared by a method based upon the methods of

Keilin[23] and Haas.[24] One hundred grams of washed fresh pig heart was ground thoroughly in a Waring Blendor with 150 ml of H_2O, care being taken to discontinue the process temporarily when the temperature began to rise above 30°C. To the ground material was added 150 ml of 0.1M NH_3-NH_4Cl buffer of pH 10.4. The buffer must not be added to the material while it is in the Blendor. From this point on, the procedure described by Haas for purifying the enzyme by precipitation and resolution was employed. No attempt was made to render the oxidase more water soluble, such as employing the ultrasonic technique of Haas.

The final cytochrome oxidase suspension contained 26 mg of material per milliliter, of which not more than 1 mg was NH_4Cl, so that the total protein present was about 25 mg/ml. Cytochrome C was prepared from fresh horse heart by the method of Keilin.[25]

(a) Acetate Buffer. Using the Warburg technique with hydroquinone as substrate, an 80 per cent inhibition of oxygen uptake was observed with the addition of 2 mg of $UO_2(Ac)_2.2H_2O$ (≏ 1,120 μg of uranium metal), and approximately 60 per cent inhibition with the addition of 1 mg of $UO_2(Ac)_2.2H_2O$ (≏ 560 μg of uranium metal). This is shown in Fig. 13.18. Figure 13.19 shows that the inhibition of oxygen uptake is overcome to some extent by the addition of more cytochrome C.

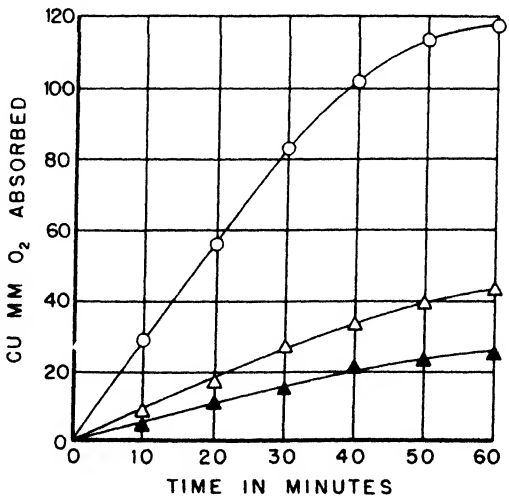


Fig. 13.18—Effect of uranyl acetate on the cytochrome oxidase-cytochrome C system using 2.0 ml of 0.02M hydroquinone as substrate in 0.1M acetate buffer at pH 6.6 to 6.8. ○, control without uranyl acetate; △, experiment with 1 mg of $UO_2(Ac)_2.2H_2O$ added (64 per cent inhibition); ▲, experiment with 2 mg of $UO_2(Ac)_2.2H_2O$ added (82 per cent inhibition).

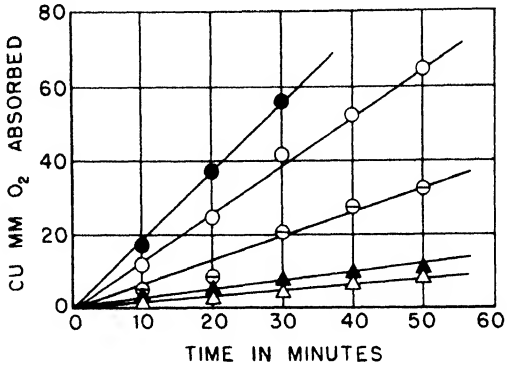


Fig. 13.19— Effect of cytochrome C concentration on the inhibition of the cytochrome oxidase-cytochrome C-hydroquinone system in acetate buffer at pH 6.3. ●, uranyl acetate, 0.2 ml of cytochrome C solution; ○, no uranyl acetate, 0.1 ml of cytochrome C solution; ⊖, no uranyl acetate, 0.05 ml of cytochrome C Solution; ▲, 1 mg of $UO_2(Ac)_2.2H_2O$, 0.2 ml of cytochrome C solution; △, 1 mg of $UO_2(Ac)_2.2H_2O$, 0.1 ml of cytochrome C solution. Inhibition of 100 per cent was found to have resulted from 1 mg of $UO_2(Ac)_2.2H_2O$ and 0.05 ml of cytochrome C solution (not on chart). All base lines (controls without enzyme) have been subtracted.

It was noticed in these experiments that uranyl acetate appeared to inhibit the autoxidation of the hydroquinone almost completely. This may be caused by the formation of a hydroquinone-U_6 complex. This observation raised a doubt as to whether the principal action of the uranyl acetate was on the reaction of cytochrome oxidase with cytochrome C, and therefore rough spectroscopic tests were run to determine the rate of oxidation of the cytochrome C alone by the oxidase in the absence of the hydroquinone substrate. It was found, using the pocket spectroscope to observe the change from reduced cytochrome C to oxidized cytochrome C, that 1 mg of $UO_2(Ac)_2.2H_2O$ caused a maximum of about 30 per cent inhibition of this reaction, and possibly much less.

Therefore it seems doubtful whether U_6 severely affects the cytochrome system itself. It might affect the coupling between the cytochrome system and dehydrogenases.

(b) Maleate Buffer. The results are shown in Fig. 13.20.

(c) Borate-Mannitol Buffer. The results are shown in Fig. 13.21.

(d) Phosphate Buffer. The results are shown in Fig. 13.22.

There was no oxygen consumption caused by the maleate or the mannitol-borate buffers.

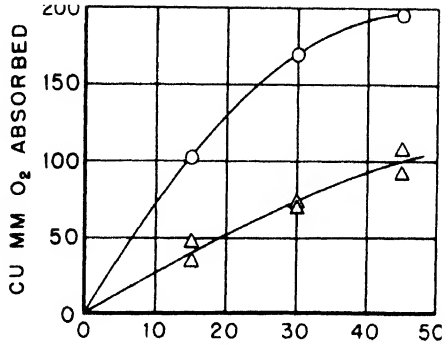


Fig. 13.20—Inhibition of the cytochrome oxidase-cytochrome C system using 2.0 ml of 0.02M hydroquinone as substrate in 0.1M maleate buffer of pH 6.8. ○, enzyme-substrate system without uranyl acetate; △, enzyme-substrate system with 1,000 μg of $UO_2(Ac)_2.2H_2O$. Two-tenths milliliter of cytochrome solution was used in all experiments.

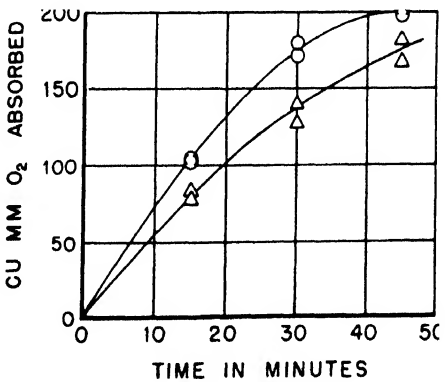


Fig. 13.21—Effect of uranyl acetate on the cytochrome oxidase-cytochrome C system using 2.0 ml of 0.02M hydroquinone as substrate in 0.1M borate-3 per cent mannitol buffer at pH 6.5. Two-tenths milliliter of cytochrome C solution was used. ○, control without added uranyl acetate; △, experiment with 1,000 μg of $UO_2(Ac)_2.2H_2O$. All base lines (controls without enzyme) have been subtracted.

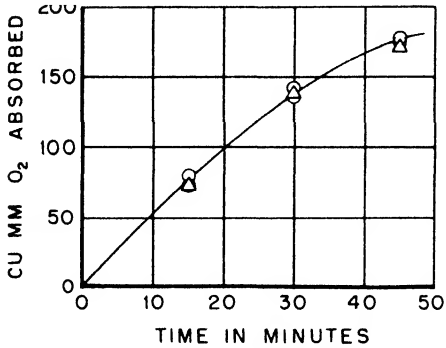


Fig. 13.22 — Effect of uranyl acetate on the cytochrome oxidase-cytochrome C system using 2.0 ml of 0.02M hydroquinone as substrate in 0.1M phosphate buffer of pH 6.8. Two-tenths milliliter of cytochrome C solution was used. ○, control without uranyl acetate; △, experiment with 1,000 μg of uranyl acetate dihydrate (zero inhibition).

Comparing all the buffers used, it is evident that the cytochrome oxidase-cytochrome C-hydroquinone system at pH 6.8 was not inhibited by 1 mg of $UO_2(Ac)_2.2H_2O$ (≏ 560 μg of uranium metal) in 0.1M phosphate buffer but was inhibited to the extent of about 70 per cent in 0.1M acetate buffer, about 28 per cent in 0.1M borate buffer containing 3 per cent mannitol, and 56 per cent in 0.1M maleate buffer.

4.10 Esterase. (Work done by Alexander L. Dounce.) A colorless and clear preparation of the enzyme was made from beef liver by fractional salting out with magnesium and ammonium sulfate. The preparation was roughly twenty-five times purer than liver brei. The solution contained 19 mg of protein per milliliter. Five-hundredths of a milliliter of the preparation turns the color of phenol red in 8 min in the test for esterase employed by Dounce.[18] This method of testing was used in all the experiments on esterase. The method can be improved slightly by observing the disappearance of the absorption band caused by the alkaline form of the phenol-red indicator, using a pocket spectroscope.

Ten milliliters of a 0.5 per cent solution of $UO_2(Ac)_2.2H_2O$ was adjusted to pH 9.3 by the addition of 0.305 ml of 1M NaOH, and 0.1 and 0.2 ml of the resulting clear solution were used in the experiments [≏500 μg and 1,000 μg of $UO_2(Ac)_2.2H_2O$, or 280 and 560 μg of uranium metal, respectively]. No more than 5 to 10 per cent inhibition of the esterase was caused even by the larger amount of uranyl acetate. Even the apparent slight inhibition that was observed probably was an artifact, since the pH of the added uranium solution was a little higher than that of the buffer substrate.

It is concluded that at the pH of the experiment esterase is not sensitive to U_6. Whether U_6 can combine with protein at such a high pH is in any case doubtful, judging from the data obtained by titration of egg albumin in the presence of U_6 (see Sec. 1).

4.11 Lactic Dehydrogenase. (Work done by Alexander L. Dounce and William M. Connors.) This enzyme was crystallized following the procedure of Straub with slight modifications.[26] Beef heart was used as source of the enzyme. The final once-recrystallized preparation consisted of a suspension of fine colorless needles slightly contaminated with amorphous impurity.

Codehydrogenase I was prepared from baker's yeast by the method of Williamson and Green.[10] Diaphorase solution was prepared by considerably shortening the procedure of Straub.[27] The principal shortening of the method consisted in complete elimination of adsorption steps. The final transparent diaphorase solution was of an intense yellow color but of unknown purity, although it must have been many times more pure than the original beef-heart suspension from which it was made.

Lactic dehydrogenase was determined by using the Warburg apparatus. The contents of the flasks were as follows: 0.02 ml of crystalline enzyme suspension was placed in a side arm; in the main compartment were placed 0.1 ml of strong coenzyme I solution (about 1 mg), 0.1 ml of the strong diaphorase solution (excess), 1.0 ml of 1 per cent *dl*-sodium lactate, and 0.5 ml of 0.04 per cent methylene blue. In the flasks containing U_6 this material was added as 0.1 ml of 1 per cent $UO_2(Ac)_2.2H_2O$ [≏1 mg of $UO_2(Ac)_2.2H_2O$ or 560 μg of uranium metal]. Finally, enough water was added to bring the total volume to 2.0 ml. No buffer other than the lactate system was employed. The pH of the lactate was adjusted so that the final pH of the mixture would be that desired (see Fig. 13.23). The concentration of lactate was kept reasonably low because of the complexing action of lactate on U_6.

It was not found necessary to add HCN to remove pyruvate (which inhibits the enzyme), apparently because too little lactate was converted to pyruvate in the determinations to prove troublesome.

The results of the experiments are shown in Fig. 13.23. Oxygen-consumption values in blank experiments (without enzyme) were so low that they have not been shown in this figure, although they have been subtracted whenever necessary from the oxygen-consumption curves for solutions containing enzymes.

At pH 7.3 there is only about 10 per cent inhibition of the enzyme system employed, but at pH 6 there is about 65 per cent inhibition, and at pH 5.5 there is about 83 per cent inhibition.

The percentage inhibition plotted against pH is shown in Fig. 13.24. It can be seen that the effect of pH on the inhibition of lactic dehydrogenase by U_6 is similar to the behavior in this respect of most other enzymes studied in this laboratory.

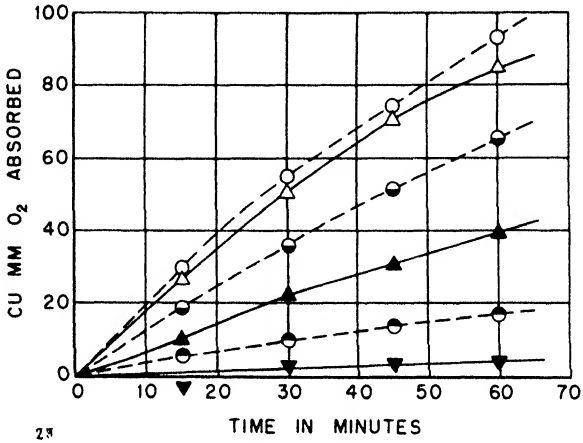


Fig. 13.23—Inhibition of lactic dehydrogenase in the presence of coenzyme I, diaphorase, and methylene blue by uranyl acetate; ○, control without uranyl acetate at pH 7.3 (average of three experiments); △, pH 7.3 with 1,000 μg of $UO_2(Ac)_2.2H_2O$ (average of two experiments); ⊖, control without uranyl acetate at pH 6.6 (one experiment); ▲, pH 6.6 with 1,000 μg of $UO_2(Ac)_2.2H_2O$ (average of three experiments); ⊖, control without added uranyl acetate at pH 5.5 (one experiment); ▼, pH 5.5 with 1,000 μg of $UO_2(Ac)_2.2H_2O$ (one experiment); all base lines (controls without enzyme) have been subtracted.

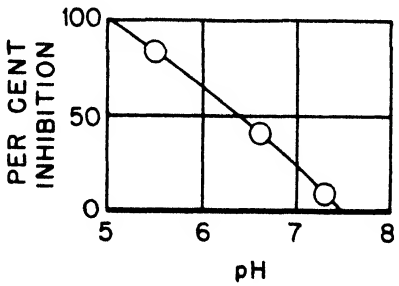


Fig. 13.24—Percentage inhibition of lactic dehydrogenase by uranyl acetate (U_6) plotted against pH.

4.12 Pepsin. (Work done by Alexander L. Dounce.) Pepsin was crystallized by the method of Northrop[9] and was kept in crystalline form in the icebox after several washings with ice-cold distilled water. Some experiments also were performed on partially purified noncrystallized pepsin.

Pepsin activity was roughly tested using Mett tubes containing approximately 5 per cent egg albumin that had been coagulated by boiling. The enzyme solution (0.1 ml) was added to 5 ml of 0.05N HCl in a small flask containing two of the Mett tubes. Sufficient enzyme was used to cause digestion of albumin from 2.5 to 3.0 mm from the ends of the Mett tubes in 5 hr.

No inhibiting action was observed when 10 mg of uranyl nitrate ($\eqsim$ 4.7 mg of uranium metal) were added to the enzyme-HCl solution. When uranyl acetate was made up in the egg albumin before coagulating it in the Mett tubes [about 2 mg of $UO_2(Ac)_2.2H_2O$ per milliliter, $\eqsim$ 1.11 mg of uranium metal] no inhibition and possibly a slight acceleration of the digestion by pepsin was noticed.

However, 2.5 mg of UCl_4 ($\eqsim$ 1.56 mg of uranium metal) added to the enzyme-HCl solution caused from 50 to 75 per cent inhibition of peptic activity as determined by the decrease in rate of digestion of the albumin in the Mett tubes.

Thus it is concluded that pepsin is very insensitive to U_6 but that it has an appreciable sensitivity to U_4. The lack of sensitivity to U_6 is probably explained by the fact that the UO_2^{++} ion does not have any great tendency to combine with proteins in acid solutions (below pH 3.0), since the carboxyl groups of the protein are too slightly ionized under these conditions to complex the UO_2^{++} ion. Evidently pepsin has no functional group with a specific affinity for UO_2^{++}.

It appears likely that the UO_2^{++} ion has a stronger affinity for carboxyl groups of pepsin at low pH than has UO_2^{++}, or else that it complexes some specific group necessary for peptic activity.

4.13 Acid Phosphatase. (Work done by Alexander L. Dounce.) Rat liver was the source of the enzyme. The liver was homogenized, and the homogenized tissue was strained and diluted in exactly the same manner as described for alkaline phosphatase in this section. The substrate was disodium phenyl phosphate with acetate buffer at pH 5.0.[28] The determinations were carried out by a slight modification[29] of Gutman's method. Amounts of $UO_2(Ac)_2.2H_2O$ varying from 100 to 500 μg ($\eqsim$ 56 and 280 μg, respectively, of uranium metal) may have produced a slight activation of the enzyme, but, when 3 mg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 1.68 mg of uranium metal) was used, an inhibition or inactivation of about 46 per cent was observed. The experiment was

repeated using malate to try to prevent the inactivating effect of the U_6. When 3 mg of $UO_2(Ac)_2.2H_2O$ and 0.2 ml of 1M malate were used, an inhibition of about 23 per cent was observed; when 5.0 mg of $UO_2(Ac)_2.2H_2O$ and 0.3 ml of molar malate were used, an inhibition of about 38 per cent was observed. Thus the malate did not prevent considerable inhibition of the enzyme, although it was very definitely helpful in reducing the amount of inhibition. It must be kept in mind that relatively large amounts of uranyl acetate were needed to produce marked inhibition of the acid phosphatase. Such concentrations would hardly be expected in the animal.

4.14 Alkaline Phosphatase. (Work done by Alexander L. Dounce.) The method of King and Armstrong,[30] with disodium phenyl phosphate and veronal buffer at pH 9.6 for substrate, was used. The source of the enzyme was a suspension of homogenized rat kidney, prepared by grinding 1 part by weight of kidney with 1 part by volume of distilled water. This suspension was strained free from fiber and was diluted 1:10 with distilled water to give a cell-free suspension, of which 0.1 ml was used in determining the enzyme. In attempting to poison the enzyme, amounts of $UO_2(Ac)_2.2H_2O$ varying from 1 to 10 mg ($\simeq$ 560 to 5,600 μg, respectively, of uranium metal) were added to the buffered substrate, and then the enzyme was added, and incubation at 25°C was carried out for accurately timed intervals of 20 to 30 min. However, no inactivation was observed in any of the experiments.

The insensitivity of alkaline phosphatase to uranyl acetate is another example of the difficulty or impossibility of poisoning enzymes with U_6 at very high pH values.

4.15 Phosphoglucomutase. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Phosphoglucomutase is one of the transphosphorylation enzymes. It converts glucose-1-phosphate to glucose-6-phosphate and therefore takes part in glycolysis. This enzyme was prepared by the method of Colowick and Sutherland,[31] with slight modifications. The substrate (Cori ester) was prepared according to Sumner and Somers.[32]

Four milliliters of Cori ester solution containing 0.1 g of the ester (glucose-1-phosphate) per 25 ml and 1 ml of veronal buffer of pH 7.4 were used as the reaction mixture. To this was added 4 drops of 3 per cent $MnCl_2$ and 0.2 ml of an appropriate dilution of the phosphoglucomutase solution. The final dilution of the enzyme varied from 1:50 to 1:125. The latter dilution was ten times greater than the dilution of the enzyme preparation used by Colowick and Sutherland, but the enzyme at this dilution still showed sufficient activity.

The activity of this enzyme is measured by determining the decrease in the amount of easily hydrolyzable ester present. Glucose-1-phosphate is easily hydrolyzed, and the inorganic phosphate that

has been liberated is then measured. The reaction product (glucose-6-phosphate) is not easily hydrolyzed under the same conditions, and therefore there is a decrease in the amount of acid-labile phosphate or an increase in the acid-stable phosphate.

The relationship of catalytic activity to enzyme concentration using optimal substrate concentration was studied. The enzyme in final dilution was diluted 1 : 50, 1 : 125, 1 : 200, and 1 : 250. The pH employed was 7.0, and the temperature was 250°C. The x-t curves, in which x is measured by determining the acid-stable phosphate, are shown in Fig. 13.25.

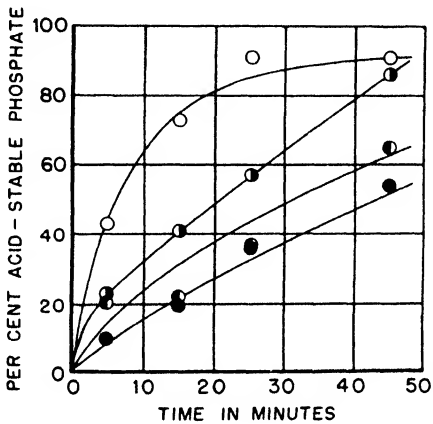


Fig. 13.25—Kinetics of phosphoglucomutase activity effect of enzyme concentration. ○, enzyme diluted 1 : 50; ◔, enzyme diluted 1 : 125; ◑, enzyme diluted 1 : 200; ●, enzyme diluted 1 : 250.

It is apparent that at the highest concentration of enzyme the reaction came to an equilibrium in 25 min, and at this time the conversion of Cori ester (acid-labile) to Robinson ester (acid-stable) was about 91 per cent. At a lower concentration the x-t curve became a straight line, and the conversion reached 86 per cent after 45 min. At the two highest dilutions (1 : 200 and 1 : 250) the conversion to the Robinson ester reached only 55 per cent after 45 min. From these results it may be assumed that the enzyme activity even at relatively high enzyme concentration follows the zero order of reaction, no doubt because of high affinity of enzyme for substrate.

The optimal concentration of substrate was determined by using different concentrations of Cori ester as shown in Fig. 13.26. Four-tenths milliliter of 0.4 per cent Cori ester solution (≏ 0.7 mg P) was considered to be the optimal substrate concentration.

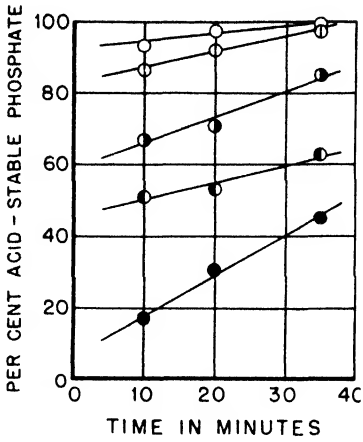


Fig. 13.26—Effect of substrate concentration on phosphoglucomutase activity. ○, 0.5 ml of Cori ester solution; ⊙, 1 ml of Cori ester solution; ◑, 2 ml of Cori ester solution; ◐, 3 ml of Cori ester solution; ●, 4 ml of Cori ester solution.

In the experiments concerned with enzyme inhibition the same substrate solution described above at optimal substrate concentration was used. The poison was added directly to the substrate solution in the form of neutralized 1 per cent $UO_2(Ac)_2.2H_2O$ or 1 per cent mercuric chloride solution. The 1 per cent uranyl acetate solution was prepared by diluting 2 per cent $UO_2(Ac)_2.2H_2O$ with an equal volume of 0.1N NaOH. The final pH of this mixture is about 5.8. The pH of the substrate-poison mixture before adding the enzyme was 6.8 to 7.0.

Experiments were made in which 0.1 ml of uranyl acetate solution [≎ 1,000 μg of $UO_2(Ac)_2.2H_2O$, or 560 μg of uranium metal; final molar concentration 0.01M] and 0.1 ml of $HgCl_2$ (≎ 1,000 μg of $HgCl_2$; final concentration 0.007M) were added to the substrate solution. After mixing the poisoning agent with the substrate solution, 0.2 ml of enzyme solution (previously diluted 1 : 8 with veronal buffer from the stock solution) was added, the reaction mixture was immediately hydrolyzed with 1N HCl, and the liberated phosphate was determined to measure the amount of Robinson ester formed. The formation of Robinson ester was then determined as described above at intervals of 10, 20, and 35 min. The pH of each mixture was checked by the glass electrode. It was found that 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal) did not cause any significant inhibition of the enzyme activity, but that 2,000 μg of $UO_2(Ac)_2.2H_2O$ (≎ 1,120 μg of uranium metal) caused 44 per cent inhibition of activity. However,

1,000 μg of $HgCl_2$ inhibited the enzyme activity to the extent of 77 to 100 per cent (Fig. 13.27).

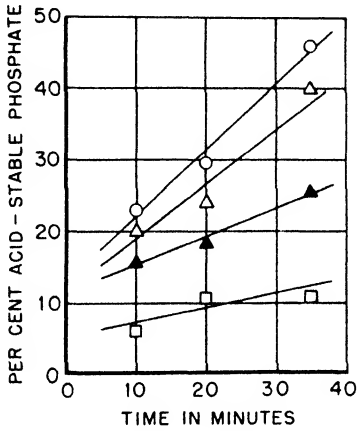


Fig. 13.27—Effects of U_6 and mercury on the activity of phosphoglucomutase. ○, phosphoglucomutase + substrate; △, phosphoglucomutase + substrate + 1,000 μg of $UO_2(Ac)_2.2H_2O$ (13.3 per cent inhibition); ▲, phosphoglucomutase + 2,000 μg of $UO_2(Ac)_2.2H_2O$ (44.0 per cent inhibition); □, phosphoglucomutase + 1,000 μg of mercuric chloride (76.5 per cent inhibition).

The effect of direct addition of uranyl acetate to the enzyme was next studied. In these experiments 3 ml of enzyme solution diluted 1 : 8 with veronal buffer from the stock solution was treated with 1.5 ml of 1 per cent uranyl acetate [≏ 15 mg of $UO_2(Ac)_2.2H_2O$ or 8.4 mg of uranium metal] of pH 6.5. A precipitate formed immediately, which subsequently was shown to contain about 80 per cent of the enzyme present. The pH of the enzyme-U_6 mixture was 6.5.

The enzyme activity was determined after the uranyl acetate and enzyme had stood from 1 to 30 min. It was found that the enzyme lost considerable activity almost immediately after the uranyl acetate was added. The experiment was repeated several times, and the same results were obtained. In one experiment, after centrifuging down the precipitate, the supernatant was tested for enzyme activity and was found to retain about 23 per cent of its original activity (Fig. 13.28).

The precipitate than was dialyzed against 0.1M citrate buffer at pH 5.8 overnight against several changes of the buffer. The dialysate was tested for its enzyme activity, and it was found that about 81 per cent of the original enzyme activity was recovered, based on the dry weight of the enzyme protein (Fig. 13.29). Thus the part of the enzyme

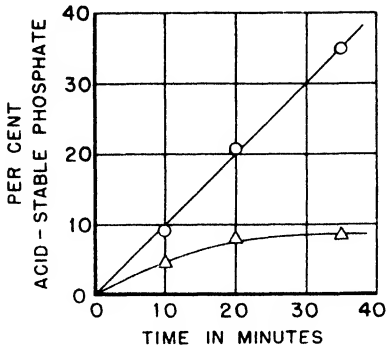


Fig. 13.28—Phosphoglucomutase activity in the supernatant from uranyl acetate-enzyme precipitation. ○, original enzyme preparation; △, enzyme activity of the supernatant from uranyl acetate-enzyme precipitation.

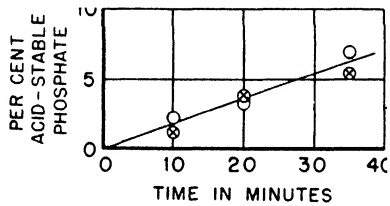


Fig. 13.29—Activity of phosphoglucomutase recovered after inactivation by uranyl acetate, by dialysis against citrate (activity of enzyme on a dry-weight basis). ○, original enzyme preparation + substrate; ⊗, enzyme recovered after inactivation.

that lost activity upon addition of the U_6 was the part precipitated by the U_6.

The difference in inhibitory action of the U_6, depending upon whether it is added to the enzyme without substrate or to the substrate before adding the enzyme, is very striking. It seems reasonable to suppose that in this case many nonspecific groups of the protein combine with the U_6 and that such nonspecific combinations cause at least part of the observed loss in activity. Whether the U_6 also blocks a prosthetic group of the enzyme remains a matter of speculation.

The most plausible explanation for the greater inactivation of the enzyme by U_6 in the absence of substrate is that the substrate is unable to dissolve enzyme precipitated by U_6 but nevertheless can prevent precipitation of enzyme by U_6. The failure of substrate to

dissolve enzyme precipitated by U_6 may be a slow-reaction-rate phenomenon; or it is possible that the enzyme in the presence of U_6 and substrate precipitates out of the solution very slowly.

The inhibitory action of U_6 on phosphoglucomutase in maleate buffer at different pH values has been determined. One thousand micrograms of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal) was used in these experiments. It was found that U_6 when added to the substrate first did not inhibit phosphoglucomutase drastically at any pH covered by this maleate buffer, although a 32.6 per cent inhibition was found at pH 6.1. The inhibition decreased as the pH became more alkaline, as has been reported previously for other enzymes. Because of the slight activity of the enzyme at pH 5.0 and 5.5, the inhibition caused by U_6 could not be accurately measured. These results are summarized in Table 13.8.

Table 13.8— Effect of pH on the Inactivation of Phosphoglucomutase by U_6

pH	Inhibition, %
5.0	Slight activity of enzyme
5.5	Slight activity of enzyme
6.1	32.6
6.5	24.8
7.2	16.2
7.6	0.0

The inhibitory action of UCl_4 on phosphoglucomutase has been studied. The same procedure was used as previously noted in studying the effect of uranyl acetate on phosphoglucomutase. One thousand micrograms of UCl_4 (≎ 630 μg of uranium metal) added to the substrate caused from 30 to 50 per cent inhibition of the phosphoglucomutase activity. When 1,000 μg of UCl_4 was added to the enzyme directly and allowed to stand, the activity was inhibited from 90 to 100 per cent. In all experiments the UCl_4 was neutralized to pH 5.0 with sodium acetate before adding it to the enzyme solution.

When UCl_4 in acetate buffer stands in the air at pH 5.0, a black precipitate forms, presumably because of hydrolysis of the UCl_4 yielding UO_2. This precipitate was tested for its inhibitory action on phosphoglucomutase, and it was found to inhibit the activity of the enzyme from 20 to 40 per cent. However, after complete oxidation by air of UCl_4 has taken place, the solution becomes yellow because of the formation of U_6. The yellow solution was tested in the manner described above and was found to cause only 2 per cent inhibition of

phosphoglucomutase activity in the presence of substrate, a result that is in agreement with results already reported for the inhibitory action of U_6.

It will be recalled that 15 mg of $UO_2(Ac)_2.2H_2O$ allowed to remain in contact with 3 ml of diluted phosphoglucomutase (see above) for 5 min in the absence of substrate caused a 70 to 90 per cent inhibition of enzyme activity. An equivalent amount of completely autoxidized UCl_4 when allowed to stand in contact with phosphoglucomutase for 5 min in the absence of substrate caused 73 per cent inhibition of enzyme activity (Fig. 13.30). These results are summarized in Table 13.9.

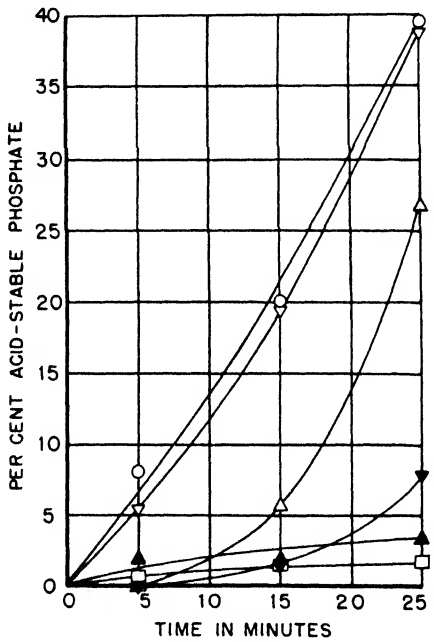


Fig. 13.30—Inhibition of phosphoglucomutase by UCl_4. ○, phosphoglucomutase + substrate alone; ▽, phosphoglucomutase + substrate + 1,000 μg of oxidized UCl_4; △, phosphoglucomutase + substrate + 1,000 μg of UCl_4 in fresh solution; ▲, substrate + phosphoglucomutase + UCl_4 allowed to stand for 5 min; □, substrate + phosphoglucomutase + black precipitate from solution of UCl_4, allowed to stand for 5 min; ▼, substrate + phosphoglucomutase + oxidized UCl_4, allowed to stand for 5 min.

These experiments show that 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≏ 560 μg of uranium metal) or an equivalent amount of UCl_4 in sodium acetate causes slight to zero inhibition of phosphoglucomutase activity when

substrate is present. However, when the uranium compound is added to the enzyme without substrate, both U_6 and U_4 cause strong inhibition of the enzyme.

It is possible that U_4 in sodium acetate has a slightly greater inhibitory action on phosphoglucomutase than has U_6 when these materials are added to the substrate before addition of the enzyme.

Table 13.9 — Effect of U_4 on Phosphoglucomutase

Presence or absence of substrate	Uranium compound used	Inhibition, %
Substrate present	Fresh UCl_4	32.2
	Oxidized UCl_4	1.27
Substrate absent	Fresh UCl_4	88.0
	Black precipitate (UO_2)	94.0
	Oxidized UCl_4	73.0

4.16 Phosphorylase. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) (a) Preparation of Crystalline Phosphorylase. The crystalline phosphorylase was prepared by the method of Green and Cori[33] with slight modifications. The rabbit was anesthetized with nembutal (2 ml for a rabbit of 3 kg body weight) and was then bled to death by cutting its carotid arteries. Only the back and leg muscles were used for the preparation. The muscle was frozen first and then was stirred in a Waring Blendor in a cold room with about an equal volume of cold water. The extracts were centrifuged and dialyzed against cold distilled water overnight, after adjusting the pH to 6.2. The pH was then adjusted to 5.8 by adding 0.03N HCl. The precipitate that formed was removed by centrifugation and filtration. The clear solution was neutralized with sodium β-glyceryl phosphate (1 g/100 ml of solution), and the enzyme was precipitated by adding saturated neutralized ammonium sulfate solution. The pH of the solution was then adjusted to 6.8. The crude enzyme was allowed to settle in a refrigerator overnight, the supernatant was removed by decantation, and the precipitated enzyme was collected by centrifugation. The paste of precipitated crude enzyme was dialyzed against cold water just to the point where all the precipitate had dissolved. This required about 30 to 60 min. Then the solution was dialyzed against sodium glyceryl phosphate-cysteine buffer of pH 6.8. The enzyme began to crystallize out after changing the buffer solution several

times. The crystalline enzyme was centrifuged down in a high-speed centrifuge and was dissolved in sodium glyceryl phosphate-cysteine buffer by warming to 30 to 35°C. The insoluble residue was centrifuged down and discarded. The clear enzyme solution was put in a refrigerator to induce recrystallization. It usually took about 30 min to crystallize the enzyme.

(b) Order of Reaction and Activity of Phosphorylase. The crystalline phosphorylase thus prepared had an activity of about 2,100 Cori units per milligram of enzyme on dry-weight basis without the addition of adenylic acid and glutathione. The activity of the enzyme was determined by measuring the inorganic phosphate eliminated from the Cori ester in the presence of glycogen:

$$\text{Glucose-1-phosphate} + \text{glycogen} = \text{glycogen-glucose} + \text{inorganic phosphate}$$

In each determination, enzyme was added to 4 ml of substrate solution in maleate buffer of pH 6.8 containing 0.016 g of Cori ester and 0.04 g of glycogen. The mixture was maintained at 25°C for a period of 45 min. During this period three or four measurements of inorganic phosphate were carried out by the method of Fiske and Subbarow at 10- or 15-min intervals. For each measurement, 1 ml of the reaction mixture was taken out carefully by pipet, and the reaction was stopped immediately by the addition of ammonium molybdate solution.

The kinetics of the reaction of phosphorylase was determined by the percentage conversion of Cori ester to inorganic phosphate at different concentrations. Two-tenths-milliliter portions of enzyme solution of four dilutions (1 : 10, 1 : 20, 1 : 40, and 1 : 60) in cysteine-sodium glyceryl phosphate buffer of pH 6.8 were added separately to 4 ml of substrate solution. The mixture was incubated at 25°C for a period of 45 min, and four determinations of free inorganic phosphate were made during this period. The results were plotted in x-t curves as in Fig. 13.31. It is apparent that the enzyme diluted 1 : 20, 1 : 40, and 1 : 60 gave a zero-order reaction up to 45 min, at which time there were 33, 23, and 17 per cent conversions of Cori ester, respectively. However, the enzyme in 1 : 10 dilution gave a conversion of 49.5 per cent of Cori ester at 45 min, and the curve was not of zero order.

All determinations on the activity of phosphorylase were made on portions of the x-t curves that were linear, corresponding to a reaction of zero order.

(c) The Inhibitory Effects of Uranium at Different Concentrations. Different buffers were tested for their complexing reaction with uranium. Among the buffers tested, maleate buffer was the most satisfactory. The substrate solution was prepared in stock solutions in 1M

maleate buffer of pH 6.8 in all the experiments except those specially described. After final dilution of the substrate, the maleate buffer was 0.1M.

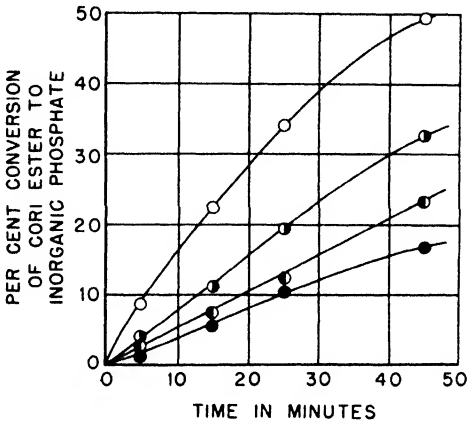


Fig. 13.31 — Effect of dilution on the activity of phosphorylase. ○, 1 : 10 enzyme dilution; ◑, 1 : 20 enzyme dilution; ◐, 1 : 40 enzyme dilution; ●, 1 : 60 enzyme dilution.

The inhibitory effect of uranium was determined by adding it to the enzyme-substrate system in different ways.

Uranium Added to the Substrate First. In this experiment, $UO_2(Ac)_2 .2H_2O$ in such amounts as 10, 20, 50, 100, 500, or 1,000 μg was added to the substrate in 0.1M maleate buffer of pH 6.8 followed by the addition of 0.2 ml of enzyme. Then the mixture was incubated, and the free inorganic phosphate was determined at different intervals for a period of 45 min. The results are plotted in Fig. 13.32.

Uranium Added to the Enzyme-Substrate Mixture. The inhibitory effect of uranium was the same as that when uranium was added to the substrate first. The results are shown in Fig. 13.33.

Uranium Added to the Enzyme First. Three different amounts of $UO_2(Ac)_2.2H_2O$ — 1,000, 500, and 100 μg (≎ 560, 260, and 56 μg of uranium metal, respectively) — were added to three equal portions of phosphorylase (0.2 mg of enzyme by dry weight), and the enzyme activity of these mixtures were then determined. One thousand micrograms of $UO_2(Ac)_2 .2H_2O$ (≎ 560 μg of uranium metal) instantly inactivated the enzyme completely; 500 μg (≎ 280 μg of uranium metal) inactivated the enzyme to the extent of 93 per cent; and 100 μg (≎ 56 μg of uranium metal) to the extent of 79 per cent. It was found that the same amounts of uranyl acetate added to the substrate before

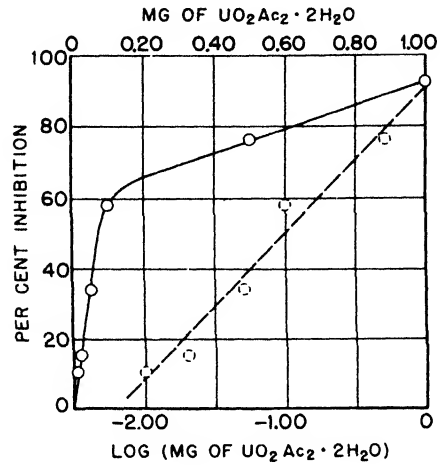


Fig. 13.32 — Inhibition of phosphorylase by adding different quantities of uranyl acetate (U_6) to the substrate first. ○, linear scale; ◌, logarithmic scale.

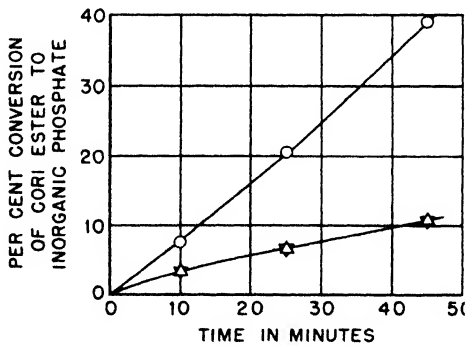


Fig. 13.33 — Inhibition of phosphorylase by adding uranium (U_6) to the enzyme-substrate mixture. ○, phosphorylase alone; △, 1,000 μg of $UO_2(Ac)_2.2H_2O$ added to enzyme substrate; ▽, 1,000 μg of $UO_2(Ac)_2.2H_2O$ added to substrate first, phosphorylase added later.

addition of enzyme inactivated the enzyme to almost the same extent. It may be assumed therefore that uranium has a greater affinity for phosphorylase than for the substrate. The results are shown in Fig. 13.34.

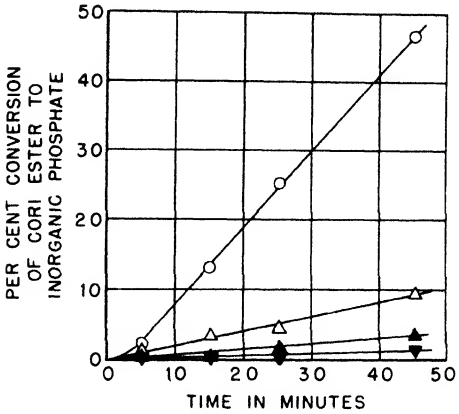


Fig. 13.34—Inactivation of phosphorylase by adding uranium (U_6) to the enzyme first. ○, phosphorylase alone; △, phosphorylase + 100 μg of $UO_2(Ac)_2.2H_2O$ (79 per cent inhibition; ▲, phosphorylase + 500 μg of $UO_2(Ac)_2.2H_2O$ (93 per cent inhibition); ▼, phosphorylase + 1,000 μg of $UO_2(Ac)_2.2H_2O$ (98 per cent inhibition).

(d) Reactivation of Phosphorylase by Dialysis. Phosphorylase was completely inactivated by adding 1,000 μg of $UO_2(Ac)_2.2H_2O$ to 0.2 ml of enzyme solution. The uranium-enzyme mixture was allowed to stand for a few minutes after mixing. The mixture was dialyzed overnight in a cellophane bag against 1M cysteine buffer of pH 7.0 containing 0.025M $NaHCO_3$, and against 1M cysteine buffer of pH 7.0 containing 0.025M sodium citrate. The volume, pH, and enzyme activity of the mixture were determined both before and after 24-hr dialysis. This was done to ensure that the pH did not change materially and to allow for the effect of dilution of the enzyme in dialysis. The results are shown in Table 13.10.

Table 13.10—Reactivation of Phosphorylase Poisoned by U_6

Complexer	Activity recovered, %	pH
$NaHCO_3$	45	7.7
Sodium citrate	12.6	7.0

It is apparent that both citrate and bicarbonate can form undissociated complexes with uranium to a sufficient extent to remove it from the enzyme and allow its diffusion through the membrane, so

that at least partial reactivation of the enzyme can occur. Further dialysis against bicarbonate and citrate solution probably would result in more complete reactivation of the enzyme. However, it is also possible that adenylic acid is lost in the dialysis, accounting in part for failure to regain all the activity of the enzyme. In this experiment the bicarbonate was more effective than the citrate. For removal of uranium from plasma protein by dialysis against 0.025M bicarbonate, see Sec. 2 on proteins.

(e) The Action of Sodium Citrate and Sodium Bicarbonate on Preventing the Inhibiting Actions of Uranyl Acetate on Phosphorylase. In these experiments, phosphorylase was added to a mixture containing 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≏ 560 μg of uranium metal) together with substrate and sodium citrate or sodium bicarbonate in concentrations calculated to give 10 or 20 molecules of protective agent per atom of uranium. The pH of the mixture was kept at 6.7. It was found that, under these conditions, sodium citrate gave better protection than sodium bicarbonate against the inhibitory effect of uranium. This is shown in Table 13.11.

Table 13.11 — Effect of Bicarbonate and Citrate in Preventing Action of U_6 on Phosphorylase

Complexer	Molecules of complexer per atom of uranium	Protection, %
Sodium bicarbonate	10	0
Sodium bicarbonate	20	7
Sodium citrate	10	12
Sodium citrate	20	28

The lack of protection by sodium bicarbonate can be explained by the fact that at pH values below 7.0 very little bicarbonate exists in the solution.

(f) Inhibitory Effect of Uranium at Different pH Values. Phosphorylase activity was determined in both maleate and cysteine-sodium glyceryl phosphate buffers at different pH values from 4.4 to 8.0 with and without the addition of uranium (Figs. 13.35 and 13.36). Phosphorylase in cysteine-sodium glyceryl phosphate showed only slightly greater activity than in maleate buffer of the same pH as can be seen from the figures. The curves rise steeply from pH 7.5 and flatten at about pH 6.8. Below pH 6.5 the activity of phosphorylase is so low that significant measurements of inactivation cannot be made. The inactivation of phosphorylase by U_6 is thus maximal at pH 6.8 and

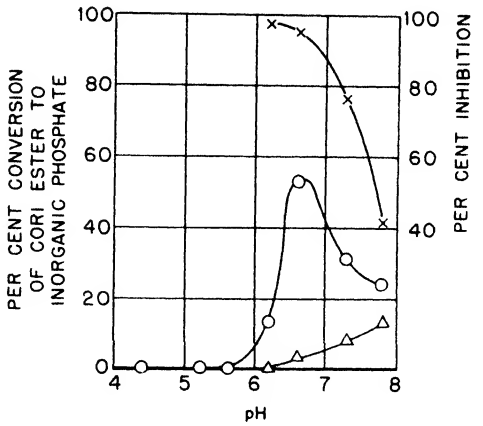


Fig. 13.35 — Inhibition of phosphorylase by uranium (U_6) in maleate buffer of various pH values. ○, phosphorylase alone; △, phosphorylase + 500 μg of $UO_2(Ac)_2.2H_2O$; ×, inhibition in percentage.

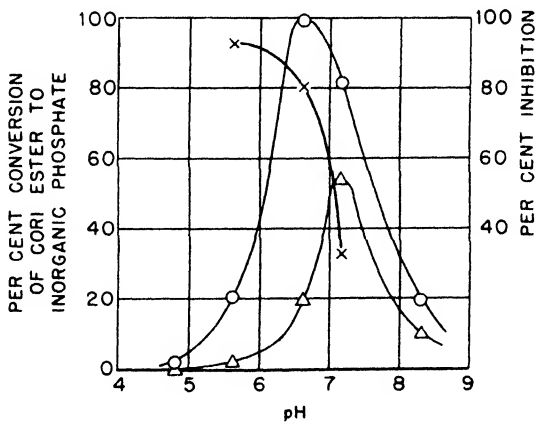


Fig. 13.36 — Phosphorylase activity with and without uranyl acetate in cysteine-sodium glyceryl phosphate buffer of various pH values. ○, phosphorylase + Cori ester; △, phosphorylase + Cori ester + 1,000 μg of $UO_2(Ac)_2.2H_2O$; ×, inhibition in percentage.

falls off rapidly as the pH increases. The tendency of U_6 to complex with the buffer anion increases with increase in pH, and the complex is not effective in inhibiting the enzyme. This result is in accordance with other pH inactivation studies reported from this laboratory.

For purposes of comparison the inhibitory effect of Hg was studied under the same condition. $HgCl_2$ (100 μg) in maleate buffer caused a poisoning effect near the optimal pH for phosphorylase similar to that of U_6 in the same buffer (Fig. 13.37). But when cysteine-sodium glyceryl phosphate buffer was used with 1,000 μg of $HgCl_2$, inhibition was negligible at the optimal pH for phosphorylase (Fig. 13.38).

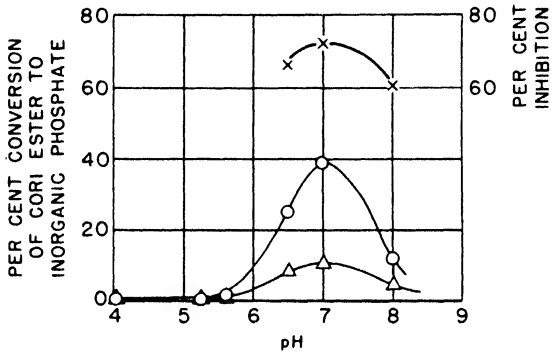


Fig. 13.37—Phosphorylase activity with and without $HgCl_2$ in 0.1M maleate buffer of various pH values. ○, phosphorylase + Cori ester; △, phosphorylase + Cori ester + 100 μg of $HgCl_2$; ×, inhibition in percentage.

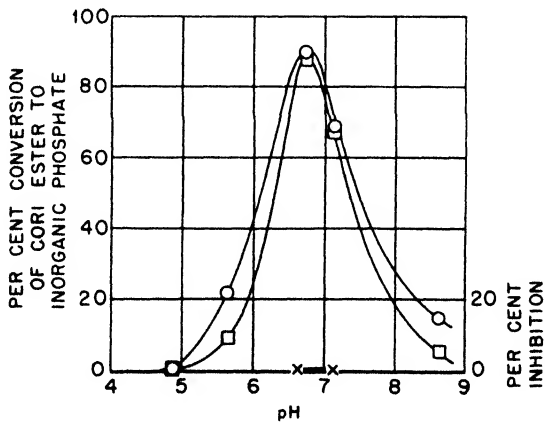


Fig. 13.38—Phosphorylase activity with and without $HgCl_2$ in cysteine-sodium glyceryl phosphate buffer of various pH values. ○, phosphorylase + Cori ester; □, phosphorylase + Cori ester + 1,000 μg of $HgCl_2$; ×, inhibition in percentage.

(g) Inhibitory Effect of U_6 at Different Concentrations of Substrate. In order to study whether or not U_6 reacts with the substrate (Cori ester) in the phosphorylase system, different amounts of Cori ester were used while the amounts of U_6 and enzyme were maintained unchanged. It was found that the inhibitory effect of U_6 decreased as the concentration of substrate increased. In this experiment 1,000 μg of $UO_2(Ac)_2.2H_2O$(≎ 560 μg of uranium metal) was used to inhibit phosphorylase activity, in the presence of either 16 or 48 mg of Cori ester. When 16 mg of Cori ester was used, the inhibition of phosphorylase was 71 per cent, but it dropped to 21 per cent when 48 mg of Cori ester was used. This indicates that U_6 forms a complex with Cori ester that is not effective as an inhibitor of the enzyme. This statement is further supported by the fact that egg albumin precipitated by U_6 can be dissolved by the addition of Cori ester.

(h) Protection of Phosphorylase against U_6 Poisoning by Different Compounds. In testing the protective effects of different compounds against poisoning of the phosphorylase system, different concentrations of such compounds were added to the enzyme mixture. The pH of the enzyme system was maintained at 6.7 in maleate buffer.

SH Compounds. Glutathione was used in ratios of 1 mole of uranium compound to 2, 5, and 10 moles of glutathione in maleate buffer of pH 6.7. Under the same condition, the effect of glutathione on the poisoning effect of Hg on phosphorylase was tested. The results indicate that, in maleate buffer, phosphorylase is protected from the inhibiting action of Hg by glutathione even when as little as 2 moles of glutathione is used for 1 atom of Hg, but glutathione did not protect phosphorylase against the poisoning effect of U_6 even when 10 molecules of glutathione is added for every atom of U_6 used. The results are shown in Fig. 13.39.

This proves that the SH of glutathione can protect phosphorylase from the poisoning effect of Hg but not of U_6. It also shows rather conclusively, when taken in conjunction with the experiments on crystalline urease to be reported, that U_6 does not act as a poison by blocking SH groups of enzymes. Although cysteine-sodium glyceryl phosphate buffer contains SH groups, it does not increase the activity of phosphorylase significantly above that in maleate buffer in the presence of U_6, but in the former buffer, 1000 μg of $HgCl_2$ did not cause any inhibition of phosphorylase, whereas it caused 98.6 per cent inhibition of maleate buffer. Sodium arsenite did not show any significant inhibiting effect either in maleate or in cysteine-sodium glyceryl phosphate buffer, even though AsO_2^- is known to have a strong affinity for SH groups and can inhibit crystalline urease. These results are shown in Fig. 13.40.

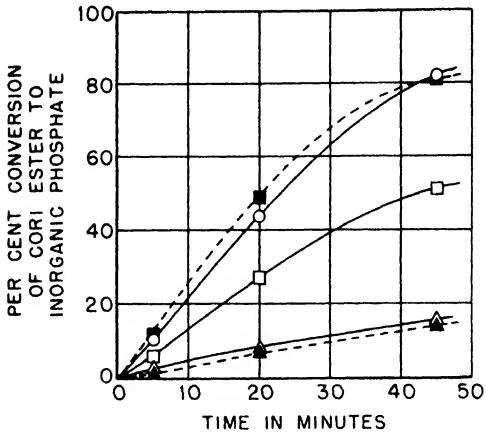


Fig. 13.39 — Effect of glutathione on the inhibition of phosphorylase by uranium (U_6) and mercury. ○, phosphorylase control; △, phosphorylase + 100 μg of $UO_2(Ac)_2.2H_2O$ (81 per cent inhibition); ▲, phosphorylase + 100 μg of $UO_2(Ac)_2.2H_2O$ + 0.6 mg of glutathione (82 per cent inhibition); ■, phosphorylase + 100 μg of mercuric chloride (37 per cent inhibition); □, phosphorylase + 100 μg of mercuric chloride + 0.6 mg of glutathione (zero inhibition).

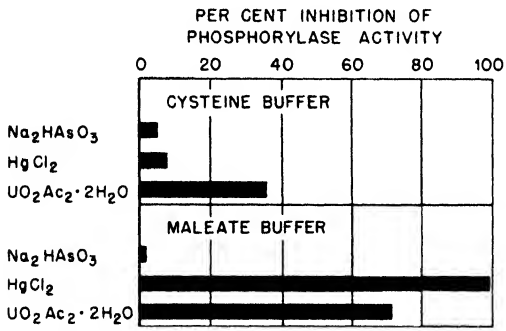


Fig. 13.40 — Inhibition of phosphorylase by uranium, mercury, and arsenic in cysteine-sodium glyceryl phosphate buffer and maleate buffer of pH 6.8.

Ascorbic Acid. The effect of ascorbic acid on the inhibition of phosphorylase by U_6 in maleate buffer at pH 6.8 was investigated. It was found that 1 to 10 mg of ascorbic acid added per each 1,000 μg of uranyl acetate failed to give any protection. This is shown in Table 13.12.

Table 13.12 — Ineffectiveness of Ascorbic Acid in Preventing Inhibition of Phosphorylase by U_6

Reaction mixture	Conversion, %	Inhibition, %
Control	62	0
1,000 μg of $UO_2(Ac)_2.2H_2O$	3.9	94
1,000 μg of $UO_2(Ac)_2.2H_2O$ + ascorbic acid, 1 to 10 mg	2.4	96
Control + ascorbic acid, 1 to 10 mg	58.5	0

Egg Albumin. In order to find out whether or not egg albumin can protect phosphorylase from poisoning by 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 560 μg of uranium metal), crystalline egg albumin was added to the enzyme system, in concentrations of 10, 15, and 25 mg, respectively, before the U_6 was added, but only slight protection was obtained even at high concentrations of albumin as shown in Table 13.13.

Table 13.13 — Effect of Albumin in Protecting Phosphorylase against Poisoning by U_6

Amount of albumin added, mg	Protection, %
10	0.0
15	7.0
25	9.5

No difference in the inhibitory effect of U_6 was observed either when albumin was added to uranyl acetate first or when uranyl acetate was added to the enzyme system first.

Amino Acids. In order to study the effects of NH_2, OH, or carboxyl groups of amino acids on the inactivating effect of U_6 on phosphorylase, different amino acids in different concentrations were added to the enzyme-substrate system containing 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 560 μg of uranium metal) at different pH values. The amino acid was dissolved in maleate buffer at the same pH as the enzyme-substrate-U_6 mixture. At pH 6.7, arginine, histidine, and tyrosine gave only an insignicant protection and none whatsoever at other pH values. The results are shown in Fig. 13.41.

B Vitamins. The effects of riboflavin, Ca pantothenate, thiamin, and choline on the poisoning effect of U_6 were studied in the same manner as amino acids. None of these vitamins gave any protection.

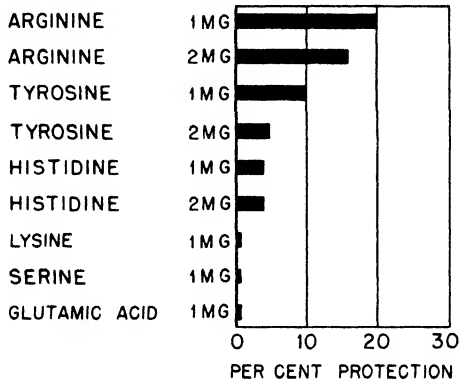


Fig. 13.41 — Protective action of amino acids against the inhibition of phosphorylase by uranyl acetate.

Inorganic Ions. Many inorganic ions have been tested for their effects on phosphorylase. These are Ag, Al, As, Bi, Co, Cu, Hg, Mg, Mn, Zn, Fe, Ca, Ba, Sr, Cr, Li, Sn, and Ce. A maleate buffer of pH 6.7 was used, and 1,000 μg of each salt was added to the enzyme-substrate mixture being tested. Results of this work, which demonstrated the relative inhibiting effects of the various ions, are shown in Fig. 13.42.

The effect of Ag^+ has also been studied in cysteine-sodium glyceryl phosphate buffer of pH 6.7. This ion caused 70 per cent inhibition in cysteine-sodium glyceryl phosphate buffer as against 94.5 per cent in maleate buffer.

The inorganic salts that caused no inhibition of phosphorylase have been investigated to determine whether any of them might affect the poisoning action of U_6 on phosphorylase. Thus As, Ca, Mn, Bi, Ba, Al, and Fe were tried for possible protective effects in the same manner as the amino acids. None of these metals protected the phosphorylase against U_6 poisoning except Bi, which showed slight protective action.

Effect of Adenylic Acid on the Inactivation of Phosphorylase by U_6. It is known that uranyl ions have a strong affinity for phosphoric acid and for organic phosphates such as glucose-1-phosphate and glyceryl phosphate. It thus seems possible that the inhibiting effect of U_6

on the phosphorylase might be due to a removal of animal adenylic acid or to a blocking of its catalytic action. Animal adenylic acid is probably the prosthetic group of animal phosphorylase.

Yeast adenylic acid, which differs slightly in structure from animal adenylic acid, does not act as a prosthetic group for phosphorylase,

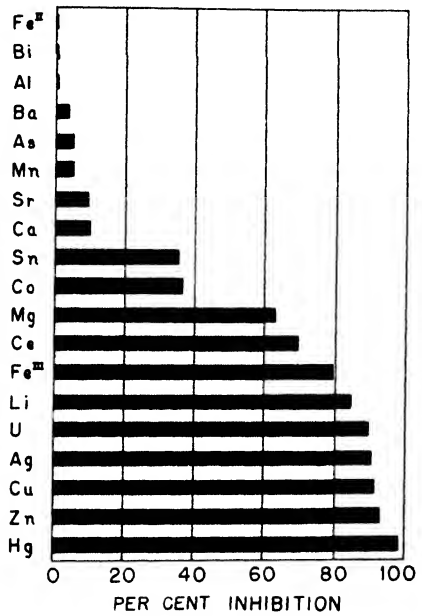


Fig. 13.42 — Effects of various inorganic ions on the activity of phosphorylase.

but it might be expected to act in relieving the poisoning of phosphorylase by U_6 if the mechanism for the poisoning suggested above is correct. Yeast adenylic acid was found to give a precipitate with very dilute U_6 at low pH values (3 to 4), but at high pH values (7 or above) this precipitate dissolved, presumably because of the formation of a soluble U_6 complex with the yeast adenylic acid. The precipitate could be dissolved at pH values below 7.0 by the addition of sodium glyceryl phosphate.

A series of experiments was carried out to test the effect of adenylic acid prepared from muscle adenosine triphosphate on preventing the inhibition of phosphorylase by U_6. It was demonstrated that animal

adenylic acid could prevent the inhibition of phosphorylase by U_6 as is shown in Fig. 13.43. Uranyl acetate was the U_6 salt used to produce the inhibition.

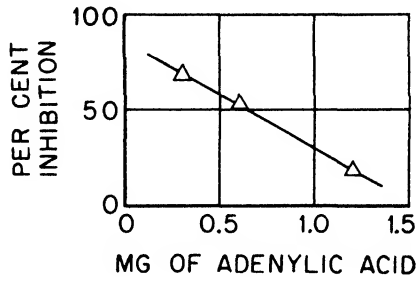


Fig. 13.43 — Effect of animal adenylic acid on the inhibition of phosphorylase by uranium (U_6).

Yeast adenylic acid was also tested, but this material did not prevent inhibition of phosphorylase by the U_6, as is shown in Table 13.14.

It is not immediately apparent why yeast adenylic acid should not prevent inhibition of phosphorylase by U_6, since it seems unlikely that there is much difference in complexing affinity for U_6 between animal

Table 13.14 — Failure of Yeast Adenylic Acid to Prevent Inhibition of Phosphorylase by U_6

Material tested	Inhibition of phosphorylase by U_6, %
Substrate + phosphorylase + 100 mg of $UO_2(Ac)_2.2H_2O$	56.6
Substrate + phosphorylase + 100 mg of $UO_2(Ac)_2.2H_2O$ + 0.3 mg of yeast adenylic acid	40.0
Substrate + phosphorylase + 100 mg of $UO_2(Ac)_2.2H_2O$ + 0.6 mg of yeast	49.9
Substrate + phosphorylase + 100 mg of $UO_2(Ac)_2.2H_2O$ + 1.2 mg of yeast	53.0

adenylic acid and yeast adenylic acid. Yeast adenylic acid may have a slight activating action on phosphorylase, as is shown in Table 13.15.

Small amounts of yeast adenylic acid plus U_6 seem to have a slightly greater inhibiting action on phosphorylase than U_6 alone. Why this should be so cannot be explained at the present time.

Free phosphate in an amount equivalent to the highest amounts of animal or yeast adenylicacid employed had no effect in preventing the inhibition of phosphorylase activity by U_6.

From the work with animal adenylic acid, which shows that this material is highly effective in preventing poisoning of phosphorylase by U_6, it appears most probable that U_6 acts specifically on phosphorylase by blocking the action of animal adenylic acid, which is probably

Table 13.15 — Activating Effect of Yeast Adenylic Acid on Phosphorylase

Material tested	Conversion of glucose-1-phosphate to Cori ester, %
Substrate + enzyme	25
Substrate + enzyme + 0.3 mg of yeast adenylic acid	27
Substrate + enzyme + 0.6 mg of yeast adenylic acid	31
Substrate + enzyme + 1.2 mg of yeast adenylic acid	34.1

the prosthetic group of the enzyme. It is quite certain that U_6 combines with the phosphate portion of adenylic acid and perhaps also with an adjacent OH group.

(i) The Effect of U_4 on Phosphorylase. Using the same procedure as described previously, the inhibitory effect of U_4 on the activity of phosphorylase has been tested. When 1,000 μg of UCl_4 ($\simeq$ 627 μg of uranium metal) previously neutralized to pH 5.0 by the addition of acetate buffer was added to the substrate first, there was no significant inhibition of the phosphorylase activity. However, when the same amount of UCl_4 was added to the phosphorylase in the absence of substrate and was allowed to stand for 2 min, the UCl_4 caused between 95 and 100 per cent inhibition of the enzyme. Finally 250 μg of UCl_4 ($\simeq$ 157 μg of uranium metal) and 500 μg of UCl_4 ($\simeq$ 314 μg of uranium metal) were allowed to stand with the enzyme for 2 min. It was found that 250 μg of UCl_4 caused 83 per cent inhibition, and 500 μg of UCl_4 caused 89.5 per cent inhibition of the activity of the enzyme. This might be due to the fact that, during the period of standing, some of the U_4 was oxidized by air to U_6, which apparently is more toxic than U_4 to the enzyme. When 0.3 molar citrate was added to one tube containing 500 μg of UCl_4 together with the enzyme, the inhibition caused by the UCl_4 was found to be only 52 per cent. Thus citrate reversed the inhibition caused by U_4 to the extent of 37 per cent. The results are shown in Fig. 13.44.

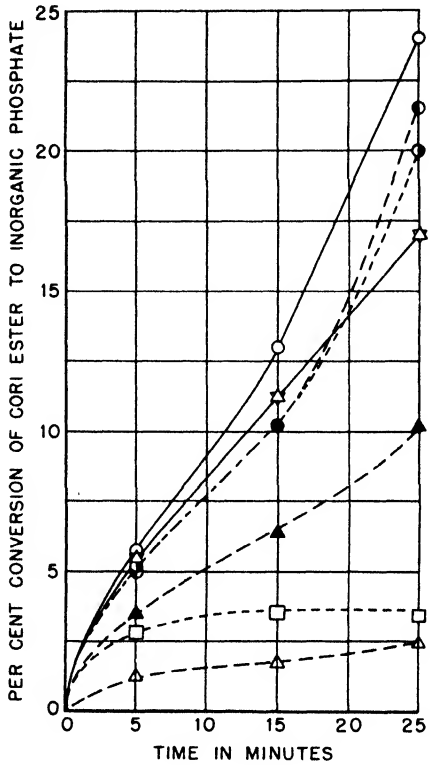


Fig. 13.44—Inhibition of phosphorylase by uranium (U_4). ○, phosphorylase + Cori ester as control for U_4 added to enzyme substrate; ◐, phosphorylase + Cori ester as control for 500 μg of UCl_4 added to enzyme first; ◑, phosphorylase + Cori ester as control for 250 μg of UCl_4 added to enzyme first; ✡, phosphorylase + 500 μg of UCl_4 added to Cori ester; ⚠, phosphorylase + 500 μg of UCl_4 allowed to stand for 2 min before adding to Cori ester; □, phosphorylase + 250 μg of UCl_4 allowed to stand for 2 min before adding to Cori ester; ▲, phosphorylase + 500 μg of UCl_4 allowed to stand for 2 min before adding to Cori ester containing citrate.

4.17 Succinic Dehydrogenase. (Work done by Alexander L. Dounce.) The enzyme preparation was exactly as described for cytochrome oxidase. A suspension of the finest particles was used in making the test.

The enzyme activity was measured by using the Warburg apparatus, with 1 per cent *dl*-sodium succinate for substrate and methylene blue for oxygen-transferring substance. Under the conditions of the experiment, 0.3 ml of enzyme suspension would cause an oxygen consumption of 40 to 45 cu mm of O_2 in 60 min.

The test was carried out as follows: 1 ml of 1 per cent disodium succinate plus 0.5 ml of 0.04 per cent methylene blue and 0.2 ml of water were added to the main compartment of a Warburg flask, and 0.3 ml of enzyme suspension was added to the side arm. Five-hundredths milliliter of 3N NaOH was used in the central well to absorb CO_2. After temperature equilibration had been established at 25°C the contents of the flask was mixed, and manometer readings were taken at 15, 30, 45, and 60 min. Usually there was an induction period lasting for 10 to 15 min.

When uranyl acetate was to be added, it was added to the substrate-methylene blue in place of the 0.2 ml of water. The final pH of the mixture varied from 6.8 to 7.1 in different experiments.

It was found that 1 mg of $UO_2(Ac)_2.2H_2O$ (≏ 560 μg of uranium metal) would cause no inhibition of the succinic dehydrogenase activity when tested in the manner outlined above. This is probably because of the presence of excess substrate (succinate), which is a good complexer for UO_2^{++}, and because of the fact that the pH was as high as neutrality in the experiments (see Fig. 13.45).

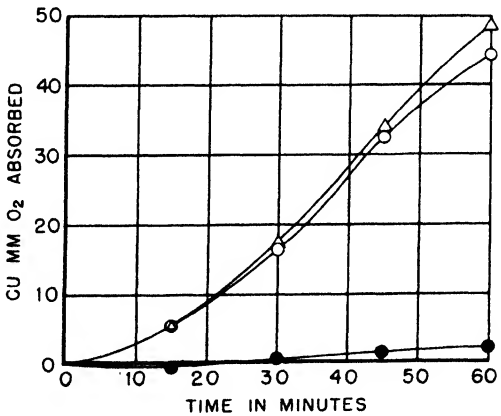


Fig. 13.45— Effect of uranyl acetate on succinic dehydrogenase. ○, control experiment without uranyl acetate: 0.3 ml of enzyme suspension + 1.0 ml of disodium succinate + 0.5 ml of 0.04 per cent methylene blue + 0.2 ml of H_2O, pH 7.1, no added buffer; △, same as control, but with 1,000 μg of $UO_2(Ac)_2.2H_2O$ dissolved in substrate; ●, control without enzyme or uranyl acetate.

Under different conditions, in which partially neutralized uranyl nitrate was added to succinic dehydrogenase preparations from pigeon breast muscle before addition of substrate, and at a lower pH (6.2), Barron found a high degree of inhibition of succinic dehydrogenase

activity by U_6 (100 per cent inhibition by 1×10^{-3} M) [University of Chicago, Atomic Energy Project Report CH. 2429 (Chicago-Rochester Conference Sept. 30-Oct. 1, 1944)]. The molarity of U_6 in the present experiment was about 1.2×10^{-3}. Barron did not report his method of determining the enzyme, and so it is difficult to compare the present results with his in a satisfactory manner. Moreover the source of enzymes in the two experiments differed, as did the pH of the determinations. In general, one would expect more inactivation at pH 6.2 than at 7.0 as already has been explained.

4.18 Thrombin. (Work done by Alexander L. Dounce.) The action of thrombin on fibrinogen was found to be inhibited by uranyl acetate nearly to the extent of the inhibition of phosphorylase by uranyl acetate. Thus the thrombin-fibrinogen system is more sensitive to uranyl acetate than most of the other enzyme systems investigated. It may be that the lengthening of clotting time in animals heavily poisoned by uranyl acetate is in part explainable on this basis.

The fibrinogen solution was prepared by making up a 2 per cent solution of lyophiled fibrinogen in water to which was added enough acetate buffer to complete the solution of the material. The final concentration of acetate was in the neighborhood of 0.1 to 0.2M, and the pH was about 7.4.

One milligram of lyophiled thrombin, stated by the University of Rochester Department of Medicine to be about 10 per cent pure, was dissolved in 5 ml of water, yielding a perfectly clear solution.

Freshly oxalated dog plasma was used.

The following experiments were performed:

1. To 0.5 ml of fibrinogen solution, 0.1 ml of 0.1 per cent $UO_2(Ac)_2 .2H_2O$ (100 μg of salt $\simeq$ 55 μg of uranium metal) was added and neutralized to pH 7, followed by the addition of 0.1 ml of thrombin solution. A control was run without uranyl acetate.

Both tubes showed complete clotting in 3 to 4 min at 25°C. There was therefore no inhibition of the clotting by the uranyl acetate.

2. The same experiment was repeated using 1 ml of dog plasma in place of the 0.5 ml of fibrinogen. The clotting time was about 3 min; hence there was no inhibition of clotting.

3. Experiment 2 was repeated except that the uranyl acetate was added to the thrombin, and then the plasma was added. Still there was no inhibition of clotting.

4. Experiments 2 and 3 were repeated, but the pH of the uranyl acetate and of the plasma was adjusted to 6.5. Molar acetic acid was used to lower the pH of the plasma. Still there was no inhibition of clotting.

Experiments 2 through 4 show that 100 μg of $UO_2(Ac)_2.2H_2O$ in 1 ml of solution is insufficient to inhibit the action of thrombin on fibrinogen in plasma under the experimental conditions employed, at pH 7.2 to 7.4 or pH 6.5. This is about 0.00024M uranyl acetate. Twice this concentration of uranyl acetate did not inhibit the action of thrombin on purified fibrinogen as shown in Experiment 1.

5. To 1.0 ml of dog plasma was added 0.1 ml of 0.5 per cent uranyl acetate (500 μg) neutralized to pH 7.0. Then 0.1 ml of thrombin solution was added. Some inhibition of clotting occurred, since clotting did not appear to be complete in less than 6 min (clotting time for control was 3 min), and the clot that was formed was not so solid as that of the control.

6. This experiment was repeated, except that the uranyl acetate was added to the thrombin, and then the fibrinogen was added. In this case complete inhibition of clotting occurred.

Experiments 5 and 6 show that 500 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 260 μg of uranium metal) in 1 ml (0.0013M) inhibit clotting partially or completely, depending on whether the uranyl acetate is added to the plasma and to the thrombin.

7. When 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 550 μg of uranium metal) was used at pH 7.2 to 7.4 and pH 6.5 in experiments carried out in a manner as described above, the clotting of plasma was completely inhibited even when the uranyl acetate was added to the plasma, followed by addition of the thrombin. In this case the concentration of the uranyl acetate was about 0.013M ($\eqsim$ 260 μg of uranium metal).

In summary, 500 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 260 μg of uranium metal). partially or completely inhibits the thrombin-fibrinogen reaction at pH 7.2 to 7.4 or pH 6.5, depending upon the order in which the constituents are added; 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 550 μg of uranium metal) always inhibits the reaction completely; and 100 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 55 μg of uranium metal) does not inhibit the reaction at all under conditions studied.

4.19 Urease. (Work done by Alexander L. Dounce with the assistance of William Connors.) Urease was crystallized according to the method of Sumner[34] and recrystallized by the method of Dounce.[35]

(a) Experiments in the Presence of Phosphate Buffer. The enzyme activity was determined, using the standard conditions of Sumner,[34] but direct nesslerization was used to determine the ammonia produced instead of aeration, in order to save time. This procedure is sufficiently accurate for enzyme-poisoning studies, provided that pure urease is used and not a crude extract.

Amounts of enzyme that varied from 0.1 to 0.3 units were employed

in the test. (A unit is the amount of enzyme that will produce 1 mg of ammonia nitrogen in 5 min at 20°C under Sumner's standard conditions.) The maximum amount of enzyme employed, on a dry-weight basis, would correspond to 2.26 μg. Amounts of uranyl acetate dihydrate (and in some cases uranyl nitrate hexahydrate neutralized with NaOH to pH 4.5) that varied from 100 to 1,000 μg (≎ roughly to 55 to 550 μg of uranium metal) were added. The minimum ratio of uranium compound to enzyme on a dry-weight basis was 440 : 1, but of course on a molecular basis this ratio would be much larger. The maximum ratio of uranium compound to urease on a dry-weight basis was about ten times this figure. The uranium compound was always added to the urea-phosphate buffer before addition of the enzyme. This urea-buffer solution contained 3 per cent urea, 5.4 per cent of K_2HPO_4, and 4.25 per cent of Na_2HPO_4.

In none of the experiments was any inactivation of the enzyme observed, which can be accounted for by the fact that a strong phosphate solution was used as buffer. The phosphate could be expected to complex UO_2^{++} ion firmly under these conditions and possibly even to precipitate most of it.

(b) Experiments with Urease Using Veronal Acetate as Buffer. A few experiments using 0.1M sodium acetate buffered to pH 6.5 with diethyl barbituric acid showed that, at this pH, 2 mg of $UO_2(Ac)_2.2H_2O$ (≎ 1.12 mg of uranium metal) caused more than 90 per cent inhibition of urease with amounts of enzyme varying from 0.1 to 0.5 unit. Using ordinary veronal buffer at pH 7.5, 2 mg of uranyl acetate gave little or no inhibition of the same amount of urease.

(c) Citrate Buffer. At pH 6.5 in the presence of 0.1M citrate buffer, it was found that 2 mg of $UO_2(Ac)_2.2H_2O$ produced no inhibition of 0.1 to 0.5 unit of urease. This was to be expected because of the strong complexing action of the citrate.

(d) Acetate Buffer. The results of poisoning experiments are shown in Fig. 13.46. It will be noted that the degree of poisoning increases sharply with decrease in pH until the pH is about 6.2. Some points in this figure represent values obtained using citrate or acetate-veronal buffer, which are shown for comparison.

(e) Maleate Buffer. The results are shown in Fig. 13.47, using uranyl acetate as the inhibiting compound. Some precipitation of the urease occurred at the lowest pH values employed. Using UCl_4 as inhibiting compound, the results are shown in Fig. 13.48. In all cases the inhibitor was added to the buffer substrate before addition of the enzyme. Three per cent urea was always used as substrate, so that in these experiments the enzyme was always saturated with substrate.

It is to be noted that the direction of the slopes of the pH-inhibition

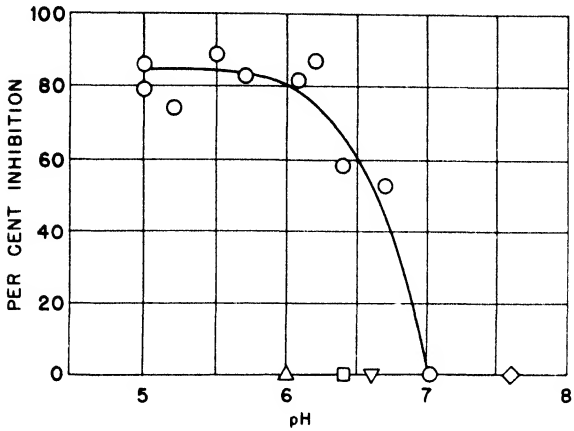


Fig. 13.46—Effect of pH on the inactivation of urease by U_s (uranyl acetate). The reaction mixture contains 0.0039 mg of urease (7.9×10^{-9} millimoles) in a concentration of 4.1×10^{-6} millimolar, 2 mg (4.8×10^{-3} millimoles) of $UO_2(Ac)_2.2H_2O$ in a concentration of 2.4 millimolar; 0.1M acetate buffer; molecular ratio of uranyl acetate to urease = 6.1×10^5. ○, experimental values from the conditions stated above; △, 0.1M maleate buffer in place of acetate buffer; □, 0.1M phosphate buffer in place of acetate buffer; ▽, 0.1M citrate buffer in place of acetate buffer; ◇, 0.1M veronal acid-veronal buffer in place of acetate buffer.

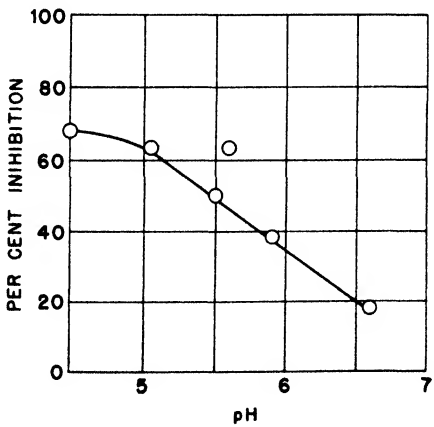


Fig. 13.47—Effect of pH on the inhibition of urease by uranyl acetate. The reaction mixture contains 2 mg of $UO_2(Ac)_2.2H_2O$ + 0.0019 mg (3.9×10^{-9} millimoles) of urease (0.25 units); 0.1M maleate buffer.

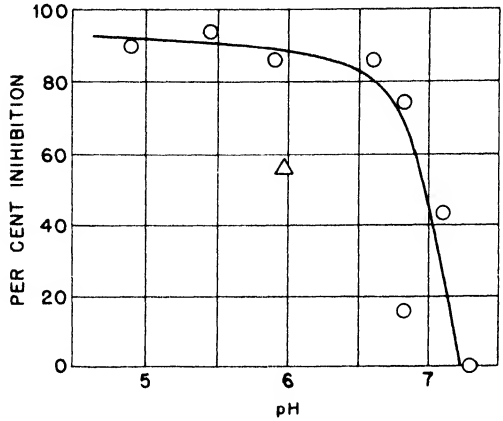


Fig. 13.48—Effect of pH on the inhibition of urease by UCl_4. ○, 100 μg of UCl_4 and 0.0019 mg (4×10^{-9} millimoles) of urease; △, 50 μg of UCl_4, 0.0019 mg (4×10^{-9} millimoles) of urease; 0.1M maleate buffer.

curves are the same for U_6 as for U_4 and that urease appears to be about twenty times more sensitive to the action of the U_4 used than to U_6. Whether contaminating heavy metals in the U_4 could have influenced this result is not known.

The action of U_6 on urease is probably nonspecific and no doubt results from blocking of large numbers of acid groups, as well as from aggregation of the enzyme particles, which results eventually in precipitation of part of the enzyme at least at the lowest pH values used.

Urease undoubtedly requires SH groups for activity. U_6 does not combine with SH groups, as has been stated in Sec. 1 of this chapter. However, U_4 appears to have some affinity for SH groups (see work on phosphorylase), unless the U_4 samples were contaminated by some other heavy metals with an affinity for SH. The greater sensitivity of urease to U_4 than to U_6 could be explained by the affinity of U_4 for SH groups.

An experiment was carried out to see whether addition of uranyl acetate to urease before addition of the buffer substrate would result in greater poisoning than addition of uranyl acetate to the buffer substrate before addition of the enzyme. Only slightly greater inhibition was found when the uranyl acetate was added to the enzyme first.

Some work was done on the inactivation of urease by uranyl acetate in the presence of sodium dithionite, $Na_2S_2O_4$, which reduces the U_6 to U_4. At pH 6.0 it was found that the addition of dithionite increased

inhibition of the urease by about 33 per cent. Suitable controls were carried out. However, this increase did not parallel the increased inhibiting effect of UCl_4 samples. Possibly the U_4 produced by the reduction of U_6 with $Na_2S_2O_4$ was not able to combine with the urease in such a way as to inactivate it as thoroughly as by adding U_4 directly (perhaps because of formation of an insoluble oxide), or possibly the UCl_4 samples may have contained enough heavy metal to increase the inactivation of the urease. Finally it is possible that the $Na_2S_2O_4$ and its decomposition products exert a certain amount of protective action against the heavy-metal poisoning of urease. The last explanation is perhaps the most plausible of the three.

Because it was felt desirable by Warren to show clearly what differences occur between UO_2^{++} and most other heavy metals in regard to their inhibitory action on enzymes, and also partly because some controversy had arisen within the project as to whether or not SH groups were involved in enzyme inhibition by U_6, a detailed study was made of the inhibition of urease by mercury, copper, silver, sodium arsenite, and sodium arsenate as well as the oxidants $KH(IO_3)_2$, KIO_4, I_2, Br_2, and H_2O_2. With some of these compounds, particularly the heavy metals, the effect of pH on the inhibition was also investigated.

Mercury has a strong inhibiting action on urease, as can be seen from Fig. 13.49, where the effects of various amounts of $HgCl_2$ on urease activity at pH 7.05 in phosphate buffer are shown. The phosphate undoubtedly tends to reduce the inhibitory action of the $HgCl_2$. The curve showing the effect of mercury on urease activity plotted

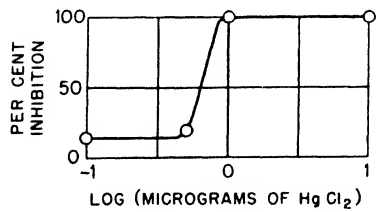


Fig. 13.49—Inhibition of urease by $HgCl_2$, at pH 7.05 in 0.1M phosphate buffer. The amount used was 0.0039 mg of urease (7.9×10^{-9} millimoles).

against pH is given in Fig. 13.50, which indicates that the direction of the curve is the same as that for corresponding curves made by plotting the inhibition of urease by U_6 or U_4 against pH. The slopes are all negative. On the other hand, the curve for the inhibition of catalase by mercury has a positive slope as would be more or less expected

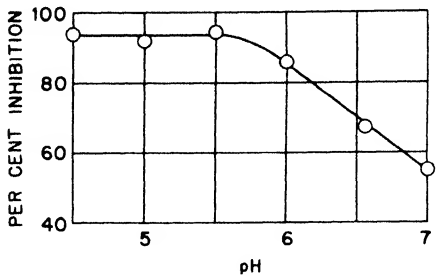


Fig. 13.50—Effect of pH on the inhibition of urease by $HgCl_2$. The reaction mixture contains 0.0039 mg of urease (7.9×10^{-9} millimoles) and 0.1 μg of $HgCl_2$; the molecular ratio of $HgCl_2$ to urease = 47.

if the Hg^{++} were acting only as a protein precipitant. These observations indicate that mercury does not inactivate urease in the same manner that it inactivates catalase (i.e., as a simple protein precipitant). As previously stated, mercury probably blocks vital SH groups in the urease. The curves for the inactivation of urease by U_6 and U_4 as functions of pH, on the other hand, have slopes of the same sign as the slope of the curve for the inactivation of catalase by U_6 plotted against pH, and the slope of this curve also is the same in sign as the slope of the curve for the precipitation of a typical protein by U_6 as a function of pH. The fact that the slopes of the curves for inactivation of urease by $HgCl_2$ and uranyl acetate as functions of pH happen to have the same signs does not indicate that $HgCl_2$ and uranyl acetate combine with the same functional groups of the urease molecule.

The inhibiting effect of $AgNO_3$ on urease was found to be even greater than that of mercury. At a ratio of 15 atoms of silver to 1 molecule of urease, inhibition of the activity of urease was practically complete. Sumner and Myrback[7] found a high degree of inactivation at a ratio of 1 gram-atom of Ag to 40,000 g of urease, or 10 atoms of silver to 1 molecule of urease.

The effect of pH on the inhibition of urease by $AgNO_3$ is shown in Fig. 13.51. The curve is so flat that it is hard to decide with centainty whether the slope is positive or negative.

The inhibition of urease by copper, shown in Fig. 13.52, was somewhat less than with Hg or Ag, but copper is nevertheless a very potent inhibitor of urease. The ratio of copper to urease used was 2,000 atoms of Cu to 1 molecule of urease.

In connection with the work on heavy metals and urease, reference is made to the work of Jacoby.[36] However, Jacoby apparently worked with very crude urease preparations.

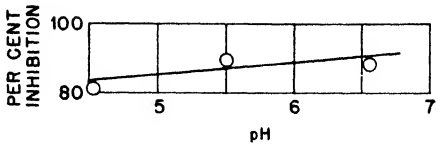


Fig. 13.51 — Effect of pH on the inhibition of urease by $AgNO_3$. The reaction mixture contains 0.0039 mg of urease (7.9×10^{-9} millimoles), 0.025 μg of $AgNO_3$, 0.1M maleate buffer; the molecular ratio of $AgNO_3$ to urease = 15.

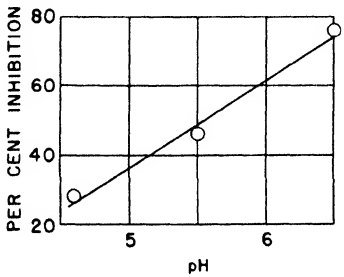


Fig. 13.52 — Effect of pH on the inhibition of urease by copper sulfate. The reaction mixture contains 0.0039 mg of urease (7.9×10^{-9} millimoles) and 5 μg of $CuSO_4.5H_2O$; 0.1M maleate buffer.

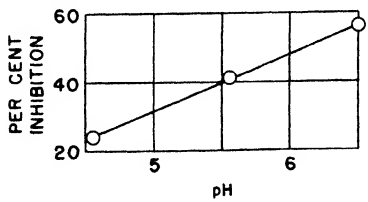


Fig. 13.53 — Effect of pH on the inhibition of urease by $NaAsO_2$. The reaction mixture contains 0.0039 mg of urease (7.9×10^{-9} millimoles) and 1 mg of $NaAsO_2$; 0.1M maleate buffer.

Urease is poisoned by the addition of sodium meta-arsenite, although it is not very sensitive to this material (Fig. 13.53). The ratio of arsenite to urease that was employed was 856,000 moles of arsenite to 1 mole of urease.

Test-tube experiments have been carried out showing that urease is very sensitive to cadmium, somewhat sensitive to the cobaltous ion, and rather insensitive to the Mn^{++} ion. These poisoning experiments were carried out at pH 7.0. It was found that 50 μg of $CdCl_2$ is required to inactivate 0.19 mg of urease, whereas 1 mg of $CoCl_2$ and 10 mg of $MnSO_4$, respectively, are needed to inactivate the same amount of urease.

Urease is quite insensitive to sodium arsenate, since 10 mg of arsenate only partially inactivates 0.19 mg of urease.

The effect of some oxidizing agents on urease was investigated. From the literature and the present experiments, it is known that urease is not very sensitive to mild oxidizing materials like $K_3Fe(CN)_6$ and porphyrindene, which should oxidize SH groups to S-S groups.[37] But excess ferricyanide does inhibit urease.[38] In one experiment, 10 mg of this material strongly inhibited 0.19 mg of urease.

Urease is quite readily inactivated by more powerful oxidizing agents, which may oxidize SH groups to $-S(=O)-OH$ or $-S(=O)_2-OH$ groups. The following experiments have been mainly concerned with the latter type of reagent.

The inhibiting effect of $KH(IO_3)_2$ has been studied quantitatively in respect to the effect of pH. One milligram of the biniodate was used to inactivate 0.0039 mg of the urease (Fig. 13.54).

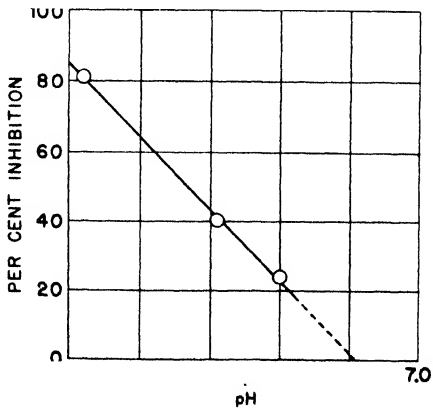


Fig. 13.54 — Effect of pH on the inhibition of urease by $KH(IO_3)_2$. The reaction mixture contains 10 mg of $KH(IO_3)_2$ and 0.0039 mg (7.9×10^{-9} millimoles) of urease; 0.1M maleate buffer.

It was found that, at pH 7.0, 1.0 ml of 0.03M KIO_4, 1.0 ml of 0.005N Br_2, or 1.0 ml of 0.005N I_2 when added to 1.0 ml of crystallized urease solution containing 0.19 mg of the enzyme, will inactivate the enzyme rapidly. Also, 10 mg of potassium persulfate will inactivate the same amount of enzyme. The inactivating effect of these substances is slightly less if they are added to the substrate instead of the enzyme solution. The inactivating effect of periodate and persulfate can be reversed at least partially if $Na_2S_2O_4$ or cysteine is added to the inactivated solutions soon enough.

Three per cent H_2O_2 will not inactivate urease in a period of ½ hr, but on standing for 12 hr in contact with the enzyme it causes inactivation.

The foregoing experiments were carried out by testing for urease activity qualitatively, using urea in the absence of buffer with phenol red as an indicator, unless otherwise stated.

Discussion of Results. The effect of pH on the inactivation of urease by heavy metal is seen to be more or less unpredictable. There are several possible explanations for this. For instance, differences in variation with pH of dissociation constants of S-M-X complexes might occur, or the buffer might combine to differing degrees with the different metal salts at different pH values (S-M-X represents the sulfhydryl complex with metallic salts of the general formula MX).

Hellerman[37] proposed the theory that urease contains free and restricted SH groups, the former having nothing to do with the activity of the enzyme, but the latter being necessary for its activity. Titration with the dye porphyrindene oxidized the "free" SH groups without affecting the activity of the enzyme, since the "restricted" SH groups were not accessible to oxidation by the dye.

The writer agrees with the concept of restricted or hindered SH groups and believes that the hindrance is caused by the molecular configuration of the complicated protein molecule. There is some doubt whether Hellerman's urease was of sufficient purity to indicate that the free SH groups belonged to the native enzyme molecule rather than to impurities or to denatured enzyme molecules, but this point is not of much importance because the free SH groups are not connected with enzyme activity.

All present results as well as all those reported in the literature are explained on the assumption of the presence of one or more sterically hindered SH groups in the urease molecule, which constitute the actual prosthetic groups of the enzyme. It is doubtful that nonprosthetic SH groups could have a great deal to do with the enzymatic activity of urease.

Heavy-metal combination with the sterically hindered SH groups of urease is conceived as follows: $U-SH + MX_2 = U-S-MX + HX$.

The action of mild oxidizing agents fails because of the impossibility of approximating a hindered SH group and a second SH group closely enough to form a disulfide linkage. However, reagents capable of oxidizing SH to $S(=O)-OH$ or $S(=O)(=O)-OH$ should readily inactivate urease.

A known example of the latter type of reaction[39] is the following:

$$R-SH + 3X_2 + 2H_2O = R-S(=O)(=O)-X + 5HX$$

Iodine and Br_2 rapidly inactivate urease. Here the steric hindrance of the SH groups has no effect in preventing oxidation, since the halogen is readily able to reach and ozidize them.

Of course it is also possible that the "hindrance" of the prosthetic SH groups is caused by the loose chemical combination with some other group in the protein molecule rather than by steric hindrance, but this seems less likely.

For additional information concerning urease and SH, see the work of Smythe.[40] For a discussion of the effect of redox potentials on urease activity see the paper of Sizer.[41]

In conclusion, it must be repeated that uranyl acetate has little or no effect on urease until a sufficient amount of this substance is added to cause incipient precipitation of the enzyme. It is most probable that uranyl acetate inactivates the enzyme by combining with acid groups, thereby causing aggregation of the enzyme molecules and finally causing precipitation. The work of Tien Ho Lan on crystalline phosphorylase very strongly supports the conclusion that uranyl acetate does not act on enzymes by blocking SH groups.

4.20 Xanthine Oxidase. (Work done by Alexander L. Dounce.) Xanthine oxidase was prepared according to the method of Ball.[42] The enzyme is an aerobic dehydrogenase containing flavine-adenine dinucleotide as a prosthetic group. It is said to be present in the livers of human beings and many animals.

The preparation made in this laboratory was about one-third as pure as the best preparation of Ball, judging by the ratio of the heights of the two principal absorption bands. This may be attributed to lack of mechanical shaking in eluting the enzyme from the fat globules.

The enzyme was tested for inhibition by U_6, using the Thunburg technique with methylene blue as hydrogen acceptor and hypothanthine as substrate. The experiments were set up as follows: 1.0 ml of hypoxanthine neutralized to pH 7.5 plus 0.1 ml of 0.04 per cent methylene blue, plus 0.1 ml of 0.5 per cent $UO_2(Ac)_2.2H_2O$ ($\simeq$ 280 μg of uranium metal) previously neutralized to pH 7.5 were placed in the

bottom of the Thunburg tube, and 0.05 ml of enzyme solution was placed in the top of the tube. Another tube was prepared containing uranyl acetate but no enzyme. The tubes were evacuted, and the contents were mixed and incubated at 25°C. The amount of enzyme present was sufficient to cause complete decolorization of the methylene blue in 20 min in both the poisoned and unpoisoned tubes. The tube without enzyme showed no decolorization.

Thus 500 μg of neutralized $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 280 μg of uranium metal) added to the substrate failed to inhibit the activity of the xanthine oxidase. The experiment was repeated using 1,000 μg of neutralized $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 560 μg of uranium metal), but still no poisoning occurred. It appears that xanthine oxidase prepared from cow's milk is insensitive to poisoning by U_6 at body pH. The pH of the contents of the tubes was found to have fallen from 7.5 to 7.2 at the end of the experiment.

The experiment was repeated using 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\eqsim$ 560 μg of uranium metal) at pH 6.0. Although the results cannot be expressed quantitatively, it was found that some poisoning of the enzyme occurred. This is in agreement with previous work on other enzymes, which have been found to be poisoned more effectively as the pH is lowered.

4.21 Respiration of Kidney- and Liver-tissue Brei of Rats in the Presence of Uranyl Acetate In Vitro. (Work done by Tien Ho Lan with the assistance of Betty Robinson.) The respiration (QO_2) of kidney- and liver-tissue brei of rats with or without the addition of uranyl acetate was measured in the Warburg apparatus at 35°C without added substrate (see Sec. 4.2 of this chapter). The animals were killed by decapitation. The animal to be killed at a given time was first fasted overnight. The tissue brei of kidney or liver was prepared by a glass homogenizer in physiological saline solution. For each gram of tissue, 3 ml of physiological saline solution was used for the preparation of the tissue brei. The time between killing the animal and the temperature equilibration of the solutions in the Warburg flasks was always kept within the limit of 20 min. Each flask contained 1.0 ml (or 0.9 ml when uranyl acetate was added) of 0.1M phosphate or 0.1M maleate buffer solution and 1 ml of tissue brei in the main compartment, with 0.05 ml of 3N KOH in the central well of the flask. The total volume of the solutions was 2.05 ml. One-tenth milliliter of a neutralized 1 per cent solution of $UO_2(Ac)_2.2H_2O$ was put in the side arm of the Warburg flask. The uranyl acetate solution was mixed with the tissue brei after temperature equilibrium had been established. Records of oxygen consumption were taken at 15-min intervals for a period of 60 min, and another reading was taken 30 min

later, so that the total time during which readings were made was 90 min. Determinations were carried out in both 0.1M phosphate buffer and 0.1M maleate buffer at pH 5.5, 6.0, 6.5, 7.0, and 7.8, respectively.

In this experiment three animals were used for each pH value of each buffer solution. It was found that 1,000 μg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 560 μg of uranium metal) did not cause any significant inhibitory action on the respiration of tissue brei of liver and kidney of normal rats either in 0.1M phosphate or 0.1M maleate buffer, except that in acid solution a slight inhibition was noticed in both liver- and kidney-tissue brei. In alkaline solution there was a slight increase in the respiration of kidney-tissue brei. The respiration of tissue brei was slightly higher in phosphate than in maleate buffer. The results are shown in Figs. 13.55, to 13.57.

5. EFFECTS OF U_6 AND U_4 ON ENZYMES IN VIVO

5.1 General Considerations and Comparison of In Vivo with In Vitro Studies. The most favorable results from the standpoint of interpretation would be to find that uranium acted upon certain enzymes in vitro in a strongly inhibitory manner and that in vivo the same enzymes were found to be diminished in activity after uranium poisoning, at least in organs where uranium is known to accumulate. However, such a situation does not appear to exist. It was at one time thought that the lack of glycogen in the livers of animals severely poisoned by uranium could be explained by the direct inhibition of phosphorylase by U_6, since Tien Ho Lan had found that the latter enzyme is quite sensitive to U_6 in vitro. However, it later developed that U_6 does not accumulate in liver to more than a very small degree, and the liver shows little or no histological damage after moderate U_6 poisoning. Moreover, Tien Ho Lan found that neither kidney, liver, nor muscle phosphorylase activity is diminished after moderate U_6 poisoning. Therefore it is necessary to look upon the lowering of liver glycogen as a secondary rather than a primary effect of U_6 poisoning. It may simply be accounted for by the fact that poisoned animals do not eat well. Robert's group found that the livers of animals poisoned by U_6 are still able to synthesize glycogen, although there appears to be some impairment of glycogen synthesis in the muscle.

On the other hand, some enzymes of the kidney that are quite insensitive to U_6 in vitro suffer marked diminution in activity after severe U_6 poisoning, particularly if poisoning has been caused by repeated injections of U_6. Examples of such enzymes are alkaline phosphatase and, to some extent, catalase. Investigators at the University of Chicago have reported a list of other enzymes that may be in this

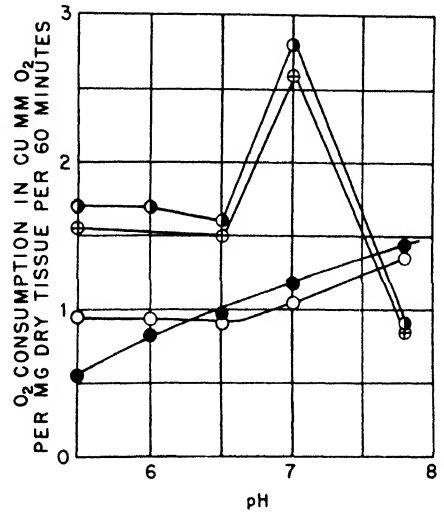


Fig. 13.55—Respiration of rat-liver and kidney-tissue brei in the presence of uranyl acetate in 0.1M phosphate-Ringer buffer at different pH values. ◑, liver-tissue control; ⊕, liver-tissue brei in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$; ○, kidney-tissue-brei control; ●, kidney tissue in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$.

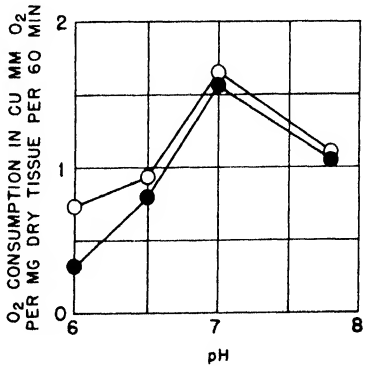


Fig. 13.56—Respiration of rat-liver-tissue brei in the presence of uranyl acetate in 0.1M maleate buffer at different pH values. ○, liver-tissue-brei control; ●, liver-tissue brei in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$.

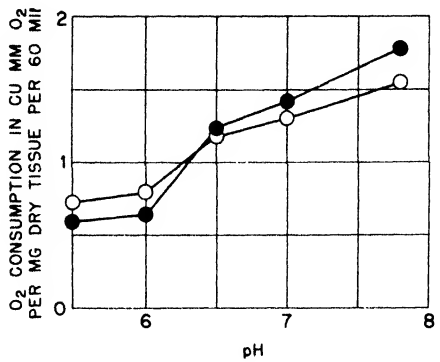


Fig. 13.57—Respiration of rat-kidney-tissue brei in the presence of uranyl acetate in 0.1M maleate buffer at different pH values. ○, kidney-tissue-brei control; ●, kidney-tissue brei in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$.

category. Here it seems inevitable that the lowering of enzymatic activity must be considered as a secondary effect rather than as a result of direct inhibitory action of U_6. Just what the mechanism of this secondary process might be is not known at the present time. It seems probable that some at least of the intracellular enzymes are not present in normal amount in the regenerated cells of the kidney after severe U_6 damage.

The question then remains, why is the kidney damaged by U_6? Can the damage be accounted for by the blocking of some vital enzymes, or must it be accounted for in some other way? A clear-cut answer cannot yet be given. The work on enzymes and proteins has led to a general mechanism for the action of U_6 on kidney that will be discussed in more detail elsewhere but is briefly as follows:

U_6 exists in plasma chiefly in the form of slightly dissociated complexes with protein and bicarbonate. The bicarbonate complex is able readily to filter through the glomerulus, whereas the protein complexes of U_6 are not able to pass the glomerular barrier. When the bicarbonate complex gets into the tubules it begins to release UO_2^{++} ions in low concentrations, which immediately combine with the lining surfaces of the tubular cells, presumably with the protein of these cell surfaces.

If the first attack of U_6 on kidney tubular cells is on the cell surfaces, it appears possible that the membrane is so altered that it may be destroyed by autolytic processes. Even if only a small defect occurred at first, this defect would have a tendency to spread to adjacent

cell surfaces and cytoplasm. Moreover it has been seen that practically without exception the effect of U_6 on enzymes becomes more pronounced as pH decreases from 7.0 to 5.5, so that any tendency toward the production of an acid urine in the distal portions of the proximal tubules would aggravate the damaging action of the U_6 on the tubular cells.

When the U_6 combines with the tubular cell surfaces, the absorptive and excretive capacity of the tubular cells for certain constituents of filtered plasma and plasma apparently is diminished. But whether cell necrosis is initiated by an attack on a vital enzyme or enzymes in the cell surface, or whether the altered physicochemical state of the cell surface produced by the action of U_6 allows intracellular enzymes to destroy the cell cannot be stated. It seems likely that, once a defect appears in the cell surface, more U_6 can continue the attack directly on the cell cytoplasm.

Nothing has been said about U_4 in discussing this mechanism of action of uranium on the kidney, but the same sort of explanation can be expected to hold for U_4 in the event that any U_4 passes through the glomerular barrier. The U_4 has far less tendency to filter through the glomerulus than does U_6, but it is likely that a certain amount can penetrate. One might expect U_4 to be somewhat worse in its effect on tubules than is U_6, provided that it reaches them at all, since it appears to have a slightly lesser affinity for bicarbonate and somewhat greater affinity for protein than has U_6. It also seems to have a somewhat greater inhibitory action on certain enzymes (urease and phosphorylase) than does U_6. It is doubtful that a sufficient reduction of U_6, which has been fixed in the tubules, can take place to cause an increase in the damaging effect of uranium materially over that which would be expected from the action of U_6 itself.

Detailed experiments dealing with changes observed in vivo in enzymes after the injection of animals with U_6 will now be described.

5.2 Experimental Material on Studies In Vivo. (a) *d*-Amino Acid Oxidase in the Liver and Kidney of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan.) Ten rats were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ ($\hat{=}$ 2.8 mg of uranium metal) per kilogram of body weight. The first rat was killed the first day after being injected, the second rat on the second day, the third rat on the third day, the fourth rat on the fourth day, and the fifth rat on the sixth day after injection. Five rats that died at various times after being injected were not studied. The same rats were studied for choline oxidase activity (see under choline oxidase in vivo). Animals that showed definite symptoms of poisoning were killed first.

The rat to be killed at a given time was fasted overnight, and tissue brei of liver or kidney was prepared by grinding in a glass homogenizer with 0.9 per cent saline. The *d*-amino acid oxidase activity of the tissue brei was determined by the method of Klein as previously described in the section on *d*-amino oxidase in vitro, with activity expressed as cubic millimeters of O_2 consumed per milligram of tissue on a dry-weight basis. It was found that the *d*-amino acid oxidase activity of the kidneys was decreased after treatment of the animals with uranyl acetate. The decrease in enzyme activity in the kidneys increased as the time after injection of the animals increased. However, the *d*-amino acid oxidase activity in the livers of the rats poisoned by uranyl acetate did not show significant change as compared with enzyme activity in the livers of normal rats (Fig. 13.58). In this figure enzyme activity, indicated by the ordinate, is represented by the oxygen consumption caused by the oxidation of *dl*-alanine in the first 30 min. The decrease in *d*-amino acid oxidase activity in the kidney following the injection of uranyl acetate might be accounted for by a decrease in apoenzyme, by a decrease in coenzyme, or a decrease in both, caused by the poisoning action on the kidney of the injected uranyl acetate.

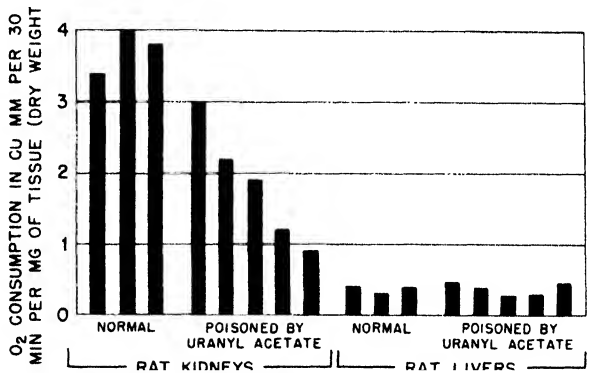


Fig. 13.58—*d*-Amino acid oxidase activity in the kidneys and livers of normal rats and of rats poisoned by uranyl acetate.

The effect of starvation on the *d*-amino acid oxidase content of the liver and kidney in rats was determined (see similar work under choline oxidase, Sec. 5.2d). It was found that starvation caused a 50 per cent drop in liver *d*-amino acid oxidase, and a smaller drop in kidney *d*-amino acid oxidase. Thus the effect of starvation on the *d*-amino

acid oxidase of liver and kidney is not quantitatively the same as the effect of uranium poisoning on this enzyme in the rat liver and kidney.

It was found that the *d*-amino acid oxidase of the kidneys of rats made tolerant to uranyl nitrate by repeated intraperitoneal injections was very low. However, the *d*-amino acid oxidase of the livers of these rats was normal, and this finding can be ascribed to the fact that the tolerant rats ate normally. This is a further indication that the lowered *d*-amino acid oxidase activities of the livers of rats acutely poisoned by U_6 is a secondary effect that may be largely attributable to the low food intake of these animals.

(b) Carbonic Anhydrase In Vivo. (Work done by Tien Ho Lan with the assistance of Marian Kaley and Paul Fanta.) The activity of this enzyme has been measured in the blood and kidney tissue of normal rats and rats previously poisoned by intraperitoneal injection of uranyl acetate.

Carbonic Anhydrase Activity of the Blood of Rats Poisoned by Uranyl Acetate. Five milligrams of $UO_2(Ac)_2.2H_2O$ (≙ 2.5 mg of uranium metal) per kilogram of body weight was injected into five rats, and subsequently one rat per day was sacrificed, starting 48 hr after the time of injection. All animals were fasted for 24 hr before being sacrificed.

The blood of each sacrificed animal was tested for carbonic anhydrase activity according to the method of Philpot and Philpot[19] as already outlined in the in vitro work. The blood of the animals was diluted 1 : 400, and 0.5 ml of this diluted blood was used in carrying out the tests for enzyme activity. The time in seconds required for the pH change to occur in the control experiment (T_c) was 70 ± 3. The amount of enzyme present in a given experiment was calculated as units. The unit is defined by the following formula:

$$E = \frac{2(T_c - T_e)}{T_c}$$

where E is the unit, T_c is the time in seconds required for the pH change to occur in the control experiment, and T_e is the time in seconds required for the pH change to occur in the experiment with enzyme added.

There are no material changes in the carbonic anhydrase activity of the blood of any of the poisoned animals, as shown in Table 13.16.

Experiments of a somewhat different nature were carried out using 20 more rats in two groups. One group of 10 rats was injected intraperitoneally with 5 mg of $UO_2(Ac)_2.2H_2O$ (≙ 2.8 mg of uranium metal)

per kilogram of body weight; the other group of 10 rats of approximately the same weight was used as a control group. Five days after the injection all the rats were sacrificed, and the blood of all the rats in the normal group was collected and pooled. The blood of all the rats in the injected group also was collected and pooled. Since there is little variation in the blood carbonic anhydrase activity of different animals, this procedure is satisfactory.

Table 13.16—Carbonic Anhydrase in Blood of Rats Poisoned by U_6

Solution tested	Time after injection, hr	Units of carbonic anhydrase per ml of undiluted blood
Normal rat blood*		798
Blood of injected rat	48	864
Blood of injected rat	72	780
Blood of injected rat	96	689
Blood of injected rat	120	711
Blood of injected rat	144	1,450
Average for injected rats		899

* Average of individual values from five normal rats averaged together with the value of the pooled blood of ten rats, the latter value being counted twice.

The kidneys of the normal and injected animals also were pooled in the same manner into two separate groups, and the carbonic anhydrase activity of each group was determined, as will be described separately.

The work just outlined then was repeated twice more, using four more groups of rats with 10 animals per group.

The results of the experiment on the carbonic anhydrase activities of the pooled blood samples of the normal and injected animals are summarized in Table 13.17.

It can be seen that any lowering of activity caused by the poison is on the average less than 26 per cent of the activity of normal rat blood. It is questionable whether any significance may be ascribed to the lowering of activity observed in the second or third experiment, especially since no red-cell counts were made.

As a further check on these results, a crude extract of carbonic anhydrase was prepared from the blood of both normal and poisoned animals. One-hundredth of a milliliter of the enzyme was used in testing for activity. The enzyme preparation from the blood of the normal rats contained 143 units per milliliter, whereas the enzyme

preparation from the blood of the poisoned rats contained 127 units per milliliter.

From all the preceding experiments it can safely be concluded that the injection of heavy doses of uranyl acetate does not materially depress the carbonic anhydrase activity of the blood of rats. This finding is not surprising, since the red cells do not appear to carry U_6, and since carbonic anhydrase is not sensitive to U_6 as demonstrated by the in vitro work.

Table 13.17 — Carbonic Anhydrase in Pooled Blood Samples of Normal and Poisoned Rats

Solution tested	Units of carbonic anhydrase per ml of undiluted blood, Expt. 1	Units of carbonic anhydrase per ml of undiluted blood, Expt. 2	Units of carbonic anhydrase per ml of undiluted blood, Expt. 3
Pooled blood of normal rats*	671	694	594
Pooled blood of poisoned rats*	671	516	465

*Ten animals were used in each group except for the poisoned animals of Expt. 1; in this case only seven animals were used.

Carbonic Anhydrase Activity of the Kidneys of Rats Poisoned by Uranyl Acetate. Preparatory to determining the carbonic anhydrase activities of the pooled kidneys of the same three groups of poisoned rats and the same three groups of normal rats, described above (see Carbonic Anhydrase Activity of the Blood of Rats Poisoned by Uranyl Acetate, second part, and Table 13.17), the kidneys were ground in a glass homogenizer with approximately 5 volumes of distilled water for every part by weight of kidney. Then a 1 : 10 dilution in water was made from this original dilution, and 0.5 ml of the final dilution was employed in testing for carbonic anhydrase activity. Also 0.5 ml of the final dilution was dried in the oven at 105°C for the determination of dry weight. The results of the carbonic anhydrase determination for the normal and poisoned rats are shown in Table 13.18.

Since some blood is retained by the kidneys, it seemed necessary to estimate the amount of blood present in the kidney brei in order to be sure that the observed carbonic anhydrase activity of the brei was not largely attributable to the blood present.

The blood was extracted from 10 ml of the original 1 : 5 dilution. It was found by a method to be described shortly that on the average only 0.0017 ml of blood was present in 1.0 ml of this kidney brei, which corresponds to a value of 0.000085 ml of blood present in the

0.5 ml of the final dilution of brei that was used in determining the enzyme activity. The various values for the amount of blood found in 1.0 ml of the 1 : 5 dilution of kidney brei that were used in obtaining

Table 13.18 — Carbonic Anhydrase in Kidneys of Normal Rats and Rats Poisoned by U_e

Tissue tested	Units of carbonic anhydrase per mg of tissue, dry wt. (Expt. 1)	Units of carbonic anhydrase per mg of tissue, dry wt. (Expt. 2)	Units of carbonic anhydrase per mg of tissue, dry wt. (Expt. 3)	Average values for three experiments
Homogenate of pooled kidneys of normal rats*	1.22	1.05	1.09	1.12
Homogenate of pooled kidneys of poisoned rats*	1.48	0.51	0.77	0.92

*Ten animals were used in each group except for the poisoned animals of Expt. 1. In this case, only seven animals were used.

the above-mentioned averaged value of 0.0017 ml are shown in Table 13.19.

From the work on normal rat blood, including the work on pooled specimens, it was found that on the average 1 ml of blood contained 798 units of enzyme. Therefore the 0.000085 ml of blood present on the average in the 0.05 ml of the final dilution of kidney brei that was used for the determination of enzyme activity would contain $798 \times 0.000085 = 0.068$ units. By averaging the results of all experiments on normal kidney brei, it was found that 0.05 ml of the final dilution of the brei contained on an average 1.12 units of carbonic anhydrase. Therefore the contribution to the enzyme activity of the brei by the blood contained therein by calculation using the foregoing figures would amount to only $(0.068 \times 100)/1.12 = 6.1$ per cent. The method of blood determination was found to give only 50 to 60 per cent recovery, but, even if the contribution of the blood to the activity of the kidney brei is doubled, it does not amount on the average to more than 13 per cent of the total activity of the brei. Therefore it seems probable that any important decrease in the carbonic anhydrase activity of the kidney tissue itself should have been easily demonstrable by the methods employed. The results given in Table 13.18 for experiment 2 show a 50 per cent decrease in the carbonic anhydrase activity of the pooled kidneys of the poisoned animals relative to that of the pooled kidneys of the normal animals. However, the values for the averages of all three experiments show a decrease of only 18 per

cent in the average carbonic anhydrase activity of the kidneys of the poisoned animals relative to the average value for the kidneys of the normal animals. This decrease is of doubtful significance.

Table 13.19 — Amount of Blood Present in Rat-kidney Brei

	Ml of blood per ml of 1 : 5 dilution of brei		
Solution tested	Expt. 1	Expt. 2	Expt. 3
Brei of normal rat kidneys	0.0030	0.00032	0.0021
Brei of kidneys of poisoned rats	0.0016	0.0012	0.0022

Method for the Determination of Blood in Kidney Brei. The method employed was the spectrophotometric determination of fluoromethemoglobin. The spectrophotometric determinations were carried out by Fanta. The method was standardized by using varying amounts of whole blood. The blood was hemolyzed by diluting with water and adding a small amount of saponin. To 5.0 ml of the hemolyzed blood of a given dilution were added 1 drop of 20 per cent potassium ferrocyanide solution and 2 drops of saturated sodium fluoride, and then the pH was adjusted to 6.5, where the intensity of the fluoromethemoglobin band in the far red is of maximum intensity, as is demonstrated in Fig. 13.59. The volume of the solution then was made up to 10.0 ml with 0.1M phosphate buffer of pH 6.5. The optical density was determined at various wave lengths of light that covered the band being measured. Finally the height of the band peak from the base line constructed as in Fig. 13.59 was measured in optical density units, and this value was used to determine the amount of blood present. The height of the absorption-band peak from the base line plotted against the dilution of the blood gives a straight line, as is shown in Fig. 13.60. For pure blood this method is easily reproducible.

In estimating the amount of blood present in tissue brei by this method, 10 ml of the brei prepared by homogenizing 1 part by weight of kidney with 5 parts by weight of distilled water was adjusted to pH 5.5 with 0.1M phosphate buffer, and the agglutinated protein was removed by centrifugation at a speed of about 18,000 rpm. The precipitated protein was washed thoroughly with distilled water, and the supernatants were pooled and adjusted to pH 6.5 with 0.1M phosphate buffer. Small amounts of $K_3Fe(CN)_6$ and NaF were added as described above. The total volume of the solution was made up to 20 ml, and

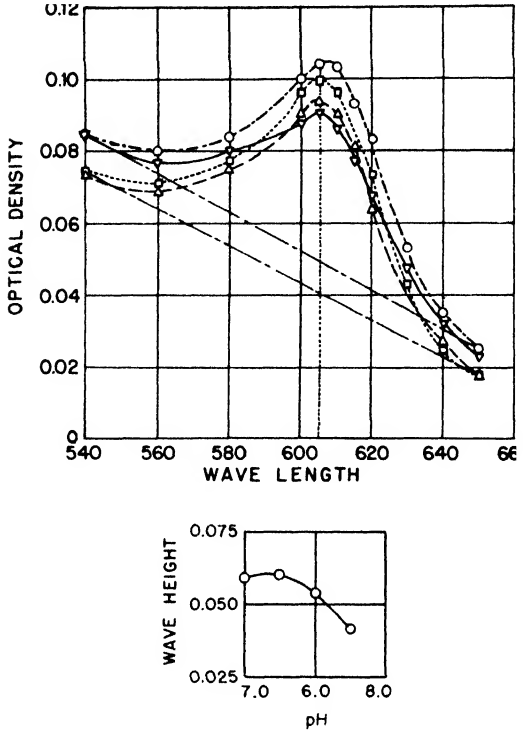


Fig. 13.59 — Above, effect of final pH on the absorption spectrum of fluoromethemoglobin. ○, pH 6.0, wave height 0.055; □, pH 6.5, wave height 0.058; △, pH 7.0, wave height 0.053; ▽, pH 7.5, wave height 0.041. Below, wave height vs. pH.

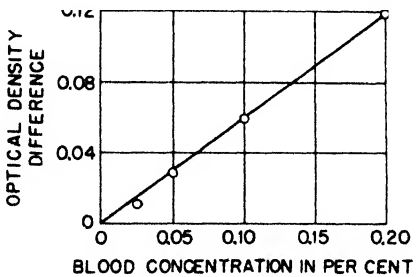


Fig. 13.60 — Calibration curve for fluoromethemoglobin determinations.

the hemoglobin was immediately determined spectrophotometrically as described above.

It was found that the recovery of known amounts of blood added to kidney brei was not quantitative, since in some cases it amounted to only 50 or 60 per cent of the added blood. In spite of this, the method was sufficiently good for the purpose of this work, as can be seen from the foregoing discussion.

(c) Kidney Catalase Levels in Normal Rats and Rabbits and in Rats and Rabbits Poisoned by Uranium Compounds. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Because of the establishment of catalasuria as a sensitive test for uranium poisoning, it became of interest to investigate the level of kidney catalase after repeated injections of relatively low doses of uranium compounds, in order to find out whether diminution in catalase level would gradually occur. It was also of interest to determine the effect on the kidney catalase level of single large doses of uranium compounds given by intravenous injection.

In all experiments kidney-tissue brei prepared in a ground-glass homogenizer was used, and the catalase activity was measured by means of the Warburg apparatus, using 2.0 ml of 0.04 hydrogen peroxide in 0.0067 phosphate buffer of pH 6.8. Figure 13.61 shows the relationship between the cubic millimeters of O_2 evolved and the time, over a 15-min interval, for two different dilutions of kidney brei.

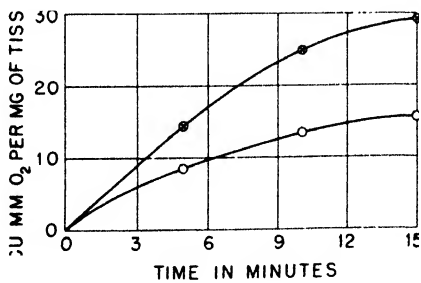


Fig. 13.61 — Catalase activity of normal rat-kidney brei at different concentrations. ○, kidney-brei dilution 1 : 200; ⊗, kidney-brei dilution 1 : 100.

In determining the catalase activity of a given sample of kidney brei, four different dilutions of the brei were made, namely, 1 : 20,000, 1 : 10,000, 1 : 7,200, and 1 : 5,000. Two-tenths milliliter of diluted brei was then used as in the determination of urinary catalase, in place of

0.2 ml of urine. The cubic millimeters of O_2 evolved were measured directly, and curves were drawn by plotting cubic millimeters of O_2 evolved at 15 min against milligrams of brei on a dry-weight basis. The slopes of these curves, two of which are shown in Fig. 13.62, are plotted as measures of enzyme activity in the vertical bar graphs that follow (Fig. 13.63 and 13.64). The slopes are expressed as cubic millimeters of O_2 evolved per milligram of tissue brei (on a dry-weight basis) in 15 min. The use of the four different dilutions of brei minimized experimental errors.

The rats were divided into five groups of animals, all of which, with the exception of Group 1, were treated by injecting uranyl acetate or uranyl nitrate intraperitoneally in various dosage schedules.

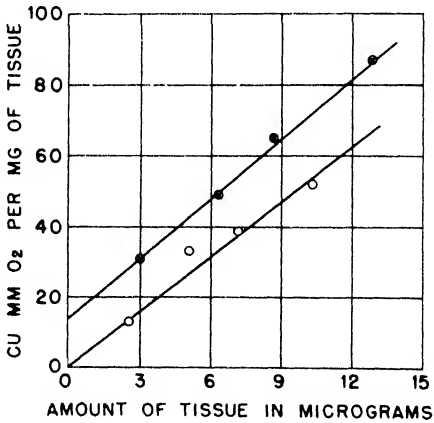


Fig. 13.62—Catalase activity of two dilutions of normal rat-kidney brei. Curve indicated by ○ has a slope of 5.17. Curve indicated by ⊗ has slope of 5.66.

Group 1 consisted of five normal noninjected animals.

Group 2 consisted of eight acutely poisoned rats. The animals in this group were injected intraperitoneally with 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per kilogram of body weight. Animals 3 and 4 were killed 3 days after injection. Animals 7 and 8 were killed 4 days after injection. Animals 10 and 11 were killed 5 days after injection. Animals 15 and 16 were killed 7 days after injection. All analyses were performed immediately after killing the animals.

Group 3 consisted of seven resistant rats. These rats had survived the intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal). Animals 1 and 2 were killed 12 days after injection;

animal 3 was killed 8 days after injection; animal 4 was killed 6 days after injection; animal 5 was killed 5 days after injection; animal 6 was killed 7 days after injection; and animal 7 was killed 10 days after injection.

Group 4 consisted of five chronically poisoned rats that had received injections of 0.27 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.15 mg of uranium metal) per kilogram every other day until 10 injections were given. The animals were killed 1 to 2 days after the last injection.

Group 5 consisted of nine tolerant rats, treated as follows: Animals 1, 2, and 3 were given 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 of uranium metal) per kilogram intraperitoneally every other day until 11 injections had been administered. Then, 2 days after the last injection, 6 mg of uranyl nitrate hexahydrate (≎ 2.9 mg of uranium metal) per kilogram of body weight was administered intraperitoneally. The animals were allowed to live for 3 weeks, and then animal 1 was sacrificed, and the kidney catalase was determined. Also one kidney each was removed from animals 2 and 3 and analyzed for catalase. One month later, animals 2 and 3 were sacrificed, and the remaining kidney in each one was analyzed for catalase.

Animals 4, 5, and 7 received 0.33 mg of $UO_2(NO_3)_2.6H_2O$ (≎ 0.16 mg of uranium metal) per kilogram of body weight every other day until 11 injections had been administered. Then, 2 days after the last injection, 6 mg of $UO_2(NO_3)_2.6H_2O$ (≎ 2.9 mg of uranium metal) per kilogram of body weight was injected. Three weeks later a second dose of 6 mg of $UO_2(NO_3)_2.6H_2O$ per kilogram was administered; and 3 weeks later a dose of 7 mg of $UO_2(NO_3)_2.6H_2O$ (≎ 3.3 mg of uranium metal) per kilogram was given. Then, after an interval of 3 weeks, the animals were sacrificed, and the catalase of the kidney was determined.

Animals 11 and 12 were treated according to the following schedule: 11 injections of 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 mg of uranium metal) per kilogram of body weight were given as in all the tolerant animals, spaced 1 day apart, so that the total period covered was 21 days. Larger doses that were injected at 3-week intervals were as follows: 5, 6, 7, and 5 mg of uranyl nitrate hexahydrate (≎ respectively 2.4, 2.9, 3.3, and 2.4 mg of uranium metal) per kilogram. Animal 11, a female, was killed, and the kidneys analyzed for catalase 3 weeks after the last injection; animal 12, a male, was sacrificed and the kidneys analyzed 8 weeks after the last injection.

All rabbits were injected intravenously with uranyl acetate.

Group 1 consisted of three normal animals.

Group 2 consisted of five acutely poisoned animals. Animals 2, 4, and 5 received 1 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.56 mg of uranium metal) per kilogram of body weight. Animals 1 and 3 received 5 mg of

$UO_2(Ac)_2.2H_2O$ (≎ 2.6 mg of uranium metal) per kilogram of body weight.

Animal 1 was killed 9 days after injection; animal 2 was killed 5 days after injection; animal 3 was killed 9 days after injection; animal 4 was killed 7 days after injection; and animal 5 was killed 4 days after injection.

Group 3 consisted of two "recovered" rabbits that had recovered from the highly toxic dose for rabbits of 0.5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.28 mg of uranium metal) per kilogram of body weight. The animals were killed, and the kidneys analyzed for catalase 8 to 9 days after injection.

Group 4 consisted of four "resistant" rabbits that had survived two doses of 0.5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.28 mg of uranium metal) per kilogram of body weight, given approximately 2 weeks apart.

Animal 1 was killed for analysis of kidneys 10 days after last injection; animal 2 was killed for analysis of kidneys 8 days after last injection; animal 3 was killed for analysis of kidneys 9 days after last injection; and animal 4 was killed for analysis of kidneys 2 days after last injection.

Group 5 consisted of six chronically poisoned animals.

Animal 4 was given daily injections of 0.01 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.0056 mg of uranium metal) per kilogram of body weight for 90 days. The kidneys were analyzed on the ninetieth day.

Animal 6 was given injections of 0.02 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.11 mg of uranium metal) per kilogram of body weight for 64 days at approximately weekly intervals. The kidneys were analyzed on the sixty-fourth day, about 1 week after the last injection.

Animal 1 was given injections of 0.02 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.11 mg of uranium metal) per kilogram of body weight for 70 days at approximately weekly intervals. The kidneys were analyzed on the seventieth day, about 1 week after the last injection.

Animal 2 was given injections of 0.1 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.056 mg of uranium metal) per kilogram of body weight at approximately weekly intervals. The kidneys were analyzed about 1 week after the last injection.

Animal 3 received 0.05 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.028 mg of uranium metal) per kilogram of body weight approximately once a week over a period of 85 days. The animal was sacrificed, and the kidneys analyzed for catalase 2 days after the last injection.

Animal 5 received 0.01 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.0056 mg of uranium metal) in seven injections at approximately weekly intervals over a period of 55 days. The kidneys were analyzed for catalase 6 days after the last injection.

All the results are summarized in the vertical bar graphs in Figs. 13.63 and 13.64. All the above-listed rats and rabbits were used in the work to be described subsequently on kidney alkaline phosphatase, with the exception of two normal rats. The analyses for kidney catalase and phosphatase were carried out at the same time. Since the groups and numbers within each group assigned to the animals are the same in both experiments, a comparison of the catalase and phosphatase values on a given animal can be made by referring to the bar

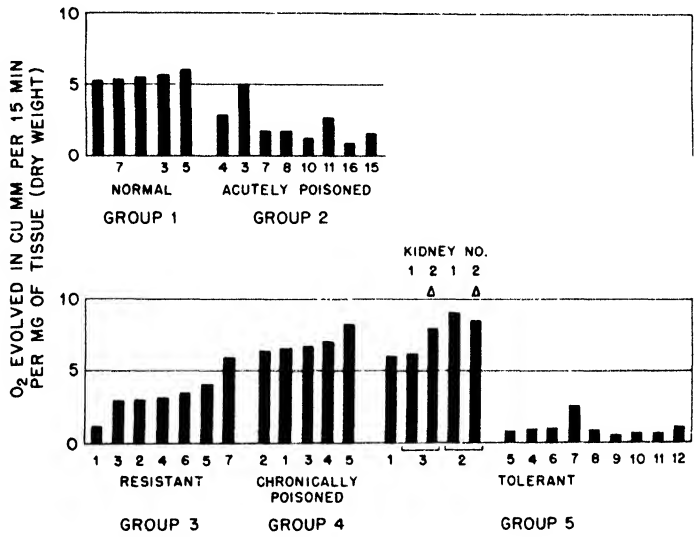


Fig. 13.63 — Rat-kidney catalase after treatment of animals with uranyl nitrate or uranyl acetate. Δ, kidney removed 1 month later.

graphs in the phosphatase experiments (Figs. 13.69, 13.70, and 13.71). Such a comparison, however, does not seem to yield information of any particular significance, except that in general the decrease in the activity of one enzyme is accompanied by a decrease in activity of the other enzyme. The decrease in the activity of kidney alkaline phosphatase seems to be more severe after treatment of the animal with U_6 than is the decrease of the activity of kidney catalase.

In the case of rats, the acutely poisoned animals in Group 2 show a definite decrease in catalase activity. The resistant rats in Group 3 show some drop in catalase activity. The chronically poisoned rats in Group 4 show, if anything, a slight increase in kidney catalase.

The tolerant rats in Group 5 show a very sharp drop in kidney catalase with the exception of animals 1, 2, and 3.

In the case of the rabbits, the only animals that show a clearly significant drop in kidney catalase are the acutely poisoned animals.

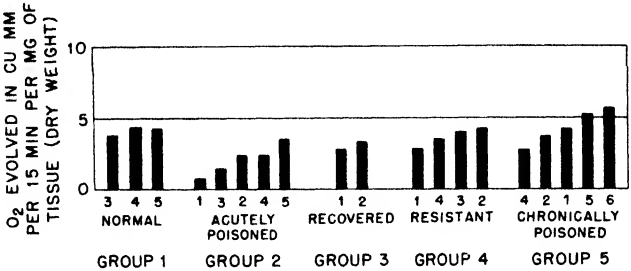


Fig. 13.64 — Rabbit-kidney catalase after treatment of animals with uranyl acetate.

Since tolerant rats show a sharp drop in both kidney catalase and kidney phosphatase, it is fairly safe to conclude that in rats, at least, tolerant animals do not have normal kidneys from the standpoint of these enzyme activities.

The drop in kidney catalase after injections of moderate amounts of uranium in repeated doses does not seem to be sufficient to explain a tendency for the urinary catalase test to decrease on repeated injections of uranium.

(d) Choline Oxidase in the Liver and Kidney of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan.) Ten rats were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 2.8 mg of uranium metal) per kilogram of body weight. One rat was killed by decapitation on the first day after being injected, another on the second day, another on the third day, another on the fourth day, and the last animal on the sixth day after injection. The remaining five animals died at various times following injection and were not studied. The first five rats were used also for studying *d*-amino acid oxidase (see under *d*-amino acid oxidase). Rats that showed definite toxic symptoms were killed first. The rat to be killed at a given time was fasted overnight, and the tissue brei of kidney and liver were prepared by grinding in a glass homogenizer with 0.9 per cent saline. The choline oxidase activity was determined by the method of Mann and Quastel as previously described in the section on choline oxidase in vitro. The oxygen consumption caused by the oxidation of choline was expressed as cubic millimeters of O_2 consumed per milligram

of tissue on a dry-weight basis. It was found that the choline oxidase activity in the kidney of rats poisoned by uranyl acetate decreased in proportion to the length of time elapsing after injection before the animal was killed. However, choline oxidase in the liver of rats poisoned by uranyl acetate increased significantly after poisoning, beginning with the third day after injection. This increase in choline oxidase activity might be due to the presence of excess substrate accumulating in the liver either as a secondary effect of kidney damage, or as a result of starvation.

The results of the experiments are shown in Fig. 13.65. The oxygen consumption caused by the oxidation of choline in the first 30 min has been used as a measure of enzyme activity and is represented on the vertical axis.

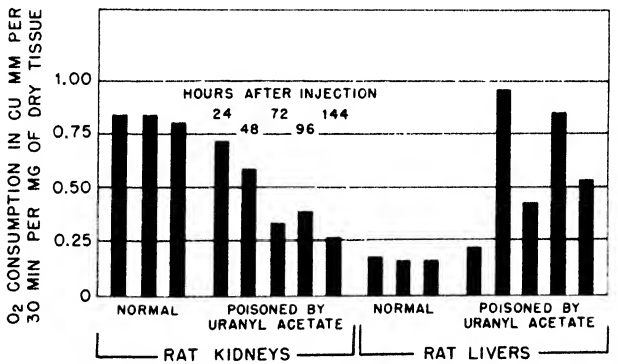


Fig. 13.65 — Choline oxidase activity in the kidneys and livers of normal rats and of rats poisoned by uranyl acetate.

It is known that animals poisoned by uranium do not eat well, and it is possible that this factor could contribute materially to an increase in the substrate for choline oxidase in the liver, since starvation is known to produce fatty livers.

In order to investigate the latter possibility, eight rats were deprived of food but were allowed free access to water. The animals were killed one at a time every other day up to 17 days after the starvation period had been started. Liver and kidney choline oxidase was determined in each animal by the method just described.

It was found that the average value for choline oxidase in the livers of these animals was ten times the normal value, a finding similar to that reported for uranium-poisoned rats. The kidney choline oxidase did not change significantly.

Thus the effect of uranium poisoning on the choline oxidase of rat liver is similar to the effect of starvation.

It was found that the choline oxidase of the kidneys of rats made tolerant to uranyl nitrate by repeated intraperitoneal injections was very low. However, the choline oxidase of the livers of these rats was normal (see *d*-amino acid oxidase in vivo, this section), and this finding can be ascribed to the fact that the tolerant rats ate normally. This is a further indication that the greatly increased choline oxidase activities of the livers of rats acutely poisoned by U_6 is a secondary effect that may be largely attributable to the low food intake of these animals.

(e) Glucose Oxidase in the Kidney and Liver of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan and Betty Robinson.) Ten rats were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per kilogram of body weight. The animals were killed by decapitation, and tissue brei of kidney and liver was prepared by grinding the tissue in 0.1M phosphate buffer of pH 7.3 (1 g of tissue in 3 ml of buffer). On the third day after injection some of the animals began to show toxic symptoms. One animal was killed each day from the third day to the seventh day after injection. Animals that showed the most serious symptoms were killed first, and the animal to be killed at a given time was previously fasted overnight. Five normal rats were killed for control determinations of glucose oxidase activity.

The activity of the glucose oxidase was determined in the Warburg apparatus by measuring the oxygen consumption of the tissue brei in the presence of glucose. The activity was expressed in cubic millimeters of O_2 per milligram of tissue, on a dry-weight basis. Two milligrams of glucose dissolved in 0.2 ml of water were put in the side arm of the Warburg flask, and 1 ml of tissue brei and 0.8 ml of 0.1M phosphate buffer of pH 7.3 were put in the main compartment. In the middle well was put 0.05 ml of 3N KOH. The total volume of the solutions was 2.05 ml. In the control flasks, 0.2 ml of phosphate buffer was added instead of glucose in order to make the total volume of the solutions 2.05 ml. The dry weight of the tissue used was about 50 to 70 mg. The oxygen consumption was recorded every 15 min for the first hour after equilibration and mixing. Then another reading was taken 30 min later so that the total time during which readings were recorded was 90 min.

The results of the experiments showed that glucose was not oxidized significantly in either the normal or damaged kidney. Glucose, however, was oxidized in normal liver, and its rate of oxidation was decreased in the livers of animals poisoned by uranyl acetate as shown

in Fig. 13.66. This may indicate that the liver is affected after the animal has been poisoned by uranyl acetate, but this effect may be secondary to the kidney damage by U_6.

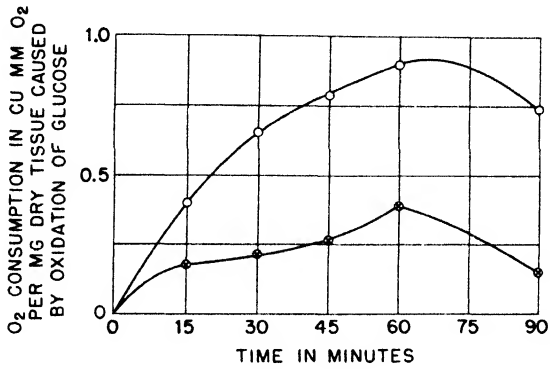


Fig. 13.66 — Oxidation of glucose by liver brei of normal rats and of rats poisoned by uranyl acetate. ○, liver brei of normal rats (average of five rats); ⊘, liver brei of rats poisoned by uranyl acetate (average of six rats).

(f) Pepsin Activity of Animals Poisoned by Injection and Ingestion of Uranyl Acetate. (Work done by A. L. Dounce with the assistance of Daphne Rothermel.) It has been found that neither injection nor ingestion of moderate amounts of uranyl acetate has any effect on gastric pepsin activity, nor for that matter on gastric pH unless the animals have been so severely poisoned that they are near death.

The in vivo results following injection of uranyl acetate probably are to be explained by the inability of U_6 to get at the cells of the gastric mucosa at a pH where it can do damage. It will be recalled that the concentration of U_6 in plasma falls very rapidly after injection of U_6. Furthermore the transition in pH between the approximately neutral condition inside and the very acid condition outside the cell membrane of HCl-secreting cells must be very sharp.

To rule out the possibility that the negative results with feeding experiments were due to a combining of uranyl acetate with the food, some experiments were done to determine the effect of introducing uranyl acetate into an empty stomach, in some instances with the pylorus tied off.

The experiments were carried out as follows: In order to obtain a sufficient amount of gastric contents, 2 ml of a 7 per cent solution of ethyl alcohol was introduced by stomach tube into young female rats weighing from 50 to 200 g. After 10 min, the tube (a soft-rubber

urethral catheter No. 8) was reintroduced, and as much of the stomach contents as could be recovered were removed at this time by means of a syringe. The pH of the contents was determined with the glass electrode. Pepsin was determined by the method of Anson,[43] except that crystalline beef hemoglobin was used for substrate instead of carboxyhemoglobin prepared by Anson's method, and 0.2 ml of gastric contents was used in place of 1.0 ml of enzyme solution.

In one group of experiments, six animals were poisoned by intraperitoneal injection of 0.5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per 100 g of rat weight in a 0.1 per cent solution. Six unpoisoned animals were studied at the same time for a control group.

All the poisoned animals died eventually. All food was removed from the cages at night so that when gastric contents were sampled the stomachs would be free of solid material. No significant changes occurred in the pH or in the pepsin activity of the gastric contents of any of the animals for 4 days after injection of the uranium. The animals died on the fifth and sixth days and showed slightly high pH values of the stomach and somewhat lowered pepsin values just before death.

In another group of experiments the animals were poisoned by feeding 1 part of $UO_2(Ac)_2.2H_2O$ by weight (≎ 0.56 part of uranium metal by weight) in 4 parts of fox chow, ad lib. Six animals were in the poisoned group, and six in the control group.

The poisoned animals all died on the seventh to ninth day after the start of the experiment. No significant changes in pH or pepsin activity were noted for 6 days after the poisoning, although the odor of the gastric contents became abnormal on the fourth day and was very foul on the sixth and seventh days. On the seventh day some of the animals showed an increase in pH and a lowering of peptic activity of the gastric contents. The foul odor may be ascribed to some change lower in the intestinal tract than the stomach. In addition, it appeared that the stomach was not emptying properly after the third or fourth day.

In order to find out whether U_6 was being retained or absorbed from an empty stomach, the duodenums were tied off at the pylorus in a group of three animals under ether. Then the animals were allowed to remain for an hour to recover from the effects of the ether, after being sewed up. Finally 2.0 ml of 0.1 per cent $UO_2(Ac)_2.2H_2O$ (≎ 1.1 mg of uranium metal) was introduced by stomach tube and allowed to remain there for 3 hr. Then the animals were killed, the esophagus was tied at the cardia, the stomach was removed, and the stomach contents were squeezed out into a measured centrifuge tube and clarified by centrifugation. The supernatant was analyzed for U_6 by the ferrocyanide method and showed losses, respectively, of 55 per cent,

63 per cent, and 77 per cent of the total of U_6 injected. There was very little solid matter centrifuged out, since the animals had been fasted overnight before the experiment. Thus uranium disappeared, but it was not determined whether it was absorbed into the circulation or whether it simply combined with the stomach mucosa.

It should be mentioned that after injection of the 2.0 ml of uranyl acetate solution by stomach tube the stomachs became greatly distended owing to an accumulation of water, so that in one animal 9.0 ml of fluid was removed from the stomach.

An experiment was conducted to show whether the inflow of water into the stomach after introduction of the uranyl acetate was caused by an irritating effect of the U_6 or by the ligation of the duodenum at the pylorus. It was found that the latter cause was operating, since in three control rats given 2.0 ml of water in place of the uranium the same increase in volume was observed.

(g) Alkaline Phosphatase in Kidneys of Animals Poisoned with Uranyl Acetate. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) An extensive study of the alkaline phosphatase activity of kidney tissue of animals poisoned by uranyl acetate and uranyl nitrate has been carried out. The method for determining phosphatase activity was an adaptation of kidney-tissue brei of a method worked out in the project laboratory for urinary phosphatase. Calcium phenolphthalein phosphate was used as substrate. Subsequent to the development of this method, a paper by Huggins[44] appeared in which sodium phenolphthalein phosphate was used as substrate in a similar method for phosphatase. Prior to the work of Huggins, sodium phenolphthalein phosphate had been used in this laboratory as substrate, but later its use was abandoned, since better results were obtained by using a saturated solution of the calcium salt in the presence of excess undissolved calcium salt. The method will now be described in detail. It is based upon a method worked out by O'Connell, Rothermel, and Dounce for determining urinary phosphatase.

Five milliliters of 2.3 per cent sodium veronal (1.3M) and 0.1 ml of homogenized tissue of the desired dilution were allowed to stand at 37° in a 25-ml Erlenmeyer flask for 15 min to establish temperature equilibrium. Then 0.5 ml of an aqueous suspension of the calcium salt of phenolphthalein phosphate containing 4 per cent of this salt was added, and the mixture was allowed to stand for 15 min with occasional stirring (the results might be improved if a slowly moving mechanical rocking device were used). At the end of 15 min the mixture was quickly placed in boiling water and allowed to remain there for 5 min to destroy the enzyme and stop the reaction. The solution was then filtered into a colorimeter tube graduated at 10 ml. The

filter paper was washed with distilled water until the volume of the original filtrate plus washings totaled 10 ml. Then 0.1 ml of 1M NaOH was added to develop maximal color, and the color was immediately read in the colorimeter, using a green filter.

It was determined at the outset of this work that the relationship between color developed and time, using a given enzyme concentration is approximately linear. This is shown in Fig. 13.67, which illustrates the results at two different enzyme concentrations. Once this point had been established, it was decided to use the colorimeter reading corresponding to a reaction time of 15 min to measure enzyme activity in all cases. It is true that the results obtained by this method are not of a highly precise nature, but the precision can be improved, as will be described shortly, to such an extent that it is possible to measure with sufficient accuracy the biological variations in phosphatase activity that were encountered.

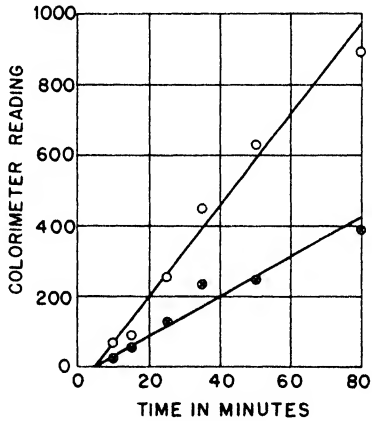


Fig. 13.67—Time curve of alkaline phosphatase activity of normal rabbit kidney at different concentrations. ○, kidney brei diluted 1 : 10 with water; ⊗, kidney brei diluted 1 :20 with water.

The colorimeter reading corresponding to a reaction time of 15 min was next plotted against the milligrams of kidney tissue employed in the test, on a dry-weight basis. The results of four such experiments at different levels of enzyme activity are shown in Fig. 13.68. It can be seen that a linear relationship holds approximately up to 6 mg of tissue; above this amount of tissue increase in colorimeter reading falls off rapidly with increase in amount of kidney brei used, possibly because of an inhibition of enzyme activity caused by free inorganic

phosphate present in the tissue, or because of absorption of the phenolphthalein by the tissue.

In determining the enzyme by this method it seemed necessary to improve the precision of the results obtained from a single determination by making several determinations on each sample of brei used. There are three obvious ways in which this could be done. A given concentration of enzyme might be used, and the determination might then be repeated several times, using the colorimeter reading at 15 min as the measure of activity. Or the curve for colorimeter reading against time might be plotted, using a single enzyme concentration with several colorimetric determinations corresponding to a set of different time values. Finally, different dilutions of kidney brei up to 6 mg per 0.1 ml might be used, and the colorimeter reading corresponding to a reaction time of 15 min then could be plotted against milligrams of tissue employed on a dry-weight basis. The slope of the curves obtained by the second or third methods would be used to determine the amount of enzyme present.

The last-mentioned method was chosen, and the slope of the curve

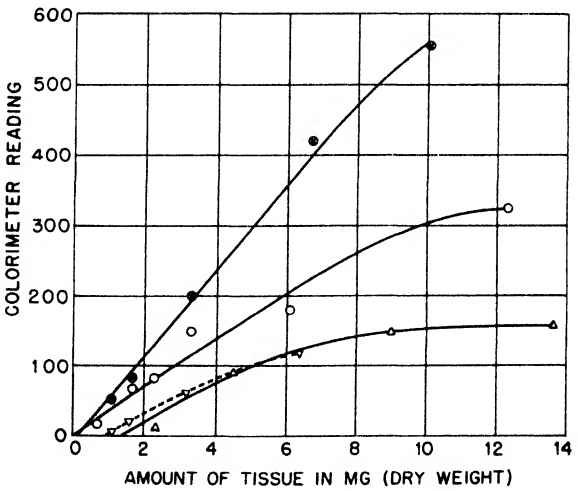


Fig. 13.68—Alkaline phosphatase activity of rat kidney in normal, poisoned, and recovered animals. ⊗, normal; ○, acute poisoning; △, chronic poisoning; ▽, recovered.

gives a measure of enzyme concentration expressed in terms of colorimeter reading per milligram of tissue employed on a dry-weight basis. To convert to King-Armstrong units of alkaline phosphatase[45]

per milligram of tissue, the colorimeter reading corresponding to a reaction time of 15 min should be multiplied by a factor of approximately 0.08.

All rats were injected intraperitoneally and were classified into different groups as described below according to the mode of poisoning.

The exact treatment of the animals in each group is summarized as follows:

Treatment of Rats. Group 1. This group consisted of eight normal rats untreated in any manner.

Group 2. In this group were 16 acutely poisoned rats, injected intraperitoneally with 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per kilogram of body weight. Animals 1, 2, 3, and 4 were killed 3 days after injection. Animals 5, 6, 7, and 8 were killed 4 days after injection. Animals 9, 10, 11, and 12 were killed 5 days after injection. Animals 13, 14, 15, and 16 were killed 7 days after injection.

Group 3. In this group were seven resistant rats that had survived 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per kilogram of body weight, injected intraperitoneally. The kidneys were tested 5 to 12 days after injection. Animals 1 and 2 were killed 12 days after injection; animal 3 was killed 8 days after injection; animal 4 was killed 6 days after injection; animal 5 was killed 5 days after injection; animal 6 was killed 7 days after injection; animal 7 was killed 10 days after injection.

Group 4. In this group were five chronically poisoned rats that had received injections of 0.27 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.15 mg of uranium metal) per kilogram every other day until ten injections had been given. The animals were killed 1 to 2 days after the last injection.

Group 5. In this group were 12 tolerant rats, treated as follows:

Animals 1, 2, and 3 were given 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 mg of uranium metal) per kilogram intraperitoneally every other day until 11 injections had been administered. Then, 2 days after the last injection, 6 mg of uranyl nitrate hexahydrate (≎ 2.9 mg of uranium metal) per kilogram of body weight was administered intraperitoneally. The animals were allowed to remain alive for 3 weeks, and then animal 1 was sacrificed and the kidney alkaline phosphatase was determined. Also one kidney was removed from animals 2 and 3 and analyzed for alkaline phosphatase. One month later animals 2 and 3 were sacrificed, and the remaining kidney in each one was analyzed for alkaline phosphatase.

Animals 4, 5, 6, and 7 were given 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 mg of uranium metal) per kilogram of body weight every other day until 11 injections were administered. Then, 2 days

after the last injection, 6 mg of uranyl nitrate hexahydrate (≎ 2.9 mg of uranium metal) per kilogram was injected; 3 weeks later a second dose of 6 mg of uranyl nitrate hexahydrate per kilogram was administered; and 3 weeks later a dose of 7 mg of uranyl nitrate hexahydrate (≎ 3.4 mg of uranium metal) per kilogram was given. Then after an interval of 3 weeks the animals were sacrificed and the alkaline phosphatase of kidneys was determined.

Animals 8, 9, and 10 were given 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 mg of uranium metal) per kilogram of body weight every other day until 11 injections had been administered; then, starting 2 days later at intervals of 3 weeks, the following doses were given in the order recorded: 5 mg of uranyl nitrate hexahydrate (≎ 2.4 mg of uranium metal) per kilogram; 6 mg of uranyl nitrate hexahydrate (≎ 2.9 mg of uranium metal) per kilogram; 7 mg of uranyl nitrate hexahydrate (≎ 3.4 mg of uranium metal) per kilogram; and 8 mg of uranyl nitrate hexahydrate (≎ 3.9 mg of uranium metal) per kilogram. After a period of 3 weeks following the last injection the animals were sacrificed, and the kidney alkaline phosphatase was determined.

Animals 11 and 12 were treated according to the following schedule: 11 injections of 0.33 mg of uranyl nitrate hexahydrate (≎ 0.16 mg of uranium metal) per kilogram were given as in all tolerant animals, spaced 1 day apart, so that the total period covered was 21 days. The larger doses, which were injected at 3-week intervals, were as follows: 5, 6, 7, and 5 mg of uranyl nitrate hexahydrate (respectively, 2.4, 2.9, 3.4, and 2.4 mg of uranium metal) per kilogram. Animal 11, a female, was sacrificed, and the kidneys analyzed for alkaline phosphatase 3 weeks after the last injection.

The rabbits were all injected intravenously. The exact treatment of the animals is summarized as follows:

Treatment of Rabbits. Group 1. In this group were five normal animals, not injected.

Group 2. In this group were five rabbits acutely poisoned by intravenous injection of uranyl acetate. Animals 2, 4, and 5 received 1 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.56 mg of uranium metal) per kilogram of body weight intravenously. Animals 1 and 3 received 5 mg of uranyl acetate (≎ 2.8 mg of uranium metal) per kilogram of body weight.

Animal 1 was killed 9 days after injection; animal 2 was killed 5 days after injection; animal 3 was killed 9 days after injection; animal 4 was killed 7 days after injection; and animal 5 was killed 4 days after injection.

Group 3. This group consisted of two rabbits that had recovered from the intravenous injection of 0.5 mg of uranyl acetate (≎ 0.28 mg

of uranium metal) per kilogram of body weight. Kidneys were analyzed 8 to 9 days after injection.

Group 4. In this group were four resistant rabbits that survived two intravenously injected doses of 0.5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.28 mg of uranium metal) per kilogram of body weight, given approximately 2 weeks apart.

Animal 1, the kidneys were analyzed 10 days after last injection; animal 2, the kidneys were analyzed 8 days after last injection; animal 3, the kidneys were analyzed 9 days after last injection; animal 4, the kidneys were analyzed 2 days after last injection.

Group 5. In this group were six chronically poisoned animals. Animal 4 was poisoned by daily injections of 0.01 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.0056 mg of uranium metal) per kilogram for 90 days. The kidneys were analyzed on the ninetieth day.

Animal 6 was poisoned by injections of 0.02 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.11 mg of uranium metal) per kilogram of body weight for 64 days at approximately weekly intervals. The kidneys were analyzed on the sixty-fourth day, about 1 week after the last injection.

Animal 1 was poisoned by injections of 0.02 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.11 mg of uranium metal) per kilogram of body weight for 70 days at approximately weekly intervals. The kidneys were analyzed on the seventieth day, about 1 week after the last injection.

Animal 2 was poisoned by injections of 0.1 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.056 mg of uranium metal) per kilogram of body weight at approximately weekly intervals for four injections. The kidneys were analyzed about 1 week after the last injection.

Animal 3 received 0.05 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.028 mg of uranium metal) per kilogram of body weight injected intravenously approximately once a week over a period of 85 days. The animal was sacrificed, and the kidney alkaline phosphatase was determined 2 days after the last injection.

Animal 5 received 0.01 mg of $UO_2(Ac)_2.2H_2O$ (≎ 0.0056 mg of uranium metal) injected intravenously seven times at approximately weekly intervals over a period of 55 days. The animal was sacrificed, and the kidney alkaline phosphatase was determined 6 days after the last injection.

The results of the experiments are as follows:

All the analytical results obtained with the rats are plotted in Figs. 13.69 and 13.70, which show at a glance that there is a tendency for the average kidney alkaline phosphatase to be low in all the animals that had received injections of U_6. This tendency seems to be least marked in the "chronically poisoned" group of animals, probably because the doses were low. However, in the group of tolerant rats

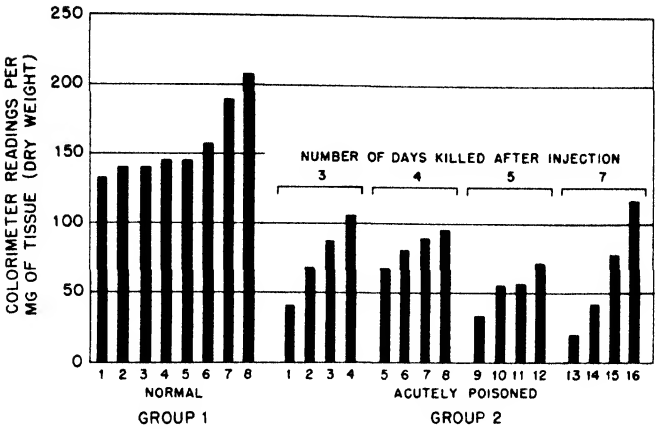


Fig. 13.69—Rat-kidney phosphatase levels after treatment of animals with uranyl acetate.

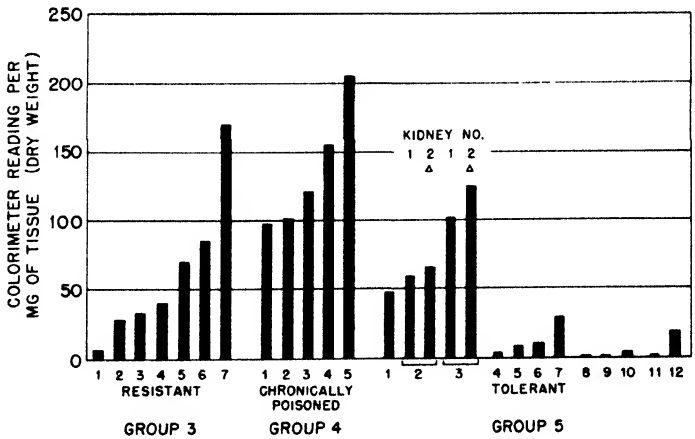


Fig. 13.70—Rat-kidney phosphatase levels after treatment of animals with uranyl acetate or uranyl nitrate. Δ, kidney removed 1 month later.

some of the results are remarkable, in that the kidney phosphatase is reduced practically to zero in certain of the animals.

In general, it can be stated that in order to reduce the kidney phosphatase to very low values in rats it is necessary to give large doses of U_6 repeatedly.

In two animals of the chronically poisoned group of rats, the alkaline phosphatase was determined in one kidney after uninephrectomy, and then 1 month later the enzyme was determined in the remaining kidney. In both cases the remaining kidney showed a slightly higher alkaline phosphatase activity per milligram on a dry-weight basis than did the kidney removed at operation (Fig. 13.70).

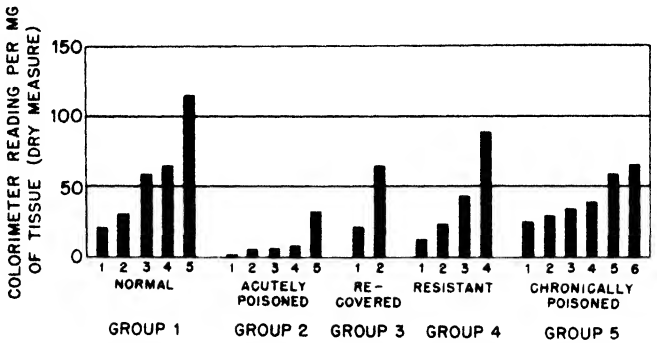


Fig. 13.71 — Rabbit-kidney phosphatase after treatment of animals with uranyl acetate or uranyl nitrate.

In the animals with the lowest kidney alkaline phosphatase values, repair presumably had taken place after the last injection of U_6, since an interval of 3 weeks was allowed to elapse before testing for alkaline phosphatase in the kidneys.

All the analytical results obtained with rabbits are plotted in Fig. 13.71. There is, as in the case of rats, a certain tendency for the alkaline phosphatase of the kidneys of animals injected with uranium to be low, but this tendency is not so marked as in the case of rats. In the acutely poisoned animals the kidney alkaline phosphatase was, however, very low in certain cases, and the average value for the acutely poisoned group was only about one-quarter of the average for the normal animals.

Summary and Conclusions. The injection of U_6 as the nitrate or acetate tends to diminish the kidney alkaline phosphatase in rats and to a lesser extent in rabbits. This diminution appeared to depend upon the amount of U_6 injected, in the case of rats. In the group of tolerant rats that had received heavy doses of U_6, the kidney alkaline phosphatase activity was almost zero. The concentration of the enzyme had a

tendency to increase toward normal after the rats for some time had received no further injections of U_6. The lowering of kidney phosphatase in rats is apparently not dependent upon the presence of "active" damage to the tubular cells, since the values were low 3 weeks after the last injection of U_6. Thus the effect of the poison is apparently not a direct effect of inhibition of the enzyme but is rather an indirect effect. The regenerated cells appear to contain less phosphatase than the original tubular cells.

(h) Phosphoglucomutase in the Liver and Kidney of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Phosphoglucomutase activity in the liver and kidney-tissue brei of rats poisoned by uranyl acetate was determined by the method of Colowick and Sutherland.[31]

Ten rats were poisoned by intraperitoneal injection of 5 mg of uranyl acetate ($\simeq$ 2.8 mg of uranium metal) per kilogram of body weight. On the third day some of the rats showed symptoms of poisoning, and one animal was killed for determination of the enzyme on this day and one or two on each day for the following 4 days. The animals were killed by decapitation, and the tissue brei was prepared in a glass homogenizer in 0.1M veronal buffer of pH 7.5, using 1 g of wet tissue to 3 ml of buffer.

In carrying out the determination of enzyme activity of the tissue brei, test tubes were set up containing 4 ml of Cori ester (0.4 per cent solution in 0.1M veronal buffer of pH 7.5), 1 ml of 0.1M veronal buffer of pH 7.5, 4 drops of 3 per cent $MnCl_2$ solution, 1,000 μg of NaF, and 1 ml of kidney or liver brei. The reaction tubes were incubated at 25°C, and the determination of acid-stable phosphate was carried out in 1-ml aliquots at 15-min intervals for a period of 45 min. Each sample was hydrolyzed with 1N HCl in a boiling-water bath for 7 min, and the protein was precipitated with 0.5 ml of 10 per cent trichloracetic acid. The protein precipitate was washed with a small amount of water. The filtrate and washings were combined, and the free phosphate was determined by the Fiske and Subbarow[15] method. A blank was run by determining the amount of free inorganic phosphate present after hydrolysis from the liver or kidney-tissue brei without Cori ester substrate, and this blank was subtracted from the amount of acid-labile phosphate present after incubation with Cori ester substrate present. The activity of phosphoglucomutase was expressed as the percentage of acid-stable phosphate formed per milligram of dried tissue.

It was found that there was no significant change in the activity of phosphoglucomutase in either the liver or the kidney of rats poisoned by uranyl acetate as compared with the liver and kidney of normal rats. In this experiment three normal rats of about the same size

were used for control determinations. The results of the experiment are shown in Table 13.20.

Table 13.20

Animals used	Phosphoglucomutase activity as %of acid-stable P	
	Kidney	Liver
Normal rats (3) rats	1.63	1.23
Poisoned rats (5) rats	1.10	1.08

(i) Phosphorylase in the Tissue Brei of Kidney, Liver, and Muscle of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan with the assistance of Marian Kaley.) Before attempting to measure phosphorylase activity in tissues, account must be taken of possible interfering enzymes. It has been shown that alkaline phosphatase does not act on Cori ester at pH 6.8. Enzymes that may interfere with the determination of phosphorylase activity are acid phosphatase and phosphoglucomutase. It has been shown that NaF can inhibit the activity of acid phosphatase, and that chloroform can inhibit the activity of phosphoglucomutase, whereas NaF and chloroform added together do not inhibit phosphorylase significantly. This was confirmed by adding NaF and chloroform to pure phosphorylase. In order to rule out the effects of acid phosphatase and phosphoglucomutase in the tissue brei, 1,000 μg of NaF and 0.2 ml of chloroform were added to the tissue brei-Cori ester mixture before attempting to determine phosphorylase activity.

Ten rats, five male and five female, were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ per kilogram of body weight. An animal was killed by decapitation at 24, 48, 72, 96, and 120 hr after injection. Two rats that lived longer than 10 days after injection were treated separately as surviving animals. Usually 80 per cent of the rats given 5 mg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 2.8 mg of uranium metal) per kilogram of body weight will die on the fifth or sixth day after injection. In this experiment, animals that showed definite toxic symptoms were killed first. The animals that died were discarded. In the control group four female and four male rats were used.

Tissue brei was prepared in 0.1M maleate buffer of pH 6.8 in a glass homogenizer by grinding 3 g of tissue (wet weight) with 10 ml of buffer. In each experiment 0.5 ml of the brei was added to 5 ml of Cori ester-glycogen mixture. The latter mixture has been described under the section on phosphorylase in vitro. To this mixture

0.1 ml of chloroform and 1,000 μg of NaF dissolved in 0.5 ml of water were added. The mixture was incubated at 25°C, and the free phosphate in 1-ml samples was determined at 15-min intervals for a period of 45 min. To determine phosphate, 1 ml of sample was treated with 1 ml of 10 per cent solution of trichloroacetic acid to precipitate the protein, and the precipitate was washed with a small amount of water. The filtrate and the washings were transferred to a 25-ml volumetric flask, and the free phosphate was determined by the method of Fiske and Subbarow.[15] The activity of phosphorylase was expressed as percentage conversion of Cori ester to free phosphate per milligram of dried tissue. Blank determinations on brei without substrate were carried out under the same conditions.

It was found that phosphorylase activity in the liver, kidney, and muscle of rats poisoned by uranyl acetate did not show any significant change as compared with the enzyme activity in these tissues of normal rats. It was also found that the liver and kidneys of female rats showed less phosphorylase activity than those of male rats.

The entire work reported above was repeated once, yielding the same results. Thus phosphorylase does not appear to be affected significantly in vivo, although this enzyme is sensitive to U_6 in vitro.

(j) Respiration without Substrate of Kidney and Liver-tissue Brei of Rats Poisoned by Uranyl Acetate. (Work done by Tien Ho Lan with the assistance of Betty Robinson.) Ten rats were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ ($\simeq$ 2.8 mg of uranium metal) per kilogram of body weight. The rats were killed on successive days over a period of 6 days. Animals that showed serious symptoms of poisoning were killed first. On the sixth and seventh days, three rats died and were discarded. The respiration of tissue brei of livers and kidneys of these rats was measured in the Warburg apparatus as already described in Sec. 4.21. In this experiment, 0.1M phosphate-Ringer buffer of pH 7.0 was used. It was found that the QO_2 (Sec. 4.2 of this chapter) of tissue brei of liver and kidney of rats poisoned by uranyl acetate increased slightly on the second day after the injection of uranyl acetate and gradually decreased to a less than normal value on the fourth and fifth day in the kidney. In the liver it decreased rapidly after the fourth day (Fig. 13.72).

(k) Tissue-slice Experiments on the Ascorbic Acid Oxidase System of Scorbutic Guinea Pigs Poisoned by Injection of U_6. The project laboratory has shown that scorbutic animals have less resistance to U_6 poisoning than normal animals (see Chap. 15). In order to find out whether or not animals poisoned by uranyl acetate have an impaired capacity to oxidize ascorbic acid, four normal and eight scorbutic guinea pigs were used in the following experiment:

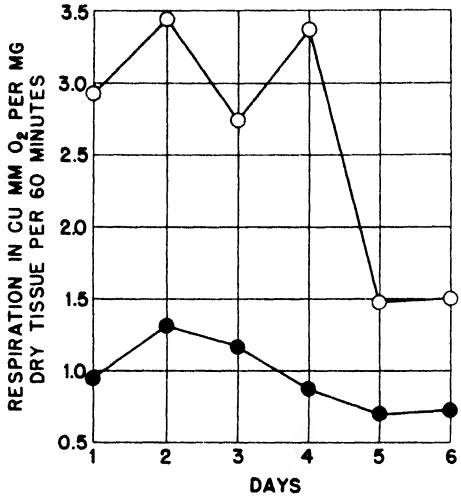


Fig. 13.72—Respiration of liver- and kidney-tissue brei of rats poisoned by uranyl acetate in 0.1M phosphate-Ringer buffer at pH 7.0. ○, liver-tissue brei; ●, kidney-tissue brei.

The scorbutic animals were prepared by putting the animals on a scorbutic diet for 3 weeks. At the beginning of the third week, four of these animals were poisoned by intraperitoneal injection of 5 mg of $UO_2(Ac)_2.2H_2O$ (≎ 2.8 mg of uranium metal) per kilogram body weight. One poisoned animal was killed by decapitation on the fifth day after the injection of uranyl acetate and one on each day for the following 3 days. The nonpoisoned normal animals were killed as convenient. Tissue slices of the kidney and liver of the animals were cut by a straight razor, and 0.1M maleate-Ringer buffer of pH 7.0 was used as the medium for the tissue slices. The rate of oxidation of ascorbic acid in vitro by the tissue slices of normal animals and of animals poisoned by uranyl acetate was measured by determining the oxygen consumption at 25°C in the Warburg apparatus. One milligram of ascorbic acid dissolved in 0.2 ml of 0.1M maleate-Ringer buffer of pH 7.0 was put in the side arm of the Warburg flask. Tissue slices were put in the main compartment of the flask with 1.8 ml of maleate-Ringer buffer, and 0.05 ml of 3N KOH was put in the middle well of the flask. The ascorbic acid was mixed in the main compartment after temperature equilibrium had been established. The manometric readings were taken at 15-min intervals for the first hour and then at 30-min intervals for the following 2 hr.

It was found that the oxygen consumption of the liver- and kidney-tissue slices of the four poisoned scorbutic guinea pigs was considerably increased in the presence of ascorbic acid, but this was not true of the tissues of the normal nonpoisoned control animals. In order to investigate this matter further, the remaining four scorbutic animals were poisoned by uranyl acetate as before, but after injection of the U_6 each animal received 20 mg of ascorbic acid daily by intraperitoneal injection for 4 to 6 days. Then one animal was killed per day, and the rate of oxidation of ascorbic acid by the tissue slices of liver and kidney were determined. It was found that the oxygen consumption of these tissues was not significantly increased by the addition of 1 mg of ascorbic acid. This probably is because the scorbutic tissues had regained a normal amount of ascorbic acid as a result of the injections of ascorbic acid. The results of these experiments are given in Table 13.21. The QO_2 values represent cubic millimeters of O_2 per milligram of dried tissue in 60 min. Each value represents an average of four results obtained using four separate animals.

Table 13.21

	QO_2 of liver		QO_2 of kidney	
Animals	Control	With added ascorbic acid	Control	With added ascorbic acid
Nonpoisoned, normal guinea pigs	4.59	5.09	8.61	8.23
Scorbutic guinea pigs, poisoned by U_6	5.27	6.58	9.44	11.78
Scorbutic guinea pigs, poisoned by U_6, then injected with 20 mg of ascorbic acid	5.28	5.38	7.13	7.64

The ability of liver- and kidney-tissue slices to oxidize ascorbic acid was found not to be imparied in the normal or the scorbutic animals, as a result of the poisoning by U_6. In fact, in the scorbutic animals it appears as though the ability of kidney slices to oxidize ascorbic acid were enhanced by poisoning the animal with U_6 injected intraperitoneally.

6. EXPERIMENTS ON THE EFFECTS OF U_6 ON TISSUE SLICES OF LIVER AND KIDNEY AS MEASURED IN THE WARBURG APPARATUS

6.1 Effect of U_6 on QO_2 of Tissue Slices of Rat Liver and Kidney without Added Substrate. (Work done by Tien Ho Lan with the assistance of Betty Robinson.) The QO_2 (Sec. 4.2 of this chapter) of

liver- and kidney-tissue slices of normal rats was determined in the Warburg apparatus with or without the addition of 1,000 μg of $UO_2(Ac)_2 .2H_2O$ at various pH values in 0.1M maleate buffer solution. These animals were killed by decapitation, and the tissue slices of kidney or liver were cut by a straight razor. The tissue slices were soaked in physiological saline solution for about 10 min before they were put in the Warburg flasks. In each flask, 2 ml of 0.1M maleate-Ringer's solution (or 1.9 ml when uranyl acetate was added) were put in the main compartment of the flask, and 0.05 ml of 3N KOH in the central well of the flask. A 2 per cent solution of $UO_2(Ac)_2.2H_2O$ was adjusted to pH 6 to 7 by adding dilute NaOH. The neutralized solution was then diluted to a 1 per cent solution. One-tenth milliliter of this solution ($\simeq$ 1,000 μg of $UO_2(Ac)_2.2H_2O$ or 560 μg of uranium metal) was put in the side arm of the Warburg flask and mixed with the tissue slices after temperature equilibrium had been established. The time between killing the animal and placing the Warburg flask in the water bath for temperature equilibration was always kept within the limit of 20 min. Readings of oxygen consumption were taken at 15-min intervals for a period of 60 min, and another reading was taken 30 min later, so that the total time during which readings were made was 90 min.

Three normal rats of the same size and age were used for each determination at each pH value. No significant change in oxygen consumption due to the addition of uranyl acetate was found in kidney slices. However, it was shown that the QO_2 of liver-tissue slices was decreased by U_6 at pH values lower than 6.8. This probably indicates the acidosis caused by any means has a tendency to heighten the action of U_6 on oxidative reactions in the liver, but whether such an effect would increase the poisoning effect of U_6 in vivo is still a question. Moreover it is also a question whether the pH of liver cells could be lowered materially by acidosis in vivo. The difference in results between liver and kidney slices cannot be explained at the present time.

The results of this experiment are shown in Figs. 13.73 and 13.74.

6.2 Effect of U_6 on the QO_2 of Normal and Scorbutic Tissue Slices. (Work done by Tien Ho Lan with the assistance of Betty Robinson.) In this experiment, six normal and seven scorbutic guinea pigs were used. The scorbutic guinea pigs were prepared by putting the animals on a scorbutic diet for 3 to 4 weeks. These animals began to show scorbutic symptoms in the third week after being fed the scorbutic diet. One animal was killed by decapitation each day in the fourth week, and the slices of kidney and liver tissue were cut by a straight razor. The QO_2 of the tissue slices with or without the addition of

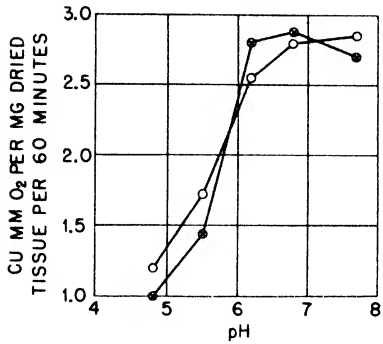


Fig. 13.73 — Respiration of rat-kidney slices in 0.1M maleate-Ringer buffer at different pH values in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$. ○, kidney slices of controls; ⊗, kidney slices in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$.

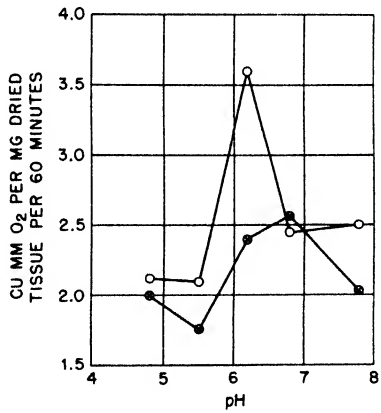


Fig. 13.74 — Respiration of rat-liver slices in 0.1M maleate Ringer buffer at different pH values in the presence of uranyl acetate. ○, liver slices of control; ⊗, liver slices in the presence of 1,000 μg of $UO_2(Ac)_2.2H_2O$.

uranyl acetate was determined in the Warburg apparatus as previously described. It was found that 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal) decreased the QO_2 of scorbutic kidney but not that of normal kidney. The added uranyl acetate did not show any inhibitory action on the QO_2 of tissue slices of either normal or scorbutic livers (Fig. 13.75).

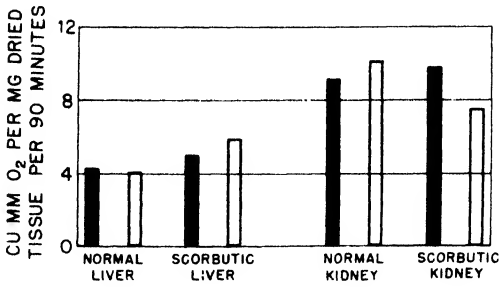


Fig. 13.75—The effect of U_6 on the QO_2 of normal and scorbutic guinea pig liver and kidney slices in 0.1M maleate-Ringer buffer of pH 7.0. □, tissue slices with 1,000 μg of $UO_2(Ac)_2.2H_2O$; ■, tissue slices without uranyl acetate.

Therefore U_6 appears to have a greater effect on scorbutic than on normal kidney tissues, possibly because of damage to intracellular substances in scurvy, which allows U_6 to gain access to the kidney-tissue cells of scorbutic animals more easily than to the cells of nonscorbutic tissue. This statement does not appear to apply to liver, and no explanation for this can be given at the present time. This finding is in agreement with the fact that scorbutic animals are more sensitive to U_6 poisoning than are nonscorbutic animals.

6.3 The Effect of U_6 In Vitro on the Oxidation of Ascorbic Acid. (Work done by Tien Ho Lan with the assistance of Betty Robinson.) In order to study the effect of U_6 on the oxidation of ascorbic acid in tissue-slice experiments, 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal) and 1 mg of ascorbic acid were added to liver- and kidney-tissue slices of both normal and scorbutic guinea pigs. The rate of oxidation of the ascorbic acid was determined in the Warburg apparatus as previously described. The uranyl acetate was prepared freshly from a 2 per cent stock solution that had been neutralized by dilute NaOH so that 0.1 ml of this solution contained 1,000 μg of $UO_2(Ac)_2.2H_2O$ (≎ 560 μg of uranium metal). One-tenth milliliter of this solution was put in one side arm, and 1 mg of ascorbic acid dissolved in 0.2 ml of 0.1M maleate-Ringer buffer of pH 7.0 in the other side arm of the Warburg flask. In the middle well of the flask was put 0.05 ml of 3N KOH. Tenth-molar maleate-Ringer buffer of pH 7.0 was added to the flasks to make the total volume of the added solutions 2.05 ml. After temperature equilibrium was established, the ascorbic acid and uranyl acetate were mixed with the tissue slices, and the oxygen consumption caused by the oxidation of ascorbic acid by normal liver- and kidney-tissue slices was measured as previously

described. It was found that the oxidation of ascorbic acid in normal liver- and kidney-tissue slices was not affected by the addition of uranyl acetate, and further that the ability of kidney slices of scorbutic animals to oxidize ascorbic acid was apparently enhanced by the addition of uranyl acetate. A good explanation for this phenomenon is lacking.

The results of experiments carried out with tissue slices from the livers and kidneys of four normal and four scorbutic guinea pigs are shown in Fig. 13.76.

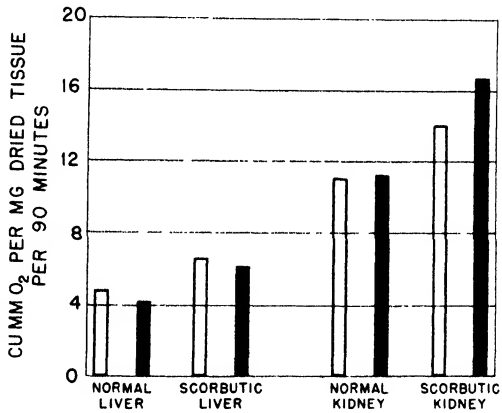


Fig. 13.76 — The effect of U_6 on the oxidation of ascorbic acid by normal and scorbutic guinea pig liver- and kidney-tissue slices in 0.1M maleate-Ringer buffer of pH 7.0. □, tissue slices + 1 mg of ascorbic acid; ■, tissue slices + 1 mg of ascorbic acid + 1,000 μg of $UO_2(Ac)_2.2H_2O$.

BIBLIOGRAPHY

1. H. Neurath, G. R. Cooper, and J. O. Erickson, J. Biol. Chem., 142: 249 (1942).
H. Neurath, G. R. Cooper, and J. O. Erickson, J. Phys. Chem., 46: 203 (1942).
D. G. Sharp, G. R. Cooper, J. O. Erickson, and H. Neurath, J. Biol. Chem., 144: 139 (1942).
F. Bernheim, H. Neurath, and J. O. Erickson, J. Biol. Chem., 144: 259 (1942).
F. W. Putnam, J. O. Erickson, E. Volkin, and H. Neurath, J. Gen. Physiol., 26: 513 (1943).
H. Neurath, J. P. Greenstein, F. W. Putnam, and J. O. Erickson, Chem. Revs., 34: 157 (1944).
A. L. Dounce, Science, 97: 21 (1943).
2. A. L. Dounce, J. Biol. Chem., 147: 685 (1943).

3. L. F. Hewitt, Separation of Serum Albumin into Two Fractions, Biochem. J., 30: 222-229 (1936).
4. G. J. Gubler and C. L. A. Schmidt, Arch. Biochem., 8: 212 (1945).
5. T. B. Johnson and H. Harkins, J. Am. Chem. Soc., 51: 1779 (1929).
6. A. J. Clark, "General Pharmacology," Vol. IV of "Handbuch der experimentellen Pharmakologie," Verlag Julius Springer, Berlin, 1937; J. W. Edwards, Ann Arbor, Mich., 1944 (Alien Property Custodian copyright, 1944).
7. J. B. Sumner and K. Myrback, Z. Physiol. Chem., 189: 218 (1930).
8. J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, Inc., New York, 1943; new edition in press.
9. J. H. Northrop, "Crystalline Enzymes," Columbia University Press, New York, 1939.
10. S. Williamson and D. E. Green, J. Biol. Chem., 135: 345 (1940).
11. J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, Inc., New York, 1943.
12. J. B. Sumner, N. Gralin, and I-B. Ericksson-Quensel, J. Biol. Chem., 125: 37(1938).
13. J. P. Greenstein, Biol. Chem., 133: 397 (1940).
14. H. O. Singher and A. Meister, J. Biol. Chem., 159: 491 (1945).
W. H. Price and C. F. Cori, J. Biol. Chem., 162: 393 (1946).
15. C. H. Fiske and Y. Subbarow, J. Biol. Chem., 66: 375 (1925).
16. H. Blaschko, J. Physiol., 90: 1 (1937).
17. J. R. Klein, J. Biol. Chem., 141: 139 (1939).
18. A. L. Dounce, J. Biol. Chem., 147: 685 (1943).
19. J. S. L. Philpot and F. J. Philpot, Biochem. J., 30: 2191 (1936).
20. N. U. Meldrum and F. J. W. Roughton, J. Physiol., 80: 113 (1933).
20a. E. R. Main and A. Locke, J. Biol. Chem., 140: 909 (1941).
21. P. J. G. Mann and J. H. Quastel, Biochem. J., 31: 869 (1937).
22. J. B. Sumner and A. L. Dounce, J. Biol. Chem., 127: 439 (1939).
23. D. Keilin and E. F. Hartree, Proc. Roy. Soc. London, B, 125: 171 (1938).
24. E. Haas, J. Biol. Chem., 148: 481 (1943).
25. D. Keilin and E. F. Hartree, Proc. Roy. Soc. London, B, 122: 298 (1937).
26. F. B. Straub, Biochem. J., 34: 483 (1940).
27. F. B. Straub, Biochem. J., 33: 787 (1939).
28. E. B. Gutman and A. B. Gurman, Proc. Soc. Exptl. Biol. Med., 47: 513 (1941).
29. A. L. Dounce and D. Beibel, Proc. Soc. Exptl. Biol. Med., 54: 22 (1943).
30. E. S. King and A. R. Armstrong, Can. Med. Assoc. J., 31: 376 (1934).
31. S. P. Colowick and E. W. Sutherland, J. Biol. Chem., 144: 423 (1942).
32. J. B. Sumner and G. Somers, Arch. Biochem., 4: 11 (1945).
33. A. A. Green and G. T. Cori, J. Biol. Chem., 151: 21 (1943).
34. J. B. Sumner, J. Biol. Chem., 69: 435 (1926).
35. A. L. Dounce, J. Biol. Chem., 140: 307 (1941).
J. B. Sumner and E. B. Sisler, Arch. Biochem., 4: 207 (1944).
36. A. L. Jacoby, Biochem. Z., 259: 211 (1939); ibid., 262: 181 (1933); ibid., 267: 167 (1933).
37. L. Hellerman, Cold Spring Harbor Symposia Quant. Biol., VII: 165 (1939).
38. I. W. Sizer and A. A. Tytelle, J. Biol. Chem., 138: 631 (1941).
39. A. Gilman, "Organic Chemistry," 2d ed., Vol. I, p. 889, John Wiley & Sons, Inc., New York, 1938.
40. C. V. Smythe, J. Biol. Chem., 114:601 (1936).
41. I. W. Sizer and A. A. Tytelle, J. Biol. Chem., 138: 631 (1941).
42. E. G. Ball, J. Biol. Chem., 128: 51 (1930).
43. M. L. Anson, J. Gen. Physiol., 22: 79 (1938).
44. C. Huggins and P. Talalay, J. Biol. Chem., 159: 399 (1945).
45. E. J. King and A. R. Armstrong, Can. Med. Assoc. J., 31: 376 (1934).

Chapter 14

CATALASURIA AS A SENSITIVE TEST FOR URANIUM POISONING

By Alexander L. Dounce, Eugene Roberts, and J. H. Wills*

1. INTRODUCTION

Breedis, Flory, and Furth[1] reported that uranyl nitrate injection resulted in elevation of the urinary concentration of alkaline phosphatase and a decrease in the concentration in the kidney as estimated by staining methods. Roberts suggested the study of phosphatasuria as a possible sensitive early test for uranium poisoning. The phosphatase work suggested to Dounce that breakdown of kidney tubular cells in uranium poisoning should result in the appearance in the urine of many or all of the intracellular enzymes of the tubular cells. Since catalase is a rugged enzyme which can be estimated quantitatively in high dilution and which occurs in the kidney in high concentration, it seemed that its appearance in the urine might constitute a very sensitive and early indicator of the action of uranium on the nephron.

Wills and Tien Ho Lan tested this hypothesis on anesthetized rabbits with cannulated bladders, finding that there was a sharp increase in urinary excretion of catalase within 3 or 4 hr after intravenous injections of moderate doses of uranium. Later Roberts found that urinary catalase increased sharply following inhalation of uranium compounds by rabbits, rats, guinea pigs, cats, and dogs. The catalase test was found to be a very sensitive indicator of damage to tubular epithelium after intravenous injection of uranyl compounds and was reproducible in the same animal on successive insults. The test gave approximately the same results in dogs and rabbits and was also applicable to cats.

* Work done by Alexander L. Dounce, Eugene Roberts, J. H. Wills, and Tien Ho Lan with the assistance of William Connors, Jean Orcutt, Marian Kaley, Betty Robinson, and Frank Smith.

The urinary concentration of catalase was studied in workers in two chemical plants in which the personnel were exposed to various concentrations of air-borne dusts of several uranium compounds. Although the average catalase values in these two plants were higher than the average for a group of laboratory workers in Rochester, all values but those of a very few individuals were well below the urinary-catalase level believed to correspond to minimal detectable tubular damage. The higher catalase averages in the two plants in question could be attributable to factors (such as urinary concentration) that are unrelated to exposure to uranium compounds.

2. METHOD

2.1 Collection of Urine. Human Urine. The urine sample was collected in a carefully cleaned glass container in which had been placed a few milliliters of a 50 per cent toluene-50 per cent chloroform mixture to act as a preservative. This is sufficient to retard bacterial growth at room temperature for 6 to 8 hr, except possibly in very hot weather. If the urine cannot be analyzed within 15 to 25 min after collection, the addition of gum arabic as a preservative is necessary to slow the decay of the enzyme. A 20 per cent solution of gum arabic (neutralized to pH 5.5 to 6.0 with 1M NaOH) is prepared, and 5 ml of this solution is added to each 95 ml of urine. When preparing the 20 per cent gum arabic, care must be taken to warm the solution very gently, or the gum will char. The rate of decay of catalase is sufficiently slowed by the gum so urine samples can be kept for 6 to 8 hr at room temperature without serious loss in activity. The dilution of the urine by the gum arabic solution can be ignored, since it will not cause a serious error when compared with the normal variation in catalase content of urine.

Urine for the catalase determination should be collected in an atmosphere free of catalase inhibitors, namely, fluoride, NO_2, H_2S, HCN, and hydroxylamine, and should be free of blood. Samples for this determination should not be taken from females within 2 days on either side of the menstrual period.

Animal Urine. Animal urine collected from metabolism cages is satisfactory for the catalase determination if (1) free of contamination with feces, (2) free of contamination with uncooked food, (3) very fresh or preserved with gum arabic and toluene-chloroform mixture (1 ml of 20 per cent gum arabic solution and 2 ml of 50 per cent toluene-50 per cent chloroform mixture are added to the collection bottle), (4) the cage contains no free copper (galvanized iron or Monel metal

may be used), and (5) the cage and collection bottles are scrubbed thoroughly with soap and hot water every day.

Urine has been collected from rabbits in many cases by gentle manual massage of the bladder. With male rabbits care must be taken that the procedure does not result in expulsion of prostatic secretion, which contains large amounts of catalase.

Urine can be collected from dogs by daily catheterization over fairly short periods (up to 21 days) without urinary infection. Female dogs with bladder fistulas (suprapubic cystotomies) have given satisfactory daily urine samples for several months, the urine being collected directly from the bladder with aseptic technique through a sterile rubber catheter or metal trocar attached to a syringe.

2.2 The Determination of Urinary Catalase. In order to determine urinary catalase the urine must be incubated with hydrogen peroxide. Then either the loss of peroxide or the increase in oxygen can be determined, since the reaction proceeds as follows:

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \tfrac{1}{2} O_2$$

Hydrogen peroxide can best be determined titrimetrically, whereas oxygen can best be determined manometrically. Oxygen also can be determined polarographically, and a polarographic method for catalase estimation has been published by Walker.[2] This method is, however, not suitable for urinary catalase determinations.

The titrimetric and manometric methods for the determination of catalase have been discussed in Chap. 5, Sec. 5.3. The manometric methods have been employed in this work almost exclusively because they are more sensitive and less subject to chemical errors than the titration procedure. The titrimetric method does not yield reliable results for catalase concentrations yielding less than 50 cu mm of O_2 by the manometric methods.

The Warburg determination constitutes a very sensitive method for the estimation of urinary catalase. Pure crystalline beef catalase diluted 1 to 10,000,000 (dry-weight basis) can be measured in this way.

Figure 14.1 illustrates the results obtained using the Warburg method.

Mention should be made of the relationship of catalase units to the cubic millimeters evolved in the Warburg method. One unit of catalase is defined as the amount of the enzyme that would give a "monomolecular" k value of 1 in the von Euler method for determining catalase.[3] In practice k must lie between 0.025 and 0.04 in order to perform a satisfactory determination by von Euler's method.[4]

$$K = \frac{1}{t} \log \frac{A}{A - x}$$

where t is time in minutes, A is initial titration value in milliliters of 0.005N $KMnO_4$ (or $Na_2S_2O_3$ if the iodometric method is used), and (A − x) is the titration value at time t, corresponding to the amount of H_2O_2 left over.

Kat. f. is defined as the ratio between K value and grams of enzyme used in the determination.[6] For crystalline beef catalase, Kat. f. = 33,000.[5] Therefore, by calculation, one k unit corresponds to about 0.00003 g, or 30 μg, of the enzyme per milliliter. For erythrocyte catalase of the cow or human being, for which the Kat. f. value is about 50,000,[7] 1 unit of enzyme corresponds to about 0.00002 g, or 20 μg, of catalase per milliliter.

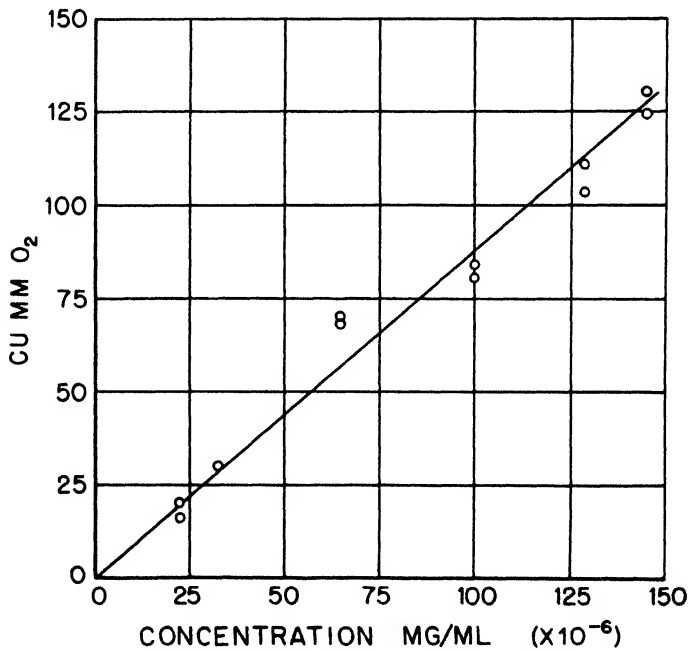


Fig. 14.1—Cubic millimeters of O_2 released from 0.04M H_2O_2-buffer mixture by various water dilutions of a standard human erythrocyte catalase preparation (Kat. f. = 55,000) in a Warburg apparatus during a 15-min period. Each point signifies a single determination on the same catalase aliquot at the given dilutions.

It is not known whether kidney catalase is similar to liver catalase or to erythrocyte catalase, but, assuming that it is similar to liver

catalase, a reading of 50 cu mm of O_2 with the Warburg determination would correspond approximately to a k value of 0.008 per milliliter. Since urine inhibits catalase to the extent of about 50 per cent, this k value would correspond to about 0.05 μg/ml. For blood catalase, a reading of 50 is equivalent to 0.032 μg/ml.

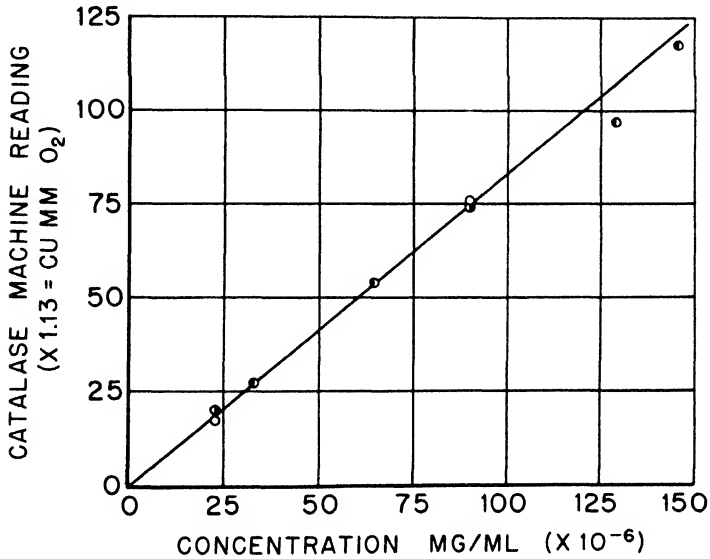


Fig. 14.2—Manometer readings of the Dounce apparatus given by 0.04M H_2O_2-buffer mixture and various water dilutions of the standard erythrocyte catalase after shaking for 15 min. Identical symbols signify single determinations on the same standard catalase aliquot at various dilutions.

Figure 14.2 shows some results obtained by means of the Dounce apparatus (Chap. 5, Sec. 5.3) with standard catalase when diluted with varying amounts of distilled water. Figure 14.3 shows results obtained using standard catalase diluted with normal human urine. It should be noted that urine inhibits the activity of purified catalase added to it to the extent of about 50 per cent. Figure 14.1 shows results from Warburg determinations calculated in cubic millimeters of O_2, using catalase diluted with distilled water. Figure 14.4 shows Warburg determinations on standard catalase diluted with human urine. Figure 14.5 shows the curve made by comparing readings on nine manometers from three catalase machines with cubic millimeters of O_2 calculated from corresponding Warburg determinations,

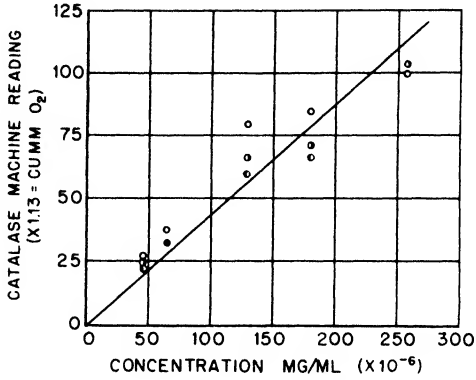


Fig. 14.3—Manometer readings of the Dounce apparatus given by 0.04M H_2O_2-buffer mixture and various dilutions of the standard catalase preparations in normal human urine after shaking for 15 min. Identical symbols signify single determinations on the same catalase aliquot at various dilutions.

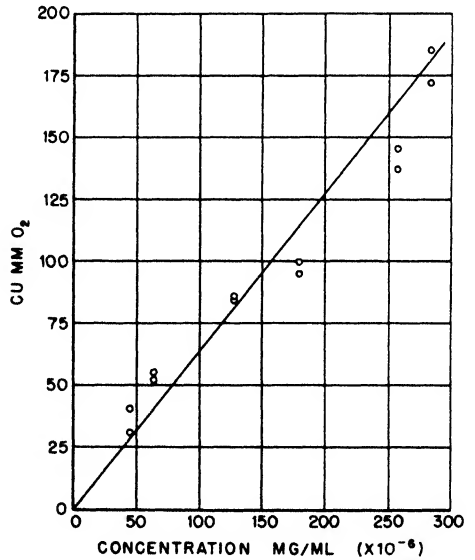


Fig. 14.4—Cubic millimeters of O_2 released from 0.04M H_2O_2-buffer mixture by various dilutions of standard catalase in normal human urine in the Warburg apparatus during a 15-min period. Each point signifies a single determination on the same catalase aliquot at the given dilution.

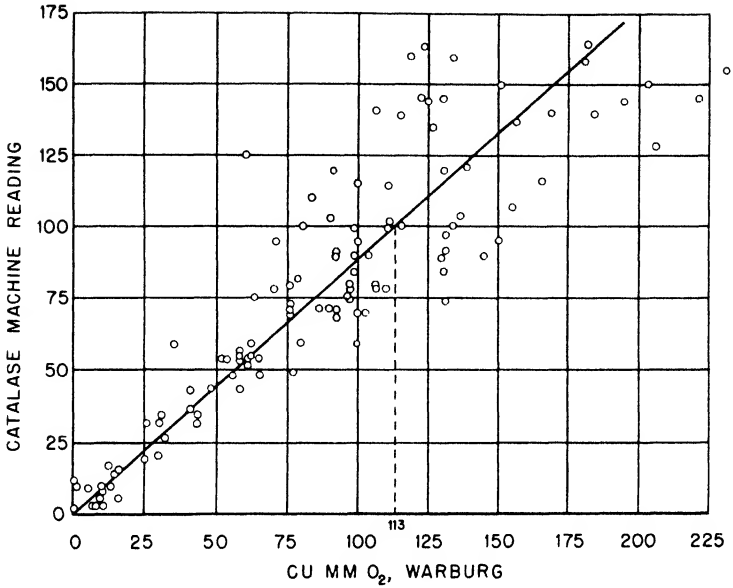


Fig. 14.5 — Simultaneous determinations of catalase activities in urine samples from rabbits exposed to uranium compounds with the Dounce catalase apparatus and the Warburg apparatus. Each point represents a single Warburg manometer reading and a single manometer reading from a Dounce catalase apparatus. A reading of 100 cu mm on the manometer of the Dounce catalase apparatus corresponds on the average to an oxygen evolution of 113 cu mm in the Warburg apparatus, giving the conversion factor used in calculating the data for Figs. 14.2 and 14.3.

using rabbit urine containing varying amounts of catalase (resulting from exposure to uranium compounds). Since no corrections have been made for size of manometer capillary bore, the curve would undoubtedly be much improved if only one manometer on one machine had been used. For best results, each manometer should be calibrated, or else capillary tubing of exact diameter should be used in constructing the manometers.

3. THE SOURCE OF URINARY CATALASE

By J. H. Wills

3.1 Method. Rabbits anesthetized with nembutal were infused through a saphenous vein with isotonic solutions at a constant rate of 1 ml/min. Other substances were injected into the lumen of the infu-

sion cannula. When clearance determinations were made, blood samples were drawn from the deep femoral artery. Urine was collected for 30-min periods.

Beef-liver catalase furnished by Dounce was once-recrystallized material prepared by the method of Sumner and Dounce.[8] Rabbit blood was hemolyzed by mixing with 2 volumes of water.

The catalase analyses were carried out by Kaley and Tien Ho Lan, using the Warburg technique.

3.2 Results. The urinary clearance of catalase varied from 0.000 to 0.645 per cent of the inulin clearance in 20 experiments during which these two determinations were made simultaneously. The mean value for the catalase clearance in percentage of the simultaneous inulin clearance was 0.111.

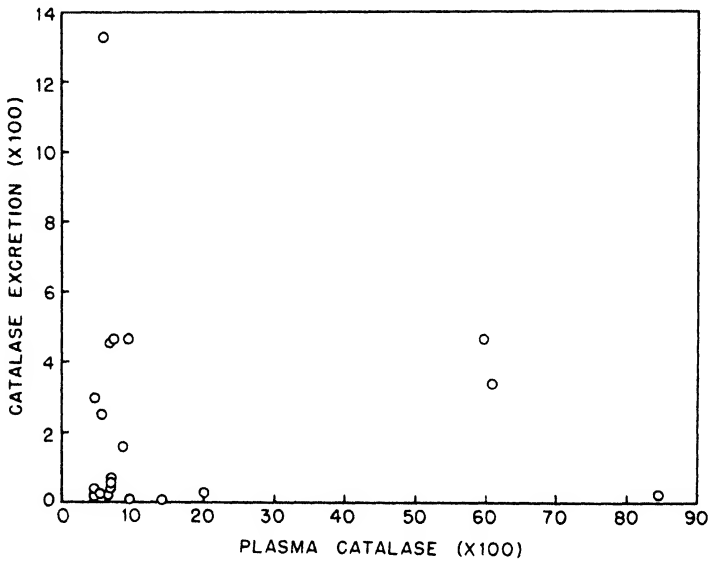


Fig. 14.6—Covariance of urinary excretion of catalase and plasma level of catalase in the rabbit.

Since inulin clearance measures the rate of glomerular filtration, this low value for catalase clearance could indicate either that catalase is filtered through the glomerulus and reabsorbed almost completely in the tubule, that it is only slightly filterable through the glomerulus, or that the urinary catalase comes directly from the kidney and not from the blood. The size of the catalase molecule

(molecular weight 250,000 as compared with 70,000 for plasma albumin) is inconsistent with the first and second hypotheses.

A plot of the values for the simultaneous clearances of catalase and inulin (in percentage deviations from the respective means) has been shown in Fig. 5.18. These data indicate that variations in the clearance of catalase are not correlated with variations in the inulin clearance. In other words, catalase excretion in normal animals does not depend on glomerular filtration.

Further evidence on this point is given in Fig. 14.6, in which catalase excretion is plotted at various plasma levels of catalase. The plasma level of catalase was varied by injection of either beef-liver catalase or hemolyzed rabbit blood. From the figure it can be seen that urinary excretion of catalase was independent of the plasma level. This shows again that catalase excretion in normal animals does not depend upon glomerular filtration.

Two rabbits poisoned 2 and 4 days previously by intravenous injection of 2 mg of uranyl acetate dihydrate per kilogram were prepared in the usual manner and injected intravenously with crystallized beef-liver catalase. When the plasma-catalase levels of these animals

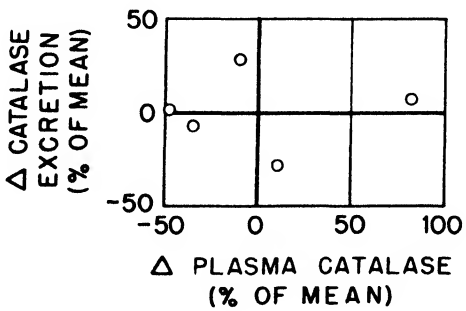


Fig. 14.7—Covariance of the deviation from the mean of the urinary excretion of catalase with that of the plasma-catalase level in two rabbits poisoned previously by intravenous injection of uranyl acetate.

were raised thus, there were no appreciable increases in catalase excretion (Fig. 14.7). This indicates that even in the kidney already damaged by uranium the glomerular membrane is still an efficient barrier to the passage of catalase into the urine from the plasma.

Glomerular filtration having been ruled out as a factor in either normal or pathological catalasuria, the only source of urinary catalase left is the kidney itself. The small amount of catalase excreted

in the urine normally may arise from the process of continual cellular replacement.* The release of catalase after insult to the kidney with heavy metals may result either from a change in the permeability of the membrane of the epithelial cell lining of the tubule or an actual breakdown of the cell structure. The first of these changes could possibly be the cause of the peak of catalasuria occurring about 2 hr after injection of uranyl uranium, whereas the second is certainly the cause of the peak found at from 2 to 5 days after injection.

Since hemoglobin is filterable through the glomerulus and has a slight catalytic effect in breaking down hydrogen peroxide, it seemed advisable to check the experiments in which hemolyzed rabbit blood was used as a source of catalase with similar ones in which crystallized beef or rabbit hemoglobin was injected. In three experiments done with beef hemoglobin, the injection produced decreases in both the urinary level of catalase and the catalase excretion; in two experiments with rabbit hemoglobin there were moderate decreases in the urinary levels of catalase but no significant changes in the catalase excretions. In two of the three experiments done with beef hemoglobin the urine flow fell to a very low level; in the two experiments with rabbit hemoglobin there was diuresis after the injection. Thus it appears that the hemoglobin injected during the experiments with hemolyzed rabbit blood probably had no effect on catalase excretion.

4. THE PRODUCTION OF CATALASURIA BY NEPHROTOXIC AGENTS IN SACRIFICE EXPERIMENTS

By J. H. Wills

4.1 Method. Rabbits anesthetized with nembutal were infused at a constant rate of 1 ml/min with isotonic solutions. Urine was collected for successive 30-min periods. After two control periods the substance or substances under study were injected into the infusion cannula through the rubber tubing, and collections of urine were made as before.

Determinations of the urinary catalase were made by Robinson, Kaley, and Tien Ho Lan, using the Warburg technique. The results have been expressed in terms of excretion rather than of urinary

* Of course large numbers of red or white cells must not be present in the urine, since red cells are rich in catalase and white cells contain some catalase. It has been found that a red-cell count of 400,000 per milliliter will not cause an appreciable elevation of the urinary-catalase level above normal, and a considerably higher white-cell count can be tolerated. Extraneous catalase can also be contributed by some kinds of bacteria.

concentration because the excretion during control periods was more constant than the urinary concentration.

4.2 Results. It was found that injection of uranyl acetate (Tables 14.1 and 14.2), uranium tetrachloride (Table 14.3), mercuric chloride (Table 14.4), and mapharsen (Table 14.1) could produce an increased

Table 14.1 — Production of Catalasuria in Rabbits by Injection of $UO_2Ac_2.2H_2O$ Alone and with Mapharsen

Dose of $UO_2Ac_2.2H_2O$, mg/kg	Dose of mapharsen, mg/kg	No. of rabbits	Max. C, % C_0*
0.3		5	166
0.3	12	2	Over 10,000
0.6		6	328
0.6	1.2	4	396
0.6	12	1	6,700

* The designation Max. C and the designation C_0 refer, respectively, to the highest rate of excretion of catalase found after injection of the test substance and to the mean rate of excretion of catalase during the control period.

Table 14.2 — Effect of Infusion of 0.87 per Cent $NaHCO_3$ on Production of Catalasuria in Rabbits by Injection of $UO_2Ac_2.2H_2O$

Dose of $UO_2Ac_2.2H_2O$, mg/kg	Without $NaHCO_3$		With $NaHCO_3$	
	No. of expts.	Max. C, % C_0*	No. of rabbits	Max. C, % C_0*
1.0	3	379	2	115
3.0	5	672	3	170

* The designation Max. C and the designation C_0 refer, respectively, to the highest rate of excretion of catalase found after injection of the test substance and to mean rate of excretion of catalase during the control period.

urinary excretion of catalase within 1 to 3 hr after the injection. Typically the catalasuria rose to a peak at 1¼ to 2¼ hr after the injection of uranyl acetate and then decreased in value (Fig. 14.8). In injection studies with intact animals it has been found that the catalasuria attains a second maximum in 2 to 5 days after the injection.

The second of the two peaks of catalasuria mentioned above is the one used in all the clinical and experimental animal work as an indicator of toxic action; the first peak is the one to be considered here

as evidence of early renal effect. It seems possible that the first peak of catalasuria may represent an effect of the metal on permeability of the tubular epithelial cell, possibly by causing aggregation of the protein units of the membrane and thereby enlarging its pores; the second peak of catalase liberation is thought to represent actual cellular breakdown.

Table 14.3 — Effect of Infusion of 0.87 per Cent $NaHCO_3$ on Production of Catalasuria in Rabbits by Injection of UCl_4

	Without $NaHCO_3$		With $NaHCO_3$	
Dose of UCl_4, mg/kg	No. of rabbits	Max. C, % C_0*	No. of rabbits	Max. C, % C_0*
1.8	1	100	1	218
7.0			1	382
7.2	1	465	1	200
10.0	1	555		

* The designation Max. C and the designation C_0 refer, respectively, to the highest rate of excretion of catalase found after injection of the test substance and to the mean rate of excretion of catalase during the control period.

Table 14.4 — Effect of Infusion of $NaHCO_3$ on Production of Catalasuria in Rabbits by Injection of 2 Mg $HgCl_2$/kg

$NaHCO_3$ solution infused, %	No. of rabbits	Max. C, % C_0*
0	2	1,510
0.87	1	1,305
1.30	1	2,900

* The designation Max. C and the designation C_0 refer, respectively, to the highest rate of excretion of catalase found after injection of the test substance and to the mean rate of excretion of catalase during the control period.

It has been found that in producing the first catalasuria peak in rabbits, four metal-containing nephrotoxic agents can be arranged in the following decreasing order of effectiveness, based upon the amount of metal injected in each case: mapharsen > mercuric chloride > uranyl acetate > uranous chloride.

There is a great discrepancy between the amounts of uranium as $UO_2Ac_2.2H_2O$ and as UCl_4 required to produce equivalent effects on the kidney, the $UO_2Ac_2.2H_2O$ being about four times as effective. This indicates that if reduction of uranyl uranium to the tetravalent form could occur in the body, this change would be a detoxifying one in so

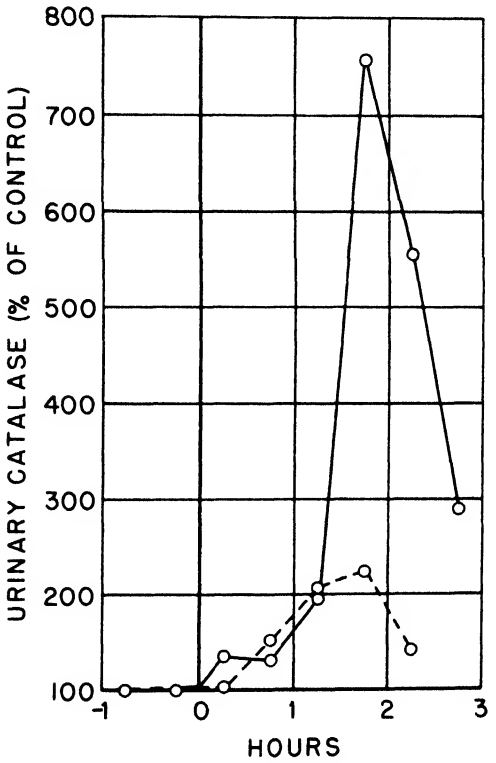


Fig. 14.8—Time courses of the urinary excretion of catalase by two anesthetized rabbits following the intravenous injection of a solution of 3 mg of uranyl acetate dihydrate (≎ 1.68 mg of uranium metal) per kilogram at zero time.

far as the kidney is concerned. The discrepancy indicates further that oxidation of tetravalent uranium to the hexavalent form cannot occur very rapidly within the body.

Data illustrative of studies of the production of the first catalasuria peak by the intravenous injection of mapharsen, mercuric chloride,

uranyl acetate, and uranous chloride are to be found in Tables 14.1, 14.2, 14.3, and 14.4.

Figure 14.9 shows the relationship between maximum excretion of catalase in the early peak to the dose of uranyl acetate. The straight line drawn through these points by the method of least squares has the equation

$$y = 194.5 + 141.3x$$

y being the maximum excretion of catalase in percentage of that during the control period and x being the dose of uranyl acetate dihydrate

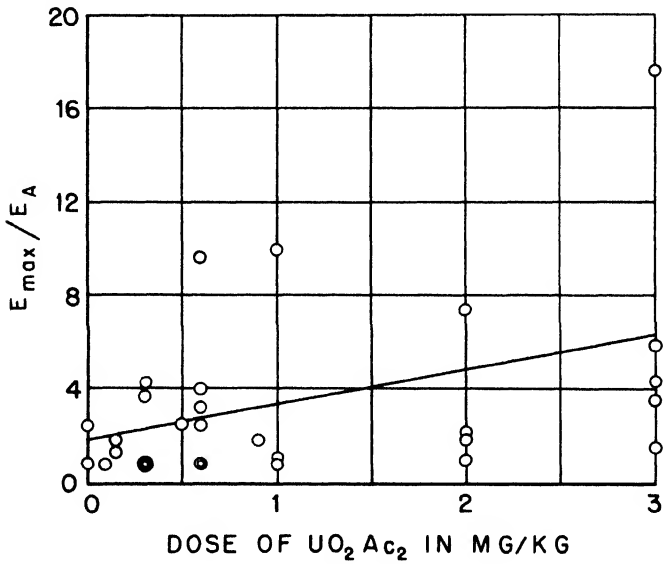


Fig. 14.9—Effect of increasing the dosage of uranyl acetate upon the ratio of the maximal urinary-catalase excretion, obtained within 3 hr after the injection, to the excretion before the injection of uranyl acetate.

E_{max} and E_A refer, respectively, to the highest rate of excretion of catalase found after injection of the test substance and to the mean rate of excretion of catalase during the control period.

in mg/kg. Since the standard error of estimate is ±330, and the coefficient of correlation is only 0.405, this curve cannot be regarded as being very significant. With doses of uranyl acetate greater than approximately 1.6 mg/kg, however, it should be possible to use the first peak of catalasuria as an index of an effect of uranyl uranium on the cells of the tubule.

Table 14.2 gives the results of some experiments in which infusion of 0.87 per cent sodium bicarbonate was studied as a means of preventing the action of uranyl uranium on the tubule epithelial cell. In a preliminary experiment it was found that 0.4 per cent bicarbonate had almost no protective action. The data of Table 14.2 show that infusion of 0.87 per cent sodium bicarbonate protected the kidney from the action of uranyl uranium signified by the early peak of catalasuria. This suggests that bicarbonate forms a firm complex with uranyl uranium in vivo as well as in vitro.

Tables 14.3 and 14.4 present some data on the effect of bicarbonate infusion upon the production of catalasuria by injection of uranium tetrachloride and mercuric chloride. Bicarbonate may have afforded slight protection against the action of uranium tetrachloride but definitely was not protective in the case of mercuric chloride. These observations check the in vitro findings that neither tetravalent uranium nor bivalent mercury is complexed strongly by bicarbonate at body pH.

5. THE PRODUCTION OF CATALASURIA BY NEPHROTOXIC AGENTS IN SURVIVAL EXPERIMENTS

By A. L. Dounce with the Assistance of Daphne Rothermel

5.1 Uranyl Acetate. At different intervals of time 4 cats, 7 dogs, and 15 rabbits were injected intravenously with various doses of uranyl acetate dihydrate. Urine was collected from cats in metabolism cages, from rabbits by manual expression, and from dogs by catheterization or from bladder fistulas. The urines were examined routinely for catalase and total protein; alkaline phosphatase and citric acid were determined in some cases. Control animals were injected with either normal saline or isotonic bicarbonate.

Of a total of 57 rabbits considered preliminary to use, 10 showed elevated catalase or protein levels in the urine before any treatment. Eight of the latter 10 animals had gross evidences of kidney involvement: injection of the medullary rays with or without cortical scarring.* Three cats out of eight studied had high protein and catalase levels in the urine during a 1-week control period. All animals having elevated urinary protein and catalase during the observation period were discarded.

Figure 14.10 shows the responses of two of the six control rabbits to repeated intravenous injections of saline or bicarbonate. It is apparent that so long as the animals remained in health there were no

* Not examined by the Pathology Division.

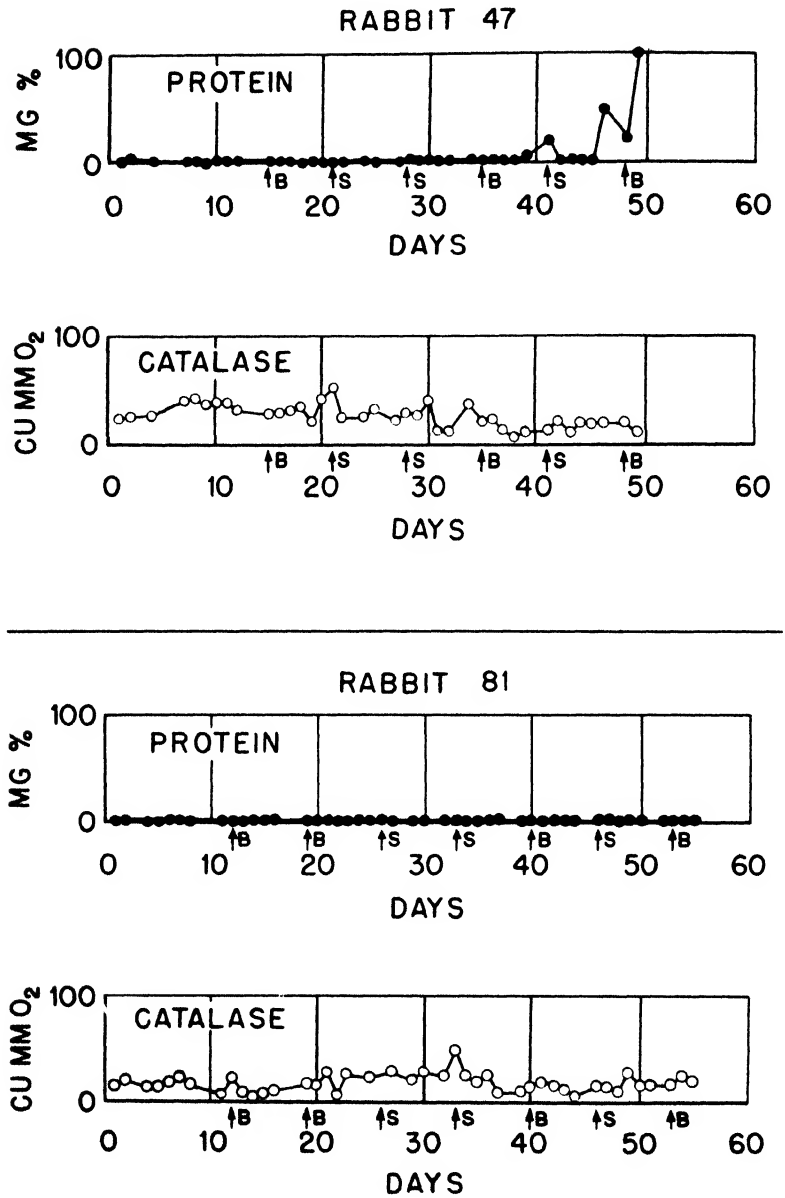


Fig. 14.10—Urinary catalase and protein levels of two control rabbits injected intravenously with isotonic bicarbonate (B) or isotonic saline (S) as indicated by the vertical arrows. Rabbit 47 developed diarrhea on the forty-sixth day of observation.

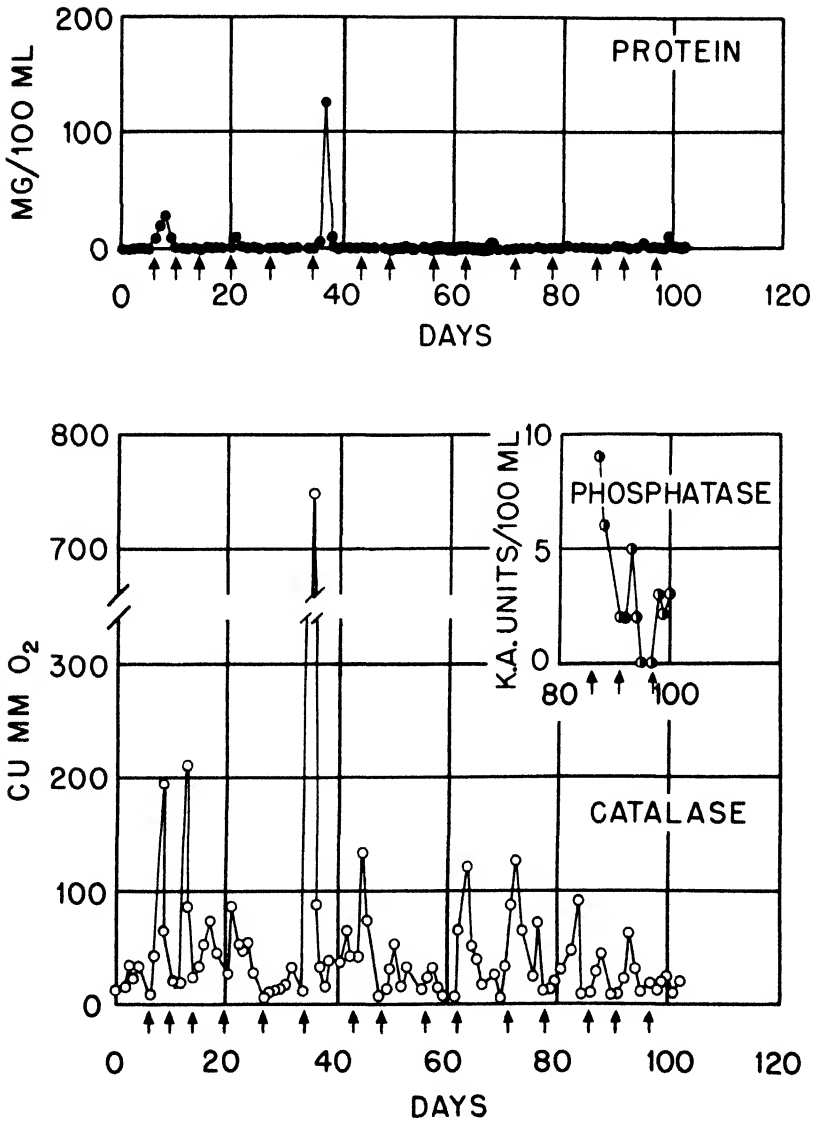


Fig. 14.11—Urinary levels of catalase, alkaline phosphatase, and protein of a rabbit injected intravenously with 0.01 mg of uranyl acetate dihydrate (≎ 0.0056 mg of uranium metal) per kilogram at times indicated by the vertical arrows.

significant alterations in the urinary concentrations of protein and catalase as a result of the experimental handling. Figures 14.11, 14.12, 14.13, and 14.14 are in sharp contrast to this picture. Here are

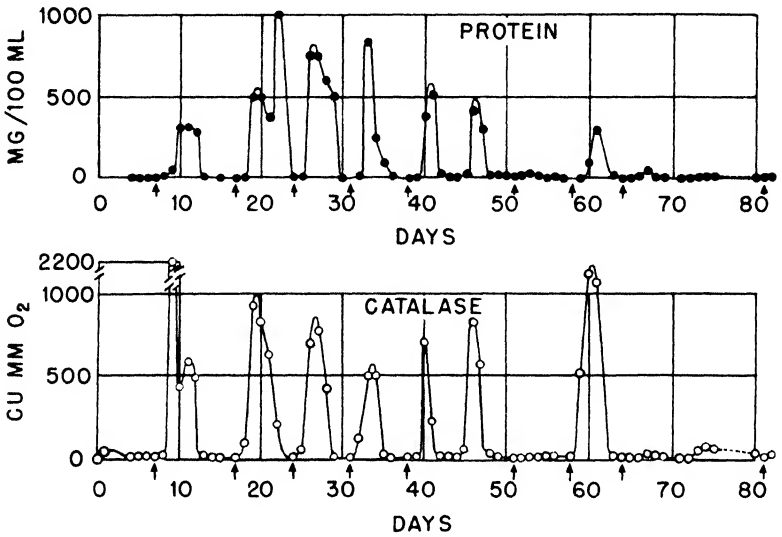


Fig. 14.12 — Urinary levels of catalase and protein of a rabbit injected intravenously with a solution of 0.05 mg of uranyl acetate dihydrate (≎ 0.028 mg of uranium metal) per kilogram as indicated by the vertical arrows.

sample records of rabbits injected repeatedly with various doses of uranyl acetate. It is evident that these injections produced great elevations in the urinary concentrations of catalase and less striking ones in the urinary protein levels. A number of deductions can be made from the latter four figures:

1. Catalasuria can occur without proteinuria (Fig. 14.11).

2. When proteinuria does occur, it reaches its peak at the same time as or later than catalasuria.

3. Higher doses of uranyl acetate bring about greater urinary excretions of both protein and catalase. This is true especially when the first response to the poison is considered, as is illustrated in Fig. 14.15 for catalase. This curve shows also that the lowest dose of uranyl acetate dihydrate that will produce a readily detectable catalasuria is between 0.01 and 0.02 mg/kg (0.006 to 0.011 mg of uranium metal per kilogram). The protein response is absent usually at a dose of uranyl acetate dihydrate of 0.02 mg/kg.

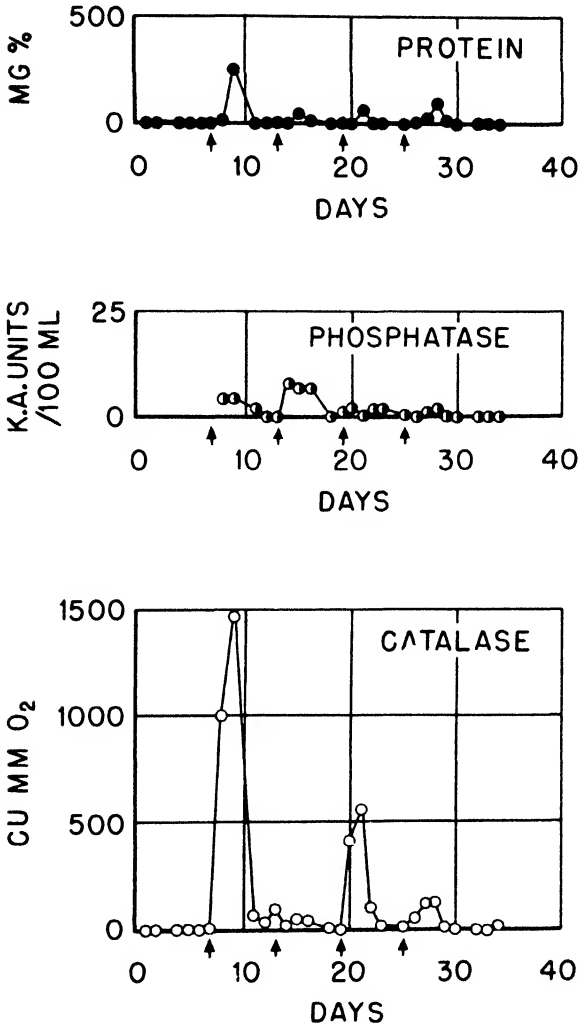


Fig. 14.13 — Urinary levels of catalase, phosphatase, and protein of a rabbit injected intravenously with a solution of 0.1 mg of uranyl acetate dihydrate (≎ 0.056 mg of uranium metal) per kilogram as indicated by the vertical arrows.

4. With the higher doses of uranyl acetate there is a distinct tendency for the responses to successive injections to decrease in magnitude. This progressive weakening of the catalase test on repeated injection of uranyl acetate dihydrate at dose levels of 0.05 mg/kg or

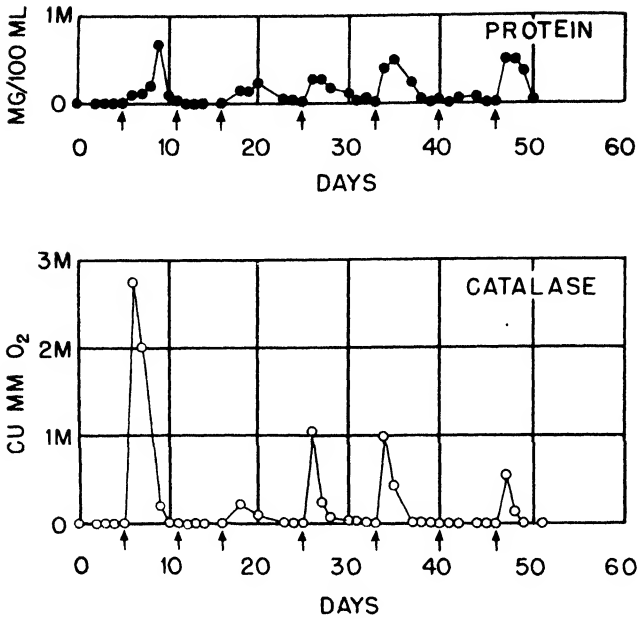


Fig. 14.14 — Urinary levels of catalase and protein of a rabbit injected intravenously with a solution of 0.5 mg of uranyl acetate dihydrate (≎ 0.28 mg of uranium metal) per kilogram as indicated by the vertical arrows.

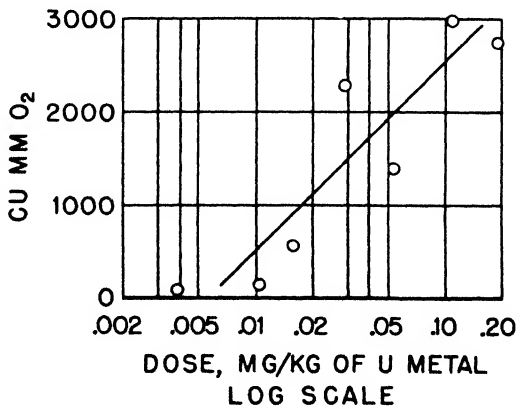


Fig. 14.15 — Effect of increasing the dose of uranium (given intravenously as uranyl acetate) upon the maximum level of urinary catalase in the rabbit in survival experiments following a single injection.

higher probably is due in part to development of tolerance of the kidney for the poison (Chap. 12) and in part to a loss of kidney catalase (see Sec. 3 of this chapter).

5. Phosphatase excretion responds less uniformly and markedly to consecutive injections of uranyl acetate than does catalase. This difference may be due to the fact that phosphatase is localized chiefly on the brush borders of the epithelial cells of the tubule, but catalase is distributed quite generally throughout the kidney. Phosphatase levels are recorded in King-Armstrong (K.A.) units.

Figures 14.16 and 14.17 show responses typical of cats and dogs to injections of uranyl acetate and show that the phenomena mentioned above for the rabbit occur also in other species.

A rat was injected subcutaneously with 1 mg of uranyl nitrate hexahydrate per kilogram, and its urine was collected over a 5-hr period on the third day after the injection. A similar collection was made

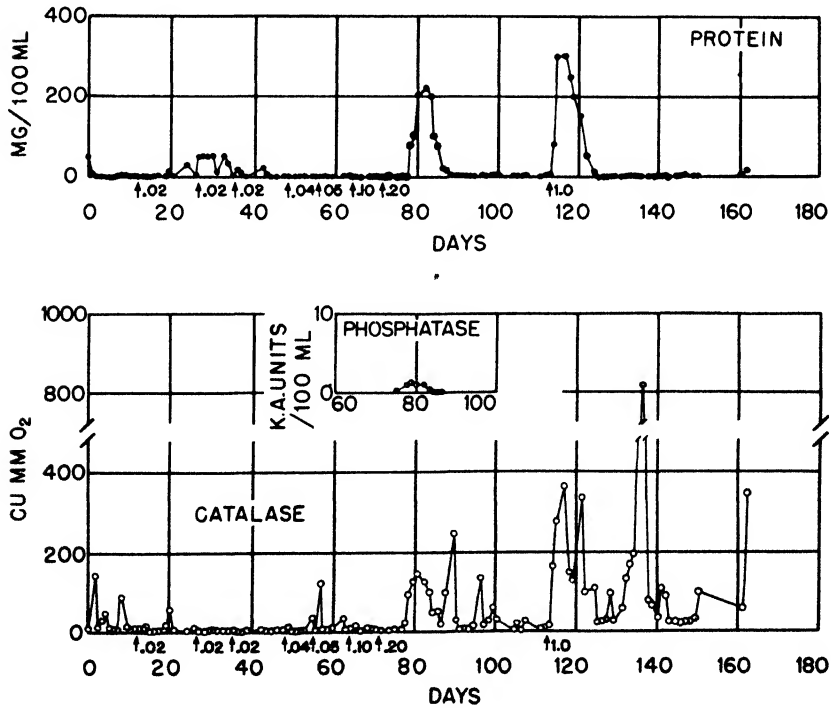


Fig. 14.16—Urinary levels of catalase, phosphatase, and protein in a cat injected intravenously with the doses of uranyl acetate indicated on the chart. Vertical arrows show times of injections.

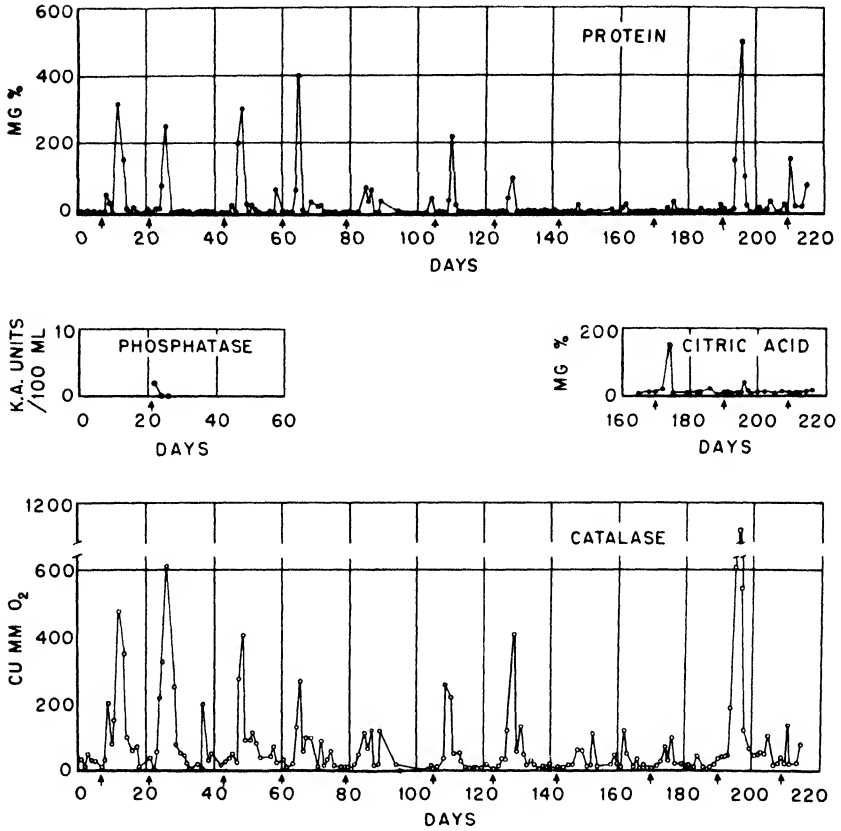


Fig. 14.17 — Urinary levels of catalase, citric acid, phosphatase, and protein in a dog injected with 0.05 mg of uranyl acetate dihydrate (≎ 0.028 mg of uranium metal) per kilogram as indicated.

from a control rat, and both urines were analyzed in duplicate for catalase. The results are shown in Fig. 14.18. It is apparent that the urine of the injected rat contained more catalase than that of the control animal, adding another species to those found to respond to injection of uranyl salts by catalasuria.

5.2 Uranium Tetrachloride. Four rabbits were injected intravenously with 0.1 mg UCl_4/kg (equivalent to 0.063 mg of uranium metal per kilogram), and the urinary levels of catalase and protein were followed. Figure 14.19 shows a representative response to such treatment. By comparison of Fig. 14.19 with Fig. 14.11 it is evident that

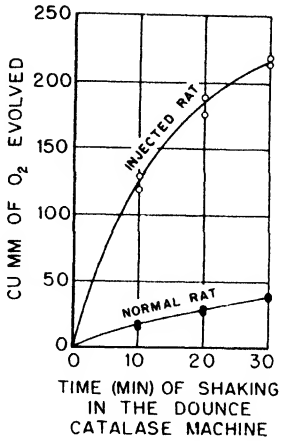


Fig. 14.18—Catalase activity of normal rat urine and urine of a rat injected intraperitoneally 3 days previously with a solution of 1 mg of uranyl nitrate hexahydrate (≎ 0.47 mg of uranium metal) per kilogram. Cubic millimeters of O_2 evolved in the Warburg apparatus is plotted against time of shaking.

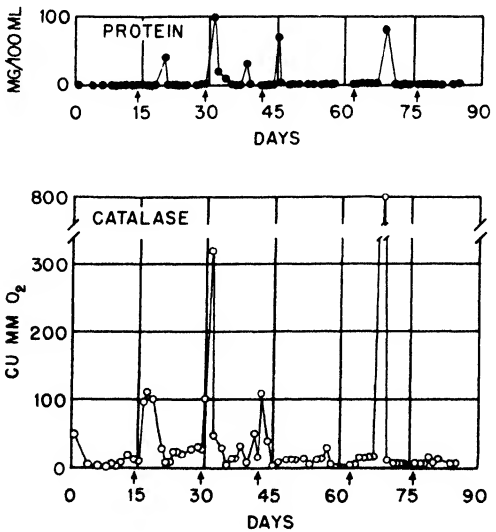


Fig. 14.19—Urinary catalase and protein levels of a rabbit injected intravenously with 0.1 mg of UCl_4 (≎ 0.063 mg of uranium metal) per kilogram in 0.1N sodium acetate. Times of injection are indicated by vertical arrows.

0.1 mg UCl_4/kg had about the same effect in producing catalasuria and proteinuria as 0.01 mg $UO_2Ac_2.2H_2O$/kg. The ultrafilterability of tetravalent uranium through collodion membranes is not more than

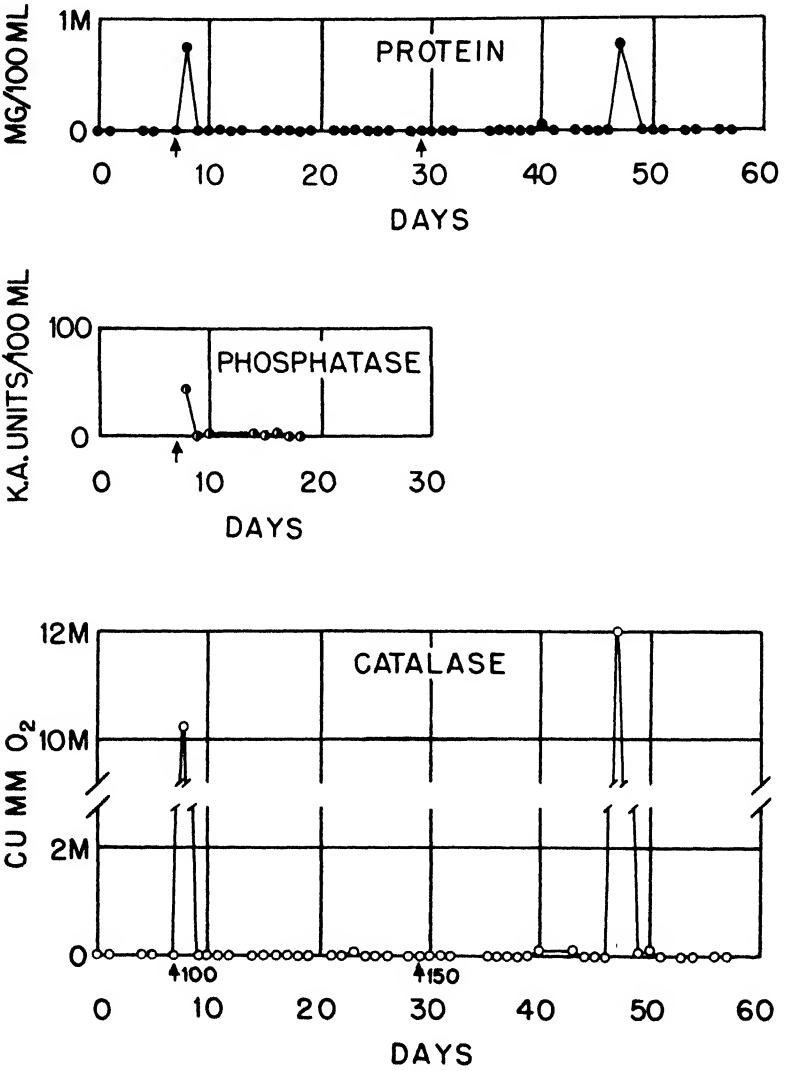


Fig. 14.20—Urinary catalase, phosphatase, and protein levels of a rabbit injected intravenously with various doses of sodium tartrate as indicated by the vertical arrows in the chart. Doses are in milligrams per kilogram.

about 2.5 per cent of that of uranyl uranium at normal values of plasma CO_2 (Chap. 1), so that it is possible that some of the toxicity of injected tetravalent uranium is caused by oxidation to hexavalent uranium in the body.

5.3 Other Nephrotoxic Agents. Two rabbits were injected intravenously with doses of sodium tartrate between 50 and 200 mg/kg. Two rabbits were injected similarly with doses of from 6 to 12 mg of potassium dichromate per kilogram. Another two rabbits were given by the same route 0.5 to 1.0 mg of mercuric chloride per kilogram. Four rabbits were given 4 to 8 mg of mapharsen per kilogram intravenously. Thirteen rabbits were injected subcutaneously with total amounts of from 2 to 10 ml of adrenaline solution (1 : 1,000), some of the doses being given in divided lots. One rabbit was injected intravenously with 4 ml of 20 per cent alcohol per kilogram.

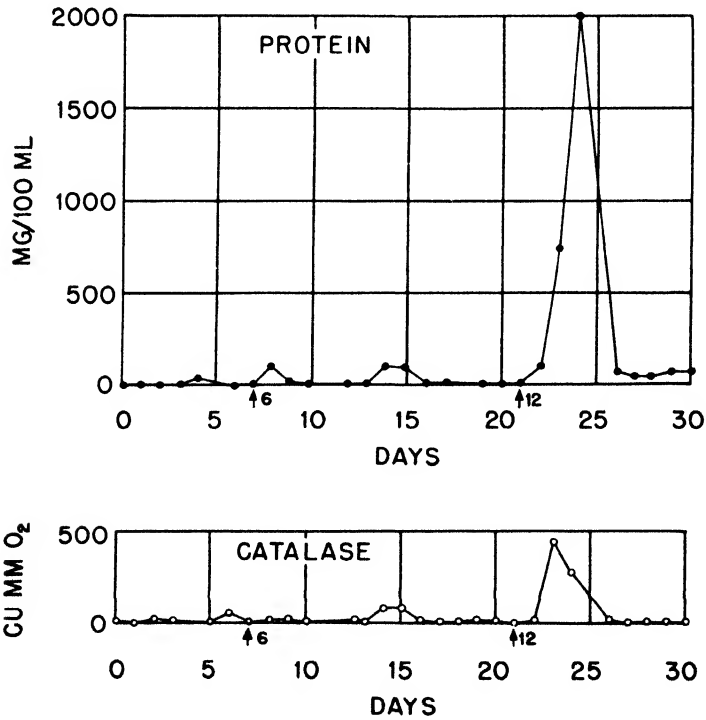


Fig. 14.21—Urinary catalase and protein levels of a rabbit injected intravenously with the doses of potassium dichromate (milligrams per kilogram) that are indicated on the chart at right of vertical arrows. Vertical arrows indicate times of injection.

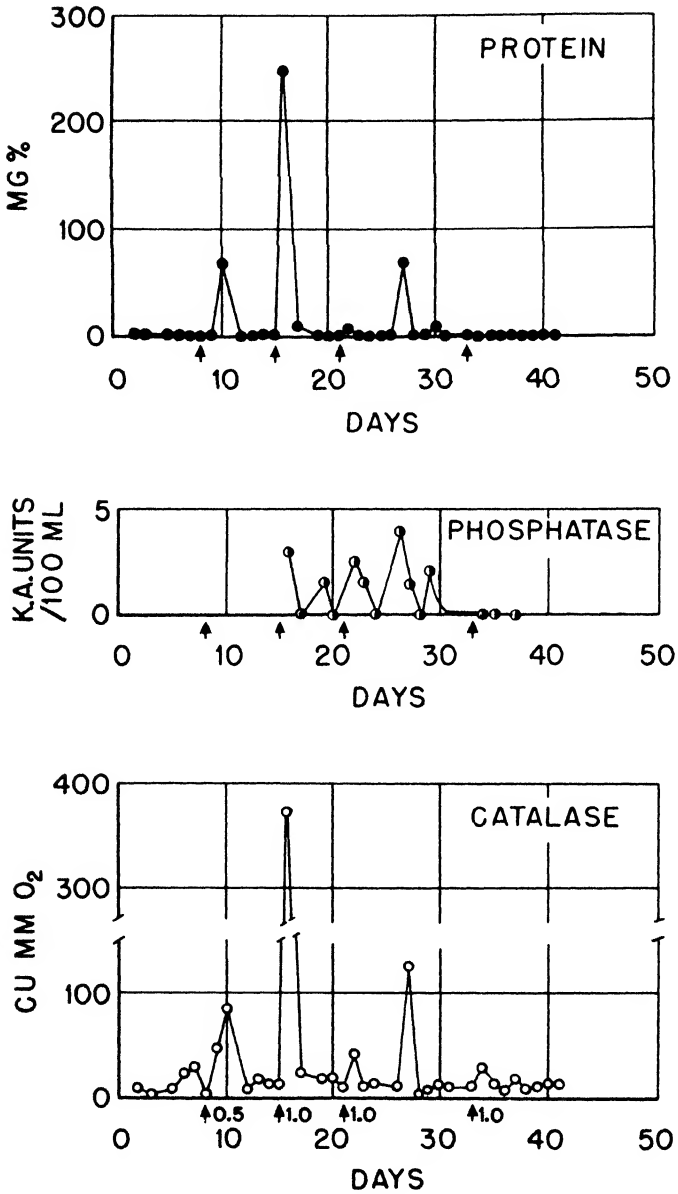


Fig. 14.22—Urinary catalase, phosphatase, and protein levels of a rabbit injected intravenously with the doses of mercuric chloride (milligrams per kilogram) indicated on the chart at right of vertical arrows. Vertical arrows indicate times of injection.

Typical effects of the injections of tartrate, $HgCl_2$, $K_2Cr_2O_7$, and mapharsen are illustrated in Figs. 14.20 through 14.23. It is evident that in sufficient amount all these substances can produce both catalasuria and proteinuria. Of the 13 rabbits injected with adrenaline, 3 were shown to have fibrotic and pitted kidneys upon autopsy and were excluded from further consideration. None of the remaining 10 animals displayed catalasuria after the injection, although 7 of them showed some proteinuria. The animal injected with alcohol developed neither proteinuria nor catalasuria.

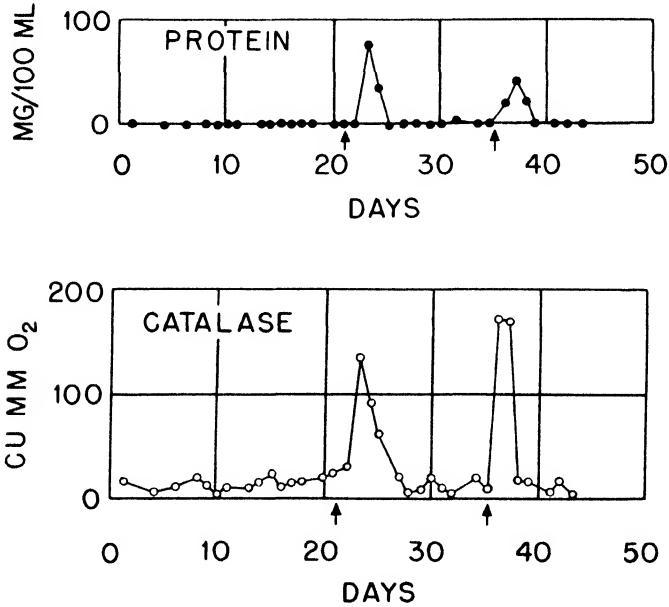


Fig. 14.23 — Urinary catalase and protein levels of a rabbit injected intravenously with 4 mg of mapharsen per kilogram at times indicated by the vertical arrows in the chart.

It is of interest that the only substance found to produce proteinuria without catalasuria is adrenaline, which acts chiefly on the glomerular circulation. The four substances that produced both proteinuria and catalasuria are all known to cause tubular damage, so that it appears that materials producing damage to the epithelial cells of the renal tubule also cause catalasuria. The catalase is released, presumably, by breakdown of the damaged epithelial cells. It is noteworthy that dichromate, which is known to exert its action on the nephron at a

higher level than the other three poisons discussed here, had much more activity in producing proteinuria in relation to its ability to cause catalasuria than the other agents (Fig. 14.21). This means, probably, that dichromate has some effect on the glomerulus as well as on the tubule.

5.4 General Comment. Thus it is seen that the catalase test can be applied to the experimental study of the action of poisons on the nephron, the urinary protein being studied along with it to distinguish glomerular effects from tubular ones. A substance having a direct effect on the glomerulus would be expected to produce proteinuria before catalasuria; if the effect were confined to the glomerulus there would be no appreciable catalasuria in the absence of hemoglobin from the urine. A substance affecting both glomerulus and tubule, such as dichromate, would have a high ratio between the simultaneous urinary levels of protein and catalase, whereas more purely tubular poisons, such as uranyl salts, mercuric salts, arsenicals, and tartrates, would have low values of this ratio.

6. CORRELATION OF CATALASURIA AND PROTEINURIA WITH BLOOD NONPROTEIN NITROGEN AND RENAL HISTOPATHOLOGY

By Eugene Roberts*

Groups of rabbits received single intravenous injections of uranyl nitrate or uranyl acetate ranging from approximately 0.005 mg to 1.13 mg of uranium per kilogram of body weight. Six of the rabbits at the 1.13-mg level were studied independently by Dounce. In the majority of instances the uranium salts were administered in isotonic saline solution. The animals were carefully screened, by biochemical tests for those constituents to be studied, and rabbits showing markedly elevated values were discarded.

In addition, 16 rabbits were selected at random from the stock colony for a study of the normal variation of urinary catalase and protein and blood nonprotein nitrogen for a period of 1 month and were then submitted for histological examination of the kidneys.

The methods for urine collection varied with the dose. In those cases where the dose was relatively high and a marked excretion of protein and catalase was expected, the urine was collected from metabolism cages. When samples were not voided at the desired time, urine was usually obtained by applying gentle pressure to the bladder.

* Catalase determinations, Tien Ho Lan and staff; pathological examination, R. G. Metcalf and staff; blood NPN determinations, F. A. Smith and staff.

At smaller dosage levels samples of urine were obtained at all times by compression of the bladder.

Food and water were placed in double feed cups so that the spilling of either into the urine was minimized in the metabolism cages. Injections were invariably made into the marginal ear vein, from which blood samples were also obtained.

Results. A control group of 16 rabbits selected at random from the rabbit colony was studied with respect to urinary catalase and protein and blood NPN for a period extending over 36 days. These animals were not subjected to the screening procedure by which the rabbits that were studied in the injected group were selected. At the end of the period the animals were sacrificed, and the kidneys were studied histologically. Most of the values for urinary variables were normal with the exception of an occasional high catalase value. However, two of the rabbits exhibited consistently high values for catalase but not for protein. All the values for NPN with the exception of the terminal value of one of the rabbits were within the normal range.

Histological abnormalities ranging from small areas of interstitial nephritis with round cell infiltration to widespread inflammatory processes accompanied by fibrosis with tubular changes were observed in 13 of the 16 animals. There did not appear to be any correlation of the urinary findings with the histological reports. Most of the histological changes observed in this group could, however, be easily distinguished from typical renal effects attributable to uranium poisoning.

The data on urinary protein and catalase excretion at the four dosage levels at which a sufficient number of animals were employed are summarized in Tables 14.5 and 14.6. Table 14.5 contains the cumulative percentage of values of urinary catalase of rabbits at and above various levels between 0 and 200 cu mm of O_2, and Table 14.6 contains the urinary protein values similarly expressed for levels between 0 and 100 mg %. In addition, the results for the uninjected control animals are included in both tables. Inspection of Table 14.5 reveals that, although there were some values above 120 cu mm of O_2 in the uninjected group, none of the screened animals that were employed in the injected studies showed any values at or above 120 cu mm of O_2 during the preinjection period and few values at 80 cu mm of O_2 or above; however, there was little difference between groups in so far as urinary protein excretion was concerned.

There was an increased intensity of response with respect to catalase from the 0.005- to 0.047-mg/kg groups; however, there was little or no difference between the 0.047 groups and those receiving higher

Table 14.5—Cumulative Percentages of Values of Urinary-catalase Activity of Rabbits at and above Various Levels between 0 and 200+ Cu Mm of O_2, Following Injection of Uranyl Acetate or Uranyl Nitrate Hexahydrate*

Catalase value, cu mm of O_2	Uninjected,† %	0.005 mg U/kg‡		0.047 mg U/kg‡		0.14 mg U/kg‡		1.13 mg U/kg‡	
		Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %
≥200	1.4	0.0	7.8	0.0	54.5	0.0	64.4	0.0	50.2
≥160	1.6	0.0	9.8	0.0	56.8	0.0	66.1	0.0	54.1
≥120	2.8	0.0	9.8	0.0	72.7	0.0	66.1	0.0	59.3
≥80	5.0	7.7	19.7	3.4	77.2	0.0	79.6	1.7	68.4
≥40	19.5	21.2	43.2	23.7	88.6	20.0	88.2	8.5	79.2
≥0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
No. determinations	414	52	51	59	48	50	59	59	77
No. animals	16	9	9	12	12	13	13	11	11

Table 14.6—Cumulative Percentages of Values of Urinary Protein of Rabbits at and above Various Levels between 0 and 100+ Mg %, Following Injection of Uranyl Acetate or Uranyl Nitrate Hexahydrate*

Protein value, mg %	Uninjected,† %	0.005 mg U/kg‡		0.047 mg U/kg‡		0.14 mg U/kg‡		1.13 mg U/kg‡	
		Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %	Pre-injection, %	Post-injection, %
≥100	0.0	0	0.0	0	8.3	0	49.2	0	41.1
≥ 80	0.0	0	0.0	0	20.9	0	62.9	0	43.6
≥ 60	0.0	0	0.0	0	22.9	0	67.9	0	46.2
≥ 40	0.3	0	0.0	0	31.3	0	69.5	0	48.8
≥ 20	0.3	0	2.0	0	41.7	0	69.5	0	56.5
≥ 0	100.0	100	100.0	100	100.0	100	100.0	100	100.0
No. determinations	394	52	51	59	48	50	59	30	39
No. animals	16	9	9	12	12	13	13	5	5

*The experimental period includes values obtained through the seventh day after the injection.
†Animals in this group, which were studied for a period of over 36 days, were selected at random from our colony without preliminary screening.
‡The animals in these groups were selected by screening from larger groups prior to inclusion in the experimental groups.

levels. It must be kept in mind that in Table 14.5 the same weight is given to all values above 200 cu mm of O_2. At the levels between 0.047 and 1.13 mg U/kg, between 68 and 80 per cent of all the post-injection catalase values were at 80 cu mm or above as contrasted to the range of 0 to 3.4 per cent for the same animals during the pre-injection period.

There was no urinary protein in any of the experimental animals during the preinjection period (Table 14.6). There was an increase in the number of values at 40 mg or higher after the injection from 0.005 mg, at which there were none, to 0.14 mg, at which 70 per cent of the values fell in this group. There was no appreciable difference between the 0.14- and the 1.13-mg level. If it is conservatively considered that the number of values for catalase activity at or above 80 cu mm of O_2 are of definite biological significance, indicating uranyl damage, and that the number of values at or above 40 mg % of protein are of similar significance in these experiments, it can be seen that, in the case of each dosage level employed, a larger percentage of the postinjection values exceeded such a catalase value than exceeded the protein value. This is especially striking in the 0.047-mg group, where the value for catalase is 77 per cent, whereas that for protein is 31 per cent. This analysis indicates that the catalase is more sensitive than the protein in so far as the total number of significantly high values is concerned.

The greater sensitivity of the catalase determination for rabbits is further emphasized in Fig. 14.24, in which the mean urinary catalase and protein and the blood NPN levels are plotted for 12 rabbits before and after the intravenous injection of 0.047 mg U/kg. The rise in catalase is seen to take place at least 24 hr before a significant rise in the urinary protein and blood NPN. The peak attained by the catalase is also higher proportionately than the peak attained by the urinary protein and the blood NPN.

The changes with time after the administration of the various doses of uranyl nitrate hexahydrate are plotted for blood NPN in Fig. 14.25. It is evident that the blood NPN does not respond until the 0.047-mg level is reached.

In so far as doses ranging between 0.047 and 1.13 mg U/kg are concerned, there was an unquestionable increase in the range exhibited both by urinary catalase and protein after the injection as compared to the preinjection period. In rabbits receiving 0.0047 mg U/kg there was an apparent increase in the range of the catalase excretion of some of the rabbits after the injection but virtually no change in the urinary protein. At this level also there was no increase in blood

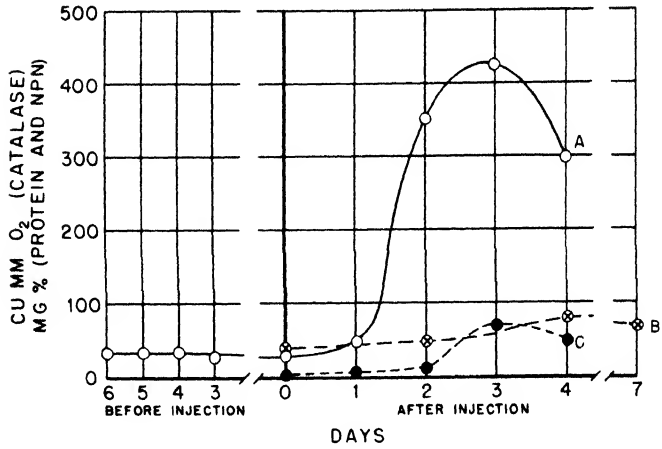


Fig. 14.24—Mean urinary catalase and protein levels and blood NPN level of 12 rabbits before and after intravenous injection of 0.1 mg of uranyl nitrate hexahydrate (≎ 0.047 mg of uranium metal) per kilogram. Curve A, catalase; curve B, NPN; curve C, protein.

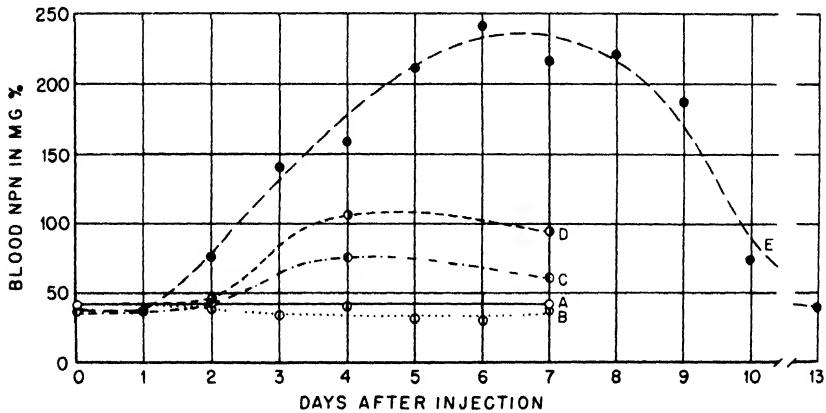


Fig. 14.25—Effect of various doses of uranyl nitrate hexahydrate upon the variation of blood NPN level with time. Curve A, control; curve B, 0.01 mg of uranyl nitrate hexahydrate (≎ 0.0047 mg of uranium metal) per kilogram; curve C, 0.1 mg of uranyl nitrate hexahydrate (≎ 0.047 mg of uranium metal) per kilogram; curve D, 0.3 mg of uranyl nitrate hexahydrate (≎ 0.14 mg of uranium metal) per kilogram; curve E, 2.4 mg of uranyl nitrate hexahydrate (≎ 1.13 mg of uranium metal) per kilogram. All animals were rabbits injected intravenously at zero time.

NPN. Because only one or two high catalase values occurred after the injection in these animals, there were probably significant differences in only two cases when the postinjection and preinjection means were tested (Table 14.7). However, in the case of four of the other animals the variability of the catalase excretion was significantly increased after the injection over the preinjection range. Thus it may be concluded that there was attained in the latter experiment a level of uranyl nitrate hexahydrate that produced a significant alteration in the catalase response of at least six of the nine injected animals but had no detectable effect with respect to the other two variables studied. One rabbit showed a significantly higher standard deviation in the control period than after the injection.

The histological examination of the kidney of the animals receiving 1.13 mg U/kg showed that this dose produced severe renal injury characterized by typical tubular necrosis and by glomerular changes. Increased evidences of repair were observed in animals the kidneys of which were submitted for study at longer intervals after the injection. There were also severe renal changes characteristic of uranyl poisoning in the 12 rabbits the tissues of which were studied at a level of 0.14 mg U/kg. In the animals given 0.047 mg U/kg the pathological changes in the kidneys, as in the previous groups, were typical of uranyl poisoning but were considerably less severe than those observed at the 0.14-mg level. In the case of one rabbit that showed a marked increase in urinary catalase and some urinary protein but no increase in blood NPN, no renal histological injury was detected. At the 0.005-mg level, the pathological findings in the kidney were minimal as regards damage definitely attributable to uranyl poisoning. Only three out of nine rabbits showed a few tubules containing necrotic cells and a small amount of epithelium of the type ordinarily thought to be regenerative in nature.

It may be concluded from this experiment that when fairly large amounts of uranyl salts are injected intravenously into rabbits the toxic response can be detected in urinary catalase and protein excretion, in the elevation of blood NPN, and in the histological evidence of typical renal tubular alteration. A decrease in dosage results in a decrease in the intensity of the response in all these variables with the increase in blood NPN disappearing before the other changes. A lower dose that produces a significant change in the catalase excretion but little or no alteration in protein excretion or in renal histology can also be established. It may also be concluded that urinary catalase is more sensitive in the case of the rabbit than the other two biochemical variables in that it appears earlier and reaches proportionately higher levels at lower dosages.

7. INHALATION EXPERIMENTS

By Eugene Roberts

7.1 Exposure of Rabbits to Inhalation of a Dusty Atmosphere Containing Approximately 0.5 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter. The biochemical studies both with respect to urinary findings and blood NPN and the results of the histological examination of the kidneys of the 12 rabbits studied at this level are discussed in considerable detail in Chap. 10, Sec. 12.9c, as illustrative of the type of procedure employed here. It can be concluded from the results that this level of exposure was injurious to the kidney of the rabbit as reflected equally well by all the criteria employed.

7.2 Response of Rabbits to 30-day Inhalation Exposure to a Dusty Atmosphere Containing Approximately 0.6 Mg UO_2F_2/cu m. Nine rabbits were exposed to a dusty atmosphere containing UO_2F_2 at a concentration of 0.6 mg/cu m for 30 days, after a 4-day preliminary study. Determinations were made of the body weight, urinary catalase, protein and phosphatase contents, blood NPN, and urea nitrogen throughout the experiment. Histologic examination of the kidneys was made at the end of the exposure.

No deaths attributable to the exposure occurred. At no time during the experiment did any of the eight surviving rabbits exhibit an abnormal elevation in blood NPN or urea.

The urinary catalase activity and protein content after the start of exposure were much less than those observed in the rabbits exposed to 0.5 mg of uranyl nitrate hexahydrate per cubic meter. No value for protein greater than 85 mg % was recorded, although every rabbit showed at least a trace of protein during the exposure period. Three rabbits had no value for catalase of 60 cu mm of O_2 or above, whereas groups of two each had one, two, and four values in that category. Two of the rabbits had catalase activity that was consistently above 60 cu mm of O_2. Only one value above 200 cu mm of O_2 was noted during the entire experiment.

The differences between the preexposure and postexposure means for catalase were not all in the same direction (Table 14.8). In two of the rabbits the postexposure means were smaller. When the differences of the seven rabbits that had higher postexposure means were tested statistically, it was found that only in two were they significantly higher than the control values, giving p values of 0.01 and 0.02, respectively. However, the p values for the differences between standard deviations were 0.01 or less for five of these animals, indicating a significant catalase response to exposure in these rabbits. In general, the animals with the highest catalase values also had the

Table 14.7—t Ratios of Differences between Preinjection and Postinjection Means and Standard Deviations of Catalase Determinations for Rabbits Injected with 0.0047 Mg of Uranyl Nitrate Hexahydrate per Kilogram of Body Weight*

Animal No.	No. of determinations A†	No. of determinations B‡	Mean A†	Mean B‡	Standard deviation A†	Standard deviation B‡	$M_B - M_A$	t ratio for means	p for means	$\sigma_B - \sigma_A$	t ratio for σ's	p for σ's
69	5	5	57.4	63.2	22.8	15.6	5.8	0.47	0.65	−7.2	−0.82	0.41
72	4	5	50.8	98.8	18.6	128.8	48.0	0.73	0.49	110.1	2.67	0.01
73	5	2	33.6	816.5	18.1	181.7	782.9	11.29	< 0.01	163.6	1.80	0.07
74	6	6	17.2	46.3	14.2	21.8	29.1	2.74	0.02	7.6	1.01	0.31
76	7	6	41.3	41.8	29.6	35.4	0.5	0.03	0.98	5.7	0.44	0.66
77	7	6	26.1	20.5	35.6	10 0	−5.6	−0.37	0.72	−25.6	−2.58	0.01
63	6	7	7.3	9.6	5.5	14.8	2.2	0.35	0.73	9.3	2.17	0.03
64	6	7	5.0	32.0	1.9	61.1	27.0	1.08	0.30	59.2	3.62	< 0.01
66	6	7	28.0	178.4	29.5	330.9	150.4	1.10	0.29	301.4	3.39	< 0.01

* Table prepared by L. S. Kogan.
† A = preinjection values.
‡ B = postinjection values.

Table 14.8—t Ratios of Differences between Preexposure and Postexposure Means and Standard Deviations of Catalase Determinations for Rabbits Exposed to UO_2F_2 at 0.6 Mg/cu m

Animal No.	No. of determinations A*	No. of determinations B†	Mean A*	Mean B†	Standard deviation A*	Standard deviation B†	$M_B - M_A$	t ratio for means	p for means	$\sigma_A - \sigma_B$	t ratio for means	p for σ's
561	4	24	7.8	5.5	12.3	7.8	−2.3	−0.50	0.62	−4.5	−1.01	0.31
562	4	24	6.0	21.5	9.5	22.6	15.5	1.34	0.18	13.1	2.79	0.01
563	4	24	7.5	34.6	10.0	22.6	27.1	2.33	0.02	12.6	2.63	0.01
564	4	24	24.8	42.3	14.0	50.7	17.5	0.68	0.50	36.7	4.15	< 0.01
565	4	20	15.2	17.1	5.9	16.1	1.9	0.22	0.83	10.2	3.13	< 0.01
566	4	15	23.0	18.7	19.7	13.9	−4.3	−0.50	0.62	−5.8	−0.79	0.43
567	4	23	13.0	15.2	12.1	31.5	2.2	0.14	0.89	19.4	3.07	< 0.01
568	4	25	13.0	13.0	16.8	19.8	0	0	1.00	3.0	0.46	0.65
569	4	25	21.0	80.5	28.9	36.7	59.5	3.08	< 0.01	7.8	0.68	0.50

* A = preexposure values.
† B = postexposure values.

highest protein values, the correlation between these two variables being 0.9. No abnormally high urinary-phosphatase values occurred.

In seven of the eight cases the kidneys showed some damage, slight to moderate in degree, which could be attributed to the exposure. The kidney of one animal was normal. Since these readings were made in a manner reported in a previous section [see Chap. 10, Sec. 12.9c, on inhalation exposures], a correlation of the biochemical variables with kidney damage ratings was possible. No significant correlation was found in any case (Table 14.9).

Table 14.9—Correlations of Biochemical Determinations with Kidney-damage Ratings

Variable	Kidney-damage rating
Catalase	0.05
Protein	−0.17
NPN	−0.29
Urea nitrogen	−0.42

The renal damage produced by the exposure in this experiment was less than that produced by 0.5 mg of uranyl nitrate hexahydrate per cubic meter. This was reflected in a decreased intensity in the response from both a biochemical and histological point of view. The smaller changes also resulted in a much lower correlation between biochemical variables and renal damage than in the 0.5-mg study.

7.3 Urinary-catalase and Alkaline-phosphatase Activity, Protein Content, and Renal Histological Changes in Rabbits Exposed for 2 to 12 Days to an Atmosphere Containing Approximately 2 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter. Preliminary experiments with rats, rabbits, and dogs exposed to dusty atmospheres containing varying concentrations of uranyl nitrate showed that this mode of administration also caused the increased excretion of catalase, phosphatase, and protein, with the catalase apparently appearing in abnormal amounts before the other two indicators.

The present experiment was designed to study further the response of rabbits with respect to urinary catalase and phosphatase activity and protein during exposure to uranyl nitrate dust, and to examine simultaneously the accompanying histological changes in the kidney.

Experimental Methods. Twenty-four rabbits were exposed by inhalation to atmospheres containing approximately 2 mg of uranyl nitrate

hexahydrate per cubic meter. The methods for the determination of the constituents studied have been detailed elsewhere (Chap. 5). Great care was taken in the collection of the samples for analysis from the metabolism cages, since contamination by food and feces renders catalase and phosphatase values falsely high. All samples in which contamination was observed were discarded. Of 36 rabbits originally available, 12 were discarded because of abnormal values for one or more of the three urinary constituents during a preexperimental screening period.

Results. Values of phosphatase, protein, and catalase were determined for each animal for 11 days prior to the first exposure. Any positive values of phosphatase or protein during the experimental period were considered significant, since in the control period none of the animals employed showed any urinary phosphatase or protein. When a value for catalase of at least 60 cu mm of O_2 or above was attained, a value higher than that reached by any of the rabbits during the control period, the animal was removed from exposure. This occurred in the case of 12 rabbits after 14 hr of exposure, in 17 animals after 16 hr, in 20 after 22 hr, and in 21 after 26 hr. Three rabbits showed no abnormal catalase values even after 40 hr of exposure, although at some time all three had values for urinary protein ranging from 10 to 25 mg %, and one of them showed a phosphatase activity of 40 units. Twelve of the animals were sacrificed at the termination of their exposure, and 12 were kept for subsequent postexposure study. Of the 12 that were not sacrificed, 4 died within 16 days of the initial exposure, whereas 8 showed a return to normal urinary values within 12 days and survived for 70 days with no outward symptoms of ill health.

Table 14.10 contains a summary of the results for all the animals. The animals are arranged in descending order of catalase values obtained on the day after the last exposure. Column 2 shows the highest values attained during the control period; the highest value for catalase shown by any of the animals was 51 cu mm of O_2. No urinary phosphatase or protein was noted during this period. From column 3 it may be seen that varying lengths of exposure were required to raise the catalase activity above 60 cu mm of O_2 for the different animals. In fact, rabbits 232, 240, and 241 never attained that level. Column 4 shows the values obtained on the day after the last exposure. Although 21 of the 24 animals showed values for catalase of 60 cu mm of O_2 or above, on the day after the last exposure, only 15 showed a trace of protein, and only 7 had positive phosphatase values. The highest postexposure values for all the animals that were not sacrificed are recorded in column 5. Ten of the twelve rabbits had values of catalase

Table 14.10--Response of Rabbits to Exposure to a Dusty Atmosphere Containing Approximately 2 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter

	Highest values obtained during 11-day control period				Values obtained on day after last exposure			Highest postexposure values			
(1) Rabbit No.	Urinary phospha-tase, K-A* units	Urinary protein, mg % (2)	Urinary catalase, cu mm O_2	(3) Total exposure Hr Min	Urinary phospha-tase, K-A* units	Urinary protein, mg % (4)	Urinary catalase, cu mm O_2	Urinary phospha-tase, K-A* units	Urinary protein, mg % (5)	Urinary catalase, cu mm O_2	(6) Pathological findings in the kidney
252†	0	0	37	13 40	80	150	> 200				Necrosis of the corticomedullary tubular epithelium and some glomerular degeneration
244	0	0	40	13 40	55	130	> 200	25	800+	200+	Died on 12th day after first exposure; moderately severe, typical uranium renal injury, 3+, with 3+ regeneration
230	0	0	21	13 40	3	120	> 200	0	250	200+	Died 16 days after first exposure; total renal damage 1+ with 1+ necrosis
233	0	0	16	13 40	25	60	> 200	3	200	200+	Died 12 days after first exposure; total renal damage 3+; 2+ necrosis and 2+ regeneration
234†	0	0	36	13 40	3	50	> 200				Interstitial nephritis; evidence suggesting uranium damage
236†	0	0	33	13 40	55	15	> 200				Necrosis and typical regeneration of the corticomedullary tubular epithelium; 2+ total damage
243	0	0	46	13 40	40	tr.	> 200	5	250	164	Died 11 days after first exposure; moderately severe typical uranium renal injury, 3+
242†	0	0	23	15 40	0	20	152				No damage characteristic of treatment
250	0	0	42	13 40	0	0	150	0	120	204	‡
237†	0	0	33	13 40	0	0	130				Mild damage consisting of mild degeneration of corticomedullary tubular epithelium
238	0	0	23	25 50	0	100	129	0	30	63	‡
248	0	0	34	15 40	0	10	126	0	50	78	‡
249†	0	0	51	15 40	0	5	124				Chronic interstitial nephritis
245	0	0	15	13 40	0	0	114	0	110	127	‡
239	0	0	25	15 40	0	tr.	98	25	120	>200	‡
247	0	0	14	13 40	0	0	98	0	160	>200	‡
235†	0	0	28	13 40	0	0	91				Chronic interstitial nephritis
246	0	0	15	22 0	0	95	70	0	10	35	‡
231†	0	0	45	15 40	0	0	64				No damage characteristic of treatment
229†	0	0	20	22 0	0	75	61				Chronic interstitial nephritis
251†	0	0	34	22 0	0	40	60				Chronic interstitial nephritis
232	0	0	51	40 30	0	0	7	0	25	44	‡
240	0	0	45	40 30	0	0	6				No damage characteristic of treatment
241†	0	0	18	40 30	0	0	5				Mild typical regeneration of corticomedullary tubules and mild degeneration; total damage 1+

* K-A represents King-Armstrong.

† Sacrificed on day after last exposure.

‡ These animals appeared to be clinically normal on the seventieth day after first exposure. All urinary values had returned to normal by 11 days after first exposure.

over 60 cu mm of O_2 during this period, and all 12 showed some protein. Only four had measurable phosphatase activity. In this, as in other experiments with rabbits, catalase tended to appear earlier in some cases than phosphatase or protein.

All four animals that died after the cessation of exposure showed phosphatase, protein, and catalase in abnormal amounts in the urine after 2 days of exposure (13 hr and 40 min total). The exact value of the catalase is not known, but in every case the values were considerably higher than 200 cu mm of O_2, values higher than any observed at a comparable time among the eight surviving animals. On the day preceding death, the protein and catalase values were still abnormal, but phosphatase was normal. All four of these animals showed definite kidney injury compatible with that ordinarily found in uranium poisoning (Table 14.10).

It is interesting to summarize the differences between the eight animals that survived and the four that died in the group of twelve that were not sacrificed. Of the eight animals surviving 70 days after the first exposure, three had at least one catalase value of 200 cu mm of O_2 or over, whereas five never reached this level. All four animals that died exceeded the 200 level at one time or another. One of the eight animals living through the experiment had at least one phosphatase determination of three or above, but seven of the eight never reached this level. All four animals that died had at least one phosphatase value of 3 or higher. None of the eight animals that lived had a protein value in excess of 160 mg %, whereas four of the animals that died before the conclusion of the experiment had at least one urinary protein value of 200 mg % or higher. Thus, there was a greater sensitivity to the effects of uranium in the animals that died than in those that survived after the cessation of the exposure as reflected in the quantities measured.

Of the 12 animals sacrificed on the day after their last exposure the three that showed catalase values over 200 and significantly high protein and phosphatase values also showed evidence of early renal changes compatible with the nature of the treatment. Two animals that showed catalase values of 64 and 152 cu mm of O_2, respectively, and 0 and 20 mg % of protein, respectively, but no phosphatase on the day after the last exposure, showed no renal damage characteristic of the treatment; whereas an animal with a catalase activity of 130 cu mm of O_2 but no other urinary changes had mild renal damage that consisted of slight degeneration of the corticomedullary tubular epithelium. Of the two rabbits showing no significant urinary catalase at the time of sacrifice, one showed mild typical degeneration of the corticomedullary tubules and mild regeneration, whereas the other

one showed no damage characteristic of the treatment. In the remaining animals the histological results were masked by the presence of a chronic interstitial nephritis. High catalase values, in general, occur before the maximal histological injury is manifest.

The urinary enzyme and the protein levels of the eight animals that survived for 70 days after the first exposure returned to normal by 12 days after the first exposure and remained within a normal range up to the thirty-third day, from which time on no further determinations were made.

This experiment proves conclusively that rabbits respond to the inhalation of uranyl nitrate dust in the same manner as to the parenteral administration of soluble uranyl compounds.

7.4 Response of Rabbits to 99-day Inhalation Exposure to a Dusty Atmosphere Containing Approximately 0.3 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter. Thirteen rabbits were employed in this study. Data are presented for a 16-day preliminary period and for 99 days following the first day of exposure. The experiment was terminated at the end of 150 days.

For the time reported, three deaths occurred. Of the 13 experimental animals, only these 3 exhibited a significant loss in weight at any time. The animal that died at 42 days (rabbit 1) exhibited an elevated blood NPN 7 days before death and a catalase value of 1,275 cu mm of O_2 6 days before death. Rabbit 12, dying on the 33rd day, was evidently severely affected by the treatment, since it exhibited extremely high urinary catalase and protein values between the 8th and 17th days, returning to normal between the 29th and 33rd day. Rabbit 13 had high protein values only on the 2 days prior to death, which occurred on the 97th day of exposure. The histologic findings in the kidneys of these animals are not available.

The remaining 10 animals appeared clinically normal on the 99th day. At no time during the experimental period were any significantly elevated values for NPN observed. Eight of these ten rabbits had no detectable urinary protein during the experimental period, whereas the other two had one and three positive values, respectively.

The catalase values for all the experimental animals were analyzed statistically in the same manner as in the experiment with 0.6 mg of uranyl fluoride (Table 14.11). The differences between the preexposure and postexposure means were all in the same direction, with the postexposure means higher. When the differences between the means were tested it was found that four individual animals had differences so large that the probability of getting a difference as large or greater than these was 1 to 7 chances out of 100. The evidence from all 13 animals indicates that this exposure to uranyl nitrate increased the

Table 14.11—t Ratios of Differences between Preexposure and Postexposure Means and Standard Deviations of Catalase Determinations for Rabbits Exposed to 0.3 mg of Uranyl Nitrate Hexahydrate per Cubic Meter*

Animal No.	No. of determinations		Mean		Standard deviation		$M_B - M_A$	t for means	p for means	$\sigma_B - \sigma_A$	t for σ's	p for σ's
	A†	B‡	A†	B‡	A†	B‡						
1	10	16	48.9	120.9	54.7	311.2	72.0	0.72	0.47	256.5	4.55	<0.01
3	9	28	26.2	30.2	15.9	23.0	4.0	0.48	0.63	7.1	1.47	0.14
4	9	28	42.8	69.6	42.1	118.7	26.8	0.66	0.51	76.6	4.10	<0.01
5	6	23	54.7	82.4	37.7	65.6	27.7	0.99	0.32	27.9	1.91	0.06
6	10	28	37.7	47.9	18.2	39.8	10.2	0.78	0.44	21.6	3.22	<0.01
7	9	29	19.7	50.9	15.0	32.8	31.2	2.74	0.01	17.8	3.20	<0.01
8	10	29	58.2	63.0	8.0	34.0	4.8	0.44	0.66	26.0	5.41	<0.01
9	10	27	31.0	63.7	29.2	66.6	32.7	1.49	0.14	37.4	3.34	<0.01
10	10	27	22.2	30.4	22.8	39.6	8.2	0.62	0.54	16.8	2.26	0.02
12	10	16	22.4	158.2	16.6	236.1	135.8	1.80	0.07	219.5	5.24	<0.01
13	10	25	22.5	49.7	24.2	59.0	27.2	1.40	0.16	34.8	3.50	<0.01
14	10	25	36.2	91.8	35.3	85.7	55.6	1.97	0.05	50.4	3.49	<0.01
16	10	29	9.3	38.4	11.7	50.2	29.1	1.80	0.07	38.5	5.42	<0.01

* Table prepared by L. S. Kogan.
† A = preexposure values.
‡ B = postexposure values.

Table 14.12—Response of Dogs to Exposure to a Dusty Atmosphere Containing Approximately 0.3 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter

Time of sample, days	Urine: Catalase, cu mm O_2	Urine: Protein, mg %	Blood NPN, mg %	Diodrast clearance, ml/min
		Animal 575, Preexposure		
82	13	0		
50	14	0		
25	2	0	38	234
12	10	0	31	230
8	0	0		
6	3	0		
		Postexposure		
2	14	0		
7	30	550	42	223
15	20	0		
22	9	0	38	194
27	37	65		
30	106	0		
35	8	0	44	347
49	106	0	34	196
63	7	0	42	177
		Animal 636, Preexposure		
81	25	25		
57	9	0		
32			52	159
18	2	0	39	189
10	0	0		
8	7	0		
		Postexposure		
5	51			
7	24	5	44	225
17	14	0		
21	18	65	40	179
35	15	0		
41	60	0	38	153
55	21	0		177
		Animal 666, Preexposure		
68	4	0		
40	10	0	29	207
13	3	0	23	237
7	36	0		
5	1	0		
3	12	0		
		Postexposure		
21*	180	200	37	233
28	75	10		
31	13	0		
35	73	0	32	318
49	40	0	39	210
63	118	0	30	201
91				

* No determinations could be made prior to this time because this animal suffered a prolapse of the uterus during heat.

catalase activity of the urine. A still better indication that this is correct is that the p values for the t ratios of the differences in the standard deviations were less than 0.01 in 10 cases, and 0.02, 0.06, and 0.14 in the remaining three. Thus the latter statistic can be used for diagnostic purposes in rabbits under conditions in which the urinary protein and blood NPN fail to show a consistent response.

In both injection and inhalation studies there has been obtained in rabbits a level of uranium intoxication that produced a response in catalase excretion but no consistently significant change in urinary protein or blood NPN.

7.5 Response of Dogs to Inhalation Exposure to Dusty Atmospheres Containing Approximately 0.3 and 1.9 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter. Blood NPN and urinary catalase and protein were studied concomitantly with renal clearances in three dogs exposed to 0.3 mg/cu m and two dogs exposed to 1.9 mg of uranyl nitrate hexahydrate per cubic meter, respectively. Urine samples were obtained at all times by catheterization with a sterile wing-tipped soft rubber catheter. The only clearance to be discussed is that of diodrast (see Chap. 5, Sec. 2.2c). The duration of the experiment was approximately 130 days, and data are presented here for 63 days of exposure.

0.3-mg Level. There were no significant changes in blood NPN in the dogs at this level, although there was an increased excretion of catalase, and one or two abnormal values were observed for urinary protein in each of the animals. The intensity and duration of the response was not striking and was certainly less than that shown by the two dogs at the 1.9-mg level (Table 14.12). In two of the dogs no decrease in diodrast clearances was observed, and, peculiarly enough, each showed one abnormally high value during the period of study. The third dog, in addition to showing an abnormally high diodrast clearance on the thirty-fifth day, had a clearance of only 78 per cent of normal on the sixty-third day.

1.9-mg Level. In two dogs at this level there were significant rises in catalase and protein excretion and decreases in diodrast clearance as a result of the exposure. Values for blood NPN were normal at all times (Table 14.13).

In dog 656 the diodrast clearance fell to approximately 21 per cent of the normal level on the 9th day after the start of exposure, at which time urinary protein was at the abnormally high level of 125 mg %, but the catalase was within the control range for this animal. Subsequently the catalase activity was high on the 13th, 20th, and 23rd days, attaining a maximal value of 260 cu mm of O_2 on the 13th day, whereas only small amounts of urinary protein were noted at these times. The

Table 14.13—Response of Dogs to Exposure to a Dusty Atmosphere Containing Approximately 1.9 Mg of Uranyl Nitrate Hexahydrate per Cubic Meter

Time of sample, days	Urine		Blood NPN, mg %	Diodrast clearance, ml/min
	Catalase, cu mm O_2	Protein, mg %		
	Animal 656, Preexposure			
71	48	20		
50	83	0		
25	22	0	59	156
14	120	20	33	154
5	5	0		
	Postexposure			
9	46	125	37	35
13	260	40		
20	183	0		
23	139	10	37	86
37	15	0	38	138
51	6	0	35	131
64	14	0	33	134
	Animal 695, Preexposure			
91	10	5		
48	6	0		
27	15	0	36	244
10	15	0	33	259
6	0	0		
4	5	0		
0	10	20		
	Postexposure			
2	25	0		
4	112	0		
7	1,785	300	39	175
14	670	150		
21	191	50	36	179
28	5	0		
35	15	0	41	192
49	2	0	37	246
63	281	100	33	203

diodrast clearance on the 23rd day was 55 per cent of that during the control period. The three subsequent determinations extending to the 64th day showed normal protein and catalase levels and a consistent diodrast clearance of approximately 87 per cent that of normal.

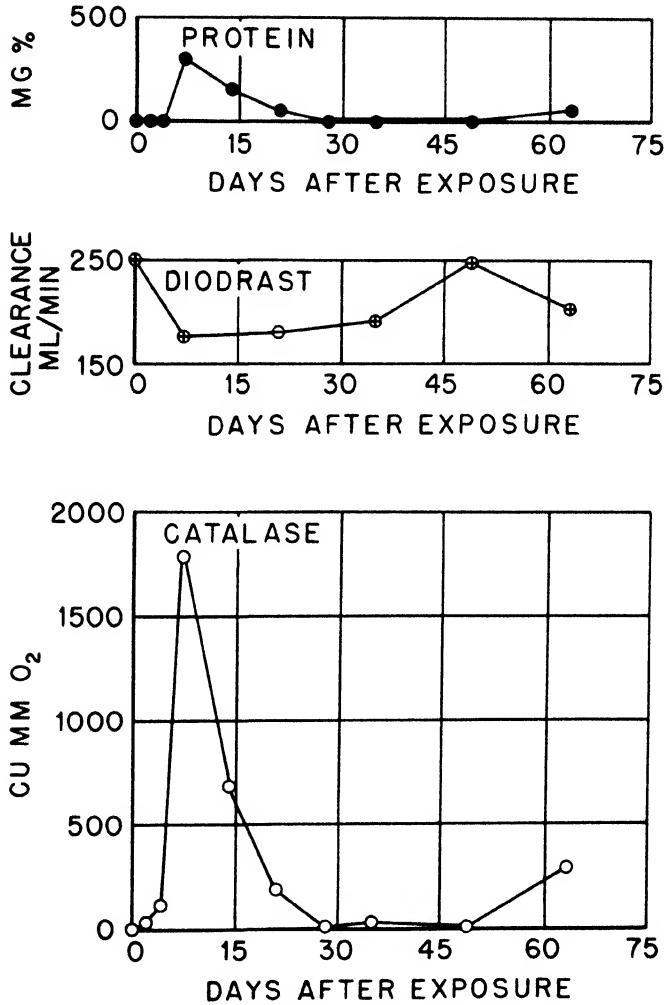


Fig. 14.26 — Urinary catalase and protein levels and diodrast clearance of a dog exposed for 8 hr/day to an atmosphere containing 1.9 mg of uranyl nitrate hexahydrate (≏ 0.89 mg of uranium metal) per cubic meter.

Dog 695 had an elevated catalase of 112 cu mm of O_2 on the 4th day of exposure, but no protein. Both the catalase and protein attained maximal values of 1,785 cu mm of O_2 and 300 mg %, respectively, on the 7th day. These values were accompanied by the lowest diodrast

clearance found during the experimental period for this animal, 69 per cent of the control value. On the 28th, 35th, and 49th days of exposure, catalase and protein were normal, and the diodrast clearance attained a normal level on the 49th day. However, on the 63rd day catalase and protein were again elevated, and the diodrast had again fallen to a value of 81 per cent of the normal. It is possible that this represents intermittent renal damage as a consequence of the exposure. The results for this animal, which showed very clearly the relations between the quantities measured, are plotted in Fig. 14.26.

Summary. The data indicate that at the 1.9-mg level there was definite functional renal change in the exposed animals as expressed by a lowered diodrast clearance and by the increased excretion of catalase and protein. This was probably accompanied by observable histologic changes, since such changes are found in other similarly exposed animals. The functional changes in the three dogs at the 0.3-mg level were minimal as reflected by the criteria employed. This is in agreement with the histologic and clinical observations in 20 other dogs exposed to this concentration of uranyl nitrate hexahydrate.

8. STUDIES OF CATALASE IN HUMAN URINE*

8.1 Normal Levels for Human Urinary Catalase. In order to evaluate and interpret the urinary-catalase activity data on the urine of workmen from various plants, catalase determinations have been made on more than 200 normal urines collected from employees of the Manhattan District at the University of Rochester. A statistical analysis was made on single samples of urine collected from 219 individuals without selection. The results are shown in Table 14.14. The study on these normal persons will be given in more detail in Sec. 9.1 of this chapter.

Table 14.14—Results of Urinary-catalase Determinations on Normal Control Group at Rochester

	Male	Female	Total
Number	150	69	219
Mean	2.63	7.63	4.12
S.D.	6.37	13.30	9.37

* Studies of persons not exposed to uranium compounds.

8.2 Urinary-catalase Levels in Patients Having Hypertension, Scarlet Fever, or Known Kidney Damage.* It was found that several patients in this group had urinary-catalase values above the normal level and that protein was also present in some cases. Urinary-phosphatase activity was essentially zero in the case of patients with questionable renal function or hypertension. From the results of experiments with dogs and rabbits it appears that occasional urinary-catalase values as high as 50 in human beings should not be considered as definitely abnormal, and even repeated values as high as 50 would presumably indicate tubular damage of a minimal amount.

Table 14.15—Catalase Activity in Patients' Urine

Patient No.	Diagnosis from history sheets	Catalase activity, cu mm O_2 in 15 min	Protein, mg %
1	Nephritis (?)	10	100
2	Renal disease	24	100
3	Glomerulonephritis, nephrotic syndrome:		
	Highest value	36,000	1,000
	Lowest value (just before death)	375	
4	Chronic pyelonephritis, NPN = 300 mg %, CO_2 = 20 vol. %	6	0
5	Hypertension, fair renal function	48	65
6	Hypertension, possible renal insufficiency	17	0
7	Hypertension, carcinoma of rectum (NPN, 58 mg %)	75	30
8	Possible hypertension	24	35
9	Hypertension	47	0
10	Urinary infection, hypertension	101	100
11	Hypertension (?)	20	0
12	Unknown (history not available)	98	60
13	Scarlet fever (two values)	6; 5	
14	Scarlet fever (two values)	28; 30	
15	Scarlet fever (two values)	28; 27	
16	Scarlet fever (two values)	248; 7	

The case of patient 3 with glomerulonephritis with nephrotic syndrome, recorded in Table 14.15, is of particular interest, since it illustrates the amount of catalase that can pass into the urine when the kidney tubules are badly damaged. Red- and white-cell counts in the urine of this patient showed that the contribution of these cells to the urinary catalase would not have corresponded to more than 200 cu mm of O_2 at any time.

* The urines of these patients were obtained by Bassett and Van Alstein.

8.3 Studies in Patients Receiving Mapharsen and Mercurial Diuretics Therapeutically. These results are given in Table 14.16. The amounts of mapharsen and salyrgan used were apparently not large enough to produce catalasuria and proteinuria. In the rabbit it was necessary to inject at least 4 mg of mapharsen per kilogram in order

Table 14.16—Catalase and Protein Levels of Urines of Patients Injected with Compounds of Mercury and Arsenic

Patient	Drug	Dosage	Duration of treatment	Urinary level	
				Catalase	Protein
B. B.	Mercuhydrin	2 ml	1 injection	16	
Y. F.	Mercuhydrin	2 ml	1 injection	4	
J. Y.	Mercuhydrin	1 ml	1 injection	24	
N. A.	Salyrgan	1 ml	1 injection	2	0
M. D.	Salyrgan	1 ml	1 injection	2	0
La P.	Salyrgan	1 ml	1 injection	2	0
La P.	Salyrgan	1 ml	1 injection	25	0
La P.	Salyrgan	1 ml	1 injection	6	0
N. S.	Salyrgan	1 ml	1 injection	19	
A. B.	Mapharsen	40 mg/day	3 days	3	0
M. B.	Mapharsen	40 mg/day	6 days	19	0
L. J.	Mapharsen	40 mg/day	1 day	9	Trace
A. M.	Mapharsen	40 mg/day	1 day	13	0
J. P.	Mapharsen	30 mg/day	1 day	10	0
J. P.	Mapharsen	60 mg/day	4 days	0	

to produce moderate elevation of catalase and protein levels in the urine. These results are of general interest, since they indicate that therapeutic doses of mapharsen and of the mercurial diuretics employed did not produce measurable damage to the kidney tubules in the cases studied.

8.4 The Effect of Exercise on Urinary Catalase and Protein.* (E. Roberts with the assistance of Paul O'Connell; catalase determinations, Tien Ho Lan and staff; statistical analysis, D. V. Tiedeman and M. J. Wantman.) In an attempt to determine what the effect of strenous exertion might be on urinary protein and catalase, the urines of football players were studied before a practice session for a game, 1 hr before the players took the field, and immediately after the game. In addition urinary alkaline-phosphatase activity, sugar, and specific

* Assistance of Mr. Wantman and Coach Elmer Burnham in making arrangements for the collection of the urine from University of Rochester football players is gratefully acknowledged.

gravity were determined. The data for the tests, as well as the playing time of each player, and in some cases the position played, are recorded in Table 14.17. An abnormal protein value was defined as any value greater than zero, whereas an abnormal catalase value was

Table 14.17 — Urinary Data* of Football Players (A) before Practice, (B) before a Game, and (C) after a Game

No.	Specific gravity			Sugar			Protein			Catalase			Phosphatase			Min. played	Position played †
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
1		1.011		0	0		0	0		0	0		0	0		0	
2		1.008	1.018	0	0	0	0	0	25	4	0	4	0	0	0	42	H.B.
3	1.023	1.022	1.017	0	tr.	tr.	0	0	20	0	0	18	0	0	0	60	H.B.
4	1.031		1.007	0		0	0	0	0	0		0	0		0	0	
5	1.029			0			0			0			0			0	
6		1.016	1.014	0	0	0	0	0	0	38	0	29	0	0	0	2	
7	1.033	1.021	1.020	0	0	0	0	0	0	0	0	0	0	0	0	53	L.E.
8	1.032		1.015	0	0	0	0	0	0	0	0	0	0	0	0	5	
9	1.026	1.023	1.019	0	0	0	0	0	0	0	6	0	0	0	0	3	
10	1.029	1.018	1.021	0	0	tr.	0	0	65	0	2	25	0	0	0	60	R.G.
11	1.026			0			0			0			0			0	
12		1.022	1.015	0	0	0	0	0	60	0	0	8	0	0	0	58	F.B.
13	1.023	1.027	1.017	0	tr.	0	0	0	0	0	4	0	0	0	0	52	R.E.
14	1.028	1.023	1.020	0	1+	0	0	0	0	0	4	2	0	0	0	5	
15	1.030		1.020	0		0	0		5	0		0	0		0	55	L.T.
16	1.033			0			0			0			0			0	
17	1.030	1.020	1.012	0	0	0	0	0	0	11	2	0	0	0	0	2	
18	1.031	1.018	1.024	0	0	tr.	0	0	80	0	3	16	0	0	0	53	
19	1.019	1.024	1.022	0	tr.	0	0	0	90	3	5	0	0	0	0	6	
20	1.029	1.024	1.021	0	0	0	0	0	5	0	3	15	0	0	0	14	
21			1.016		0	0		0	100+		24	48		0	0	58	R.T.
22		1.017	1.002		0	0		0	0		1	0		0	0	3	
23		1.018	1.011		0	0		0	0		3	9		0	0	0	
24			1.017		0	0		0	0		1	0		0	0	0	
25			1.020		tr.	0		0	20		0	0		0	0	10	

* Unit for sugar is the Benedict qualitative designation; unit for protein is milligrams per cent; unit for catalase is cubic millimeters of oxygen evolved; unit for phosphatase is King-Armstrong units; "tr." indicates only a trace was present.

† H.B., halfback; L.E., left end; R.G., right guard; F.B., fullback; R.E., right end; L.T., left tackle; R.T., right tackle.

defined as a value greater than any previous value. The protein excretion was significantly higher after the game than before the game or before practice. Before-game and before-practice values were identical from individual to individual. The t ratios of the differences in mean catalase values at the three time periods showed that the

prepractice level of catalase was not significantly different from either the pre-game or the post-game levels. However, the post-game level may possibly be higher than the pre-game level. Correlations between the urinary variables and time in the game were found to be

Table 14.18 — Effect of Mild Exercise (Running) on Urinary Catalase and Protein of Three Subjects

	Before exercise		After exercise	
Subject	Protein, mg %	Catalase, cu mm O_2	Protein, mg %	Catalase, cu mm O_2
J. J. R.	0	1	15	3
P. O.	0	4	15	10
G. T.	0	3	25	4

.40 for catalase and .49 for protein. Both correlations may possibly be different from a true correlation of zero. This is an indication that catalase may be affected by exercise. However, the highest catalase value of 48 cu mm of O_2 found after the game was not one that would cause undue concern, whereas values of 20 mg % or more of protein, of which there were eight after the game, would cause some concern if they were found in spot urine samples and would definitely require further checks. It thus appears that, whereas the protein values can be raised by exercise to values which if found in a person employed in handling a nephrotoxic agent would require further study, none of the catalase values were out of the normal range.

Three people were studied with regard to the effect of mild exercise (running) on urinary protein and catalase. In all three cases there was an increase in urinary protein from 0 to 15 – 25 mg % and a slight increase in catalase values. However, the highest catalase value recorded after the exercise was 10 cu mm of O_2 (Table 14.18), a value within the normal range. These experiments have brought out the fact that it is possible to produce by exercise an increase in urinary protein, which, if produced by nephrotoxic agent, would be medically significant. Exercise does not bring the levels of urinary catalase beyond the normal range. The combined use of the catalase and protein tests is therefore a much more powerful screening tool for studying the effects of nephrotoxic agents, since a significant response in both is highly significant, whereas a response in protein alone may be questionable. In view of the fact that it has been established in rabbit experiments that there is a range of injury at which catalase can be

made to appear without the appearance of protein, the greater sensitivity of the catalase as well as its possibly greater specificity make it a useful addition to the group of established monitoring devices.

9. FURTHER STUDIES OF CATALASE IN HUMAN URINE*

9.1 Urinary-catalase and Protein Levels in Persons Having No Contact with Uranium Compounds and in Those Having Such Contact. (E. Roberts; catalase determinations, Tien Ho Lan and staff; statistical analysis, D. V. Tiedeman and M. J. Wantman.) The purpose of this experiment was to compare the catalase and protein levels of urines of a group of people exposed to uranium salts with those of an unexposed population to determine whether any significant differences in the excretion of these substances existed. In addition, the unexposed group was studied with respect to the excretion of urinary alkaline phosphatase, and the uranium content of the urines of one of the industrial groups was also determined by the fluorophotometric method (Chap. 2).

Table 14.19—Mean Urinary-catalase Values for Personnel at the University of Rochester and at Company A

	Rochester	Company A
Number of persons	219	46 (123 determinations)
Mean catalase value, cu mm O_2	4.12	16.71
Standard deviation	9.37	13.43
t ratios of difference in means	−7.04	
p value	<0.01	

The "unexposed" population consisted of 219 people employed by the Manhattan District in the University of Rochester, from each of whom one spot sample of urine was obtained. No selection was exercised in obtaining the samples. The "exposed" population consisted of 46 people employed by Company A, from whom 123 spot urine samples were obtained over a period of 2 months and shipped by railway express to Rochester. No information was obtained relative to the degree of exposure of these people to uranium compounds or to other nephrotoxic agents.

*Studies of persons exposed to uranium compounds.

The mean urinary-catalase values for the personnel of Rochester and at Company A are recorded in Table 14.19. The mean catalase value for the Rochester group was 4.12 cu mm of O_2 as compared to 16.71 for the industrial group. The difference between results from the Rochester and the Company A groups was sufficiently large to make the probability that it occurred by chance less than 0.01.

However, it is not believed that this difference is of medical significance so far as exposure to uranium is concerned. This conclusion has been reached from the following considerations:

1. The urines from the control group in Rochester were analyzed immediately after collection, whereas the industrial samples were analyzed only after a period of several hours.

2. High urinary-catalase values might be dependent in part on the concentration of the urine, which is known to be increased by physical exertion.

3. The plant workers were not tested before exposure to uranium, so that some positive findings (i.e., urinary catalase values greater than 50) may have preexisted.

The mean urinary-protein values for the two groups are recorded in Table 14.20. It is obvious that there is no difference between the two groups so far as urinary protein is concerned.

Table 14.20 — Mean Urinary-protein Values for Personnel at the University of Rochester and at Company A

	Rochester	Company A
Number of persons	219	46 (123 determinations)
Mean protein value, mg %	1.75	0.07
Standard deviation	13.93	0.22
t ratios of difference in means	0.93	
p value	0.35	

9.2 Second Test of Personnel at Company A. (Experimental work supervised by A. L. Dounce; analyses performed by Paul O'Connell at the plant.*) Urinary-catalase determinations were carried out using urine samples from 54 workers at the plant. Forty-six persons presumably exposed to inhalation of uranium in some form comprised the "exposed" group, and eight laboratory workers who had minimal

* It is desired to express appreciation for the excellent cooperation and assistance provided by the management at this plant, without which this study would have been impossible.

Table 14.21 — Urinary-catalase and Protein of Low- or Zero-exposure Group at Company A

Sex	Catalase, cu mm O_2	Protein
M	8	+
M	12	0
M	4	+
M	14	0
M	3	0
M	10	0
M	7	+
F	7	+

Table 14.22 — Urinary-catalase and Protein Values of Exposed Group at Company A

Sex	Catalase, cu mm O_2	Protein	Sex	Catalase, cu mm O_2	Protein
M	27	0	M	6	
M	33	Trace	M	23	0
M	29	0	M	17	0
M	21	Trace	M	8	0
M	14	0	M	10	0
M	16	0	M	9	0
M	12	0	M	8	0
M	4	0	M	3	0
M	9	0	M	6	0
M	6	0	M	5	0
M	15	0	M	4	Trace
M	5	0	M	46	Trace
M	18	0	M	10	0
M	7	0	M	16	Trace
M	8	0	M	9	0
M	6	0	M	5	0
M	13	0	F	11	0
M	9	0	F	19	Trace
M	11	+	F	8	+
M	17	+	F	26	0
M	7	Trace	F	12	+
M	15	0			
M	11				
M	13	0			
M	14	0			

or no exposure to inhalation of uranium compound were used as a small control group. Of the exposed group, five were female; of the unexposed group, one was a female.

The catalase determinations were carried out using one of the early forms of the catalase machine. Experimental results from this machine cannot be related to the corresponding results as determined by the Warburg apparatus. It is probable that all the results were somewhat below the correct values.

The results of the determinations are included in Tables 14.21 and 14.22 and show that the difference between the exposed and unexposed groups is probably not significant. Although some of the exposed persons showed a positive urinary-protein test, some positive urinary-protein tests were obtained also from the unexposed group.

The study of this plant was unsatisfactory in several respects, but it demonstrated at least that no appreciable elevation in urinary catalase occurred among the workers tested.

9.3 Test of Personnel at Plant B. (Experimental work supervised by A. L. Dounce, Tien Ho Lan, and W. F. Neuman; analyses by Paul O'Connell, Marian Kaley, Betty Robinson, and Daphne Rothermel; statistical analysis of the results by M. J. Wantman, D. V. Tiedeman, and L. S. Kogan; exposure ratings by Tybout.*) A relatively large number of workers in this plant were tested. Some were exposed and some were not exposed to uranium compounds. Besides urinary catalase, urinary protein and urinary uranium also were determined, and some urinary-phosphatase determinations were made. At the beginning of the experiment, work was done at the plant with the catalase machine, but this proved unsatisfactory for reasons to be discussed later, and therefore all the catalase determinations that have been recorded were performed at Rochester with the Warburg apparatus on samples of urine transported to Rochester from the plant by train or automobile. Gum arabic was used to prevent catalase decay, and toluene-chloroform mixture was used as preservative. All samples were analyzed within 6 hr of the time of their collection. In general, the results should be correct or slightly low. Since control samples were run on persons at the plant, slight inaccuracies caused by the 6-hr interval between time of collection and analysis should be of no consequence.

There were two reasons for the failure to carry out good analyses at the plant. One was that the air was contaminated with catalase inhibitors. The second reason was that the catalase machine, which

* It is desired to express appreciation for the cooperation of the nursing staff, the personnel, and the management of Plant B, without which this study would have been impossible.

had been fairly satisfactory for rabbit urine, showed a high and unsuspected cutoff in sensitivity for human urine. This situation was remedied by a slight change in design of the machine, but not in time for more work at this plant. That the machine was finally made satisfactory for work with human urine was later demonstrated by numerous control analyses and two satisfactory field performances.

In a study of Plant B, urine specimens from 169 employees were collected in order to determine whether or not abnormally high values of catalase and protein, as well as measurable amounts of uranium, would be found in individuals who were exposed to various uranium compounds at various low levels of atmospheric contamination.

Table 14.23—t Ratios of the Differences in Mean Urinary Catalase, Protein, and Uranium Concentrations of Highly Exposed and Nonexposed Plant B Employees

	Nonexposed*		Highly exposed†	
Test	Mean	Standard deviation	Mean	Standard deviation
Catalase, cu mm O_2	14.35	12.49	15.36	12.57
Protein, mg %	0.82	3.87	0.36	1.34
Uranium, mg/liter	0.026	0.023	0.057	0.028

* Group composed of 49 persons.
† Group composed of 14 persons; exposure rating >70 on any compound.

Test	Mean difference	t	p
Catalase	1.01	0.27	0.79
Protein	0.46	0.43	0.67
Uranium	0.032	4.33	< 0.01

The results presented in Table 14.23 indicate that the amount of uranium in the urine does not seem to be related to the amount of catalase or protein in the urine. The correlations between urinary uranium and urinary catalase and protein were −.01 and +.01, respectively.

However, when persons with the highest and lowest levels of urinary uranium were segregated, it was found that 12 males with urinary uranium > 0.1 mg/liter had an average urinary catalase of 26.1, whereas 15 cases (11 male and 4 female) with urinary uranium < 0.01 mg/liter had an average urinary catalase of 14.1. It was calculated that the probability that this difference is caused by chance alone is 0.07. In view of the fact that the urinary protein was essentially zero in both groups and that relatively few individuals were in each group,

the significance of this urinary-catalase difference between the two groups is doubtful. The results of this t test are summarized in Table 14.24.

Because of the limited range of exposure and because of the limitations of the exposure rating as the criterion (see below) a t test of the difference between the mean urinary values for the highest exposure (exposure ratings of 70 or higher) was made. The results of this test are shown in Table 14.23. The mean urinary-catalase and protein values for the 14 persons with the highest exposures are not significantly different from the mean urinary-protein and catalase values

Table 14.24—t Test for Mean Catalase Difference between High- and Low-urinary-uranium Groups

	High group	Low group
Mean	26.1	14.1
Standard deviations	23.4	10.0
Number of values	13.0	15.0

$M_h - M_l = 12.0$
$t = 1.8$
$p = 0.07$

of the 49 unexposed persons, but the urinary uranium was significantly different in these two groups. Here the difference was numerically small, but, owing to the uniformity of the values, the probability that the difference was due to chance alone was less than 0.01.

Criterion of Exposure. The exposure data used in this study were obtained from Tybout, who indicated that the figures on the tables are the product of the following factors:

1. The percentage of the time that the worker is in extensive contact with the particular uranium compound encountered in any given operation. "Extensive contact" occurs under those conditions where above the average level of uranium dust per cubic meter of air is present.

2. The number of hours per day spent on each operation.

The product of factors 1 and 2 was divided by 10 in each case to obtain a number of more convenient size, e.g., the loaders in their operation were in extensive contact 100 per cent of their working time. Since they worked 8 hr per day as loaders, their exposure figure is 8×100, or 800. Dividing this by 10 gives 80, the highest exposure it was possible to receive.

Limitations of Study. The general exposure variable was simply a differentiation of individuals into exposed and nonexposed categories. Therefore the correlations of the other variables with general exposure are point-biserial correlations, i.e., one variable is a continuous one; the other (general exposure) is dichotomized.

Protein values were not reported as unit values but were reported as being between certain limits. Under these circumstances it was necessary to assign to each determination the mid-point value of the range recorded.

Table 14.25—Individual Values for Urinary Catalase and Protein of the High- and Low-urinary-uranium Groups*

Low-urinary-uranium group (less than 0.01 mg/liter as uranium metal)				High-urinary-uranium group (more than 0.1 mg/liter as uranium metal) §			
Sex	Protein, † mg %	Catalase, ‡ cu mm O_2	Uranium, mg/liter	Sex	Protein, † mg %	Catalase, ‡ cu mm O_2	Uranium, mg/liter
F	< 10	0	< 0.01	M	0	26	> 0.1
F	0	27	< 0.01	M	0	21	> 0.1
F	0	21	< 0.01	M	0	10	> 0.1
F	0	8	< 0.01	M	0	5	> 0.1
F	0	0	< 0.01	M	0	11	> 0.1
M	0	8	< 0.01	M	0	47	> 0.1
M	0	1	< 0.01	M	0	10	> 0.1
M	0	12	< 0.01	M	0	10	> 0.1
M	0	18	< 0.01	M	0	29	> 0.1
M	0	8	< 0.01	M	0	21	> 0.1
M	0	20	< 0.01	M	0	40	> 0.1
M	0	17	< 0.01	M	Trace	87	> 0.1
M	0	33	< 0.01	M	0	6	> 0.1
M	0	14	< 0.01				
M	0	24	< 0.01				

* Plant average for catalase, 14.2 cu mm of O_2.
† Average protein, 0 mg %.
‡ Average catalase, 14.1 cu mm of O_2.
§ Average catalase, 26.1 cu mm of O_2.

Individual High Values Recorded at the Plant. In Table 14.25 are recorded the values of urinary catalase, protein, and uranium that were used in calculating the p value of 0.07 in Table 14.24.

If a urinary-catalase value of 50 is taken as the minimal value indicating appreciable active tubular damage sufficient in degree to be of possible medical interest, it can be seen that there are only three

individuals in the high-urinary-uranium group whose catalase values approach or exceed this value. This is a very low percentage of the total number of employees tested (1.8 per cent). Nevertheless it would have been of interest to examine these individuals critically by using clearance tests to determine whether any other sign of kidney involvement could have been discovered.

In the case of at least one of these three persons, a high urinary catalase was found more than once.

Conclusions. In conclusion it can be stated that in general neither the urinary-protein nor the urinary-catalase test showed measurable active tubular damage among the employees of Plant B who were tested. In 3 out of a total of 169 cases tested values were found suggesting minimal active tubular damage might have been occurring at the time the tests were made. However, it was impossible to investigate these three cases further to attempt to confirm or refute this possibility.

9.4 Test of Ten Soldiers Exposed to Uranium Compounds at Site C. Ten soldiers exposed to uranium compounds by inhalation at Site C were examined thoroughly for signs of uranium poisoning. The urinary protein, tested on 3 different days, was zero in all cases, and all urinary-catalase values were below 10 with an average value of 1.5. Thus it can be safely assumed that there was no active tubular damage at the time the tests were made. The urinary-uranium concentrations were all less than 0.01 mg/liter except for two cases, in one of which two values (0.04 and 0.02 mg/liter) were found, and in the other of which one value (0.02 mg/liter) was found. With such a low average uranium excretion it is doubtful whether the exposure of these soldiers immediately preceding the tests was significant. Moreover, very low urinary-fluoride concentrations were found.

In this study the only determinations that may have been different from the normal were those of tubular resorption of chloride, but even these changes were slight and could be due to conditions unrelated to occupation.

The following additional tests were made on these soldiers: blood NPN, blood urea, blood chloride, serum calcium, plasma inorganic phosphorus, total plasma bilirubin, electrophoresis of plasma proteins, urinary sugar (qualitative), urinary amino acid nitrogen, urinary creatinine, urinary protein, and urinary catalase.

The results of all these determinations were normal. The unusually low urinary-catalase average of these men is probably attributable to the low urinary specific gravity of the urinary samples.

Test of 31 Workers at Site C. A second test at Site C of 31 workers exposed to uranium compounds by inhalation was carried out. Only the results of the urinary-catalase studies are available. The average

urinary catalase was 4.6, which compares favorably with the average urinary catalase of the Rochester control group, already mentioned. The observed values are listed in Table 14.26.

Table 14.26 — Urinary Catalase of Workers Exposed to Uranium Compounds at Site C

Sex	Catalase, cu mm O_2	Sex	Catalase, cu mm O_2
M	0	M	7
M	26	M	13
M	0	M	9
M	0	M	0
M	0	M	1
M	0	M	0
M	0	F	0
M	2	F	2
M	0	F	0
M	1	F	2
M	0	F	6
M	0	F	30
M	1	F	0
M	2	F	38
M	2	F	3
		F	0

In this table there are only three values that deviate from the average, and all these values are below 50.

In conclusion it may be said that the catalase test on these workers did not indicate any measurable active tubular damage.

10. COMPARISON OF CATALASURIA WITH PHOSPHATASURIA AND PROTEINURIA

From the data presented in this chapter it can be seen that in rabbits the urinary-catalase test is nearly always more sensitive than the urinary-protein test after the first injection of the uranium compound, but after repeated injections the urinary-protein test sometimes becomes more sensitive than the urinary-catalase test. However, this difference is more marked at the higher dosage levels, whereas if the dose is 0.01 or 0.02 mg of uranyl acetate dihydrate per kilogram (equivalent to 0.0056 or 0.011 mg of uranium metal per kilogram) the urinary-catalase test is usually more sensitive than the urinary-protein test.

In dogs the urinary-catalase test and the urinary-protein test are of comparable sensitivity. In one injected animal the protein test was

more sensitive than the catalase test, while in two animals the catalase test was more sensitive than the protein test. In the inhalation work the catalase was a trifle more sensitive. In the cats studied the catalase test was found to be more sensitive than the protein test for the first injection of uranium, but the protein test appeared somewhat more sensitive on subsequent injections of the uranyl compound.

The phosphatase test in rabbits is not quite so sensitive as the urinary-protein test, but after low doses of uranyl acetate it is very definitely reliable upon repetition. This is not true in cats and dogs, where the phosphatase test seems definitely less useful than either the catalase or the protein test, owing largely to the fact that it does not give reliable results upon repetition following repeated doses of U_6. Hence, among the animals studied, the phosphatase test is to be recommended only for rabbits, using rather low doses of uranyl acetate dihydrate not greater than 0.05 mg/kg (equivalent to 0.028 mg of uranium metal per kilogram).

The fact that kidney phosphatase is considerably lowered following injections of uranyl acetate in rats but not in rabbits explains, no doubt, why the test is applicable to rabbits but not to rats.

The conclusions concerning the relative values of the urinary-phosphatase, catalase, and protein tests are as follows:

1. The phosphatase test as applied to human beings is of unknown value but probably would be less sensitive than the catalase or the protein test.

2. The catalase test, in the absence of urinary erythrocytes or large numbers of pus cells or bacteria, is a very sensitive test for tubular damage by uranium and other toxic agents for tubules.

3. The urinary-protein test is a very sensitive test for kidney damage by uranium but of much less specificity than the catalase test, since transient effects of heavy exertion and possibly fright and alcohol can produce proteinuria as a result of glomerular leakage. These conclusions are based upon the results of studies with human beings as well as with animals.

In regard to kidney damage by tubular poisons other than uranium it can be stated that the urinary-phosphatase test appears to be inferior to both the protein and catalase tests, even in rabbits. The protein and catalase tests are of comparable sensitivity in most cases.

Inhalation experiments with rabbits also indicate that the urinary-phosphatase test is applicable to rabbits in inhalation studies. The phosphatase test employed when this work was done had only about

one-tenth the sensitivity of the final improved test* developed later. The results, therefore, would have been more favorable to the phosphatase test if the final procedure had been applied in the above work.

11. SUMMARY AND CONCLUSIONS

1. It has been demonstrated that an abnormally high excretion of catalase occurs in the urine of rabbits, dogs, cats, and probably rats after the poisoning of these animals with high or low doses of uranium compounds, whether administered by parenteral injection or by inhalation.

2. The increase in catalasuria after uranium poisoning coincides in time with the appearance of demonstrable amounts of protein in the urine. The urinary catalase and the protein values go through maxima usually from 3 to 6 days after the administration of soluble U_6 compound by injection. If the uranium compound is administered by inhalation, these maxima may be delayed and of longer duration.

The catalase maxima occur at the time of maximum breakdown of renal tubular cells and result from an escape of the catalase from the damaged cells. An earlier maximum has been observed in acute injection experiments, which appears within 2 to 3 hr after the intravenous injection of large amounts of uranium compounds. The significance of this earlier maximum is not certain.

3. The appearance of catalase in the urine after exposure to uranium compounds in animals is an extremely sensitive test for kidney tubular damage by uranium. In rabbits the catalase test is more sensitive than the urinary-protein test, and this is probably true in

* A solution for controlling pH is prepared by dissolving 11.5 g of sodium diethyl barbiturate in water and diluting to 500 ml. This gives a satisfactory final pH when mixed with urine (9.2±), but if purified enzyme preparations are used the pH will be too high. Five-tenths milliliter of urine and 5.0 ml of the sodium diethyl barbiturate solution are mixed and allowed to come to temperature equilibrium at 37°C. Then 0.5 ml of a well-shaken 4 per cent suspension of calcium phenolphthalein phosphate [E. J. King, J. Path. Bact., 55: 311 (1943)] is added by means of a 1-ml graduated pipet with the tip ground off. The tubes are well shaken and are allowed to stand at 37°C for 1 hr. No doubt results could be improved by using a slowly moving mechanical shaking device. At the end of the hour, the supernatant liquid is removed by decantation or by rapid centrifugation and to it is added 0.1 ml of 1.5N NaOH. If the urine has caused a clouding, the solution must be centrifuged at this point. Readings are taken immediately in the Klett colorimeter, using the green filter. The zero value for the colorimeter is obtained by using a blank (centrifuged if necessary) containing urine, sodium diethyl barbiturate solution, NaOH, and water in place of substrate.

To convert colorimeter readings to King-Armstrong phosphatase units per 100 ml of urine, the colorimeter readings should be multiplied by a factor of approximately 0.042.

For application of the method to tissues, see Chap. 13, Sec. 5.2g.

cats also. In the case of dogs the catalase test is more sensitive than the protein test in some animals and less sensitive in other animals. The catalase test is preferable to the phosphatase test because it is more reproducible after repeated doses of uranium compounds.

4. In general, catalasuria and proteinuria are the most sensitive indicators of the acute stage of tubular damage to the kidneys. The combination of the catalase and protein tests furnishes an advantage over the protein test alone, not so much because of the greater sensitivity but rather because of the greater specificity.

The appearance of abnormally high catalasuria is a specific indication of tubular damage to the kidneys, whereas the appearance of proteinuria may be an indication of glomerular damage alone, or glomerular plus tubular damage. Even in rather severe uranium poisoning, plasma catalase, because of its high molecular weight, does not filter through the glomerulus.

5. Other agents besides uranium that attack the tubules, such as mercuric chloride, mapharsen, sodium tartrate, and potassium dichromate, have been found to produce catalasuria. Adrenaline, on the other hand, does produce a slight proteinuria without any catalasuria. The same is true of heavy exercise, fright, and probably alcohol.

6. Patients with nephritis may or may not show abnormal catalasuria, depending upon the acuteness of the condition and the degree of tubular involvement. An abnormal catalasuria is not a test for the amount of degeneration in a kidney. It is rather a sensitive test for acute tubular damage.

7. No evidence of renal-tubular damage due to industrial exposure to uranium has been found. Although some urinary-catalase values above 50 associated with elevated urinary-protein levels have been found, it is impossible to say what was the cause of these changes.

8. Since the catalase test is a sensitive test for tubular damage, it should be useful in testing the action of various drugs on the kidney. Thus far it appears that certain organic mercurial diuretics (salyrgan and mercuhydrin) and mapharsen in therapeutic doses do not cause damage to the tubules, as indicated by their failure to cause abnormal catalasuria in human beings.

BIBLIOGRAPHY

1. C. Breedis, C. M. Flory, and J. Furth, Arch. Path., 36: 402 (1942).
2. B. S. Walker, J. Am. Chem. Soc., 63: 2015 (1941).
3. E. Tria, J. Biol. Chem., 129: 377 (1939).
4. J. B. Sumner, Advances in Enzymol., 1: 163 (1941).
5. J. B. Sumner and A. L. Dounce, J. Biol. Chem., 136: 343 (1940).
6. H. von Euler and K. Josephson, Ann., 452: 158 (1927).
7. M. Laskowski and J. B. Sumner, Science, 94: 615 (1941).
8. J. B. Sumner and A. L. Dounce, J. Biol. Chem., 121: 417 (1937).

Chapter 15

THE MECHANISM OF ACTION OF URANIUM COMPOUNDS IN THE ANIMAL BODY

By Alexander L. Dounce*

1. INTRODUCTION

The purpose of this chapter is to furnish explanations for the mode of primary action of uranium compounds on the body. Such information is needed to evaluate the results obtained from toxicological studies and to search intelligently for antidotes to uranium poisoning. This chapter is restricted principally to explanations of the primary action of uranium compounds in producing toxic effects. A brief discussion dealing with such effects as the production of acidosis following uranium poisoning is appended, since these phenomena are secondary to the initial damage.

In regard to the cause of toxicity of uranium, only chemotoxic effects will be considered. Apparently it is highly improbable that enough uranium could accumulate in the body to cause general radiation damage, and nothing is known as to whether individual cells here and there might be affected by radiation as the result of the accidental accumulation of very high concentrations of uranium in their vicinity. The latter possibility could become important in its relationship to carcinogenesis, but there is thus far no evidence that uranium compounds are carcinogenic.

It is not intended to attempt explanations for the toxic action of massive doses of uranium on the animal, such as would cause death in 12 hr or less. In the case of administration of such massive doses of uranium compounds, many tissues and organs may receive direct damage from the uranium (such as intestine, liver, and spleen), possibly as the result of blocking vital enzyme action or of markedly

*Work done by Alexander L. Dounce, J. H. Wills, William F. Neuman, Tien Ho Lan, Frances Haven, Margaret Neuman, and Eugene Roberts.

changing the physical properties of the proteins in the affected cells. The ideas presented in this chapter therefore apply chiefly to doses of uranium compounds that produce slightly to moderately toxic but nonlethal effects. The magnitudes of such doses presumably depend in individual cases upon the rate at which the compound in question can gain access to plasma and the rate of its solubility in plasma. The valence state of the compound in question also must be considered, since U_4 as such is apparently less toxic than U_6.

All work thus far carried out on toxicity of uranium compounds indicates strongly that those compounds which do not contain other toxic elements act chiefly in a toxic manner on the kidney tubules. Therefore the main emphasis of mechanism studies has been placed on the manner in which uranium affects the kidney tubules, and other studies have been more or less preliminary to or secondary to this principal study. There are still considerable gaps in knowledge of such processes as the mechanism of absorption of uranium from the gastrointestinal tract, the mechanism of its absorption through skin, and the mechanism of its deposition in bone, which can be filled in by future studies.

The mechanism of action of uranium on the kidney tubules has been sufficiently elucidated so that it is apparent that uranium filtered through the glomerulus is deposited largely on tubular cell surfaces in the distal half of the proximal convoluted tubules, whereby it damages or destroys these cells. The exact initial chemical damage that takes place in these cells as a result of the deposition of uranium upon their surfaces cannot be stated at the present time, but there is good evidence that proteins and perhaps enzymes in the cell surfaces are altered by combining with the uranium.

2. LOCAL EFFECTS OF URANIUM COMPOUNDS

Local damage of the eye and lung, and possibly to a slight extent of the skin, can be produced by exposure to uranium compounds. It is likely that these local effects are not caused so much by the uranium in the compounds as by the effect of acidity, or in certain cases by the presence of another toxic element, such as fluorine.

Most of the readily soluble uranium compounds, such as uranyl nitrate, uranyl acetate, UCl_4, and UCl_5, hydrolyze to produce acid. The compounds UCl_4 and UCl_5 produce acid, as can be seen from the following equations:

$$UCl_4 + H_2O \rightarrow UOCl_2 + 2HCl$$

$$2UCl_5 + 3H_2O \rightarrow UO_2Cl_2 + UOCl_2 + 6HCl$$

Both $UOCl_2$ and UO_2Cl_2 themselves are acidic compounds, and therefore UCl_4 and UCl_5 produce more acid per molecule than do uranyl compounds. This statement applies especially to UCl_5. Even 0.1M uranyl nitrate in aqueous solution has a pH of 4.0 or lower. Effects of HF on the eye are well known, and hence care must be taken in interpreting eye damage when caused by UO_2F_2, because hydrolysis may liberate HF locally.

Serious lung damage might readily be produced by HCl resulting from hydrolysis of UCl_4 particles lodging there, and the liberation of HF in the lung might be even more serious. Insoluble uranium compounds accumulate in the lung after inhalation and may be expected to act locally as inert material, because their rate of solution is very slow or negligible, and they are chemically inert until dissolved.

Recent work has indicated that the particle size of relatively insoluble uranium compounds is related inversely to the toxicity of these compounds by inhalation. This may be because the fine particles have a higher rate of solubility than the coarse particles in plasma (see Chap. 10).

These points have been emphasized, not with the idea of explaining all local toxic effects of uranium compounds, but rather to show that the toxic effects probably can be attributed to other factors than uranium per se. There is no evidence that properly neutralized uranyl or uranous compounds have a serious local effect on the eye, lung, or skin unless massive doses are employed.

3. MECHANISM OF ENTRY OF URANIUM INTO THE BODY

Most of the views about absorption of uranium compounds by various routes are deductions from general chemical and biochemical knowledge, and there is little direct experimental work bearing on the matter.

3.1 Absorption from the Eye. In rabbits it is easy to get enough absorption of U_6 into the blood stream to prove fatal after introducing a U_6 compound into the eye. It is assumed that in this case the absorption of a soluble complex such as the bicarbonate complex occurs in the conjunctival membrane and possibly also in the nasal mucosa.

Since tears contain very little protein, the U_6 is free to combine with any complexers such as bicarbonate or anions of organic acids that may be present, and in addition with the proteins of the lining membranes. That the latter type of combination does occur is proved by finding relatively large concentrations of uranium in the eyeball some days after introducing the U_6 compound into the conjunctival sac.

3.2 Absorption of U_6 through the Skin. Very little is known of the mechanism of skin absorption. In the case of compounds such as

uranyl nitrate, which are somewhat soluble in lipid, it is likely that the lipid plays a role as a vehicle for transporting U_6 to the deeper layers of the skin. According to one theory of absorption through the skin, the material in question must possess both lipid and plasma solubility. The absorption of organic acid anion complexes of U_6, and especially absorption of the bicarbonate complex, must also be considered as probable once the U_6 has penetrated into the deeper layers of the skin.

Presumably U_4 is oxidized by the air to U_6 before skin absorption occurs, but this point has not been investigated.

3.3 Lung Absorption of Uranium. It seems most probable that the bicarbonate complex of U_6 is the chief form of uranium absorbed in the lung. Presumably U_4 is oxidized to U_6 in the lung before appreciable absorption can occur.

The problem of solubility in plasma of the various uranium compounds tested for toxicity by inhalation is of immediate concern in a consideration of the mechanism of absorption of uranium compounds from the lung. In the case of relatively insoluble compounds the particle size may be very important. It should be emphasized that all the chemical evidence at hand indicates that once U_6 has dissolved in the plasma it is carried in the blood as bicarbonate and as protein complexes, no matter in what form it was originally introduced. The U_4 compounds very likely are oxidized to U_6 before they dissolve appreciably in plasma.

The first attempt to determine solubilities of various uranium compounds in plasma was made by the inhalation group, who unfortunately used citrated plasma, the importance of citrate as a complexer of U_6 and U_4 not having been realized at the time the work was carried out. It is likely that the citrate greatly enhanced the solubilities of the uranium compounds, particularly of certain moderately soluble U_6 compounds, such as UO_3. As reported in Chap. 10 concerning the inhalation experiments, uranium compounds may be grouped roughly into these three distinguishable classes: a very soluble class, uranyl nitrate and uranyl fluoride; a very insoluble class, U_3O_8; and an intermediate class, uranates, etc. This solubility classification paralleled the toxicity by inhalation to a considerable extent, whereas the solubilities in distilled water did not seem to be well correlated with toxicity by inhalation.

In order to avoid the use of a complexer of U_6 such as citrate or oxalate, it was decided to repeat this work on solubility using serum in place of citrated plasma. Loss of the fibrinogen could hardly introduce a serious error. In order to be certain that the bicarbonate content was in the normal range, equivalent to about 45 to 55 vol. %

CO_2, a mixture of 5 per cent CO_2 in 95 per cent nitrogen was passed into the serum samples before adding the uranium compound to be tested. The serum was analyzed, using a Van Slyke apparatus, to be sure that the resulting CO_2 content was within the acceptable range.

The polarographic method of analysis was used to determine the U_6 that had dissolved in the serum. The method as applied to serum is summarized briefly in the appendix to this chapter.

Owing to the lack of time available when the second study was made, it was not possible to work out the method in final form soon enough to obtain completely reliable results on all the samples tested. The results presented are the best approximations of the rate of solubility that could be obtained under the circumstances. The values obtained at the end of ½ hr and at the end of 24 hr have been averaged, and the results have been interpreted (approximately) as the rate of solubility per 12 hr. All the compounds, except possibly the acetate, appeared to dissolve to a greater extent after 24 hr than after ½ hr. These data are shown in Fig. 15.1. It can be seen that uranyl acetate, nitrate, and fluoride are very soluble. Indeed it is even possible that all the added sample may have dissolved in the ½-hr period. However, all the other compounds have strictly limited rates of solubility in serum.

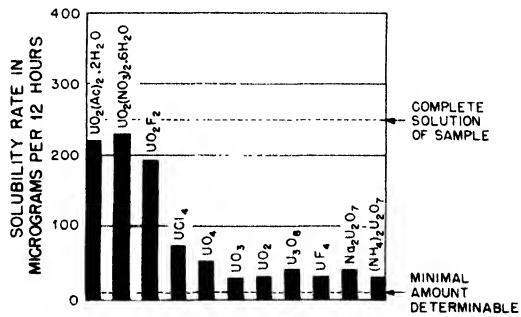


Fig. 15.1 — Rate of solubility of various uranium compounds in serum.

It is well to keep in mind that the rate of solubility of a uranium compound in serum is undoubtedly determined to a large extent by the rate of its reaction with bicarbonate to form the bicarbonate complex. The complex, once formed, comes into equilibrium with the U_6-protein complex, but it is doubtful whether most uranium compounds in the solid phase would react as rapidly with protein in a direct manner as with bicarbonate. Hence any loss of CO_2 from the serum that might occur in experiments such as have been described would constitute a

uranyl nitrate, which are somewhat soluble in lipid, it is likely that the lipid plays a role as a vehicle for transporting U_6 to the deeper layers of the skin. According to one theory of absorption through the skin, the material in question must possess both lipid and plasma solubility. The absorption of organic acid anion complexes of U_6, and especially absorption of the bicarbonate complex, must also be considered as probable once the U_6 has penetrated into the deeper layers of the skin.

Presumably U_4 is oxidized by the air to U_6 before skin absorption occurs, but this point has not been investigated.

3.3 Lung Absorption of Uranium. It seems most probable that the bicarbonate complex of U_6 is the chief form of uranium absorbed in the lung. Presumably U_4 is oxidized to U_6 in the lung before appreciable absorption can occur.

The problem of solubility in plasma of the various uranium compounds tested for toxicity by inhalation is of immediate concern in a consideration of the mechanism of absorption of uranium compounds from the lung. In the case of relatively insoluble compounds the particle size may be very important. It should be emphasized that all the chemical evidence at hand indicates that once U_6 has dissolved in the plasma it is carried in the blood as bicarbonate and as protein complexes, no matter in what form it was originally introduced. The U_4 compounds very likely are oxidized to U_6 before they dissolve appreciably in plasma.

The first attempt to determine solubilities of various uranium compounds in plasma was made by the inhalation group, who unfortunately used citrated plasma, the importance of citrate as a complexer of U_6 and U_4 not having been realized at the time the work was carried out. It is likely that the citrate greatly enhanced the solubilities of the uranium compounds, particularly of certain moderately soluble U_6 compounds, such as UO_3. As reported in Chap. 10 concerning the inhalation experiments, uranium compounds may be grouped roughly into these three distinguishable classes: a very soluble class, uranyl nitrate and uranyl fluoride; a very insoluble class, U_3O_8; and an intermediate class, uranates, etc. This solubility classification paralleled the toxicity by inhalation to a considerable extent, whereas the solubilities in distilled water did not seem to be well correlated with toxicity by inhalation.

In order to avoid the use of a complexer of U_6 such as citrate or oxalate, it was decided to repeat this work on solubility using serum in place of citrated plasma. Loss of the fibrinogen could hardly introduce a serious error. In order to be certain that the bicarbonate content was in the normal range, equivalent to about 45 to 55 vol. %

CO_2, a mixture of 5 per cent CO_2 in 95 per cent nitrogen was passed into the serum samples before adding the uranium compound to be tested. The serum was analyzed, using a Van Slyke apparatus, to be sure that the resulting CO_2 content was within the acceptable range.

The polarographic method of analysis was used to determine the U_6 that had dissolved in the serum. The method as applied to serum is summarized briefly in the appendix to this chapter.

Owing to the lack of time available when the second study was made, it was not possible to work out the method in final form soon enough to obtain completely reliable results on all the samples tested. The results presented are the best approximations of the rate of solubility that could be obtained under the circumstances. The values obtained at the end of ½ hr and at the end of 24 hr have been averaged, and the results have been interpreted (approximately) as the rate of solubility per 12 hr. All the compounds, except possibly the acetate, appeared to dissolve to a greater extent after 24 hr than after ½ hr. These data are shown in Fig. 15.1. It can be seen that uranyl acetate, nitrate, and fluoride are very soluble. Indeed it is even possible that all the added sample may have dissolved in the ½-hr period. However, all the other compounds have strictly limited rates of solubility in serum.

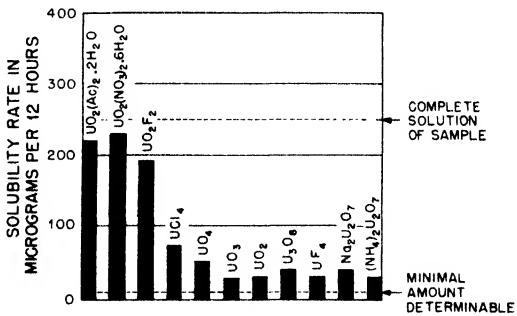


Fig. 15.1—Rate of solubility of various uranium compounds in serum.

It is well to keep in mind that the rate of solubility of a uranium compound in serum is undoubtedly determined to a large extent by the rate of its reaction with bicarbonate to form the bicarbonate complex. The complex, once formed, comes into equilibrium with the U_6-protein complex, but it is doubtful whether most uranium compounds in the solid phase would react as rapidly with protein in a direct manner as with bicarbonate. Hence any loss of CO_2 from the serum that might occur in experiments such as have been described would constitute a

source of error. Moreover the introduction of an acid uranium compound into serum would have a tendency to lower the pH and thus drive off CO_2. For this and other reasons, the problem of determining the rate of solubility of uranium compounds in serum is complicated and technically difficult, and the problem of obtaining equilibrium values for the total solubility of soluble uranium compounds in plasma is virtually meaningless, owing to the great change in the composition of the plasma that is brought about by the addition of any considerable amount of soluble uranium compound. In any event the rate of solubility at very low uranium concentration is the important factor in determining solubility in the lung, and this factor is very hard to measure accurately.

3.4 Absorption from the Gastrointestinal Tract. It is not known whether uranium is absorbed from the stomach. If it is, the UO_2^{++} ion present at the low pH of gastric juice might penetrate to the blood stream, where it would then become complexed with bicarbonate and protein as usual. It seems likely that in the intestine the bicarbonate complex of U_6 may be the form that is absorbed. Ingested U_4 could hardly be appreciably absorbed in the intestine unless previous oxidation to U_6 took place. If any U_4 were absorbed in the stomach, it would no doubt be in the form of the UO^{++} ion.

It is known that only a small percentage of uranium mixed with the diet is absorbed from the gastrointestinal tract. The unabsorbed portion is probably combined with protein, protein breakdown products, and perhaps other food residues and is excreted in the feces.

3.5 Absorption of U_4 Compounds. It has been concluded from all work on U_4 compounds that direct absorption of U_4 by any other route than direct intravenous injection into the blood stream is unlikely. This is chiefly because of the great insolubility of U_4 at pH values of 6.0 or above except in the presence of relatively high concentrations of complexing agents such as certain organic acid anions. Also, soluble U_4 compounds are rather readily oxidizable by molecular oxygen. The U_4 does not have so great an affinity for bicarbonate as has U_6, and, even in the presence of most organic acid anions, protein would tend to bind the U_4 rather completely, thereby fixing it locally until oxidation could occur. U_4 precipitated as oxide or hydrous oxide will undergo slow oxidation by the oxygen of the air.

4. FATE OF URANIUM IN BLOOD

4.1 Transportation of U_6 and U_4 in Plasma. From what has been done on the formation of complexes of U_6 and U_4 with various organic anions and proteins (see Chap. 1), it can be deduced that U_6 is transported in plasma chiefly in the form of complexes with protein and

bicarbonate. The blood cells do not carry significant amounts of U_6, as has been shown by direct analysis. The distribution of U_6 between protein and bicarbonate depends upon the CO_2-combining power of the plasma as has been stated in Chap. 1. The higher the bicarbonate concentration, the more U_6 is carried by bicarbonate and the less by protein. Equilibrium between U_6 bicarbonate and U_6 protein seems to be set up rapidly, i.e., in a period of minutes at the longest.

U_4 has less tendency to complex with bicarbonate than has U_6, and in plasma it appears to be carried partly as a complex with protein and possibly also as a colloidal oxide or hydroxide stabilized by blood colloids. It is not carried to a significant degree by the blood cells, as was ascertained by direct analysis.

The U_6 bound to protein, as well as U_4 bound to protein or present as colloidal oxide, is not filterable through the glomerulus, but U_6 bound in plasma to bicarbonate is readily filterable.

Even though U_4 is only present to a small extent in plasma in a filterable form, an appreciable amount might pass through the glomerulus, provided that a sufficiently rapid equilibrium were established between the filterable and nonfilterable portions.

Mercury presents a case analogous to the case of U_4. Ultrafiltration experiments carried out by Fanta on mercury in serum indicated that mercury is not filterable to a greater extent than 5 per cent and possibly is filtered to a lesser extent than this. Nevertheless, sufficient mercury can get into the tubules in mercury poisoning to cause severe or fatal damage. The mercury that gets to the tubules must either be filtered through the glomerulus or excreted by the tubules, and the latter possibility does not appear probable.

4.2 State of Oxidation of Uranium in Blood Plasma after Injection of U_6 and U_4. Results of studies on valence state, which have been outlined in Chap. 1 on chemistry, can be summarized as follows:

1. U_3 is very unstable, owing to its great ease of autoxidation, and presumably cannot exist in the body for any appreciable length of time.

2. U_5 is very unstable because of dismutation to $U_6 + U_4$ and could not exist in appreciable concentration in the body.

3. The potential E_0' for the U_6,U_4 system at pH 7.0 will be approximately +0.05 volt, based on hydrogen in 0.1M acetate buffer. This result was obtained by means of the platinum electrode and the polarograph and has been confirmed roughly using the indicator-dye method with methylene blue.

4. The potential for the U_6,U_4 system in 0.5M bicarbonate buffer at pH 7.0 as determined at the platinum electrode is about +0.14 volt based on hydrogen. The potential is established fairly rapidly.

5. The potential for the U_6,U_4 system in 0.05M citrate at pH 5.0 is about +0.19 volt based on hydrogen. This potential appears to be independent of $[H^+]$ or [citrate]. The system poises the platinum electrode very weakly, and the equilibrium potential is reached quite slowly.

6. The lactate-pyruvate system in the presence of lactic dehydrogenase, coenzyme I, and methylene blue will not reduce U_6 (Chap. 13). On the other hand, U_4 will cause deoxygenation of hemoglobin rather readily at pH 5.0 and is oxidized by molecular oxygen in acetate at pH 7.0.

The work on oxidation-reduction potentials on the U_6,U_4 system makes it probable that U_6 could be reduced in the body only if it should penetrate cells and if sufficient time were allowed to elapse. But thus far there is no evidence that U_6 penetrates cells in the body in appreciable quantities. All the evidence at hand indicates that U_6 is perfectly stable in plasma.

It appears likely that U_4 could be appreciably oxidized in plasma over a period of from a few minutes to 1 or 2 hr. It has been found by Tien Ho Lan that protein retards but does not prevent the oxidation of U_4 by molecular oxygen. Glutathione, and especially glutathione plus ascorbic acid, apparently can slow the autoxidation of U_4 to a negligible rate, but the glutathione of blood is mainly in the erythrocytes. Ascorbic acid alone is without effect in this respect.

From what has been stated it is evident that any conversion of U_4 to U_6 that may occur in plasma is slow, and from what is known of the rate of removal of U_4 from plasma (see Sec. 5 and Chap. 11) it seems that the major portion at least of injected U_4 escapes oxidation while in the plasma.

5. RESULTS OF DISTRIBUTION STUDIES*

Much of the distribution work has been done on rats following rather large intravenous doses of soluble uranium compounds (3.0 to 5.0 mg of uranyl nitrate hexahydrate, equivalent to 1.8 to 2.5 mg of uranium metal per kilogram). However, some work on animals exposed to inhalation of uranium dust has served as a check on the injection experiments.

5.1 Distribution of U_6 in Body Organs and Tissues. Using uranyl nitrate, it has been found that the two chief sites of deposition of U_6 are the bone and kidneys. However, the uranium in the kidney after exposure has stopped decreases fairly rapidly, whereas the concentration of uranium in bone decreases only very slowly, indicating that bone is the most important site of storage in the body.

*Carried out by William Neuman and collaborators.

5.2 General Distribution of U_4 in Body Organs and Tissues. After the injection of a U_4 compound, such as UCl_4 partially neutralized with sodium acetate, it is found that uranium accumulates in the liver and spleen as well as in bone and kidney, but the soft tissues all lose uranium so much faster than bone that bone again represents the chief ultimate storage site.

5.3 Blood Levels of U_6 and U_4. After injection of uranyl nitrate or acetate the blood level falls very rapidly, so that after 1 hr over 95 per cent of the injected uranium has left the blood stream and gone into bone or kidney. After the injection of U_4 the blood level remains appreciably high for longer periods, but even in this case it has fallen to low levels after 4 hr. The effect of nephrectomy on the blood curve of uranium after injection of U_6 into a rabbit has also been studied. Even after removal of the kidneys the bone takes up U_6 so fast that the blood level falls rapidly (see Chap. 11).

5.4 Local Distribution of Uranium in the Kidney. (a) Results of Autoradiograph Studies, Carried Out by J. Henry Wills and William Neuman to Show Mode of Distribution of Uranium in the Kidney. Rats were used in this work. Autoradiographs were prepared using radioactive uranium diluted with ordinary uranium. Animals were injected with 5 mg of uranyl nitrate hexahydrate ($\Rightarrow$ 2.4 mg of uranium metal) per kilogram containing uranium 232, equivalent to 2 microcuries/kg. From 1 to 6 hr after injection, the animals were killed; the kidneys were removed and fixed in 50 per cent absolute alcohol-50 per cent chloroform. Paraffin sections 5 to 10 μ in thickness were fixed on microscope slides, which were then placed section side down on Wratten metallographic plates and allowed to stand in the dark for 10 days to 3 weeks. The plates were then developed and enlargements were made of the autoradiographs. The sections on the slides were stained with hematoxylin-eosin, mounted in the usual manner, and photographed at the same enlargement as the autoradiographs. Composite photographs were then made of the autoradiographs and stained sections.

The results of this work showed that the radioactive uranium was concentrated in certain areas of the kidney more than in others and that, in particular, some nephrons appeared to have heavy concentrations of uranium compared to others. Although most glomeruli did not show much uranium, occasional glomeruli appeared to show heavy concentrations. Superimposed upon this strong localization of uranium was a general background effect that was rather uniform in distribution. It is probable that, if the doses of uranium administered to the animals had been lower, the localization would have been even more pronounced, and the general background effect less marked.

(b) Microstaining Reactions for Localizing Uranium in the Kidney. (Carried out by Macelyn Anders and Priscilla Hutchens.) Rats and a few rabbits were used in this work. Uranium can be located in microscopic sections by staining reactions using ferrocyanide to produce the characteristic red compound, or by using the small amount of uranium present to act as a mordant locally for hematoxylin. The results of using these two techniques are in good agreement (Anders).

Relatively high doses of uranium, 3 to 5 mg of uranyl nitrate hexahydrate (≃ 1.4 to 2.4 mg of uranium metal) per kilogram, must be administered to get satisfactory microstaining reactions. When such doses are employed, it is found in general that the uranium tends to be located in intensely staining rings or collars on the inside surfaces of some of the tubules; in addition there is a rather generalized weak staining of some of the tubular epithelial cells. Uranium is not detected in the glomeruli. In general the staining is not uniform throughout the section but is rather patchy, and, in sections showing weak uranium staining, definitely stained localities in the section must be searched for.

Judging from this type of localization of uranium, one might suspect that local damage to some groups of nephrons would exceed damage to other groups. However, in the absence of serial sections, this conclusion is unwarranted from this study alone. Damaged areas of the kidney may not be stained, and stained areas may appear not to be damaged, whereas sometimes the stained and damaged areas coincide fairly well.

6. STUDIES ON THE EXCRETION OF URANIUM COMPOUNDS

6.1 General Routes of Excretion. In all animals so far studied (rats, rabbits, cats, and dogs) the only route for appreciable excretion of U_6 appears to be by way of the urine. Rats fed on a fox chow diet excrete on the average about 60 per cent of injected U_6 in the urine during the first 3 days after an intravenous injection of $UO_2(NO_3)_2$. Excretion of uranium thereafter is very slow indeed.

After intravenous injection of UCl_4 partly neutralized with sodium acetate, only about 10 per cent of the injected dose shows in the urine in the course of 3 days, and thereafter the rate of excretion in urine is very low. However, an appreciable fecal excretion of uranium begins at about the second day after injection and continues for some time. Presumably this is the result of excretion in the bile of uranium that has accumulated in the liver. Distribution and excretion studies thus show that U_4 behaves somewhat differently in the body from U_6.

6.2 Urinary Excretion of Uranium in Sacrifice Experiments as Influenced by Various Factors. (a) Experiments on Rabbits. (By J. Henry Wills with the assistance of Daniel Adler and Edna Main.) This work has been covered in some detail in Chap. 5. Table 5.1 of that chapter shows that the pH of the urine, as influenced by diet or by the nature of the infusion, is of some importance in limiting or promoting the urinary excretion of uranium following the intravenous injection of a standard dose of uranyl acetate. Infusion of bicarbonate was shown to have a particularly great effect in increasing the excretion of hexavalent uranium, and administration of equal amounts of base (sodium) as lactate or citrate had less effect, even though the urinary pH went higher than with bicarbonate infusion. The principal action of bicarbonate infusion is therefore to be ascribed to the effect of the bicarbonate anion.

(b) Experiments on Rabbits and Cats. (By Alexander L. Dounce and Robert W. Fleming.) The principal aim at the time these experiments were carried out was to study the effect of the pH of urine on the rate of excretion of uranium by the kidney after the injection of U_6 or U_4 compounds. It had been predicted from chemical studies that an acid urine would cause the retention of uranium by the kidney and that an excess of bicarbonate would cause an enhanced excretion after the administration of U_6 intravenously. On the other hand, it was felt that the pH of the urine would have little effect on the excretion of U_4, since this material should not filter appreciably through the glomerulus.

The work with animals confirmed the predictions made from chemical studies and in addition demonstrated that an increase in pH of the urine is much less effective in causing rapid excretion of uranium by the kidney than is flooding the animal with bicarbonate.

In the experiments with rabbits the urinary pH was lowered by keeping the rabbit on an oat-and-water diet for several days, since it was found impossible to lower the pH during an experiment by infusion of even such a strong acid as 0.1N HCl. In the experiments with cats the urinary pH was lowered by feeding the animal a diet of water and meat mixed with ammonium chloride for a few days previous to the experiment. All experiments were carried out under nembutal anesthesia. The trachea, the saphenous vein, and the bladder were cannulated. The results of the experiments using rabbits are presented in Table 15.1.

The results indicate that rendering the urine acid causes retention of U_6 by the kidney and that infusion with bicarbonate enables the animal to excrete a large fraction of the injected U_6 in a relatively short time, with only a very small retention of uranium by the kidney.

It is also seen in the case of the two animals studied that U_4 does not filter well through the glomerulus in the rabbit, since uranium shows in the kidney or urine only in low concentrations after injection of U_4,

Table 15.1 — Excretion and Retention of U_6 and U_4 by Rabbits

Rabbit No.	Dose of uranium compound, mg/kg of body wt.	Infusion fluid	Urinary pH range during 4-hr period	Percentage of dose excreted in 4-hr period	Total urine volume for 4-hr period, ml
1	3 mg of uranyl acetate*	0.85% NaCl	7.4–8.0	17.8	608
2	3 mg of uranyl acetate*	0.85% NaCl	7.6–8.4	8.9	106
3	3 mg of uranyl acetate*	0.85% NaCl	7.1–7.7	14.2	306
4	3 mg of uranyl acetate*	0.85% NaCl	4.0–6.0 (oat diet)	3.6	397
5	3 mg of uranyl acetate*	0.85% NaCl	5.0–5.8 (oat diet)	3.6	140
6	10 mg of uranyl acetate†	0.85% NaCl	4.5–5.4 (oat diet)	3.6	213
7	10 mg of uranyl acetate†	0.85% NaCl	6.7–7.8	23.2	365
8	10 mg of uranyl acetate†	0.87% $NaHCO_3$	8.0–8.6	71.2	405
9	10 mg of UCl_4 neutralized to pH 6.5 with sodium acetate‡	0.85% NaCl	7.3–7.7	1.6	365
10	10 mg of UCl_4 neutralized to pH 5.5 with sodium acetate‡	0.87% $NaHCO_3$	8.1–8.4	4.8	517

*Equivalent to 1.67 mg of uranium.
†Equivalent to 5.61 mg of uranium.
‡Equivalent to 6.30 mg of uranium.

and that infusion with bicarbonate does not change this situation markedly. These results are in agreement with the work of Wills (Sec. 6.2). Prior to U_4 injection, Wills used sodium hydrosulfite to reduce the 5 per cent of U_6 that contaminated all the UCl_4 samples used, and, since

this was not done in the experiments just outlined, it is not surprising that his results showed an even lower filtration of uranium after the injection of U_4.

A few experiments with cats are summarized in Table 15.2. The results obtained using cats on an ordinary diet of meat scraps and food leftovers show that the excretion of U_6 after bicarbonate infusion is far higher than after saline infusion, although cats on an ordinary diet appear to excrete a somewhat higher percentage of uranium than do rabbits on an ordinary diet of commercial rabbit food. In the case of cats on an acid-producing diet of meat and ammonium chloride, it is seen that the urinary pH was not reduced materially, although the blood alkali reserve of the animal was materially depleted. Hence it is not surprising to find somewhat more uranium excreted in the urine of the two "acid" cats than in the urine of the three "acid" rabbits. The effect of the acid-producing diet on the two cats is, however, very marked in causing retention of uranium by the kidneys.

Somewhat discordant results were obtained with the cats in the case of U_4. One animal showed a kidney retention of uranium about the same as in the rabbits, but another animal showed about ten times this retention. In the second animal, hydrosulfite was used to remove the 5 per cent U_6 present in the UCl_4. It cannot be stated without more work whether this second result should be attributed to experimental error.

The excretion of uranium after injection of U_4 appears to be somewhat higher in cats than in rabbits. It is likely that this difference, if it is real, means that U_4 is slowly but appreciably oxidized to U_6 in the plasma of the cat, whereas in the rabbit such oxidation is inappreciable.

In order to show the time course of urinary excretion of uranium in cats after the injection of U_6 and U_4, Figs. 15.2 to 15.5, which are sufficiently self-explanatory, are included.

Cat 7 in Table 15.2 requires special comment. After a period of 4 hr, one kidney was quickly removed, and the infusion medium was changed from 0.85 per cent NaCl to 0.87 per cent $NaHCO_3$ to determine whether uranium deposited in the remaining kidney could be washed out. That such washing out can occur is demonstrated by estimated values for uranium in the kidneys and urine at the end of 4 and 8 hr. Approximately 25 per cent of the uranium retained by both kidneys at 4 hr would have been washed out by 8 hr if both kidneys had been left in the animal, on the assumption of approximately equal distribution in both kidneys, which thus far has always been found to be the case.

Figure 15.6 shows the cumulative percentage excretion of uranium in the urine in this cat for the total 8 hr of the experiment. The sharp

Table 15.2—Excretion and Retention of U_a and U_s by Cats

Cat No.	Wt., kg	Dose, mg of uranyl acetate per kg of body wt.	Uranium equiv., mg	Infusion fluid	Urinary pH range	% of dose retained by kidneys	% of dose excreted in urine	Total urine volume, ml
1	3.7	11.1	6.22	0.85% NaCl	7.5–7.4 (first 4 hr)		27.4 at 4 hr	554 at 4 hr
2	3.7	22.2	12.5	0.85% NaCl	8.3–7.4		39.0 at 6 hr	439 at 6 hr
3	3.6	21.6	12.1	0.85% NaCl	8.3–7.8		18.5 at 4 hr	154 at 4 hr
4*	2.8	6	3.37	0.85% NaCl	8.0–7.1	3.3 at 8 hr	17.5 at 4 hr 19.5 at 8 hr	121 at 4 hr 188 at 8 hr
5†		6	3.37	0.85% NaCl	8.3–6.8		19 at 4 hr	170 at 4 hr
6‡	2.85	6	3.37	0.85% NaCl	7.4–7.0	72 at 8 hr	5.8 at 4 hr 7.9 at 8 hr	168 at 4 hr 370 at 8 hr
7	2.6	6	3.37	0.85% NaCl for first 4 hr	8.3–8.1 for first 4 hr	45¶	14 at 4 hr (one kidney then removed)	148 at 4 hr (one kidney then removed)
				0.87% $NaHCO_3$ for second 4 hr (after removal of one kidney)	8.3–8.2 for second 4 hr	33**	26 at 8 hr	353 at 8 hr
8	3.1	6	3.37	0.87% $NaHCO_3$	8.4–7.7	0.8 at 5 hr	64 at 4 hr 66 at 5 hr	205 at 4 hr 282 at 5 hr
9§	2.25	UCl_4 brought to pH 5.5 with $NaC_2H_3O_2$	3.37	0.85% NaCl	7.4–7.0	0.77 at 8 hr	5.6 at 4 hr 7.8 at 8 hr	175 at 4 hr 316 at 8 hr
10	2.97	UCl_4 brought to pH 5.5 with $NaC_2H_3O_2$, then enough hydrosulfite added to keep all U in U_4 state	8.32	0.85% NaCl		7.2 at 6 hr	8.2 at 4 hr 10.2 at 6 hr	110 at 4 hr 236 at 6 hr

*At 1 hr, uranium clearance = uU/pT = 3.2; plasma concentration = 3.4 μg U/ml. At 8 hr, uranium clearance = 1.6 ml/min; plasma concentration = 0.34 μg U/ml (u = concentration of uranium in urine; U = rate of flow of urine; p = concentration of uranium in plasma; T = time in minutes).

†At 0 time, plasma CO_2-combining power = 40 vol. %; at 6 hr = 32 vol. %; at 8 hr = 27 vol. %. At 8 hr, uranium clearance = 2.8 ml/min; plasma concentration = 0.22 μg U/ml. Diet consisted of meat plus NH_4Cl (acid producing).

‡At 0 time, plasma CO_2-combining power = 38 vol. %. Diet consisted of meat plus NH_4Cl (acid producing).

§At 0 time, plasma CO_2-combining power = 42 vol. %. At 8 hr, uranium clearance = 0.07 ml/min; plasma concentration = 8.2 μg U/ml.

¶Estimated for first 4 hr from analysis of removed kidney.

**Estimated for second 4 hr from analysis of remaining kidney.

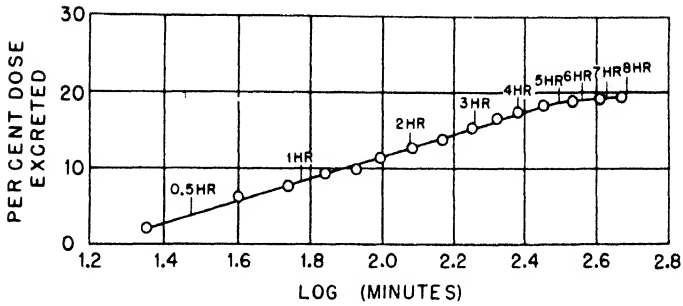


Fig. 15.2—Saline-infused cat 2, normal diet; percentage of dose excreted (cumulative) plotted against logarithm of time (minutes). Dose, 6 mg of uranyl acetate (≎ 3.37 mg of uranium metal) per kilogram.

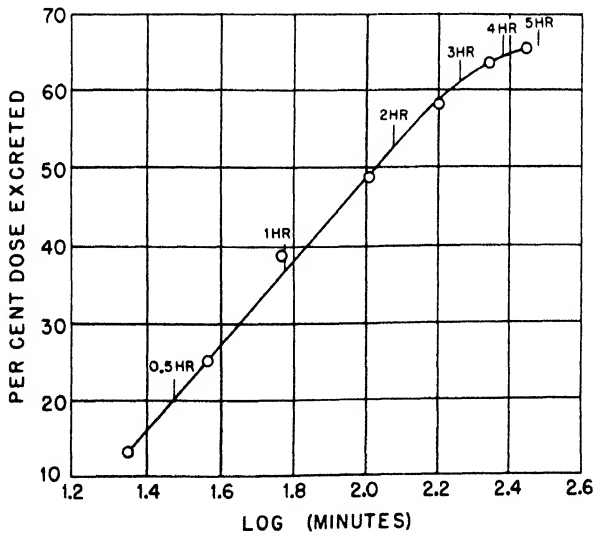


Fig. 15.3—Bicarbonate-infused cat 1, normal diet; percentage of dose excreted (cumulative) plotted against logarithm of time (minutes). Dose, 6 mg of uranyl acetate (≎ 3.37 mg of uranium metal) per kilogram.

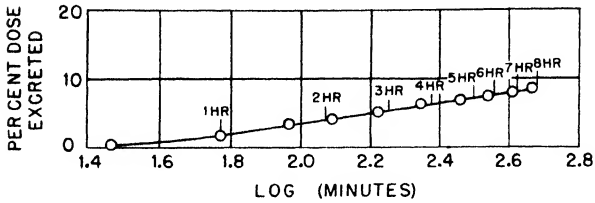


Fig. 15.4—Saline-infused cat 4, acid diet, lowered plasma bicarbonate; percentage of dose excreted (cumulative) plotted against logarithm of time (minutes). Dose, 6 mg of uranyl acetate (≎ 3.37 mg of uranium metal) per kilogram.

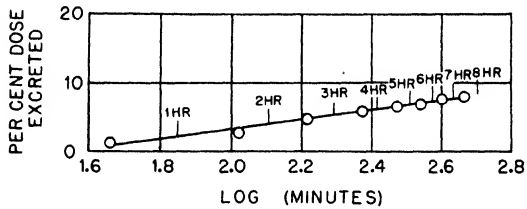


Fig. 15.5—Saline-infused cat 5, normal diet; percentage of dose excreted (cumulative) plotted against logarithm of time (minutes). Dose, 5.38 mg of UCl_4 (≎ 3.37 mg of uranium metal) per kilogram, partially neutralized with 1M sodium acetate.

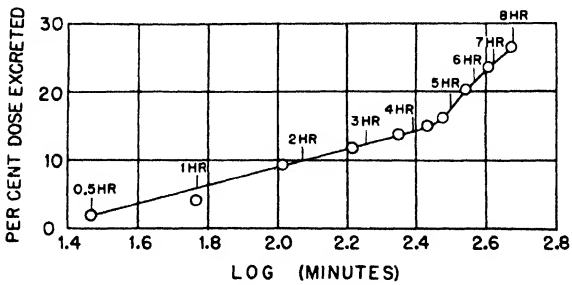


Fig. 15.6—Uninephrectomy experiment, cat 7. Percentage of dose excreted (cumulative) plotted against logarithm of time (minutes). One kidney removed at end of 4 hr; saline infusion for first 4-hr period; bicarbonate infusion for second 4-hr period. Dose, 6 mg of uranyl acetate (≎ 3.37 mg of uranium metal) per kilogram, intravenously; acid diet; lowered plasma bicarbonate.

break in the curve shortly after the removal of one kidney and commencement of the bicarbonate infusion is to be noted.

7. THE MECHANISM OF THE TOXIC ACTION OF URANIUM ON THE KIDNEY

7.1 Introduction. It has been concluded from all available information that the primary toxic action of U_6, however administered, is restricted mainly to an effect on the kidney, and more specifically to an effect on the renal tubules. The distal portions of the proximal convoluted tubules seem to receive the major portion of the damage in uranium poisoning. As has already been stated, this may not hold for massive doses of uranium.

Less is known about the primary toxic effects of U_4. It seems, however, fairly certain that appreciable amounts of U_4 cannot enter the blood stream except by direct injection. U_4 injected intravenously into rabbits appears to act like a smaller dose of intravenously injected U_6. In rats the acute toxicity by intravenous injection of U_4 may be as high as that of U_6, but the toxicity by intraperitoneal injection is lower than that of U_6. It must be kept in mind that U_4, which is oxidized in the plasma to U_6, can pass in this form the glomerular barrier and get into the kidney, but it is also possible that some U_4 as such may be filtered. The urinary catalase response after injection of U_4 supports the argument that U_4 generally acts when injected like a smaller dose of U_6 (see Chap. 14). After intravenous injection, U_4 accumulates in the liver as well as in the kidney and bone, but it is not known whether the liver is thereby damaged.

7.2 Details of the Mechanism Explaining the Action of U_6 on the Kidney. A simple mechanism to explain the action of U_6 on the kidney has been formulated as the result of a careful consideration of the major part of the research summarized in this chapter. This mechanism is as follows:

(a) The U_6-bicarbonate complex filters readily through the glomerulus. As this happens, more U_6 is transferred from protein to bicarbonate in the plasma until finally almost all the U_6 that has not been deposited in the bone has passed through the glomerulus in the form of the U_6-bicarbonate complex.

(b) Once in the tubule, the bicarbonate complex under the conditions ordinarily obtaining there begins to break down, liberating UO_2^{++}, which for the most part immediately combines with protein in the tubular cell walls. The factors that seem to operate in breaking down the U_6-bicarbonate complex are as follows:

1. A redistribution of U_6 between the bicarbonate and protein must occur in the tubules, since the major portion, at least, of the protein of the plasma has been left behind when glomerular filtration occurred.

2. Absorption of bicarbonate, which in mammals apparently occurs in the proximal as well as in the distal tubules, lowers the free HCO_3^- concentration to a point where the mass-law effect operates to promote decomposition of the U_6-bicarbonate complex, according to the following equation:

$$U_6\text{-bicarbonate complex} \rightleftharpoons UO_2^{++} + \text{bicarbonate}$$

3. Absorption of water by the tubule causes a concentration of U_6, which probably increases the likelihood that any free UO_2^{++} that is present will combine with the tubular cell walls.

4. A drop in pH to 6.5 or lower in the tubules will at once cause decomposition of any U_6-bicarbonate complex remaining, with the liberation of UO_2^{++}, most of which will combine with tubular-cell protein. A small fraction of the UO_2^{++} can, however, combine with organic acids, especially citrate, in the tubules and will be excreted in the urine. If HCO_3^- is present in the urine, the U_6-bicarbonate complex will be excreted. It will be recalled that in rabbits with acid urine, a high amount of uranium accumulates in the kidney in the first 4 to 8 hr after intravenous injection of uranyl acetate, although very little appears in the urine during this time. However, when the animal is flooded with bicarbonate administered by infusion so that the bicarbonate ion in the tubules is high, there is very little accumulation of uranium in the kidney after intravenous injection of uranyl acetate, but a large excretion of uranium in the urine occurs in 4 to 8 hr following the injection.

Increasing the urinary pH without flooding the animal with bicarbonate has much less effect in preventing uranium from "sticking" to the tubular cells, apparently because the affinity of U_6 for ordinary organic acids even at high pH is not great enough in comparison with its affinity for protein.

The evidence presented earlier in this chapter appears to favor such a mechanism very strongly, and nothing has yet been discovered to contradict it. There is a possibility, however, that eventually some refinements may have to be added. The mechanism probably can be applied to U_4 if this valence form actually passes the glomerular barrier without oxidation. The major difference to be expected between the action of U_6 and U_4 on the tubule is that U_4 would probably tend to combine somewhat more firmly with the protein of the tubular-cell walls than would U_6.

7.3 Action of Uranium Combined with Tubular-cell Walls. It may now be asked why the combination of uranium with the tubular-cell surfaces eventually causes damage or destruction to the cells. This question has not been answered in a clear-cut manner.

From the enzyme studies it seems somewhat doubtful that the damaging action is caused by blocking of any one specific enzyme system. If the cause of the damage is enzyme poisoning, it seems likely that a number of enzymes may be affected. But it is not certain that enzyme poisoning is the cause of the cell damage. However, it is fairly safe to say that, if uranium were to damage enzymes anywhere in the body, the only location where such damage might be extensive at relatively low concentrations of uranium would be the renal tubules.

It seems possible that uranium deposited on the cell surfaces of the renal tubules may damage these cells by combining with nonenzymatic protein, thereby causing a physicochemical change that eventually can lead to cell destruction. Such a possibility is perhaps favored by the lag period that occurs between deposition of the uranium on the cell surfaces and the appearance of toxic symptoms. A poison such as cyanide, which is known to inhibit certain metallo-enzymes very powerfully, does not show a delayed toxic action on the animal but instead acts in a drastic manner immediately after absorption.

7.4 Additional Material Not Yet Presented That Has a Bearing on the Mechanism of Action of Uranium on the Kidney. (a) General Results of Studies in Pathology Which Have a Bearing on the Mechanism Problem. (R. G. Metcalf and collaborators. See Chap. 4.) The chief organ affected by the administration of moderately toxic amounts of soluble uranium compounds appears to be the kidney. Damage to the kidney is chiefly in the form of necrosis of the cells of the tubular epithelium, although mild glomerular damage is reported by some investigators who use special staining methods. It seems probable that with moderately toxic doses of uranium compounds the damage to the tubule lies in the distal portion of the proximal tubules, but that with severely toxic doses the damage may extend into the descending loop of Henle and perhaps even beyond this as far as the first portions of the distal convoluted tubule. Whether there is rather uniform damage to all nephrons or whether some are affected more than others cannot yet be stated from the results of histopathological work alone, though the latter seems to be nearer the truth.

Following the initial dose of or exposure to uranium, the kidney damage becomes apparent only after a lag period that varies from a number of hours to a day or more, depending upon the dose. The tubular lining cells break down, the tubules tend to become filled with

cellular debris, and free cell nuclei may appear in the urine among the tubular casts that are present. After a period of 3 to 5 days, depending upon the size of the single dose, tubular-cell regeneration begins, and repair is usually complete in a period of 10 days to 2 weeks if the animal survives. The regenerated cells do not always have the identical microscopic appearance of the original cells that they have replaced but may appear more flattened. The regenerating cells also have different staining characteristics from the original cells, and in cases where clear-cut regeneration is dubious, because of minimal damage, a state of heightened cellular activity may be observed in which the cells have the appearance of being in early prophase.

The regenerated cells, which replace those originally present in the distal portions of the proximal convoluted tubules before the damage, may grow up from the descending portion of the loop of Henle, but it is equally possible that they arise locally as the result of the division of small numbers of undamaged cells in the damaged area.

(b) The Locus of Action of Uranium on the Nephron as Defined by Clearance Studies. (J. H. Wills.) 1. Uranium Clearance. It has been shown already in Table 5.3 that the clearance of uranyl uranium by the kidney is decreased by acidification of the urine and increased by alkalinization with bicarbonate. It has also been shown (Table 5.1) that increased urinary pH is not the only significant factor increasing the urinary excretion of uranyl uranium but that the nature of the infused anion is important also. Bicarbonate was shown to produce an especially large increase. The action of bicarbonate was shown to increase excretion and to reduce retention of uranium in the kidney. From the curve for the effect of bicarbonate on the ultrafilterability of hexavalent uranium from plasma it appeared that increased uranium excretion during bicarbonate infusion is due not only to decreased retention of the metal by the kidney but also to increased filtration through the glomerulus (Table 5.4).

Table 5.2 shows that bicarbonate has also a slight effect on the excretion of tetravalent uranium in the urine. The curve for the effect of bicarbonate on ultrafilterability of tetravalent uranium from plasma shows that in this case there is only a slight effect on glomerular filtration, and the increase in the dose excreted from 2.41 to 4.27 per cent is reflected in the decrease in retention by the kidney from 3.50 to 1.14 per cent of the metal injected.

Thus it appears that bicarbonate has something to do with transport of hexavalent and probably of tetravalent uranium along the tubular lumina. The work of Walker *et al.*[1] suggests that some bicarbonate is reabsorbed in the proximal tubule, and that of Pitts and Lotspeich[2]

seems to show that the major reabsorption of bicarbonate is in the proximal convolution. On this basis, uranium would be expected to be deposited in the proximal convolution, especially in the latter half of that segment. Presumably the metal would exercise its deleterious effect wherever it is deposited in the nephron.

2. Other Clearances. The possibility of change in the physical properties of the nephron within a few days after poisoning by uranyl salts[3] makes it advisable to use only immediate effects on renal function in attempting to identify the locus of uranium action on the kidney. The following compilation shows the average effects of intravenous injection of 3 mg of uranyl acetate (≈ 1.7 mg of uranium metal) per kilogram upon various clearances by the rabbit kidney within 2 hr and 10 min after injection.

Substance	Clearance after poisoning, % of control
Creatinine	95
Inulin	94
Xylose	104
Chloride	99
Urea	99
Glucose	307
Amino acid	180
Diodrast	45
Phenol red	84

Of the nine substances studied by the clearance technique, three—creatinine, inulin, and xylose—have been used as measures of glomerular filtration.[4] It is probable that the changes in these clearances after the injection of uranium are not significant, thus indicating no interference with glomerular filtration.

Two of the other substances studied, namely, chloride and urea, show clearly insignificant effects on their clearances. Both of these substances are thought to be reabsorbed partially in the proximal convoluted tubule and partially in the more distal portions of the nephron. It is likely that interference with some localized tubular region would be compensated for by increased passage through other sites, so that it is not surprising that the clearances of these two chemicals give no information about the site of action of uranium.

Of the remaining four substances studied, two are reabsorbed in the tubule—glucose and amino acid—and two are secreted by the tubule—diodrast and phenol red.[4] Glucose is stated to be reabsorbed almost completely in the first half of the proximal convolution.[1] Thus

the comparatively large effect of uranium on the clearance of glucose indicates interference with the function of the last part of the proximal convolution. Complete interference with glucose reabsorption would result in an increase of glucose clearance to about 70,000 per cent of the control value.

The site of amino acid reabsorption is not known definitely, but it is known that reabsorption of creatine and of amino acids occurs by the same mechanism.[5] Furthermore it has been found that phlorizin decreases reabsorption of creatine[6] and that administration of glucose increases the urinary output of creatine.[7] The latter finding was attributed by the authors to some unknown effect of the hexose on metabolism, but it appears possible that it results from competition of glucose and creatine for some reabsorptive mechanism in the tubule. If this chain of reasoning is correct, amino acids are reabsorbed at least to some extent in the same part of the proximal convolution as is glucose. Then the increase in amino acid clearance after injection of uranyl acetate also indicates interference with the function of the more distal portions of the proximal convolution.

Both phenol red and diodrast are believed to be secreted into the proximal tubule.[4] The decreased renal clearance of these two substances after poisoning with uranyl acetate indicates, therefore, interference with the function of the proximal tubule.

3. Summary. All the physiological evidence summarized here indicates, where it gives any definite information, that the action of uranium on the nephron is not on the glomerulus and that in the tubule it is localized probably in the latter one-half to two-thirds of the proximal convolution.

(c) Evaluation of Special Tests Used in Uranium Poisoning. 1. The Urinary Catalase Test. The urinary catalase test is one of the most sensitive early tests for uranium poisoning. It has been outlined briefly in Chap. 5 and described in detail in Chap. 14. A detailed application of the test has shown that very small doses of uranium, as low as 0.01, or 0.02 mg of uranyl acetate ($\simeq$ 0.006 or 0.012 mg of uranium metal) or of uranyl nitrate hexahydrate ($\simeq$ 0.005 or 0.01 mg of uranium metal) per kilogram intravenously injected, can exert a damaging effect on the kidney tubules, as measured by the increase in urinary catalase within a period of 2 to 4 days in dogs and rabbits. The test has been extended in less detail to cats and rats.

The test is reproducible after repeated exposures to uranium, and only grossly toxic doses, administered singly or repeatedly, lower the kidney catalase of animals to a serious extent. This test indicates that uranium has an effect on the kidney tubules, since it has been

demonstrated (see Chap. 14) that the source of urinary catalase in uranium poisoning is the damaged tubular cells.

2. The Urinary Phosphatase Test. Phosphatase appears in the urine, especially in rabbits, after administration of a moderately small dose of uranium, as was discovered by Furth and coworkers,[8] but, unlike the catalase test, the phosphatase test tends to become unrepeatable after repeated exposures to uranium. An important reason for the lack of repeatability of the phosphatase test seems to be that the kidney alkaline phosphatase can be materially lowered by moderate doses of uranium given repeatedly.

Since the alkaline phosphatase is known to be localized in the proximal convoluted tubules in most animals and to be highly concentrated in the brush border of these cells, the appearance of this enzyme in the urine in uranium poisoning is a direct indication of damage to the proximal convoluted tubule of the kidney. The lowering of phosphatase in the kidney after uranium poisoning is a good indication that newly regenerated cells of proximal tubules are different in this respect from the cells in the original undamaged tubules.

3. The Urinary Protein Test. The urinary protein test is nearly as sensitive as the urinary catalase test for renal damage by uranium. It has been determined that a sizable fraction of the urinary protein following uranium poisoning is plasma albumin. Alling found by electrophoresis of the urine of rats poisoned by U_6 that the urine contained plasma albumin and also various plasma globulins. Fanta found, after injecting crystallized beef- or horse-serum albumin into rabbits that had been previously poisoned with uranyl acetate, and then treating the diluted urine of the injected animals with an appropriate antiserum, that the injected albumin, whether beef or horse, quickly appeared in the urine of the poisoned animals. Crossover reactions were ruled out by testing blanks and controls. Beef-serum albumin and horse-serum albumin antisera were prepared by injecting these proteins into rabbits until a high antibody titer was obtained. Uranyl acetate was administered intravenously in doses of 0.1 mg/kg of body weight ($\simeq$ 0.06 mg of uranium metal per kilogram).

The question remains as to how the plasma albumin gets into the urine. Since the peak of urinary protein coincides with or slightly follows the peak of urinary catalase, and since histologically detectable glomerular damage is minimal or absent after the injection of reasonably low doses of uranium, it might be surmised that urinary protein leaks through the capillaries surrounding the damaged tubules. This idea is supported by the fact that protein appearing in the urine in uranium poisoning usually is not massive in amount. However, it

is also possible that the glomeruli are affected secondarily by the tubular damage to a sufficient extent so that some leakage of plasma protein can occur. It is also possible that normally a small amount of protein passes through the glomerulus but is reabsorbed and that in uranium poisoning this small amount of filtered protein is not reabsorbed.

Since catalase is a conjugated protein that gets into the urine in uranium poisoning from the damaged tubular cells, it is very likely that other protein in the urine in uranium poisoning comes from the damaged tubular cells. How much protein comes from this source in comparison to the amount coming from the plasma cannot be stated at present.

(d) The Effect of Uranium Compounds on Proteins and Enzymes. This subject is covered in considerable detail in Chap. 13. Only a brief summary will be given here.

It is quite certain that U_6 combines with COOH groups of proteins to form complexes, as in the case of a simple organic acid like acetic acid.

It appears likely that precipitation of protein by U_6 involves an aggregation or polymerization, together with a neutralization of charge on the aggregates. In the case of proteins that require the presence of salt to be precipitated by uranyl compounds, it seems possible that polymerization may have occurred without complete neutralization of charge. In general, proteins appear to be best precipitated by U_6 at pH regions near their isoelectric points. Elevation of the pH to 7.5 or higher or lowering of the pH to 3 or below usually causes proteins precipitated by U_6 to dissolve. U_4 combines rather strongly with proteins and precipitates them, probably in a manner similar to the action of U_6.

Another functional group that may occur in proteins and may be important in uniting U_6 with protein is the phosphate group. U_6 will form strong complexes with organic phosphates such as glyceryl phosphate, and this might occur in proteins, particularly if an OH group were close to the phosphate group.

As has been shown in Chap. 13, the combination of U_6 with proteins does not appear to cause denaturation, and this is also true of U_4, at least in some cases, provided always that the pH be kept within a range that the protein in question can tolerate without denaturation. Protein denaturation that could be attributed to U_6 has never been encountered and this also applies to U_4, although fewer proteins were tested for denaturation with U_4 than with U_6.

The work on enzymes has indirectly led to an understanding of the general mechanism of action of uranium on the kidney, but it has not

furnished decisive evidence as to whether or not the immediate cause of uranium damage to the kidney is inactivation of certain enzymes. In general it has been found that at physiological pH, particularly in the presence of bicarbonate, U_6 is not a very powerful enzyme poison. U_4 appears to be somewhat more effective as an enzyme poison than U_6, but there is little likelihood of a sufficiently rapid accumulation of U_4 in the kidney as a result of reduction of U_6 to account for the tubular cell damage. Furthermore U_6 presumably is the form of uranium that enters the blood stream unless the method of direct intravenous injection is employed for introducing U_4.

The enzyme work has led to the concept that, in order to have enzyme inactivation by U_6, there must be a potential source of UO_2^{++} ions. The complexes of U_6 themselves appear to be without effect as enzyme inhibitors at moderate concentrations. In some respects this has interfered with the study of the inhibition of enzymes by U_6 compounds, since as a rule either the substrate or the buffer combines so firmly with uranyl ion that little free UO_2^{++} is available to complex with the enzyme, and therefore the inhibiting action of the U_6 is diminished. However, in other respects this is not a great disadvantage, since the body fluids contain many complexers for U_6.

If it is the uranyl ion that inactivates the enzyme, it follows that a drop in pH should increase enzyme inhibition in most cases, because, by forming undissociated acid, a drop in pH generally reduces the amount of U_6 complexer present as anion. It is the anion of a complexer, not the undissociated acid, which can react with U_6, as has been explained in Chap. 1. Experimentally it has been found that a drop in pH does cause an increase in the degree of inhibition of enzymes by U_6, with one apparent exception, i.e., pepsin, which is not appreciably inhibited by moderate amounts of U_6 at pH 2.0. But it has already been stated that protein precipitated by U_6 will dissolve at pH 3 or lower, since there are no longer enough ionized COO^- groups left to complex the UO_2^{++} ion. Since un-ionized COOH groups do not form such complexes, the very low pH tends to prevent combination of UO_2^{++} ions with protein. Apparently the uranyl ion does not combine with pepsin (or its substrate) at pH 2.0 and hence has no inhibiting action.

Much of the enzyme inhibition caused by U_6 probably can be explained by the formation of U_6 complexes with ionized acid groups of the protein component, especially when the pH is lowered sufficiently so that aggregation or precipitation of the enzyme can occur. This type of action could occur in the kidney tubules. With an enzyme like phosphorylase, which is very sensitive to U_6, this explanation probably cannot account for the inhibition, and it is likely that blocking of an essential phosphate group of the prosthetic group occurs. It will be

recalled, however, that the phosphorylase of the kidney is not materially inactivated by injection of U_6 into animals intravenously, in spite of its in vitro sensitivity to U_6.

In regard to coenzymes and prosthetic groups, any such group that contains a phosphate radical should be suspected of being a potential complexer for U_6. Such complexing of U_6 might or might not cause inhibition of the reaction normally catalyzed by the coenzyme or prosthetic group in question. For example, the action of codehydrogenase I does not appear to be blocked by complexing with U_6. On the other hand, the action of phosphorylase is blocked by U_6, very likely because of complexing between U_6 and the phosphate of adenylic acid, which is probably the coenzyme of phosphorylase.

Unfortunately, enzymes containing pyridoxal phosphate have not been investigated in this laboratory with respect to inhibition by uranium in vitro. Such enzymes are amino acid decarboxylases and transaminases. Glutamic acid transaminase is said to be insensitive to U_6 in vitro but to be lowered in the kidney after subcutaneous injection of uranyl nitrate in rabbits.[9]

Only two enzymes especially sensitive to U_6 have been found in the Rochester laboratory. These are phosphorylase and thrombin.

8. ATTEMPTED PREVENTION AND THERAPY OF URANIUM POISONING

By William Neuman, Frances Haven, Alexander L. Dounce, Tien Ho Lan, and Eugene Roberts

8.1 General Results of Attempted Therapy Using Known Complexers of U_6. It has been possible to prevent or treat acute uranium poisoning with a fair degree of success by the administration of various chemical agents intraperitoneally, by stomach tube, or by incorporating the agent in the diet of the animals. In general, better results are obtained if the therapeutic agent is injected than if it is administered in the diet.

Therapeutic agents that have yielded satisfactory results by the route of intraperitoneal injection or by forced feeding are as follows: sodium bicarbonate, trisodium citrate, disodium malate, sodium lactate, disodium fumarate, disodium succinate. All these agents presumably act in the same way; the organic anions of all the compounds listed except bicarbonate are burned in the body leaving an alkaline residue, which takes the form of sodium bicarbonate. This sodium bicarbonate, if in sufficient concentration, will appear in the urine and will keep U_6 that has passed through the glomerulus in a complexed and therefore nontoxic form. Moreover an increase in plasma bicarbonate has a

tendency to cause the excretion of citrate in the urine, and this citrate also can act to keep the U_6 in a complexed and therefore nontoxic state. The appearance of citrate under these circumstances might furnish a better detoxifying mechanism than the excretion of bicarbonate, since a fairly high plasma bicarbonate is required to cause appreciable additional excretion of bicarbonate in the urine.

None of the above-mentioned compounds, however, show any better preventive or therapeutic action in U_6 poisoning than bicarbonate itself. Even in the case of citrate, the injected citrate anion is to a considerable extent burned by body tissues. Moreover bicarbonate is the least toxic of the above-mentioned substances and hence is the compound to be preferred in attempting to prevent or treat uranium poisoning. The effect on uranium excretion and retention of infusing animals with bicarbonate has already been mentioned.

In an attempt to ascertain whether a U_6 complexer that was not burned by the body could be used in therapy, the salt of disodium *d*-malate was prepared and tested in rats. Although insufficient experiments were carried out to be certain of the results, it appeared that the *d*-malate which is not readily metabolized by the body was no more effective than the *l*-malate in preventing U_6 poisoning.

It has been found that U_6 poisoning can be ameliorated if poisoned rats are treated with bicarbonate starting as late as 12 hr after the administration of the U_6, but the bicarbonate must be administered repeatedly. This is in agreement with the finding, reported elsewhere in this chapter, that infusion of a U_6-poisoned animal with bicarbonate causes some of the U_6 fixed in the kidney to be washed out. However, the best effects produced by bicarbonate are obtained if the animal is pretreated by feeding or by injecting bicarbonate by stomach tube for 2 or 3 days before the injection of the uranium. Under these circumstances it is possible to reduce the mortality from 80 to 0 per cent if sufficient bicarbonate is used to produce a moderate alkalosis. The aim is to give as much bicarbonate as can well be tolerated by the animal and as often as possible. Since bicarbonate injected intraperitoneally may cause local reactions, it is preferable to administer it by vein or by stomach tube.

It is unlikely that pH has any considerable direct effect on the prevention of poisoning by U_6, since animals with high urinary pH have nevertheless shown severe poisoning by uranium. However, an increase in pH may indirectly have a beneficial effect in uranium poisoning by causing an increase in the ionized fraction of any possible U_6 complexers that may be present in the urine. This has been explained in detail in Chap. 1.

In addition to causing a complexing of uranium by citrate or bicarbonate, the use of bicarbonate in uranium poisoning may have indirect beneficial effects such as keeping the damaged kidney tubule free of plugging by cellular debris and aiding the process of tubular-cell repair. Such secondary beneficial effects are reported in the following paragraphs, in which bicarbonate employed was insufficient to exert an appreciable effect in detoxification by complexing U_6.

8.2 Bicarbonate Action in Uranium-poisoned Rats.[10] (By Eugene Roberts and Charles J. Spiegl.) Eighty rats were employed, comprising four experimental groups of 18 animals each and one control group of eight. Determinations were made of urinary volume, pH, creatinine, urea, and protein, and also of the blood nonprotein nitrogen, urea nitrogen, and creatinine.

The usual effects of uranium poisoning were noted in all the animals whether or not they received bicarbonate after the injection of 3 mg of uranyl acetate (≈ 1.7 mg of uranium metal) per kilogram. In each case a marked rise in urinary protein occurred and was followed by a sharp fall in the creatinine excretion. At the time that the blood nonprotein (NPN) and urea nitrogen attained maximal values the creatinine excretion reached a minimum. In each case the urinary volume rose on the first day after injection and reached a maximum a short time after the blood NPN began to fall. The animals receiving bicarbonate after the injection exhibited a greater total output of urine than did the other groups, although bicarbonate administered to uninjected rats exerted no influence on urinary volume. In the group that received bicarbonate after the injection of uranium the protein excretion rose to significantly higher levels than that of the nonbicarbonate group, whereas the blood NPN of the latter group reached a higher level than that of the former. At no time were there any significant pathological differences observed between the animals that did or did not receive bicarbonate.

It is concluded from these findings that sodium bicarbonate when administered in the diet under the conditions of this experiment does not prevent the kidney damage caused by uranium poisoning but rather aids the recovery of the poisoned animals by alleviating secondary effects, such as acidosis, and possibly the precipitation of protein material in the renal tubules.

8.3 High Water Intake as a Therapeutic Measure in Uranium Poisoning. A high water intake appears to be beneficial in uranium poisoning, especially if given in conjunction with bicarbonate treatment. This can be inferred from acute physiological experiments in which catalasuria is used as a criterion of uranium damage to the renal tubules. A high rate of infusion that causes a high rate of urine

production tends to prevent the development of catalasuria in these experiments. Direct evidence of the beneficial effect of a high water intake in rats poisoned by U_6 was obtained by W. F. Neuman (see Chap. 11).

8.4 Possible Therapeutic Action of Ascorbic Acid in Uranium Poisoning. Tien Ho Lan has found that scorbutic guinea pigs show an enhanced susceptibility to U_6 poisoning and that the administration of ascorbic acid is beneficial in uranium poisoning, both in scorbutic and nonscorbutic guinea pigs. This apparently is not true in rats, according to Haven. The mechanism of the beneficial action of ascorbic acid is not known, and its action appears to be less powerful than that of sodium bicarbonate. However, it would be advisable to use it in conjunction with sodium bicarbonate in the prevention or therapy of acute uranium poisoning.

8.5 Beneficial Effect of a Low-salt, High-protein Diet in Uranium Poisoning. It has been found by W. F. Neuman that the use of a diet low in salt and fat and high in protein is beneficial in aiding recovery in rats poisoned by U_6. The high protein intake presumably aids cell repair. The effect of a low-salt diet might possibly be related to the enhanced precipitating action of uranium on proteins, caused by salt (see Chaps. 1 and 13).

Table 15.3—Substances That Are Harmful or without Effect in Uranium Poisoning

No effect	Harmful
Phosphate*	Choline chloride*
$CaCl_2$*	Cysteine†
Tyrode's solution*	Acid diet**
NaCl*	
Cysteine†	
Yeast extract‡	
Liver extract‡	
Thiamin‡	
Niacinamide‡	
Tocopherol*	
Pituitrin§	
Desoxycorticosterone*,‡	
Cortalex*,‡	
Suprarenal cortex liquid*,‡	
Desiccated thyroid*,‡	

*Haven.
†Dounce.
‡Tien Ho Lan.
§Miller.
**W. F. Neuman.

8.6 Measures That Are Harmful or without Benefit in Uranium Poisoning. Substances in this class are summarized in Table 15.3.

8.7 Summary. It appears that the best procedure at present for the treatment of acute uranium poisoning is the continued administration of as much alkali as can be tolerated for a period of several days at least, together with a high intake of water. The diet should be low in salt and fat and high in protein of good biological value; it probably should contain a large amount of vitamin C.

At the present time no complexers for uranium that are more effective than bicarbonate or citrate have been found, so that nothing comparable to British anti-Lewisite (BAL) exists for use in uranium poisoning. Since U_6 does not complex with SH compounds, it appears doubtful that BAL or similar compounds would be effective in uranium poisoning. However, U_4 appears to have some affinity for SH groups. Therefore BAL might be worth a trial, since it is conceivable that it would reduce U_6 to U_4 and then complex U_4 firmly.*

The only measures that could be recommended for prevention of possible chronic uranium poisoning would be avoidance of acidosis, reasonably high water intake, and an adequate diet, low in fat, high in vitamin C, and high in protein of a high biological value.

9. A STUDY OF THE METABOLIC DISTURBANCES PRODUCED BY URANIUM POISONING

9.1 Introduction. In general it seems fairly safe to state that metabolic disturbances occur secondarily to the primary kidney damage caused by the uranium and that they might be expected after damage of the kidney tubules by other agents. It is very doubtful that uranium in relatively low doses has any direct effect on metabolism in general. This statement probably does not apply to the case of massive doses of uranium.

The chief metabolic disturbances produced by uranium are summarized very briefly under the following topics: carbohydrate metabolism, lipid metabolism, nitrogen metabolism, phosphorus metabolism, vitamin C metabolism, citric acid metabolism, phenol metabolism, body temperature, and water balance.

9.2 Carbohydrate Metabolism. (Eugene Roberts.) Exposure of rats and dogs to 20 mg of uranyl nitrate hexahydrate dust per cubic meter produced a decrease in glucose tolerance in both species. In rats this disturbance was accompanied by a decrease in the ability to

*Later experiments have shown that BAL is of no value in uranium poisoning in rats.

form muscle glycogen but by no change in the ability to form liver glycogen. In the dog there appeared to be some abnormality in blood lactic acid levels as well as in glucose levels during the tolerance experiments (see Chap. 10).

9.3 Effect of Injected U_6 on Lipid Metabolism. (a) Phospholipid Metabolism. (Frances Haven and Ruth Crossland.) In rats there is a tendency to develop fatty livers following acute doses of uranyl nitrate given intraperitoneally. Furthermore the average degree of unsaturation of the phospholipid fatty acids of the livers of rats poisoned by acute doses of uranyl nitrate hexahydrate (2.5 to 5 mg/kg of body weight, ≈ 1.2 to 2.4 mg of uranium metal per kilogram) is increased from 13 to 15 per cent over the normal average. There is a corresponding and simultaneous decrease in the degree of unsaturation of the fatty acid components of the kidney phospholipids. This decrease amounts to a drop from the normal average of about 25 per cent in animals given 5 mg of uranyl nitrate per kilogram. These findings refer to animals still in the acute stages of poisoning. Later on, during or after repair of the kidney damage, different results may be found.

Since the degree of unsaturation of the fatty acids of the blood phospholipids also increases during the acute stages of uranium poisoning in rats, the question immediately arises whether there is a transport of phospholipids with highly unsaturated fatty acid components from the damaged kidneys through the blood stream to the liver. Such a preferential transport of phospholipid would cause a relative increase in the kidneys in the percentage of phospholipid having fatty acid components with a low degree of unsaturation. Whether such a transport of phospholipid, and also perhaps of other lipid, actually occurs is not yet certain, but it is plausible in view of the likelihood that changes in the liver in uranium poisoning are secondary to the kidney damage rather than the result of primary toxic effects of uranium on the liver.

(b) Cholesterol Metabolism. (Frances Haven and Jean Box.) The total cholesterol content of the adrenals of both male and female rats was decreased in animals that died of uranium poisoning. In male rats the total cholesterol decreased in the livers. In female rats the kidneys showed an increase in total cholesterol, which could be accounted for by an increase in the cholesterol ester. In male rats no change in the total cholesterol content of the kidneys could be found.

Small amounts of cholesterol are found in the urine of rats acutely poisoned by uranyl nitrate. This urinary cholesterol probably is derived from tubular-cell debris.

9.4 Nitrogen Metabolism. A considerable amount of work on nitrogen metabolism in uranium poisoning has been carried out by various members of the project, but it will suffice here to state that

the total blood NPN and urea nitrogen fractions increase significantly in moderate and acute uranium poisoning, undoubtedly as the result of the damage to the kidney tubules. This phenomenon occurs quite generally in kidney damage produced by a variety of causes.

9.5 Phosphorus Metabolism. (William F. Neuman.) It was found by the use of radioactive phosphorus as a tracer that some retention of phosphate occurs in uranium poisoning. However, the specific point where a blocking of phosphorus metabolism occurs could not be found.

Phosphate retention occurs quite generally in severe kidney damage produced by a variety of causes.

9.6 Vitamin C Metabolism. (a) In the Guinea Pig. (Work done by Tien Ho Lan.) It was found (Chap. 10) that intraperitoneal injection of uranyl acetate in normal guinea pigs increased urinary excretion of vitamin C, but this was not true in scorbutic guinea pigs. When vitamin C saturation tests were carried out in such animals by injecting ascorbic acid intraperitoneally into normal and mildly scorbutic guinea pigs, it was found that the scorbutic animals excreted nearly as much ascorbic acid as the nonscorbutic animals. This result is contrary to the findings in animals not poisoned by U_6, in which case it is usual to find a much greater retention of injected vitamin C if the animal has scurvy than if it does not have scurvy. It is not known why a scorbutic guinea pig loses its ability to retain injected vitamin C after it has been poisoned by U_6.

It was also found that scorbutic guinea pigs are more susceptible to U_6 poisoning than are nonscorbutic guinea pigs and that tissue slices of scorbutic animals in the Warburg apparatus show a greater sensitivity to added U_6 than do tissue slices of nonscorbutic animals, when inhibition of respiration without added substrate is used as a criterion. The latter finding may be related to a damage of intercellular substance caused by the scurvy, which permits better access of the added U_6 to the tissue cells.

(b) Influence of Uranyl Acetate Injection on Vitamin C Metabolism in the Rat. (Work done by Eugene Roberts and Charles J. Spiegl.) Rats injected intraperitoneally with uranyl acetate (0.3 mg or 0.6 mg/100 g body weight, $\simeq$ 0.17 or 0.34 mg of uranium metal per 100 g) showed a pronounced alteration in ascorbic acid metabolism. Uranyl acetate injection stimulated animals at a low urinary level of vitamin C to a daily excretion as high as eight times that of the control rats. On the other hand, when uranyl acetate was administered to rats that had been stimulated by sodium phenobarbital to produce large amounts of urinary vitamin C, a marked decrease in urinary vitamin followed. The excretion after administration of the uranium was approximately

one-half of that maintained by uninjected controls and one-half of that exhibited by the injected animals prior to injection.

9.7 Citric Acid Metabolism. (Work done by Frances Haven and Challiss Randall.) There is an increased excretion of citric acid in the urine of rats moderately or acutely poisoned by intraperitoneal injections of uranyl nitrate, which excretion coincides in time approximately with the increase in urinary catalase and protein. Later a second period of urinary citrate excretion occurs, during and immediately following the period of cellular repair. These findings probably apply to dogs as well as to rats (see Chap. 12).

The source of the urinary citric acid that appears following uranium poisoning is probably the kidney. There is no indication that citric acid metabolism is affected by U_6 poisoning outside the kidney. The tolerance to uranium no doubt can be attributed, at least to a considerable extent, to this enhanced citric acid production by the kidney, since the concentration of citrate in the urine is ample to keep U_6 in a complexed and therefore nondamaging state. The reader is referred to Chap. 12 for a detailed study of urinary citrate in uranium poisoning.

9.8 Phenol Metabolism. (Work done by Frances Haven and Jack O'Leary.) An increase in free and total urinary phenols occurs in uranium poisoning. This increase in urinary phenol is directly related to the dosage of U_6 administered and occurs at a time when catalasuria and proteinuria occur. Therefore it is probably the direct result of damage to the tubular cells of the kidney. The percentage of conjugated phenol is not affected; hence the conjugation mechanism must be intact. The biochemical precursor of this urinary phenol is unknown, but the phenomenon of phenol excretion in the urine following uranium poisoning is interesting and merits further study. A significant increase in urinary phenol occurs after the intraperitoneal injection of as little as 0.3 mg of uranyl nitrate hexahydrate per kilogram of body weight in the rat.

9.9 Body Temperature. (Work done by William Neuman.) There is a rather consistent fall, particularly from 12 to 24 hr before death, in the body temperature in rats severely poisoned by intraperitoneal injections of uranyl nitrate or acetate. If the rectal temperature of a rat falls below 35°C following injection of uranyl nitrate, the animal will invariably die. This fall in body temperature evidently is a reflection of a decrease in over-all metabolism, but whether this is accounted for by a general toxic effect of uranium or rather more specifically by kidney damage is still uncertain. In view of the known specific action of uranium on the kidney the latter explanation is more plausible.

9.10 Water Balance. (Work done by Frances Haven and collaborators.) There is a marked increase in urinary volume in the early stages of moderate or severe uranium poisoning, which may be followed by a period of oliguria or anuria. The latter invariably occurs in animals that later die from the poisoning. These effects are caused by damaged renal tubules. In addition, there is a tendency for the animals to show some edema of the liver and kidney, and in severe poisoning ascites often occurs, perhaps because of an increased capillary permeability. Generalized edema usually is absent. In very severe and fatal uranium poisoning, plasma clots may be found in the peritoneal cavity or in tissue spaces. This points to an increase in capillary permeability, but whether this is the result of a primary toxic action of uranium on the blood vessels or rather results from the heavily damaged kidneys cannot be stated at present.

10. THE IN VITRO UPTAKE OF URANIUM BY BONE SALTS

By Margaret W. Neuman and William F. Neuman

10.1 Introduction. Some in vitro studies have been made on the factors influencing the uptake of uranium by bone. The following section presents a summary of the information obtained at this writing.

Methods. Most of the data to be presented have been obtained by shaking finely ground (60-mesh) glycol-ashed rabbit bone with uranium solutions in a bicarbonate buffer. The uranium was used in all cases as uranyl acetate, with 1 extra mole of sodium acetate added per mole of uranyl acetate. The initial and final solutions were analyzed for uranium, and the amount of uranium taken up by the bone was calculated. In a few cases whole bone was used instead of bone ash, and some experiments were carried out using serum, serum ultrafiltrate, or water instead of bicarbonate solution.

The amount of uranium taken up by bone ash proved to be influenced by many factors, such as uranium concentration, buffer concentration, amount of solution, amount of bone, and time. Consistent results were obtained only if all the known variables were kept constant except the one being studied. The established routine at present is to add to a centrifuge tube 10 mg of bone salt (which can be weighed and transferred with an error of less than 3 per cent) and 15 ml of solution, containing a known concentration of uranium and buffer. The suspension is shaken for 48 hr at room temperature. In every case the bicarbonate solution is brought into equilibrium before shaking with a mixture of 5 per cent CO_2 in 95 per cent O_2. The tube is stoppered with a paraffin-coated stopper during the shaking. No significant

amounts of CO_2 are lost during 48 hr. After shaking, the tube is centrifuged, and an aliquot of the supernatant is taken for analysis.

10.2 Comparison of Uranium Uptake from Bicarbonate Buffer with That from Ultrafiltrate and from Serum. The fluid that carries the uranium to bone in vivo presumably is the extracellular portion of the blood. Serum ultrafiltrate was obtained by a pressure filtration, through a collodion membrane, of serum to which uranium had been added. Serum was used instead of plasma to avoid the use of anticoagulants, which would complex the U_6. The loss of fibrinogen is not of importance, since only a small fraction of U_6 could be carried in plasma by this protein. A comparison was made of the uranium uptake from the ultrafiltrate and that from a bicarbonate solution containing approximately equal concentrations of uranium and CO_2. There was a slightly lower concentration of uranium and CO_2 in the ultrafiltrate. The results of the experiments are shown in Table 15.4.

Table 15.4 — A Comparison of the Adsorption of Uranium from Bicarbonate, Serum Ultrafiltrate, and Serum

		Bicarbonate buffer				Ultrafiltrate			
Solution, ml	Bone, mg	Initial solution, conc. U, μg/ml	Final solution, conc. U, μg/ml	Bone, conc. U, μg/g	Amount removed by bone, %	Initial solution, conc. U, μg/ml	Final solution, conc. U, μg/ml	Bone, conc. U, μg/g	Amount removed by bone, %
10	3	24.3	20.6	11,965	15	20.8	18.8	6,666	9.6
5	10	24.0	4.1	9,755	83	18.6	3.9	7,206	79.0
5	10	24.0	4.1	9,786	83	21.2	3.9	8,480	86.0
		Serum				Ultrafiltrate			
3	30	102.0	18.8	8,222	81	140.0	13.0	12,800	90.0

For each set of values two or three samples were averaged. It is clear from these results that bone ash has a remarkable affinity for uranium, since the data show that it can hold as much as 2,000 times as much uranium as can be held by the buffer. Bone ash removes about the same quantities of uranium from ultrafiltrate as from bicarbonate buffer.

Unfortunately the results listed in Table 15.4 do not represent equilibrium values, since the bone-ash suspensions remained in the shaking apparatus for only 3 hr, which is not a sufficient length of time for establishment of equilibrium. Since it is possible that the distribution of uranium between solution and bone ash might be the same at equilibrium for bicarbonate buffer, serum, and serum ultrafiltrate, it is probably not profitable to speculate at this point about the differences in results obtained with the various solutions.

Because bicarbonate and protein are known to be the most important complexing substances in plasma for U_6, and because the bicarbonate complex is known to be easily filterable and diffusible, bicarbonate has been chosen as the first medium to be studied in obtaining fundamental chemical information from studies in vitro on the deposition of uranium in bone. The rest of the experimental work deals mainly with the uptake of uranium by bone ash or bone from bicarbonate solutions.

10.3 Time Curves for Uranium Uptake by Bone Ash. In order to determine the time at which an equilibrium state would be obtained, a series of samples were equilibrated for various time intervals. In Fig. 15.7 are presented the time curves for the uptake of uranium from water and from 0.025M bicarbonate solutions. In both cases equilibrium was nearly reached in 24 hr. Thereafter there was only a slight gradual increase in the uptake of uranium by bone ash. More uranium was taken up at equilibrium by the bone ash from water than from bicarbonate solution.

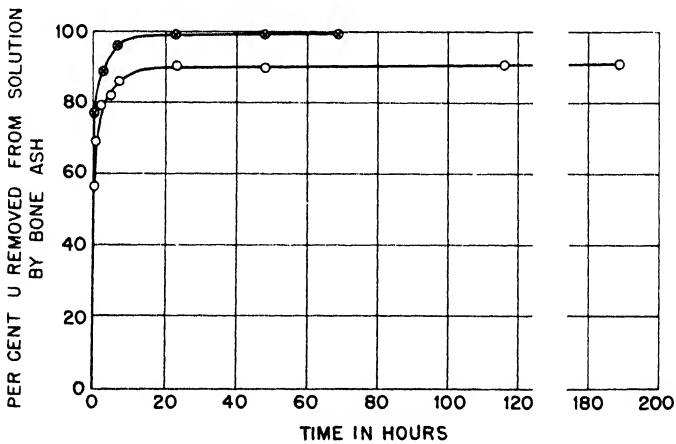


Fig. 15.7—Uranium uptake by bone ash plotted against time. ○ = 1×10^{-4}M uranium in 2.5×10^{-2}M HCO_3^-; ⊗ = 9.37×10^{-6}M uranium in H_2O.

10.4 Uranium Uptake by Bone Ash as a Function of Concentration of Uranium. In these experiments the bicarbonate concentration was maintained at 0.025M. With increasing concentrations of uranium in solution, greater quantities of uranium, but smaller percentages of the total amount present, were taken up by bone ash. This is in accordance with the adsorption isotherm of Freundlich, which is a parabola. On a log-log scale this parabola becomes a straight line.

In Fig. 15.8 the data are shown to fit a straight-line curve when plotted on a log-log scale.

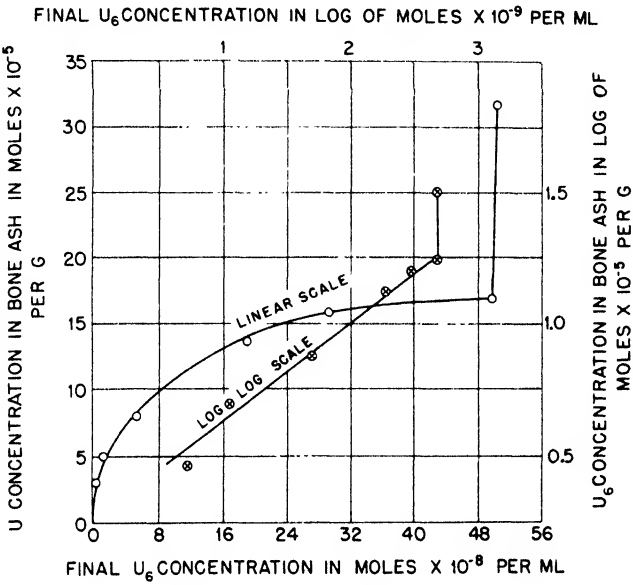


Fig. 15.8 — Effect of increasing U_6 concentration on the uptake of uranium (U_6) by bone ash. ○, linear scales; ⊗, logarithmic scale.

The point at which 31×10^{-5} moles of uranium were taken up per gram of bone ash is out of line with the rest of the results, considerably more so than can be accounted for by the error in the methods used. A sudden change in slope was also observed at a lower uranium concentration in a similar experiment that was carried out for only a 3-hr period. The adsorption curve will be extended to include higher concentrations of uranium to determine the possible significance of the sudden change in slope. It has been suggested that this change to a vertical slope represents a sudden precipitation of the slightly soluble UO_2HPO_4, initiated by the presence of the solid bone particles. There is always phosphate in the solution, which dissolves from the bone. Such a precipitate might be rapidly and completely adsorbed on the bone particles.

10.5 Uranium Uptake by Bone Ash as a Function of Bicarbonate Concentration. If bicarbonate is the only variable, the curve is similar to that shown in Fig. 15.9. With increasing concentrations of bicarbonate, smaller quantities of uranium as well as smaller percentages of total uranium present were taken up by the bone. However,

this curve is a composite of the influence of pH as well as bicarbonate concentration. The pH changed from 6.55 to 7.9 over the range of bicarbonate concentrations employed.

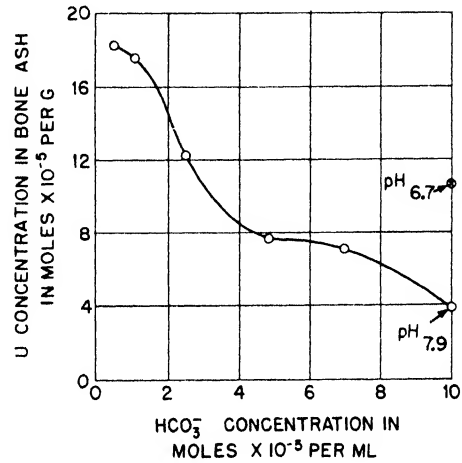


Fig. 15.9—Curve showing effect of increasing HCO_3^- concentration on the uptake of uranium (U_6) by bone ash. ○, pH 7.9; ⊘, pH 6.7.

In an additional experiment the pH of the solution of highest bicarbonate concentration (0.1M) was lowered from 7.9 to 6.7 by equilibration with 100 per cent CO_2, a procedure that would not materially increase the HCO_3^- concentration. In this case, as shown in Fig. 15.9, uranium uptake by bone was increased but not to the level observed at a similar pH but with a lower bicarbonate concentration. It appears that, at this concentration of bicarbonate, about one-half of the reduction in adsorption was due to pH and one-half to change in bicarbonate concentration. The extent to which the changes shown in Fig. 15.9 are due to pH and to HCO_3^- may be investigated by equilibrating the solutions with varying concentration of CO_2 while maintaining a constant pH or HCO_3^- concentration.

10.6 Discussion. It appears from the results at hand that the mechanism of the uranium uptake by bone is complex. The curve obtained with varying uranium concentrations meets the requirements for a surface-limited reaction. This could mean an adsorption. It is possible, however, that there is a chemical combination involved and that the combining sites in bone become exhausted as the uranium concentrations are increased, giving a parabolic curve on a linear scale. The sudden change in slope at the high concentration suggests

a precipitation of some insoluble uranium compound. The situation is complicated by the fact that bone salt itself is not entirely insoluble.

Bicarbonate exerts a significant influence on the uptake of uranium by bone. With increasing concentrations of bicarbonate, more uranium is held in solution and less is taken up by bone. This phenomenon is not a simple competition between bone and bicarbonate for the uranium, because in all cases bicarbonate was present in considerable excess in respect to uranium. The lowest molecular ratio of bicarbonate to uranium was 11 : 1.

It is possible that an exchange takes place between the uranium bicarbonate complex in solution and the bicarbonate compounds in bone. As the bicarbonate is increased in the solution, a smaller percentage of the total uranium reaches the bone.

Of significance in the therapeutic action of bicarbonate in uranium poisoning is the steep slope in the curve shown in Fig. 15.9, at the physiological concentration of bicarbonate (approximately 0.025M). This indicates that small changes in blood-bicarbonate concentration above or below the physiological level would result in significant changes in the amount of uranium depositing in bone.

All phases of the problem should be extended by future experiments. The influence of pH and bicarbonate concentration as single variables should be investigated. The adsorption curve should be extended and repeated to study the sudden change in slope at high uranium concentrations and to determine what, if any, insoluble compound precipitates. The effects of variations in the relative amounts of solution and bone should be clarified. In other experiments the influence of other blood buffers, particularly of HPO_4^{--}, which is also a strong uranium complexer, should be studied. Dried whole bone should be used instead of bone ash. Preliminary experiments have given evidence that the organic material of bone, presumably the protein, actively combines with uranium.

10.7 Summary. The results of studies, not completed at this writing, on in vitro uptake of uranium by bone ash have been described. The results are briefly as follows:

1. Bone ash has a remarkable affinity for uranium. It adsorbs uranium almost equally well from serum, serum ultrafiltrate, and bicarbonate buffer.

2. With increasing concentrations of uranium in solution, greater quantities of uranium but smaller percentages of the total uranium are taken up by bone ash in a manner described by the Freundlich adsorption isotherm. However, a sharp loss in uranium from solution appears to occur at high uranium concentrations, owing possibly to the precipitation of some insoluble uranium compound.

3. With increasing concentrations of bicarbonate at constant concentrations of uranium, smaller quantities as well as smaller percentages of the total uranium present are taken up by bone ash.

4. With increasing pH, the uptake of uranium by bone ash decreases.

The possible significance of these results in regard to physiological findings is pointed out.

APPENDIX — POLAROGRAPHIC METHOD FOR THE DETERMINATION OF URANIUM IN SERUM

Five milliliters of serum was pipeted into a platinum dish and after being dried under an infrared lamp was ignited strongly over a Méker burner. The residue was dissolved in concentrated nitric acid and then was evaporated carefully to dryness. This procedure was repeated once. Then the resulting uranyl nitrate was dissolved in 1 ml of concentrated nitric acid and warmed slightly. Then 10 ml of saturated calcium nitrate solution was added while the platinum dish was still warm.*

The solution was next transferred to a 25-ml separatory funnel, allowed to cool, and was extracted twice with 5-ml portions of ether. The combined ether extracts were then extracted twice with 5-ml portions of distilled water. The resulting solution was diluted to 20 ml and then was analyzed polarographically without the addition of supporting electrolyte, which is probably unnecessary in such a strongly acid solution. In every case the U_6,U_5 cathodic wave was clearly registered without interference from other waves.

The uranium concentration in the sample being tested was measured by comparing the wave height with the wave height of a known sample of uranyl nitrate dissolved in 1N nitric acid, which was roughly the same as the concentration of the acid in the unknown samples.

In order to dissolve the uranium compound to be tested in serum, a sample of the compound, weighed out on an analytical balance so as to contain 10 mg of uranium as metal, was added to 40 ml of serum at 37°C in a ground-glass homogenizer. The sample was carefully and throughly blended into the serum. The mixture was then transferred to a 50-ml Erlenmeyer flask with a rubber stopper and was placed in a shaking machine in a warm room at 37°C. A small crystal of thymol was added to prevent bacterial action. Since alcohols and phenols do not complex U_6, this procedure was considered to be safe. Samples

*The addition of calcium nitrate at this step to drive the uranyl nitrate more completely into the ether was done as the result of information obtained at Dr. Furman's laboratory at Princeton University.

were taken for analysis at ½ hr and 24 hr after placing the material in the warm room at 37°C.

Before ashing the samples for analysis it was necessary to remove the excess undissolved uranium compound. This was accomplished by centrifuging the material for about 5 min at about 15,000 rpm.

After centrifuging, the serum was found to be separated into four fractions. The excess uranium compound was on the bottom; a small layer of lighter solids was just above this; clear serum was above this; and a small layer of fatty material was on the top. The clear portion of the serum was pipeted off for analysis.

BIBLIOGRAPHY

1. A. M. Walker, P. A. Bett, J. Oliver, and M. C. MacDowell, Am. J. Physiol., 134: 580 (1941).
2. R. F. Pitts and W. D. Lotspeich, Federation Proc., 5: 82 (1946).
3. M. E. Bobey, L. P. Longley, R. Dickes, J. W. Price, and J. M. Hayman, Jr., Am. J. Physiol., 139: 155 (1943).
4. H.W. Smith, "The Physiology of the Kidney," Oxford University Press, New York, 1937.
5. R. F. Pitts, Am. J. Physiol., 140: 535 (1944).
6. R. F. Pitts, Am. J. Physiol., 109: 542 (1934).
7. J. Haldi and G. Bachman, Am. J. Physiol., 115: 364 (1936).
8. C. Breedis, C. M. Flory, and J. Furth, Arch. Path., 36: 402 (1943).
9. E. D. Vyshepan, Biokhimiya, 8: 45 (1943).
10. E. Roberts and C. J. Spiegl, The Mechanism of Bicarbonate Action in Uranium-poisoned Rats, Univ. Rochester, Atomic Energy Project Rept. M-1632.

Chapter 16

STUDIES ON HUMAN EXPOSURES TO URANIUM COMPOUNDS

By Joe W. Howland*

1. INTRODUCTION

As has been stated in the introductory chapter of this volume, the primary purpose of this entire study on the toxicology of the uranium compounds was directed toward the protection of both workers and scientists engaged in carrying out that work necessary for the success of the Manhattan District operation in its development of atomic energy for military purposes. During the course of these activities numerous observations were made on the possible toxic reactions of human beings to these compounds either as the result of acute exposure or following long-continued chronic exposure. In this chapter these observations are summarized as completely as possible. Obviously since all these individuals were engaged either in research on uranium purification processes or in the processes themselves, much detail of their work must be omitted for security purposes.

Following a brief review of the related literature on human exposure to uranium compounds, a discussion will be given of the clinical effects of the few accidental acute exposures, a résumé of the clinical studies made in several installations on the effect of known chronic uranium exposure, and finally a review of the laboratory observations made on the industrial medical program carried out in the manufacturing laboratories and plants in various parts of the country.

In an examination of the following data it is quite obvious that, except for the acute exposures in which the individual was heavily contaminated with the uranium compound, no evidences of a chronic toxicity for any of the uranium compounds could be demonstrated by the methods used in the examination.

*This work is a summary of a portion of the control activities of the medical section of the Manhattan District.

2. REVIEW OF THE LITERATURE

There is very little scientific literature on the effect of uranium and its compounds on the human body. The few papers that have appeared are primarily reports on the therapeutic effect of uranium compounds on diabetes mellitus, dermatological disorders, and neoplastic diseases.

Kennedy,[1] West,[2] Walsh,[3] Tylecote,[4,5] and Wilcox[6] treated large numbers of diabetics orally with uranyl nitrate hexahydrate in doses ranging from ½ to 60 grains daily (0.011 to 3.9 g) for periods up to 1 year. The occurrence of transient albuminuria was noted by Tylecote only.

Gailleton,[7] Clark,[8] and Truttwin[9] used uranyl nitrate topically in the treatment of a variety of skin disorders, including lupus, psoriasis, eczema, trychophytosis, and senile atrophy. No toxic symptoms were noted.

Clark[8] and Hocking[10] used uranium or uranium-thorium preparations in the treatment of neoplastic growths with supposedly successful results. No urinary changes were noted. Pack and Stewart[11] studied the effects of intravenous colloidal uranium-thorium preparations in eight patients with carcinoma, all receiving approximately 60 ml of a 1 per cent solution of each of these metals. Questionable benefit was noted in one case. Two patients showed evidence of renal damage, although at autopsy no changes were noted in the kidneys of the single case examined.

Only a single paper is found in the literature on the potential hazard of uranium compounds used in industry. DeLaet and Meurice[12] reported four cases in a Belgian ceramics plant in which changes in the peripheral blood (anemia, leucopenia) were interpreted as due to the effect of the uranium compounds. No urinary abnormalities were noted.

3. EXPOSURE OF PERSONNEL TO URANIUM COMPOUNDS IN THE LABORATORIES AND PLANTS OF THE MANHATTAN PROJECT

The ultimate value of an industrial toxicological program can be measured only by the success obtained in the application of the laboratory findings for the protection of workers in industry. Hence an attempt is made in this final section to present a concise over-all picture of the type of exposures to uranium compounds that occurred in all phases of the experimental and industrial work and the results obtained in the study of exposed personnel for positive evidences of exposure.

In the preceding chapters many data have been presented on the toxicological findings following animal exposures to various uranium compounds by inhalation, ingestion, and skin absorption. It is quite important to state early in this account that such observations can be applied to man only in a relative fashion, inasmuch as the human being is anatomically, physiologically, and biochemically somewhat different from the animal, and his susceptibility to toxic substances may be different.

As has been stated, the urgency of the project demanded that work on the isolation and purification of various potentially toxic uranium compounds be rushed to completion as rapidly as possible. Hence it was not feasible to defer such activity until preliminary toxicity tests could have been conducted. Again, the earlier work on the toxicity of uranium had been conducted largely for the production of experimental toxic kidney diseases in order to study the physiology of this condition. Consequently, little was known about the relative toxicity of varied doses of even uranyl nitrate, the most studied compound. Nothing was known about the toxicity of doses absorbed through the lung, the most common industrial exposure. Obviously, if even temporary protection was to be effected, some sort of a value for the maximum allowable concentration of the metal suspended in air was necessary. Hence, after careful study, the data originally obtained on the toxicity of lead, a heavy metal of atomic weight 207, were used for establishing a temporary maximum allowable concentration of these compounds in air. This amount was 150 μg of uranium dusts per cubic meter of factory air. Following the adoption of this provisional standard the experimental program was rushed to completion to determine whether this level was within safe limits. At the same time the concentration of the dusts in factory air was reduced to this recommended value by means of improvements in existing ventilation, housekeeping, etc.; redesign of processes and handling methods; and, in areas where concentrations continued to exceed this value, the use of recommended protective equipment. The results of the experimental program as given in previous sections indicate that this predicted safe allowable concentration of uranium compounds in air was indeed a wise one.

It cannot be too strongly emphasized that the evaluation of the total exposures to uranium dusts in factory and in laboratory air is entirely different from evaluation of the exposures of animals by inhalation of uranium dusts. In the toxicological chambers the animal is exposed constantly 6 hr daily to a known concentration range of a single uranium dust of high purity, unmixed with appreciable nuisance dust. The particle size of the dust and resultant absorption are, relatively, known. The humidity and temperature are controlled. The animals

are healthy specimens of known age, sex, and weight. Even the normal reaction of the animal to incarceration in exposure chambers is controlled by a similar study in a chamber to which no uranium dusts are added.

The worker in a uranium-processing plant undergoes an entirely different experience. In any given location under operating conditions, concentration of uranium dusts may vary many thousandfold (from approximately 100 μg to 100 mg of the heavy uranium dust per cubic meter of air). Owing to the nature of the work in a chemical purification process, the dust is usually not a pure compound but is composed of a mixture of the dusts from various processes being carried out in that building or buildings. The particle size of the compounds will vary over a very wide range and fluctuate constantly. Humidity and temperature are usually uncontrolled. In addition to all these physicochemical variables, the problem of the worker himself is encountered, perhaps the most unstable factor of the entire group. All ages and races are employed; both sexes are employed; selectivity as to a state of health, except for the elimination of obvious pathology, is rarely allowable if employment is to be maintained. The action of individuals in following safety and precautionary rules for the prevention of exposure, such as the use of gas masks and other protective equipment, is always unpredictable.

With the foregoing considerations in mind, it becomes important to state the comparative value of animal toxicological data as applied toward the protection of the worker. The relative toxicity of a compound for a group of animal species is in almost all instances well above the recommended 150 μg/cu m of air. This difference between the recommended and toxic levels is called a "factor of safety" for the compound in question and will give a wide margin of protection for another species (including the human) that might be unusually susceptible. Again, animals in their exposure chambers undoubtedly receive much higher concentrations of the toxic compound than do workers in the processing plants and laboratories, owing to the use of preventive devices, shifting of work stations, and the psychological fear of a possibly dangerous material. Finally, the relative toxicity of a series of similar compounds usually shows that the susceptibility or resistance of any species bears the same relationship to other species, the sensitive species to one compound (such as the rabbit to nitrate) being again the sensitive species to a related compound.

The general aim of the toxicological program is of course to protect against the insidious development of toxicity from a chronic exposure. That acute exposures will and do occur is an expected event in most

industrial medical programs, and the prevention of such acute exposures (usually to high concentrations of material) falls within the realm of the combined medical and safety departments. In the following discussion of human exposures to uranium compounds, the only two instances of acute exposure encountered will first be given in some detail, followed by a general account of observations made on an over-all survey of chronically exposed personnel.

3.1 Acute Uranium Poisoning. Fortunately, in spite of the very large numbers of individuals working on uranium compounds, the acute toxic effects have been observed in only two instances. Both of these exposures were accidental in nature and involved the same compound, uranium hexafluoride. Inasmuch as this is the first report of any acute injury to the human body by uranium compounds, the details and findings will be discussed at considerable length.

A. Report of First Accident. An accident occurred at an experimental laboratory where a very large amount of uranium hexafluoride escaped, resulting in the death of two employees, serious injury to three others, and slight injury to thirteen additional persons.

(a) Description of the Accident. The cause of the accident was the sudden rupture of a large tank containing the hexafluoride as a gas with the simultaneous rupture of high-pressure steam lines. This resulted in a very dense cloud of the compound and its hydrolysis products, uranium oxyfluoride and hydrofluoric acid, plus steam released from the broken lines.

One of the two engineers was working 5 to 6 ft away from the site of the explosion, and the other was observing the procedure from immediately behind him.

The two fatally injured were directly in the path of the released chemical cloud. One was unable to find his way out of the laboratory for about 5 min and expired 10 min later; the other escaped quite rapidly and died in about 70 min from the time of the accident. Two of the three seriously injured men were in the vicinity of the fatal cases; the third was just outside the building within a few feet of the place where the tanks containing the hexafluoride were stored.

The three seriously injured cases required 10 to 14 days of hospitalization before recovery was complete. The 13 other cases received only minor injuries and required little or no treatment other than that which could be given in a dispensary.

A study revealed that the exposure of the various individuals differed remarkably, but, in general, injury was in direct proportion to the intensity of the exposure. The duration of the exposure was relatively short in all cases. It is estimated that the average exposure

was about 17 sec. The characteristics of the individual exposures varied somewhat because of the different actions taken on the part of the individuals, e.g., holding the breath, closing the eyes, and removal from contaminated areas. A further variation in exposure resulted from the individuals' actions in showering and removal of clothes.

There was a considerable variation in the CT level (concentration times time of exposure) that the different individuals experienced. In general the most severe injuries occurred in those nearest the site of the explosion. There appeared to be less variation in the length of exposure than in the concentration of the exposure.

The steam lines were broken by the explosion of the storage tank and the atmosphere of the room contained a mixture of uranium hexafluoride, hydrofluoric acid, uranium oxyfluoride, and live steam. The combination of the moisture and heat undoubtedly facilitated the hydrolysis of the uranium hexafluoride and from a toxicological viewpoint aggravated both the systemic and vesicant action of these substances.

(b) Case Reports of Fatally Injured Workers. (1) Case No. 1. Clinical Observation. He was the more seriously injured of the two fatal cases and nearer to the point of explosion. He was in extremis when first seen, apparently having been sprayed with live steam containing liquid, solid, and gaseous material in large quantities. He died 16 min after the accident.

Gross Pathological Findings. The skin revealed extensive third-degree burns, and over 70 to 80 per cent of the body surface showed some degree of injury. The areas covered by his underwear (shorts only) were free from burns except for the skin above the anus and that of the scrotum. The hair on the scalp also seemed to afford protection. The areas of mottled blue, green, and cyanotic red distributed in the skin of all parts of the body were thought to be related to chemical irritants and to the fluid ("citrated-like") character of the blood. The conjunctivas were edematous and injected. The corneas were frosted. The oral and pharyngeal mucous membranes were edematous and gray-green in color. There was generalized edema of the subcutaneous tissue beneath the burns, varying considerably in degree. This is typical of chemical burns as opposed to thermal burns (suggesting the invasiveness of the chemical-type burn). Other positive findings were:

1. Small petechial hemorrhages in the epicardium.
2. The pulmonary tissue was bulging into the intercostal spaces. The surfaces of the lungs were dark red, and the organs only partially floated in water. On section, the bronchial mucosa was a dirty-gray necrotic mass with partial desquamation. The parenchyma was unchanged except for increased fluid content.

3. The blood resembled citrated blood.

4. The intima of the blood vessels was tinted with hemoglobin.

5. The external surfaces of the neck organs (thyroid, larynx, trachea, and proximal esophagus), together with the surrounding fascia, had a green-blue fluorescent sheen. The mucosa of the larynx and trachea was necrotic, dirty green in color, and sloughing. The submucosa was edematous. The mucosa of the esophagus down as far as the region of bifurcation of the trachea was likewise necrotic and sloughing. Upon section the thyroid parenchyma was a dark reddish green in color and appeared to be necrotic.

Microscopic Pathological Findings. 1. Skeletal muscle taken from superficial areas showed considerable edema.

2. The lungs showed marked vascular congestion and patchy alveolar edema. Red cells were present in the alveoli.

3. The kidneys showed tubular swelling and marked vascular and glomerular congestion. The tubular epithelium was detached in some areas from the basement membrane. Coalescence of the epithelium was present in many tubules.

Table 16.1 — Uranium Analysis on Autopsy of N.E.*

Sample	In alcohol, μg U/g	In formalin, μg U/g
Lung	800	570
Bronchi	750	320
Tongue	25	
Heart		22
Kidney	18	35
Liver	17	
Stomach wall	42	4
Spleen		0
Ribs		0
Esophagus		1
Testis		0
Fresh skin, outer layer	270	
Fresh skin, inner fat layer	35	
Urine	0.10	
Alcohol (blank)	0	
Formalin (blank)		0

*Separate portions of organs were preserved in alcohol and in formalin.

Laboratory Findings. The autopsy material was analyzed for uranium by spectrographic means (see Table 16.1).

(2) Case No. 2. Clinical Observations. Following the accident his condition appeared to be good. A short time after admission to the

hospital (20 min) he developed a progressive respiratory distress associated with considerable generalized pain. He expired 70 min after the explosion.

Gross Pathological Findings. 1. Approximately 70 to 80 per cent of the body surface was burned. Most of the burns appeared to be third degree in type. The scalp was not involved, being protected by the hair, which was brittle and dry.

2. The conjunctiva of the right eye was edematous and red.

3. The mucosa of the mouth and nasopharynx was necrotic and gray-red in color.

4. Numerous petechial hemorrhages were noted in the epicardium.

5. The blood was fluid and of a citrated-like nature.

6. The intima of the arteries was stained with hemoglobin.

7. The bronchi were filled with undigested food. The bronchial mucosa was intensely injected but not necrotic. The lung parenchyma was diffusely red and bloody without any focal lesions.

8. Lobulation of the liver was indistinct.

9. Petechial hemorrhages were present in the right kidney.

10. The laryngeal, tracheal, and esophageal mucosa were injected and edematous but not necrotic.

Microscopic Pathological Findings. 1. The superficial skeletal muscle was edematous.

2. The heart showed myocardial fragmentation and an area of subepicardial hemorrhage.

3. The lungs showed marked vascular congestion, moderate alveolar edema, and some alveolar hemorrhage.

4. The kidneys showed tubular swelling, marked vascular and glomerular congestion, and a separation of the tubular epithelium from the basement membrane. Coalescence of the epithelium was observed in many tubules.

Laboratory Findings. Analysis for uranium in the autopsy material was carried out by the spectrographic method (Table 16.2).

(c) Description of Findings in the Nonfatal Cases. In addition to the two fatal cases previously described, 19 men were exposed to the huge chemical cloud that enveloped almost everything within a radius of approximately 100 yd of the center of the explosion. This cloud required several minutes to dissipate.

Three of these individuals developed no symptoms. Fourteen were immediately hospitalized for observation and treatment (besides the one that died almost immediately and the other who died in slightly more than 1 hr). Two additional men reported to the dispensary 36 hr

after the incident because of minor symptoms. Except for three men who were more seriously injured, all the hospitalized patients were ready for discharge within 48 hr after hospital admission.

Table 16.2 — Uranium Analysis on Autopsy of B.R.*

Sample	In alcohol, μg U/g	In formalin, μg U/g
Lung	0	0
Bronchi	0	0
Tongue	0	
Heart	0	
Kidney	0	0
Liver	0	1.1
Stomach wall	1.3	0.3
Spleen		0
Ribs		0
Esophagus		0
Testis		0
Fresh skin, outer layer	310.0	
Fresh skin, inner fat layer	8.0	
Alcohol (blank)	0	
Formalin (blank)		0

*Separate portions of organs were preserved in alcohol and in formalin.

(1) Clinical Findings. For brevity a résumé of the effects of the chemical exposure on the various body structures and systems is given.

Eyes. Eleven of 16 surviving patients experienced an intense burning sensation of the eyes with profuse lacrimation. A chemical conjunctivitis occurred in 8 of these 11 patients. A severe corneal ulceration occurred in one case and was described by the ophthalmologist as a chemical burn of the entire corneal epithelium. A slit-lamp examination of the eyes of this patient 2 months later revealed a diffuse haze of the epithelium of the lower half of both corneas. No staining or change in sensitivity was noted. Prognosis was given for the complete disappearance of this pathology.

Respiratory Tract. Of the 16 surviving patients, nine complained of throat irritation, six of hoarseness, and six of nasal "stuffiness." Respiratory distress of a transient nature occurred in 10 of the 18 patients. This was described as a shortness of the breath, inability to "catch a breath," or just as an uncomfortable sensation in the chest. In 10 patients severe coughing spells occurred, often of paroxysmal

nature. On examination nine patients showed numerous râles in the chest, suggestive of a chemical bronchitis; one, a typical pulmonary edema; and one, a suggested pulmonary edema. The patient with pulmonary edema raised blood-streaked sputum for 3 days after the accident. This sputum had a glistening reddish-orange appearance and in areas unstained by blood resembled hydrolyzed uranium hexafluoride.

Skin. Ten patients complained of burning of the skin shortly after exposure. Some of these individuals noted intense pain in the scrotum and penis and around the anus (probably because of the hydrolysis of the hexafluoride in these moist areas and a release of the irritant hydrofluoric acid). Eight patients showed definite second-degree burns. Two had coagulative necrosis of the skin of the legs, which healed very slowly, more than 1 month elapsing before complete epithelialization took place. The burned regions at that time were pigmented and of a parchmentlike appearance. Most of the severe burns were on the lower legs, the area of the burn starting abruptly at the top of the shoes. The burns were treated with vaseline gauze dressings with mild compression. No calcium gluconate (prescribed for hydrofluoric acid burns) was necessary.

Gastrointestinal Tract. Six of the 16 patients had nausea and vomiting in some degree. Three of these had in addition abdominal cramps with or without some distention. The symptoms disappeared spontaneously in 12 to 24 hr. No diarrhea was observed in any patient.

Urinary Tract. Two of the three seriously injured individuals showed transient albuminuria. One excreted from 39 to 100 mg of albumin (per 100 ml of urine) daily for a period of 7 days after the accident. Thereafter he showed only an occasional trace of albumin, which disappeared entirely by the time of his discharge from the hospital. All three of the seriously injured had a 3-day period of transitory urinary suppression. One showed a mild rise in the blood urea and nonprotein nitrogen level. These blood constituents were still somewhat elevated 3 weeks after the accident, after which a gradual reduction to normal levels was noted. Five of the group of 16 continued to excrete uranium 18 days after the accidental exposure.

Nervous System. All the seriously injured individuals were unusually nervous and apprehensive for 4 to 5 days after the accident. One individual was definitely overstimulated for about 3 days, exaggerating all facial expressions and being unusually verbose and talkative. At times he was almost incoherent. The other seriously injured patient, although normally quiet and placid, became very apprehensive with a similar tendency toward the exaggeration of statements. The opinion of all observers held that the mental reactions were more than could possibly be explained on a fear-reaction basis.

(2) Laboratory Data. The laboratory data collected on these cases consisted of complete blood counts with differential smears, blood urea nitrogen and nonprotein nitrogen, blood chlorides, serum cholesterol, plasma carbon dioxide, total proteins, and complete urine analysis, including frequent analysis for the uranium content of the urine by the spectrographic method.

The significant findings follow:

1. Temporary fall in the plasma protein level (mild hemodilution).
2. Transitory type of albuminuria.
3. Elevation of blood nonprotein and urea nitrogen.
4. Uranium present in urine in amounts from 0.00 to 0.51 mg/liter (Table 16.3). The persons having the highest exposure showed the

Table 16.3 — Uranium Analyses of Urines on Five Nonfatal Cases

Date	Initials of victim	Uranium, mg/liter	Total urinary output, ml
Sept. 4	T.O.	0.51	1,000
5	T.O.	0.15	2,370
6	T.O.	0.18	3,900
9	T.O.	0.10	?
10	T.O.	0.10	?
11	T.O.	0.07	?
12	T.O.	0.14	?
20	T.O.	0.00	?
Sept. 16	H.A.	0.07	?
Sept. 20	B.L.	0.05	?
Sept. 4	L.E.	0.15	1,395
5	L.E.	0.09	1,800
6	L.E.	0.00	2,150
9	L.E.	0.00	1,725
10	L.E.	0.00	1,775
11	L.E.	0.21	?
12	L.E.	0.16	?
20	L.E.	0.02	?
Sept. 4	K.R.	0.00	820
5	K.R.	0.05	2,655
6	K.R.	0.15	2,000
9	K.R.	0.06	1,635
10	K.R.	0.11	1,765
11	K.R.	0.10	1,925
12	K.R.	0.11	?
20	K.R.	0.05	?

highest uranium excretion. In addition their urinary abnormalities were the most severe, consisting of albuminuria plus red cells and casts in the urinary sediment, and their blood urea and nonprotein nitrogen were elevated for several weeks.

The roentgenographic films of the exposed individuals showed a diffuse inflammatory process of mild nature radiating out from the hilus of each lung. Involvement of the upper lung fields was more marked. Findings present in the lower lobes suggested those seen in mild pulmonary edema with an increase in the prominence of the lung markings. No consolidation was noted.

Bacteriological cultures of the sputum contained only normal flora.

B. Report of Second Accident. The patient became suddenly surrounded by vapor as the result of a sudden development of a leak on a cylinder containing uranium hexafluoride. In the cloud he was unable to find an exit rapidly so that the period of his exposure in the cloud may have been as much as 10 min. When taken to the dispensary he complained of burning of his eyes and throat. Coughing and retching were quite marked with vomiting on one occasion.

Discussion of Findings. He was immediately hospitalized, and during the following month his clinical course was studied in considerable detail. The important findings were related to the respiratory tract, urinary tract, and the eyes.

The lower respiratory tract showed clinical evidence of a chemical pneumonitis. Râles were present throughout the chest, and the patient produced a thick tenacious black sputum for about 10 days. The X-ray findings immediately after the accident showed no abnormalities but 5 days later revealed an increase in the density of the bronchovascular markings and hilus shadows. Ten days after exposure the chest was clear both clinically and radiographically. There was a hemorrhagic involvement of the left arytenoid cartilage and vocal cords on laryngoscopic examination. Thirteen days later this involvement was resolving favorably.

There was a rapid rise in the quantity of the solid elements in the urine followed by a gradual decrease as the patient improved. The single constituent appearing in largest amount was a coarsely granular cast. Epithelial cells of various types were noted. Occasional large mononucleated phagocytes containing red cells and cellular debris were also found.

The eyes showed a chemical conjunctivitis with necrosis of the corneal epithelium. Within 5 days under conservative treatment the corneal epithelium had almost completely regenerated. Visual acuity at the time of discharge was normal.

The mental status for the first 5 or 6 days following the accident was marked by general sluggishness with transient periods of restlessness, irascibility, and nervous tension with occasional silliness and loss of contact. All symptoms of this type cleared up within a week after admission to the hospital.

C. Discussion. The typical picture of the acute poisoning of human subjects by the inhalation of a soluble uranium compound (uranium hexafluoride) is one of an initial chemical injury to the respiratory tract followed almost immediately by evidences of kidney injury. At the onset there is almost immediate development of marked weakness and prostration. The patient complains of suffocation and retrosternal pain and on examination exhibits dyspnea, cyanosis, and numerous wet râles and rhonchi throughout the chest. The latter findings are associated with the development of an annoying irritative cough, often producing a greenish-gray sputum that frequently contains blood. In severe exposures, signs of pulmonary edema are often seen. X rays of the chest show a soft infiltration along the bronchovascular markings and an increased width of the intercostal spaces. At times areas of consolidation may be noted. The circulatory system immediately following the exposure shows signs typical of the onset of traumatic or secondary shock plus secondary signs related to the cyanosis or pulmonary edema. The body temperature is usually elevated for a period of 12 to 72 hr, the highest observation being 103°F. The burns of the skin resemble those following hydrofluoric acid exposure but are almost without exception less severe. A similar latent period of two or more hours between exposure and development of the chemical burn is noted. With conservative treatment the burns are self-limiting in type, although as much as 6 weeks may be required for the healing of the injured area. The exposure is usually associated with a change in sensorium, states of depression or agitation being quite common in all individuals. Corrosive action of the chemical results in a coagulation necrosis of the epithelium of the cornea, at times accompanied by deeper ulceration. Healing is spontaneous and complete. The renal changes as observed in human beings have been entirely those of laboratory observation and have never been associated with clinical findings such as anuria, costovertebral pain, colic, or the like. Maximum changes as observed have been transient albuminuria, increase in solid elements in the urine, and moderate retention of nitrogenous products in the blood.

An analysis as to the probable cause of the various clinical findings indicates that in all probability the injurious effects observed on the skin, eye, mucous membranes of upper respiratory tract, esophagus, larynx, and bronchi were all directly caused by the action of the fluoride ion on the exposed tissues. The uranium as such had its only effect in the production of the transient urinary-tract changes.

D. Conclusion. The acute effects of exposure to high concentrations of uranium hexafluoride have been demonstrated to consist of corrosive changes in the skin, eyes, and respiratory mucosa, probably

caused by direct action of the contained fluoride, and transient kidney changes related to toxic action of the absorbed uranium.

3.2 Chronic Exposure to Uranium Compounds. The previously discussed reports of accidental exposures to uranium hexafluoride are the only known instances of acute high-concentration exposures to uranium compounds. The most important problem, however, to the medical group supervising such activities in the Manhattan District, was the possibility of the existence of a chronic toxicity to uranium compounds that might develop in workers in the important laboratories and plants of the project.

After operations on these possibly toxic substances had been carried out for several months, it was evident either that no toxic changes had occurred or that their development was of such an insidious nature that the methods used for their detection were insufficiently sensitive to show changes. Hence it was necessary to set up some plan of examination that could detect the presence of abnormalities as they developed.

In the following section the problem will be stated, followed in turn by discussion of the type of examination, method of analysis, and experimental observations.

The Problem. The selection of the tests used in the exposure of workers in industry followed these criteria: (1) the test should use the most sensitive index of injury to the human organism; (2) it should be of the highest reliability and applicable to all types of individuals; (3) all known and specific types of damage known to be caused by uranium compounds should be tested for; (4) the test should not alarm the subject psychologically so that he would suspect presence of damage where none exists; (5) the test should be sufficiently simple to be capable of being performed in a routine clinical laboratory.

A review of the available data indicated that at that time the only definite damage known to be caused by uranium compounds was restricted to the development of a toxic necrosis, with resultant damage to kidney tubules in varying degrees depending, in so far as could be determined, on the susceptibility of the animal species and the size of the dose. Suitable tests for this type of damage had already been known and consisted simply of tests on the urine for excreted albumin as well as evidences of the actual tubular damage in the form of excreted cells and cellular debris either singly or in the composite casts. Other tests such as urinary specific gravity were also of value in predicting renal abnormalities. Therefore use of the common urinary tests satisfied the foregoing conditions for the detection of any kidney abnormality. Inasmuch as uranium, like other heavy radioactive materials, was suspected of being deposited in excessive amounts

in bone, it was possible that the small amount of radiation to which the bone marrow would be exposed over an extended period of time might result in a depression of the red and white marrow coupled with a production of abnormal white-cell elements. Inasmuch as this possibility could exist, suitable tests were necessary in order to detect this possible pathological development. As to the nature of these events, the only evidence that could be used was the development of changes in the cells of peripheral blood. For this reason, routine analyses of the circulating blood elements, namely, red cells, hemoglobin, and white cells, were necessary plus a differential examination of the white-cell elements. Serial examination of these should indicate the development of any depressive state or abnormality that might occur following radiation exposure from the deposit of uranium.

It was suspected also that some liver damage might occur. However, in the absence of suitable clinical tests it was decided that the clinical evidence of liver disease should suffice as an index of the development of this condition.

Hence the examination of personnel exposed to uranium compounds resolved itself into serial examination of the blood and urine at specific intervals. Development of any unusual state or abnormality in an individual was checked upon with additional, more specific tests as will be outlined below.

Examination of Human Subjects. Before employment of an individual in any of the laboratories or industries using or engaging in the production of uranium compounds, a preliminary screening for obvious physical abnormalities was carried out. This consisted of a complete physical examination by a competent observer, X ray of the chest, a Wasserman test, and special tests thought to be necessary for any clinical purpose. In addition a laboratory examination of the blood and urine was carried out. The blood examination consisted of a hemoglobin test by the Sahli method, erythrocyte count, leukocyte count, and a complete differential examination of the white-cell elements. The urine examination consisted of the testing for specific gravity, the reaction (acid or alkaline), the qualitative analysis for albumin and sugar, and a microscopic examination of the centrifuged sediment for cells and abnormal elements.

Inasmuch as the war emergency had caused marked diminution in available labor throughout the entire country, it was necessary to accept individuals for employment whose physical status would not meet the requirements for peacetime industry. In addition, certain individuals, particularly scientists, were of such value to the project that their presence was necessary in spite of obvious physical defects. Hence it was necessary to limit the refusal of possible employees to

the minimum, and disqualification was made only in those showing obvious kidney pathology, extreme cardiovascular disease, and various pulmonary conditions, particularly chronic bronchial asthma. The latter condition is well known in industry to result in considerable difficulty, particularly when noxious materials and gases are present.

Following the screening examination the individual was employed and placed in the laboratory or industrial process where needed. At intervals, urine and blood examinations were carried out for the detection of changes. Although there was variation in the frequency of these examinations at first, the following schedule was soon decided upon: urines were examined once a month, and, inasmuch as blood changes were presumed to develop relatively slowly, a 3-month interval was used between blood tests.

Special examinations were later carried out after an individual had been exposed to uranium compounds for an extended period of time. One consisted of an analysis at suitable intervals of a 24-hr specimen of urine by the fluorophotometric method for uranium content. It was soon discovered that only the individuals exposed to high concentrations of the soluble compounds showed an increased urinary excretion. Considerable difficulty was encountered in this type of examination because of the instances of contamination of the urine from dust on the clothing and skin. For this reason, accurate analyses of excreted uranium were limited largely to those individuals showing only high exposure over an extended period of time. Where possible, analyses of teeth and also of bone samples for deposited uranium were made. The latter study, although very limited, tended to show that little deposition was occurring in the processing plants where the instituted precautionary measures had been followed. This observation agreed with the presence of low uranium excretion in these individuals.

All individuals in the laboratories and plants were examined according to the following schedule whether exposed or unexposed to uranium compounds. The exposed personnel might be regarded as an experimental group and, as detailed below, were subdivided according to the degree and type of exposure. The unexposed personnel corresponded to a control group representative of the population in the locality. The studied control group of one locality was not compared with the experimental or control groups from another locality.

Method of Analysis. The data on both physical examinations and the laboratory studies were submitted to a central office for study. The physical examinations were first reviewed for obvious abnormalities. The serial laboratory data were printed in such a form as to facilitate comparison and were subjected to a final screening for

detection of gross errors. Any markedly abnormal finding in the laboratory study was checked back with the laboratory where the finding originated in order that any possible pathological cause for this variation would be eliminated. At times it was necessary to repeat physical examinations and related studies to solve the problems. Final data were grouped in such a form as to facilitate statistical evaluation. The data were punched on cards and analyzed according to the methods given in Chap. 3 on Statistics.

Experimental Observations. The program described above was carried out in all the laboratories and processing plants working on hazardous uranium compounds. Inasmuch as these sites were extremely varied in type and purpose it would be difficult to submit the findings collectively. Hence a subdivision of the various types of work has been made with a representative example of each group selected for exposition. The various types selected included (1) laboratories, (2) small uranium-processing plants, (3) large ore- and uranium-processing plants, and (4) large uranium-processing plants. A brief description of each of these installations will be made including statements as to the types of work, the physical status of the workers, the types of exposure, and, finally, the results of the laboratory data obtained in a study of such a group. The exposition related to the process itself must be rather general, inasmuch as both the processes and specific materials used in them must be kept secret for purposes of security.

(1) Laboratories. Type of Work. The laboratory selected for extensive study was one in which several hundred people were engaged in the study of various uranium compounds.

Physical Status of Workers. Employed in this installation was a large group of professional and academic scientists either directing large groups or acting as leaders of smaller research sections. Under them were routine laboratory workers engaged in the actual operation of the specific processes. In addition maintenance and housekeeping workers were employed.

Type of Exposure. The exposures were to uranium compounds both wet and dry with resultant hazard by inhalation and skin absorption. The amounts of material used were so small as to eliminate possible toxicity by ingestion. The types of exposure may be classified in several ways. Many workers were exposed to several, and others to only single, compounds. Many had a relatively heavy exposure as gauged by the many hours of work daily with these compounds. Others had only moderate or casual exposures, the latter term being used to indicate less than 1 hr daily. Still others had no exposure whatever and were used as controls.

The medical program was closely combined with a safety program to ensure the use of all protective methods known for the prevention of injury due to specific uranium compounds as well as those other materials ordinarily used in large laboratory and industrial practice.

The Results of the Analyses. The serial analyses of data obtained on this group of individuals can best be described, because the amount of data accumulated on these workers over a period of 2 years of observation is so voluminous that it would be impossible to tabulate sufficient data to illustrate the study even briefly. Although several hundred individuals were employed at all times, serial examinations on all individuals were obviously not possible owing to the continual turnover of labor and transfer of personnel common to all war installations. However, sufficient analyses were obtained covering an extended period of time to demonstrate conspicuously the fact that no pathological abnormalities that were within the range of statistical significance did occur. This finding is highly important, inasmuch as it is obvious that statistical analysis of data on sufficiently large groups of people may indicate changes that are not apparent on direct examination of the data of a single individual. Urinary abnormalities were not observed even after the heaviest exposure. Blood variations that occurred were well within the normal range of individuals in the studied locality. In conclusion it can be said that, within the limits of the method employed, no detectable changes were observed following chronic exposure of these individuals to uranium compounds.

(2) Small Uranium-processing Plants. Type of Work. The work carried out in this installation involved the processing of chemically pure uranium metal. The larger portion of the work is that classed as heavy labor in a metallurgical plant and involves exposures to a limited extent to the radiation from the metal itself as well as inhalation and ingestion of insoluble uranium compounds.

Physical Status of Workers. Several professional chemical engineers assisted by routine laborers engaged in the plant processing were employed. The office staff in this installation was used as the control group. A small number of individuals were working in an analytical laboratory engaged in the analysis of the insoluble uranium compounds. Much direct handling of the metal was encountered.

The Types of Exposure. The types of exposure encountered were (1) the dust of insoluble compounds in a relatively limited locality and (2) direct exposure to the uranium metal by skin contact. All the material used was in a dry form and insoluble as well, so that the possibility of ingestion again was considerably reduced. The safety program and medical program to all intents and purposes were identical and were under the supervision of the plant chief.

Analysis of Data. The statistical analysis of the laboratory data obtained from these individuals again showed no conspicuous change in either urine or blood. An additional study of the urines of this group was carried out with the catalase method as discussed in a previous section. Results of this study indicate that even to this very delicate test little change is noted.

This installation was also subjected to another type of study not mentioned previously. This is the study of changes in the contour of the fingerprints as caused by the exposure to beta radiation from handling of the uranium metal itself. Such changes were observed in individuals exposed to continuous radiation for long periods either from X rays (particularly fluoroscopy) or the direct handling of radium. These changes require months for development, but within the period covered by this study they would form a positive index of this type of exposure. The results of the fingerprint examination for the detection of this type of skin injury were completely negative.

In conclusion this study indicates that, again within the limits of the method, no detectable damage to any individual at this installation could be demonstrated.

(3) The Large Ore- and Uranium-processing Plant. The Type of Work. The type of work carried out at this installation consisted of the extraction of uranium from uranium ore (pitchblende) containing varying amounts of radium. The uranium was purified by a series of processes employing both alkali and acids, resulting in the formation of such compounds as uranyl nitrate (acid) and sodium diuranate salt (alkaline) plus many intermediaries. The processes involved solution of uranium compounds in many different ways with the precipitation, extraction, and filtration of the collected salts. Hence the hazards were not only from the dusty ore but also from the wet filter cakes and final dried compounds. Both soluble and insoluble compounds were included in the processing stages.

The Physical Status of the Workers. The physical status of the workers, except for a few professional engineers working in a supervisory capacity, was that consistently found in the group classed as routine laborers. A small laboratory was maintained.

The Type of Exposure. The type of exposure was, as in the large laboratory, a mixed one with exposures to the ore and to a large number of uranium compounds existing in both wet and dry states. Much individual handling of the wet compounds took place, which made possible exposure by ingestion and skin absorption in addition to inhalation of the dried material suspended in the plant air. Exposures to potentially dangerous acids (particularly hydrofluoric) and alkali used in the process also occurred. A full-time safety program was

maintained at this installation. It cooperated in the enforcement of specific handling methods for special uranium compounds and ore. Monitoring schedules both for uranium and for radiation (largely for radium and radon) were carried out for the over-all control of the amount of hazardous material to which workers in each of the plant localities were exposed.

Analysis of Data. Analysis of the data from these installations is complicated by turnover of labor and shifting of personnel so that, although several hundred individuals were employed at all times, a consecutive record of laboratory data covering 2 years of study is available on only a small percentage of this group. In spite of the fact that a certain number of individuals were also exposed to radium radiation (and possible deposition in bone) no significant abnormalities in the examination of blood laboratory data could be attributed to the exposure. Additional analyses were made on the urinary excretion of uranium in selected individuals receiving heavy exposure. These results were not abnormal. Urinary-catalase-excretion studies were also carried out and, as shown elsewhere, gave no positive results in the absence of obviously chronic renal abnormalities from causes unrelated to the exposure. These included chronic nephritis, cardiorenal disease, and the like.

In conclusion it must be stated that, again within the limits of the method, no demonstrable changes that could be attributed to the uranium exposure were observed.

(4) Large Uranium-processing Plant. Type of Work. The type of work carried on in these plants differed from that described previously in that only one or two uranium compounds were used, and, more important, the operating procedures were such that very little material was allowed to escape into the factory air. Hence, although many people were employed, actual moderate to heavy exposure occurred in relatively few individuals. Such exposure was for the most part accidental in type. In addition to the routine plant processing, a research laboratory was maintained for the investigation of changes in processing methods as well as analyses of the product. This involved problems somewhat similar to those already discussed in Sec. 3.2 (1).

Physical Status of the Workers. The physical status of the workers for the most part was that characteristic of any labor population recruited from a large portion of the United States and brought with it certain specific problems that would influence any analysis of laboratory data. Such a problem was exemplified by the presence of

hookworm disease in individuals recruited from the South, and the effect of this parasitism in producing abnormalities in the white-blood-cell count. A high percentage of women was employed here also. This is somewhat different from the condition existing in the other smaller installations.

Type of Exposure. The type of exposure was confined to inhalation and skin absorption of a single compound, plus the exposure to certain special compounds occurring as by-products. Certain special materials that in themselves are toxic and capable of producing kidney abnormalities were developed for the processes. Combined engineering and special safety procedures reduced all hazards to a minimum. Coupled with this was the high monetary value of the purified uranium, which made it imperative that all steps be taken against its loss in the plant in any way and therefore reduced the secondary exposure of the plant personnel.

Analysis of Data. The analysis of the data obtained on these thousands of individuals has been carried out elsewhere. Results, however, show that any existing abnormalities occurring in either blood or urinary examination would be completely explainable on the basis of extraneous causes and cannot be attributed to the uranium. No instance has been found in this area of injury to an individual's health primarily due to specific uranium toxicity.

Elaborate monitoring procedures have been carried out. These included the regular scheduled examination of air for concentration of uranium dust by the electrostatic precipitator or filter methods. Analyses of the 24-hr concentration of uranium in the urine have also been carried out on possible high-exposure cases. Correlations between the degree of exposure by inhalation and the urinary uranium content have been attempted. No consistent relationship has been demonstrated in the studies carried out to date.

In conclusion it is again evident that, within the limits of the method, no demonstrable changes that could be attributed to the uranium exposure were observed.

4. SPECIAL EXAMINATIONS AND STUDIES

A series of studies of more extensive type have also been carried out on groups of individuals employed in the various processing plants discussed in the previous section. Some of these, the catalase studies, have been described elsewhere in connection with the reports on the animal experiments (Chap. 14). All such studies demonstrate that

injury by uranium compounds to the normal kidney is almost nonexistent in the uranium-processing plants of the Manhattan District.

Two other studies that have not been mentioned were also carried out.[1] It was obvious that the routine laboratory studies carried out on all personnel exposed to hazardous compounds would not include many of the elaborate analytical procedures used by clinical medicine and physiology to demonstrate minimal changes in the function of the kidney, blood-forming organs, and liver. Studies of this type were necessary. The general plan consisted of the selection of a group of individuals who had received known heavy exposure to certain uranium compounds over a known period of time, and the use of all the most selective tests on them to determine whether any damage could be demonstrated. Two such studies were carried out and will be discussed in detail.

4.1 Study 1. The purpose of this first study was to evaluate as completely as possible the physiological status of 10 men who had been working for 5 to 9 months in an industrial plant processing hexafluoride. As has been discussed under the acute cases reported in this chapter, this compound has hazardous properties, probably largely caused by its hydrolysis products, uranium oxyfluoride and hydrofluoric acid. The number of men used in this study was small, but, inasmuch as they held a variety of positions in the plant, they could be considered to roughly represent what might be termed a small cross section of the employees. The men were hospitalized for 2 days and the following procedures carried out:

a. Medical histories
b. Physical examinations
    1. Dental examinations
c. X rays of chest
d. Electrocardiograms
e. Hematopoietic system evaluation
    1. Hemoglobin
    2. Erythrocyte count
    3. Leucocyte count
    4. Differential examination
    5. Hematocrit
f. Liver-function evaluation
    1. Electrophoretic study of serum protein
    2. Prothrombin time
    3. Bromsulfalein liver excretion test
    4. Total plasma bilirubin
    5. Fasting sugar

g. Renal function
   1. Blood metabolites
      Nonprotein nitrogen
      Urea nitrogen
      Calcium
      Phosphorus
      Chlorides
   2. Metabolites in urine
      Urinary amino acid nitrogen
      Urinary creatinine excretion
   3. Routine urinary analysis
   4. Renal-clearance tests
      Chloride clearance
      Urea clearance
      Creatinine clearance

h. Special tests
   1. Urinary catalase
   2. Quantitative uranium content of urine excreted in a 24-hr period
   3. Quantitative fluoride content of urine excreted in 24-hr period

An analysis of these studies showed no discernible serious toxic effects that might have occurred as a result of exposure to the uranium hexafluoride.

It might be of interest to mention that the concentration of uranium in the urine ranged from 0.01 to 0.04 mg/liter per 24-hr period. From these figures it seems very doubtful that these men incurred high exposures to the uranium.

The urinary fluoride concentration ranged from 0.22 to 0.44 mg/liter per 24-hr period. This is well below the upper limit of the normal range of fluoride in urine (1.5 mg/liter).

4.2 Study 2. This study concerned itself with the examination of 31 individuals who had been exposed to uranium trioxide, tetraoxide, or tetrachloride in dust form for periods of more than 1 year. During a portion of this time all were exposed to concentrations of these compounds in excess of the recommended 150 μg of uranium per cubic meter of factory air.

The following studies were carried out:

a. Medical histories
b. Physical examinations
c. X rays of chest

d. Hematopoietic system evaluation
    1. Hemoglobin
    2. Erythrocyte count
    3. Differential examination
    4. Leucocyte count
    5. Sedimentation rate
e. Renal function
    1. Blood metabolites
        Nonprotein nitrogen
        Urea nitrogen
        Chlorides
        Total protein
    2. Routine urinary analyses
    3. Renal-clearance tests
        Chloride clearance
        Urea clearance
        Creatinine clearance
f. Icteric index
g. Special tests
    1. Urinary catalase
    2. Urinary uranium

An analysis of these studies revealed no abnormal findings that could be considered to be due to chronic exposure of these workers to uranium compounds.

Mention must also be made of the analyses of teeth and bone removed from exposed workers for uranium content. These indicate that the amount of uranium absorbed and stored by the human being working in such industry is quite small. The hazard that might occur from such storage is in all probability negligible.

5. CONCLUSION

In conclusion this study indicates that, within the limits of the methods employed, no specific evidence of chronic injury to workers employed in any phase of the uranium industry has occurred as the result of the peculiar toxicity of uranium. It is of course possible that changes may develop in some individuals at a later date, but the experience already gained in animal observations indicates that this is unlikely.

Hence the standards set for the industry on the basis of 150 μg of uranium dust per cubic meter of factory air and the maintenance of this by suitable engineering and safety procedures have resulted in the protection of the industrial worker in the uranium industry as far as can be determined by the most selective methods.

BIBLIOGRAPHY

1. Kennedy, Case of Diabetes Mellitus Treated with Uranium Nitrate, Lancet, 1: 835 (1874).
2. S. West, The Treatment of Diabetes Mellitus by Uranium Nitrate, Brit. Med. J., II: 467-721 (1895).
3. D. Walsh, Multiple Medication with Uranium, Trans. Med. Press, 128: 444 (1904).
4. F. E. Tylecote, The Pharmacology and Therapeutics of Uranium, Med. Chron. Manchester, 40: 379 (1904).
5. F. E. Tylecote, The Action of Uranium Salts, Edinburgh Med. J., 58: 459 (1904).
6. R. W. Wilcox, The Therapeutics of Uranium Nitrate, Med. Record, 92: 361 (1917).
7. Gailleton, Lupus Treated with Uranium Nitrate, Lyon Med., 103: 123 (1904).
8. A. Clark, The Use of Uranium as a Radioactive Therapeutic Agent, Brit. Med. J., II: 716 (1912).
9. H. Truttwin, The Use of Uranium in Dermatology, Arch. Dermatol. u. Syphilis, 160: 132 (1930).
10. F. D. M. Hocking, The Employment of Uranium in the Treatment of New Growths, Internl. Conf. on Cancer, pp. 253-258, Wright, Bristol, England, 1928.
11. G. T. Pack and F. W. Stewart, Uranium-Thorium Colloid in the Treatment of Carcinoma, J. Cancer Research, 14: 152 (1930).
12. M. DeLaet and C. Meurice, Ing. chim., 9: 247 (1925).

PART II

FLUORINE AND HYDROGEN FLUORIDE

Chapter 17

TOXICITY FOLLOWING INHALATION OF FLUORINE AND HYDROGEN FLUORIDE

By Herbert E. Stokinger*

1. INTRODUCTION

These studies were begun at the request of the Medical Section of the Manhattan District to provide information on the type and degree of toxicity resulting from the exposure of laboratory animals to elementary fluorine vapor and to gaseous hydrogen fluoride, the knowledge thus derived to serve as a basis for a better understanding of toxicity problems resulting from human exposure. The studies were accordingly designed so as to indicate to those entrusted with the health of fluoride workers the probable nature and extent of the hazard resulting from two types of exposure to these gases, namely, (1) overwhelming concentrations for short periods and (2) relatively small concentrations for protracted periods. For engineers the studies were to provide a guide to assist in the choice of upper limit of permissible concentration for exposures of personnel under daily working conditions and to indicate what measures should be undertaken to control, minimize, and, if possible, eliminate human exposures to fluorine gases. The toxicity studies of hydrogen fluoride were also to furnish critical data for the interpretation of results of inhalation-exposure studies of uranium hexafluoride in which hydrogen fluoride plays a significant role.

Part II is subdivided into two sections, each dealing separately with inhalation toxicity resulting from exposures to (1) commercial fluorine gas and (2) gaseous hydrogen fluoride. Pertinent information

* Work done by N. Eriksen, B. Andur, A. L. Shannon, N. Glover, N. Kaplan, M. Schlamowitz, P. Hoch, N. Murphy, C. W. LaBelle, J. A. Orcutt, Nancy Smith, E. W. Same, and I. Slotnik; work directed by H. E. Stokinger.

is also presented on the special methods of handling these toxic vapors. The methods of performing these studies were similar to those used for the uranium-dust exposures and have been fully described in Chap. 10, Parts A and B. Differences in procedure only will be noted in the appropriate sections here.

1.1 Problems of Handling Gaseous Fluorine Products. In the preparation for the toxicologic studies, both gases presented certain problems requiring special engineering techniques. Most of these problems centered about the peculiarities in either the physical or chemical properties of the gases.

1.2 Fluorine. Fluorine, an extremely powerful oxidant with highly corrosive effect on most material, demands extreme care in handling. Special instructions are required for those involved with this material in order to eliminate serious accidents. The transfer of pure fluorine gas from commercial cylinders at pressures of 400 lb/sq in. to suitably reduced pressures for toxicologic work demands a well-isolated building constructed of noninflammable materials. The transfer of the gas is conducted with a 6-in. cement wall between the worker and the high-pressure tank. Absolute absence of moisture in the flow lines is essential, and, despite precautions, it is not infrequent that combustion of the lines, valves, and gauges occurs. Thus, in the light of the difficulties in handling fluorine gas, it is not unreasonable that no previous animal-exposure studies have been undertaken. Moreover, it has been only comparatively recently that fluorine gas has been commercially available. Such knowledge as exists of fluorine-gas intoxication has been obtained through general plant experience with this material and provides only the crudest indication of its toxicity. Pilot studies, to be described later, have been performed and have established the approximate limits of toxicity from fluorine-gas exposures. Four short-term exposure studies, performed at air concentrations based on the results of the pilot studies, have furnished a more precise delineation of the toxicity picture resulting from fluorine exposures.

1.3 Hydrogen Fluoride. Hydrogen fluoride gas is somewhat less troublesome to handle than is fluorine gas. Partly for this reason, a certain amount of information[1,2] was available on the toxicity of hydrogen fluoride vapor for certain animal species at the time this work was started, and this information supplied a convenient guide to approximate toxicity from exposure to this material. For the present demands, however, these results were in need of amplification.

In preparation for the toxicologic studies of hydrogen fluoride gas, the greatest concern was given to devising a practical method of introducing the gas into the exposure chamber in the monomolecular

state. Considerable time was spent on this problem. At room temperatures at which the animals are exposed, molecular aggregation of hydrogen fluoride is considerable and subject to variation with change in temperature and pressure. Under the conditions of exposure, the gas is molecularly associated with a weight in the neighborhood of 50; monomolecular hydrogen fluoride is 20. The association results in condensation of the material in the lines and introduces an uncertainty in the amount of fluoride metered into the exposure unit. Metering the hydrogen fluoride through an orifice at one temperature introduces into the exposure unit a different amount of hydrogen fluoride than that at another temperature, and toxic response thus becomes a function of the temperature of the entering hydrogen fluoride gas, an unallowable situation.* Further, it does not permit a strict comparison with the exposure levels of fluorine where temperature changes create no such difficulties. Moreover, the possibility exists that each molecular species of hydrogen fluoride possesses separate toxicity. Elimination of these problems was finally attained by introducing the hydrogen fluoride gas with steam tracer lines† at 100°C, at which temperature the molecular association is negligible. It was assumed that, at the subsequent attenuation and lowered temperature in the chamber, reassociation is negligible.

Two 30-day studies only were performed with five animal species to amplify and extend the previously reported studies with this compound.

1.4 General Problems of Metering and Estimation of Fluorine-vapor Concentrations. The problem of maintaining a uniform concentration of vapor within the exposure chamber was easy by comparison with the problems associated with maintaining a uniform concentration of high-density dust (described in Chap. 10, Part A). However, values obtained for the vapor concentration were open to serious question. One problem, always present, was that of adsorption. Adsorption of the fluorine and fluorine-containing gases occurred on the metallic walls of the chamber, on the animal cages, and on the fur of the animals. Therefore the question arose as to the precise meaning of the values of concentration derived from samples of the ambient air near the animals.

Another limitation imposed upon the studies of fluorine vapor was the problem of analysis. This is recognized by microanalysts as one

* This factor has not been considered in previously reported exposures to HF.

†Near the conclusion of the work a report[3] appeared in the literature describing a method similar to that devised by the engineering group of this section.

of the most difficult in the category of elemental analyses. The problem was accentuated in these studies by the necessity for analyzing extremely small amounts of the vapor in the chamber atmospheres. Although this was less true of hydrogen fluoride than of elemental fluorine, the latter presented also a problem of sampling. Despite textbook opinion to the contrary, fluorine gas is but slowly absorbed in aqueous solution, and much effort was spent in finding an approximately satisfactory means of absorbing minute amounts of fluorine. Thus the levels of fluorine exposures are stated with less assurance than those of hydrogen fluoride, for which analytical problems have been better resolved.

Although undue emphasis may appear to have been placed on the difficulties associated with the toxicologic studies of fluorine materials, such statements are made to stress differences in the problems of vapor and dust exposures. Actually the relative magnitude of the problems was smaller in the present instances than in those associated with uranium-dust exposures, both intrinsically and extrinsically. Apart from the indicated differences in the fluorine-vapor exposures, basically the inhalation procedures of both vapor and dust exposures were similar. Both were alike as regards use of similar types of exposure chambers, cage equipment, species, variety and diet of animals, and type and duration of exposure. The measures used for evaluation of toxic response also followed the pattern of those used for uranium-dust exposures. Procedures differed, obviously, as to the method of introducing the toxic materials into the exposure chambers. Certain factors related to the fluorine problem have also been studied, namely, the storage of fluoride within the animal body and the dental effects of fluorine exposure. The nature of the fluorine burn has been given special consideration in a separate section, owing to its accidental occurrence in practice. Included also are results of tests of respirators with the certification of some as suggested methods of protecting the worker from injurious exposure.

2. EXPOSURES TO ELEMENTAL FLUORINE GAS

Fluorine has found wide application in the chemical technology associated with the production of uranium. Never before had such quantities of elemental fluorine gas been handled daily. The handling of large volumes of hazardous gas under pressure made the danger of sudden release of large quantities a real one and the continuous exposure to low concentrations from unavoidable losses from the equipment was a source of considerable concern.

In an effort to determine the effects, both external and internal, that may be anticipated from exposures to overwhelming concentrations of fluorine vapor, the following studies were made: (1) a dermal exposure of animals to pure fluorine gas, (2) pulmonary exposures for brief durations at concentrations varying from 10,000 to 100 ppm, and (3) four short-term animal-inhalation exposures at concentrations ranging from 16 to 0.5 ppm (25 to 0.8 mg/cu m) to demonstrate the effects of continual exposure at low concentrations of fluorine. Because the dermal exposure was simpler than the pulmonary exposures and involved a different technique, it will be discussed first.

2.1 Dermal Exposures, Nature of the Fluorine Burn. (Work performed by James A. Orcutt and Nancy B. Smith.) Contact of the skin of rabbits with pure fluorine vapor under pressure was made to determine whether the resulting response resembled a thermal or a chemical type of burn (like HF) or a combination of the two. A comparison was therefore made with the thermal burn produced by the combustion of oxyacetylene and with a predominantly chemical burn produced by aqueous hydrogen fluoride.

The shaved backs of four New Zealand rabbits were exposed to pure fluorine gas under 40 lb pressure. The rabbits were anesthetized with intravenously administered sodium pentobarbital and were exposed for periods of 0.2, 0.3, 0.4, and 0.6 sec* at distances of 1, 0.5, 1, and 1.5 in., respectively.

The exposure of 0.2 sec duration produced a small ischemic area approximately ¼ in. in diameter, resembling a blister, surrounded by an erythematous area. The changes that occurred during a subsequent period of 13 days consisted of superficial eschar that sloughed off by the fourth day disclosing normal epidermis.

The three exposures of 0.3, 0.4, and 0.6 sec produced similar responses, all of which were more marked than that of the exposure of 0.2 sec duration and were characterized by a flash of flame that did not occur during the shortest exposure. The immediate effects of the flash exposure were (1) combustion of hair, singeing, and erythema of the epidermis of the area covered by the flash that involved an area several times that of the primary burn (see Fig. 17.1), and (2) coagulation necrosis of the burned area with charring of the epidermis. Subsequent observation for a period of 13 days revealed the following

* The length of exposure was controlled by a solenoid-operated valve that was in turn operated by a Microflex timer, with a total operating lag of no more than 0.05 sec. The design of this and the jet exposure unit was made by F. W. Doughty and L. Baurmash under the direction of G. E. Goring.

sequence in the healing process: (1) development of a thick dark-brown elevated eschar at the site of exposure and edema of surrounding and underlying tissues, (2) development of a scaliness over the area of the flash burn and rapid return to normal of the epidermis of the outlying area, (3) subsidence of edema and erythema, (4) gradual separation of the eschar over the exposed area, beginning at the periphery and extending centrally, and (5) disclosure of new skin, normal except for a mild hyperemia.

No immediate reaction was noted following the application to the shaved surface of a rabbit's back of 0.2 ml of 47 per cent hydrogen fluoride (aq.) held within a ring of lanolin 1¼ in. in diameter and left to dry for 24 hr. Within a few days, however, erythema of the epidermis with small dark spots of liquefaction necrosis appeared. Healing occurred more slowly than in the burns from fluorine and was characterized by a multiple eschar formation in the necrotic areas. The eschar did not separate from the underlying tissues until the twenty-seventh day, a period, it is to be noted, some 2 weeks after the complete healing of the fluorine burn, Fig. 17.2.

The burn resulting from the exposure to the oxyacetylene flame was the same as that resulting from the flash exposures of fluorine of duration greater than 0.2 sec as determined by both immediate examination and subsequent 13-day observation of the healing process, Fig. 17.3.

It is clear from a comparison of the separate responses of a pure thermal type of burn and a chemical burn that pure concentrated fluorine vapor under pressure produces a flash burn that is identical with that produced by an oxyacetylene flame and may be classed as a second-degree thermal burn.

2.2 Pulmonary Exposures. Exposure Equipment. As pointed out in the Introduction of this chapter, the transfer of the pure concentrated fluorine gas from a commercial cylinder under pressure of 400 lb/sq in. to a pressure convenient for toxicological work required special equipment.

Outhouse for Transfer of Fluorine. An outhouse was constructed for this purpose, made of cement and equipped with lines and valves operable through a 6-in. concrete wall. A diagrammatic sketch of the means used to reduce fluorine pressures is shown in Fig. 17.4. In this way nitrogen-diluted fluorine mixtures were prepared that varied in fluorine concentration from 50 to 5 per cent, based on metering from calibrated orifices. Chemical analyses of these mixtures were not performed.

Exposure Chambers. Flood-concentration exposures were carried out in a steel rectangular exposure chamber (approximately 27 cu ft capacity) of the full-exposure type described in Chap. 10, Sec. 6.1e.

Fig. 17.1 — Burn produced by fluorine gas under pressure.

Fig. 17.2 — Burn produced by 47 per cent hydrogen fluoride.

Fig. 17.3 — Burn produced by oxyacetylene flame.

Access to the chamber was provided by a movable door, which comprised one side and which was clamped into position by means of vertical bars. The recurrent daily exposures to lower concentrations were performed in a larger chamber, approximately 270 cu ft capacity, also of the same full-exposure type and design. The chamber was, however, equipped also with a wooden unit designed for exposing the heads of rabbits and guinea pigs to fluorine atmospheres while at the same time allowing the bodies of the animals to be in a fluorine-free atmosphere.

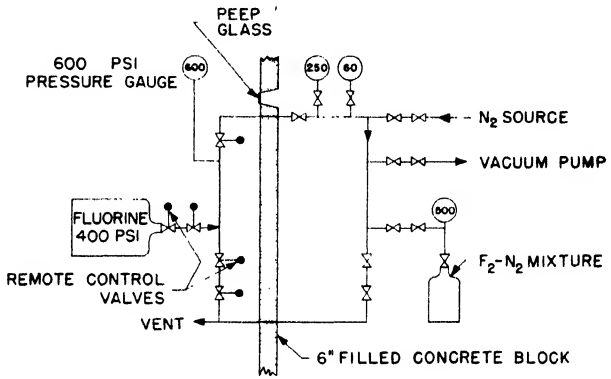


Fig. 17.4 — Fluorine-nitrogen blending system (schematic).

Operation of Chambers. The preparation and maintenance of fluorine in atmospheres were similar in both the pilot and short-term studies. Fluorine-containing atmosphere was drawn through the chamber directly from the tank containing the fluorine-nitrogen mixture, through ports at the top of the chamber by means of an exhaust fan. In the pilot exposure chamber the rate of flow was 25 cfm, through the larger chamber, 145 cfm. Circulation of the fluorine atmosphere within the chambers was aided by a centrally located fan. The chamber atmosphere, exhausted through ports at the lower corner of the chamber, was passed through an alkali scrubbing tower before being discharged into the outside atmosphere.

Concentration of Fluorine. For the control of the fluorine concentration in the pilot studies, which ranged from 10,000 to 100 ppm, reliance was placed solely on metering, assuming ideal gas conditions; no analysis of the fluorine-air mixtures was made. The gas was metered at room temperature.

On certain of the short-term studies, chemical analyses of the chamber-air mixtures were attempted but were later abandoned when

results were considered unsatisfactory because of inability to find means of accurate sampling. Dependence was placed on metered concentrations for the most part.

(a) Flood-concentration Exposures. (Toxicological procedures performed by Nils Eriksen, E. W. Same, and I. Slotnik.) Pilot animal-inhalation-toxicity studies of brief duration at high atmospheric concentrations of fluorine gas were performed in order to supplement the information obtained from flash, high-pressure dermal exposures and that obtained from repeated daily pulmonary exposures at lower concentrations. Five pilot exposure studies were performed at 10,000; 1,000; 500; 200; and 100 ppm of fluorine (15,750; 1,575; 790; 315; and 160 mg F_2/cu m, respectively). The duration of exposure ranged from 5 min at the highest concentration to 7 hr at the lowest. This selection yielded a concentration-time product (CT value) very similar for all exposures. The toxicologic effects of fluorine-gas inhalation were tested on four species of animals. Approximately the same numbers of animals in each species were used at each level, namely, 8 rabbits, 20 guinea pigs, from 40 to 50 rats, and from 40 to 50 mice. The variety and the weight range of the animals were the same as those described in Chap. 10, Part B. Because of the survey nature of the study, mortality and gross examination of the animals was made at death or autopsy, and histologic examination was made only of the critical tissues of two rabbits and two rats exposed at the 10,000-, 1,000-, and 100-ppm level.

Mortality. Exposures to fluorine were uniformly fatal to each of four species at the concentration range of 10,000 to 200 ppm inclusive (CT values of from 50,000 to 30,000). Only at the lowest concentration level studied, 100 ppm, was there survival of certain numbers of animal species following a 7-hr exposure (Table 17.1). In Table 17.1 it is seen that all animals were dead after 5 min of exposure at a concentration of 10,000 ppm, with the exception of one rabbit that died the following day. At successively decreasing concentrations, less immediate and devastating mortality was produced during the exposure, although all animals of all species except the guinea pig ultimately succumbed by the fourteenth day at exposure levels as low as 200 ppm. Oddly, the guinea pigs, which died soonest after the start of exposure of all species at the three highest levels, showed at the end of the 14-day observation period less mortality at the 200-ppm level and no mortality whatsoever at the 100-ppm level.

The difference in the mortality produced at four of the levels of exposure is graphically illustrated in Fig. 17.5. This figure represents the over-all mortality response of the entire group of animals exposed at a given level and shows that fluorine-gas exposures at widely

Table 17.1 — Mortality Following Exposures to Fluorine Gas at High Concentrations

Animals		Concentration of gaseous fluorine		Duration of exposure	Mortality, %		
					At end of exposure	24 hr after exposure	14 days after exposure
Species	No.	Mg/cu m	Ppm				
Rat	45	15,750	10,000	5 min	100	100	100
Mouse	45				100	100	100
Guinea pig	20				100	100	100
Rabbit	8				88	100	100
Rat	45	1,575	1,000	30 min	87	100	100
Mouse	45				47	73	100
Guinea pig	20				100	100	100
Rabbit	8				33	63	100
Rat	50	790	500	1 hr	90	94	100
Mouse	50				88	96	100
Guinea pig	20				100	100	100
Rabbit	8				25	63	100
Rat	45	315	200	3 hr	71	96	100
Mouse	50				58	100	100
Guinea pig	20				65	85	90
Rabbit	8				13	63	100
Rat	50	160	100	7 hr	20	50	54
Mouse	45				45	96	96
Guinea pig	20				0	0	0
Rabbit	8				0	0	88

different concentrations, but for which the CT product is approximately constant, produce distinguishable differences in mortality.

Pathology. The respiratory tract was the chief site of damage. Severe and consistently occurring changes were observed in six animals examined. Respiratory failure resulting from the acute pulmonary damage appeared to be the sole cause of death.

Rabbit. Pulmonary changes consisted of severe edema of the alveoli that in some cases involved the perivascular spaces, and hemorrhage was a common finding in this species. In certain animals necrosis of the bronchial epithelium, metaplasia of the alveolar epithelium, and emphysema of the air-containing portions of the lungs were noted.

More hemorrhage was observed in the animals exposed at the 10,000-ppm level than at the lower levels, and the severity of the tissue changes was greater at this level, but the type of change was of a similar nature at all levels. In some of the more severe cases that survived for longer periods, infectious processes in the lungs were in evidence.

Rat. Rats exposed to 10,000 ppm for 5 min showed changes similar in nature but slightly less severe than those exposed for 30 min to 1,000 ppm. One of the rats that died on the day following exposure to 100 ppm showed changes essentially the same as those in animals exposed at higher levels. In one rat that was killed 19 days after exposure to 100 ppm, pulmonary changes were insignificant.

In general, the extent of pulmonary damage in the rat was similar to that in the rabbit with the difference that, whereas pulmonary hemorrhage was absent or extremely slight in the rat, it constituted an important component of the damage in the rabbit.

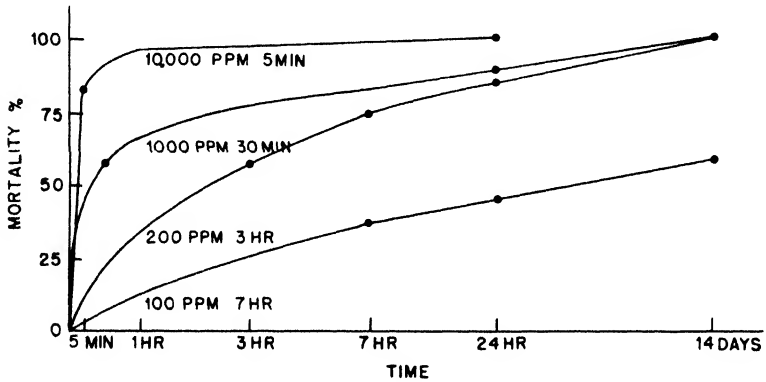


Fig. 17.5—Mortality of animals exposed to four levels of fluorine gas.

Discussion. The acute toxicity of inhaled fluorine was observed to be the result of the corrosive action of the gas on the respiratory system. In those animals that were able to withstand this severe effect, the dosage of fluorine was apparently not high enough to cause death by its latent action in the form of fluoride.

Summary. Three hours of exposure to elemental fluorine gas at a concentration of 200 ppm by volume as well as shorter exposures at correspondingly higher concentrations are invariably fatal to the rabbit, guinea pig, rat, and mouse. Exposure at 100 ppm results in an over-all mortality of 60 per cent for these four species within 2 weeks after exposure.

Severe damage to the respiratory tract was the only pathology consistently observed.

(b) Short-term Inhalation-exposure Studies of Fluorine Gas. (Toxicological procedures performed by Nils Eriksen, B. H. Amdur, and A. L. Shannon; dental studies by P. Dale.) Studies of the toxicologic

effects of fluorine-gas exposures were made at four levels of concentration—25, 8, 3, and 0.8 mg/cu m (16, 5, 2, and 0.5 ppm, respectively). Exposure of the animals covered a period of 35 days with the exception of that of the highest level where death of the animals foreshortened the exposure to 21 days (Table 17.2). For the most part, the animals received a total exposure of the order of 170 hr.

Table 17.2—Concentration of Gaseous Fluorine in Exposure Atmospheres and Length of Exposures

Weighted-mean concentration of gaseous fluorine		No. of concentration samples taken	Length of exposure		
Mg/cu m	Ppm		Calendar days	Exposure, days	Exposure, hr
25	16	19*	21	18	95
8	5	44*	35	29	160
3†	2		35	31	176
0.8†	0.5		35	31	178

*In these samples the chemical and metered values were in reasonable agreement.

†No samples were taken for chemical analysis at these levels; fluorine was metered to yield these concentrations.

For control of exposure concentrations, dependence was placed on accurate metering of the fluorine-gas-air mixtures after difficulties in sampling of the chamber air quantitatively for fluorine proved insurmountable. In addition to the usual criteria of toxicity employed in the uranium-inhalation studies (Chap. 10, Part A), the following determinations were used to evaluate the toxicologic response to repeated daily exposures to fluorine gas. The fluoride content of teeth, primarily, and of certain bones was determined in order to establish whether inhaled fluorine gas is deposited as fluoride in the osseous structures of animals. For similar reasons a correlative study of the dental changes in rats was made. In order to form an estimate of the degree of retention and excretion of fluorine, the amount of urinary fluoride in rabbits was determined. The possible effects of fluorine intake on calcium in the blood and on blood-coagulating time were appraised by a study of the blood-calcium levels and clotting time in dogs and rabbits.

Signs of Toxicity. The outward symptoms of toxicity from inhaled fluorine gas consisted of a coarsening and stiffening of the fur and irritation of eyes and of nasal and buccal mucosa. This was confined to rats exposed at the two highest levels. These symptoms appeared to a less marked degree in the rats exposed at the 3-mg level and were absent at the 0.8-mg level.

The dogs exposed at the two higher levels exhibited irrational seizures, which were usually followed by death. No outward manifestations of toxicity appeared in the dogs at the two lower levels. Symptoms similar to those of the rats appeared in the remaining species exposed, although to a slighter degree.

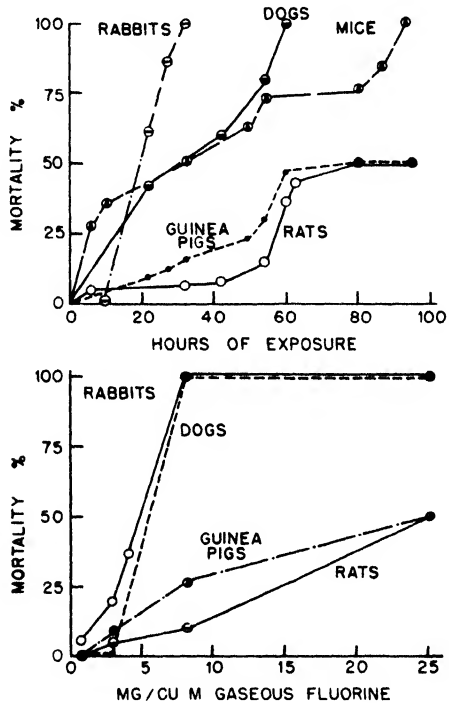


Fig. 17.6—Mortality of animals exposed daily to gaseous fluorine. Upper, fluorine concentration 25 mg/cu m; lower, fluorine concentrations from 0.8 to 25 mg/cu m.

Mortality. Animal mortality, a prominent feature of exposure to gaseous fluorine at the two highest levels, was less in evidence at the two lower levels (3 and 0.8 mg). Mortality ranged from 75 per cent at the highest level to 4 per cent at the lowest level. Figure 17.6, upper, shows the mortality rate of five species exposed to 25 mg/cu m. The dog, rabbit, and mouse were most susceptible to fluorine intoxication, each showing a 100 per cent mortality rate at the highest level (Fig. 17.6). The rat, guinea pig, and hamster (not shown) were the least susceptible species, the rat and guinea pig showing a 50 per cent mortality at the highest exposure level and the hamster showing no

mortality at the 8-mg level. Some rabbits and guinea pigs underwent only head exposure to the gas; allowing for accidental death from strangulation, the mortality among these animals was less than for the totally exposed animals (Table 17.3).

Table 17.3 — Mortality of Animals Exposed to Gaseous Fluorine

Concentration of gaseous fluorine, mg/cu m	No. of deaths per no. exposed							
	Totally exposed						Head exposed	
	Rabbit	Dog	Mouse	Rat	Guinea pig	Hamster	Rabbit*	Guinea pig
25	18/18	5/5	49/49	24/48	15/30		9/9	4/12
8	19/19	5/5	10/50	4/34	7/26	0/30	9/10	2/12
3	2/10	0/5		1/21	1/12		3/10	0/12
0.3	1/18	0/5		0/18	0/12		1/12	1/12

* An undetermined number also died possibly from strangulation, in the head-exposure cages.

Weight Changes. Exposure to gaseous fluorine resulted in weight losses uniformly in all animals exposed at the 25-mg level but in only certain species at the 8-mg level. Maximal average losses at the highest level amounted to 10 per cent and were confined to the rat and guinea pig chiefly. At the next two lower levels no weight losses were incurred, and weight increased or was maintained (dog) at the lowest exposure level in the normal manner (Table 17.4). In general the head-exposed animals lost less weight during the exposure than did the animals that were fully exposed.

Table 17.4 — Weight Change in Animals Exposed to Gaseous Fluorine

Concentration of gaseous fluorine, mg/cu m	Weight change, % of original weight							
	Totally exposed						Head exposed	
	Rat	Guinea pig	Mouse	Hamster	Dog	Rabbit	Rabbit	Guinea pig
25	−10	−7						−1
8	+16	−13	+12	+2				−6
3	+8	+22			+3	−5	+14	+28
0.8	+25	+62			−3	+24	+20	+44

Biochemistry. Biochemical determinations of three constituents of the blood, namely, nonprotein nitrogen, urea, and calcium, and two constituents of the urine, namely, amino acid nitrogen and fluoride, made on the dog and rabbit at the lowest levels showed significant

changes from the normal in only one constituent, that of urinary fluoride. A 150 per cent increase in urinary fluoride excretion occurred in the rabbit exposed at the 3-mg level.

A preliminary study to determine the effect of fluorine exposures on the clotting time of blood of dogs and rabbits was made at the 3-mg level of exposure. The results showed that although average values obtained on both the dogs and the rabbits increased after the first week of exposure, the variation of individual determinations was so great as to make the observed average increase of doubtful significance (see Table 17.10).

Hematology. Hematologic studies performed on dogs, rabbits, and rats at four levels of exposure yielded no changes of value for appraising the toxicity from fluorine-gas exposure.

Table 17.5 — Pathology of Animals Exposed to Gaseous Fluorine

Concentration of gaseous fluorine, mg/cu m	Dog	Rabbit	Rat
25	Moderate to moderately severe hemorrhage in lungs in 4 of 4. Livers congested in 4 of 4	Pulmonary damage similar to that of dogs in 4 of 4	Rather severe pulmonary irritation in many cases in 15 of 29, testicular degeneration in 20 of 29; gross and histologic buccal changes
8	Red discoloration of lungs, mild bronchitis, and bronchiectasis in 4 of 5	Moderate pulmonary irritation in 4 of 5, slight liver damage in 4 of 5	
3	Pulmonary hemorrhage and edema in 2 of 5	Mild bronchial inflamation in 3 of 4	No apparent damage upon gross examination
0.8	No consistent significant damage	Little or no pulmonary damage	

Pathology. Histological changes resulting from the exposure to gaseous fluorine consisted chiefly of pulmonary damage to all species and testicular damage in some. Pulmonary damage in the dog was of a severe to a moderately severe degree at the highest fluorine concentration. At the 8-mg level four of five dogs were observed to have

mild bronchitis and bronchiectasis, and at the 3-mg level two dogs were found to have a slight pulmonary hemorrhage and edema. At the lowest level no pulmonary damage in dogs was observed (Table 17.5).

The rabbits showed a similar pattern of pulmonary response at the four levels of exposure and in addition showed slight hyperemia of the liver at the 8-mg level.

Rats, in addition to showing rather severe pulmonary irritation at the 25-mg level, showed definite testicular degeneration.

Gross examination of rats exposed at the 25-mg level showed dehydration, irritation, erythema, and occasional ulcerous lesions of the oral mucosa. Histological changes in the oral mucosa consisted of hyperplasia, acanthosis, hyperkeratosis, and necrosis.

Dental Changes in Rats and Tissue Deposition of Fluoride. Dental fluorosis and hyperplasia occurred at the 25-mg level. Deposition of fluoride was large and increased progressively with increase in exposure time. The deposition was greatest in osseous tissue that metabolized most actively. The average fluoride content of incisor roots was nearly fifteen times that of control animals; incisor crowns, five times that of control animals (Table 17.6). The increase in the degree of fluorosis in the teeth of rats with time is shown in Fig. 17.7 (lower). Curves showing time increase of fluoride content of osseous tissues of rats exposed at the 25-mg level are given in Fig. 17.7 (upper). It is seen from a comparison of the two graphs that the increase in severity of the fluorosis parallels the increased fluoride uptake by the bone.

A successive decrease in fluorosis and fluoride storage in bone was noted with decreasing exposure levels until at the 0.8-mg level only questionable fluorosis was apparent, although the average fluoride content was still from two to three times normal in rat bone. Pigmentary changes were just perceptible in animals exposed at the 3-mg level, but no histological evidence of disturbed dental formation and calcification was present such as was seen in a mild form at the 8-mg level. In dogs exposed at the 8-mg level the fluoride content of the maxillary and mandibular alveolar bone following exposures of no longer than 45 hr (during 8 days), increased over 50 per cent of preexposure values obtained on biopsied material (Table 17.7). In longer exposures of 176 hr at 3 mg, the fluoride content in the same bone of the dog increased on the average of approximately 120 per cent.

A summary of the results of each of the 4 weeks of exposure is given separately in Tables 17.8 to 17.11.

Table 17.6—Fluoride Content of Dried Incisors and Femurs of Control and Exposed Rats

Concentration of gaseous fluorine, mg/cu m	Total exposure, hr	No. of animals	Incisor				Femur			
			Crown		Root		Crown		Root	
			Ppm	% of control	Ppm	% of control	Ppm	% of control	Ppm	% of control
25	95	5	1,386	453	4,042	1,480	3,978	673	2,708	558
3	176	9	850	278	1,540	564	1,427	241	964	198
0.8	178	10	554	181	746	273	1,192	202	864	178
0 (controls)	0	9	306		273		592		485	

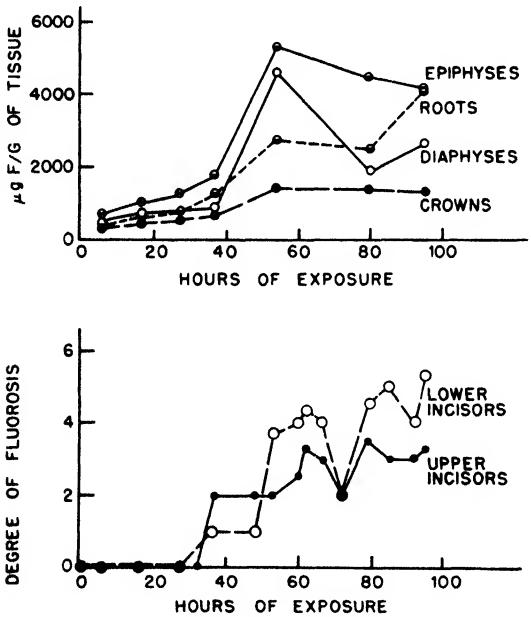


Fig. 17.7—Changes in osseous tissues of rats exposed to fluorine gas at 25 mg/cu m. Upper, fluoride content; lower, degree of fluorosis.

Summary. Fluorine by daily inhalation was highly toxic to animals at 3 mg/cu m and above. At 0.8 mg few toxic effects were observed. A summary of the important effects observed at exposure levels of 25, 8, 3, and 0.8 mg/cu m is given in Tables 17.8 to 17.11.

1. On continued exposure to fluorine gas the fur of the animals became coarse and stiff; the eyes and nasal and buccal mucosa became irritated, especially in the rat; and irrational seizures occurred in

Table 17.7 — Fluoride Content of Maxillary and Mandibulary Alveolar Bone of Dogs Exposed to Gaseous Fluorine

Concentration of gaseous fluorine, mg/cu m	No. of dogs	Exposure time, hr	Preexposure				Postexposure					
			Right maxilla	Right mandible	Left maxilla	Left mandible	Left maxilla	Left mandible	Right maxilla	Left maxilla (new bone)	Right mandible	Left mandible (new bone)
8	83	24	580	550			570	400				
	91	45	400	370			500	870				
	92	45	51	300			860	730				
Mean Values												
			343	407			643	667				
None (control)	94	0	400	320			580	510	500*		520*	
3	106	176			235	263			3,320	3,400	3,500	3,540
	107	176			910	960			1,100	2,100	1,200	1,300
	108	176			790	450			658	729	595	467
	109	176			610	440			930	820	560	980
	110	176			1,000	780			774	892	784	1,030
Mean Values												
					709	579			1,356	1,588	1,328	1,463

*New bone regenerated from site of biopsied structures.

Table 17.8 — Animals Exposed for 95 Hr in 18 Days to Gaseous Fluorine at the 25-mg Level (Summary of Results)

1. Mortality. Exposure to gaseous fluorine resulted in high mortality in all species.

Species	No. of deaths per no. exposed	Mortality, %
Mouse	49/49	100
Rabbit (head exposure)	9/9	100
Rabbit	18/18	100
Dog	5/5	100
Rat	24/48	50
Guinea pig	15/30	50
Guinea pig (head exposure)	8/12	33

2. Weight Changes. In general, weight losses occurred in surviving species.

Species*	Mean orig. wt., g	Wt. change, %
Rat	228	−10
Guinea pig	488	−7
Guinea pig (head exposure)	413	−1

*Early and high mortality precluded recording of weight changes in many species.

3. Pathology.
   Dog: Moderate to moderately severe hemorrhage in lungs; congested liver.
   Rabbit: Pulmonary damage similar to that of dogs.
   Rat: Moderately severe pulmonary irritation in some cases; testicular degeneration in two-thirds of animals examined; gross and histologic buccal-mucosa changes.
4. Hematology. No significant changes.
5. Retention of Fluoride in Tissues of Exposed Rats. Fluoride deposition in teeth and bone occurred, increasing with exposure time. A parallel increase in the severity of dental hypoplasia and fluorosis was noted.

No. of rats	Fluoride, % of control			
	Incisor		Femur	
	Crown	Root	Epiphysis	Diaphysis
5	453	1,480	673	558

6. Symptoms.
   Dog: Irrational seizures; stiff and coarse fur; irritated nasal mucosa.
   Rat: Stiff and coarse fur; irritated nasal and buccal mucosa and palpebral fissures.

the dog, often followed by death. These symptoms of fluorine toxicity appeared with decreasing intensity at the three highest levels; at the lowest level outward signs of toxicity were absent.

Table 17.9 — Animals Exposed for 160 Hr in 29 Days to Gaseous Fluorine at the 8-mg Level (Summary of Results)

1. Mortality. Gaseous fluorine was highly lethal for most species.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit	19/19	100
Dog	5/5	100
Rabbit (head exposure)	9/10	90
Guinea pig	7/26	27
Mouse	10/50	20
Guinea pig (head exposure)	2/12	17
Rat	4/34	11
Hamster	0/30	0

2. Weight Changes. Weight gain was retarded in most species, with a definite weight loss occurring in the guinea pig.

Species*	Mean orig. wt., g	Wt. change, %
Guinea pig	483	−13
Guinea pig (head exposure)	400	−6
Hamster	63	+2
Mouse	20	+12
Rat	133	+16

*Certain species excluded because of early high mortality.

3. Pathology.
   Dog: Mild pulmonary irritation; mild bronchitis and bronchiectasis.
   Rabbit: Moderate pulmonary irritation, slight liver hyperemia in four of five.
4. Hematology. No significant findings.
5. Deposition of Fluoride in Teeth and Bones. Very mild to mild dental fluorosis and hypoplasia were observed in the rats after exposure, and moderate deposition of fluoride in the maxillary and mandibulary alveolar bone of exposed dogs.
6. Symptoms. Similar to those observed at the 25-mg level but to a less marked degree.

2. At the highest level of exposure, fluorine gas was almost invariably fatal for all animal species; at the lowest level of exposure, deaths occurred but rarely. The dog and rabbit were most susceptible to fluorine; the rat and hamster, least susceptible.

3. The inhalation of fluorine gas produced moderate to moderately severe pulmonary irritation in the dog, rabbit, and rat at all levels down to and including 3 mg/cu m. The rat showed, in addition, a high incidence of testicular degeneration at the 25-mg level.

4. Severe dental fluorosis and hypoplasia of dental tissue resulted in the rat from exposure at the 25-mg level; this manifestation decreased to insignificance at the 0.8-mg level.

Table 17.10 — Animals Exposed for 176 Hr in 31 Days to Gaseous Fluorine at the 3-mg Level (Summary of Results)

1. Mortality. Moderate mortality observed in animals exposed to gaseous fluorine.

Species	No. of deaths per no. exposed	Mortality, %
Rabbit (head exposure)	3/10	30
Rabbit	2/10	20
Guinea pig	1/12	8
Rat	1/21	0
Guinea pig (head exposure)	0/12	0
Dog	0/5	0

2. Weight Changes. All species gained weight except the rabbit.

Species	Mean orig. wt., g	Wt. change, %
Rabbit	2,950	−5
Dog	7,100	+3
Rat	284.0	+8
Rabbit (head exposure)	2,700	+14
Guinea pig	365.0	+22
Guinea pig (head exposure)	325.0	+28

3. Pathology.
   Dog: Pulmonary hemorrhage and edema.
   Rabbit: Mild bronchial inflammation.
   Rat: Essentially normal; gross examination only.
4. Biochemistry.
   Dog: No changes in blood NPN and urea N levels.
   Rabbit: Doubtful change in blood NPN level; no change in blood urea N level; increase in urinary fluoride, 150%.
5. Hematology. No abnormal findings.
6. Blood-clotting Time. A slight rise, of doubtful significance, was observed in dogs and rabbits.

	Calendar days relative to start of exposure							
Species	−13	−6	+2	+9	+16	+23	+30	+37
Dog*	2.5	2.5	2.25	3.25	3.0	3.75	2.75	3.5
Rabbit†	2.25	2.5	2.5	3.0	3.0	3.0	3.25	2.75

   *Four values were averaged on days +16 and +30, five on the other days.
   †Nine values were averaged on day +16, ten on preceding days, eight on following days.

7. Dental Changes and Fluorine Deposition.
   Rats: Very mild dental fluorosis; moderate deposition of fluoride in osseous tissues.
   Dogs: Mean fluoride content of mandibulary and maxillary alveolar bone increased 120%.
8. Symptoms.
   Rat: Occasional nasal and palpebral irritation. Slight stiffening of fur.
   Other species: Slight stiffening of fur.
9. Retention of Fluoride in Tissues of Exposed Rats.

No. of rats	Fluoride, % of control			
	Incisor		Femur	
	Crown	Root	Epiphysis	Diaphysis
9	278	564	241	198

Table 17.11 — Animals Exposed for 178 Hr in 31 Days to Gaseous Fluorine at the 0.8-mg Level (Summary of Results)

1. Mortality. Very little mortality.

Species	No. of deaths per no. exposed	Mortality, %
Guinea pig (head exposure)	1/12	8
Rabbit (head exposure)	1/12	8
Rabbit	1/18	6
Guinea pig	0/12	0
Rat	0/18	0
Dog	0/5	0

2. Weight Changes. All species gained weight with the exception of the dog.

Species	Mean orig. wt., g	Wt. change, %
Dog	9,100	−3
Rabbit (head exposure)	2,500	+20
Rabbit	2,500	+24
Rat	140.0	+25
Guinea pig (head exposure)	294.0	+44
Guinea pig	306.0	+62

3. Pathology.
   Dog: No consistent significant damage.
   Rabbit: Little or no pulmonary damage.
4. Biochemistry.
   Dog: No changes found in the blood NPN and blood-calcium levels.
   Rabbit: No significant changes in the concentration of NPN and calcium in the blood, and amino nitrogen in the urine.
5. Hematology. No significant changes in the hemogram of the exposed animals.
6. Dental Changes and Deposition of Fluoride in Bones and Teeth. Moderate deposition in teeth and bone, with no dental hypoplasia in rats.
7. Symptoms. No outward signs of toxicity were observed.
8. Retention of Fluoride in Tissues of Exposed Rats.

No. of rats	Fluoride, % of control			
	Incisor		Femur	
	Crown	Root	Epiphysis	Diaphysis
10	181	273	202	178

5. The deposition of fluoride in osseous tissues of the dog and rat occurred at all four exposure levels. Fluoride increments were as high as fifteen times that of normal incisor crowns of rats at the 25-mg level and 1½ times in the alveolar bone of the maxilla and mandible of the dog at the 8-mg level.

6. Urinary-fluoride excretion increased 150 per cent in rabbits exposed at the 3-mg level.

3. EXPOSURES TO HYDROGEN FLUORIDE GAS *

Hydrogen fluoride gas, HF, was a critical intermediate in the wartime production of uranium. Its large-scale use presented an industrial-hygiene problem of considerable magnitude, exposures to HF (from uranium hexafluoride, UF_6) constituting possibly the greatest single source of minor incapacitation of workers.[4] For this reason and also because it was desired to learn what part hydrogen fluoride played in exposures to UF_6, two 30-day animal-inhalation-exposure studies were performed. Guided by the previous results of Machle,[1,2] levels of 25 and 7 mg HF/cu m (33 and 8.6 ppm, respectively) were chosen. The high level was calculated to produce considerable mortality, the lower level little or none. Thus it was hoped that two levels of exposure would prove sufficient to delineate the border line between toxic and "safe" concentrations to hydrogen fluoride gas. Five species of animals — dog, rabbit, rat, guinea pig, and mouse — were used for the tests at both levels. Both exposures were of 5 weeks duration, a total of 166 exposure-hours. Exposure occurred daily, except Sunday, for a period of 6 hr.

Exposure Chamber and Operation. The animals were exposed in a full-exposure unit of the type described in Chap. 10, Sec. 6.1e, with certain modifications to allow for the corrosive nature of hydrogen fluoride gas.

The chamber in which the animals were exposed was a rectangular enclosure having a capacity of approximately 270 cu ft. The walls and ceiling were lined with sheet copper and the floor with transite. The fourth wall was fitted with a removable door that was held in position from the outside by horizontal bars. At the center of the ceiling was an electrically driven fan to promote distribution of the hydrogen fluoride in the chamber.

In operation the chamber contained two metal cages with compartments of sizes suitable for the accommodation of the various species of animals. An arrangement was provided within the chamber to allow the head of the rabbit to be exposed to atmospheres containing hydrogen fluoride, while the body was bathed in HF-free air.

The hydrogen fluoride-containing atmosphere was drawn continuously through the chamber by means of an exhaust fan. Anhydrous HF vapor was released into the air-inlet duct on top of the chamber.

* Toxicologic procedures performed by Nils Eriksen, N. Glover, N. Kaplan, and A. L. Shannon.

The air stream entered the chamber through four openings in the ceiling and was withdrawn at the lower corners of the chamber. The exhaust gas was passed through a scrubbing tower before being released into the outside atmosphere.

The HF vapor was obtained from a 6-lb cylinder containing anhydrous liquid hydrogen fluoride. The cylinder was housed in a wooden enclosure that was heated by means of an electric-light bulb in order to maintain a positive vapor pressure of a few pounds per square inch in the cylinder.* A section of the HF line between the cylinder and the chamber was equipped with a steam jacket to heat the HF to 100°C, at which temperature the degree of association between HF molecules is negligible. The heated HF passed through a small orifice equipped with a differential manometer and thence through another heated section of copper line to the air-inlet duct of the chamber (Fig. 17.8).

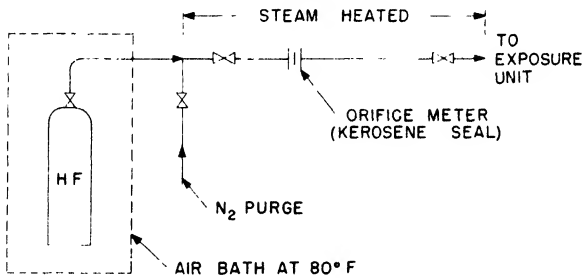


Fig. 17.8—Hydrogen fluoride-gas feed unit (schematic).

The flow of air through the chamber was maintained at 145 cfm. The required flow of HF, in terms of the manometer reading, was determined empirically by analysis of the chamber atmosphere before exposure was begun.

Concentration of HF in Chamber. Samples of chamber atmosphere were taken hourly and analyzed for their content of fluoride. Samples were taken in partially evacuated copper vessels of approximately 9 liters capacity, to which was later added 50 ml of 0.005N KOH for absorption of the gas. After 10 min of agitation to allow for thorough absorption, the contents of the vessel was transferred to a flask, and the fluoride-ion concentration of an aliquot portion was determined by the "modified" method of Willard and Winter (described in Chap. 2, Sec. 7, of this book). The results of this analysis were used to deter-

*The normal boiling point of HF is 19.4°C.

mine the concentration of HF in the exposure unit after correction for atmospheric pressure was made and after subtraction of a "blank" value.

The concentration of fluoride ion in air samples was checked periodically by a sensitive biologic method that involved the quantitative measurement of the inhibition of liver esterase activity by fluoride.

Table 17.12 — Concentration of HF in Exposure Atmosphere

Weighted-mean concentration of HF		Deviation,	Percentage of samples
Mg/cu m	Ppm	mg/cu m	in given range
25.1*	33	25.1 ± 2.8	38
25.1	33	25.1 ± 5.7	68
25.1	33	25.1 ± 11.4	95
7.2†	8.6	7.2 ± 0.8	38
7.2	8.6	7.2 ± 1.6	68
7.2	8.6	7.2 ± 3.2	95

* Desired concentration, 25 mg HF/cu m; standard deviation, 5.71; number of samples, 161.

† Desired concentration, 7 mg HF/cu m; standard deviation, 1.59; number of samples, 160.

Table 17.13 — Temperature and Humidity of Exposure Atmospheres

	Temperature, °F		Humidity, %	
Weighted-mean concentration of HF, mg/cu m	Weighted mean	Range*	Weighted mean	Range*
25.1	72.7	83.8–66.7	55.5	48.1–66.0
7.2	72.3	80.4–66.5	56.8	46.8–97.2

*Computed from daily means.

Agreement between the two methods was good. Details and results of the method, developed by a member of the Inhalation Section, are given in Chap. 2, Sec. 8.1 and Sec. 7.

The average concentration of 161 samples analyzed by the chemical method (weighted for exposure time) and the percentage of samples at a given deviation from this concentration are given in Table 17.12. A blank, approximately 1.5 mg HF/cu m, a mean of 19 blank samples taken at intervals throughout the exposure period, was subtracted to give the values appearing in the table. From Table 17.12 it may be

seen that approximately two-thirds of the samples were within ± 25 per cent of the average concentration. Table 17.13 shows the weighted mean of the temperature and of the relative humidity for both 30-day exposures.

Signs of Toxicity. What appeared to be subcutaneous hemorrhages developed in rats within a few days after the start of exposure. The hemorrhages were particularly noticeable around the eyes and on the feet. These hemorrhagic areas may have contained porphyrins. Mice were similarly affected but to a lesser degree. In dogs an inflammation of the scrotal epithelium became apparent after the third day of exposure. These findings were confined principally to the 25-mg level, but hemorrhagic areas, less severe in nature, did develop in the feet of rats at the 7-mg level during the latter half of the exposure period.

Table 17.14 — Mortality of Animals Exposed to HF

Species	At conc. of 25.1 mg HF/cu m		At conc. of 7.2 mg/cu m	
	No. of deaths per no. exposed	Mortality, %	No. of deaths per no. exposed	Mortality, %
Totally exposed:				
Rat	29/29	100	0/15	0
Mouse	18/18	100	0/20	0
Guinea pig	0/20	0	0/10	0
Rabbit	0/18	0	0/10	0
Dog	0/4	0	0/5	0
Head exposed:				
Rabbit			0/12*	0

* Two head-exposed rabbits died early in this experiment, apparently from accidental injury.

Mortality. Deaths occurred only at the 25-mg level of exposure, and this was found exclusively in the rat and mouse. In these species all the exposed animals died at the highest level of exposure (Table 17.14). Deaths occurred among rats throughout the entire exposure period; in mice, however, all animals died by the seventieth hour of exposure.

Weight Changes. At the 25-mg level the rats showed a pronounced loss in weight just prior to death; the rabbits showed only a slight loss in weight, the dogs showed no change, and the guinea pigs, after a consistent gain, lost weight following the third week of exposure. Approximately normal gains in weight were observed in animals exposed at the 7-mg level (Table 17.15).

Biochemical Findings. Determinations of calcium and of alkaline phosphatase activity in the blood, and of protein, reducing sugar, and fluoride in the urine of dogs and rabbits, showed no changes of significance at either level of exposure of any constituent save that of urinary fluoride. Overnight excretion of fluoride at the 7-mg level increased approximately 1 ½ times over control values.

Table 17.15—Mean Weight Change of Animals Exposed to HF*

Concentration of HF, mg/cu m	Mean weight change, % of initial weight					
	Totally exposed					Head exposed, rabbit
	Rabbit	Dog	Guinea pig	Rat	Mouse	
25.1	−8	+3	+12	−11		
7.2	−17	+4	+31	+21	+9	+7

* Initial weights of animals are found in Tables 17.8 and 17.9.

Table 17.16—Results of Blood-coagulation Tests* on Animals Exposed to HF

Concentration of HF, mg/cu m	Species	No. of animals	Constituents of blood-coagulation mechanism			
			Prothrombin time		Fibrinogen level	
			Pre-exposure, sec	Post-exposure, sec	Pre-exposure, mg %	Post-exposure, mg %
25.1	Dog	4	24.7	23.7	286	404
	Rabbit	6	26.9	22.7	316	528
	Rat	5	36.5	37.9	378	830
0 (control)	Dog	1	29.5	28.3	247	212
	Rabbit	1	26.9	24.6	340	392

* Average values.

Blood-coagulation Studies. The determination of prothrombin-clotting time and blood-fibrinogen levels in dogs and rabbits exposed at the high level showed changes in fibrinogen level only (Table 17.16).

Pathology. Tissue changes following exposure to hydrogen fluoride gas were noted in the dog, rabbit, and rat, the three species examined. Degenerative testicular changes, ulceration of the scrotum, and moderate hemorrhage and edema of the lungs were found in the dog. Similar pulmonary damage, but to a lesser degree, was found in the rabbit and rat. In the latter species renal-cortical degeneration and necrosis were noted. The above changes were found at the high level only. Insignificant changes were noted in the dog, rat, and rabbit

exposed at the lower level with the exception of localized hemorrhagic areas in the lungs of one dog (Table 17.17).

Hematology. Hemograms of 20 variables determined in the dog, rabbit, and rat exposed at the 25-mg level showed no changes of consequence.

Table 17.17 — Pathology of Animals Exposed to HF

Concentration of HF, mg/cu m	Dog	Rat	Rabbit
25.1	Degenerative testicular changes in 4 of 4, moderate hemorrhage and edema of lungs in 3 of 4, ulceration of the scrotum in 4 of 4	Moderate hemorrhage and edema and marked capillary congestion in lungs in 20 of 30; renal cortical degeneration and necrosis in 27 of 30	Slight pulmonary hemorrhage in 4 of 10
7.2	Localized hemorrhagic areas in lungs in 1 of 5	Gross examination revealed no changes	

Table 17.18 — Fluoride Content of Osseous Tissues of Rats Exposed to HF

Concentration of HF, mg/cu m	No. of rats	Exposure hr	Fluorine, ppm			
			Incisor		Femur	
			Crown	Root	Epiphysis	Diaphysis
Serial Analyses During Exposure						
25.1	1	25	197	2,220	3,830	2,290
	1	43	1,160	3,650	6,500	3,590
	2	52	1,110	4,155	4,725	3,920
	1	64	2,440	6,910	6,520	5,030
	1	70	1,860	5,500	8,260	5,980
	1	75	1,930	6,480	6,300	6,710
	1	95	2,640	8,480	7,200	5,370
0 (control)			247*			
Analyses at Termination of Exposure						
7.2	10	166	2,955	3,835	4,070	3,165
0 (control)			337†			
0 (control)				286‡	625‡	500‡

*Average of 18 analyses.
†Average of 67 analyses.
‡Average of 11 analyses.

Deposition of Fluoride in Osseous Tissues. (Work done by Ann Tarbell and Marion J. Voss.) A study of the fluoride deposition in the teeth and femurs of rats exposed at 25- and 7-mg levels was made. At the 25-mg level analyses were performed serially on animals exposed from 25 to 95 hr; at the 7-mg level analyses were made at the end of exposure only. The fluoride deposition was also studied in jawbones of dogs exposed at both levels at the end of an exposure period of 166 hr.

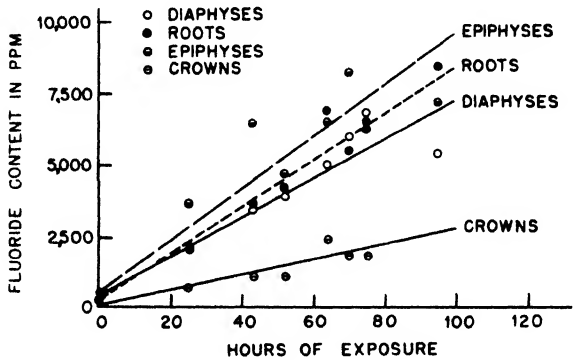


Fig. 17.9—Increase in fluoride content of osseous tissues of rats following increasing exposure to 25 mg of hydrogen fluoride gas per cubic meter.

Table 17.19—Fluoride Content of Maxillary and Mandibular Alveolar Bone of Dogs Exposed to HF

Concentration of HF, mg/cu m	Exposure, hr	No. of dogs	Fluorine, ppm					
			Maxilla			Mandible		
				Postexposure			Postexposure	
			Pre-exposure	Old bone	New bone*	Pre-exposure	Old bone	New bone*
25.1	166	4	795	1,307	2,675	825	1,163	2,050
7.2	166	3	884	1,386	2,016	770	1,366	2,243
0 (control)		1	1,220	914	903	1,110	1,010	840

*Callus at site of previous biopsy.

Table 17.18 shows that a progressive increase of fluoride occurred in four bony structures of rats exposed at the 25-mg level. Greatest increases were observed in the incisor roots, somewhat less in the epiphyses and diaphyses of the femur, and least in incisor crowns (Fig. 17.9). Maximal increases over control values of fluoride in the

root were twenty-five-fold; in the crown, tenfold. The fluoride content of the same structures of animals exposed at the 7-mg level for 166 hr was on the average somewhat lower than that found for animals exposed at the higher level. In other words an exposure to 25 mg for a period of 20 days resulted in a fluoride deposition greater than that

Table 17.20—Animals Exposed for 166 Hr in 29 Days to HF at the 25-mg Level (Summary of Results)

1. Symptoms.
   Dog: Inflammation of scrotal epithelium.
   Mouse: Subcutaneous hemorrhages around eyes and in feet.
   Rat: Same as the mouse, but to a more severe degree.
2. Mortality. High for the rat and mouse; low for the guinea pig, rabbit, and dog.

Species	No. of deaths per no. exposed	Mortality, %
Rat	29/29	100
Mouse	18/18	100
Guinea pig	0/20	0
Rabbit	0/18	0
Dog	0/4	0

3. Weight Changes. Only the rat and rabbit lost weight.

Species	Original wt., g	Wt. change, %
Rat	212	−11
Rabbit	3,700	−8
Dog	9,700	+3
Guinea pig	496	+12

4. Biochemistry.
   Dog: No significant changes in blood-calcium and alkaline phosphatase levels.
   Rabbit: No significant changes in blood-calcium and in urinary-protein and reducing-sugar levels.
5. Blood-coagulation Studies. Dog, rabbit, and rat showed a significant increase in prothrombin time and fibrinogen level.
6. Pathology.
   Dog: Degenerative testicular changes and moderate hemorrhage and edema of lungs; ulceration of the scrotum.
   Rabbit: Slight pulmonary hemorrhages.
   Rat: Moderate hemorrhage and edema of lungs; renal-cortical degeneration and necrosis.
7. Hematology. No changes of significance in hemograms of the dog, rabbit, and rat.
8. Deposition of Fluoride in Osseous Tissues.
   Dog: Fluoride content of maxillary and mandibular alveolar bone increased from two to three times that originally present.
   Rat: Fluoride content of incisal crowns and roots, epiphyses, and diaphyses increased from 10 to 30 times during exposure. Moderate pigmentation effects on incisors.

from exposure to a 7-mg level for a period of 30 days. At approximately equal CT values, 1,200 and 1,162 (50 hr at 25 mg and 166 hr at 7 mg, respectively) fluoride deposition was essentially the same.

Table 17.19 shows a comparison of the fluoride content of the jawbones of dogs determined (1) before exposure (on biopsied tissue), (2) following 30-day exposure, on bone regenerated at the site of biopsy (new bone), and also on (3) the opposite side (old bone). A 200 per cent increase of fluoride was found in old bone at both levels following 166 hr of exposure; in new bone, a 300 per cent increase occurred.

Table 17.21 — Animals Exposed for 30 Days (166 Hr) to HF at 7-mg Level (Summary of Results)

1. Symptoms.
   Rat: Subcutaneous hemorrhages in feet.
2. Mortality. No mortality in any of the exposed species.
3. Weight Changes. All species gained weight.
4. Biochemistry. Overnight output of urinary fluoride increased fivefold over control values in rabbit.
5. Blood-coagulation Studies. No significant changes in rats.
6. Pathology.
   Dog: Localized hemorrhagic areas in lung found in one case.
   Rabbit and rat: Gross examination revealed no changes.
7. Deposition of Fluoride in Osseous Tissues.
   Dog: Fluoride content of maxillary and mandibular alveolar bone increased to a maximum of threefold.
   Rat: Incisal crowns and roots and epiphyses and diaphyses increased in fluoride content from five to twelve times original content.

A summary of the individual criteria obtained for each of the two exposure levels is given in Tables 17.20 and 17.21.

Summary. Hydrogen fluoride gas inhaled for a total of 166 hr in repeated daily exposures by animals was highly toxic at 25 mg/cu m and was only midly irritant at 7 mg. The typical responses in animals exposed at these two levels follow:

1. Hydrogen fluoride was lethal solely at the 25-mg level and then only to the rat and mouse. Larger species such as the dog, rabbit, and guinea pig did not die from such exposures.

2. On autopsy all species showed evidence of pulmonary damage; the dog, degenerative testicular changes; and the rat, renal damage in instances in which the survival time was of more than 2 weeks' duration.

3. Clinical symptoms of hydrogen fluoride exposures consisted of subcutaneous hemorrhages around the eyes and on the feet. In dogs the scrotal epithelium was inflamed.

4. The fluoride content of the teeth of rats increased as much as 300 per cent in exposures at the 25-mg level for periods up to 95 hr.

Somewhat smaller increases were found in the femoral diaphyses and epiphyses of these animals. In the jawbones of dogs the increases under similar conditions of exposure were only from 200 to 300 per cent.

3.1 A Biological Method for the Quantitative Estimation of Fluoride Ion. (Method developed by Max Schlamowitz.) A need exists for a rapid quantitative method for the estimation of small amounts of fluoride ion. The present method represents the attempt to fulfill that need. The principle upon which the method is based is the specific inhibition of activity of liver esterase by the fluoride ion. Loevenhart and Pierce[5] have previously demonstrated the great sensitivity of liver esterase toward fluoride.

The method that has thus far been developed applies to solutions containing pure fluoride ion and for solutions containing fluoride ion in which uranium is a contaminant and has been adapted solely for the analysis of fluoride in exposure-chamber atmospheres.

Results. Samples of animal-exposure-chamber atmospheres containing either HF or HF and UO_2F_2 were taken for quantitative analyses. Aliquots of the same sample were analyzed for comparison by the "modified" fluoride method of Willard and Winter (Chap. 2 of this book). The samples were collected by absorption in water or in 1N or 2N NaOH.

Fluoride in HF Atmospheres. Twenty-nine samples were analyzed by the enzymatic procedure and compared with those obtained on aliquots by the chemical method. Analyses by the enzymatic method were usually performed in duplicate. The analytical data for both methods are presented in Table 17.22, as well as the calculation of the percentage error. Analyses agreed in 73 per cent of 29 cases tested within the maximal allowable error of both methods (approximately 12 to 13 per cent). Fifteen of the 29 cases (52 per cent) agreed to within ± 3 per cent, and 26 of 29 samples (90 per cent) fell within 16 per cent, which is ± 4 per cent outside the allowable error. The estimated allowable error for each method is approximately ± 6 per cent. The maximal allowable error in the comparison of the two methods is therefore approximately ± 12 per cent. The total number of positive and negative errors was such as to indicate a probability distribution. In the calculations of the error of the results the assumption has been made that the chemical method for the determination of fluoride ion is valid for its analysis in the air samples.

F^- in Atmospheric Hydrolysis Products of UF_6. Fifteen analyses of chamber atmospheres containing the hydrolysis products of UF_6, HF, and UO_2F_2 were made by the enzymatic method; these were compared with analyses obtained by the chemical method after investigation into the effect of the uranyl ion as uranyl nitrate on the inhibition

of esterase by fluoride showed that the effect was negligible if the amount of uranyl ion was not greater than fifty times that of fluoride. Thus, 100 ppm of uranyl ion caused the same percentage inhibition of esterase as did 0.025 ppm of fluoride ion.

Table 17.22—Comparison of Results of Enzymatic and Chemical Methods of Analysis of Fluoride Ion

Sample No.	By enzymatic method,* μg/ml	By chemical method,* μg/ml	Error, %†
34	7.20	7.13	+1.0
38	5.50	5.60	−1.8
39	3.81	3.68	+3.3
44	5.95	6.13	−2.9
45	6.00	5.95	+0.8
46	4.32	4.04	+6.8
47	5.88	6.00	−2.0
48	4.00	4.01	−0.3
49	5.76	6.68	−13.8
50	4.28	4.53	−5.5
51	3.51	3.22	+9.0
52	5.49	5.85	−6.2
53	1.82	1.78	+2.2
54	3.49	4.14	−15.7
55	9.37	10.9	−14.0
56	5.17	6.14	−15.8
57	3.83	3.33	+15.0
58	8.50	8.22	+3.4
59	6.03	7.59	−20.6
60	5.07	6.66	−23.8
61	7.85	6.92	+13.4
62	2.45	2.29	−7.0
63	5.81	5.85	−0.7
64	17.5	17.8	−1.7
65	1.20	1.74	−31.0
66	8.21	7.99	+2.8
67	9.60	9.70	−1.0
68	3.00	3.03	−1.0

* Samples of the gas taken at the mixing unit showed 214 μg/ml when analyzed by the enzymatic method and 213 μg/ml when analyzed by the chemical method. The percentage of error was +0.5.

† Percentage error = $\frac{\text{enzymatic value} - \text{chemical value}}{\text{chemical value}} \times 100.$

The results of the enzymatic measurements on the air samples containing both uranyl ion and HF are given in Table 17.23. Values by both the chemical and enzymatic methods showed an agreement of ± 5 per cent.

Table 17.23 — Comparison of Results of Enzymatic and Chemical Methods of Analysis of Fluoride Ion in the Presence of Uranyl Ion

Sample No.	Absorbing medium*	By enzymatic method, μg/ml	By chemical method, μg/ml	Error, %
1	H_2O	7.90	7.87	+0.4
2	H_2O	2.67	2.71	−1.4
3	H_2O	4.65	4.68	−0.6
4	H_2O	4.60	4.45	+3.3
5	H_2O	4.42	4.53	−2.4
6	H_2O	4.46	4.38	+1.8
7	H_2O	5.18	5.50	−5.8
8	H_2O	6.43	6.44	−0.2
9	H_2O	4.65	4.54	+2.4
10	H_2O	6.93	6.94	−0.1
11	H_2O	6.96	6.85	+1.6
12	H_2O	5.35	5.25	+1.9
13	1N NaOH	4.23	4.35	−2.8
14	1N NaOH	2.42	2.53	−8.0
15	1N NaOH	1.12	1.19	−5.9

* A relatively pure esterase, prepared from liver extract by fractional precipitation with ammonium sulfate, was employed for the analysis of all samples in which water was the fluoride-absorbing agent. The use of the purified enzyme permitted a sharper end point in the acid-base titration than would otherwise be possible.

The data reported for fluoride samples collected in NaOH were obtained with a crude preparation of the enzyme, one comparable to the preparation reported in Table 17.22.

4. DISCUSSION OF THE TOXICOLOGY OF THE FLUORINE GASES

Of the two gases, HF and F_2, the F_2 was the more toxic, the tolerated exposure concentrations for each of the two gases being, respectively, 7 and 1 ppm. This is equivalent to 6 and 1.7 mg of fluoride ion per cubic meter.

A comparison of the toxicologic results of F_2 and HF gas shows that F_2 produces a toxicity distinct from that of HF. This occurs despite the reactivity of F_2 with body fluids to form HF. Evidence for the distinct response of F_2 lies not only in its definitely greater toxicity but also in the fact that dogs and rabbits are killed by F_2 while rats and mice survive the same exposures. On the other hand, rats and mice are very susceptible to HF, but dogs and rabbits are not. These species responses give striking evidence of the independent toxicity of F_2 and HF. Furthermore, less fluoride ion is retained in the bones

of animals exposed to F_2 than in those exposed to an equivalent concentration of HF. In connection with deposition of fluoride in bones, it is interesting to note that more fluoride is retained in new bone regenerated from biopsied mandible and maxilla of the dog than in comparable undisturbed structures (Sec. 2.2b of this chapter).

It has been pointed out that distinct differences exist in the action of the two gases as indicated by differences in over-all toxicity, species response, and the rate of fluoride deposition in the bone. One major characteristic, however, common to both materials, is the marked toxic action on the respiratory system. At lethal levels, death invariably resulted from severe pulmonary damage, and at nonlethal levels, if any damage occurred, it was usually pulmonary damage. Although fluoride gases are chiefly pulmonary irritants, they affect other organs to a lesser extent. There was considerable inflammation of the buccal membranes, eyes, and scrotum, the latter accompanied by testicular degeneration at the levels used. High concentrations of fluorine under pressure are extremely hazardous, producing second-degree burns not unlike those from an oxyacetylene flame (Sec. 2.1).

There is a marked accumulation of fluoride in the bones and also in the teeth. In fact, fluorosis of the teeth serves as a good indicator of fluorine exposure. In the case of the 25-mg level of HF, renal-cortical degeneration was observed in the rats but not in the dogs or rabbits. The presence of renal damage in the case of F_2 is questionable, because in most cases only gross observations on dying animals were made.

One additional factor in the toxicity of fluorine-containing gases is concerned with the contamination of the fur. In the case of those animals that lick their fur, considerable material could be ingested. Also there was a prolongation of exposure by evolution of gases into the ambient air from the fur following removal of animals from the exposure chamber. These conditions resulted in a more severe toxic response in animals that were bodily exposed than in those which had only their heads exposed (Sec. 2.2b). In the case of fluorine gas, absorption by the fur was so great that its texture was affected.

5. ACUTE TOXICITY OF SODIUM FLUORIDE ADMINISTERED INTRAPERITONEALLY TO ALBINO RATS

Several investigators (see Bibliography, references 6 to 10 and Table 17.24) have reported acute toxicity doses for NaF in dogs, cats, rabbits, guinea pigs, and rats. The lethal dose varied from 25 to 300 mg/kg.

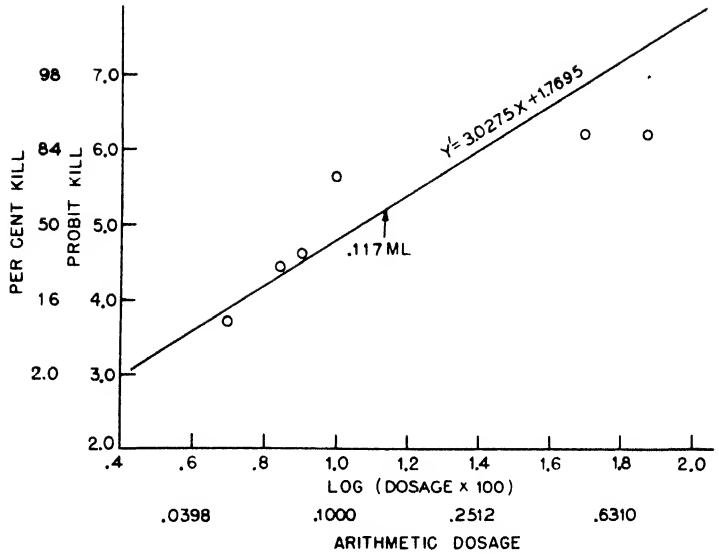


Fig. 17.10—Relation of dosage of NaF, intraperitoneally injected, to mortality of 100- to 200-g rats.

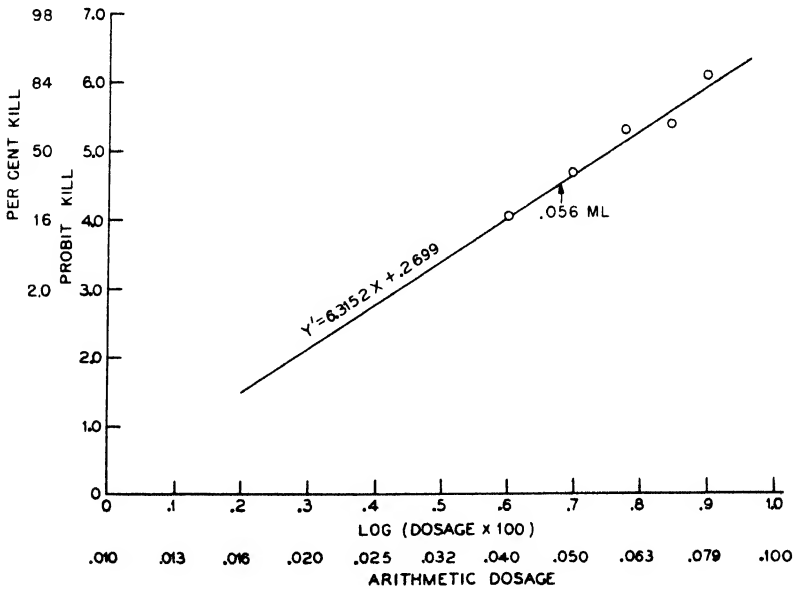


Fig. 17.11—Relation of dosage of NaF, intraperitoneally injected, to mortality of 200- to 300-g rats.

In a study of the acutely toxic dose of NaF, approximately 300 female rats were divided into two main groups according to the body weight, i.e., 100 to 200 g and 200 to 300 g. Each of the weight groups was subdivided into a number of groups containing 7 to 40 rats each. These smaller groups were given doses of 4 to 300 mg NaF/kg. In Fig. 17.10 are shown the results of study on 100- to 200-g rats; the

Table 17.24 — Toxicity of Sodium Fluoride and Calcium Fluoride

Investigator	Compound	Animal	Route of administration	Dose, g/kg	Effect
Tappeiner*	NaF	Rabbit	Intraven.	0.15	Fatal
Muehlberger*	NaF	Rat	Subcut.	0.046	Fatal
Schultz*	NaF	Rabbit	Subcut.	0.2 – 0.4	Lethal dose
Schultz*	NaF	Dog	Subcut.	0.3	Lethal dose
Kolipinski*	NaF	Cat	Intraven.	0.19	Fatal
Greenwood[7]	NaF	Mammal	Oral	0.5	Fatal
Greenwood[7]	NaF	Mammal	Intraven. or hypodermically	0.08 – 0.15	Fatal
Simonin, Pierron[8]	CaF_2	Guinea pig	Ingestion	0.025	Fatal
Simonin, Pierron[8]	CaF_2	Guinea pig	Subcut.	0.040	Fatal

*Listed by De Eds.[6]

probit mortality is plotted against the logarithm of the dosage. The LD_{50} for the 24-hr mortality was of the order of 47 mg/kg. In Fig. 17.11 a similar graph is shown for the results on 200- to 300-g rats. Here the LD_{50} was found to be about 24 mg/kg.

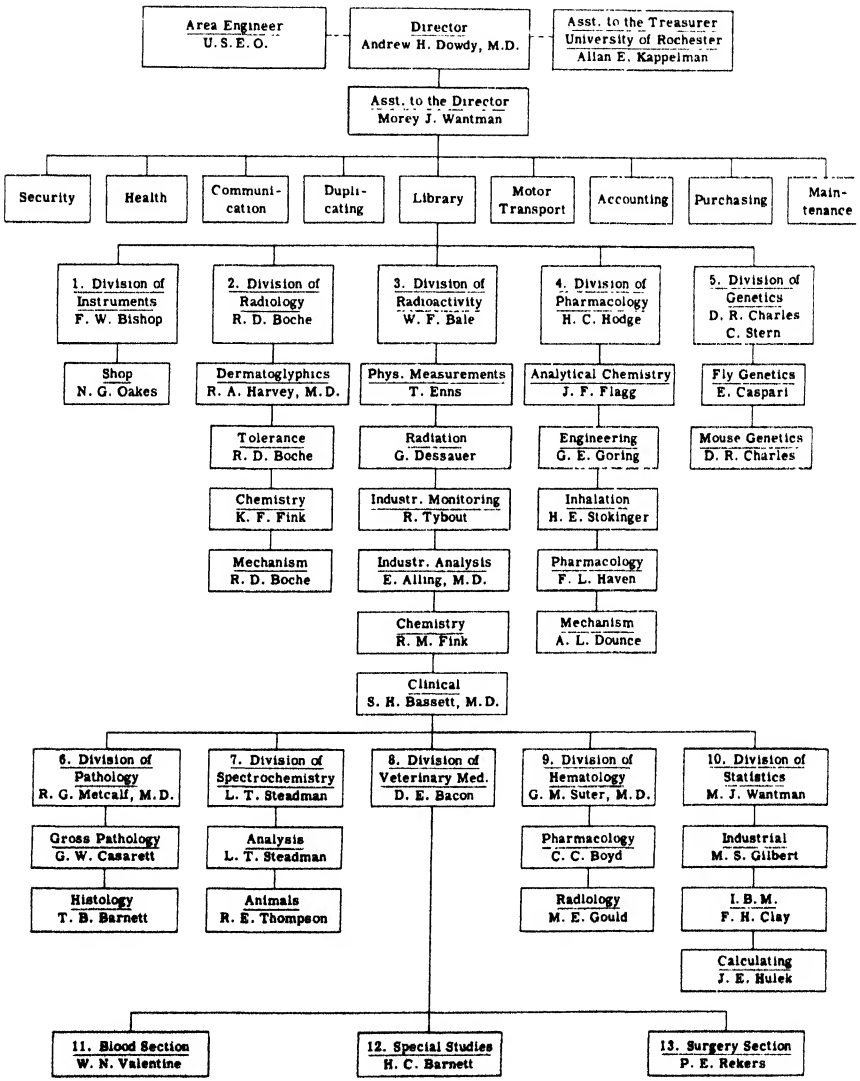
The NaF is thus shown to be a fairly toxic substance. The increase in toxicity with increasing age of rats has not, so far as is known, been previously observed. This constitutes a substantial difference. On a milligram-per-kilogram basis only half as much NaF is required to kill the average rat, weight 200 to 300 g, as is lethal for a 100- to 200-g female rat. These values are of the same order as reported by Muehlberger following subcutaneous administration of NaF to rats. He reported that 46 mg/kg was a fatal dose. Table 17.24 gives the results obtained by various authors.

BIBLIOGRAPHY

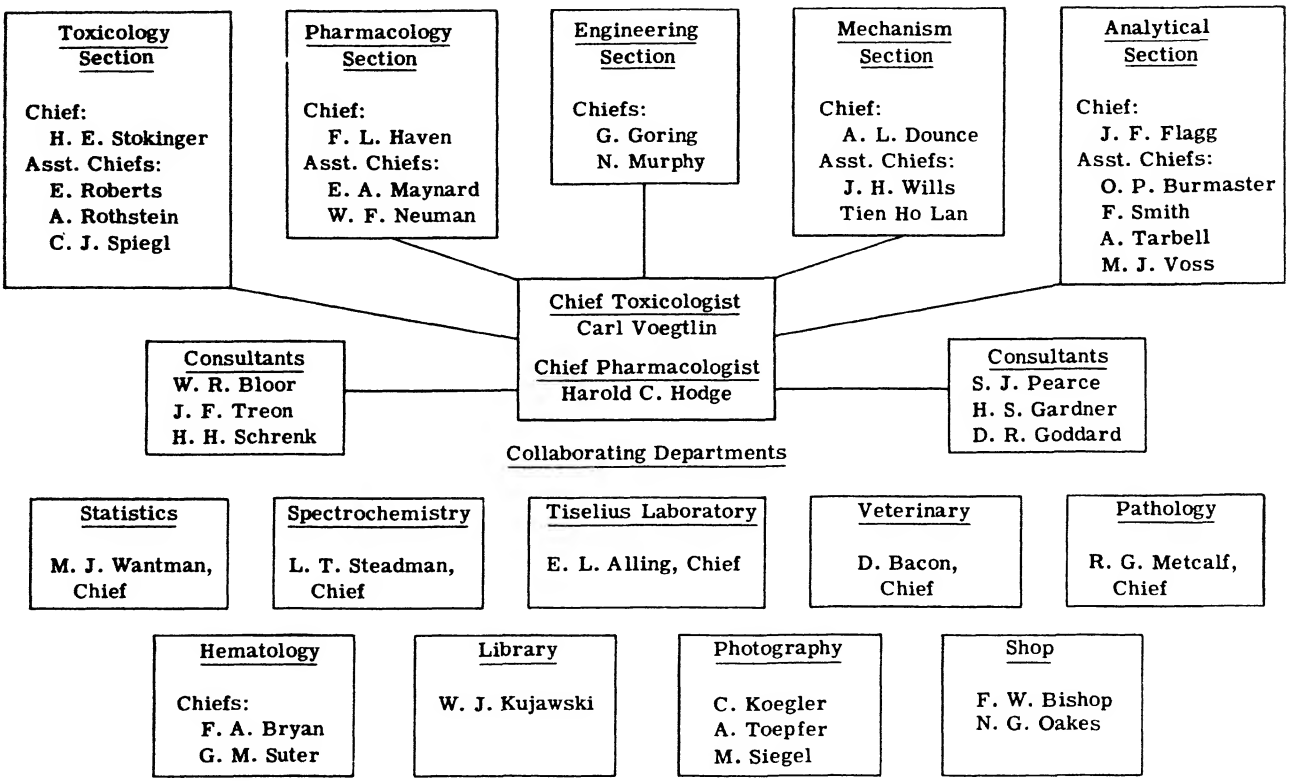
1. W. Machle, A. Thaman, K. Kitzmiller, and J. Cholak, The Effects of Inhalation of Hydrogen Fluoride. I. The Response Following Exposures to High Concentration, J. Ind. Hyg., 16: 129 (1934).
2. W. Machle, A. Thaman, K. Kitzmiller, and J. Cholak, The Effects of Inhalation of Hydrogen Fluoride. II. The Response Following Exposure to Low Concentration, J. Ind. Hyg., 17: 223 (1935).
3. W. S. Petterson, Flowmeter for Gaseous Anhydrous Hydrogen Fluoride, Anal. Chem., 17: 54 (1945).
4. F. A. Bryan, Statistical Study of Uranium Hexafluoride and Fluorine Exposures, Univ. Rochester, Rochester Area File No. 5498 (March, 1946).
5. A. S. Loevenhart and G. Pierce, The Inhibiting Effect of Sodium Fluoride on the Action of Lipase, J. Biol. Chem., 2: 397 (1906).
6. F. De Eds, Summary of Acute Toxicity Data, Medicine, 12: 1 (1933).
7. D. A. Greenwood, Physiol. Rev., 20: 582 (1940).
8. P. Simonin and A. Pierron, Compt. rend. soc. biol., 124: 669 (1937).
9. T. H. Sollmann and P. J. Hanzlik, "Fundamentals of Experimental Pharmacology," 2d ed., J. W. Stacey, San Francisco, 1940.
10. K. Roholm, "Fluorine Intoxication," H. K. Lewis and Co., Ltd., London, 1937.

APPENDIX

Organization Chart of the Manhattan Department, University of Rochester



Organization Chart of the Pharmacology Division, Manhattan Department, University of Rochester



PHARMACOLOGY DIVISION PERSONNEL

(June 1943 - July 1946)

Division Chief — H. C. Hodge, June 1943 -

Chief Toxicologist — C. Voegtlin, Oct. 1943 -

Pharmacology Section

Section Chief — F. L. Haven, Sept. 1943 -

Assistant Chiefs:

E. A. Maynard, Oct. 1943 -
W. F. Neuman, July 1943 -

Staff:

R. S. Crossland, Sept. 1943 -
P. P. Dale, June 1943 -
R. Fleming, July 1944 -
H. B. McCauley, July 1943 - Feb. 1945
M. W. Neuman, Oct. 1943 -
J. A. Orcutt, Apr. 1944 -

Assistants:

J. E. Box, Aug. 1943 - July 1945
A. B. Carlson, Jan. 1944 -
V. Edwards, Jan. 1945 - Aug. 1945
F. Furth, July 1943 -
J. D. Miller, Feb. 1944 - Mar. 1946
P. W. O'Connell, Nov. 1943 -
J. F. O'Leary, Mar. 1944 -
J. C. O'Leary, Mar. 1944 - Feb. 1945
J. Orcutt, June 1944 - Nov. 1944
C. Randall, Nov. 1944 -
I. W. Thomas, July 1943 - May 1944
M. Wenning, June 1945 - May 1946
A. Wing, Feb. 1944 - Apr. 1945

Technical Assistants:

B. J. Clark, Aug. 1944 - Apr. 1946
A. Cobb, July 1943 -
W. L. Downs, Jan. 1944 -
W. J. Maier, Jan. 1944 - Mar. 1946
B. J. Mulryan, June 1943 -
J. Pagano, June 1943 -
M. W. Potter, Sept. 1944 -
L. B. Proctor, June 1945 -
N. Smith, Oct. 1944 - June 1945; Sept. 1945 - May 1946

Toxicology Section

Section Chief — H. E. Stokinger, Sept. 1943 -

Assistant Chiefs:

E. Roberts, Sept. 1943 -
A. Rothstein, Dec. 1943 -
C. J. Spiegl, Dec. 1943 -

Toxicology Section (Continued)

Staff:

B. H. Amdur, May 1944–
D. C. Brodie, Sept. 1944–Sept. 1945
J. G. Cobler, May 1944–Oct. 1945
B. Craver, Oct. 1944–Sept. 1945
H. P. Dygert, Nov. 1943–
N. Eriksen, July 1944–Dec. 1945
J. B. Field, Feb. 1945–
P. Hoch, May 1944–May 1945
C. W. LaBelle, Nov. 1945–
S. Laskin, Dec. 1944–
U. C. Pozzani, Feb. 1945–
J. J. Rothermel, May 1944–
M. Schlamowitz, June 1944–Nov. 1945
G. F. Sprague, Jr., Jan. 1944–
C. Weil, Oct. 1943–Oct. 1945
F. W. Wichser, Dec. 1944–Apr. 1946
R. C. Baxter, Dec. 1945–
H. L. Berke, Mar. 1944–
C. W. Bishop, Apr. 1944–
J. H. Brinkman, Nov. 1944–July 1945
L. B. Cohenour, Jan. 1944–Feb. 1946
W. D. Davis, May 1944–
D. Dittman, Dec. 1943–May 1946
A. Fiorica, Oct. 1945–
N. Glover, June 1944–
W. M. Harrison, Jan. 1944–
N. R. Hofschneider, Aug. 1945–
C. A. Horton, Apr. 1944–Oct. 1945
N. Kaplan, Oct. 1944–Jan. 1946
G. Laush, May 1944–
M. Lubelle, Nov. 1944–
R. Maier, Mar. 1944–June 1945
J. Minor, June 1944–
H. A. Oberg, Dec. 1943–Mar. 1946
M. P. Reid, July 1945–
R. G. Sanford, May 1944–
R. Scott, Sept. 1945–Mar. 1946
A. Shannon, Nov. 1944–
A. Schepartz, May 1944–
G. Siegel, Oct. 1943–June 1945
I. Slotnik, July 1943–Feb. 1945
R. H. Todd, Nov. 1945–
J. A. Tornaben, June 1944–
H. Wilson, Dec. 1944–

Analytical Section

Section Chief—J. F. Flagg, July 1944–

Assistant Chiefs:

O. P. Burmaster, July 1943–Apr. 1945
F. Smith, June 1944–
A. Tarbell, Nov. 1943–Sept. 1945
M. J. Voss, Jan. 1944–

Staff:

J. Adolph, Feb. 1945–Nov. 1945
J. L. Bleasdale, May 1944–June 1944
M. D. Browning, Apr. 1945–Sept. 1945
M. I. Crossmon, June 1944–
A. Dulabahn, July 1944–Mar. 1945
R. Lobene, July 1944–Oct. 1945
D. Lucas, April 1945–
A. D. Morabito, July 1944–
D. J. O'Malley, Aug. 1944–Sept. 1945; Mar. 1946–
E. Smith, Nov. 1943–Dec. 1945
N. B. Smith, Oct. 1944–June 1945; Sept. 1945–May 1946
V. S. Sullivan, June 1944–
D. M. Valenti, Nov. 1943–Mar. 1945
E. Ware, Aug. 1944–July 1945

Mechanism Section

Section Chief—A. L. Dounce, Aug. 1943–

Assistant Chiefs:

Tien Ho Lan, Oct. 1943–
J. H. Wills, Jr., Oct. 1943–

Staff:

D. L. Adler, Jan. 1944–Aug. 1945
P. E. Fanta, Mar. 1944–
E. R. Main, Jan. 1944–
A. A. Sunier, June 1944–Sept. 1945
G. H. Tishkoff, Mar. 1944–

Assistants:

W. Connors, Nov. 1943–
M. W. Kaley, Apr. 1944–Apr. 1946
P. W. O'Connell, Nov. 1943–
B. Robinson, Sept. 1944–July 1945
D. L. Rothermel, Oct. 1944–

Engineering Section

Section Chiefs:

G. E. Goring, Jan. 1944–Feb. 1946
N. Murphy, Dec. 1943–Mar. 1946

Staff:

L. Bauermash, Jan. 1944–Mar. 1946
F. W. Doughty, Jan. 1944–July 1945
R. Garson, Jan. 1944–Aug. 1944
W. J. Maier, Jan. 1944–Mar. 1946
E. W. Same, Mar. 1944–Mar. 1946
R. Wilson, Oct. 1945–
W. Wolf, Mar. 1944–Apr. 1946

Assistants:

M. Cedorbaum, Jan. 1944–Jan. 1945
D. Lehman, Jan. 1945–May 1945
C. J. Monahan, Dec. 1944–Mar. 1945

Consultants

Walter R. Bloor, Department of Biochemistry, University of Rochester; fluorophotometry, Feb. 1944–

Howard S. Gardner, Department of Engineering, University of Rochester; chemical engineering, Mar. 1944–

David R. Goddard, Department of Biology, University of Rochester; biological systems, July 1944–

Consultants (Continued)

Seldon J. Pearce, U.S. Bureau of Mines, Pittsburgh, Pa.; respiratory protective devices, Mar. 1944–

Helmuth H. Schrenk, U.S. Bureau of Mines, Pittsburgh, Pa.; respiratory protective devices, Apr. 1944–

Joseph F. Treon, Kettering Laboratories, University of Cincinnati; industrial toxicology, Apr. 1944–

COLLABORATING DIVISIONS

Statistics

Chief — M. J. Wantman, Sept. 1943–

Associate Chief — D. V. Tiedeman, June 1944–

Section Heads:

L. S. Kogan, Mar. 1946–
F. H. Clay, Sept. 1944–
E. R. Street, July 1944–

Unit Heads:

M. S. Arcesty, Jan. 1944–
H. Baldwin, Mar. 1946–
R. B. Tilden, Jan. 1944–
J. E. Hulok, Jan. 1944–
D. Kuhnert, Jan. 1944–
M. G. Yaroslow, Sept. 1944-

Spectrochemistry

Chief — L. T. Steadman, May 1943–

Assistants:

H. E. Thompson, May 1943–
G. E. Reinhardt, Oct. 1944–
G. Sandall, May 1944–Mar. 1946
H. Frankel, Sept. 1944–May 1946
P. Pruett, Jan. 1944–Sept. 1945
H. Ulrich, May 1943–Sept. 1945
E. Sprague, May 1943–

Tiselius Laboratory

E. L. Alling, Sept. 1943–

Veterinary Medicine

D. Bacon, June 1944–Apr. 1946
L. J. Desson, Nov. 1943–
R. P. Ball, June 1944–

Pathology

Chiefs:

R. G. Metcalf, Nov. 1943–May 1946
T. B. Barnett, July 1944–

Associates:

M. V. Anders, Apr. 1944–Aug. 1945
F. A. Inda, Oct. 1945–
R. P. Kennedy, Feb. 1945–Oct. 1945
G. M. Suter, Dec. 1943–Feb. 1945

Assistant—G. W. Casarett, Sept. 1943–

Hematology

Chiefs:

F. A. Bryan, Aug. 1943–Nov. 1945
G. M. Suter, Sept. 1945–

Assistant:

C. Boyd, Sept. 1943–Mar. 1946

Library

W. J. Kujawski, July 1944–
E. Smith, Jan. 1944–Apr. 1944
E. Stern, Mar. 1944–

Photography

C. Koegler, Jan. 1945–
A. Toepfer, Sept. 1943–June 1944
M. Siegel, Mar. 1944–May 1946
R. Hay, May 1946–

Shop

Chief—F. W. Bishop, June 1943–

Head—N. G. Oakes, Oct. 1943–

Assistant—C. Utter, Feb. 1944–

INDEX

A

D

G

H

N

O

P

Q

R

S

T

U

V

W