ADVANCES IN
BIOLOGICAL AND MEDICAL PHYSICS

VOLUME I

ADVANCES IN BIOLOGICAL AND MEDICAL PHYSICS

Edited by

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VOLUME I

ACADEMIC PRESS INC., PUBLISHERS

NEW YORK, 1948

ACADEMIC PRESS INC.
111 Fifth Avenue
New York 3, N. Y.

First printing, 1948
Second printing, 1957

PRINTED IN THE UNITED STATES OF AMERICA

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FOREWORD

The recent developments in nuclear physics, which culminated in the release of atomic energy, have excited the interest of both the general public and the scientists to an unprecedented degree. The initial applications of nuclear physics to the medical and biological sciences appeared soon after the discovery of natural radioactivity at the end of the nineteenth century. Radium and several other naturally occurring radioactive elements have found considerable use in medicine, primarily as therapeutic aids in the treatment of neoplasms. The discovery of artificial radioactivity in 1933 and the coincidental development of the cyclotron immediately opened new vistas through which scientific knowledge could be extended. The use of radioactive isotopes of stable elements to study the mechanism of life processes was a concept soon to be applied as a new and powerful research tool to almost all of the medical and biological sciences. Parallel to this rather spectacular development was the similar use of heavy stable isotopes as tracers. Before the entry of the United States into World War II, the applications of these new physical methods to fundamental biological research, clinical investigation, and actual therapeutic trial had proceeded at a rapidly increasing rate. The impact of the war served to accelerate rather than retard the progress in this new field. During the war period, the emphasis was directed towards health problems associated with radiations of various types resulting from the release of atomic energy, but radioactive isotopes proved of great value in studies on shock, blood preservation, respiratory and circulatory physiology, and in numerous other war-time medical investigations. During the war a number of factors were introduced which most certainly altered the predictable pattern which this new field may assume in the immediate future.

The first, and probably the most significant, of the factors has been the discovery of the chain reacting uranium pile. This device can produce continuously a tremendous intensity of neutrons which in turn may be employed to prepare almost limitless quantities of a large number of radioactive isotopes. Included among the radioactive materials that can be produced by neutron irradiation from the pile are the radioactive isotopes of a large proportion of the elements that are known to be essential to some or all forms of life—hydrogen, carbon, sodium, phosphorus, sulfur, chlorine, potassium, calcium, iron, cobalt, copper, zinc, and iodine. Second, the release of nuclear energy is associated with the formation of incredible quantities of a large series of radio-elements called the fission products, as well as considerable amounts of several radioactive heavy elements, notably, neptunium and plutonium. The adequate protection of workers who come in contact with these materials in the peaceful applications of atomic energy has presented a number of problems in the field of industrial medicine. The grim significance of the atomic bomb and radioactive warfare does not require further emphasis—the unpleasant possibilities are all too clear. Third, the wartime developments in nuclear energy demanded a rapid extension of training of workers in all of the fields of applied nuclear physics, including those in the biological sciences and medicine. Fourth, this interval has seen a rapid and extensive development in many of the practical techniques for the use of artificial radio-elements, which includes the ready availability of reliable devices for the detection and measurement of radioactivity, equipment for the protection of people working in this field, special films and emulsions for autoradiography, and many other technological advances. Over and above these factors, there is a widespread and intense public interest in the many aspects of atomic energy and, in particular, its applications to the advancement of knowledge in the medical sciences. It can be readily appreciated that the availability of almost limitless supplies of radioactive tracers, the rapid increase in the number of scientific workers trained in this new field, the new medical problems presented by the toxicological factors arising from the release of atomic energy, and the widespread public interest that is manifested both by moral and material support also have a great accelerating effect upon the future developments in this field.

Obviously the many important phases of this new field cannot all be adequately reviewed in a single volume. The Editors have attempted to select a number of salient topics for this first volume in order to present a few aspects of the keystones of the field; namely, the fundamentals of radioactivity, instrumentation, techniques of application, and radiation protection.

The Editors wish to thank the authors of the several chapters in this first volume for their contributions and for their patience in awaiting the publication of them. Special thanks are due to our colleagues, Dr. Cornelius A. Tobias for his constructive criticism of the manuscripts, and to Dr. Ellsworth C. Dougherty for compiling the Subject Index. The support of the publishers is gratefully acknowledged.

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April 15, 1948

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I. INTRODUCTION

Since most of the chapters of the present volume are concerned with the application of isotopes, it seems desirable to present in the first chapter a general orientation and background statement on the medical usefulness of isotopes with a summary of some of the important literature. We are treating the heavy and artificial radioactive species in much greater detail than the naturally occurring radioactive isotopes since, in general, the applications of the latter are well known. We are, furthermore, only touching upon that subject matter covered in more detail in several of the other chapters, *i.e.*, iron metabolism, iodine metabolism, *etc.*

The first of the following sections is devoted to a general discussion of the methods by which isotopes can be employed in medical and biological problems and to a consideration of the factors that determine or influence the choice of isotopes that have been and may be used in biological work. For the principal characteristics of most of the radioactive species (half-lives, types of radiation, *etc.*) the reader may consult Chapter 3 by Cohn in this volume.

✓ Radioactivity has played a role in biology and medicine almost from the beginning of our knowledge of radioactive phenomena. Four years after Becquerel, in 1896, had discovered the radioactive properties of uranium ($_{92}U$) salts and only two years after the Curies, in 1898, had identified radium ($_{88}Ra$) and polonium ($_{84}Po$), Walkhoff (1900) published observations, made the year before, on the effects of the radioactivity of radium and its decay products on living tissue; he, in fact, made the first record of a radium burn of human skin. In 1901 radium was first employed in an attempt to treat a human disease—lupus erythematosis—by Danlos and Bloch (1901). Shortly thereafter radium was first applied in the treatment of malignancy.

However, the most important application of radioactivity as a tool for fundamental biological investigation came much later. Thus, Hevesy, in 1923, was the first to employ a radioisotope in a "tracer" experiment, that is, one in which the fate of an element (in this case lead—$_{82}Pb$) was followed in a living organism (a plant) by measuring in various samples of the organism the radiations of a radioisotope, radium D (RaD, or $_{82}Pb^{210}$), substituted for the stable form of the element. He was thus able to follow the distribution of minute amounts of lead in the plant. ✓

The existence of more than one stable isotope for many elements was suggested by Soddy in 1910. Much evidence for this view accrued in the ensuing years and was finally confirmed beyond question for elements unrelated to the natural radioactive series by the work of Aston in 1920. But the "heavy" isotopes—so-called because, in most cases, the one or more rarer stable isotopic species of a given element have heavier nuclear masses than the most abundant form—were long in finding biological application, inasmuch as techniques had to be developed by which different forms of an element could be separated—forms that were, for most practical purposes, chemically identical, but differed in the masses of the atomic nuclei. Ultimately, however, in 1933, Lewis, after successfully separating heavy hydrogen or deuterium ($_1H^2$ or D) from ordinary hydrogen, employed it in the first biological experiment with a stable heavy isotope. He investigated the effect of almost pure deuterium oxide, or "heavy water", on living cells and organisms and found that it acted as a poison, presumably because of the different metabolic behavior resulting from the considerable difference in the nuclear masses of normal and heavy hydrogen. Barnes (1933) shortly followed with studies on the biological effects of dilute solutions of heavy water; he claimed to demonstrate a stimulating effect on the growth of microorganisms, but subsequent research has supplied conflicting data, and there is at present no definitive answer to this question. In the field of isotopic tracing the use of deuterium has been very extensive. Hevesy with a co-worker, Hofer (1934a, b) used it to investigate the exchange of water in and out of the vertebrate body. They found that, in the case of the goldfish, the exchange of heavy water atoms between the animal and the surrounding medium was very rapid, an exchange equilibrium being reached within a few hours; however, in this time hydrogen atoms bound to carbon in the goldfish tissues were exchanged little, or not at all with the deuterium atoms in the body water. This sort of study would have been quite impossible without an isotopic tracer.

✓ Essentially, the great developments in the use of isotopes, both stable and radioactive, followed the discovery of artificial radioactivity by the Joliot-Curies in 1933, and the application of an artificial isotope—in this case, of phosphorus (${}_{15}P^{32}$)—to a biological problem by Chievitz and Hevesy (1935, 1937). These workers traced the distribution and excretion of phosphorus in rats and showed that it was deposited in the bones not only of the growing, but of the adult animal as well. Subsequently, there has been a flood of literature in which is reported a great number of experiments involving the use of both radioactive and heavy isotopes in biology and medicine. Particularly noteworthy has been work with heavy isotopes by Schoenheimer (1942) and his collaborators, who have demonstrated by ingenious experiments the dynamic state of body constituents. Also of great importance has been a number of studies demonstrating the actual or potential value of certain radioactive isotopes, particularly of phosphorus and iodine, in clinical investigation, diagnosis, and therapy.

Recently a comprehensive treatment of radioactive isotopes in biology has appeared in book form by M. D. Kamen (1947). However, not all phases of medical application were treated fully therein, particularly the clinical application of radioisotopes. The use of stable isotopes in biology, although the subject of a number of review papers, has not as yet been comprehensively treated in book form. However, Kamen (1947) has devoted considerable discussion to the use of deuterium. Among the other chapters of the present volume only that by Vennesland on carbon and nitrogen isotopes deals in detail with the use of stable isotopes.

II. The Medical Significance of Isotopes

1. A Classification of the Use of Isotopes

The role of isotopes in basic medical research falls into two main categories, which can be usefully summarized and subdivided as follows:

I. Use as labeling agents ("tracers") of normal and abnormal physiology.
  A. As tracers of metabolism
    1. Of intrinsic body constituents
      a. Water
      b. Minerals, including trace elements
      c. Organic substances
    2. Of extrinsic substances (abnormal to the body in form or amount)
      a. In pharmacological studies
      b. In toxicological studies

B. As tracers of non-metabolic processes
  1. Involving cells and other intrinsic complex constituents
  2. Involving foreign substances
    a. Dissolved gases
    b. Colloids and particles of greater than colloidal size
    c. Foreign cells and organisms
II. Use in the study of the behavior and effects of isotopes *per se*
  A. Differential fates of isotopes
  B. Effects of isotopes
    1. Of abnormal concentrations
    2. Of radiation (including radiotherapy)

2. A Survey of Useful Isotopes

Several important clinical applications have derived from fundamental studies in both of the foregoing categories. These are discussed in the third section of this paper. Many further clinical uses are to be anticipated. Diagnostic procedures may conceivably develop from all of the subcategories listed. Radiotherapeutic applications are already well recognized for certain isotopes and undoubtedly will be extended to others. The field of clinical application of isotopes is in its infancy and seems destined for much expansion in the coming years.

In the broadest sense medically useful isotopes include all those species that can be used, at least potentially, as tracer substances in the study of living processes, both normal and abnormal. Inasmuch as the same modified isotope ratios that make study of the effects of abnormal isotopic concentrations possible also provide the basis for tracer studies, and as the same radioactive properties that permit unstable isotopes to be applied to the biological study of radiation and in radiotherapy also make them useful as tracer agents, it is not necessary to distinguish between the two main categories of application in a preliminary survey of valuable isotopes.

The isotopes that have been used in fundamental investigations in medicine and related fields are listed with their principal applications in Table I. Elements known or believed to be essential to life and naturally occuring isotopes thereof are marked with an asterisk [*].

It may be pointed out that it is quite impossible to separate stable and radioactive isotopes into different broad categories of use. Both types have application to tracer work, and both can be used to study the behavior and effects of isotopes *per se*. It is even possible to employ ordinarily stable species in the study of radiation effects. For example, the rarer of the two stable isotopes of lithium—$_3Li^6$—and of boron—$_5B^{10}$—respectively, undergo disintegration when bombarded with slow neutrons, and this has made possible the study and use of localized radiation effects by concentrating lithium and boron into selected sites, such as tumors, in the bodies of experimental animals and then subjecting these to neutron beams (Zahl *et al.*, 1940). Nevertheless, it is useful in a preliminary consideration to take up stable and radioactive isotopes as separate groups.

There are 81 elements for which stable isotopes are known (Seaborg, 1944). Of these elements, 21 have a single species, and 60 have two or more. Altogether, 278 stable species have been reported.

Among the 21 elements having but one stable species are five—sodium ($_{11}$Na), phosphorus ($_{15}$P), manganese ($_{25}$Mn), cobalt ($_{27}$Co), and iodine ($_{53}$I)—of the 18 elements known or thought to be essential to mammalian life. The normal role of these elements in metabolism can thus never be studied by stable isotopic tracing since there is no way to distinguish such atoms when introduced into a living system from those already there.

Of the 197 less abundant species of the 60 elements existing in more than one stable form, seven have thus far found biological application—namely, hydrogen ($_1$H), lithium ($_3$Li), boron ($_5$B), carbon ($_6$C), nitrogen ($_7$N), oxygen ($_8$O), and sulfur ($_{16}$S). Except for lithium and boron, which, so far as known, are not essential in animal nutrition, these are essential elements biologically. The rare isotopes, $_1$H^2 (D), $_6$C^{13}, $_7$N^{15}, $_8$O^{18}, and $_{16}$S^{34}, have proved of varying value in the study of basic processes of intermediary metabolism. It may be noted that these species are all of light elements. For one reason or another they have been able to compete as tracer substances with unstable species of the same elements. It is possible that stable isotopic tracing will be extended in the near future to additional elements despite the fact that considerable expense is involved in isolating the components of isotopic mixtures for elements heavier than the first few of the periodic system. All elements above the first 14 have convenient and relatively much cheaper radioisotopes, but there are several light elements possessing no satisfactory radioactive species, *e.g.*, magnesium ($_{12}$Mg), for which stable tracing has not yet been explored; furthermore it is quite conceivable that the effects, even of minimal radioactivity, may prove undesirable in certain types of future experiments. If such is the case, non-radioactive tracing, especially for the remaining 8 essential elements that have more than one stable isotope, may become of great value. Even in the case of elements with useful radioactive species, stable isotopes may find important application in multiple labeling experiments.

There are a few elements for which the less abundant one or more isotopes all constitute appreciable fractions of the whole, *e.g.*, magnesium, bromine ($_{35}$Br), *etc.* A rarer isotope of such an element is limited as a potential tracer substance for normal metabolism because of the limited dilution to which it can be subjected before it is no longer accurately measurable with available techniques. Even with these isotopes, however, important experiments are possible.

There are about 600 known radioactive isotopes (Plutonium Project, 1946; Seaborg, 1944); in fact, one or more unstable forms have been discovered for all known elements. Of these, over 70 have thus far found application in biological and medical work, including species of most of the essential elements. However, the unstable species of certain elements—most importantly, nitrogen, oxygen, and magnesium—can have but limited biological and medical application because of shortness of half-life. √

It may be noted from Table I that several of the lighter elements have, in both stable and radioactive categories, isotopes that have been used in biological research. Of these, the isotopes of hydrogen and carbon in particular offer valuable features in both their stable and unstable forms. For all of the elements known to be essential to life either stable or radioactive isotope tracing has been employed.

3. Isotopes as Tracers

Although the various uses of isotopes as tracers have in common the feature of marking an element, compound, colloid, *etc.*, and thus permitting the material so marked to be followed in the living organism, rather varied theoretical considerations apply to the various types of marking experiments.

(*a*) *As Tracers of Metabolism.* The usefulness of both stable and radioactive isotopes as tracers in metabolic studies is dependent on the fact that all the isotopes of a given element behave in metabolism in practically the same manner—with the notable exception of the hydrogen isotopess—yet can be followed by virtue of their nuclear properties. However, an important distinction exists between the two isotopic classes.

For all elements of biological importance unstable species are known that do not exist in nature in detectable amounts, or, if detectable, are so rare as to be insignificant for all practical purposes. On the other hand all known stable isotopes are found in nature. This means that tracing the normal metabolic fates of an abundant essential element with a radioisotope is not limited by the natural isotopic constitution of the system, whereas tracing with a stable isotope of the element may be in various ways limited or even rendered impossible thereby. For example, if an essential element exists in one stable form only, stable tracing of its normal metabolism is not possible. If more than one form exists, stable tracing is limited by the amount of dilution to which the tracer isotope or isotopic mixture can be subjected in the system before the mass ratios in the resulting mixture of tracer and naturally occurring isotopes approach so closely to the normal ratios for the element that the magnitude of the difference between them approximates the same order as the magnitude of error in the measuring technique. Dilution of rarer stable species can be much greater than of more abundant forms. In other words, for normal metabolic

TABLE I

Important Stable and Radioactive Isotopes That Have Been Used in Basic Medical and Related Investigations‡

*Element	§Isotope		Some Problems in Which Isotope Has Been Used†
	Stable	Radioactive	
*Hydrogen (${}_{1}\text{H}$)	*${}_{1}\text{H}^{1}$		High speed proton irradiation (suggested) (143)
	*${}_{1}\text{H}^{2}$(D)		Intermediary metabolism (74, 118); body water turnover (66–68) and content (68, 100)
		${}_{1}\text{H}^{3}$(T)	Body water content (105); photosynthesis (74)
Lithium (${}_{3}\text{Li}$)	${}_{3}\text{Li}^{6}$ (in natural element)		Slow neutron therapy of experimental tumors (78, 145)
Beryllium (${}_{4}\text{Be}$)		${}_{4}\text{Be}^{7}$	Absorption, distribution, and excretion (55a)
	${}_{4}\text{Be}^{9}$ (natural element)		Fast neutron irradiation and therapy (1, 129)
Boron (${}_{5}\text{B}$)	${}_{5}\text{B}^{10}$ (in natural element)		Slow neutron therapy of experimental tumors (145)
*Carbon (${}_{6}\text{C}$)		${}_{6}\text{C}^{11}$	Intermediary metabolism (74); CO distribution and elimination (137); photosynthesis (74)
	*${}_{6}\text{C}^{13}$		Intermediary metabolism (139)
		${}_{6}\text{C}^{14}$	Photosynthesis (8); other preliminary tracer work (10)
*Nitrogen (${}_{7}\text{N}$)		${}_{7}\text{N}^{13}$	Respiratory gas exchange (71); supposed nitrogen fixation by barley (116)
	*${}_{7}\text{N}^{15}$		Intermediary metabolism (139)
*Oxygen (${}_{8}\text{O}$)	*${}_{8}\text{O}^{18}$		Photosynthesis (117); bacterial fractionation (25)
Fluorine (${}_{9}\text{F}$)		${}_{9}\text{F}^{18}$	Bone and tooth physiology and chemistry (141)

TABLE I—*Continued*

Element	Isotope		Some Problems in Which Isotope Has Been Used
	Stable	Radioactive	
*Sodium ($_{11}$Na)		$_{11}Na^{22}$	Retention in congestive heart disease (111); effect of dietary chloride deficiency (19)
		$_{11}Na^{24}$	Electrolyte metabolism (36, 37, 47, 50, 54, 58, 73, 93, 140); adrenal physiology (3–5); therapy of leukemia (88, 135)
*Magnesium ($_{12}$Mg)		$_{12}Mg^{29}$	Photosynthesis (115a)
*Phosphorus ($_{15}$P)		$_{15}P^{32}$	Mineral metabolism (16, 17, 65); intermediary metabolism (14, 63); labeling of erythrocytes (103); therapy of chronic leukemias, polycythemia vera, etc. (31, 32, 70, 80, 81, 83, 84, 114) and skin lesions (89, 91); diagnosis of malignancy (90, 92); colloids localizing in the reticuloëndothelial system (2, 22, 23, 82)
*Sulfur ($_{16}$S)	*$_{16}S^{34}$		Intermediary metabolism (27)
		$_{16}S^{35}$	Intermediary metabolism (96, 97, 131–134); thiamine metabolism (11); mineral metabolism (28, 126)

Element	Radioactive Isotope	Some Problems in Which Isotope Has Been Used
*Chlorine ($_{17}$Cl)	$_{17}Cl^{38}$	Mineral metabolism (50, 94)
Argon ($_{18}$A)	$_{18}A^{41}$	Respiratory gas exchange; blood circulatory pattern (71)
*Potassium ($_{19}$K)	$_{19}K^{42}$	Mineral metabolism (35, 47, 50, 104) adrenal physiology (3–5)
*Calcium ($_{20}$Ca)	($_{20}Ca^{41}$) $_{20}Ca^{45}$	Mineral metabolism (46)

TABLE I—*Continued*

Element	Radioactive Isotope	Some Problems in Which Isotope Has Been Used
*Manganese ($_{25}$Mn)	$_{25}$Mn^{52} ($_{25}$Mn^{54})	Distribution of colloidal MnO_2 in reticuloëndothelial system (53, 123)
	($_{25}$Mn^{52}) $_{25}$Mn^{54}	Mineral metabolism (48)
	$_{25}$Mn^{56}	Mineral metabolism (10a)
*Iron ($_{26}$Fe)	$_{26}$Fe^{55}	Mineral metabolism (51, 52, 99); blood physiology (40–42) and preservation (38, 39, 115)
	$_{26}$Fe^{59}	
?*Cobalt ($_{27}$Co)	$_{27}$Co^{56} ($_{27}$Co^{57}) $_{27}$Co^{58}	Mineral metabolism (48)
*Copper ($_{29}$Cu)	$_{29}$Cu^{64}	Mineral metabolism (144)
?*Zinc ($_{30}$Zn)	$_{30}$Zn^{63}	Interstitial injection of pectin-protected colloid (102a)
	$_{30}$Zn^{65}	Mineral metabolism (122)
Arsenic ($_{33}$As)	$_{33}$As^{74}	Absorption, distribution, and excretion (26, 69); experimental filariasis (85)
Selenium ($_{34}$Se)	$_{34}$Se^{83}	Absorption, distribution, and excretion (95)
?*Bromine ($_{35}$Br)	$_{35}$Br^{82}	Electrolyte exchange (47, 50); thyroid physiology (107); distribution of brominated dyes (101)
Krypton ($_{36}$Kr)	$_{36}$Kr$^{79, 81}$	Respiratory gas exchange; blood circulatory pattern (71, 136)
Rubidium ($_{37}$Rb)	$_{37}$Rb^{86}	Electrolyte exchange (47)
✓ Strontium ($_{38}$Sr)	$_{38}$Sr^{85}	Absorption, distribution, and excretion (55a)
	$_{38}$Sr^{89}	Bone physiology (46, 55, 106)
	$_{38}$Sr^{90}	Absorption, distribution, and excretion (55)
Yttrium ($_{39}$Y)	$_{39}$Y^{88}	Absorption, distribution, and excretion (55)

TABLE I—*Continued*

Element	Radioactive Isotope	Some Problems in Which Isotope Has Been Used
Yttrium ($_{39}Y$) (con.)	$_{39}Y^{91}$	Absorption, distribution and excretion (55); colloids in reticuloëndothelial system (22, 23)
Zirconium ($_{40}Zr$)	$_{40}Zr^{89}$	Absorption, distribution, and excretion (55)
	$_{40}Zr^{95}$	Absorption, distribution, and excretion (55); colloids localizing in reticuloëndothelial system (22, 23)
Columbium ($_{41}Cb$)	$_{41}Cb^{95}$	Absorption, distribution, and excretion (55); colloids localizing in reticuloëndothelial system (22)
Molybdenum ($_{42}Mo$)	$_{42}Mo^{99}$	Absorption, distribution, and excretion (103a)
Ruthenium ($_{44}Ru$)	$_{44}Ru^{103}$ $_{44}Ru^{106}$	Absorption, distribution, and excretion (55)
Antimony ($_{51}Sb$)	$_{51}Sb^{124}$	Absorption, distribution, and excretion; experimental filariasis (12)
Tellurium ($_{52}Te$)	$_{52}Te^{121}$	Absorption, distribution, and excretion (21)
	$_{52}Te^{127}$ $_{52}Te^{129}$	Absorption, distribution, and excretion (55)
*Iodine ($_{53}I$)	$_{53}I^{128}$	Iodine metabolism and thyroid physiology (86)
	$_{53}I^{130}$	Iodine metabolism and thyroid physiology (61, 86); therapy of thyrotoxicosis (15, 56, 60, 62), and metastatic thyroid carcinoma (120, 121); absorption of insulin (113)
	$_{53}I^{131}$	
Xenon ($_{54}Xe$)	$_{54}Xe^{127}$	Respiratory gas exchange; blood circulatory pattern (71); narcotic effects (83a)
	$_{54}Xe^{133}$	Absorption and distribution (55)
Caesium ($_{55}Cs$)	$_{55}Cs^{134}$	Absorption, distribution, and excretion (55a)

TABLE I—*Continued*

Element	Radioactive Isotope	Some Problems in Which Isotope Has Been Used
Caesium (${}_{55}$Cs) (con.)	${}_{55}Cs^{136}$	Absorption, distribution, and excretion (55)
Barium (${}_{56}$Ba)	${}_{56}Ba^{133}$	Absorption, distribution, and excretion (55a)
	${}_{56}Ba^{140}$	Absorption, distribution, and excretion (55)
Lanthanum (${}_{57}$La)	${}_{57}La^{140}$	Absorption, distribution, and excretion (55)
Cerium (${}_{58}$Ce)	${}_{58}Ce^{141}$	Absorption, distribution, and excretion (55)
	${}_{58}Ce^{144}$	Absorption, distribution, and excretion (55)
Praseodymium (${}_{59}$Pr)	${}_{59}Pr^{143}$	Absorption, distribution, and excretion (55)
Element 61	61^{147}	Absorption, distribution, and excretion (55)
Gold (${}_{79}$Au)	${}_{79}Au^{198}$	Absorption, distribution, and excretion (30); chrysotherapy in arthritis (9); colloidal therapy (51a, 122a)
Mercury (${}_{80}$Hg)	${}_{80}Hg^{197}$	Mercury vapor as an industrial health hazard (43)
Lead (${}_{82}$Pb)	${}_{82}Pb^{210}$(RaD)	Absorption, distribution, and excretion (18, 102)
	${}_{82}Pb^{212}$ (ThB)	Absorption, distribution, and excretion (21a).
Bismuth (${}_{83}$Bi)	${}_{83}Bi^{210}$(RaE)	Absorption, distribution, and excretion (77)
✓ Astatine (${}_{85}$At)	${}_{85}At^{211}$	Thyroid physiology (57)
Radon (${}_{86}$Rn)	${}_{86}Rn^{220}$(Tn)	Elimination in breath (127)
	${}_{86}Rn^{222}$	Numerous studies on uptake and elimination in breath, distribution, etc.; treatment of various malignancies and dermatoses

TABLE I—*Concluded*

Element	Radioactive Isotope	Some Problems in Which Isotope Has Been Used
Radium ($_{88}Ra$)	$_{88}Ra^{226}$	Numerous studies on distribution, toxicity, etc.; treatment of various malignancies and dermatoses
	$_{88}Ra^{228}(MsTh_1)$	
Thorium ($_{90}Th$)	$_{90}Th^{228}(RdTh)$	Absorption, distribution, and excretion (55)
	$_{90}Th^{232}$ (natural element)	Numerous studies on distribution, toxicity, etc.
	$_{90}Th^{234}(UX_1)$	Absorption, distribution, and excretion (55)
Protoactinium ($_{91}Pa$)	$_{91}Pa^{233}$	Absorption, distribution, and excretion (55)
Uranium ($_{92}U$)	Natural element	Numerous studies on distribution, toxicity, etc.
	$_{92}U^{235}(AcU)$	Production of slow neutrons (125); effect of fission from colloids localizing in reticuloëndothelial system (136)
Neptunium ($_{93}Np$)	$_{93}Np^{239}$	Absorption, distribution, and excretion (55)
Plutonium ($_{94}Pu$)	$_{94}Pu^{239}$	Absorption, distribution, and excretion (55)
Americium ($_{95}Am$)	$_{95}Am^{241}$	Absorption, distribution, and excretion (55)
Curium ($_{96}Cm$)	$_{96}Cm^{242}$	Absorption, distribution, and excretion (55)

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‡ See also the book by Govaerts (1945) on the use of stable isotopes and the recent book by Kamen (1947) on the use of radioisotopes in biology.

* Elements known or believed to be essential in mammalian nutrition based on the book by Shohl (1939); those for which unanimity is lacking are also marked with a question mark (?).

§ An isotope is placed in parentheses if it occurs together with another isotope (of the same element) used for tracer work, but is itself not significant for the tracer purposes to which the mixture may be applied. Two or more isotopes are connected by a brace if they occur together in tracer preparations and are of about equal importance.

† References are here cited by number.

studies the accuracy of radioactive tracing is limited by the sensitivity of apparatus for detecting absolute numbers of radioactive disintegrations whereas the accuracy of stable isotope tracing is limited by the sensitivity of apparatus for measuring differences in the relative concentrations of isotopes. In general, the former techniques have a higher order of sensitivity.

In studies on the major elements comprising protoplasm, the use of stable tracers has a theoretical advantage over the use of radioisotopes. The former may be used in relatively much greater concentrations than the latter since (with the exception of deuterium as compared with ordinary hydrogen) the differences in metabolic behavior between isotopes by reason of their nuclear mass differences are negligible for most purposes, whereas the radiation effects of nuclear disintegrations place definite limits on the concentrations of an unstable tracer that can be used for studying normal metabolism. For example, it is theoretically possible to grow plants, and possibly animals as well, in such a manner that one of their constituent elements is represented almost entirely by a single one of its stable isotopes. The growing of such organisms will make possible the biosynthesis of complex labeled substances such as proteins, which then can be used in tracer experiments. For the main elements of protoplasm and body fluids—namely, the organic elements (${}_{1}H$, ${}_{6}C$, ${}_{7}N$, ${}_{8}O$, ${}_{15}P$, and ${}_{16}S$) and the mineral elements (${}_{11}Na$, ${}_{12}Mg$, ${}_{17}Cl$, ${}_{19}K$, ${}_{20}Ca$)—normal metabolism has been extensively studied by isotopic tracing except in the case of ${}_{8}O$, with which little work has been done, and ${}_{12}Mg$, for which only one article has been published (Ruben, Frenkel and Kamen, 1942).

In tracer studies on the normal metabolic role of elements and compounds, both essential and non-essential, that occur in trace amounts in a living system the greater sensitivity of the radioisotopic techniques makes the application of unstable isotopes preferable, where possible. In the case of many trace elements, unstable tracing is necessary because only radioactive measuring techniques offer sufficient sensitivity for following the movement of the trace amounts normally present.

It has been possible to make intensive studies of the metabolism of two important trace elements—iron (${}_{26}Fe$) and iodine (${}_{53}I$)—with the use of radioisotopes. Preliminary work has also been done with the rarer trace elements known (${}_{25}Mn$ and ${}_{29}Cu$), or thought on strong evidence (${}_{27}Co$, ${}_{30}Zn$, and possibly ${}_{35}Br$), to be essential to mammalian life. Even where rarer stable species exist—as with all essential trace elements except ${}_{25}Mn$, ${}_{27}Co$, and ${}_{53}I$,—it is possible that these will not find important uses for some time to come.

Radioisotopic tracer studies have also been carried out with a number of other elements known to be normally present in the body in minute amounts but not proved to be essential—in fact thought of, at least in most cases, merely as contaminants. It is possible to measure accurately such tiny amounts of these in their radioactive form that in some experiments they can probably be regarded as being studied within normal limits of abundance. Under such conditions it may be said that their "normal" metabolism is being investigated. For such studies a largely "carrier-free" radioisotope is necessary—*i.e.*, an unstable species accompanied by few or no stable atoms of the same element.

Pharmacological studies and toxicological studies with many substances abnormal to the body in form or amount can be carried out by techniques either of stable or radioactive isotope tracing, depending upon the element to which the labeling atoms belong.

In studying the metabolism of trace substances there exists an insensible intergradation between normal processes and manifestly abnormal toxicological ones that obtain when the amount of a trace element or compound is increased to a level at which its metabolic fate is markedly changed from normal. In cases of the latter extreme it may be possible to trace the fate of the element or compound with its natural isotope or isotopes in their normal ratios. In other words absolute boundaries cannot be drawn between isotopic tracing and simple distribution studies of an element or its compounds measurable by many different chemical and physical means.

It is, however, desirable to think of isotopic tracing as a group of techniques in which minute quantities of either a stable or an unstable species are used to study metabolic and other physiological processes. Thus, even though these techniques intergrade into other types, it is nevertheless possible to recognize, for example, the application of radiophosphorus—${}_{15}P^{32}$—to the study of phospholipid metabolism, and of deuterium to the study of intermediary fat metabolism, as typical examples of the isotopic tracing method. These considerations apply to the study both of normal metabolic processes and of the metabolic fate of therapeutic or toxic compounds. Minute quantities of tracer substances can be used to follow their distribution and, even though other methods of marking such compounds exist, some of which intergrade with the typical isotopic techniques, the criterion of relatively minute quantities is a generally sound one. It should be remarked, however, that because most stable isotopic ratios can be greatly altered without changing in any appreciable manner the behavior of a metabolic system this criterion may break down in unusual cases of stable isotope tracing.

(*b*) *As Tracers of Non-Metabolic Processes.* Inasmuch as the term metabolism designates chemical processes of living organisms, it cannot be applied inclusively to the fate of chemically inert substances, which may include dissolved gases, colloids, particles of greater than colloidal dimensions, cells, and even foreign organisms. Past work has amply demonstrated that radioactive isotopes are particularly well suited for the labeling of such substances and structures, and thus for the following of such non-metabolic physiological processes as they may undergo in the body.

Labeled substances and cells have been extensively employed not only in distribution studies *per se*, but also as agents in the study of physiological processes that are involved in such distribution. Important examples in the latter category are the studies of Jones (in press) and Dobson *et al.* (1947). They have used radioactively labeled inert gases to evolve laws of reapiratory exchange and blood tissue perfusion rates and applied radioactively labeled colloids to the measurement of circulation and the determination of blood volume.

Most labeled substances of this nature are, however, only relatively and not absolutely inert. Colloids and other particulate substances may slowly dissolve, their constituent element or elements, including tracers, entering the general metabolic pool. Cells of both intrinsic and extrinsic origin, and also foreign organisms, undergo metabolic exchange with the body and, as a rule, ultimately break down, releasing their constituents. For the most part, it is thus not possible to distinguish in a definitive manner non-metabolic from metabolic processes since the latter are often inseparably connected with the former.

4. The Behavior and Effects of Isotopes

Although the greatest value of isotopes probably lies in their tracer application, the importance of their differential behavior and effects in living organisms must not be overlooked.

It has already been stated that for most practical purposes all isotopes of a given element except hydrogen behave in essentially the same manner in metabolism. However, evidence is accruing that under natural conditions there is a small difference in the use of different isotopes, at least by certain organisms. Thus ${}_8O^{16}$ has been shown by Dole, Hawkings and Barker (1947) to be used slightly more rapidly than ${}_8O^{18}$ by certain bacteria. Metabolic studies of the future may well be directed toward detecting similar differences in human metabolism. These differences do not, however, effectively limit tracer studies of normal metabolism, because such differential effects are well within the range of biological error for present day experiments.

The difference in the behavior of isotopes may be approached from the converse standpoint, namely, the effect of abnormal isotopic concentrations on biological systems. It has already been pointed out that "heavy water" in large concentrations behaves as a poison (Lewis, 1933). This has been demonstrated on organisms from simple cells to mice. Similar studies are yet to be made with other elements.

By far the most important effect of isotopes *per se* is that of the radiations accompanying radioactive species. Unstable nuclei give off ionizing radiation at the time of their decay; and the ionization in the tissue, if sufficiently intense, will cause certain irreversible changes in protoplasm. The subject of the biological effect of radiation is one of great importance—from the fundamental standpoint because there are many interesting effects to be studied and particularly because the mechanism by which mutations occur and hence evolution is effected may have an intimate connection with ionizing radiations in nature, and from the applied standpoint because ionizing radiation has been known for about 50 years to have therapeutic value in certain human diseases. In the application of radioisotopes as tracers far too little attention has been paid to the possibility that the metabolic process studied may be influenced by the radiations emitted (Dobson and Lawrence, in press).

Ionization produced by the emanations of radioisotopes is similar to the ionization produced by X- and gamma-rays from extrinsic sources and by accelerated nuclear particles. However, in one respect radioisotopes have a unique application, namely, in that they can take part in metabolic processes and therefore be distributed and localized in the body in various ways, depending upon the fate of chemical substances in which they are incorporated before administration or become incorporated thereafter.

With radioactively labeled substances that localize to a greater or lesser degree in given parts or tissues of the body it has been possible to effect selective irradiation of those parts or tissues. The rationale of radioisotopic therapy is based on such selective localization. With radiophosphorus and radioiodine it has been possible to treat leukemia and thyrotoxicosis with a certain measure of success because, as discussed more thoroughly in Section III, phosphorus is taken up in greater concentrations in rapidly growing tissues and in bone than in more slowly metabolizing and non-osseous tissues, and because iodine is concentrated under natural conditions at least several hundred times in a unit volume of thyroid gland over a unit volume of any other tissue of the body.

5. *Problems in Selection of Isotopes*

In determining what isotope or isotopes are appropriate for a projected experiment a number of considerations enter into the choice. First, of course, it must be decided whether a suitable isotope or isotopes exist theroretically. Then the actual use of these depends upon absolute and relative problems in production, application, and measurement.

(*a*) *Production.* The preparation from a mixture of stable isotopes of a single species in pure form or of a mixture enriched with a rarer species differs basically from the preparation of a radioisotopic species. In the case of stable isotopes it is necessary to separate atoms that for almost all practical purposes are chemically identical, but vary among themselves by existing in two or more different states of nuclear mass. Except with the first few elements of the periodic system these differences constitute a very small proportion of the masses of individual nuclei. In the case of radioisotopic species, however, many are made by transmutative processes and therefore can be separated in physically pure form (except for possible, and usually small, contamination with other isotopes of the same element), or need at most only a minute amount of added stable carrier for complete separation from the parent isotope. Even when one has a mixture of stable and radioactive isotopes of a desired element—such as results from neutron capture,—there need not necessarily be a problem in application, for the specific activity—in other words, the radioactivity per unit weight of the element (which, of course, is in turn a function of the ratio of radioactive to stable atoms)—can be small and still permit accurate tracing.

The various methods of separating the stable isotopes of an element are treated in the Smyth report (1945). These methods all depend on the physical and chemical differences resulting from the different masses of the isotopes. As one ascends the periodic system, the ratios of differences in mass to total mass naturally become increasingly small. The nucleus of heavy hydrogen or deuterium (${}_1H^2$) is almost exactly twice as heavy as that of normal hydrogen (${}_1H^1$), whereas the difference between the two most abundant uranium isotopes (${}_{92}U^{238}$ and ${}_{92}U^{235}$) is only $\frac{3}{238}$ of the heavier isotope.

Most methods of separation take advantage of the small statistical differences in physical and chemical behavior of molecules identical except for the nuclear masses of the component atoms. In general, the chemical differences between isotopes are exceedingly small by comparison with those between two different elements. In all such processes the separation factor is small, and many stages are required. In the Smyth Report (1945) the principal statistical separation methods given are six in number: (1) gaseous diffusion, (2) distillation, (3) centrifugation, (4) thermal diffusion, (5) exchange reactions, and (6) electrolysis. Of these, (2), (5), and (6) are better for elements of low atomic weight, and (1), (3), and (4) for those of high atomic weight.

Another group of separation methods, however, does not take advantage of the statistical effect. These are the electromagnetic techniques, which are made possible by the relative differences in the paths of ions traveling in a magnetic field due to variations in nuclear mass. The magnitude of the effect of a field on the path of heavier ions is less than that on the path of lighter ions, and this fact has been exploited in a number of techniques for the electromagnetic separation of isotopes. In fact the University of California Radiation Laboratory group working with the calutron made the first usable amounts of ${}_{92}U^{235}$ by this method.

A problem in stable isotope preparation lies in the considerable expense involved in all methods of separation. However, mass production techniques have already made possible costs within the means of scientific institutions for "heavy" isotopes of certain important elements—*e.g.*, ${}_{1}H^{2}$, ${}_{6}C^{13}$, and ${}_{7}N^{15}$.

The production and processing of radioactive isotopes is discussed in this volume in the chapter by Cohn. A few words are, nevertheless, appropriate here.

The problems involved in the preparation of radioisotopes are more varied than in the preparation of stable species because in the latter case one is dealing with isotopes already existing in nature, whereas in the former one must, for the majority of elements, first create an isotope before considering the matter of its separation. Exceptions are, of course: (1) the naturally occurring radioisotopes of the elements in the uranium, actinouranium, and thorium series, of which thallium (${}_{81}Tl$), lead (${}_{82}Pb$), and bismuth (${}_{83}Bi$) also have stable species; and (2) certain isotopes of at least four other elements—${}_{19}K^{41}$, ${}_{37}Rb^{87}$, ${}_{62}Sm^{148}$, and ${}_{72}Lu^{176}$—for which both stable and radioisotopes exist in nature.

Radioactive isotopes are manufactured at the present time in either the cyclotron or the uranium pile. For many species the pile is a cheap and abundant source. However, others cannot be produced by pile reactions and must be made by the more versatile cyclotron. ✓

Problems in production of certain radioisotopes may operate to render a theoretically useful species difficult or impractical of application. There are three principal types of such problems. One difficulty is related to the proportion of a given target that consists of an isotope or isotopes appropriate for the production of a desired radioactive species. If, for example, the target isotope for a desired reaction is a rare stable species of its element, then the yield of the desired product is usually low when the normal element is used as a target. One possible way of correcting this is to separate out or concentrate the rare species by stable isotope techniques. However, in most instances under present conditions this would make the cost of the ultimate product prohibitive for general purposes. A second difficulty is related to the ease with which the desired nuclear reaction takes place under available conditions of bombardment. If, for example, the reaction is very inefficient, then significant yields of the desired product can only be obtained by prolonged bombardment. Certain important isotopes of such elements as iron—${}_{26}Fe^{55}$, ${}_{26}Fe^{59}$—and calcium—${}_{20}Ca^{45}$—have relatively inefficient yields, whereas others, of such elements as sodium—${}_{11}Na^{24}$—and chlorine—${}_{17}Cl^{38}$—have very efficient yields under the appropriate conditions of bombardment. A third difficulty lies in preparing targets for certain desired nuclear reactions; there may be practical difficulties of either a chemical or a physical nature that, in turn, limit the yield possibilities.

The relative ease of preparation under existing conditions of radioisotopes as compared with the separation of stable species of the same element is one of the principal barriers to an extension in the application of stable isotopes to biological tracer work. As already noted, however, certain stable species have been produced in quantity. Two of these—${}_6C^{13}$ and ${}_{16}S^{34}$—have been able in the past to compete with radioisotopes—${}_6C^{14}$ and ${}_{16}S^{35}$. Now, however, that ${}_6C^{14}$ and ${}_{16}S^{35}$ can be produced in quantity in the uranium pile, they will find much wider use.

(*b*) *Application.* Once a desired isotope has been produced, it is necessary to prepare it in a form appropriate for the experiment.

The separated stable isotopes may be used as such or changed into a new chemical form, as experimental requirements direct. The experiment must be planned so that measurement, as discussed in the next subsection, can be carried out with the mass spectrometer, or in the case of deuterium and heavy oxygen by certain additional techniques.

However, with radioactive species it is usually first necessary to separate them by chemical processes, as described in Chapter 3 by Cohn, from the elements originally included in the target and from such isotopes of other elements than that desired as may be formed in side reactions. In the case of non-transmutative reactions (n, γ; d, p; *etc.*) the new isotopes cannot ordinarily be obtained free of non-radioactive species of their elements unless they are separated by the techniques applied to stable isotopes. So far, this last procedure has not proved practical and for many purposes it is not even theoretically desirable as a radioactive species can often be satisfactorily employed even when one or more non-radioactive isotopes of the same element constitute most of the preparation. However, for some purposes "carrier-free" species are very valuable, even essential. As already noted, this is most important in the study of essential trace element metabolism. For the production of such isotopes, transmutative reactions (n, p; p, n; d, n; *etc.*) are necessary.

✓ The most important factor in the application of radioisotopes is the half-life. Certain species are very short-lived and can only be used for short-term experiments; radioactive oxygen and nitrogen isotopes are severely limited for this reason. Other species combine long half-lives with metabolic fates that lead to their deposition for long periods in bone and thus limit their application, particularly in humans, wherein there is danger of chronic radiation effects; many of the fission products are in this category. However, species of convenient half-life for biological purposes exist for the majority of elements.

${}_7N^{15}$ and ${}_8O^{18}$, the heavy stable species of oxygen and nitrogen, are successful competitors of the radioactive isotopes of these elements because of the half-life limitations of the latter. In the case of nitrogen this is true despite the fact that excellent cyclotron yields of ${}_7N^{13}$ (of 10.2 minutes' half-life) are available. For similar reasons the rarer stable isotopes of magnesium will probably prove ultimately to have much wider applicability than the short-lived radioactive species.

Another important problem in the experimental application of radioisotopes is the protection of personnel from over-exposure to radiation either by external contact or by the assimilation of radioactive materials. Health protection features are discussed at length in Chapter 5 by Parker.

(c) *Measurement.* Highly successful procedures for measuring both stable and radioactive isotopes have been developed in the past four decades. For the two classes rather different principles are involved.

The measurement of stable isotopes is based upon the techniques of *mass spectrum analysis*, which are reviewed in a recent book by Aston (1942). In the *mass spectrometer* it is possible to accelerate ionized atoms and molecules electrically and then subject them to electrical and magnetic fields in such a way as to focus ions with a given ratio of mass to charge at the same point on the collecting plate of the instrument. In this way these ions are measurable in groups having the same ratio of mass to charge. Almost all stable isotopes measurements are carried out with the mass spectrometer. Only for deuterium and heavy oxygen are additional methods of stable isotope analysis in use (Keston *et al.*, 1937).

Measurement of radioisotopes is discussed at length in Chapter 4 by Evans.

In dealing with problems in which humans are the subjects, an important consideration in the application of radioisotopes is the *safe tracer dose.* As yet there is no conclusive evidence as to what constitutes the maximum allowable radiation either for the body as a whole or for given organs and regions of the body. It has been commonly assumed, however, for practical purposes that 0.1 r.e.p. (roentgen equivalent physical) is the maximum allowable dosage for total body irradiation. On this basis, it is possible to define arbitrarily the safe tracer dose as the amount of a given radioisotope that can be administered without conferring more than 0.1 r.e.p. per day in any part of the body. For example, in administering radioiodine this dose would be that amount radiating the thyroid at a level not exceeding 0.1 r.e.p. during the first day. Doctor C. A. Tobias has derived such values for a number of biologically important isotopes administered by intravenous injection, and his calculations are reproduced in Table II. For simplicity he has assumed that no excretion occurs. Morgan (1947) recently published much more elaborate data for calculating radiation from isotopes administered by various routes. Where the half-life of an isotope is quite short (*e.g.*, $_{11}\text{Na}^{24}$) a higher dose than 0.1 r.e.p. might be permissible. On the other hand, where an isotope has a long half-life, and is retained for long periods in the body, it may be that a smaller amount should be given. In this connection there is much yet to be learned about the chronic effects of radiation from unstable isotopes.

TABLE II

Constants of Medically Important Radioactive Isotopes after a Single Intravenous Administration

DEFINITIONS: 1 mc = 3.7×10^7 disintegrations/sec; 1 roentgen equivalent physical ≈ 83 ± 1 ergs/g. = 5.25×10^{13} ev/g. ≈ 1.92×10^{-6} g.-cal./g.

Isotope	Radiation	Half Life T	g. of active material/millicurie	Dose in roentgens due to 1 mc./g. tissue/day	Amount conferring 0.1 r.e.p./day in any part of body*	Usual tracer sample	E_{max} of β-rays in kev
${}_1H^3$	β⁻ only	31 years	2.6×10^{-7}	3.0×10^2	16 mc	1.5 mc	15
${}_6C^{14}$	β⁻ only	5000 ± 1000 years	1.9×10^{-4}	4.7×10^3	1.5 mc	0.1 mc	154
${}_{11}Na^{24}$	β⁻, γ	14.8 hours	1.13×10^{-10}	0.96×10^4	187 μc	50 μc	β⁻ 1400 γ 1300 γ 2780 in coincidence
${}_{15}P^{32}$	β⁻ only	14.3 days	3.40×10^{-9}	4.14×10^4	sol. phosphate 168 μc colloid. phosphate 20 μc	25 μc	1710
${}_{26}Fe^{59}$	β⁻, γ	47 days	2.14×10^{-8}	7.36×10^3	75 μc	5–30 μc	1 {β⁻ 460, γ 1100} 2 {β⁻ 260, γ 1300}
${}_{26}Fe^{55}$	K, e⁻	5 years	7.7×10^{-7}	3.6×10^2	1.5 mc	50–300 μc	K X-ray 5.9
${}_{53}I^{131}$	β⁻, γ	8 days	7.66×10^{-7}	1.25×10^4	0.2 μc	0.2 μc	β⁻ 595 γ 367 γ 80

* Calculations based on 70 kg. man; no excretion is assumed. ${}_1H^3$, ${}_6C^{14}$, ${}_{11}Na^{24}$, and ${}_{15}P^{32}$ are assumed to be evenly distributed in body (except colloidal phosphate, which is concentrated in liver and spleen); ${}_{26}Fe$ isotopes, in total blood volume; and ${}_{53}I^{131}$, in thyroid.

Three general categories of measurement are available for use with radioisotopes. These are the *in vitro*, *in vivo*, and autoradiographic methods.

With the *in vitro* method, samples are removed from the experimental subject and measured quantitatively by techniques appropriate to the isotope.

In the case of the *in vivo* method, however, the experimental subject is submitted to direct measurement. A Geiger-Müller counter may be applied to a tissue, organ, structure, or entire organism, and with a precise knowledge of the geometry of the counting procedure quantitative measurements may often be obtained. This technique is particularly applicable to human subjects. Furthermore, it has great practical advantage over the *in vitro* technique in that repeated measurements can be made on the same organism, organ, or tissue, and individual curves obtained. Thus a single experimental subject may provide values for which many subjects would be needed with the use of *in vitro* methods. Recently a small mica-window counter has been described by Strajman (1946). His instrument should prove of great practical utility, as it is of a size that may permit its incorporation into many clinical instruments, such as bronchoscopes, rectoscopes, *etc.* ✓

Finally the autoradiographic technique has shown itself to be very useful in largely qualitative measurements of isotopic distribution (Gross and Leblond, 1946). By approximating a section of the tissue, organ, or structure to be measured to the surface of a radiosensitive emulsion, it is possible to obtain a photographic "imprint" of the section, patterned according to the relative concentrations of the isotope therein. A recent technique has been described by Evans (1947), whereby tissue slices can be permanently mounted directly on photographic emulsions, thus permitting the simultaneous observation of tissue and its autoradiograph.✓

Of these three methods by far the most important for purposes of basic reasearch is the *in vitro* method. However, both *in vivo* and autoradiographic techniques have had, and will continue to have, important roles in experimentation. In general, the *in vivo* technique is the best adapted to experimentation and diagnostic procedures with humans since it permits measurements on the intact subject.

III. CLINICAL APPLICATIONS OF ISOTOPES

1. General Considerations

Radium and radon, and their decay products, have been used for over 45 years in the treatment of various malignancies and dermatoses. These applications are familiar to the medical profession as a whole and need not be detailed here. They are reviewed in such books as that of Ellinger (1941), and others dealing with roentgen and radium therapy.

As indicated in Table I artificial isotopes of two elements, ${}_{15}P$ and ${}_{53}I$, have thus far been employed in therapy with definite success. Others—${}_{11}Na$, ${}_{25}Mn$, ${}_{30}Zn$, ${}_{38}Sr$, ${}_{39}Y$, ${}_{40}Zr$, ${}_{41}Cb$, and ${}_{79}Au$—have received preliminary trial, but therapeutic techniques with these elements are still in the experimental stage.

A number of isotopes have also been employed for diagnostic purposes. These are discussed in the following subsections.

The isotopes that so far have shown definite value in therapy and diagnosis have been those that demonstrate a fair degree of selective localization, either as the result of their normal fate in metabolism or of their fate as special compounds or colloids. Thus phosphorus concentrates to a small degree in rapidly growing tissue (particularly in the nucleoprotein) and in bone; iodine localizes in the thyroid gland (at least several hundred times the concentration in the rest of the body); and certain colloidal preparations can be made that localize in various parts of the reticuloëndothelium.

So far, all techniques of radiation with isotopes, except in the case of the treatment of hyperthyroidism with radioiodine, have suffered from the limited selectivity of uptake by the diseased tissue over normal tissues. For example, in the treatment of diseases of the bone marrow and lymphatic system with radioactive phosphorus, it is impossible to avoid to a greater or lesser extent the irradiation in various parts of the body of those normal cells that metabolize rapidly and others in the immediate vicinity of the latter. Actually, in the treatment of polycythemia vera it would be desirable if irradiation could be delivered only to the nucleated red cells and in leukemia and lymphosarcoma only to the offending leukocytes or lymphosarcoma cells, and not to the normal cells, especially the platelet- and red cell-producing centers. Some additional progress may be anticipated in the management of these blood dyscrasias by the application of labeled colloids. The work of Jones, Wrobel and Lyons (1944), and the more recent work of Dobson *et al.* (1947), shows that radiation may be limited largely to the liver and spleen by the use of anhydrous colloidal chromic phosphate labeled with ${}_{15}P^{32}$, which localizes selectively in the reticuloëndothelial cells of the liver and spleen when introduced intravascularly in a highly dispersed form. Similar properties have been found by Dobson *et al.* (in press) for a number of other colloids—of yttrium (${}_{39}Y$), zirconium (${}_{40}Zr$), and columbium (${}_{41}Cb$)—which may be labeled with radioisotopes of these respective elements. Some of the colloids can be concentrated to a high degree in the bone marrow, others in the liver and spleen.

However, for neoplastic diseases in general, localizing substances of adequate selectivity that can be labeled and used for radiotherapy have not been discovered. If such are found, it would be desirable that they carry a radioactive isotope which emits a very soft ray, such as $_1H^3$ or $_6C^{14}$. Even these emit beta-rays that traverse several cell diameters; however, the half penetration of the $_1H^3$ beta-ray is less than the radius of the average cell.

2. Radiophosphorus ($_{15}P^{32}$) in Therapy and Diagnosis

The first radioisotope to be used in therapy was $_{15}P^{32}$. Although applied as early as 1936 it was not until 1939 that Lawrence, Scott and Tuttle (1939), who employed it in the study of chronic leukemia, recommended its clinical use. Lawrence (1940) gave the first clinical summaries. A number of studies followed, which, for the period through 1945, have been quite thoroughly reviewed in a recent paper by Reinhard *et al.* (1946). In the past two years several important papers have appeared on $_{15}P^{32}$ therapy. Aside from the paper by Reinhard *et al.*, in which the results of treating a large series of patients suffering from leukemias, lymphoblastomata, and other malignant conditions were reported, there have been papers by Erf (1946a, b) attesting the usefulness of P^{32} in the treatment of polycythemia, of Jacobson *et al.* (1947) comparing the use of radiophosphorus and the alkylamines (nitrogen mustards) in the treatment of neoplastic and allied diseases of the hematopoietic system, of Low-Beer (1946a, b) on the external application of $_{15}P^{32}$ to warts, basal cell carcinomata, *etc.*, and of Lawrence (1947, in press) and of Lawrence *et al.* (1948) reporting the results of 11 years' experience with the largest reported series of patients with leukemia and polycythemia.

$_{15}P^{32}$ in soluble form (in labeled isotonic Na_2HPO_4) given by mouth or vein is used in the treatment of blood dyscrasias. The determination and calculation of dosage, routes of administration, and supportive treatment of patients receiving this type of therapy are discussed in most of the works cited.

The general findings of workers using soluble $_{15}P^{32}$ therapy have been as follows:

1) It is at the present time the treatment of choice for many cases of polycythemia (rubra) vera (Erf, 1946a, b; Lawrence, 1947, in press; Reinhard *et al.*, 1946) (*i.e.*, primary polycythemia, erythremia, or Osler-Vaquez's disease). In most patients complete hematological remission and almost complete symptomatic relief can be effected. A single course of treatment may bring about a remission lasting from six months to several years.
2) For the treatment of chronic leukemia, both lymphatic and myelogenous, it is at least as satisfactory in producing hematological and symptomatic remissions as X-ray (Lawrence, 1947, in press; Lawrence *et al.*, 1948; Reinhard *et al.*, 1946) and may prolong life somewhat longer (Lawrence, 1947, in press; Lawrence *et al.*, 1948). It is thus

of *palliative*, and not curative value. There is another definite advantage of $_{15}P^{32}$ over X-ray, namely, that the former practically never produces radiation sickness; in the few cases reported this has been very mild.

3) $_{15}P^{32}$ therapy has little effect on the course of patients with acute or subacute lymphatic, myelogenous, and monocytic leukemia (Reinhard *et al.*, 1946). It is possibly of the same limited value as X-radiation.

In none of these serious diseases has there been observed later the induction of other tumors as a result of the radiation therapy.

Complications of soluble $_{15}P^{32}$ therapy are leukopenia, thrombocytopenia, and anemia, and these constitute limiting factors in the dosage. In a few cases X-radiation has value in conjunction with $_{15}P^{32}$ therapy in reducing more rapidly the size of lymph nodes in lymphatic leukemia and in combating splenomegaly, particularly in myeloid leukemia. It has been common experience among workers concerned that therapy with $_{15}P^{32}$ requires a considerable degree of individualization. The limitations of $_{15}P^{32}$ in the form of the soluble phosphate for blood diseases are brought out in a recent article by Graff, Scott and Lawrence (1946).

$_{15}P^{32}$ in the treatment of skin lesions has been described by Low-Beer (1946a; 1947). He soaks a small piece of blotting paper in a labeled phosphate solution and applies this to the surface of the tumor or other skin lesion. The beta-rays from $_{15}P^{32}$ have a "half-penetration" of about 1 mm. in tissue, *i.e.*, ½ of the particles are absorbed in the first millimeter of tissue, ½ of the remaining in the second millimeter, and so on. This suggests $_{15}P^{32}$ may prove superior to X-ray or radium in the treatment of certain radiosensitive superficial skin lesions by avoiding irradiation of the deeper, uninvolved tissues.

$_{15}P^{32}$ has also been used for experimental therapeutic purposes, in an insoluble colloid, chromic phosphate. Exploratory work during the past few years on the therapy of the splenomegaly and hepatomegaly of chronic leukemia and other diseases with this material has been carried on (Lawrence *et al.*, unpublished information). Another possible application of labeled chromic phosphate and colloids with similar properties is their use in the interstitial infiltration of chemically inert radioactive material into malignant growths. This has been suggested by the work of Allen, Hempelmann and Novack (1945), on the injection of chromic phosphate into the tissues around transplanted mouse tumors. They showed that chromic phosphate remains at the point of injection and that the radioactive material can cause regression of mammary tumors under a certain size. Clinically this technique has recently been used for the injection of enlarged superficial lymph nodes in leukemia (Lawrence *et al.*, unpublished information). It is possible that a similar procedure may prove of some value in the treatment of malignancies in inoperable sites.

The use of ${}_{15}P^{32}$ as a possible diagnostic tool has been suggested by Low-Beer (1947) and Low-Beer *et al.* (1946). They have found in a few cases that, if small doses of labeled Na_2HPO_4 are given to women with suspected breast carcinoma, the activity of the superficially located, malignant lesion (except for slowly growing mucoid carcinomata) is 25% or more above that of the surrounding tissue of the skin. Benign lesions in their series showed less than 25% greater activity. For such work the Strajman counter (1946) should have considerable value, particularly if localizing compounds emitting gamma-rays can be found.

The use of ${}_{15}P^{32}$ has thus been shown to have considerable therapeutic value. Further research may well expand its clinical usefulness in the form of other inorganic or organic compounds, both in therapy and diagnosis.

3. *Radioiodine* (${}_{53}I^{130}$ *and* ${}_{53}I^{131}$) *in Therapy and Diagnosis*

The therapeutic use of radioactive iodine was reported upon for the first time simultaneously in 1942 by Hertz and Roberts (1942a) and Hamilton and Lawrence (1942) for the treatment of hyperthyroidism (*i.e.*, thyrotoxicosis, Grave's disease, Basedow's disease, *etc.*). Comprehensive reports on two series of patients with hyperthyroidism appeared in 1946, one by the former authors (1946) and the other by Chapman and Evans (1946).

For therapeutic purposes carrier-free radioiodine in the form of an aqueous NaI solution is used, being administered by mouth. In the two most recent papers (Chapman and Evans, 1946; Hertz and Roberts, 1946), the iodine has consisted of a mixture of ${}_{53}I^{130}$ and ${}_{53}I^{131}$, of which the former has been the clinically more important component. The determination and calculation of dosage are given both by Hertz and Roberts and by Chapman and Evans.

Hertz and Roberts (1946) have found that about 80% of their patients having thyroids of 60–75 g. in size, and treated with an appropriate dose of radioiodine have been effectively cured. Chapman and Evans (1946) have also reported encouraging results. A complication of this form of therapy has been myxedema, resulting from an excessive depression of thyroid function, but as yet there have been no other post-irradiation effects such as the induction of carcinoma. The ultimate evaluation of this form of therapy, as compared with other methods must await more extensive work and the passage of time.

In the last few years workers have also reported a few cases in which treatment of iodine-metabolizing thyroid malignancy has met with partial or considerable success when radioiodine has been used. Seidlin *et al.* (1945; 1946) have recently reported in detail on a case of metastatic thyroid adenocarcinoma in which metastases took up iodine. This patient responded favorably to radioiodine therapy administered on three occasions—in

March, 1943, April, 1944, and March, 1945. It would, therefore, appear that such treatment can be of value for those thyroid malignancies in which iodine is concentrated.

As a diagnostic tool for the determination of the level of the thyroid function, radioiodine appears to offer good clinical possibilities. So far, most of the publications dealing with thyroid physiology as studied with radioiodine have been in the nature of fundamental investigations rather than of diagnostic developments. Hertz and Roberts (1942b), however, have shown that radioiodine can be used in distinguishing between simple hyperthyroidism and the ophthalmopathic type.

In general the use of radioiodine for the treatment and diagnosis of certain thyroid diseases appears highly promising.

4. Other Isotopes in Therapy and Diagnosis

The use of isotopes of sodium and strontium in therapy has been reported by various workers in the last few years. In addition, recent work with phosphate, manganese, zinc, yttrium, zirconium, columbium, and gold colloids shows considerable promise.

Radiosodium ($_{11}Na^{24}$) as labeled NaCl has been applied to the treatment of chronic leukemia by Thygesen, Videback and Villaume (1944) and by Lindgren (1944). The former workers observed favorable results in 7 cases of chronic lymphatic leukemia, but the report extended only over a period of 150 days. Lindgren, who treated five cases of leukemia with radiosodium and radiophosphorus, found that $_{15}P^{32}$ gave a much better response. A more recent paper by Evans and Quimby (1946) on the effect of $_{11}Na^{24}$ on normal and leukemic mice suggests that this form of therapy may prove comparable to total body roentgen radiation. These workers found that the hematological response in normal animals was roughly the same with $_{11}Na^{24}$ as with equivalent roentgen irradiation. They did not, however, compare roentgen irradiation with $_{11}Na^{24}$ therapy in their leukemic animals, which appeared to show after administration of the radiosodium a more rapid, although less prolonged, fall in the leucocyte count than did the normal. There are as yet insufficient published data for an evaluation of radiosodium therapy in chronic leukemia, but it is to be expected that the results would be similar to those following total body irradiation with X-rays, since there is no selective localization of sodium.

The usefulness of radiosodium as a diagnostic tool is evident from a recent review by Quimby (1947). She has reported that, in a number of circulatory diseases (arteriosclerosis, Raynaud's disease, *etc.*) and injuries (trench- and immersion-foot, frost-bite, *etc.*) of the extremities, it has proved of considerable value in determining the status of the circulation. Since $_{11}Na^{24}$ can be measured by applying a Geiger-Müller counter to the surface of the skin and thus detecting its penetrating gamma-rays, its distribution in the blood and extracellular tissue fluids can be followed. With this technique of measuring tissue vascularity it has been possible to assist in evaluating the blood supply prior to amputation. Similar investigations with the radioactive inert gases will be reported by the work of Tobias, Lawrence and Hamilton (unpublished information.)

The use of radiostrontium ($_{38}Sr^{89}$) for the therapy of bone malignancies has been suggested on several occasions because of the concentration of this element in bone analogously with calcium. Its use was first suggested by the late Dr. Charles Pecher (Lawrence, 1942; Pecher, 1942). A comprehensive report on this material is still to be published. Actually, however, it is unlikely that diffuse irradiation of bone and marrow for relatively localized malignant growths will prove of much value, particularly in view of the danger of marrow depression. Nevertheless, beneficial results have been obtained in the treatment of metastatic prostate cancer in the way of slowing down the malignant process and controlling pain (Lawrence, 1942).

A number of radioactive colloids have been used in preliminary therapeutic studies. These include, as already mentioned, anhydrous chromic phosphate (Dobson *et al.*, 1947; Jones *et al.*, 1944; Lawrence *et al.*, unpublished information), which localizes almost entirely in the liver and spleen, colloidal MnO_2 protected by gelatin (Hahn and Sheppard, 1946; Sheppard and Hahn, 1946), zinc suspended in a suitably prepared solution of pectin (Müller, 1946), colloids of several elements, radioisotopes of which occur as fission products ($_{39}Y$, $_{40}Zr$, $_{41}Cb$) (Dobson *et al.*, 1947, in press), and colloidal sols of gold (Sheppard, Goodell and Hahn, 1947; Hahn *et al.*, 1947). The fission colloids may, depending upon the method of preparation, concentrate upon intravenous administration almost exclusively in the liver and spleen or go in equal concentration into the bone marrow (Dobson *et al.*, in press). Because of the relative abundance of yttrium, zirconium, and columbium in fission, there is a potentially rich source of their radioactive species for such investigations and for possible widespread clinical application. Investigations with radiomanganese as labeled colloidal manganese dioxide in an aqueous medium with gelatin as a protective colloid have recently been reported upon by Hahn and Sheppard [1946; 1946(123)]. They suggest the possibility that this material may be localized upon intravenous injection in the lymphoid reticuloëndothelium. More recently Sheppard, Goodell, and Hahn (1947) have suggested the use of gold sols labeled with $_{79}Au^{198}$ for this same purpose.

In addition to the intravascular use of these colloids there are also, as mentioned, possible applications in interstitial infiltration of malignant growths (Allen *et al.*, 1945; Müller, 1946). Müller (1946) has recently reported on the use of $_{30}Zn^{63}$, a short-lived isotope of 38.3 minutes' half-life, in the interstitial injection of uterine carcinomata (2 cases) with a pectin-protected suspension of zinc; he reports the strict localization of the isotope in the region of inoculation during the period of its effective activity and a tissue reaction similar to that following radium therapy. Hahn *et al.* (1947) have given encouraging accounts of the clinical application of gold sols containing ${}_{79}Au^{198}$ and given interstitially and intraperitoneally in the treatment of certain malignant growths. In general the use of labeled colloidal materials is promising and deserves thorough clinical trial.

Although ${}_{15}P$ and ${}_{53}I$ radioisotopes are the only artificial species for which therapeutic applications have been developed, it seems quite likely that in a few years a number of different isotopes will have well accepted clinical uses. In addition to the elements already mentioned, there are important possibilities in the labeling of organic compounds with long-lived radiocarbon—${}_{6}C^{14}$—and radiohydrogen or tritium—${}_{1}H^{3}$ or T. If radiation is ever to prove a vital tool for the palliation or cure of malignancy, the only possible way to accomplish this end would seem, from our present perspective, to be the discovery of organic substances which undergo highly selective localization in various types of cancer cells in contrast to those of normal tissue, and which can be labeled with a suitable radioisotope. Tritium with the low penetrating power of its beta-rays and its rapid turnover in the body is particularly interesting in this regard. If it can be put in relatively stable positions in localizing compounds, it will expend most of its ionizing power within the concentrating cells. Potentially it is an ideal radiotherapeutic agent.

A discussion of radiotherapy with isotopes would not be complete without mention of fissionable isotopes—*i.e.*, of uranium—${}_{92}U^{235}$—and plutonium—${}_{94}Pu^{239}$—and of other isotopes that release large amounts of ionizing radiation upon being bombarded with slow neutrons—*i.e.*, of lithium—${}_{3}Li^{6}$—and boron—${}_{5}B^{10}$. If these materials can be localized in given tissues and organs and the region bombarded with slow neutrons, a significantly higher radiation can be conferred on the isotope-containing tissue than on the normal or involved tissue through which the neutrons also must pass. Kruger (1940) has shown that pieces of transplantable mouse tumor soaked in boric acid solution and then irradiated *in vitro* with slow neutrons before inoculation into new hosts showed a significantly lower percentage of "takes" than control tumor pieces soaked in boric acid, but not irradiated. Zahl, Cooper and Dunning (1940) have shown that, if lithium or boron salts are infiltrated into mouse tumors *in vivo* and the host animals bombarded with slow neutrons, there is a significant increase in tumor regression over that in bombarded, but uninjected controls. Recent work of Tobias, Weymouth, Wasserman, and Stapleton (1948) on the effect of colloidal UO_2 enriched with ${}_{92}U^{235}$ concentrated in the liver and spleen of mice and there subjected to slow neutron bombardment also shows clearly the enhanced effect of radiation when nuclear disintegration processes of high energy are induced in tissues. It is interesting to note that these workers observed a greater biological effect per unit of tissue ionization with fission recoils than with beta particles. There seems little chance, however, that such studies will ever lead to therapeutic applications because of the short mean free path of thermal neutrons, the damaging effects of their infiltration and the toxic effects of fissionable materials and their products.

Finally, there should be mentioned the possible therapeutic use of charged nuclear particles—protons, deuterons, and alpha particles—of high energies from the cyclotron and other heavy particle accelerators. Such would be an isotopic application of sorts inasmuch as the nuclei of single species of hydrogen and helium are being employed. None of these has been used clinically as yet. But recently Wilson (1946) has suggested the possibility that very high energy protons—of 100 Mev or more—produced by the giant cyclotrons might find use in direct beam radiotherapy. The 100 Mev proton would produce its maximum ionization some centimeters beneath the skin and thus might be used to radiate deep-lying lesions, especially where the skin tolerance is a limiting factor. A comparable, although non-isotopic application of accelerated particles might be the use of high energy electrons from the betatron as suggested by Kerst (1944) and Quastler (1946). Also deuterons may be used to produce fast neutrons from beryllium ($_4$Be); these and other neutrons have been used in a number of studies on the biological effects of irradiation (Aebersold and Lawrence, 1942; Stone and Larkin, 1942).

In summary it may be observed that the role in therapy of radioactive isotopes and of those stable species that can also be adapted to the production of ionization in tissues is still in a primitive stage of development. Unquestionably, the future will see great expansion in our knowledge of their usefulness and practical application. The use of the new types of radioactivity must, however, be approached with caution. It has long been known that ionizing radiation, in addition to its acute effects, may have such chronic manifestation as the induction of malignant change in tissue. Radium-induced bone sarcomata have been observed for many years. Recently Brues, Lisco and Finkel (1946) have shown that both radiostrontium—$_{38}Sr^{89}$—and plutonium—$_{94}Pu^{239}$—can produce bone tumors in mice, particularly the former. This has particular significance for the possible use in man of long-lived isotopes of elements that concentrate in bone —as for example, many of the fission product elements. And finally the possibility of genetic mutations induced by radioisotopes in the metabolic pool must be borne in mind.

IV. SOME APPLICATIONS OF ISOTOPES IN EXPERIMENTAL MEDICINE

1. General Considerations

In the preceding section we have emphasized the clinical aspects of certain investigations with isotopes. In this last section we propose to review some of the basic studies that have no direct clinical application as yet. These are all in the field of tracer research.

Isotopes in tracer work are tools for the elucidation of physiological processes, not objects of study in themselves. Thus a truly comprehensive survey of tracer applications must cut across all the experimental fields of biology and inevitably separate in a somewhat artificial manner isotopic from non-isotopic studies of the same phenomena. It is not our purpose, therefore, to consider all the many medically important experiments reported in the literature in which isotopes have been used. Rather, representative studies have been selected that illustrate the applicability of the various isotopes so far employed.

To be sure, the use of isotopes in some experimental fields, as for example that of intermediary metabolism, has been so revolutionary and so important that a discussion of recent work is essentially an account of studies with isotopes. Chapter 2 by Vennesland on carbon and nitrogen isotopes illustrates this point well. In other fields, especially those dealing with non-metabolic studies, isotopes may play only a casual role.

For orientation in the various physiological studies of medical interest Table I can be referred to. It includes not only references to works of strictly medical significance, but also to a few of the fundamental studies in the basic biological fields, *i.e.*, on photosyntheses, *etc.* A number of important isotopes are thoroughly or partly covered by other chapters. Radiophosphorus, which has been the most widely employed isotopic tracer, is treated in Chapter 7 by Chaikoff in connection with phospholipid metabolism and in Chapter 9 by Hevesy in connection with nucleoprotein metabolism. The heavy isotopes of nitrogen and carbon are treated in Vennesland's chapter. Tracer studies on the metabolism of two important trace elements—iron and iodine—are treated in the Chapters 6 and 8, by Hahn and Leblond, respectively. Also, a chapter by D. H. Copp on isotopic tracers, in the study of the mineral metabolism of the elements in bone—$_{9}F$, $_{11}Na$, $_{15}P$, $_{20}Ca$, $_{38}Sr$, *etc.*—and of the essential trace elements (other than iron and iodine)—$_{25}Mn$, $_{27}Co$, $_{29}Cu$, $_{30}Zn$—is scheduled for the second volume of *Advances in Biological and Medical Physics*; also a chapter by H. B. Jones on inert gas exchange studied with radioisotopes. In the latter case a report of such studies will also appear elsewhere (Jones, in press). These subjects are, therefore, not treated here except for the inclusion of references in Table I to the more important papers.

In connection with elements treated in the other chapters a few studies not discussed therein are particularly worthy of mention here for their clinical significance.

Næslund and Nylin (1946) have shown by labeling erythrocytes with $_{15}P^{32}$ that the human placenta in rare cases is permeable to these cells. Labeled cells introduced into the maternal circulation before parturition were later detected in the fetal cord blood. This study may have great significance in relation to the Rh problem.

Of considerable clinical importance has been the work with radioiron on blood preservation. Gibson *et al.* [1947 (38, 39)] have made extensive tests of various blood preservatives in relation to the survival of transfused cells after *in vitro* storage. They have found that refrigeration, the addition of dextrose to the citrate anticoagulant, the maintenance of a slightly acid reaction of the diluted plasma or resuspension fluid, and optimal dilution are essential for prolonged preservation. In certain acid-citrate solutions whole blood can be safely preserved with up to 70% viability for three weeks. Cells preserved by the simple addition of citrate are almost entirely broken down within 24 hours in the new host after only two weeks of preservation *in vitro*, as first shown with radioiron by Ross and Chapin (1943). Other significant studies have been the determination of red cell and plasma distribution and volume with radioiron-labeled erythrocytes by Gibson *et al.* [1946 (40, 42), 1947 (41)] and the demonstration by Moore *et al.* (1944) and Hahn *et al.* (1945) that ferrous iron is better absorbed from the gastro intestinal tract than ferric iron.

Reiner *et al.* (1943) have been able to study the absorption rates of insulin labeled by coupling it with diazotized iodoaniline containing radioiodine. A correlation was found between the differences in absorption rate and the intensity of hypoglycemic action produced. In the preparations used, absorption was found to be in the following order: insulin > globin insulin (with zinc) > protamine zinc insulin.

In the following subsections are considered some of the more important studies conducted with isotopes not considered in other chapters in this volume.

2. *Deuterium and Tritium*

There has been extensive work by Schoenheimer and his school on intermediary metabolism using deuterium-labeled fats and other organic compounds (Kamen, 1947; Schoenheimer, 1942). A review of these studies is, however, beyond the scope of this chapter. In addition, deuterium has been used in the determination of the turnover rate of water in the vertebrate body. Hevesy and Hofer's early work with the goldfish (Hevesy and Hofer, 1934a, b) was followed shortly by their studies on the turnover rate of body water in man (Hevesy and Hofer, 1934c). They found that, while a little deuterium appears in the urine within half an hour after ingestion by mouth, the bulk of water in the body, as determined by labeling with heavy water, has a half-life of about 9 days, or, otherwise stated, that a given water molecule spends, on the average, about 13 days in the body. They further made the first determination of total body water with the isotopic dilution technique (Hevesy and Hofer, 1934c).

Recently Moore (1946) with deuterium, and Pace *et al.* (1947) with tritium, have also determined total body water in man. In view of the fact that there is no other reliable method for the determination of body water, these isotopes certainly will find wide usefulness in this connection.

Tritium as an organic labeling agent has so far been used only in studies on photosynthesis (Kamen, 1947). However, it, as well as deuterium, doubtless has great potentialities in the further unraveling of the vast complexities of intermediary metabolism.

3. *Radiocarbon*

A considerable body of the literature on the tracer application of carbon isotopes is covered by Vennesland in her chapter. However, she deals almost entirely with heavy carbon, and, thus, a few important studies with radiocarbon merit discussion here.

Short-lived radiocarbon—${}_{6}C^{11}$ (of 20.5 minutes' half-life)—has had its most important use in studies of photosynthesis and the utilization of CO_2 by various heterotrophic organisms (Kamen, 1947). In problems of mammalian physiology it has been used otherwise only in the study of carbon monoxide metabolism. Tobias *et al.* (1945) have shown that $C^{11}O$ is not converted to $C^{11}O_2$ *in vivo* in the human during a period of two hours. An advantage that C^{11} has over C^{14} is the fact that the penetrating annihilation gamma-rays of the former permit convenient *in vivo* measurements whereas the weak beta-rays of the latter permit *in vitro* counting only, which for reasonable accuracy must have a rigidly controlled geometry.

Long-lived radiocarbon—${}_{6}C^{14}$ (of $>$ 4,000 years' half-life)—has only recently become sufficiently abundant—from pile sources—to permit its widespread use. Consequently its scientific exploitation has scarcely begun. Promising to be one of the most important of tracer isotopes for use in studying intermediary metabolism, it can be incorporated by appropriate syntheses into the carbon chains of carbohydrates, fats, amino acids, vitamins, and other organic compounds. As examples of the types of syntheses that will become increasingly common may be mentioned the recent work of Reid (1947) in synthesizing labeled tyrosine, of Heidelberger *et al.* (1947) in synthesizing labeled dibenzanthracene (an important aromatic carcinogen), and Melville, Rachele and Keller (1947) in synthesizing methionine with C^{14} in the methyl radical. Recently Bloom, Curtis and McLean (1947) have shown that carbon administered as carbonate may be laid down at least for several weeks in bone without showing any change in concentration. It is, however, too early to predict the limitations that bone-deposition may play on the use of ${}_6C^{14}$ for tracer studies in man, but, because of this problem, its application to human experiments must be approached with caution.

4. Heavy and Radioactive Sulfur

Sulfur has great significance as one of the most important organic metabolites. In addition, as sulfate, it is one of the less important anions involved in the electrolyte balance of the body. Most work with labeled sulfur has been directed toward elucidating its role in intermediary metabolism, particularly in the form of sulfur-containing amino-acids.

Heavy sulfur—${}_{16}S^{34}$, the most abundant of the three rarer isotopes of sulfur—has thus far been produced in small amounts only and consequently has had very limited use. Du Vigneaud *et al.* (1944) have synthesized labeled methionine using sulfur slightly enriched with ${}_{16}S^{34}$ as well as ${}_6C^{13}$ (heavy carbon) in the β-position; these workers were able to show with this doubly labeled material that the carbon chain of methionine is not utilized in the *in vivo* conversion of methionine to cystine.

Radiosulfur—${}_{16}S^{35}$, the only unstable species of sulfur of convenient half-life for biological use—has also had limited application, although somewhat more extensive than ${}_{16}S^{34}$. As with ${}_6C^{14}$, it is now available in much larger quantities from the pile than it has previously been from the cyclotron. Its use has been reviewed by Kamen (1947). A number of studies have been carried out in both plant and animal sulfur metabolism. Particularly important has been the work of Tarver and his associates (Melchior and Tarver, 1947a, b; Tarver and Schmidt, 1939, 1942, 1947; Tarver and Reinhardt, 1947), on the synthesis of sulfur-containing amino acids (Melchior and Tarver, 1947a; Tarver and Schmidt, 1939, 1942) and the metabolism of these and of elemental sulfur in mammals, including the *in vivo* conversion of methionine to cystine (Tarver and Schmidt, 1939) and taurine (Tarver and Schmidt, 1942) and the distribution of sulfur fed as methionine in the body protein (Tarver and Schmidt, 1942). Most significant of the results of their work and the work of other biochemists using the sulfur label has been the further verification of the dynamic state of body constituents, for a rapid turnover of protein sulfur has been demonstrated, even in the fasting animal (Tarver and Schmidt, 1942). Neither elemental sulfur nor sulfate can be used to form sulfur-containing amino-acids (Tarver and Schmidt, 1939, 1942). Soluble sulfides are mostly converted to sulfate and thus share the fate of the latter, although a small amount of sulfide may be incorporated into protein as shown by Smythe and Halliday (1942) and by Dziewiatkowski (1946).

Borsook *et al.* (1940) have studied the metabolism of vitamin B_1 (thiamine) labeled with ${}_{16}S^{35}$. They have demonstrated that, just as in the case of the major organic metabolites, it is in a state of dynamic equilibrium and has a rapid turnover in the body.

The use of sulfur isotopes as metabolic tracers will doubtless give much further vital information on intermediary metabolism—particularly of the proteins.

5. Isotopes for Body Electrolytes

The subject of electrolyte metabolism—one aspect of mineral metabolism—has not received the intensive study with tracers that has been the case with intermediary metabolism. Nevertheless, there is a considerable body of literature on the use of isotopes in the study of the uptake, transport, and elimination of electrolytes. Although the subject of electrolyte dynamics is not treated elsewhere in this volume, it is too broad a subject to cover exhaustively in this chapter. However, a few of the more salient studies are cited here.

Kamen (1947) has reviewed the available radioisotopes that may be used in the study of electrolytes. The principal elements involved are in the form of the cations: ${}_{11}Na^+$, ${}_{12}Mg^{++}$, ${}_{19}K^+$, ${}_{20}Ca^{++}$; and the anions: ${}_{17}Cl^-$, ${}_{6}C$ as carbonate and bicarbonate, ${}_{15}P$ as the acid phosphates, and ${}_{16}S$ as sulfate—as well, of course, as ${}_{8}O$, which forms a part of all complex anions. Of these ${}_{8}O$ and ${}_{12}Mg$ do not, as already indicated, have radioactive species of general usefulness.

Of the main essential cations, ${}_{20}Ca$ and ${}_{12}Mg$ are also important bone minerals and organic metabolites. All of the elements involved in the essential anions, with the exception of ${}_{17}Cl$, also play a role in the intricate dynamics of intermediary metabolism. In addition, phosphate and carbonate are, with ${}_{20}Ca$ and ${}_{12}Mg$, the principal constituents of bone. Therefore, of the elements occurring as electrolytes, it is ${}_{11}Na$, ${}_{19}K$, and ${}_{17}Cl$ that are of interest in metabolism largely because of their status as electrolytes. Most isotope research on the role of electrolytes has been done with isotopes of these three elements and our discussion here will be largely limited to them.

The use of isotopes provides the only method whereby the absolute values for the time of normal penetration and turnover of electrolytes in tissue and cell spaces can be determined. Much of the work so far has been directed toward elucidating these hitherto unsolvable problems.

Two papers of particular significance for the general study of ionic equilibria have been those of Hahn and Hevesy (1941) and of Greenberg *et al.* (1943) on the rate of penetration of ions through the capillary wall; the latter workers have in addition studied the permeability of the blood-cerebrospinal barrier to ions. Hahn and Hevesy (1941) found with labeled chloride, bromide, sodium, potassium, and phosphate ions injected into the circulation of rabbits, that potassium was by far the fastest to penetrate the capillary wall; from tissue measurements they also concluded that the capillary endothelium in muscle is much more permeable to sodium and phosphate than is the endothelium in the brain. Greenberg *et al.* (1943) used labeled sodium, potassium, phosphate, and iodide, which are normal to the body, and rubidium, strontium, and bromide, which they considered foreign. Disappearance of these ions from the plasma was in the order: $K^+ = Rb^+ >$ phosphate $> I^- > Na^+ = Br^- > Sr^{++}$. In the cerebrospinal fluid the increase in concentration followed the order: $K^+ > Na^+ > Br^- > Rb^+ > Sr^{++} >$ phosphate $> I^-$.

Moore (1946) has presented data on the determination of various fluid and tissue volumes of the human body with radioactive tracers of sodium, chlorine, and potassium, as well as with deuterium oxide.

Study of the uptake, distribution and excretion of sodium with the short-lived radioactive isotope, ${}_{11}Na^{24}$ (of 14.8 hours' half-life) was first carried out by Hamilton and Stone (1937) and by Hamilton (1937). In man, absorption of sodium taken by mouth was observed to begin within a few minutes and to be completed, in most cases, in between three and ten hours. In that period excretion was negligible.

Since this initial work there have been a number of studies on the movement of sodium across various body and cell membranes. Visscher *et al.* (1944) have studied in detail the interchange between the sodium ion of the intestinal lumen and the blood and have concluded that the turnover between intestine and blood is very large, being equal to the total plasma sodium in approximately 83 minutes. Flexner and his associates have studied in several species the transfer of sodium across the placental barrier, including that of the human placenta (Gellhorn, Flexner and Hellman, 1943). In general, they have found that the transfer of sodium per unit weight of placenta increases greatly as gestation proceeds.

Sodium is of particular interest as it is predominantly an extracellular cation. Manery and Bale (1941), in rats and rabbits, and Kaltreider *et al.* (1941), in man, have shown that within 9–12 hours complete equilibration of injected sodium is achieved, its spread from the plasma being rapid for the first two or three hours, and thereafter much slower. Kaltreider *et al.* (1941) have given evidence for the view that the volume of fluid involved in the initial spread—averaging about 25% of the body weight in man—represents the extracellular fluid volume.

Of particular clinical interest has been the study done with ${}_{11}Na^{24}$ by Fox and Keston (1945) on the mechanism of shock from burns and trauma in mice. These workers have shown that the sodium content of injured skin is greatly increased, exceeding the gain in water (edema), and that under conditions of severe injury approximately one-half of the total amount of extracellular sodium is side-tracked and rendered unavailable to normal body interchange. They have concluded that the conventional extracellular fluid space has been modified into much larger "sodium space" including extracellular edema fluid and intracellular water containing excess sodium. Fox and Keston also noted a great influx of sodium into burned human tissues as determined by *in vivo* measurements at the bedside.

Some use of long-lived radiosodium—$_{11}Na^{22}$ (of 3.0 years' half-life)—has also been made. Reaser and Burch (1946) have used it in the study of the retention of sodium in congestive heart failure, and Cuthbertson and Greenberg (1945) were able to demonstrate the increase of sodium space in the rat body under conditions of dietary chloride deficiency. This isotope should prove highly useful in many long-term studies of sodium turnover.

There is but a single useful isotope of radiopotassium—$_{19}K^{42}$ (of 12.4 hours' half-life). It has been used in a number of studies on the uptake, distribution, and excretion of potassium. In contrast with sodium, potassium is present predominantly as an intracellular cation. Noonan *et al.* (1941) have shown that, in rats injected with a labelled 1% potassium chloride solution, potassium ion penetrates rapidly into most tissues of the body, the rate being highest for liver, heart, kidney, lung, diaphragm, and gastrointestinal tract, intermediate for the general musculature and skin, and low for testes, erythrocytes, and brain. In tissues with rapid penetration, the total potassium has a relative activity higher than the simultaneous plasma value during the first one to two hours; this is particularly marked after intraperitoneal injection. Fenn *et al.* (1941), in a subsequent paper, verified these observations in the rabbit, cat, and dog. They concluded that an excess of potassium in the plasma is quickly removed by the viscera, together with an anion, and is slowly released to the muscles.

Studies with radiochlorine have been carried out thus far with one of the short lived isotopes—$_{17}Cl^{38}$ (of 37 minutes' half-life). The long-lived species —$_{16}Cl^{36}$ (of about 1,000,000 years' half-life)— now available from the pile, may be expected to prove of considerable value. Manery and Haege (1941) have studied the penetration of labeled chloride, administered as lithium chloride, into the tissues of the rat and rabbit. They have found that, after intravenous administration, equilibrium is reached within a few minutes in such organs and tissues as the kidney, liver, muscles, cartilage, and tendon, but that $_{17}Cl^{38}$ does not enter the entire chloride-containing phase of testes and pyloric mucosa even in one hour.

In addition to their use in the study of normal electrolyte dynamics, radiosodium and radiopotassium have been used in studying the physiology of the adrenal cortex (Anderson and co-workers, 1939, 1940).

From the studies cited it can readily be seen that the use of tracers has a vital role to play in the elucidation of problems in electrolyte dynamics.

6. Isotopes of Elements not Known to be Essential in Animal Nutrition

Of the 96 known elements, only 15 are universally accepted as essential in mammalian nutrition ($_1H$, $_6C$, $_7N$, $_8O$, $_{11}Na$, $_{12}Mg$, $_{15}P$, $_{16}S$, $_{17}Cl$, $_{19}K$, $_{20}Ca$,

${}_{25}\mathrm{Mn}$, ${}_{26}\mathrm{Fe}$, ${}_{29}\mathrm{Cu}$, and ${}_{53}\mathrm{I}$), although for two or three others (${}_{27}\mathrm{Co}$, ${}_{30}\mathrm{Zn}$, and possibly ${}_{35}\mathrm{Br}$) there is strong evidence that they also play an indispensable role. Thus, at least 78 elements are not believed directly essential for mammals, although the need of various plants for other elements, especially boron (${}_{5}\mathrm{B}$) and molybdenum (${}_{42}\mathrm{Mo}$), has been conclusively demonstrated, also the need of certain lower animals for such elements as vanadium (${}_{24}\mathrm{V}$), silicon (${}_{14}\mathrm{Si}$), *etc.* A minority of these 78 elements has been studied in mammals with isotopic tracers—in connection with their behavior when present either in trace or toxicological amounts. It is interesting to speculate as to how many elements will eventually be found to be essential in trace amounts.

For over 45 years there has been interest in the biological effects of the naturally occurring radioactive elements, particularly ${}_{88}\mathrm{Ra}$, ${}_{90}\mathrm{Th}$, and ${}_{92}\mathrm{U}$. In the case of radium in particular, there have been many studies on its distribution after injection or ingestion in mammals; assays for the contents of these elements in tissues have, in most cases, been made with various techniques of measurement for radioactivity. The literature on radium and uranium up to 1932 has been reviewed in detail in the book by Stoklasa and Pěnkava (1932). However, the main emphasis of these studies has been investigation of the toxic and radiation effects of the heavy radioactive elements rather than tracing their fate in the body. One important fact deriving from these studies has been that radium, like many of the heavy metals, is deposited in bone and, by its chronic radiation effects there, may induce malignant change (*cf.* Brues *et al.*, 1946, in the case of strontium and plutonium). These effects are reviewed by Stoklasa and Pěnkava.

Recently there has been considerable interest in the fate *in vivo* of trace amounts of elements now thought to be nonessential or foreign to the mammalian body. In such experiments the amount of the labeled element introduced should be so small as to have no manifest toxic or pharmacological effect and no detectable radiation effect on the organism as a whole. So far at least 33 non-essential elements have been studied on this basis: beryllium (${}_{4}\mathrm{Be}$), fluorine (${}_{9}\mathrm{F}$), argon (${}_{18}\mathrm{A}$), arsenic (${}_{33}\mathrm{As}$), selenium (${}_{34}\mathrm{Se}$), krypton (${}_{36}\mathrm{Kr}$), rubidium (${}_{37}\mathrm{Rb}$), strontium (${}_{38}\mathrm{Sr}$), yttrium (${}_{39}\mathrm{Y}$), zirconium (${}_{40}\mathrm{Zr}$), columbium (${}_{41}\mathrm{Cb}$), molybdenum (${}_{42}\mathrm{Mo}$), ruthenium (${}_{44}\mathrm{Ru}$), antimony (${}_{51}\mathrm{Sb}$), tellurium (${}_{52}\mathrm{Te}$), xenon (${}_{54}\mathrm{Xe}$), caesium (${}_{55}\mathrm{Cs}$), barium (${}_{56}\mathrm{Ba}$), lanthanum (${}_{57}\mathrm{La}$), cerium (${}_{58}\mathrm{Ce}$), praseodymium (${}_{59}\mathrm{Pr}$), element 61, gold (${}_{79}\mathrm{Au}$), lead (${}_{82}\mathrm{Pb}$), bismuth (${}_{83}\mathrm{Bi}$), astatine (${}_{85}\mathrm{At}$), radon (${}_{86}\mathrm{Rn}$), thorium (${}_{90}\mathrm{Th}$), protoactinium (${}_{91}\mathrm{Pa}$), neptunium (${}_{93}\mathrm{Np}$), plutonium (${}_{94}\mathrm{Pu}$), americium (${}_{95}\mathrm{Am}$), and curium (${}_{96}\mathrm{Cm}$). Citations for these studies are included in Table I under the respective elements.

Certain of the foregoing studies have particular medical significance. Hamilton (1947; unpublished information), who has studied the distribution in rats of a large number of the above elements administered in trace amounts, has demonstrated with radioisotopes the selective localization of many in bone—particularly of $_{39}Y$ and $_{58}Ce$ (fission products) and $_{94}Pu^{239}$ (an end-result of $_{92}U^{238}$ neutron capture), these elements are excreted very slowly and have biological half-lives in the body of from one to three years. When ingested, they are not taken up in significant amounts from the gastroïntestinal tract; however, if inhaled, they are retained for long periods in pulmonary tissue. In view of their long half-lives and their abundance as pile products, they thus constitute a potential health hazard for the personnel of atomic energy installations.

The use of radioisotopes has been of considerable value in showing the distribution of nonessential elements used in pharmacology. For example, Bertrand and Tobias (in press) have demonstrated with radiogold that the uptake of gold sodium thiosulfate in the synovia and synovial fluid of a patient suffering from rheumatoid arthritis, and of rabbits in which chemical (turpentine) arthritis has been induced, was very marked, while, in the case of control animals, uptake was somewhat less. Such studies are of value in relation to the use of gold salts in rheumatoid arthritis. The use of radioantimony by Brady *et al.* (1945), in the study of *Dirofilaria immitis* infection (filariasis) in dogs, has shown that sodium antimony xylitol is taken up in significant amounts by the adult worms, which concentrate it only less than the thyroid and liver. Similar studies with radioarsenic by Lawton *et al.* (1945) on *Litosomoides carinii* infection (filariasis) of the cotton rat, have shown the concentrating of sodium arsenite by the parasites. Such studies can be expected to aid in our understanding of the mechanism of many pharmacological agents and may possibly point the way toward the development of new techniques.

Bromine, an element that possibly plays an essential role in animal nutrition, has been used in radioactive form to label a number of dyes. For example, Moore and Tobin (1942) have been able to show by this means the selective localization of labeled dibrom trypan blue in inflammatory lesions.

In summary, it may be noted that, in the application of radioactive isotopes of the majority of elements to biological problems, we are still in a primitive stage. Before the wide adoption of these new techniques can take place, a new generation of workers must be trained. The field has just opened up, and the future will see much further work and many important discoveries.

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Nitrogen and Carbon Isotopes: Their Application *in Vivo* to the Study of the Animal Organism

> "Déduire les phénomènes qui se passent dans l'organisme de l'analyse des matériaux qui le traverse, ce serait prétendre connaitre ce qui se passe dans une maison en analysant les aliments qui entrent par la porte et la fumée qui sort par la cheminée."
>
> "Avant d'etudier chacune de ces phases de l'activité vitale, la *destruction* organique, la *création* organique, il importe de mettre en lumière et de bien ètablir . . . le rapport étroit qui unit indissolublement les deux termes de notre division des phénomènes vitaux . . . ces phénomènes se produisent simultanément chez tout être vivant, dans un enchaînement qu'on ne saurait rompre."
>
> Claude Bernard, 1878(9)

By BIRGIT VENNESLAND

CONTENTS

I. INTRODUCTION

The last decade has witnessed the preparation of isotopes of the biologically important elements, nitrogen and carbon, and the application of these isotopes as tracers in the study of intermediary metabolism. There has been little or no lag in the utilization of the isotopes for biological purposes, once they were made available; although the limited distribution has prevented many laboratories from participating in this field.

The stable isotope of nitrogen of mass 15 (N^{15}) was first concentrated by Urey and coworkers in 1937 (138, 139) and was almost immediately employed by Schoenheimer and his associates (116, 119, 120) to study hippuric acid synthesis *in vivo.* The Columbia group rapidly developed the basic methods used in applying this isotope to animal studies, and the bulk of the work with N^{15} to date has been done in that laboratory.

Although the useful isotopes of nitrogen are limited to one, the N^{15} mentioned above, there are three isotopes of carbon, C^{11}, C^{13} and C^{14}, which can be applied profitably to biological studies. The first of these to be so employed was the unstable radioactive isotope, C^{11}, which was used by Ruben, Hassid and Kamen in 1939 (102) in a study of photosynthesis. In the same year Urey and coworkers (99) described the preparation of concentrates of the stable isotope of carbon, C^{13}. Within the next year Wood, Werkman, Hemingway and Nier (163) used C^{13} to study CO_2 fixation in autotrophic bacteria, and Rittenberg and Waelsch (98) used C^{13} to study urea formation. The long life radioactive isotope of carbon, C^{14}, was described by Ruben and Kamen in 1941 (105). It has not yet been employed in biological studies to the same extent as C^{13}, though it shows promise of being the isotope of choice in future developments. For a description of other isotopes of carbon and nitrogen, the reader is referred to the literature (97).

This paper will be limited to N^{15}, C^{11}, C^{13}, C^{14}, and their application to *in vivo* studies of the animal organism. A section on methods is included for the benefit of workers without experience in the field.

Because N^{15} has been employed primarily in *in vivo* studies, its application will be covered fairly completely. The last comprehensive review of work done with N^{15} was that of Schoenheimer (109). Since the interpretation of more recent data is dependent on knowledge obtained in earlier work, an effort will be made, in the tables particularly, to compile for comparative purposes data from both earlier and later experiments. This necessitates the repetition of much which has already been well stated. It is emphasized here, that many of the general principles were developed in their present form by 1941, under the guidance of Schoenheimer, and that Schoenheimer and the Columbia group are responsible for much of the groundwork underlying the application of isotopes to *in vivo* studies.

In the case of carbon isotopes, a much wider variety of biological material has been employed, and several recent reviews covering various portions of the field are available. Thus, the use of isotopically marked carbon in the study of intermediary metabolism was summarized by Buchanan and Hastings in 1946 (32). Later in the same year, Wood (158) covered the fixation of carbon dioxide and the interrelationships of the tricarboxylic acid cycle. A review of acetate metabolism by Bloch (15) appeared in 1947. The application of carbon isotopes to the study of photosynthesis has been described recently by Rabinowitch (84).

The discussion in the present paper will be limited to those carbon isotope experiments conducted with the intact animal. This choice is made with the object of attempting such correlations as may be possible among the various data that have accumulated from *in vivo* experiments done with N^{15} or carbon isotopes. There is also a hope that a compilation of data now available may stimulate further correlation of N^{15} and carbon isotope studies. The chosen restrictions will necessitate omission of much material which is pertinent to any complete discussion of our present knowledge of intermediary metabolism. For example, the application of deuterium to *in vivo* studies has contributed much, but will be touched on only as necessity demands, since it is outside the scope of the paper. Furthermore, the enormous contributions of *in vitro* studies, both with and without isotopes, to the elucidation of data obtained *in vivo*, though given little space, are not thereby implied to be of secondary importance.

II. METHODS

1. *Preparation and Measurement of Isotopes*

The deliberately superficial treatment of procedures in this section is intended only for orientation of those whose interests in the field have no practical motivation. References are omitted, since a more detailed discussion of all topics under this heading and further references can be obtained from one source (157).

(*a*) ***Preparation of N^{15} and C^{13}.*** The carbon isotope of mass 13 constitutes 1.1% (by number) of all naturally occurring carbon atoms, the remaining 98.9% being the more abundant isotope of mass 12. The nitrogen isotope of mass 15 constitutes 0.38% (by number) of naturally occurring nitrogen, whereas 99.62% is nitrogen of mass 14. (These figures are termed natural abundance.) Wherever the elements occur in nature, whether free or combined, the relative proportions of these isotopes are, for practical purposes in biological tracer work, the same. The small fluctuations observed do not seem to be of sufficient magnitude to warrant more than incidental consideration (62, 66, 117, 137).

The heavy isotopes, C^{13} and N^{15} are separated from the naturally occurring mixture in the form of simple compounds such as CO_2, NaCN and NH_3. In practice, complete separation is not achieved, nor is it necessary. The concentration of isotope required for a given problem is a function of the degree of dilution which the isotope undergoes during the course of the experiment, since both these factors determine the accuracy of the final analyses desired.

Since isotopes of the same element have virtually identical chemical properties, separation of the heavy isotopes from the more abundant light ones cannot be accomplished by ordinary chemical means. Use can be made, however, of the small differences which do exist in physical properties. For preparing substances enriched in N^{15} or C^{13}, the most widely applied procedure has been the exchange method. This method is based on the fact that there are slight but definite differences between the chemical reaction rates of the light as opposed to the heavy isotopes. As an example we may consider the equilibrium reaction

$$\underset{\text{(gas)}}{HC^{12}N} + \underset{\text{(sol'n)}}{C^{13}N^-} \rightleftarrows \underset{\text{(gas)}}{HC^{13}N} + \underset{\text{(sol'n)}}{C^{12}N^-}$$

If the velocity constant of the reaction from left to right is compared with the velocity constant of the reaction from right to left, a small difference will be found. The equilibrium constant, α, will be slightly greater than 1.

$$\frac{[HC^{13}N][C^{12}N^-]}{[HC^{12}N][C^{13}N^-]} = 1.012 \text{ at } 25°C.$$

Expressed qualitatively, this means that the amount of C^{13} in gaseous HCN is slightly greater than that in the ion CN^- with which it is in equilibrium. The difference is too small to be of value for preparative purposes, unless the process is repeated many times in succession. In practice, a long column is constructed with a solution of CN^- flowing downward and a stream of HCN gas flowing up. The gas becomes enriched with C^{13} as it rises. Carbon thirteen is prepared also by utilizing the exchange reaction:

$$\underset{\text{(gas)}}{C^{13}O_2} + \underset{\text{(sol'n)}}{HC^{12}O_3^-} \rightleftarrows \underset{\text{(gas)}}{C^{12}O_2} + \underset{\text{(sol'n)}}{HC^{13}O_3^-} \qquad \alpha = 1.012 \text{ at } 25C^\circ$$

In a similar fashion, N^{15} is prepared by using the reaction:

$$\underset{\text{(sol'n)}}{N^{14}H_4^+} + \underset{\text{(gas)}}{N^{15}H_3} \rightleftarrows \underset{\text{(sol'n)}}{N^{15}H_4^+} + \underset{\text{(gas)}}{N^{14}H_3} \qquad \alpha = 1.031 \text{ at } 25^\circ C$$

In addition, C^{13} can be prepared by thermal diffusion. This method is based on the fact that a gas molecule such as methane diffuses at a different rate under the influence of a temperature gradient if the carbon has a mass of 12 than it does if the carbon has a greater mass, 13.

(*b*) *Measurement of N^{15} and C^{13} with the Mass Spectrometer.* The utilization of stable isotopes as tracers in biological work is dependent upon a method of convenient and accurate analysis. The mass spectrometer is the instrument most suitable for this purpose. In the form in which it is used at present, this instrument is a device which measures the relative abundance of the heavy and the normal isotope in molecules of a suitable gaseous compound containing the isotope mixture to be analyzed. The first step in the analysis must, therefore, be the conversion of the sample to the suitable gaseous form. Compounds containing N to be analyzed are first converted into ammonia by Kjeldahl digestion and the ammonia is then converted to gaseous N_2 by treatment with alkaline hypobromite. In the case of carbon, the compound is prepared for analyses by oxidation to CO_2. About 1 or 2 ml. of gas are used for an analysis. The principles on which the mass spectrometer operates may be summarized in a descriptive fashion as follows: The molecules of the gas (in our case, either N_2 or CO_2) are converted into positively charged ions by a stream of electrons. Then, by the imposition of electric and magnetic fields, these ions are set into motion, in such a manner that the path of each singly charged ion is a function of its mass only. Ions of different masses will have different paths, and are thereby separated into a kind of spectrum.

Data obtained in this way give the ratio of the amounts of different molecules. Thus, with nitrogen gas, the mass spectrometer gives the ratio of the number of molecules of $N^{14}N^{14}$ of mass 28 to the number of molecules of $N^{14}N^{15}$ of mass 29. With CO_2, the relative abundance of $C^{13}O_2$ and of $C^{12}O_2$ is determined.

(*c*) *Preparation and Measurement of C^{11} and C^{14}.* The two useful radioactive isotopes of carbon, C^{11} and C^{14}, are both produced by nuclear reactions. The instrument originally employed was the cyclotron. C^{11} was made by bombarding boron, usually in the form of boric oxide, or boric acid, with deuterons. C^{14} was made by treating nitrogen in the form of saturated solutions of NH_4NO_3 with slow neutrons. At present C^{14} is produced and distributed for research by government laboratories. [See Addendum.]

In the case of C^{11}, the short half-life of about 20 minutes imposes severe restrictions on its use, because of the limited time within which the experiments must be completed. For the same reason C^{11} can be employed only by laboratories close to the means of production. However, C^{14} has a very long half-life, about 1×10^4 years, so that decay presents no problem, and C^{14} will presumably largely replace C^{11} for future biological tracer work.

Both C^{11} and C^{14} may be measured by means of a Geiger-Müller counter or an electroscope, the former being the usual instrument of choice. The counter consists of a tube filled with a suitable gas mixture containing a wire and an outer case across which a high voltage is imposed. This tube is sensitive to ionized particles in the sense that they cause an electric discharge to take place. These discharges are detected by means of an amplifier and their number is recorded by a suitable recording system. Such results then are obtained as number of counts in a given time. It is not necessary in biological tracer experiments to know the absolute amount of radiation, but only to determine the relative amount. Because C^{14} has a very soft radiation it is somewhat more difficult to count than C^{11}, but a correction for decay must be made for the latter.

2. Special Chemical Methods in Isotope Work

(*a*) *Synthesis of Compounds Containing Isotopes of Carbon and Nitrogen.* The isotopes of carbon and nitrogen are made available to the biological chemist in the form of simple compounds such as cyanide, ammonia and carbon dioxide. The latter two substances are often utilized directly in biological tracer work. When, however, it is desirable to use a tracer as part of a more complex molecule, these complex molecules must be synthesized from the simpler forms of the isotope. In such cases, special methods of synthesis may be required, because considerations apply which need not be taken into account in ordinary organic synthetic work. Thus, it is usually necessary to work with relatively small quantities and to devise procedures which involve minimum waste of the costly tracer.

Considerable work has already been done to develop suitable synthetic methods for preparing many labeled substances of biological interest. To afford a convenient view of the scope of this work, compounds which have been synthesized containing carbon and nitrogen isotopes have been listed in Table I, together with references to the papers which describe the syn-

TABLE I

Synthetic Compounds Containing Isotopes of Carbon and Nitrogen
(Amino acids)

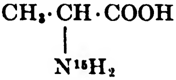
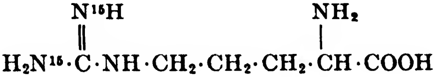
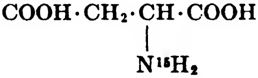
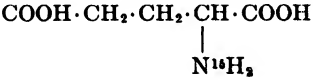
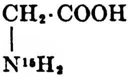
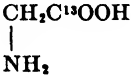
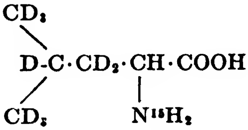
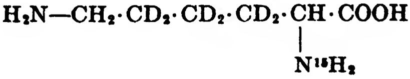
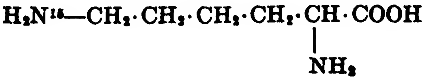
Name	Formula	Reference
DL-Alanine	 $CH_3\cdot\underset{N^{15}H_2}{\underset{\vert}{CH}}\cdot COOH$	(110, 124)
L(+)-Alanine	As above	(124)
DL-Arginine; also L(+)-arginine	 $H_2N^{15}\cdot\overset{N^{15}H}{\overset{\Vert}{C}}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot\overset{NH_2}{\overset{\vert}{CH}}\cdot COOH$	(23)
DL-Aspartic acid	 $COOH\cdot CH_2\cdot\underset{N^{15}H_2}{\underset{\vert}{CH}}\cdot COOH$	(110)
DL-Glutamic acid	 $COOH\cdot CH_2\cdot CH_2\cdot\underset{N^{15}H_2}{\underset{\vert}{CH}}\cdot COOH$	(110)
L(+)-Glutamic acid	As above	(124)
Glycine	 $\underset{N^{15}H_2}{\underset{\vert}{CH_2}}\cdot COOH$	(110, 149)
	 $\underset{NH_2}{\underset{\vert}{CH_2}}C^{13}OOH$	(82, 108)
DL-Leucine		(110)
L(−)-Leucine	As above	(112)
D(+)-Leucine	As above	(112, 86)
DL-Lysine; also L(+)- and D(−)-lysine	 $H_2N—CH_2\cdot CD_2\cdot CD_2\cdot CD_2\cdot\underset{N^{15}H_2}{\underset{\vert}{CH}}\cdot COOH$	(155)
DL-Lysine	 $H_2N^{15}—CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{NH_2}{\underset{\vert}{CH}}\cdot COOH$	(53)

TABLE I.—(*Continued*)

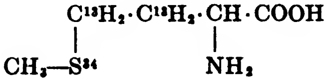
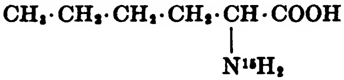
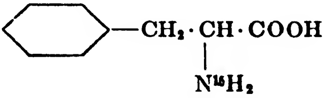
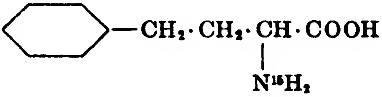
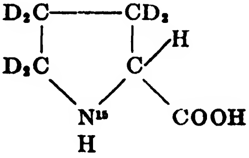
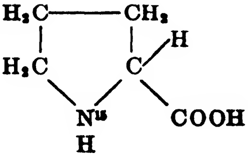
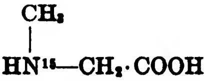
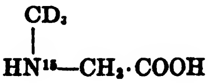
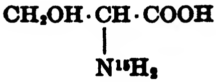
Name	Formula	Reference
DL-Methionine	 $\underset{\underset{CH_3—S^{34}}{\vert}}{C^{13}H_2}\cdot C^{13}H_2\cdot \underset{\underset{NH_2}{\vert}}{CH}\cdot COOH$	(65)
DL-Norleucine	 $CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot \underset{\underset{N^{15}H_2}{\vert}}{CH}\cdot COOH$	(110)
DL-Phenylalanine		(110)
DL-Phenylamino-butyric acid; also L(+)- and D(−)-phenylaminobutyric acid		(146)
DL-Proline; also L(−)-proline		(136)
		(124)
Sarcosine	 $\underset{}{HN^{15}}{\overset{\overset{CH_3}{\vert}}{}}—CH_2\cdot COOH$	(20)
	 $\overset{\overset{CD_3}{\vert}}{HN^{15}}—CH_2\cdot COOH$	(150)
DL-Serine	 $CH_2OH\cdot \underset{\underset{N^{15}H_2}{\vert}}{CH}\cdot COOH$	(133)
L(−)-Serine D-(+)-Serine	As above	(133, 124)

TABLE I.—(*Continued*)

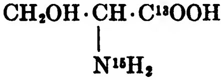
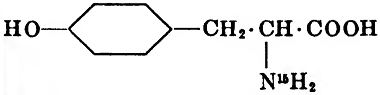
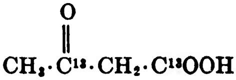
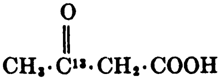
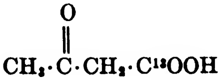
Name	Formula	Reference
DL-Serine...........	 $CH_2OH\cdot\underset{\underset{N^{15}H_2}{\vert}}{CH}\cdot C^{13}OOH$	(124)
DL-Tyrosine.........		(110)
Carboxylic acids (other than amino)		
Acetic acid..........	$CH_3\cdot C^{11}OOH$	(34)
	$CH_3\cdot C^{14}OOH$	(6)
	$CH_3\cdot C^{13}OOH$	(108, 137)
	$CD_3\cdot C^{13}OOH$	(19)
	$C^{13}H_3\cdot COOH$	(2)
Acetoacetic acid....	 $CH_3\cdot\overset{\overset{O}{\Vert}}{C^{13}}\cdot CH_2\cdot C^{13}OOH$	
	 $CH_3\cdot\overset{\overset{O}{\Vert}}{C^{13}}\cdot CH_2\cdot COOH$	(108)
	 $CH_3\cdot\overset{\overset{O}{\Vert}}{C}\cdot CH_2\cdot C^{13}OOH$	
Butyric acid........	$CH_3\cdot CH_2\cdot CH_2\cdot C^{11}OOH$	(34)
	$CH_3\cdot CH_2\cdot CH_2\cdot C^{14}OOH$	(6)
Caproic acid........	$CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot C^{14}OOH$	(6)
Fumaric acid........	$HOOC^{11}\cdot CH{=}CH\cdot C^{11}OOH$	(1)
Lactic acid..........	$C^{11}H_3\cdot C^{11}H(OH)\cdot COOH$	(44, 143)
	$CH_3\cdot CH(OH)\cdot C^{11}OOH$	(44)
Oxalic acid..........	$HOOC^{11}\cdot C^{11}OOH$	(71)

TABLE I.—*(Continued)*

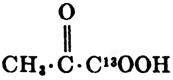
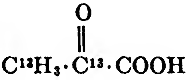
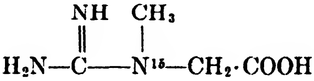
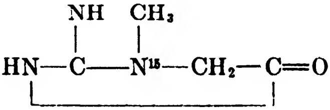
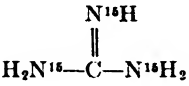
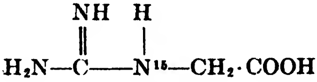
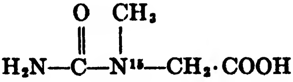
Name	Formula	Reference
Propionic acid.......	$CH_3\cdot CH_2\cdot C^{14}OOH$	(78)
	$CH_3\cdot CH_2\cdot C^{11}OOH$	(34, 77)
β-Hydroxypropionic acid........	$CH_2OH\cdot CH_2\cdot C^{11}OOH$	(77)
Pyruvic acid........	 $CH_3\cdot\overset{\overset{O}{\|}}{C}\cdot C^{13}OOH$	(108)
	 $C^{13}H_3\cdot\overset{\overset{O}{\|}}{C^{13}}\cdot COOH$	(108)
Succinic acid.......	$HOOC^{11}\cdot CH_2\cdot CH_2\cdot C^{11}OOH$	(1)
	Creatine, urea and related compounds	
Creatine............	 $H_2N^{15}{-}\overset{\overset{N^{15}H}{\|}}{C}{-}\overset{\overset{CH_3}{\vert}}{N^{15}}{-}CH_2\cdot COOH$	(24)
	 $H_2N{-}\overset{\overset{NH}{\|}}{C}{-}\overset{\overset{CH_3}{\vert}}{N^{15}}{-}CH_2\cdot COOH$	(20)
Creatinine..........		(20)
Guanidine..........	 $H_2N^{15}{-}\overset{\overset{N^{15}H}{\|}}{C}{-}N^{15}H_2$	(83)
Guanidinoacetic acid.....	 $H_2N{-}\overset{\overset{NH}{\|}}{C}{-}\overset{\overset{H}{\vert}}{N^{15}}{-}CH_2\cdot COOH$	(23)
Hydantoïc acid.....	 $H_2N{-}\overset{\overset{O}{\|}}{C}{-}\overset{\overset{H}{\vert}}{N^{15}}{-}CH_2\cdot COOH$	(23)
Methylhydantoic acid.............	 $H_2N{-}\overset{\overset{O}{\|}}{C}{-}\overset{\overset{CH_3}{\vert}}{N^{15}}{-}CH_2\cdot COOH$	(23)

TABLE I.—*(Continued)*

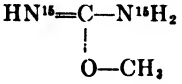
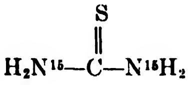
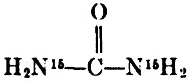
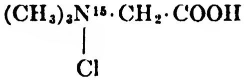
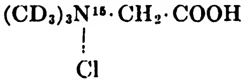
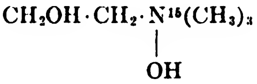
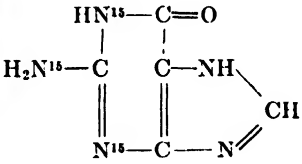
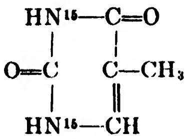
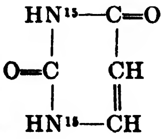
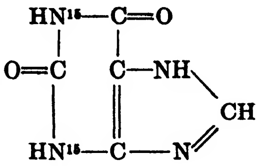
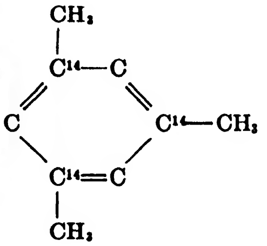
Name	Formula	Reference
O-Methylisourea....	 $HN^{15}{=}\underset{\underset{O-CH_3}{\vert}}{C}{-}N^{15}H_2$	(23)
Thiourea............	 $H_2N^{15}{-}\overset{\overset{S}{\Vert}}{C}{-}N^{15}H_2$	(83)
Urea................	 $H_2N^{15}{-}\overset{\overset{O}{\Vert}}{C}{-}N^{15}H_2$	(23)
Betaine.............	 $(CH_3)_3\underset{\underset{Cl}{\vert}}{N^{15}}\cdot CH_2\cdot COOH$	(132)
	 $(CD_3)_3\underset{\underset{Cl}{\vert}}{N^{15}}\cdot CH_2\cdot COOH$	(149)
Choline............	 $CH_2OH\cdot CH_2\cdot\underset{\underset{OH}{\vert}}{N^{15}}(CH_3)_3$	(23, 29)
Ethanolamine.......	$CH_2OH\cdot CH_2{-}N^{15}H_2$	(23)
Guanine............		(83)
Thymine............		(83)
Uracil..............		(83)

TABLE I.—(*Continued*)

Name	Formula	Reference
Xanthine		(83)
Acetylene	$HC^{11}\equiv C^{11}H$	(44)
Barium carbide	BaC^{11}_2	(44)
Cyanamide	$N\equiv C—N^{15}H_2$	(24)
Cyanide	$KC^{11}N$	(44)
1,3,5-Trimethylbenzene		(57)

In instances where more than one atom in a molecule is labeled, the actual isotope abundance may be sufficiently low (this is true mainly in the case of C^{11} and C^{14}) so that very few single molecules contain the isotope in more than one position. Thus, the α-β labeled lactate $C^{11}H_3 \cdot C^{11}HOH—COOH$ actually consists largely of a mixture of molecules labeled in the α-position and molecules labeled in the β position. Furthermore, if a molecule contains isotope in one of several symmetrical positions, although each single molecule may have only one position labeled, a large number of such molecules may be regarded as having all positions labeled, since it is not possible to distinguish between them. Thus, there is no practical difference between $C^{11}OOH \cdot CH{=}CH \cdot COOH$ and $C^{11}OOH \cdot CH{=}CH \cdot C^{11}OOH$.

thetic procedure. An effort has been made to be complete, but most intermediates have not been listed because of space limitations. Furthermore, the table does not include substances prepared by biological synthesis, since such compounds have not yet been employed extensively in tracer work.[1]

As indicated in Table I, several of the amino acids labeled with nitrogen

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[1] In biological synthesis, the living organism may be employed to produce the desired compound. The possibilities of using procedures of this type have been recognized (5, 6, 73), and biological synthetic methods may well be used more extensively in the future, especially when the isotopes are more abundantly available.

have been resolved into both isomers. Such resolutions are often difficult to achieve chemically without large losses. The procedure employed in these cases deserves some mention, since it is peculiar to the use of isotopically labeled compounds. It is known as the "washing out" technique, and can be employed wherever it is desirable to separate one labeled component completely from another labeled component. As the name implies, the undesirable component is "washed out", by first diluting it with a suitable quantity of the same substance in unlabeled form. Partial separation of the components by chemical or physical methods is then employed, followed by as many repetitions as are necessary. It does not matter if the total quantity of the undesirable component is undiminished, since each dilution results in a diminution of the labeled impurity. This procedure may be used not only to prepare substances for experiments, but also to insure the absence of any undesirable impurity from compounds which are later isolated for analysis. For example, in work on CO_2 fixation, where positive proof was desired that CO_2 had been converted to organic form, the absence of isotopic CO_2 itself from the examined material could be insured by adding normal CO_2, flushing it out, and demonstrating that any number of repetitions of this procedure had no effect on the isotope content of the substance tested.

(*b*) *The Isotope Dilution Technique.* Another procedure peculiar to the use of isotopically labeled compounds has been developed by Rittenberg and Foster (92) and independently by Ussing (141), for the quantitative determination of substances present in mixtures. It has been called the isotope dilution technique, since it depends, in principle, only on a determination of the amount of dilution which occurs when a substance which contains any stably bound isotope is mixed with an unknown amount of the same substance free from isotope. The procedure is applicable in any case in which it is possible to prepare the given compound in suitably labeled form, and to isolate an analytically pure sample of the compound from the mixture to be analyzed. The great advantage of this method is that it is not necessary to make a quantitative separation of the substance to be analyzed. Any amount of highly purified sample will do, provided it is sufficient for isotope determination. The amount of the substance (y) in the mixture to be analyzed can then be calculated from the equation

$$y = \left(\frac{C_o}{C} - 1\right) X$$

where X is the amount of the original substance added and C_o is its isotope content, whereas C is the isotope content of the substance isolated. Amounts should be expressed as mole units, calculated with due consideration for the effect of the isotope on the molecular weight. If such effect is small, weight units may be employed.

The isotope dilution technique shares with some biological analytical methods the advantage of being applicable to a determination of one of a pair of optical isomers. It has been used to measure the amount of D- and L-glutamic acid in malignant tumors (56) and has indicated that reports of the existence of extensive amounts of D-glutamic acid in tumor tissue were erroneous (96).

The nature of the nitrogenous constituents of tissue phosphatides has also been studied by the isotope dilution technique (36, 37). Phospholipides from a variety of sources were analyzed for choline and ethanolamine. These analyses indicated the presence of unknown nitrogenous constituents in several phosphatide preparations since the choline content was lower than the total non-amino nitrogen, and the ethanolamine content was lower than the total amino nitrogen. Analysis of amino acids present in crystalline bovine serum albumin and human serum albumin (123) as well as hemoglobin and β-lactoglobulin (54) have also been reported. The isotope dilution technique has likewise been applied to the determination of the total amount of a given amino acid in an animal body under circumstances where such data were desired in connection with the interpretation of the data obtained in labeled amino acid feeding experiments.

Within the last 2 years, Keston, Udenfriend and Cannan (64) have devised a very sensitive modification of this analytical procedure, and described its application to the analysis of amino acids in a mixture. In the modified method, a labeled substance which will form a stable derivative of the compound to be analyzed is added first. The isotope content is C_r. A great excess, W, of unlabeled derivative is then added, and the derivative is purified to constant isotope concentration, C_c. The amount of derivative initially present is then equal to $W\ (C_c/C_r)$.

3. *Some General Considerations of the Tracer Technique in Relation to Carbon and Nitrogen*

The basic principle of the tracer technique as applied to the intact animal organism may be stated as follows. A substance containing an isotopic label is administered, and, after a suitable length of time, the tissues and excreta are examined for their isotope content. Entire organs may be examined, if this is possible, or various chemical fractions of any desired and feasible degree of purity may be separated for isotope analyses. Results obtained in such fashion provide conclusive evidence that an isotopic element administered in a given form may be converted in the animal organism to the particular form in which the isotope is found.

Proper use of the isotope technique can, however, yield much more information. Subsequent sections of this paper will describe procedures and calculations from the data, in order to show in some detail how various conclusions have been drawn in regard to the chemical reactions which occur *in vivo* in the animal organism.

The use of each isotope presents its own peculiar problems. Each has its own advantages and disadvantages. In general, one may say that the principal tracer for organic compounds in biological systems is a carbon isotope, since this element provides the structural skeleton of the organic molecules found in living matter. When the biochemist asks whether one compound is a precursor of another, he usually means, is the carbon skeleton of the one converted into the carbon skeleton of the other. Such a question can be answered most directly by labeling with carbon isotopes.

When nitrogen is used as a label, it can be used to trace the nitrogen alone, unless there is reason to suppose that nitrogen remains attached to the carbon atom to which it was originally anchored. If it does not remain attached, it is of interest to know whether the break can be attributed to biological action, or may be expected to occur spontaneously. It has been demonstrated by direct tests with N^{15}, that the nitrogen to carbon bond is stable under ordinary *in vitro* conditions in the absence of enzymes and that breaks which occur may, therefore, be regarded as biological reactions (63). These reactions occur with such frequency however, that nitrogen cannot be regarded as a tracer for the carbon chain except in occasional circumstances. There is an advantage, often, in labeling a compound with both tracers.

A discussion of the application of heavy hydrogen, D, to biological problems is not included in this paper, except in the cases where deuterium has been used in conjunction with N^{15} and C^{13}. For special considerations which have to be taken into account in using D, the reader is referred to the literature (118). It should be pointed out that all isotopes, N^{15}, C^{11}, C^{13}, C^{14} and D may be used to label the same molecule. The judicious use of combinations of labels may give far more information than the use alone of any single one.

The isotope tracer technique cannot be regarded as a tool which will solve all problems in intermediary metabolism. By itself it could solve very few, especially when the finer details of chemical mechanisms are sought. It is common practice to bring other information to bear in the interpretation of data obtained with labeled compounds. The isotope tracer technique has, however, one advantage which gives it a preëminent position as a biochemical tool. It can be applied under conditions which are completely physiological. In the case of the heavy isotopes, the organism is accustomed to C^{13} and N^{15} in normal amounts. The organism does not differentiate between the light and heavy isotopes. There is no reason to suppose that the organism will handle N^{15} any differently from N^{14}, or C^{13} differently from C^{12}. The course of these labeled atoms through the body must, therefore, be regarded as normal. In the case of the radioactive isotopes, although they are foreign to the body, there is also no reason to suppose that they are handled in a manner different from that for their normal counterparts. In short, wherever the isotope tracer technique can be applied to demonstrate the occurrence of a particular reaction or reaction sequence in the intact organism, the technique itself is the arbiter of whether the results obtained are "truly physiological".

4. Methods of Calculating and Presenting Data

An examination of the literature reveals that the data obtained in biological tracer experiments have been expressed in a variety of units. Such differences often entail difficulty in comparison of data. Since this paper will contain compilations of results from many different experiments, these results will be expressed in one form only in order to facilitate comparison. The form chosen is the *per cent* of the isotope present, calculated on the basis of the isotope in the administered compound taken as 100. In the case of the stable isotopes, only excess over normal is considered. The significance of this choice may be explained best in terms of a presentation of the various units in which results have been expressed in the literature.

In the case of the stable isotopes, the mass spectrometer analyses are recalculated first to give *atom-%* of a heavy isotope.

$$\text{Atom-\% } N^{15} = \frac{\text{Number of } N^{15} \text{ atoms} \times 100}{\text{Number of } N^{15} \text{ atoms} + \text{number of } N^{14} \text{ atoms}}.$$

$$\text{Atom-\% } C^{13} = \frac{\text{Number of } C^{13} \text{ atoms} \times 100}{\text{Number of } C^{13} \text{ atoms} + \text{number of } C^{12} \text{ atoms}}.$$

Results obtained in biological experiments are sometimes expressed directly in this manner. The advantage of this mode of presentation is that the *per cent* error may be seen directly. Figures for N^{15} are accurate to a value of about 0.003 (93). Figures for C^{13} are accurate to a value of 0.01 (157). A further advantage of this form of presentation lies in the fact that no error is introduced by the assumption of incorrect values for normal abundance of the heavy isotope. Such errors cannot be large. There are, however, small differences in the normal abundance figures reported in the literature, especially for carbon (62). A disadvantage of the method exists, on the other hand, in the fact that only the excess isotope content above the normal is significant in the interpretation of results. For this reason it is more frequently customary to subtract the normal heavy isotope abundance, and give results as *atom-% excess*.

Atom-% N^{15} excess = atom-% N^{15} found, minus atom-% N^{15} in normal nitrogen.

Atom-% C^{13} excess = atom-% C^{13} found, minus atom-% C^{13} in normal carbon.

In normal nitrogen the atom-% N^{15} excess has been measured to be 0.368. In naturally occurring carbon the atom-% C^{13} excess has been reported to be 1.06 to 1.09. Figures for atom-% excess still show the error directly.

Both these modes of presentation have the disadvantage that the figures vary directly with the isotope content of the administered compound. To compare experiments in which different isotope concentrations are employed, it is necessary to eliminate the variable introduced in this fashion. A convenient procedure is to calculate the excess isotope content on the basis of concentration in the administered form taken as 100. Such figures represent the value of the isotope concentration relative to the isotope concentration in the labeled compound in which the isotope is introduced. These values are expressed as *per cent.*

Per cent excess N^{15} (or C^{13}) on the basis of the administered form taken as 100, $= 100 \times \frac{C}{C_o}$, where C is the atom-% N^{15} (or C^{13}) excess found in the substance analyzed, and C_o is the atom-% N^{15} (or C^{13}) excess in the administered form.

Such figures no longer show the error directly. They do, however, indicate the *per cent* of the atoms in the substance analyzed, which must have been derived from the administered labeled atoms. Thus, a value of 5 or 10 for N^{15} indicates that 5% or 10%, respectively, of the nitrogen in the examined compound is derived from the administered N^{15}. Unless otherwise specified, all results given in this paper are expressed in such units.[2]

Data obtained with radioactive isotopes are often given as counts per minute, as total radioactivity in absolute units, or as *per cent* of a standard. Since C^{11} and C^{14} are not present normally, no consideration of normal amounts applies. Results obtained with the radioactive isotopes will be given in the same units as were chosen for the heavy isotopes, provided

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[2] To avoid ambiguity, the following convention will be adopted. The isotope content of a particular compound will be averaged over the entire molecule. Thus, though a compound labeled with carbon may have the label only in certain positions, the calculations will be made for the average isotope content of all the carbon atoms in the molecule. Similarly, the isotope content of a compound having more than one nitrogen atom, will be given as the average isotope content of all the nitrogen, even though the label is known to be present in only one position. Exceptions to this convention will be explicitly indicated.

recalculation of the data in the literature is possible. *Per cent* carbon in substance analyzed derived from administered labeled carbon is equal to

$$100 \times \frac{\text{Amount radioactivity (in any units)/mole carbon in substance isolated}}{\text{Amount radioactivity (in the same units)/mole carbon in the administered labeled compound}}.$$

Such a calculation can only be made, of course, if the amount of administered and isolated carbon is known. Since it is not necessary to determine concentration of radioactivity per atom in order to measure it, these figures may not always be available. Measurement of the heavy isotopes necessitates determination of concentration per atom, because of the presence of the normal amounts. This difference in methodology is mainly responsible for the different forms in which the data have been given.

Comparison of data all calculated in the same fashion necessitates a note of warning, however. Expression of results in the same units still does not indicate directly comparable significance. It is obvious, perhaps, that in animal experiments, the condition of the animal and the length of time of the experiments will produce differences in the *per cent* of an isolated compound derived from the administered compound. Another variable which must be considered is the total quantity of the labeled compound administered in relation to the weight of the animal. This may have very profound effects on results, since it determines the extent to which a labeled compound and its derivatives are diluted by nonisotopic molecules of the same sort in the animal body. To be strictly comparable, therefore, experiments must employ animals in the same condition for the same length of time, and the same amount of labeled substance must be fed per unit weight. Use can be made of measurements of the dilution to determine how much of a given metabolite the animal produces in a certain time. Such methods will be shown in the subsequent section.

III. The Metabolism of Nitrogen Compounds

1. *The Rapid Transfer of Nitrogen in the Living Organism—A General Phenomenon*

(*a*) *Transfer after Ammonia Administration.* The various organisms which have been used for experiments with N^{15} include the rat, mouse, rabbit, guinea pig, dog, human, pigeon, the tobacco plant and buckwheat plant, and the nitrogen-fixing bacterium, *Azotobacter vinelandii.* Though attention is focused, in this review, on the intact animal organism, it seems appropriate to point out that in plants and bacteria, as well as in animals, there is a fundamental similarity in the general fate of administered ammonia nitrogen. The data in Table II have been collected to illustrate this point.

This table shows that when N^{15} is administered as an ammonium salt to the mature or the immature rat, the rabbit, the pigeon, the tobacco plant or the bacterium, *Azotobacter*, the labeled nitrogen can subsequently be found widely distributed in the amino acids of the proteins of the organism. Results are given (as previously mentioned) in such units that they represent the minimum *per cent* of each fraction which must have been derived from the labeled NH_3. The table shows only some of the amino acids which have been examined. Although not all protein constituents have been accounted for in any experiment, it should be apparent that the following generalization holds in all species investigated: there must be mechanisms in every living organism which provides for the ready conversion of ammonia nitrogen to protein nitrogen, either in the form of amide groups, of amino groups present in the protein as peptide bonds, or possibly in other positions of nitrogen-containing amino acid residues.[3]

TABLE II

Distribution of N^{15} in Constituents of Various Organisms after Administration of $N^{15}H_3$

(All results calculated on basis of 100% N^{15} excess in compound administered)

Species	Immature rat (55)	Mature rat (95)	Rabbit (153)	Pigeon (7)	Tobacco plant (145)	*Azotobacter vinelandii* (35)
Duration	5 days	9 days	2½ hrs.	4½ days	72 hrs.	15 minutes
Form of N^{15} given	Ammonium citrate	Ammonium citrate	Ammonium citrate	Ammonium citrate	Ammonium chloride	Ammonium sulfate
Organ examined	Total body	Liver protein	Liver protein	Mixed internal organ protein	Leaf protein	Whole organism
Compound examined:						
Protein—N	....	1.1	0.07	....	8.2	
Amide—N	9.4	1.1	0.11	6.2	17.9	3.0
Glutamic acid	7.0	2.3	0.17	2.0	10.6	7.9
Aspartic acid	5.5	1.5	....	2.7	9.4	2.5
Arginine	2.7	1.3	....	0.7	7.1	2.0
Histidine	1.0	....	....	0.6	7.4	1.9
Glycine	4.1	0.9				
Lysine	0.2	....	....	....	....	2.4
Cystine	....	....	0.03			

Numbers in parenthesis under species give references to papers from which data were obtained.

This result would be expected in the case of the tobacco plant and *Azoto-*

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[3] There are some exceptions to the positions in which the $N^{15}H_3$ is distributed. For example, in Table II, the figure for lysine nitrogen in the immature rat is too low to be significant. Such exceptions will be discussed later.

bacter since it is known that both these species can derive all their nitrogen requirements from ammonia, *i.e.*, they can grow readily on media containing ammonium salts as the only source of nitrogen. There was no reason to be certain in the case of the rat, rabbit, and pigeon, however, that the fate of administered ammonia would be qualitatively similar to that in plants and bacteria. The results shown in Table II indicate that such is actually the case.

These conclusions are of a qualitative nature. When the data in Table II are examined from a quantitative point of view, it must be remembered that the experiments with the various species are not directly comparable. The time of the experiment (as indicated in the table), and the amount of $N^{15}H_3$ administered per unit weight of each species, varied considerably. Thus, the low figures observed in the case of the liver proteins of the rabbits as compared to those in the liver proteins of the rats can be attributed in large part to the difference in the length of time of the experiments (2.5 hours as compared with 5 and 9 days) and do not necessarily indicate that there is a real difference in the rate at which ammonia nitrogen is converted to protein nitrogen in the two species. In the case of the plant and bacteria, furthermore, the high values obtained, even in a short time, are probably due in part to the fact that the labeled ammonia administered represented a higher proportion of the total N available to the organism than was the case in the animal experiments. This, of course, does not mean that part of the differences observed between species may not be due to large differences in metabolic rates. It simply means that the experimental data available are insufficient to warrant quantitative conclusions of such a nature. One generalization of a semi-quantitative nature may, however, be drawn for all species examined, namely, that the nitrogen isotope is distributed at a rapid rate even when there is no increase of protein nitrogen. This fact is particularly obvious in the case of the mature rat, rabbit and pigeon. These animals were in nitrogen equilibrium so that no net protein synthesis occurred. The immature rats were on an inadequate diet and actually lost weight during the course of the experiment. Yet ammonia nitrogen entered the proteins at a sufficient rate to indicate a considerable protein turnover. The significance of this conclusion will be discussed in more detail in the section on protein synthesis (page 67). Furthermore, differences observed among the various amino acids of the proteins of each species, especially the high values generally obtained for the amide group, glutamic acid, and aspartic acid, will also be discussed in some detail in a later section.

(*b*) *Transfer after Administration of Amino Acids.* The animal organism obtains most of the nitrogen in its food in the form of various amino acids. Some of these are essential and must be furnished in the diet if the animal is to grow and survive. Others are non-essential and need not be furnished in the diet, indicating that the animal is able to synthesize them from other precursors. Provided it is on an adequate diet, the mature healthy animal is in nitrogen equlibrium, *i.e.*, it excretes as much nitrogen as it consumes. It is of interest, under these circumstances, to examine the fate in the organism of nitrogen furnished to the animal in the form of various amino acids, both essential and non-essential.

A large body of data bearing on this point has been accumulated by the Columbia group. Some of their results have been compiled in Table III. The organism used in every case was the rat and most of the experiments were done under comparable conditions. Equivalent amounts of the different amino acids were fed for a 3-day period, except in the case of DL-tyrosine, which was fed for 10 days, and lysine, which was fed for 4 days. The rats fed lysine were immature but all others were mature rats on an adequate stock diet. The N^{15} was present in the amino group of the fed amino acid in every case except arginine, in which the amidine group was labeled.[4] The data shown in the table give only a portion of the results obtained in these amino acid feeding experiments. They are sufficiently extensive, however, to indicate the experimental basis for the following conclusions which the authors have drawn relative to the fate of amino acid nitrogen in the animal body.

First, the distribution of N^{15} was qualitatively similar to that observed after ammonia feeding, *i.e.*, N^{15} introduced in the form of a particular amino acid found its way into most of the amino acids examined. It should be emphasized that these amino acids are present in the body as protein constituents and that the process of N^{15} distribution occurs under conditions in which there is no net synthesis of protein.

Secondly, proteins of various tissues showed marked differences in the rate at which the administered label entered the protein molecule. In general, it can be seen from Table III that proteins of the liver and of the other internal organs incorporated the label much more rapidly than the proteins of the carcass, *i.e.*, muscle, skin, bone and connective tissues.

Finally, after the feeding of naturally-occurring or L-amino acids, the protein constituent which contained the highest N^{15} concentration was invariably the amino acid which was fed. The next highest isotope content was generally found in the amide nitrogen, and in the aspartic and glutamic acid. On the other hand, when the unnatural amino acids were administered the corresponding natural amino acid isolated from the tissues did not contain a higher isotope content than the other amino acids of the tissues, but the highest isotope contents were generally found in the amide nitrogen and in the aspartic and glutamic acids.

(*c*) *The Role of Amide Groups, Glutamic Acid and Aspartic Acid in Nitrogen*

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[4] Some of these amino acids were also labeled with deuterium. Formulae for all amino acids employed in the experiments summarized in Table III can be found in Table I.

TABLE III

Distribution of Nitrogen in Protein Constituents of Rats Fed Amino Acids Labeled with N^{15}

(All results calculated on basis of 100% N^{15} excess in compound administered)

Compound fed	DL-Tyrosine (111)		DL-Serine (133)	Glycine (85)		L(−)-Leucine (112)		D(+)-Leucine (86)		L(+)-Lysine (155)	D(−)-Lysine (87)	L(−)-Proline (136)		L(+)-Arginine (13)	
Tissue examined	Liver	Carcass	Internal organs†	Liver	Carcass	Liver	Carcass	Liver	Carcass	Total body	Total body	Liver	Internal organs‡	Liver	Internal organs‡
Compound isolated from protein:															
Protein—N	1.9	0.6	1.2	1.40	0.29	0.93	0.46	0.89	0.24	1.98	0.62	0.4	1.5	0.33	0.51
Amide—N	2.2	0.6	1.9	1.44	0.56	0.78	....	2.45	0.77	1.18	1.18	0.47	0.78	0.17	
Glutamic acid	2.7	0.5	1.0	0.89	0.27	1.85	0.89	1.33	0.17	1.52	0.44	0.52	1.60	0.36	
Aspartic acid			....	0.73	0.20	1.16	0.70	1.03	0.24	1.00	0.62	0.39	1.21	0.21	
Arginine	1.5	....	....	0.78	0.09	0.89	0.24	1.19	....	0.78	0.48	0.34	0.95	1.13	3.03
Glycine	....	....	....	8.86	1.04	0.76	....	0.55	0.14						
Tyrosine	15.5	4.9	....	0.47	0.13	0.50	0.20	....	0.11	....	....	0.34			
Lysine	0.1	....	....	....	....	0.06	....	....	....	13.0	0.18	....	....	....	0.07
Leucine	....	....	....	....	....	7.92	1.90	0.77	0.25						
Cystine	....	....	5.1	....	....	....	....	....	....	....	....	0.34			
Proline	....	....	....	....	....	....	....	....	....	....	....	3.36	15.6		
Histidine	0.7	....	....	....	....	....	....	....	....	....	....	....	....	....	0.12
Hydroxyproline	....	....	....	....	....	....	....	....	....	....	....	0.82			
Hydroxy-amino acids*	....	....	2.4												

* NH_3 liberated by periodate.

† Liver, gastrointestinal tract, kidney, spleen, testes, heart, lungs.

‡ Heart, lungs, spleen, testes.

Carcass protein is muscle, skin, bones and connective tissue.

Rats fed lysine were immature. All others were mature rats on adequate stock diet.

DL-Tyrosine was fed for 10 days. L(+)Lysine was fed for 4 days. All other substances were fed for 3 days. Amount labeled compound fed was in every case about 1.5–1.7 m*M*/rat/day.

Numbers in parenthesis after compound fed give references to papers from which data were obtained.

Transfers. The relatively high rate at which N^{15} appears in amide nitrogen and in the nitrogen of the dicarboxylic amino acids indicates that the rate at which the nitrogen-carbon bond is broken and reformed in these particular substances, must be higher than that which obtains in other amino acids. Such a high turnover rate is consistent with a central role of these substances in nitrogen transfer, a fact already indicated by *in vitro* studies which have shown that glutamic acid and aspartic acid are important constituents of transaminating systems (31, 85) and that the amide grouping may represent an important stage in ammonia uptake in the living organism (3). The data in Tables II and III are not sufficient, however, to warrant any conclusion about the precise function of the groups discussed. They simply indicate a high rate of breakdown and resynthesis.

More extensive data on the turnover of these groups in comparison with that of other nitrogenous compounds have been obtained by Shemin and Rittenberg (126) who measured the N^{15} content of various tissue constituents over a period of 7 days after cessation of the regular 3-day feeding period. The labeled amino acid fed to the rats was glycine. Some of the results obtained are shown in Figs. 1 to 4. The first 3 Figures indicate the change with time of the isotope levels observed in various fractions of the liver (Fig. 1), the internal organs (Fig. 2), and the carcass (Fig. 3). It is apparent that in the liver and the internal organs where initial isotope values were high, there was a general tendency for high levels to fall, and low levels to rise or to fall only slightly. In the carcass, where initial values were low, all fractions showed a tendency to increase, with the exception of the non-protein nitrogen. In all tissues, however, the N^{15} content of glycine remained higher than that of any other constituent. Next came the N^{15} of the non-protein nitrogen and amide nitrogen, which fluctuated in relation to each other but always gave values higher than the remaining constituents. Next in order of decreasing N^{15} content came glutamic acid and total protein nitrogen. These values showed an interesting tendency to run parallel. Since the protein nitrogen value includes the high N^{15} content of the glycine, it is apparent that glutamic acid must have contained a considerably higher N^{15} concentration than the average of the remaining amino acids, other than glycine. This was borne out by the considerably lower values observed for arginine, tyrosine, and histidine.

2. The Synthesis and Breakdown of Proteins

The transfer of nitrogen from ammonia and from amino acids to the various amino acids of the body proteins indicates that the proteins of the living animal must be labile molecules subject to constant breakdown and resynthesis (88, 109, 113). Otherwise, it would be impossible for the N^{15} to enter, since these reactions occur when the total amount of protein is not increasing. One must picture a mechanism involving continuous rupture and reclosure of peptide bonds to free the amino groups, and constant

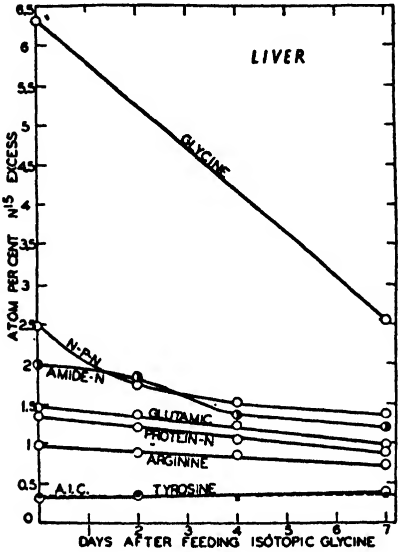

FIG. 1.

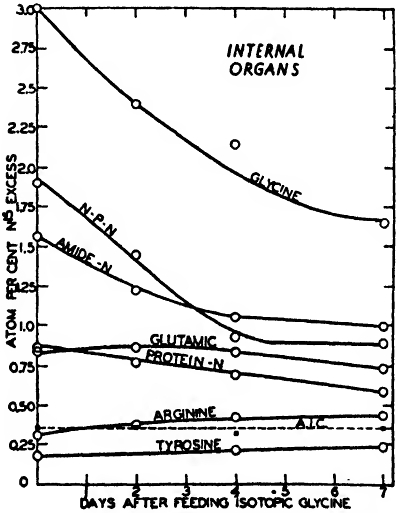

FIG. 2.

deamination followed by reamination of the amino acid residues. The detailed mechanism involved cannot be determined from the isotope data, but it is possible to obtain some information about the rate at which the reactions occur. The data in Table III have already indicated certain minimum values. Except in the case of serine, there are figures which show, for each amino acid fed, the N^{15} content of that same amino acid in the tissue proteins. These figures indicate directly the *per cent* of the protein amino acid which must have been derived from the dietary amino acid. They may be regarded as minimum figures for protein regeneration in the sense that at least this proportion of the total protein must have been broken down and resynthesized in order to admit the amino acids. Only the naturally occurring amino acids are considered, since those of unnatural configuration are necessarily deaminated before incorporation into protein, as will be discussed later. We find then, 8.88% for glycine, 7.92% for L(−)-leucine, 3.36% for L(−)-proline, and 1.13% for L(+)-arginine in the liver proteins, with considerably lower figures for carcass proteins. After feeding L(+)-lysine, 13% of the lysine in the total protein of the body was derived from dietary lysine.[5] Except in the case of lysine, these figures are all for a 3-day period. Such figures are known, however, to be too low, since they do not take into account the amount of isotope which is lost from the amino acid

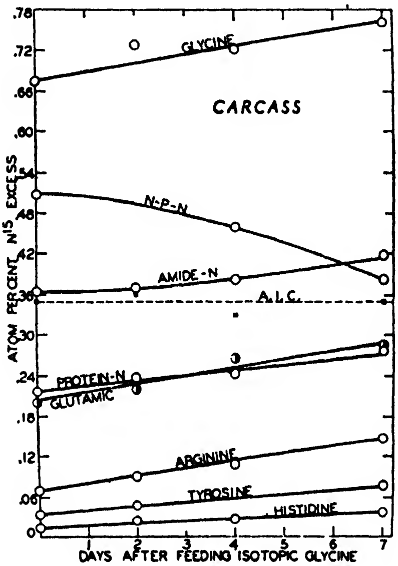

Fig. 3.

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[5] Since immature growing rats were used in this experiment, results are not directly comparable with others. Lysine occupies, however, an exceptional position among the amino acids, which will be indicated later.

by preliminary deamination and reamination before incorporation into the protein, nor the dilution of the administered labeled amino acid in the same unlabeled amino acid of the diet.

A better approximation of the protein turnover was obtained in the experiment with L(−)-leucine. This amino acid was labeled with deuterium as well as with N^{15}. Leucine is an essential amino acid. The rat cannot synthesize the carbon chain of leucine. It can, however, deaminate and reaminate the carbon chain. The deuterium content of the carbon chain of the leucine may therefore be regarded as a better label for this amino acid than is the N^{15} of the amino group. In the experiment listed in Table III, the deuterium content of the leucine which the rat consumed was 1.8 atom-% (taking into consideration the dilution in unlabeled leucine in the diet). The leucine isolated from the liver contained 0.44 atom-% D. This indicated that at least 24% $\left(\frac{0.44 \times 100}{1.8}\right)$ of the leucine originally present in the liver had been replaced by dietary leucine. Similar calculations for the leucine of carcass proteins indicated 7% replacement of leucine. To compare these figures with those derived from the N^{15} content of leucine, the values in Table III must be multiplied by 2 to take account of the fact that the animals consumed in their diet an amount of unlabeled leucine equal to the amount of labeled leucine which was added. One then finds that 15.8% of the nitrogen of liver leucine and 3.8% of the nitrogen of carcass leucine is derived from the dietary leucine.

It is also possible in an experiment of this sort, to calculate what proportion of the dietary amino acid is deposited as such in the body, provided the actual amount of leucine in the diet and in the body is known. The latter figure was determined by the isotope dilution method and found to be 183 milliequivalents (total in 4 rats). Seven *per cent* of the leucine of carcass protein (liver can be ignored since it represents a small proportion of the total N of the animal) is equivalent, therefore, to $183 \times .07 = 12$ milliequivalents of leucine. A total of 39 milliequivalents were fed. $12/39 \times 100 = 31\%$ of the total dietary leucine was, therefore, deposited directly in the body proteins in a 3-day period.

The calculation of protein regeneration rates described above is applicable only in the case of a labeled carbon chain which the animal cannot synthesize. Even then, the figures obtained assume that the deuterium content of the dietary leucine represents the deuterium content of the leucine available to the body for incorporation into protein.

Another procedure which can be applied with the feeding of non-essential amino acids avoids such an assumption. It is based on the same principle which has been applied to study creatine turnover by Bloch, Schoenheimer and Rittenberg (24) (see p. 78). The labeled amino acid is fed until adequate

N^{15} levels are established in the protein. Feeding is then discontinued, and the rate of disappearance of the label from the protein is determined. In the glycine experiments of Shemin and Rittenberg (127) where N^{15} levels were followed for 7 days after termination of glycine feeding (Figs. 1–4) the data obtained were sufficient to make an estimate of protein regeneration rates. This could be done both on the basis of the changes in N^{15} content of the glycine in the protein of a particular organ, or on the basis of the change in N^{15} content of the total protein nitrogen of an organ. For these calculations, assumptions must be made in regard to the isotope content of glycine available for replacement (in the first case) or of the average nitrogen available for replacement (in the second case). It was assumed that these figures were those of the carcass glycine and the carcass protein-nitrogen respectively.

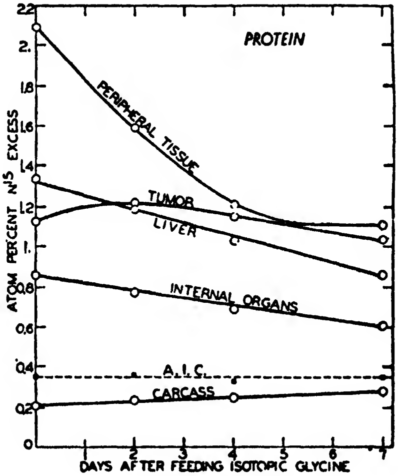

FIG. 4.

In Fig. 4, the changes in N^{15} content of liver protein are shown. It is estimated that when the N^{15} content of liver protein reaches 0.84 atom-% N^{15} excess (halfway from the initial value of 1.34 to the average of 0.35), approximately half of the N^{15} will have been transferred to the other organs and in turn be replaced by nitrogen of other organs. The time for this is about 7 days, a value in agreement with the figure for liver protein regeneration obtained with L(−)-leucine. From the glycine data graphed in Fig. 1 for liver, a half-time of 5–6 days, is estimated in similar fashion. The calculations are probably not sufficiently accurate to warrant the conclusion that this figure is significantly different from the 7 day half-life calculated on the basis of the total nitrogen. Whether these two figures, *i.e.* (1) regeneration rate with respect to a particular amino acid, and (2) regeneration rate with respect to all the amino acids, represent the same process or not, must, of course, be regarded as an open question. The process whereby the protein breaks down and is resynthesized may involve complete rupture of all peptide bonds at once, rupture of one peptide bond at a time, or any of all the intermediate possibilities. The isotope data indicate that the ruptures occur, and their average rate, but not the manner in which they occur. Calculations of rates are based on assumptions which cannot be completely correct, but the order of magnitude of the figures obtained cannot be much in error.

It must be kept in mind, however, that all calculations of protein regeneration rate hitherto described represent average values for a very complex mixture of different proteins. There is evidence (127), and indeed one would expect, that different proteins in the same tissue might show marked differences in regeneration rates. In order to determine a regeneration rate for one particular protein, it is necessary to be able to separate that protein from the other protein of a tissue. When carried out on a small scale such separations often involve great technical difficulties. In one instance, however, it has been possible to examine a protein which may be regarded as reasonably pure. This is the antibody protein to *pneumococcus* polysaccharide, Type III. Preliminary investigation by Schoenheimer, Ratner, Rittenberg and Heidelberger (114), of the rate at which plasma proteins incorporated N^{15} after the feeding of labeled amino acids indicated that the rate was not significantly different from that of liver and other internal organs. This rate, it will be remembered, is considerably larger than that of the carcass proteins. The fibrinogen, euglobulin, pseudoglobulin and albumin fractions were examined separately and also found to display no marked differences among themselves. The similarity of data obtained with plasma proteins and with liver is compatible with the view that the liver is the site of origin of the plasma proteins. When a rat was immunized with hemocyanin, the antibody protein, separated by precipitation with the antigen, showed an N^{15} uptake similar to that of the other serum proteins (115). A careful, detailed study of an antibody protein was then done with rabbits which were immunized to *pneumococcus* Type III. The nitrogen-free specific carbohydrate from the microorganism was used to precipitate the antibody. Labeled glycine was fed for a 3-day period when the total circulating antibody was declining. The results obtained are shown in Fig. 5. It is apparent that, even though the total amount of antibody was decreasing, the uptake of N^{15} during labeled glycine feeding, and the subsequent rapid drop, indicated that antibody protein was regenerated in a fashion similar to other serum proteins. The concentration of N^{15} fell from a maximum of 1.6% to half that value in about two weeks. This was regarded, then, as approximately the half-life time of the antibody protein in the rabbit. The half-life time of the other serum proteins was about the same.

In marked contrast to the results obtained in the actively immunized animal, Heidelberger, Treffers, Schoenheimer, Ratner and Rittenberg (61) found that antibody formed by one animal and injected into another to produce passive immunization, showed no interaction with dietary nitrogen. This contrast between active and passive antibody was most strikingly demonstrated in an experiment in which a rabbit fed marked glycine was rendered actively immune to Type III *pneumococcus* and passively immune to Type I. The respective specific polysaccharides were used to precipitate each antibody. The serum proteins and the Type III antibody showed a high N^{15} content, whereas the N^{15} present in the Type I antibody was negligible.

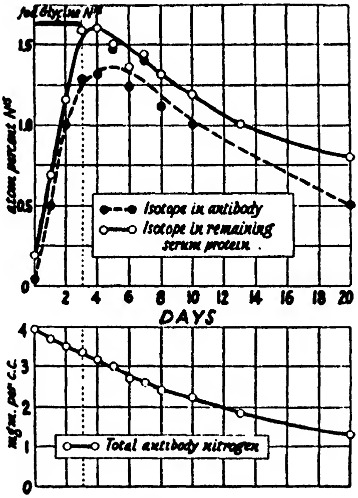

Fig. 5.

As has long been known, injected antibody disappears from the blood stream at a considerably more rapid rate than antibody formed by active immunization. This rate of disappearance seems to follow a course similar to that of injected labeled serum proteins. Fink *et al.*, in Whipple's laboratory (53), have prepared labeled plasma proteins by feeding a dog lysine labeled with N^{15} in the ϵ position (see Table I). The dog incorporated this indispensible amino acid into its plasma proteins. These were then removed from the animal. Subsequently, the labeled protein was injected into another dog and its rate of disappearance was followed. One of the advantages of employing ε-labeled lysine, was that the N^{15} concentration in the lysine could be determined directly on the nitrogen liberated by treatment of the protein with nitrous acid.

The experiments indicated that there was a rapid initial disappearance of injected protein which continued for about 48 hours. This was followed by a slower rate of fall. (With animals in a state of shock, results similar to the normal were obtained.) Whipple and his associates attribute the initial rapid phase to mixing with a labile pool of tissue proteins, and the slow phase to actual metabolic destruction. They point out that injected antibody (Heidelberger's data, (61)) showed a similar disappearance.

Since the disappearance of the N^{15} from the plasma protein was nonlogarithmic, no estimate of half-life was made. However, one can calculate roughly from the data given for the slow phase, a half life of 5–8 days for the serum proteins of the dog. In view of the inaccuracies involved, this half-life cannot be regarded as significantly different from the regeneration rate of serum and antibody proteins in the rabbit, and serum proteins in the rat. It is not meant to imply, here, that the figures are more than rough estimates. One is inclined to suspect, however, that injected antibody is handled in a similar fashion to normal serum protein, that both are subject to a breakdown of the molecule and resynthesis of the constituents back to protein, but that, in the resynthesis, the specific configuration of the antibody is lost, since the inducing antigen is absent.

3. The Synthesis and Breakdown of Purines and Pyrimidines in Nucleic Acids

When ammonium citrate was administered to pigeons and rats and the purines and pyrimidines were examined, Barnes and Schoenheimer (7) found that the turnover of these nucleic acid constituents was just as rapid as the protein turnover. The labeled nitrogen was found to be about equally distributed among both purines and pyrimidines, and both ring and amino groups were labeled.

Plentl and Schoenheimer (83) ascertained, in addition, that if purines and pyrimidines labeled with N^{15} were fed to the animals, these units were not incorporated into nucleic acids but were metabolized. The inability of the body to incorporate fed purines and pyrimidines may be due to impermeability of membranes, but the possibility cannot be excluded that the organism synthesizes purines and pyrimidines only in combination with other nucleic acid constituents. The excretory products of purines and pyrimidines will be discussed in Section *4*. What we are concerned with emphasizing here is that the nucleic acids show a rapid synthesis and breakdown in terms of their purine and pyrimidine constituents, and that even the rings are constantly opening and closing. These reactions proceed at a rate comparable with the rate of protein regeneration. The nucleic acid turnover is fastest in the liver and more rapid in intestinal tract, gonads, kidneys and pancreas than in heart and lungs. These differences are shown in Table IV. The relatively low values observed for blood make it seem unlikely that there is a transport of the nucleic acid constituents from organs where they are synthesized to organs where they are not. It would seem, rather, that all organs can carry on these syntheses.

Recent work in genetics and related subjects (58, 76, 122) has assigned to the nucleic acids a significant role in protein synthesis. It has been suggested that genes are nucleoproteins and are responsible agents for the synthesis of enzymes. The term enzyme has even been applied to a gene.

TABLE IV

Distribution of N^{15} in Purines of Various Organs of the Pigeon after Administration of $N^{15}H_3$ (7)

	Per Cent Nitrogen Derived from Administered $N^{15}H_3$
Mixed purines of:	
Lungs	1.5
Heart	1.4
Blood	1.1
Pancreas	3.7
Kidneys	3.9
Gonads	4.7
Gastrointestinal tract	7.1
Liver	8.9
Uric acid on last day	21.1

In the absence of experimental evidence, speculation may be a sterile approach. Certain points may, however, be made in regard to the terminology employed in these matters. First, in speaking of protein synthesis it is customary to imply that the process is "enzyme catalyzed". This may be correct or incorrect, depending on the definition of an enzyme. The danger spot in the classical definition seems to the author to lie in the statement that an enzyme is a catalyst. A catalyst is usually regarded as a substance which emerges unchanged from the reaction it causes. The fact that those enzymes we know are apparently real catalysts, in the sense that one enzyme molecule causes the chemical conversion of a very large number of substrate molecules, without suffering any net change during the process, does not by any means make it certain that all agents responsible for syntheses in protoplasm function in a similar way. It may not be unfruitful to take the point of view that the nucleic acids are quite directly concerned with protein synthesis, and to keep in mind that the mechanism of this synthesis with regard to interaction of agent and substrate may be quite different from the mechanism of enzyme-catalyzed reactions as we hitherto have known them. The high regeneration rate of both protein and nucleic acid may imply a great variety of possibilities of actual chemical interactions between the two groups.

The notion that nucleic acids (with or without accompanying proteins) act as agents for protein synthesis does not derive alone from genetic implications or from demonstrations that rapid protein synthesis in protoplasm is accompanied by increase in nucleic acid content (76). The alternate assumption that the synthesis of each protein in the body must be catalyzed by another protein may be reduced to an absurdity. If every protein requires another protein to catalyze its synthesis, an infinite series of proteins which catalyze the synthesis of other proteins is implied. At some place the speculator must break this hypothetical series, and it seems simpler to break it sooner rather than later. In other words, it is not unreasonable that all proteins may be synthesized by mechanisms quite different from those involved in enzyme catalyses as we know them at present, and that the nucleic acids are intimately involved in these chemical mechanisms.

4. The Formation of Nitrogenous Excretory Products

The main nitrogenous excretory products of the rat are urea, ammonia, creatinine and allantoin. By far the largest proportion, about 90%, of the nitrogen excreted is in the form of urea. In the amino acid feeding experiments, which have previously been described (Table III), it was found that very roughly one-half of the isotope fed appeared in the urine, and most of the remaining isotope was retained in the tissue proteins. The actual distribution observed is indicated in the last two columns of Table V. The figures show that the N^{15} administered in the form of D-amino acids or as the amidine group in arginine was excreted to a considerably greater extent than the N^{15} administered in the form of L-amino acids. In both cases, however, urinary urea contained an appreciable concentration of N^{15}.

(*a*) *Mechanism of Urea Formation.* Experiments conducted with the nitrogen isotope have helped considerably to clarify present views of the mechanism of urea formation. On the basis of tissue slice experiments, Krebs (69) originally proposed a mechanism which involved the combination of ammonia, CO_2 and ornithine to form citrulline, the further conversion of citrulline to arginine, and the hydrolysis of arginine to urea and ornithine, thus completing a cycle whereby the net change consisted of a combination of ammonia and CO_2 to form urea. There has been considerable objection to this proposed mechanism, partly on the basis of other tissue slice experiments, and also, perhaps, because of a reluctance to apply conclusions derived from *in vitro* experiments to the situation *in vivo.* Experiments with N^{15} labeled compounds in living rats have, however, confirmed the essential features of the mechanism.

The evidence from isotope experiments conducted by the Columbia group may be summarized as follows: (1) A large proportion of deutero-labeled ornithine fed to the mouse could be recovered as tissue arginine (38). (2) In rats, the amidine group of arginine was found to take up labeled nitrogen rapidly after ammonia or amino acids labeled with N^{15} were fed (85, 112). Comparison of the N^{15} content of the amidine group of arginine with that of the excreted urea has been made in these experiments and found to be compatible with the conversion of one to the other. For example, in the experiment in which the N^{15} content of various fractions was determined for 7 days after cessation of labeled glycine feeding, the average isotope concentration of the amidine group of arginine and of excreted urea

TABLE V

Distribution of N^{15} in Urinary Urea and Ammonia and in Body Proteins of Rats Fed Various N^{15} Labeled Compounds

(All results calculated on basis of 100% N^{15} excess in compound administered)

Substance administered	Collecting period (3 days unless otherwise indicated)	N^{15} concentration in urinary constituents			Total N^{15} in urine	Total N^{15} in body protein
		NH_3	Urea	$N^{15}H_3$/Urea N^{15}		
					Per cent	*Per cent*
Urea	....	0.34	8.09	0.043	Estimate > 95	Too low for accurate estimate
Ammonia	....	4.50	11.7	0.384		
DL-Tyrosine	....	9.85	3.35	2.94	50–60	
DL-Serine	3rd day	29.2	3.0	9.7		
Glycine*	....	1.80	2.00	0.90	40.6	44.3
L(−)-Leucine	....	3.90	2.43	1.60	27.6	57.5
D(+)-Leucine	....	15.7	4.19	3.75	58.3	34.4
L(+)-Lysine	....	2.6	2.4	1.08	23.9	65.8
D(−)-Lysine	....	4.3	1.0	4.3	69.1	21.4
L(−)-Proline	1st day	2.6	2.7	0.96		
	2nd day	3.2	3.1	1.03		
	3rd day	3.9	3.2	1.22	39.7	59.8
L(+)-Arginine	1st day	0.46	3.8	0.12		
(Amidine group	2nd day	0.55	4.8	0.11		
labeled)	3rd day	0.65	5.3	0.12	52.2	28.9

* Additional data are available in paper of Shemin and Rittenberg (126).

Data on urea obtained from reference (13). All other data obtained from corresponding references given in Tables II and III.

were estimated to be nearly the same (126). (3) When rats were fed L(+)-arginine labeled with N^{15} in the amidine group, there was a prompt excretion of almost all the labeled N as urea (13). The results obtained with arginine feeding indicated, in fact, that the amidine group of arginine contributed very little to the metabolic pool of nitrogen. Ingested urea has likewise been shown to be unavailable for body nitrogen (13) (see Table V). The formation of urea in the body is an irreversible process. Finally, it should be mentioned that in tissue slice experiments, the carbon of urea has been shown to be derived from CO_2 (50, 98).

(*b*) *Source of Urinary Ammonia.* The amount of nitrogen excreted by the mammal in the form of ammonia is variable, and small, relative to the urea nitrogen. It has been shown conclusively by isotope experiments, that urea cannot be regarded as a source of urinary ammonia. Dietary ammonia can also be excluded. The amino acids, on the other hand, apparently function as urinary ammonia precursors, probably by acting as substrates for deaminating reactions in the kidney. There is evidence from other sources to support this view (8). Some of the evidence derived from isotope experiments in regard to the source of urinary ammonia has been summarized in Table V.

This table gives the *per cent* of urea nitrogen and of ammonia nitrogen which were found to be derived from the labeled substance fed. The ratio of these values, *i.e.*, $\frac{N^{15}\text{ concentration in }NH_3}{N^{15}\text{ concentration in Urea}}$ will indicate the relative ability of the various substances fed to serve as precursors of urinary ammonia. The higher the ratio, the better the precursor. From the ratios listed in the table, it can readily be seen that the D-amino acids are the best precursors. The L-amino acids come next. The amidine group of arginine and urea are excreted as urea to a great extent, and contribute little to urinary ammonia. Even fed ammonia is converted more readily to urea than to urinary ammonia.

(*c*) *Creatine as the Source of Urinary Creatinine, and the Sources of Creatine.* Perhaps one of the most clean-cut applications of the isotope tracer technique has been made by Bloch and Schoenheimer (20, 21, 23) and Bloch, Schoenheimer and Rittenberg (24), who have used N^{15} as a label to determine the origin and fate of body creatine and of urinary creatinine. Results with the tracer technique show:

(a) All urinary creatinine is derived directly from body creatine, and the conversion of creatine to creatinine is irreversible in the body. The rate of this reaction can be expressed as 2% body creatine decomposed to creatinine per day (24).[6]

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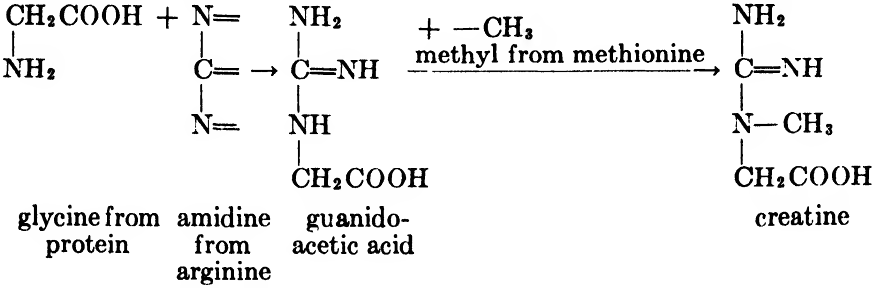
[6] Because the only change undergone to any significant extent by body creatine was its irreversible conversion to excretory creatinine, an ideal opportunity was

(b) The sources of creatine are glycine, the amidine group of arginine, and the methyl group of methionine (23).[7]

(c) Guanidoacetic acid is a precursor of creatine; sarcosine is not. (Sarcosine in the body is very rapidly demethylated to glycine (22).)

The formation of creatine may therefore be indicated as follows:



$$\begin{array}{l} CH_2COOH \\ | \\ NH_2 \end{array} + \begin{array}{l} N{=} \\ | \\ C{=} \\ | \\ N{=} \end{array} \rightarrow \begin{array}{l} NH_2 \\ | \\ C{=}NH \\ | \\ NH \\ | \\ CH_2COOH \end{array} \xrightarrow[\text{methyl from methionine}]{+\ -CH_3} \begin{array}{l} NH_2 \\ | \\ C{=}NH \\ | \\ N{-}CH_3 \\ | \\ CH_2COOH \end{array}$$

The above experiments were done with rats. The evidence for the identity of the creatine precursors is summarized in Table VI. The synthesis of creatine in the pigeon has also been examined more recently by Bloch (14), and a similar mechanism has been found to operate, though there are some quantitative differences. Table VII shows some of the results obtained with the two different species. It is apparent that the pigeon, like the rat, converts the amidine group of arginine to the amidine of creatine. Also, in the pigeon as in the rat, the sarcosine N of creatine is derived from glycine. The two species show a large divergence in respect to the extent to which administered NH_3 is converted to creatine. This is in accordance with the fact that arginine is an essential amino acid in the bird. Its amidine group contained no N^{15} after $N^{15}H_3$ feeding nor was there any after feeding $N^{15}H_3$ and citrulline. Since, however, administered $N^{15}H_3$ entered the sarcosine moiety of creatine it appears that the pigeon can synthesize glycine, though the growing chick requires added glycine in the diet.

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afforded for determination of creatine turnover by the isotope technique. Labeled creatine was fed to rats for 6 days, after which the animals were kept for 43 days on the same creatine-free diet. The labeled creatine originally administered formed an inseparable mixture with the body creatine, the isotope content of which was reflected in the isotope content of the excreted creatinine. The rate of decrease of isotope in the creatinine followed a logarithmic course, as would be expected if the amount of isotope excreted in this form is directly proportional to the isotope content of the body creatine. The decrease was equivalent to 2% per day. This figure represents both the rate of synthesis of new creatine and the rate of its conversion to creatinine. It was found to be in close agreement with the amount of creatine excreted, *i.e.*, the creatinine excreted per day in the urine was equivalent to 2% of the total body creatine.

[7] The transfer of the methyl group of methione to creatine was demonstrated by du Vigneaud *et al.* (147) (see p. 90).

The conclusions outlined above regarding the nature of the precursors of creatine are entirely consistent with results obtained by Borsook and Dubnoff (25, 26, 27) in *in vitro* studies. Both the *in vitro* work and the *in vivo* work with N^{15} were done almost simultaneously.

TABLE VI

Fraction of Newly Formed Creatine Nitrogen Derived from Respective Test Substance (23)

Material administered	Fraction of N derived from test substance		
	Of total creatine	Of amidine group	Of sarcosine group
	Per cent	*Per cent*	*Per cent*
DL-Tyrosine	0		
L-Leucine	2.3	2.8	
DL-Glutamic acid	3.3		
Ammonia	2.5		
Glycine	11.1	2.2	25.9
Glycine	14.4	3.0	
Sarcosine	7.7		
Sarcosine	10.2	3.0	
Arginine	22.0	26.4	0.6
Hydantoic acid	0		
Methylhydantoic acid	0		
Urea	0		
Choline	0		

Guanidinoacetic acid is not included in the table, as its administration is known to result in extra creatine formation. The efficiency of this compound calculated by employing the same factor would be 66.7%.

TABLE VII

Sources of Muscle Creatine in Rats and Pigeons (23, 14)

Material administered	Pigeon		Rat	
	Amidine N of creatine	Sarcosine N of creatine	Amidine N of creatine	Sarcosine N of creatine
Arginine	1.00	0.04	2.21	0.05
Glycine	0.17	1.35	0.02	2.26
Ammonia	0.15	0.86	2.0	0.64

Results calculated on the basis of 100% N^{15} excess in the administered compound.

The pigeon excretes small quantities of urea, *ca.* 5% of the urinary N being present in this form. Urea isolated from pigeon droppings after labeled arginine feeding showed a high isotope concentration but, in view of the relatively much lower level found in the liver and kidney arginine, it has been suggested that such urea may be of bacterial rather than metabolic origin.

(*d*) *Sources of Uric Acid and Allantoin.* It has long been known that uric acid is the main excretory form of nitrogen in birds. In accordance with this fact, Barnes and Schoenheimer (7) found that uric acid isolated from pigeons which had been given $N^{15}H_3$ contained large quantities of isotope (Table IV). Creatine and guanidoacetic acid were ineffective as purine precursors in both rats and pigeons, *i.e.*, there is no connection between creatine metabolism and purine and pyrimidine metabolism. The purines are allantoin precursors in the rat and uric acid precursors in the pigeon. Pyrimidines form NH_3 and urea. Although those conclusions could not be reached exclusively on the basis of the work with isotopes, all results were consistent with them. Previously suggested purine precursors such as urea, histidine and arginine could be excluded.

TABLE VIII

The Biological Precursors of Uric Acid Carbon in the Pigeon (130)

Substance administered	Rate given	Per cent carbon derived from administered carbon					
		Uric acid carbon No.					
		2	8	4	5	6	Respiratory CO_2
	mM/hr.						
$C^{13}O_2$	0.75	0.0	0.0	0.9	0.0	3.1	3.4
$CH_3\cdot C^{13}OOH$	1.00	34.7	36.1	1.2	0.0	3.8	4.5
$CH_3\cdot CHOH\cdot C^{13}OOH$	0.50	0.0	0.0	4.2	0.0	3.0	2.8
$C^{13}H_3\cdot C^{13}HOH\cdot COOH$	0.50	1.9	1.9	1.3	2.6	1.7	2.0
$NH_2CH_2C^{13}OOH$	0.50	0.0	0.0	22.3	2.7	2.1	2.5

Sonne, Buchanan and Delluva (130) have recently employed C^{13} to study the precursors of uric acid carbon in the pigeon. Their results are shown in Table VIII. They conclude from this data that carbon atoms 2 and 8 of uric acid are derived from the carboxyl group of acetic acid, carbon atom 4 is derived from the carboxyl group of glycine, carbon 5 from the α or β carbon in lactate, and carbon 6 from CO_2. The distribution of carbon isotope observed in these experiments will be discussed in more detail in the section on carbohydrate and fat metabolism.

5. *Metabolism of Individual Amino Acids*

(*a*) *The Essential Amino Acids.* Throughout the development of the application of N^{15} to the study of amino acid metabolism in the rat, there has been a gratifying agreement with results obtained by feeding experiments in regard to the essential nature of some of the amino acids. Feeding experiments have shown that histidine is an essential amino acid for the rat. Its carbon chain cannot be synthesized in the body. The corresponding α-hydroxy or keto acid (43, 60) as well as the unnatural isomer (40, 42) may, however, be substituted for L-histidine in the diet. In agreement with these facts, Schoenheimer, Rittenberg and Keston (121) found that nitrogen could be transferred to histidine from administered $N^{15}H_3$ (see Table II), but all the isotope was located in the α-amino group. None was in the nitrogen of the imidazole ring. Histidine, in other words, can be freely deaminated and reaminated. Inversion of the unnatural isomer probably involves such deamination and reamination, but the imidazole ring cannot be synthesized in the body of the rat.

Lysine is another amino acid essential for the rat, but neither the corresponding α-hydroxy acid (α-hydroxy-ϵ-amino-n-caproic acid) nor the unnatural D-isomer can substitute for L-lysine in the diet (74). In accordance with this, it has been found that no nitrogen is transferred to lysine by any substance investigated. The figures shown for the N^{15} content of lysine (Tables II and III) after administration of labeled ammonia or other amino acids are all too low to be significant. Furthermore, after feeding D-lysine labeled with both deuterium and nitrogen, no D or N^{15} could be detected in the L-lysine of the tissues. In sharp contrast is the ready transfer of N^{15} from ammonia to lysine observed by Burris and Wilson (35) in *Azotobacter* (Table II) which can readily synthesize this amino acid.

The essential amino acid leucine presents a somewhat different picture. Isotope experiments show it can be freely deaminated and reaminated in the body. This is in accordance with the fact that the corresponding α-hydroxy and α-keto acids may substitute for leucine in the diet. The feeding experiments indicate, however, that the D-isomer is not an adequate substitute for L-leucine in the growing rat (101). Since isotope experiments of Ratner, Schoenheimer and Rittenberg (86) (which will be described below) have demonstrated the deamination and inversion of D-leucine in the adult rat, one may conclude that the rate of this process is apparently insufficient to supply the whole growth requirement of the young animal.

(*b*) *Amino Acids of Unnatural Configuration.* It has already been shown (Table III) that the nitrogen of D-amino acids is available for nitrogen transfers in the animal body. The tissues of the rat can metabolize D-amino acids, though to a limited extent.[8] In some instances D-amino acids can be inverted to the corresponding L-form. This process has been studied with the essential amino acids, lysine and leucine, labeled with N^{15} and with stably bound deuterium in the carbon chain (86, 87, 112, 155). The animal cannot synthesize these carbon chains, and the deuterium may therefore be regarded

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[8] D-Amino acids are partly excreted unchanged in the urine. (87, 125).

as an adequate label for it. This assumption seems justified by the relatively large amounts of D which appear in the L-lysine and L-leucine of the body proteins after feeding of the corresponding deuterium labeled L-amino acids. (These experiments are the same as those for which N^{15} data have been given in Table III). The relative amounts of D and N^{15} which appear in the L-amino acids of the protein may then be used as a measure of the extent to which the nitrogen to carbon bond of the α-amino group is broken before the amino acid is incorporated into protein. Data of this type can be expressed as the ratio of D to N^{15} calculated on the basis of a value of 1 for the administered substance. Results obtained are given in Table IX, together with similar data obtained with proline. The ratio of 1 observed in the tissue lysine after feeding of L-lysine, indicates that once the L-lysine is deaminated it cannot be reaminated. This fits with the previously mentioned observation that no nitrogen is transferred to lysine in the body of the rat. No D or N^{15} appears in the L-lysine of the body after feeding of D-lysine labeled with these two isotopes.

TABLE IX

Ratio of Deuterium to N^{15} in Natural Amino Acids Isolated from Proteins of Rats after Feeding Amino Acids Labeled with Both Isotopes

(Both isotope concentrations calculated on the basis of 100% excess in administered compound. Ratio D/N^{15} in administered compound is therefore always 1)

Compound isolated	L(−)-leucine		L(+)-lysine	L(−)-proline	
Tissue source	Carcass	Organs	Total body	Carcass	Organs
Amino acid fed	D/N^{15}	D/N^{15}	D/N^{15}	D/N^{15}	D/N^{15}
L(−)-Leucine	1.54	1.76			
D(+)-Leucine	12.7	6.1			
L(+)-Lysine	....	....	1.04		
D(−)-Lysine	....	....	*		
L(−)-Proline	....	....	....	1.0	1.1

* No appreciable isotope present.

When L-leucine labeled with D and N^{15} is fed, on the other hand, the D/N^{15} ratios observed in the L-leucine isolated from carcass and organ proteins are 1.54 and 1.76 respectively. These figures indicate that about two out of three L-leucine molecules are incorporated into protein without previous deamination and reamination. One-third of the leucine molecules lose their N^{15} label before incorporation into protein. When D-leucine labeled in similar fashion is fed, the L-leucine of the body proteins contains a much larger proportion of the deuterium than of the N^{15}. The high ratios of 12.7 and 6.1 indicate extensive deamination and reamination must be associated with inversion. In fact, the actual amounts of N^{15} found are sufficiently small to be accounted for entirely in terms of a reintroduction into the molecule by amination from a source containing a low N^{15} concentration. In other words, inversion of D-leucine involves a rupture of the nitrogen to carbon bond.

It should be emphasized that interpretation of the isotope ratios observed in experiments of this type requires consideration of other factors than the actual value of the ratios themselves. Data obtained by feeding proline labeled with D and N^{15} have been included to illustrate this point (136). The proline isolated from the tissues showed a D/N^{15} ratio of 1, like lysine, but the interpretation of the results obtained in the two cases are somewhat different. Unlike lysine, proline is not an essential amino acid. It can readily be formed in the animal organism. One might have expected all nonessential amino acids to show evidence for deamination and amination of the carbon chain. But in proline the amino group is secondary and so may be expected to remain attached to the rest of the molecule until the ring is opened. Opening of the ring may then involve further degradation and loss of deuterium. In any case the data demonstrate that ring opening followed by rapid deamination and amination and subsequent ring closure does not occur to any great extent unless losses of deuterium are also involved in the process.

Inversion of D-amino acids has also been studied with an amino acid foreign to the body. Du Vigneaud, Cohn, Brown, Irish, Schoenheimer and Rittenberg (146) have fed rats D- and L-phenylaminobutyric acid labeled with N^{15} and determined the N^{15} content of the acetyl-L-phenylamino acid excreted in the urine. Their results indicated that the *in vivo* conversion of D-phenylaminobutyric acid to acetyl-L-phenylaminobutyric acid involved a loss of practically all the original nitrogen of the fed compound, but that much of the nitrogen remained attached when the L-form was converted to the acetyl derivative. These experiments were done with rats which had been fed heavy water to raise the D content of the body fluids to about 2.5 atom-%. Analyses showed that the acetylphenylaminobutyric acid excreted in the urine had taken up one atom of D in the α position of the amino acid after both D- and L-phenylaminobutyric acid had been fed. The reader is referred to the original paper for a detailed discussion of the interpretation of these findings.

Though ample evidence exists that the body can metabolize and invert many amino acids of unnatural configuration, there is still no conclusive evidence that D-amino acids are formed in the animal body. Application of the isotope technique to a study of this question has indicated, in the case of glutamic acid and tyrosine, that if the body can form the D acids, the amount so formed must be very small. The procedure employed was first applied by Bernhard (11) to prove that the long chain dicarboxylic acids are not normal intermediates in fat metabolism. It has been stated as follows by Shemin and Rittenberg (125): "If a substance, labeled with an isotope, is administered to an animal and can subsequently be isolated either from the tissues or excreta, then the isotope concentration of the recovered compound will indicate whether or not it has been diluted by the same non-isotopic substance formed from the dietary constituents."

When DL-tyrosine and DL-glutamic acid labeled with N^{15} were fed to vitamin B_2-deficient rats, appreciable amounts of the corresponding D-amino acids were excreted in the urine. Isolation and analysis for N^{15} indicated that the isotope content of the excreted D-acids was identical with the N^{15} content of the fed acids. There was no evidence, in other words, for the synthesis of the D-amino acids. In the case of glutamic acid it was estimated that the method was sensitive enough to detect 1% racemization in the glutamic acid synthesized in the body of the rat. Further evidence for the fact that D-amino acids are not synthesized was obtained by feeding unlabeled DL-tyrosine together with $N^{15}H_3$. The D-tyrosine isolated from the urine contained no N^{15}.

(*c*) *Relations between Arginine, Ornithine, Proline, Hydroxyproline, and Glutamic Acid.* As previously mentioned in the discussion of the mechanism of urea formation, free ornithine is readily converted into arginine in the animal body. Ornithine, as such, does not exist in detectable amounts in the proteins. It is present in the sense that it is a constituent of the arginine molecule.

Clutton, Schoenheimer and Rittenberg (38) demonstrated the conversion of ornithine to arginine by feeding deutero-ornithine to mice. The deuterium content of the arginine subsequently isolated from the tissue proteins showed that 7.5% of the arginine had been derived from the fed ornithine. Roloff, Ratner and Schoenheimer (100) found that about 2% of the hydrogen of the proline and 0.5% of the hydrogen of the glutamic acid were similarly derived.

In subsequent experiments, proline labeled with both D and N^{15} was fed to rats (136), and significant amounts of deuterium were found in the ornithine moiety of the tissue arginine and in the glutamic acid. The amounts of N^{15} found in these tissue constituents were insufficient to indicate a direct conversion of the carbon chains. The hydroxyproline of the tissues contained sufficient N^{15} as well as D, to indicate it had been derived from proline

These demonstrated conversions of proline to ornithine, to glutamic acid and to hydroxyproline, and of ornithine to proline and to glutamic acid, are in harmony with results from *in vitro* experiments, which have indicated relations between these amino acids (67, 136, 154). The exact mechanism of the various interconversions is not known, but isotope studies of the intact animal, combined with *in vitro* studies, have sufficed to serve as a basis for an interesting hypothesis regarding the interrelationship.

The main contribution of the isotope studies to this hypothesis, apart from the demonstration of the conversions already mentioned, derives from a study of the distribution of N^{15} in arginine isolated from the tissues after feeding N^{15} in other forms. The amidine group of arginine takes up N^{15} considerably faster than the remainder of the molecule. This is to be expected

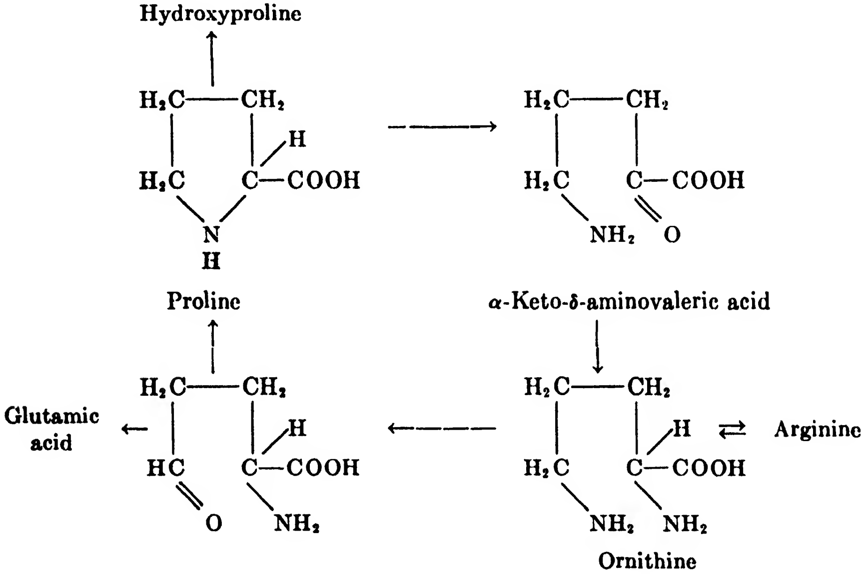

Diagram I
Schematic representation of postulated interrelationships between proline, hydroxyproline, ornithine, arginine and glutamic acid. (Not all intermediates have been included).

because of the function of the amidine group in urea formation, a process which occurs with considerable rapidity in the body. The ornithine moiety of the arginine also takes up N^{15}, although at a slower rate. When the N^{15} contents of the two amino groups of ornithine were determined separately by Shemin and Rittenberg (126, 127), it was found that they were equal. This led to the conclusion that neither arginine nor ornithine could be reversibly deaminated and reaminated. Otherwise such a condition would not obtain. It will be recalled that proline is likewise not subject to such a process (Table IX). To account for these facts Shemin and Rittenberg have postulated that the conversion of proline to ornithine and of ornithine to proline proceed by two different pathways, so that a cyclic rather than a direct interconversion is involved, as indicated in Diagram I. Examination of the diagram will reveal that every nitrogen atom in the δ position passes through the α position, with the result that any N^{15} present in one group would eventually become equally distributed in both groups. This phenomenon has been referred to by the authors as the "intramolecular nitrogen shift". The mechanism also indicates how deamination and reamination of proline would proceed similarly by a cyclic process during which all the hydrogen in the molecule would be labilized, thus accounting for the D/N^{15} ratio of 1 (Table IX).

(*d*) *Formation of Cysteine from Serine and Methionine.* By the combined use of the isotopes S^{34} and C^{13}, du Vigneaud, Kilmer, Rachele and Cohn (148) have established that the sulfur of cysteine and cystine in the body can be derived from methionine, but that the carbon of methionine is not converted to the carbon of cysteine. This was done by feeding growing rats a diet in which the essential amino acid, methionine, was furnished as a DL preparation containing S^{34} and C^{13} (in β and γ positions). Early in the experiment the hair of the rats was removed with a depilatory and the new hair which grew in during the course of the labeled methionine feeding (12 days) was used to furnish a sample of cystine for isotope analysis. This cystine was found to have derived 80% of its sulfur, but no significant amount of its carbon, from the methionine.

The source of the carbon chain of cysteine was found by Stetten (133) to be serine. The data which demonstrate this fact are shown in Table III. When DL-serine containing N^{15} was fed to rats, 5.1% of the cystine of the internal organs was found to have been derived from the dietary serine. This value is sufficiently higher than the amounts introduced into other amino acids by nitrogen transfer, and the amounts introduced into cystine from other nitrogen sources, to indicate that a conversion of the carbon chain of serine to the carbon chain of cysteine must have occurred.

(*e*) *Conversion of Serine to Glycine.* One of the first biological reactions to be studied with the aid of N^{15} as a marker was the conjugation of benzoic acid with N^{15} glycine. Rittenberg and Schoenheimer (94) showed that the hippuric acid isolated from the urine contained only enough N^{15} to account for about one-third of the glycine fed. In other words, the bulk of the glycine in the excreted hippuric acid was not of dietary origin. The excretion of body glycine in the form of hippuric acid has been used in later experiments by Shemin (124) to study the origin of glycine.

The procedure may be described as follows: The feeding of benzoic acid to rats results in hippuric acid excretion. The body can synthesize the necessary glycine, and the glycine in the hippuric acid can, therefore, be regarded as a sample of body glycine. Since the body can incorporate N^{15} administered in the form of NH_3 or of other amino acids into its body glycine, the feeding of any labeled N^{15} compound which contains the label in such a form that it may be regarded as part of the nitrogen pool, will result in the presence of N^{15} in the excreted hippuric acid. N^{15} administered as labeled glycine should be expected to result in the highest N^{15} concentration in the hippuric acid, since such N^{15} would be diluted only by the body pool of glycine, partly by actual mixing, partly by deamination and reamination. This degree of dilution can be expressed as a dilution factor C_o/C (C_o = N^{15} in administered compound, C = N^{15} in hippuric acid). Results obtained in such experiments with both the rat and the guinea pig

TABLE X

Utilization of Nitrogen-Containing Compounds for Glycine Formation (124)

N^{15} labeled compound administered	Dilution factor C_o/C	
	Rat	Guinea pig
Glycine	2.8	2.4
Ammonia (as citrate)	425	21
L-Serine	5.5	3.9
D-Serine	158	148
L-Glutamic acid	45	10
D-Glutamic acid	1500	450
*DL-Glutamic acid	64	13
*DL-Aspartic acid	58	19
L-Alanine	94	21
*DL-Proline	47	23
L-Leucine	120	54
Ethanolamine	334	57

* DL compounds administered as 0.35 m*M* of the L + 0.35 m*M* of the D/100 g. body weight. All other compounds administered as 0.35 m*M*/100 g. body weight.

All animals received, in addition to the N^{15} labeled compound, 0.35 m*M* benzoic acid/100 g. body weight.

are shown in Table X. As would be expected, the dilution factor of glycine is lower than that of any other substance tested. The dilution factor of ammonia is high, on the other hand, since ammonia can enter glycine only by transference to a carbon chain. Substances with dilution factors between those of ammonia and glycine may be regarded as potential glycine precursors in the sense that the carbon chain of the substance with its attached nitrogen may be converted to the carbon chain of the glycine. Of all the substances tested, L-serine showed by far the lowest dilution factor, next to glycine. Serine may be regarded as a direct glycine precursor on this basis. For some of the other amino acids the evidence is inconclusive. Glutamic acid, particularly, may quite possibly contribute its carbon skeleton to glycine.

Corroboration of the direct conversion of serine to glycine, together with some facts about the mechanism of the reaction was obtained by feeding serine containing N^{15} in the amino group and C^{13} in the carboxyl group. The glycine isolated from the hippuric acid contained C^{13} in appreciable amounts, and the N^{15}/C^{13} ratio in the isolated glycine was about equal to that in the administered serine. The conclusion may be drawn that L-serine is converted into glycine by splitting off the β-carbon atom (124).

(*f*) *Glutathione Metabolism.* The metabolism of the tripeptide glutathione has been studied by Waelsch and Rittenberg (151, 152, 153), who determined the isotope present in the tissue glutathione after administration of N^{15}-glycine to rats and rabbits as well as $N^{15}H_3$ and N^{15}-glutamic acid to rabbits. The half-life time of glutathione in the livers of both species was calculated to be very short, about 2–4 hours. The possibility that the tripeptide might function as a precursor of hippuric acid glycine was excluded, but the extremely rapid turnover of the compound was regarded as suggestive of active metabolic function, possibly in amino nitrogen transfer.

(*g*) *Glycine as a Precursor of Porphyrin.* Shemin and Rittenberg (128) have fed glycine labeled with N^{15} to the human. Hemin isolated from blood samples was analyzed for its isotope content over a period of 99 days. From the rate of loss of isotope after maximum content had been reached, the average half-life of the hemin molecule was estimated to be 100 days. From the estimated half-life and the isotope content of the hemin on the 18th day, while the isotope content was still increasing, it was concluded that only the administered amino acid, glycine, could have had a sufficiently high N^{15} content to act as the nitrogen precursor of the porphyrin. Bloch and Rittenberg have also found significant amounts of deuterium in the hemin of rat blood after deuterio-acetate feeding (19).

6. Metabolism of the Nitrogenous Constituents of Phospholipides

The origin and fate of choline and of ethanolamine in tissue phospholipides have been studied by Stetten (131, 132) with the aid of N^{15}. Labeled ammonia, betaine, glycine, ethanolamine and choline were fed to rats, and the isotope concentrations of choline and of ethanolamine in the body were determined after a suitable time interval. Results showed that both these substances in the tissues were rapidly replaced by the corresponding substance in the diet. In other words, tissue phospholipides, like proteins, have a high regeneration rate with respect to the nitrogenous component, even when there is no net change in the total amount present. In three days, at least 28% of the ethanolamine and 21% of the choline in the tissues were replaced by dietary ethanolamine and choline, respectively. Ethanolamine was also found to be a precursor for the biological synthesis of choline, glycine was found to be a precursor of tissue choline, and the ingested betaine was found to be demethylated by the body to glycine.

In another series of experiments Boxer and Stetten (29) have determined the effect of dietary choline on the rate of phosphatide choline turnover in the tissues. The half-life of choline in normal rats was about 6 days. Rats on a choline-deficient diet showed a half life of 18 days for tissue choline at the time when fatty livers were developing. Thus, choline deficiency induced a marked decrease in the turnover of this substance, although the actual quantity of choline in the tissues showed little change. The formation of fatty liver seems to be related to the rate at which dietary or newly synthesized choline enters the tissue phospholipides.

The interesting phenomena of transmethylation have not been discussed in this paper, because the isotope applied in these studies has usually been deuterium. However, du Vigneaud, Simmonds, Chandler and Cohn (149) and du Vigneaud, Simmonds and Cohn (150) have recently employed N^{15} together with deuterium, to examine the transfer of methyl groups from dietary sarcosine and betaine to tissue choline and creatine. The use of both N^{15} and D made possible the conclusion that neither sarcosine nor betaine was converted to choline as a whole. The previous conclusion of Bloch and Schoenheimer (22) that sarcosine was demethylated to glycine before conversion to creatine was also confirmed (150).

IV. CARBOHYDRATE AND FAT METABOLISM

1. *Carbon Dioxide Fixation and the Tricarboxylic Acid Cycle*

When the isotopes of carbon were applied to the study of metabolic phenomena, it soon became apparent that CO_2 could not be regarded only as a metabolic end product, but that all the various forms of life could utilize the carbon of CO_2 to form other organic compounds.

The first evidence that forms other than photosynthesizing organisms and autotrophs could utilize CO_2 had been furnished by Wood and Werkman on the basis of chemical balance studies with heterotrophic propionic acid bacteria (161, 162). Then, when Ruben and his coworkers applied C^{11} to a study of photosynthesis and discovered that CO_2 uptake did not require light, but occurred in the so-called dark reaction (102, 103, 106, 107), they tested a variety of tissues for their ability to fix CO_2. In these tests $C^{11}O_2$ was incubated with the tissues for a given amount of time, then removed by acidification, boiling, and flushing with unlabeled carbon dioxide. Residual radioactivity in the solution could then be taken as an indication that the radioactive carbon had been converted into some form other than CO_2 or its salts. On the basis of such tests, Ruben and Kamen reported in 1940 (104) that ground rat liver, as well as yeast and heterotrophic bacteria, could fix CO_2. Their experiments did not indicate the nature of the compound in which CO_2 was fixed. Later in the same year, the conversion of CO_2 to urea by liver tissue was demonstrated (50, 98) and evidence was presented from two different sides, that CO_2 participates in the intermediary metabolism of carbohydrates in animal tissues. First, Evans, who had found, while working in Krebs' laboratory, that pyruvate could form α-ketoglutarate in the presence of malonate-poisoned pigeon liver (47), demonstrated, in collaboration with Slotin (49, 51), that the α-ketoglutarate contained C^{11}, if the isotope was added to the incubation mixture as $C^{11}O_2$. Then the Harvard group (39, 129) showed that rats could incorporate carbon administered as $NaHC^{11}O_3$ into newly formed liver glycogen *in vivo*.

The phenomenon of CO_2 fixation has subsequently been studied extensively. Only those fixation reactions which are involved in carbohydrate metabolism in animal tissues will concern us here. These are (1) the addition of CO_2 to pyruvic acid to form oxaloacetic acid and (2) the addition of CO_2 to α-ketoglutaric acid to form oxalosuccinic acid. The latter reaction, which has been studied by Ochoa (79, 80, 81), has only recently been demonstrated with the aid of isotopic carbon (56a). The addition of CO_2 to pyruvate, the Wood-Werkman reaction, has, however, been extensively investigated with C^{11} and C^{13} (48, 52, 142, 160, 164, 165). It occupies a strategic position in current schemes of both fat and carbohydrate oxidation, since it is thought to be the mechanism whereby three-carbon units derived from carbohydrate are converted to four-carbon dicarboxylic acids.

To facilitate the subsequent discussion, it seems convenient to present at this point, without experimental evidence, an outline of current hypotheses concerning the interrelationships of the various intermediates in the mechanisms whereby fat and carbohydrate are metabolized in the animal body. These interrelationships are indicated in abbreviated fashion in Diagram II. In essence the scheme postulates that a three-carbon unit, pyruvic acid, occupies a pivotal position in carbohydrate metabolism, and that a two-carbon unit, which is closely related to acetate, occupies a similar position in fat metabolism.[9]

Both the three-carbon unit and the two-carbon unit are oxidized by

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[9] The conversion of fatty acids to the two-carbon unit, as well as the conversion of pyruvic acid to glucose or glycogen, are both regarded as reversible, at least in the sense that they proceed in both directions though not necessarily by identical paths. The reactions of glycolysis (conversion of glucose to pyruvate and lactate) are known in some detail. The reactions involving breakdown and synthesis of the fatty acids have been less studied. The conversion of carbohydrate to fat, which has not been indicated on the scheme, is thought to proceed by way of a decarboxylation of pyruvate to the 2-carbon unit.

similar mechanisms. These are essentially the reactions of the tricarboxylic acid cycle. Oxaloacetic acid is formed by addition of pyruvate and CO_2.

Simplified Representation of Present Hypotheses Concerning Interrelationships of Fat and Carbohydrate Metabolism

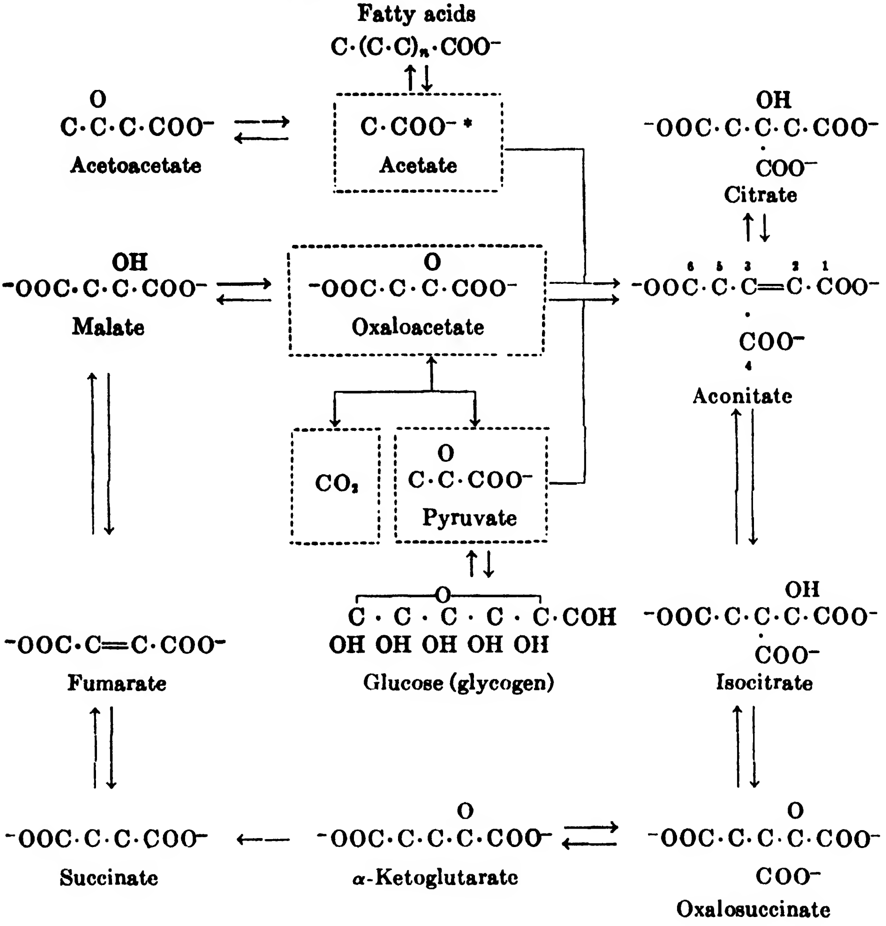

Diagram II

* Two-carbon unit, probably not acetate itself but closely related.

The hydrogen atoms attached to carbon have been omitted from the formulae. They can easily be filled in to complete a valence of four for each carbon.

In the condensation, carbons 1, 2, 3, and 4 of aconitate are derived from oxaloacetate. Carbons 5 and 6 are derived from the β and α carbons of pyruvate, respectively, or from the α and carboxyl carbon of acetate. (This mode of condensation is not certain, but seems most probable.)

The oxaloacetate then condenses with pyruvate to form the 6-carbon aconitic acid after oxidation and loss of CO_2, or it condenses with acetate to form a similar six-carbon intermediate. By a series of successive oxidations and decarboxylations, the aconitate is reconverted to a four-carbon dicarboxylic acid, which may then reenter the cycle. The net effect is the complete oxidation of the carbohydrate (in the form of pyruvate), or of the fat (in the form of the two-carbon unit). The four-carbon dicarboxylic acid is regenerated during the cycle.

Much of the evidence for the detail of the above scheme has been derived from *in vitro* experiments which are outside the scope of this review. It should be kept in mind that all the various reactions do not necessarily occur in every tissue of the body, but some may be restricted to a particular organ. For a more detailed and critical discussion of the individual reactions, the reader is referred to the review by Wood (158). Meanwhile, it should be clearly understood that the scheme is regarded as a working hypothesis, not as a completely established fact. There are parts of it which are still highly controversial, and there are alternate hypotheses. To the author, however, the relations indicated in Diagram II seem to be the simplest manner of correlating most of the known facts.

In the following sections the results obtained with *in vivo* administration of fat and carbohydrate precursors labeled with carbon isotopes will first be described. Interpretation of these results requires that all the reactions of fat and carbohydrate metabolism be kept in mind. There is evidence, as will be seen later, to indicate that these reactions occur very rapidly. As a result, the entire store of intermediates of this type, together with their potential precursors in the body, may be regarded as a unit, or "body pool", at least in the sense that a carbon atom introduced in the form of one of the constituents may be converted readily to carbon in the form of any of a large variety of other constituents. The possible conversions are limited however, and the discovery of the limitations has had, and will have, much to do with elucidation of the detail of the interrelationships.

2. *Synthesis of Carbohydrate*

It has been known for some time that the liver of the rat is rather uniformly depleted of glycogen on fasting and that the feeding of even relatively small amounts of carbohydrate-formers results in a deposition of liver glycogen, usually within several hours. This phenomenon has been well standardized since it has been used extensively to determine whether or not a given substance is glycogenic and to what extent (41). In the case of good glycogen-formers, the amount of glycogen deposited is often 70–90% of the maximum amount which the substance could form theoretically. This occurs even though the rat is simultaneously metabolizing its reserves at a rate considerably faster than that at which the glycogen is deposited.[10]

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[10] The fasting rat may be presumed to be forming glucose at a rate sufficient to

The deposition of liver glycogen in the fasted rat has been studied extensively with C^{11} by Hastings and his coworkers at Harvard (34, 39, 129, 143, 144). Since C^{11} has a half-life of only about 20 minutes, the experiments were designed to take as short a time as possible. Two to 2.5 hours was the usual length of time allowed for glycogen formation. Lactate was synthesized to contain the label either in the carboxyl position or in the α and β position, and measurements were made of the amount of C^{11} in the liver glycogen deposited after feeding the labeled lactate (39, 143). In addition, acetic, propionic and butyric acids were prepared containing C^{11} in the carboxyl group. These were fed to rats together with glucose to stimulate glycogen production[11] (34) and the amount of C^{11} in the newly formed glycogen was measured. In Table XI, the results obtained have been compiled and expressed as *per cent* C^{11} per unit of carbon, on the basis of the administered compound taken as 100. The concentrations are, in all instances, calculated to average out over the carbon in the entire molecule.

Table XI shows that an average of 6% of the glycogen carbon was derived from fed lactate carbon when the label was present in the carboxyl position, and 14% of the glycogen carbon was derived from lactate carbon when the label was present in the α and β position. Such results indicate immediately that the liver glycogen deposited after lactate feeding is only derived in part from the administered lactate even when the latter is the stimulus for the glycogen deposition. The difference between the results observed with the label in the carboxyl position and in the α and β position also indicates that the carboxyl group is, in part at least, split from the remainder of the molecule during the conversion of carbon in the form of lactate to carbon in the form of glycogen.

The relatively small amount of C^{11} which appears in the glycogen in

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maintain its blood sugar, so that the disappearance of liver glycogen does not indicate a lack of ability to form carbohydrate. Under these circumstances, a reasonable explanation of the deposition of liver glycogen would seem to be that an exogenous carbohydrate precursor tends to induce a change in the dynamic state of the carbohydrate-forming system, and that this is compensated for by the temporary removal of carbohydrate precursor as an equivalent amount of stored glycogen. In other words, the metabolic interrelationships in the fasted rat are such that the introduction of exogenous carbohydrate does not immediately reduce the formation of endogenous carbohydrate. (See also p. 102 paragraph 5).

[11] Neither acetate nor butyrate have ever been shown to induce glycogen deposition in the livers of fasted rats. Propionic acid, on the other hand, has been shown to be glycogenic, under these circumstances, but 6–7 hours are required before considerable quantities of glycogen appear (45, 46). Lactate, however, is an excellent glycogen former under these conditions. Glucose was therefore administered to induce glycogen formation when acetate, propionate and butyrate were fed, but not when lactate was fed.

either case may be interpreted in terms similar to those which apply to the situation already described for nitrogen. Thus, although the urea excreted after protein feeding is equivalent in amount to the ingested nitrogen, the nitrogen of the urea is not identical with the nitrogen fed. Labeled nitrogen ingested in one form is partly diluted by similar unlabeled molecules and partly distributed among components of the nitrogen "pool" in the animal body. In a similar manner, it seems that labeled carbon introduced in the form of a particular carbohydrate intermediate should be expected to be partly diluted by unlabeled molecules of the same substance formed in the body and partly distributed by chemical transformation into a variety of other forms. A body "pool" of carbohydrate precursors may be postulated. The size of the "pool", estimated on the basis of the dilution of the α and β carbons of the lactate, would be about 100/14 or seven times the amount of substance fed. Since an average of a little over one millimole of lactate was administered, one can estimate the pool to be equivalent in 2.5 hours to about 10 millimoles of α and β carbon lactate in a rat weighing about 100 g. The word "pool" should not be taken in a static sense. It has a dynamic significance, and the size of the pool represents the *rate* at which the body can accomplish the various reactions which result in the observed dilution.

TABLE XI

Incorporation of Labeled Carbon into Newly-Formed Liver Glycogen in Rats (*39, 143, 34*)

Substance administered	$CH_3CHOHC^{11}OOH$		$C^{11}H_3C^{11}HOHCOOH$		$CH_3C^{11}OOH$ + Glucose		$CH_3CH_2C^{11}OOH$ + Glucose		$CH_3CH_2CH_2C^{11}OOH$ + Glucose	
	Amt. fed mM	Glycogen-C^{11} %	Amt. fed mM	Glycogen-C^{11} %	Amt. fed mM	Glycogen-C^{11} %	Amt. fed mM	Glycogen-C^{11} %	Amt. fed mM	Glycogen-C^{11} %
Exp. No.:										
1	0.56	5	1.28	21	2.58	1.1	1.27	3.4	1.95	5.2
2	1.16	2	1.08	19	3.20	2.5	2.23	3.4	2.01	6.7
3	0.90	6	1.09	10	1.75	2.5	2.31	1.1	1.88	6.9
4	1.30	34*	0.92	14	1.90	0.05*	2.38	10.5	1.67	5.5
5	1.90	12	1.12	16	2.06	1.4	2.02	6.3	1.45	3.1
6	1.40	4	1.10	6	2.30	1.9	1.39	5.9		
Average		6		14		1.9		5.1		5.5

* Omitted from average.

C^{11} concentration expressed as per cent of amount in labeled substance fed.

400 mg. glucose fed. Rats weighed about 100–130 g.

The discussion thus far has been concerned with results obtained only after lactate feeding. Table XI indicates that when glucose is the stimulus for glycogen production, C^{11} administered as the carboxyl group of acetate, of propionate and of butyrate, appears in the glycogen of the liver. The glycogen cannot, therefore, necessarily be regarded to be formed directly from the fed glucose. Rather, it appears that acetate, propionate and butyrate must all be regarded as components of the pool of precursors from which the glycogen is actually formed. It is interesting that the carboxyl groups of lactate, of propionic acid and of butyric acid were found to contribute to the glycogen carbon to approximately the same extent.

TABLE XII

Incorporation of $C^{11}O_2$ into Liver Glycogen of Fasted Rats after Lactate and Glucose Feeding (130, 144)

Unlabeled compound fed	m*M* C^{11} injected	m*M* Glycogen carbon formed	C^{11} in glycogen		
			Per cent total C^{11} administered	Per cent glycogen carbon derived from administered CO_2*	Per cent glycogen carbon derived from total CO_2 of body†
Lactic acid, *150 mg. to all.*	0.39	2.1	1.08	0.20	15.6
	0.61	2.2	0.42	0.12	9.7
	0.19	2.2	0.31	0.27	7.6
	0.76	2.0	0.80	0.30	13.9
	0.64	2.8	0.80	0.18	6.9
	0.65	1.6	1.06	0.43	14.2
	0.76	2.6	0.44	0.13	11.9
					Average = 11.4
Glucose, *mg.*					
600	1.01	4.2	0.84	0.20	8.1
400	0.74	2.9	1.52	0.39	17.3
400	trace	3.1	0.99	Very small‡	6.6
300	trace	0.7	0.55	Very small‡	20.4
400	trace	0.8	0.47	Very small‡	13.2
					Average = 13.1

* Calculated on the basis of C^{11} concentration in the administered CO_2 taken as 100.

† Calculated on the basis of C^{11} concentration in the expired CO_2 taken as 100.

‡ Cannot be calculated since the amount of CO_2 in which the C^{11} was originally furnished is not known.

Before attempting any discussion of these data, it is necessary to consider another set of experimental findings. These relate to the fixation of carbon dioxide in the newly formed glycogen. The experiments summarized in Table XII show that when lactate or glucose is administered to fasted rats to stimulate glycogen formation, C^{11} administered in the form of

$C^{11}O_2$ is incorporated into the glycogen molecule. Carbon dioxide must, in other words, be regarded as a member of the pool of glycogen precursors, which has already been shown to contain ketogenic as well as known glycogenic members.

In the case of some of the $C^{11}O_2$ experiments, a difficulty arises in connection with the units in which results are expressed. The table includes three different ways of giving the data. The first of these, amount of C^{11} in glycogen as *per cent* of the total C^{11} administered, reflects the form in which the results were first obtained. The second column, gives the C^{11} concentration in the glycogen carbon calculated on the basis of the C^{11} in the administered CO_2 taken as 100. This might seem adequate at first approach until one considers the last three experiments. In these, the $C^{11}O_2$ was prepared without addition of carrier, so that a very high concentration of C^{11} was present initially. The actual amount of CO_2 in which this C^{11} was present was not determined, since it is apparent that the total amount of C^{11} fixed was not influenced by the high initial concentration of the C^{11}. This indicates, as one might have expected, that the $C^{11}O_2$ which was injected intraperitoneally as a sodium bicarbonate solution must have been diluted in the body before it entered the glycogen to such an extent that the amount of CO_2 added had no appreciable effect on the total amount involved. Similar situations should, of course, be expected to arise in any other experiments if the label were administered as a small amount of substance containing a very high C^{11} content. It happens that the extreme situation is most readily produced with $C^{11}O_2$, since this tracer can be prepared directly in this form.

If the average figure in the second column is taken as 0.3, a dilution of $\frac{100}{0.3}$ or about 330-fold for the conversion of the injected CO_2 into glycogen, may be calculated. In the 2.5 hours of the experiment the rats excreted about 12.5 m*M* CO_2, however, which contained about 50% of the total C^{11}. If the injected CO_2 were first diluted in the body CO_2, an average initial dilution of 0.5 millimole into 25, or 50-fold would occur before the CO_2 entered the glycogen. Though complete mixing of the respiratory CO_2 with the injected CO_2 is an assumption which cannot be completely justified on the basis of the data in these experiments, it can confidently be regarded as a closer approximation of the truth than the assumption that no dilution of the injected CO_2 occurred. The experiments with a trace of injected CO_2 indicated that this latter assumption is completely in error. On the assumption of complete mixing then, the *per cent* glycogen carbon derived from CO_2 may be calculated on the basis of the C^{11} concentration in the respiratory CO_2 taken as 100. Such figures are given in the last column of Table XII.[12]

These figures indicate that an average of 13% of the glycogen carbon was derived from carbon dioxide, whether the stimulus for glycogen formation was glucose or lactate.

When the data given in Table XI were considered with this possibility in mind, it was found that the amount of C^{11} which appeared in the glycogen after feeding glucose and carboxyl-labeled acetate could be accounted for on the assumption that the acetate was first oxidized to CO_2 and then reincorporated into glycogen. In all other cases, the amount of C^{11} incorporated into liver glycogen was significantly greater than could be accounted for by such indirect incorporation.

Several additional facts were demonstrated in these experiments. Thus, it was shown that muscle glycogen gave little evidence of turnover during the 2.5 hrs. of the experiments, and that the liver glycogen of an unfasted rat likewise showed little exchange with the carbohydrate pool. Once glycogen has been deposited in liver or in muscle (muscle glycogen does not show the marked fluctuations with fasting and feeding that liver glycogen shows) it apparently does not exchange rapidly with its precursors. This stability is only relative, however, to the rapid mixing which occurs in the pool of carbohydrate precursors. These experiments were of short duration, 2–2.5 hours. There is no reason to suppose that examination of glycogen depots over a 3-day period would not reveal as rapid a turnover as the body proteins undergo. Experiments done with N^{15} were usually carried out over a considerable period of time. Though protein regeneration rate was termed rapid, with an average half-life of about 5 days, it can be seen that it is slow relative to the rate at which carbohydrate precursors can be

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[12] Justification for the assumption of rapid mixing may be regarded to rest at present on the following facts.

(a) After injection of $C^{11}O_2$ into the blood stream of dogs, Ball *et al.* (4) found that the C^{11} concentration of the bicarbonate of the pancreatic juice reflected the C^{11} concentration of the blood bicarbonate, indicating rapid mixing of CO_2 in the body fluids.

(b) When glycogen was formed from pyruvate by liver slices *in vitro*, about the same proportion of glycogen carbon was calculated to be derived from CO_2 as was found *in vivo* (33).

(c) In recent experiments of Sonne, Buchanan and Delluva (130), where the precursors of uric acid in the pigeon were examined, carbon atom 6 of uric acid was shown to be formed from CO_2. The C^{13} content of the respiratory CO_2 and of carbon atom 6 were virtually identical, whether the C^{13} was initially administered as $C^{13}O_2$, as the carboxyl group of acetate, lactate or glycine, or as the α and β carbon of lactate.

Consequently, though the figures in the last column of Table XII may rightly be regarded as subject to considerable error, they nevertheless should reflect the correct order of magnitude of the amount of glycogen carbon derived from CO_2.

handled in the fasted rat. In the section on acetate metabolism, observations of Rittenberg and Bloch (90, 91) on the incorporation of C^{13} (administered as acetate) into liver glycogen will be discussed. These experiments were carried out under conditions more nearly comparable to those used in most of the N^{15} experiments with amino acids.

The study of glycogen formation with C^{11} was hampered by the limited time available because of the rapid decay of the tracer. Consequently, it was impossible in these experiments to determine how the C^{11} which entered the glycogen molecule was distributed among the various carbon atoms of the glucose units. Wood and his collaborators have contributed significantly to the problem of the mechanism of liver glycogen formation in the rat by degrading the glycogen formed and determining the position of the labeled carbon. C^{13} was used in these experiments. When $C^{13}O_2$ was administered with glucose, the C^{13} was found only in carbon atoms 3 and 4 of the glucose in the glycogen (159). (See Addendum.)

TABLE XIII

Incorporation of $C^{13}O_2$ into Liver Glycogen of Fasted Rats after Glucose Feeding (159)

Unlabeled compound fed	mM $C^{13}O_2$ injected	mM glycogen carbon formed	C^{13} in glycogen				
			At.-% C^{13} excess in C − 3 + 4	Per cent glycogen carbon derived from administered CO_2*		Per cent glycogen carbon derived from total CO_2 of body†	
				C − 3 + 4	Av. in all 6 carbons	C − 3 + 4	Av. in all 6 carbons
600 mg./100 g. body weight.	2.3	3.2	0.03	0.57	(0.19)	10.0	(3.3)
	9.1	4.1	0.10	2.16	(0.72)	15.4	(5.1)

* Calculated on the basis of C^{13} concentration in the administered CO_2 taken as 100.

† Calculated on the basis of C^{13} concentration in the expired CO_2 taken as 100.

First experiment done as in Table XII. In second experiment rat was fasted 48 hours instead of 24, and liver glycogen was removed after 3.5 hours instead of 2.5 hours.

Table XIII gives some of the data obtained in two of these experiments. The first experiment was performed under conditions comparable to those of Table XII. In the second experiment, conditions were somewhat different, as indicated in the table. The glucose of the glycogen isolated from the livers was degraded, and the C^{15} was found only in positions 3 and 4. The C^{13} content of these carbon atoms is given first as the atom-% of C^{13} excess actually measured. These figures are then calculated both on the basis of the administered $C^{13}O_2$ taken as 100, and on the basis of the respiratory $C^{13}O_2$ taken as 100. In addition, they are expressed in terms of what the C^{13} content would be if the excess present in positions 3 and 4 were averaged over all the carbon in the glucose molecule. It is these latter figures which can be compared directly with data shown in Table XII. The results obtained by Wood are lower than the average observed in the C^{11} experiments. Considerable variation was observed in the latter, however, and, in view of the possible experimental errors, the results cannot be regarded as contradictory. The $C^{13}O_2$ data coincide with the lower range of the data obtained with $C^{11}O_2$.

The localization of labeled carbon in positions 3 and 4 of glucose was also observed when C^{13} was administered in the form of the carboxyl group of acetate, of propionate, and of butyrate (70, 158). On the other hand, when acetate labeled with C^{13} at both carbon atoms was administered with glucose, the C^{13} was found to be present in all six carbon atoms of the glucose in the newly formed liver glycogen (72). Table XIV shows the distribution of the C^{13} in the various positions. It is apparent that some differences are observed, but they are not marked.

TABLE XIV

Position of Labeled Carbon in Glycogen after Feeding $C^{13}H_3C^{13}OOH$ to Rats (72)

Position	Biological degradation per cent C^{13}	Position	Chemical degradation per cent C^{13}
3 + 4	5.8	3	6.7
2 + 5	4.7	6	5.6
1 + 6	3.3	1 + 2 + 4 + 5	5.8

C^{13} concentration expressed as per cent of amount in acetate fed.
2.5 m*M* acetate + 400 mg. glucose/100 g. of rat.

No data are available at the time of the writing, concerning results obtained after feeding $CH_3 \cdot C^{13}H_2 \cdot CH_2 \cdot C^{13}OOH$, except the statement that the label is found predominantly in positions 3 and 4 of the glucose obtained from the liver glycogen (158). There is no doubt that publication of more extensive data on the distribution of C^{13} in the glucose molecule after administration of the label in various forms, will bring much helpful information to bear on the problem of the chemical reactions whereby carbohydrate precursors are eventually converted into liver glycogen. Meanwhile, the possible reactions will be discussed in the light of our present knowledge.

As has already been mentioned, the evidence that even good glycogen formers, such as lactate and glucose, do not necessarily enter newly formed liver glycogen directly, indicates that one must postulate a metabolic pool from which the carbon precursors of glycogen are drawn. The constituents of this pool are regarded as being in dynamic interchange, *i.e.*, the members interact with each other in a variety of ways. Consequently, the glycogen formed from its precursors, which are members of the pool, will not reflect the isotope content of the initially administered substance, but rather the isotope content of the immediate precursor from which the glycogen is formed.

Even when glucose is the stimulus for glycogen formation, the incorporation of considerable amounts of CO_2 carbon into the glycogen, indicates that the fed glucose is not necessarily the immediate precursor of the liver glycogen. This should not be taken to mean, however, that glycogen cannot be formed from glucose without a breakdown of the glucose molecule. Such is not the case. *In vitro* experiments with rabbit liver slices which can convert glucose to glycogen indicated that the process occurs without appreciable incorporation of CO_2 into the glycogen (144). Under the conditions of these *in vitro* experiments, however, a three-carbon unit such as lactate, could form virtually no glycogen at all. However, when liver slices were incubated under such conditions that pyruvate could induce glycogen formation, the amount of CO_2 incorporated was the same as that observed *in vivo* (33). One is led to the interpretation that the incorporation of CO_2 is associated with glycogen formation under conditions in which the 6-carbon hexose unit is readily formed from lactate or pyruvate. Such conditions obtain *in vivo*. The hexose must then be regarded as being equilibrated very rapidly with its 3-carbon precursors. It is the latter which introduce the incorporated CO_2 into the hexose molecule.

The manner in which CO_2 is incorporated can best be explained by referring to Diagram II. Here it is indicated that pyruvate and CO_2 combine to form oxaloacetate. Since oxaloacetate can be reversibly converted to the symmetrical molecule, fumarate, any label present in one carboxyl group can be distributed in both. Pyruvate formed by decarboxylation of oxaloacetate thus labeled, will contain labeled carbon in its carboxyl group. When pyruvate is converted to hexose units, the label will be present in positions 3 and 4, since these are derived from the carboxyl carbon of the pyruvic acid. The incorporation of CO_2 into glycogen is thereby explained. Since the amount of CO_2 introduced was the same with both lactate and glucose feeding, one must assume complete equilibration of the hexose unit with its three-carbon precursor. The extent to which CO_2 enters the carboxyl position of pyruvate does not, however, indicate complete equilibration of pyruvate with CO_2. If this were the case, the carboxyl carbon of pyruvate should have the same isotope content as the CO_2, and 33% glycogen carbon should be found to be derived from CO_2, since two out of six carbon atoms in hexose are derived from the carboxyl group. The figure observed, 13%, indicates an average value somewhat less than half the maximum value theoretically possible. This was initially interpreted to indicate that one out of six carbons in glucose is derived from CO_2. However, incomplete equilibration could easily account for the discrepancy. This incomplete equilibration is indicated also by the fact that glycogen formed after feeding carboxyl-labeled lactate contained considerably more labeled' carbon than could be accounted for by CO_2 incorporation.

There is still some question about the mechanism whereby pyruvate is carboxylated and phosphopyruvate, which is presumably necessary for hexose synthesis, is formed. The detail of the evidence for these reactions will not be discussed here, however.

Up to this point, the condensation of oxaloacetate with pyruvate to form a 6-carbon dicarboxylic acid, has not been discussed. Examination of Diagram II will indicate that the regeneration of the four-carbon dicarboxylic acid from the six-carbon tricarboxylic acid by the indicated cycle involves the loss, by decarboxylation, of those two carbon atoms initially present as carboxyl groups of oxaloacetate. Similarly, the carboxyl group of pyruvate is also lost when the latter compound is condensed with oxaloacetate. The α carbon of lactate and the carboxyl carbon of acetate are converted, during the cycle, to carboxyl groups of the dicarboxylic acids. This explains why labeled carbon introduced as the carboxyl group of acetate should be introduced into the same positions of glucose as CO_2 carbon. It also affords a possible explanation for the apparent equilibration observed between the carboxyl group of acetate and CO_2. The methyl carbon of acetate and the β carbon of lactate should likewise, by going through the cycle, be distributed between the two methylene carbons of the dicarboxylic acids and thereby enter positions 1, 2, 5 and 6 of glucose.

It should be clearly understood that the conversion of CO_2 carbon or acetate carbon into glycogen carbon, by the route outlined above, does not by any means imply a net formation of glycogen from these precursors. In fact, the scheme in Diagram II indicates such net conversion would not occur. Acetate carbon can only enter the tricarboxylic acid cycle by a route which provides for the loss, as CO_2, of an amount of carbon equivalent to that in the acetate added. Thus, there is no conflict between the newer isotope data and the older data from feeding experiments which indicate that certain substances, like acetate, are not glycogenic, but that others, like lactate, are. Most of the substances classified as glycogenic by feeding experiments, contain a three- or four-carbon chain which could enter the scheme, like lactate itself, in such a way as to be available for actual glycogen synthesis.

There is an implication in this last statement that the stimulus for glycogen deposition is furnished by introduction of those precursors which can cause a net increase in the three-carbon precursor of the hexose molecule. It has been pointed out by Wood (158) that the stimulus or lack of stimulus may be more indirect. For example, the ability of a substance to induce glycogen formation may be related to some property associated with high-energy phosphate formation. This, of course, is a possibility. Since, however, the relations outlined in Diagram II afford an apparently adequate mechanism to account for the known facts, further discussion of alternate mechanisms is dispensed with.

To avoid misunderstanding, the present status of the reactions in Diagram II as a working hypothesis is again emphasized. The discussion in this paper has been designed only to indicate what may be regarded as the simplest and most plausible explanation of results obtained when liver glycogen formation *in vivo* has been studied with carbon isotopes. The reader has already been referred to the literature for a discussion of the nature of the *in vitro* evidence on the basis of which the scheme has initially been formulated (48, 68, 156, 158).

There has been no consideration, up to this point, of the relations between the components of what may be called the fat and carbohydrate pool with the amino acids. The glycogenic amino acids would be expected to enter the pool by routes about which some information is available. Thus, the reactions of transamination (31, 96) can easily account for the conversion of alanine to pyruvate, of aspartate to oxaloacetate, and of glutamate to α-ketoglutarate, as well as the corresponding reverse reactions. The ready transfer of nitrogen among the amino acids, which has been demonstrated *in vivo* with N^{15}, bears out the occurrence of such transformations.

Carbon isotopes have also been applied, but only to a limited extent, to study the conversion of amino acids into carbohydrate. Thus, Gurin and Wilson (59) found that, after feeding alanine labeled with C^{13} in the carboxyl position to phlorhizinized rats, about 3% of the carbon of the extra sugar excreted was derived from the administered alanine. In a fashion similar to that employed in the study of glycogen formation in rats, Olsen, Hemingway and Nier (82) have studied glycogen formation after glycine administration to mice. The results are shown in Table XV. It can be seen that the proportion of glycogen carbon derived from glycine labeled in the carboxyl position is of the same order of magnitude in mice as was observed in rats fed other precursors. The experiments are not strictly comparable, however. The rats were fed 1–2 m*M* precursor/100 g. body weight. The mice received 8.5 m*M*/100 g. body weight. The duration of the experiments was 2–2.5 hours for the rats and 16 hours for the mice. Consequently, it is probably unwise to attempt more than casual comparison of the results. Insufficient data are on hand to warrant conclusions about the manner in which the two-carbon unit, glycine, is converted into carbohydrate. Perhaps it should be pointed out that, though glycine induces considerable glycogen deposition in the livers of fasted rats, the action is delayed, with maximum deposition 14 hours after ingestion, whereas other glycogenic substances usually cause a much more rapid response (75).

The recent results obtained in the study of the carbon precursors of uric acid in the pigeon may be recalled in connection with the conversion of glycine carbon to glycogen carbon. The reader is referred back to Table VIII. The authors have made several interesting conclusions from these data regarding the relationships of the uric acid precursors. Thus, glycine carboxyl carbon is not converted to acetate carboxyl carbon. Otherwise, some C^{13} should have appeared in positions 2 and 8 after glycine administration, since these carbons are clearly derived from the carboxyl groups of acetate. The distribution of C^{13} in positions 4 and 5 is curious. The authors suggest that carbon atom 4 may be derived from the carboxyl group of lactate or glycine and carbon 5 from the α (or β) carbon of lactate. Consequently, lactate may be converted to glycine or a metabolic derivative of glycine by loss of the β carbon in a fashion comparable to the conversion of serine to glycine (see p. 87).

These findings are of interest in connection with the evidence previously described (see p. 88) that glutamic acid may be a precursor of glycine, since the interconversion of lactic acid and glutamic acid can easily be accounted for by way of the tricarboxylic acid cycle.

TABLE XV

Glycogen Formation from Glycine in Mice (82)

Glucose in Liver Glycogen, *Mg.*	C^{13} in Liver Glycogen Calculated on Basis of 100% Excess in Administered Glycine
19.8	5.5
13.0	6.4
7.0	9.2
17.2	8.6

Liver glycogen isolated 16 hours after feeding $CH_2NH_2 \cdot C^{13}OOH$ to mice. 125 mg. glycine/20 g. mouse.

Glycogen formation in the intact animal has likewise been studied by Ussing (140) and by Boxer and Stetten (28, 30) and Stetten and Boxer (134, 135) with the aid of deuterium. If the deuterium content of the body water of rats is kept at a suitably high level, the glycogen formed during the period incorporates stably bound deuterium. The results are entirely in accord with those obtained with carbon, *i.e.*, that such glycogen must be formed from particles with few carbons in the chain and that hydrogen is labilized during the interconversions.

3. Formation and Fate of Acetate

The experiments discussed in the previous section, which showed that acetate carbon appeared in newly formed liver glycogen, indicated, at the same time, that ingested acetate is rapidly metabolized in the rat. This finding was entirely in harmony with conclusions previously drawn on the basis of feeding experiments without isotopes. The ketogenic character of acetate has been confirmed also by Swenseid *et al.* (137), who demonstrated that the ketone bodies excreted by fasting rats fed acetate labeled with C^{13} contained appreciable quantities of the isotope. In these experiments controls with $C^{13}O_2$ excluded the possibility that the C^{13} found in the ketone bodies could be indirectly derived from CO_2 carbon.

The metabolism of acetate in the animal organism has been studied extensively by Bloch and Rittenberg (12, 16, 17, 18, 19) and Rittenberg and Bloch (89, 90, 91). The sources of acetate, the fate of acetate, and the rate of acetate formation *in vivo* were investigated by the combined use of deuterium and C^{13}.

The procedure employed to study the precursors of acetate was similar in principle to that previously described for determining the precursors of glycine (see p. 87). When foreign amino acids or amines are fed to the rat, the corresponding acetyl derivatives are excreted in the urine. Bernhard had shown that such acetyl derivatives contained significant amounts of deuterium if deuterioacetate was administered (10). Bloch and Rittenberg confirmed this finding, and employed it as a test for acetate precursors (18). Substances labeled with deuterium were administered to rats together with phenyl amino butyric acid, and the deuterium concentration of the excreted acetyl derivative was determined. Presence of deuterium in concentrations significantly above that of the body water was then taken to indicate that the fed compound could be regarded as an acetate precursor. By this procedure ethanol, butyric acid, alanine, *n*-valeric acid and myristic acid were found to be converted to acetate by the animal. No deuterium appeared in the acetylphenylaminobutyric acid, however, when deuterio propionic acid or 10,11-dideuterioundecylic acid was fed.

The same authors showed further (19) that when acetate labeled with deuterium in the methyl group and C^{13} in the carboxyl group was fed together with phenylaminobutyric acid, the ratio of the isotopes in the acylamino acid excreted was identical with that in the compound fed. This indicated that acetate itself acted as a direct acetylating agent in the body. The acetylation reaction involved no labilization of hydrogen in the acetate. Consequently, the deuterium alone could be regarded as an adequate tracer for the acetate carbon chain.

Using deuterioacetate in this fashion, the authors were able to estimate the total amount of acetate formed by the rat in a given amount of time. The reasoning in back of the procedure was that the dilution of the fed acetate, as measured by the ratio $\frac{\text{D in acetate fed}}{\text{D in excreted acetyl group}}$, should indicate how much endogenous acetate had been mixed in the body with the fed deuterioacetate. There is an implied assumption here, of course, that complete mixing of endogenous and exogenous acetate occurs.

A selected group of the data obtained in these experiments are presented in Table XVI. The results with *dl*-phenylaminobutyric acid indicate that the dilution factor varies inversely with the dosage, as it should. The product of dosage times dilution factor is constant. This product represents the mM acetic acid formed/day/100 g. rat. The table shows that approximately the same figures were obtained when the D and L isomers of phenylaminobutyric acid were fed separately. Furthermore, the method was independent of the foreign substance fed, since the aromatic amines, sulfanilamide and *p*-amino benzoic acid, gave the same results as phenylaminobutyric acid. On the basis of the collected data, the conclusion was reached that the rat forms acetic acid at the rate of 15–20 mM/day/100 g. body weight.

TABLE XVI

Acetylation of Foreign Amines by the Rat (19)

Duration of feeding, days	Deuterio acetate fed per day mM/100 g. body wt.	Other substance fed	Dilution factor	Dosage × dilution factor
5	0.078	DL-phenylaminobutyric acid	309	24.1
10	0.81	"	28.8	23.3
4	1.52	"	13.1	19.9
5	1.68	"	11.5	19.3
8	1.60	"	11.8	18.9
8	1.60	L(+)-Phenylaminobutyric acid	13.1	21.0
8	1.51	D(−)-Phenylaminobutyric acid	10.8	16.4
8	0.91	Sulfanilamide	20.5	18.7
8	1.57	*p*-Aminobenzoic acid	11.8	18.5

$$\text{Dilution factor} = \frac{\text{D in acetate fed}}{\text{D in excreted acetyl group}}$$

In another set of experiments Rittenberg and Bloch (89, 90, 91) fed labeled acetate to rats and to mice, and determined the distribution of label in the body fat, in glycogen, and in glutamic and aspartic acids. The results of these experiments are presented in Table XVII. The acetate fed to the mice was labeled with both D and C^{13} in the carboxyl group, but that fed to rats contained only C^{13}. The isotope concentrations in the substances isolated from the tissues of the rats were lower than those of the mice, because of the relatively short duration of the feeding (3 as opposed to 8 days). The data obtained on mice were more extensive and reliable, but no significant differences were observed between the two species.

From these experiments it was concluded that acetate can be converted to fatty acids and cholesterol as well as to aspartic acid, glutamic acid and glycogen. The experiments on the conversion to fatty acids will be discussed first.

The total fatty acids of both liver and carcass of mice contained significantly higher concentrations of D and C^{13} than the body water and respiratory CO_2 respectively. It was concluded, therefore, that both the methyl group and carboxyl group of acetate was used for fatty acid synthesis. This utilization was direct, *i.e.*, did not proceed by way of CO_2 or body water. Acetate entered liver fat more rapidly than body fat, and saturated fatty acids more rapidly than unsaturated. Degradation of the oleic acid to azelaic acid and pelargonic acid revealed that the isotopes were distributed about equally in the two halves of the oleic acid molecule. Furthermore, the carboxyl group of the fatty acids contained about twice as much C^{13} as the average found in all the carbons of the fatty acid molecule.

These findings were interpreted to indicate that the fatty acids were formed from acetate by a fairly direct condensation of two carbon units, resulting in the presence of C^{13} in the carboxyl carbon and in all alternate carbons in the molecule.

The liver cholesterol of mice also contained more C^{13} and D than could have been introduced by way of CO_2 and H_2O. These results were in harmony with previous studies of the conversion of acetate to cholesterol in rats (17). Since deuterium alone was used in these latter experiments they will not be discussed here. Together with the data in Table XVII, however, they substantiate the conclusion that cholesterol is formed in rats and mice by a condensation of two-carbon units derived directly from acetate.

The incorporation of carbon derived from the carboxyl group of acetate into glycogen and into the amino acids, aspartic and glutamic acids, is also shown in Table XVII. In both rats and mice the amount of C^{13} in liver glycogen and in aspartic acid is low, being somewhat over half the value obtained for respiratory CO_2 (for rats, the C^{13} content of urea may be regarded as equivalent to the C^{13} content of the respiratory CO_2). Somewhat higher amounts were found in glutamic acid. Decarboxylation of this latter substance revealed also that not all the C^{13} was present in the α-carboxyl carbon, but that some must also be in the rest of the molecule.

The data are not out of harmony with the results obtained in previous short term studies of glycogen formation. Similar interpretations of the manner of entry of the labeled carbon into glycogen carbon may apply. Glutamic and aspartic acids are protein constituents present in considerable amounts in the animal body, so that high dilutions of incorporated C^{13} would be expected. The entry of the C^{13} into these amino acids might well be by way of the corresponding keto acids, α-ketoglutarate and oxaloacetate, which are constituents of the tricarboxylic acid cycle.

In conclusion, it may be pointed out that the amount of carbon derived from fed acetate in the carbohydrate and protein constituents of rat liver examined in these experiments is considerably smaller than the amount of nitrogen generally derived by liver amino acids from similar amounts of fed compounds labeled with N^{15}. The experiments with rats in Table XVII are not strictly comparable with the amino acid feeding experiments of Table III, since the rats fed acetate were immature, growing animals, whereas mature animals were employed in most of the amino acid feeding experiments. Nevertheless, the differences may reflect a more rapid dilution undergone in the body by carbon in the form of acetate, than by nitrogen in the form of an amino acid. Compare also, in this regard, the greater rate of dilution of fed acetate in body acetate than, for example, fed glycine in body glycine (Table X).

TABLE XVII

Distribution of C^{13} and D in Body Constituents of Mice and Rats Fed Acetate Labeled with C^{13} in the Carboxyl Group and D in the Methyl Group (89, 90, 91)

Substance isolated	Mice, fed $CD_3 \cdot C^{13}OOH$ for 8 days				Rats, fed $CH_3 \cdot C^{13}OOH$ for 3 days	
	Carcass		Liver		Carcass	Liver
	C^{13}	D	C^{13}	D	C^{13}	C^{13}
Total fatty acids	0.83	0.17	1.05	0.42	0.41	
Carboxyl carbon of fatty acids	....	....	....	....	0.92	
Saturated fatty acids	1.03	0.31	1.63	0.55		
Carboxyl carbon of saturated fatty acids	1.83	....	2.96			
Azelaic acid* from "oleic" acid fraction	0.92	0.18				
Pelargonic acid† from "oleic" acid fraction	0.72	0.19				
Cholesterol	0.48	0.31	0.89	0.87		
Glutamic acid	....	....	....	0.82	....	0.36
α-Carboxyl of glutamic acid	...	....	....	0.98	....	0.42
Aspartic acid	....	....	....	0.39	....	0.23
Glycogen	. .	....	....	0.39	....	0.20
Amidine carbon of arginine	....	....	....	0.82	....	
Respiratory CO_2	0.67					
Urinary urea	....	....	....	....	0.34	
Body water	....	0.12				

All figures calculated on basis of isotope content of administered acetate taken as 100.

Both rats and mice given 1.5 m*M* labeled acetate/100 g. body weight/day for period indicated. Mice were mature. Rats were immature, weighing about 50 g.

* Carbons 1 to 9.

† Carbons 10 to 18.

This large dilution of carbon of acetate is consistent with the fact that the two-carbon acetate particle is a constituent of a system by way of which a large proportion of the carbon chains of the body are metabolized.

Whatever may be the errors of the scheme presented in Diagram II, and there may be many, its value as a working hypothesis rests not only on its ability to account for the observed experimental facts, but also on its service as a framework for correlating fat, carbohydrate and protein metabolism.

V. CONCLUSION

The material which has been discussed represents only a small fraction of the evidence which has been brought to bear on many of the problems of intermediary metabolism in animal tissues. Restriction of the subject matter to *in vivo* experiments with nitrogen and carbon isotopes has necessitated the omission of many pertinent facts. Conclusions drawn from *in vivo* studies with deuterium may be mentioned particularly, since this isotope of hydrogen has been applied as a tracer for fat, carbohydrate, and protein metabolites under conditions similar to those described for N^{15} and C^{13}. The contributions of comparative biochemistry have also been slighted, as has the great body of *in vitro* work both with and without isotopes. The latter is in most instances more informative in regard to detail of mechanism than the experiments *in vivo* can ever be.

In spite of these omissions, the material presented in this review represents an attempt to give a fairly complete picture of our present concepts of the interrelationships between protein, fat, and carbohydrate constituents in the animal body, insofar as they have been studied with the isotopes of carbon and nitrogen. The "dynamic state of the body constituents", the rapid breakdown, resynthesis and interchange, which the application of isotopes to biochemistry has revealed, is certainly more extensive than previous balance studies led investigators to expect. The conclusions from isotope studies have not been entirely revolutionary, however.

Preceding the extensive application of isotypes to the study of the living organism, the study of enzymes *in vitro* revealed the reversible nature of many enzyme-catalyzed reactions. This fact alone entailed the conclusion that isotopes must be expected to reveal a considerable amount of chemical interchange among the components of a living system without simultaneous net changes. Subsequently, *in vitro* analyses of the detail of biochemical processes have gone hand in hand with *in vivo* isotope studies to give a rapidly expanding understanding of the underlying chemical bases of the facts of physiology and of all biological phenomena. Throughout this development, the agreement observed between conclusions derived from both types of approach, the *in vivo* and the *in vitro* methods, lends mutual strength to the validity of both procedures as experimental tools in biology.

The author is grateful to Dr. E. A. Evans, Jr., Dr. K. E. Bloch, Dr. H. S. Anker and Dr. K. E. Hite, who have made valuable suggestions and criticisms during the preparation of the manuscript.

ADDENDUM

Rapid development of the field covered in this review has occurred within the last year. This is particularly true in regard to the description of techniques employed with C^{14}. This isotope has been actively distributed recently by the Isotopes Branch of the United States Atomic Energy Commission (190, 193). The following references are listed with the hope of bringing the bibliography partially up to date.

Kamen (181) has published a book on tracer methodology which contains, among much other useful information, a description of the methods employed with C^{14}. A recent review (182) on the fundamental aspects of the use of isotopes in biochemical research and several publications (168, 177, 184, 188) relating to the technique of measuring C^{14} have appeared. The natural occurrence of C^{14} has been reported (167, 176). Additional studies of the isotope dilution technique (170, 175, 181) are also available.

A system of nomenclature of isotopic compounds has been suggested (191), and methods of synthesis for the following labeled compounds of biological interest have been described: histidine (N^{15}) (197), methionine (Methyl C^{14}) (187), phenyl alanine (α and carboxyl C^{14}) (178), tyrosine (β C^{14}) (192), nicotinic acid (carboxyl C^{13}) (189), cholestenone (198) and testosterone (199) (both with C^{14} in ring A), and 1, 2, 5, 6-dibenzanthracene (C^{14} in 9-position) (179).

A new, simple procedure for making $NaC^{14}N$ from $BaC^{14}O_3$ (166), the conversion of carbon dioxide to acetylene on a micro scale (169), the preparation of $C^{14}O$ from $BaC^{14}O_3$ (183), and more information on isotopic acetate and acetoacetate synthesis (200) are available. The biosynthesis of C^{13}-labeled starch has also been described (185).

In the biological field, Shemin and Rittenberg have continued their studies of the fate of N^{15}-labeled glycine in the human. The function of glycine as a nitrogenous precursor of the protoporphyrin of hemoglobin has been established (194) and a calculation of the life span of the human red blood cell has been made, from the data on the rate of entrance and disappearance of the isotope (195). It has also been shown that the uric acid excreted by the human obtains its N in position 7 directly from the amino group of glycine (196). This reinforces and supplements the previous report of Sonne, Buchanan and Delluva concerning the precursors of uric acid in the pigeon. Buchanan and Sonne (171) have extended their very interesting study of this process and shown that carbon atoms 2 and 8 of uric acid are derived from formate. Since these carbons were previously shown to be derived from the carboxyl group of acetate, it appears that acetate carbon may be converted to formate carbon in the pigeon.

Tesar and Rittenberg (197) have studied the fate, after ingestion, of the γ N of histidine in the rat. Gurin and Delluva (178) have demonstrated the conversion of phenylalanine to adrenalin in the intact rat by a route which involves no apparant change in the carbon structure except a loss of CO_2 from the side chain. Mackenzie *et al.* (186) have shown that the methyl group of methionine is oxidized *in vivo*. Kalckar and Rittenberg (180) have demonstrated a rapid turnover *in vivo* of the 6-amino N of adenylic acid in skeletal muscle. Friedberg (173) and Friedberg, Winnick and Greenberg (174), have studied protein and peptide synthesis *in vivo*. Delluva and Wilson (172) have used C^{13} to study CO_2 assimilation in the living rat and shown that the carbon appears in urea, in the amidine group of arginine, and in the carboxyl carbons of glutamic and aspartic acid, in accordance with expectations.

Recent results of Wood and his collaborators will appear in the report on the Isotope Symposium held at the University of Wisconsin, September, 1947. Wood's work represents a systematic study of the distribution, in newly formed liver glycogen, of isotopic carbon administered in the form of a number of glycogenic and ketogenic substances. The results can be explained fully by postulating that the reactions of glycolysis and of the tricarboxylic acid cycle operate *in vivo* in the manner deduced from *in vitro* studies.

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The Nature and Production of Artificial Radioactivity

By WALDO E. COHN

CONTENTS

I. THE NATURE OF RADIOACTIVITY

1. Isotopy, Stability and Terminology (2, 3, 5, 6)

(*a*) *Isotopy.* Perhaps the two most important generalizations which came as a consequence of the discovery of radioactivity in uranium by Becquerel in 1896 were the "transformation" theory of Rutherford and Soddy, postulating an inherent instability in radioactive elements which results in spontaneous disintegrations accompanied by the expulsion of particles, and the so-called "displacement" law of Russell and of Soddy and Fajans, relating the chemical properties of parent and product atoms to the Periodic Table of the elements. From the displacement law and the experimental inseparability of atoms which have arrived independently at the same place in the Periodic Table, the concept of "isotopes"—atoms of identical chemical properties but of differing mass—was established by Soddy well in advance of the demonstration by Aston that many elements contain (or can be caused to contain) atoms of varying atomic weight. Those which, in nature, contain atoms of but one mass are termed "monoisotopic" elements (*e.g.*, P, Na).

(*b*) *Stability.* The fundamental assumption is made that stable nuclei (for the phenomenon of radioactivity is now known to be exclusively a property of the atomic nucleus) are those which exist in nature and in which radioactivity has not been discovered. A radioactive atom may best be regarded as one whose existence is liable to sudden and unannounced termination by a spontaneous nuclear transformation. Its existence up to the instant of such decay is no different, chemically or physically, from that of its "stable" brethren, except for the slight differences due to its increased or diminished mass (and perhaps to difference in spin). Radioactivity, occurring in the natural and in the artificial or induced "radioisotopes," is considered as evidence of nuclear instability which, in turn, depends upon the forces and particles in the particular nucleus under consideration.

(*c*) *Nuclear Particles.* Although high speed electrons or β-rays (termed "negatrons" if negative and "positrons" if positive, and denoted by the symbols β^- and β^+, respectively) are known to be ejected in many radioactive disintegrations, it is currently believed that protons and neutrons are the fundamental building blocks of atomic nuclei, and that the aforementioned particles are formed at the instant of decay. The proton is the nucleus of the "light" hydrogen atom ("mass number" $\equiv A = 1$) carrying a charge equal to that of the electron and positive in sign. The neutron is essentially the same particle but electrically uncharged. The mass number is defined as the integral number nearest the actual mass[1] of the nucleus in question and is, therefore, by definition, the sum of the number of neutrons and the number of protons.

The number of protons in a nucleus defines the total positive charge of that nucleus, which decides the number of extranuclear electrons in the neutral atom; this, in turn, defines the chemical identity of the atom. The number of protons is given by the "atomic number" of the element, symbolized by Z. Thus, any nucleus (with the exception of isomers, which are discussed below) may be defined, without ambiguity, in terms of two numbers, Z, the atomic number or number of protons, and A, which is the total number of neutrons plus protons (sometimes known collectively as "nucleons").

(*d*) *Nomenclature.* It is clear that the atomic number and the chemical symbol of an element are identical and either is sufficient to set the other. Hence, it is customary to refer to a given nuclear species by the chemical name or symbol and the mass number. For example, the nitrogen isotope of mass number 14 is commonly referred to as N^{14} (in some prints as N 14, to avoid superscripts, and in others as ${}^{14}N$, to permit the placing of valency symbols in the usual position). In writing nuclear reactions, however, it has been customary, even though unnecessary, to specify both the chemical symbol and Z, thus leading to the obvious redundancy ${}_7N^{14}$, *etc.*, in the reaction ${}_7N^{14} + {}_0n^1 \rightarrow {}_1p^1 + {}_6C^{14}$. This reaction indicates that a neutron ($Z = 0$) has reacted with the nucleus of N^{14} to produce a carbon nucleus of mass 14 and a proton (which could equally well be designated as ${}_1H^1$). A simplified designation of this same reaction is $N^{14}(n, p)C^{14}$. This and other reactions will be discussed later.

(*e*) *The Neutron: Proton Ratio.* An examination of a table of the known stable nuclei reveals the fact[2] that only a few of the many possible combinations of neutrons and protons are included and that any other combination is either known to be radioactive or has not been found (this is presumptive evidence that such nuclei, if ever formed, are so unstable that they decom-

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[1] The actual masses of these nuclear particles are expressed in terms of the mass of the O^{16} atom, which is defined as having mass 16.0000. . . . On this scale, the H^1 atom has a mass of 1.00813, the neutron one of 1.00893, the electron one of 0.000549.

[2] Other facts revealed: those of even A have an even Z (except H^2, Li^6, B^{10} and N^{14}); those of odd A have an odd or even Z and no "isobar" (nuclei of the same A but differing Z) exists (except in the cases of Cd^{113}-In^{113}, In^{115}-Sn^{115}, and Sb^{123}-Te^{123}); those of odd Z have no more than two stable isotopes each, in many cases but one; and those of even Z have many stable isotopes.

pose before they can be detected). For example, the carbon nucleus has, by definition, 6 protons but only those varieties containing 5, 6, 7 or 8 neutrons have been found. Of these combinations, only those containing 6 and 7 neutrons are stable (non-radioactive) and are found in nature, while those containing 5 and 8 exist, after their production, as unstable nuclei foredoomed to disintegration. A closer inspection of the ratio of neutrons to protons $\frac{(A - Z)}{Z}$ in the known stable nuclei discloses the facts that (1) this ratio is less than 1 in only two cases, the rare ($10^{-5}\%$) isotope of ${}_2He^3$ and the proton (${}_1H^1$) itself; (2) this ratio tends to rise with increasing Z so that ratios of over 1.5 are found in the region of Pb and Bi; (3) nuclei possessing a ratio which does not occur in nature and which are hence, by experience, unstable, remove that instability by one or more steps of changing neutrons into protons or protons into neutrons, ultimately producing a ratio which is stable. These steps involve releases of energy which, per atom, range up to millions of times those involved in chemical reactions. Hence, we conclude that the naturally occurring nuclei are energetically more stable than the radioactive ones, and also that the neutrons, while electrically neutral, participate in the stabilization of nuclei. From the failure to find He^2 (which would have a nucleus of 2 protons with no neutrons), the scarcity of He^3 (with less than 1 neutron per proton) and the increased number of neutrons per proton in the heavy elements (where high proton concentrations exist), it is logical to consider the neutrons as binders or diluents which help to overcome the repulsive (Coulomb) forces between the nuclear protons.

2. Radioactive Decay and Growth (2, 3, 5, 6)

(*a*) *Spontaneous Nuclear Disintegrations* (20). Radioactive decay can occur in five ways, four of which give rise to a nucleus which differs chemically from the parent (displacement law). (1) The emission of energetic He^4 nuclei (α particles), *e.g.*, ${}_{92}U^{238} \rightarrow {}_{90}Th^{234}$ (known as UX_1) $+ {}_2He^4$, is characteristic of the elements with Z greater than 82 but is practically non-existent in elements below this atomic number. (2) *Negatron* decay (generally called "β" decay), the emission of high speed negative electrons, results from the conversion of a nuclear neutron into a proton ($n \rightarrow p^+ + \beta^-$), leaving a nucleus of the same mass but of atomic number $Z + 1$; an example is ${}_6C^{14} \rightarrow {}_7N^{14} + {}_{-1}\beta^0$. (3) The emission of high speed positive electrons, or *positrons* (β^+), by the conversion of a nuclear proton to a neutron ($p^+ \rightarrow n + \beta^+$), leaves a nucleus of atomic number $Z - 1$; *e.g.*, ${}_6C^{11} \rightarrow {}_5B^{11} + {}_1\beta^0$. (4) *Orbital electron capture*, generally termed "K capture," by which is meant the movement of an orbital (usually K) electron *into* the nucleus, accomplishes the conversion of a proton into a neutron just as does positron emission[3]; for example, ${}_{18}A^{37} + {}_{-1}e^0 \rightarrow {}_{17}Cl^{37}$. One frequent consequence of

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[3] A positron emitted means, in addition to the loss of this particle, an electron lost to the decomposing atom since Z diminishes by one unit; hence, the total energy lost

this type of decay, a fortunate one from the standpoint of detection, is the emission of X-rays due to the movement of another electron into the vacancy in the K shell. (5) If the nucleus formed as a result of any of the above mentioned types of disintegration is not formed in its lowest energy state, or is produced initially as an excited state ("isomer") of a stable neutron-proton combination, one or more γ-rays, which are high energy photons or electromagnetic quanta, may be emitted to accomplish a reduction in energy content. Thus γ emission may accompany any of the above modes of disintegration. Nuclei which are the excited isomers of more stable states usually decay to those states solely by γ-ray emission; this mode of decay is termed "*isomeric transition*" (abbreviated I.T.).[4] In certain cases, the γ- or X-rays from such a disintegration may cause the ejection of K or other electrons (distinguished from the nuclear electron or β-ray by the symbol (e^-)) from the extranuclear electron shells of the radiating atom itself. This process is termed "internal conversion" since some of the γ-ray energy is converted into electron kinetic energy. It should be noted, however, that X-ray emission and electron emission, while coincident with and caused by the nuclear change, originate in the extranuclear part of the atom.[5]

(*b*) *Multiple Decay.* Although the decay of a nucleus always results in a loss of energy, and hence in the formation of a nucleus of lower energy content, this does not imply that the "daughter" nucleus is stable or that, if unstable, it will have a longer life span than its parent. As a matter of fact, in the many cases of radioactive "chains," which are most common in the naturally occurring radioelements and in those species created as a result of nuclear fission, it is quite common to have daughters with shorter lives than their "parents." A decay chain will arise whenever conversion of more than one neutron to proton (or *vice versa*) is required in order to achieve lasting stability. Similarly, it is occasionally possible for an unstable nucleus to disintegrate in more than one way. Sometimes, as in the case of the "branched" decay chain,

$$\begin{tikzcd}
& 51\text{m } Y^{91} \arrow[dd, "\text{I.T.}"] & \\
Sr^{91} \arrow[ur, "\beta^-"] \arrow[dr, "\beta^-"'] & & \\
& 57\text{d } Y^{91} \arrow[r] & \text{stable } Zr^{91}
\end{tikzcd}$$

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is at least that equivalent to two electron masses, or about 1 Mev. However, K-capture can take place whether this amount of energy is available or not and sometimes competes with positron emission (*e.g.*, Cu^{64}) (*cf.* p. 122).

[4] The precautions to be observed in using such an isotope as a tracer are discussed under Recoil Phenomena (p. 144).

[5] The properties of nuclear radiations and their interaction with matter are discussed in the chapter concerned with their detection and measurement.

which involves an isomeric pair, the two products may come together to a common species. In other cases the branching is permanent; for example, Cu^{64} can decay either to Zn^{64} or Ni^{64} (and to the latter by either positron emission or K-capture) both of which are stable:

$$\text{stable}\ {}_{28}Ni^{64} \underset{K}{\overset{\beta^+}{\leftleftarrows}} {}_{29}Cu^{64} \xrightarrow{\beta^-} \text{stable}\ {}_{30}Zn^{64}$$

(*c*) *Half-life* (10, 11). Regardless of the number of steps involved in achieving final stability, or of the relative rapidity with which these are accomplished, the nucleus which is involved shows no chemical or physical differences from its isotopic brethren during the period between disintegrations (or between creation and disintegration) save those due to the mass and spin differences. The theory of radioactive decay assumes only (a) that the lifetime of an unstable nucleus may have any value from zero to infinity and (b) that it is independent of the previous length of life of the nucleus. The lifetime of a given atom thus may be expressed only as a probability, which has no practical significance except when large numbers of atoms are being considered. As is true of such purely statistical (19) phenomena, the accuracy of prediction goes up with the number of cases and, since one normally deals with large numbers of atoms, it is possible to state the "half-life" of a given radioactive species quite accurately. The necessity for dealing in terms of half-life arises as a natural consequence of the fact that some of the atoms in a given sample of radioactive element will live forever. However, the half-life, by which is meant that time during which half of the atoms in a given sample will have decayed, is finite for all elements in which radioactive decomposition can be detected. The half-life is often used descriptively, *e.g.*, 4 year Fe^{55} (or 4y Fe^{55}); this is also one way of specifying a particular member of an isomeric pair, as in the decay scheme of Sr^{91} given in the preceding section.

(*d*) *Decay Rate*. From the basic assumption that any atom of a radioactive element may disintegrate at any instant regardless of its age, and from the demonstration that this rate is not to be influenced by ordinary chemical or physical conditions, we can state that the number of such decompositions ($-dN$) occurring within a given time interval (dt) is proportional (λ) to the number of atoms (N) of the species being considered:

$$-\frac{dN}{dt} = \lambda N \tag{1}$$

The "decay constant" is, by definition, the fraction of the sample decomposing per unit time. From this equation we derive the basic law of radioactive decay,

$$\frac{N_2}{N_1} = e^{-\lambda(t_2-t_1)} \tag{2}$$

(usually written as $N_t = N_o e^{-\lambda t}$, where N_o = atoms present at $t = 0$) where N_1 is the number of nuclei present at time t_1 and N_2 is the number present at time t_2. Since our fundamental assumption is that the rate of decay is proportional to the number of atoms present, it is clear that the above equation can be used to relate either numbers of atoms or rates of decay.

(*e*) λ *and Mean Life.* The relationship between λ, the decay constant, and the half-life ($t_{\frac{1}{2}}$) by which most radioelements are designated is easily derived from equation (2) as follows: $\frac{N_2}{N_1} = 0.5 = e^{-\lambda t_{\frac{1}{2}}}$ whence

$$t_{\frac{1}{2}} = \frac{0.693}{\lambda} \tag{3}$$

λ is not only the fraction of atoms decaying in unit time but is also (a) the reciprocal of the time required for the disintegration rate to drop to $\frac{1}{e}$ of its initial value, (b) the reciprocal of the time in which all the atoms initially present would disintegrate if the initial decay rate were to be maintained constant, (c) the reciprocal of the average or mean life, t_m. This last is, by definition, the sum of the lengths of all the individual lives in a sample divided by the number of lives in that sample (*i.e.*, the number of atoms). The number of atoms which will decay in the period between t and $t + dt$ is equivalent to $\lambda N dt = \lambda N_o e^{-\lambda t} dt$, where N_o is the number present initially. Since each atom in this group lives for time t, the sum of the lengths of all the lives in the group is $t(\lambda N_o e^{-\lambda t} dt)$. Hence,

$$t_m = \frac{1}{N_o}\int_0^\infty \lambda N_o t e^{-\lambda t}\, dt = \frac{1}{\lambda} = \frac{t_{\frac{1}{2}}}{0.693}\,. \tag{4}$$

(*f*) *Parent-Daughter Relationships.* When a radioactive element decays, another element (called the "daughter") is formed. A parent-daughter relationship can be derived relating the rate at which the daughter (which may be stable or radioactive) appears, to the rates of decay of both daughter and parent. If P and D are the *numbers of atoms* (not radioactivity) of parent and daughter, respectively, present at time t, then the rate at which D increases is the difference between the rate of decay of P, each atom of which gives rise to an atom of D, and the disappearance of the previously formed atoms of D, which will be going on at an ever increasing rate since it is proportional to the number of atoms of D present. Thus, if λ_D and λ_P represent the decay constants of daughter and parent, respectively,

$$\frac{dD}{dt} = \lambda_P P - \lambda_D D \tag{5}$$

But, from (2),

$$P = P_o e^{-\lambda_P t}$$

Whence

$$D_t = P_o \frac{\lambda_P}{\lambda_D - \lambda_P} (e^{-\lambda_P t} - e^{-\lambda_D t}) \tag{6}$$

The *radioactivity* (indicated by the asterisk*) of the D atoms is given by

$$D_t^* = D_t \lambda_D , \qquad \text{while} \qquad \lambda_P P_o = P_o^* ;$$

Hence

$$D_t^* = P_o^* \frac{\lambda_D}{\lambda_D - \lambda_P} (e^{-\lambda_P t} - e^{-\lambda_D t}) \tag{7}$$

From this, several interesting formulae are derived.

1. Growth of Radioelement from a Constant Source (*i.e.*, a parent which remains essentially constant during the period of observation, such as uranium or a pile or cyclotron).

Here $\lambda_P = 0$ and (7) becomes

$$D_t^* = P_o^*(1 - e^{-\lambda_D t}) \tag{8}$$

P_o^* is simply D_∞^*, the so-called "saturation" activity of the daughter element, which is attained only after a time very long compared to the half-life. This gives the standard "growth" formula, used to calculate yields of radioelements from cyclotrons, piles, *etc.*, as well as that of $Th^{234}(UX_1)$ from U^{238}, *etc.*,

$$\frac{D_t^*}{D_\infty^*} = 1 - e^{-\lambda_D t} \tag{9}$$

From this it is seen that growth from a "constant" source is the mirror image of decay. After one half-life has elapsed, 50% of the saturation activity will be attained; after two half-lives, 75%; after three, 87.5%, *etc.*

2. Time of Maximum Production of Daughter Radioelement from a Diminishing Source. When the parent or source of the daughter radioelement decays at an appreciable rate during the growth period, the daughter element will increase to a maximum activity and then diminish in amount. From equation (7) we can derive the expression for $t_{\max}$, the time when D^* is at a maximum, as follows:

$$\frac{dD_t^*}{dt} = [-\lambda_P e^{-\lambda_P t} + \lambda_D e^{-\lambda_D t}] \frac{\lambda_D P_o^*}{\lambda_D - \lambda_P} = 0$$

Whence

$$t_{\max} = \frac{1}{\lambda_P - \lambda_D} \ln \frac{\lambda_P}{\lambda_D} \tag{10}$$

In the special case where $\lambda_P = \lambda_D$,

$$t_{\max} = \frac{1}{\lambda} \tag{11}$$

From Eq. (5), it is obvious that, at $t_{\max}$, $\lambda_D D = \lambda_P P$, or

$$D^*_{t_{\max}} = P^*_{t_{\max}} \tag{12}$$

In other words, at the time of maximum D activity it is equal to the P activity.

3. Amount of Shorter-Lived Daughter in "Equilibrium" with Longer-Lived Parent. Substituting for P_o^*, from equation (2), in (7), we find that

$$\frac{D^*_t}{P^*_t} = \frac{\lambda_D}{\lambda_D - \lambda_P} (1 - e^{-(\lambda_D - \lambda_P)t}) \tag{13}$$

It is clear from this equation that, if the daughter is more long-lived than the parent ($\lambda_D < \lambda_P$), its activity will eventually become infinitely greater than the latter, since the parent will decay away to a negligible value, leaving only the daughter activity. However, if the parent is more long-lived than the daughter, we will eventually find a constant ratio of daughter to parent activity, that of the daughter being the greater, and the daughter will seem to decay with the half-life of the parent. If we set $t = \infty$ in (13), we find

$$\frac{D^*_\infty}{P^*_\infty} = \frac{\lambda_D}{\lambda_D - \lambda_P} \tag{14}$$

A finite, positive value of this ratio is obtained for all cases where $\lambda_D > \lambda_P$ (*i.e.*, the daughter decays more rapidly than the parent).

If $\lambda_P = 0$ (constant source or parent), we see again that the saturation activity of the daughter is equal to that of the parent, which is also a consequence of (8).

4. Amount of Daughter Produced from Parent at Any Time after End of Growth of Parent. It can also be shown, by the use of (2) and (5), that the amount of radioactive daughter co-existing with its radioactive parent after a growth period (t_1), during which the parent was being created from a constant source (pile, cyclotron, very long-lived radioisotope, *etc.*), followed by a period of decay (t_2) is given by

$$D^*_{(t_1+t_2)} = \frac{P^*_\infty}{\lambda_D - \lambda_P} [\lambda_D e^{-\lambda_P t_2}(1 - e^{-\lambda_P t_1}) - \lambda_P e^{-\lambda_D t_2}(1 - e^{-\lambda_D t_1})] \tag{15}$$

where P^*_∞ is the saturation value of P^* ($t_1 = \infty$, $t_2 = 0$).

3. Specifications of a Radioisotope (3, 3a, 4)

(*a*) *Labeling.* In producing a radioisotope there usually exist clear specifications as to the type and characteristics of the product desired. These will be set by the use to which the radioisotope will be put. From the standpoint of biology, there are only three kinds of uses and these are not always independent of each other: tracers, analytical aids, and sources of radiation. The tracer use, which is by all odds the most important, has been defined best as "the study of the exchange of atoms of identical chemical properties." This use depends upon changes in the isotopic abundance in the particular element or compound being studied. Any element in which the ratio of one isotope to another has been altered from that which is found in nature may be considered "labeled" and capable of use as a tracer. The extent of its use (*i.e.*, the dilution of the label with the naturally occurring isotopes which can be permitted) is limited by the natural ratio and by the sensitivity of the detecting device. It is for the former reason that the radioactive isotopes are so much more versatile than the enriched stable isotopes as tracers; since they do not exist in nature, a much greater dilution ratio is possible without loss of the label.

(*b*) *Chemical Properties* (*Z*). It is clear that the tracer use involves the study of a particular chemical element. Hence, if a radioisotope is to be used as a label for a particular element, it must be isotopic with that element, *i.e.*, it must have the same *Z*. Isotopy is not always required when the radioisotope is to serve simply as an analytical aid without tracer function. For example, if a radioactive tracer is to be used to determine the extent to which one sample of water is diluted by another, it is clear that radioactive Na or K or Cs or any completely soluble element can be used; the sole criterion is that of complete and even diffusion throughout the aqueous medium. In such a case it is only the *general* chemical nature of the isotope which is involved, which gives us some choice in the matter of *Z*. The same argument can be applied to the use of radioisotopes as sources. If the sources are to be handled physically and not chemically, the *Z* of the source is not important. On the other hand, the present use of radiophosphorus or radioiodine as internal sources in experimental therapy is completely dependent upon the chemical properties of P and I, respectively; hence, usage of this type is as dependent on *Z* as is the tracer use.

(*c*) *Nuclear Properties.* Having decided upon the atomic species, or *Z*, of the radioisotope desired, the next specifications to be considered are those associated with the particular radioactive isotopes of this atomic number. In most of the cases of biological interest there is no choice. For example, there is but one reasonably long-lived radioisotope of phosphorus; the same can be said of sulfur, potassium, copper and many others. In the case of Fe, however, there is both the 4 year Fe^{55}, which decays by K-capture, and the 44 day Fe^{59}, decaying by β and γ emission, to be considered. Some investigations may make it desirable to use one and some the other; in many cases both can be used together without complications arising, while in others the use of both as independent labels is desired. The choice of half-life and of radiation characteristics can be more than just a matter of convenience, as, for example, in the use of radioiodine as an internal source. The total irradiation given by an iodine source to the tissue in which it is deposited is determined by its half-life and its radiation types and energies. There are six radioactive iodine isotopes with half-lives varying from 2.4 hours to 13 days, with β energies varying over a factor of 2 and with miscellaneous associated γ activities, all of which must be considered.

(*d*) *Total Activity.* Having set the Z and the A of the desired radioisotope by such considerations as those listed above, we can now turn our attention to certain other characteristics which are more variable and more easily controlled, such as the total amount of the radioisotope desired and the amount of stable isotopic material with which it can be mixed initially without losing its usefulness. The total amount available will depend upon the efficiency of the nuclear reaction used, the half-life of the radioisotope to be produced, the length of irradiation and the power of the producing device (*cf.* equations (8) and (18)). The total amount of radioisotope which can be made available is sometimes of critical importance (for example, in a therapeutic experiment).

(*e*) *The Specific Activity,* which is the ratio of active isotope to all material of identical Z present in the sample (*i.e.*, the isotope abundance), can vary from the pure radioisotope itself, which we designate as "carrier-free"[6] material, to that which is so diluted with stable isotopes of the same species as to be useless (natural K is in the latter category). During each step of the (irreversible) dilution process, from one extreme to the other, the isotope becomes less and less generally useful. The importance of seeking the highest specific activity possible in isotopic material arises from the fact that it can be added to a reacting or living system as a label for a particular species without changing the mass or concentration of that species present. If the radioisotope is created in material which is isotopic with it and not by the transmutation of an element of differing Z, carrier-free material is not obtained (except in the special case of a Szilard-Chalmers reaction; *cf.* page 144). In such cases the specific activities attained are functions of the total activities and hence of the factors that determine total activity.

(*f*) *Radiochemical Purity.* Since the radioisotope desired is to be detected by means of radiation-measuring instruments, it is necessary to take pre-

[6] *Cf.* footnote p. 142.

cautions against measuring any other source of radiation in the samples. The safest procedure is to have only one radioactive isotope present in the starting material. Extraneous or undesired radioactivities which may contaminate the radioisotope initially are: radioactive daughters (*e.g.*, I^{133} has a radioactive daughter), radioisotopes arising from other nuclear reactions, radioisotopes arising from chemical impurities and from associated material (*e.g.*, the anion or cation associated with the desired element), *etc.* A radioisotope having a radioactive isomer to which it decays must be used with caution, for the transition from the upper state to the lower (which is usually the state measured) may have chemical consequences as serious as those resulting from β-decay (*cf.* p. 145).

(*g*) *Chemical State and Purity.* The final criteria are those of the chemical state of the radioisotope and the chemical purity in which it can be obtained. If the radioisotope, once having been created, cannot be purified of toxic heavy metals or other undesirable elements, or cannot be converted into a desired form (for example, carrier-free P^{32} into PCl_3) another method of creation which lends itself to the necessary conversion must be found.

All of the above criteria must be taken into account before complete specification of the radioisotope desired and the method of obtaining it can be made. An example of how some of these considerations are met in practice is given on page 140.

II. NUCLEAR REACTIONS

1. General Principles (3, 4, 9, 20)

(*a*) *Reactants.* Nuclear reactions are stated in the same form as is used for chemical reactions, the reactants being placed on the left side and the products on the right. However, instead of the double arrow or equal sign so common in chemistry, the reactants and products of a nuclear reaction are connected by an arrow pointing in one direction. This non-reversibility precludes equilibrium or Mass Law considerations.

In order to produce a reaction, the reactants must be brought together and this is done by firing one (the projectile) at the substance to be activated (the target). Under ordinary (chemical) conditions, their electron shields keep atoms at distances of over 10^{-8} cm. from each other and, since the nuclei are on the order of 10^{-12} cm. in diameter, there is no chance for any nuclear interaction. Hence, a nuclear reaction will be accomplished only if one of the reactants has no charge (neutron, γ-ray) or is accelerated to a velocity sufficient to overcome the repulsive (Coulomb) force which arises between it and the nucleus with which it is to react.

(*b*) *Energetics* (17). Having taken appropriate steps, discussed in greater detail below, to bring the reactants together, we may examine the nature of the stationary or target nuclei and see what factors influence the course of the reaction. A nucleus which consists of several particles has been compared most effectively to a drop of a liquid ("drop" model), chiefly for the reasons that its constituent particles (protons and neutrons) are of about the same density, they seem to be free to move about within the nucleus and they are apparently close enough to interact with each other (8). The binding forces between nuclear particles extend over ranges of about 2×10^{-13} cm. and, throughout most of the Periodic Table ($A > 15$), are about 7–8 Mev/neutron or proton. The total binding energy[7] is therefore roughly proportional to A and not to A^2, which indicates that the interaction is not completely random but is, on the average, between specific particles in the nucleus. (This is analogous to the situation in chemisrty where only certain numbers and kinds of atoms interact.) The repulsion between protons, which is overcome by the increased neutron:proton ratio and by the loss in mass known as the "packing fraction," goes up as the 5/3 power of A (being proportional to

$$\frac{Z(Z-1)}{1.5 \times 10^{-13} A^{\frac{1}{3}}},$$

where the denominator is the nuclear radius).

Even without a knowledge of binding energies and of the repulsion of particles we might still expect that the reaction producing a radioisotope would involve much energy because of the amount which is released subsequently by the radioisotope in the process of decay. Actually, the energy involved in a nuclear reaction is usually greater than the energy of decay and is greater than the kinetic energy of the projectile by the large unit particle binding energy; thus a large part of the energy consumed initially is liberated immediately after the reaction (analogous to an activation energy).

For example, the absorption of a "slow" neutron (one with only a fraction of an electron-volt of kinetic energy) by a P^{31} nucleus results in the formation of P^{32}. It is known that the decay of P^{32} to S^{32} by β decay involves a maximum energy loss of 1.7 Mev (the maximum energy of P^{32} β-rays; no γ-rays are emitted). Hence, the P^{32} atom has a maximum mass of $31.9809 + .0018 = 31.9827$ (S^{32} atomic mass + 1.7 Mev in mass units), which is 0.0105 mass units *less* than the mass of P^{31} plus one slow neutron ($30.9843 + 1.0089$). This excess mass (binding energy of the added neutron) is lost by γ emission immediately ($< < 10^{-6}$ sec.) after the absorption of the neutron. If the original incident neutron had possessed 2 Mev of kinetic energy,

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[7] The total binding energy of a nucleus is given by the difference between the sum of the mass of the particles (neutrons and protons) involved and the actual experimental mass, expressed in energy units (1 atomic mass unit is equivalent to 931 Mev).

the total energy to be lost by such γ emission would have been 2 Mev larger. (The masses of the electrons could have been omitted in the above calculation as they are equal in number throughout.)

The above example illustrates three general principles: (1) the absorption of a particle into a nucleus results in an energy increase equal to the unit particle binding energy plus the kinetic energy of the particle; (2) the "compound nucleus" (8) thus formed dissociates immediately with the loss of most of the gained energy; (3) the rest of this energy appears subsequently as radioactive decay energy and/or as a permanent mass difference in the stable isotope which ends the overall process.

In charged particle reactions, kinetic energy must be imparted to the projectile in order that it may overcome the repulsive (Coulomb type) force which arises between it and the target nucleus at close range (9), a force which does not exist in the case of the neutron. The repulsive force which prevents amalgamation of projectile and target nucleus is known as the "barrier" and was originally thought to be that kinetic energy of projectile below which no nuclear reaction could take place (hence the term "classical threshold energy"). It has been shown both theoretically and experimentally, however, that there is a certain probability that a charged projectile will penetrate a nucleus even when its kinetic energy is less than the barrier height; the *probability* of this "leak" is finite at all energies of projectile, rising exponentially with increasing energy and becoming unity when the barrier height is reached (12, 13). That is to say, at a kinetic energy equal to the barrier height, all projectile nuclei can (if aimed correctly) enter the target nuclei. Conversely, to be sure of leaving the nucleus, a charged particle (proton, usually) must possess energy greater than the barrier height.

Once a particle has penetrated the target nucleus, a compound nucleus is formed which dissociates in one manner or another, the probability of a given mode of dissociation depending upon the energy which it can release in so doing. The amount of energy released in such a reaction can be calculated in the same manner as for the $P^{31}(n,\gamma)P^{32}$ reaction discussed above. Consider the reaction $T(i,j)R$. The sum of the rest masses of T and i may be greater than, equal to, or less than that of $R + j$. But mass is indestructible (bearing in mind the equivalence of mass and energy), so we write

$$M_T + M_i = M_j + M_R + Q$$

where the symbols represent the rest masses of the atoms T and R and of the particles i and j, and Q is the amount of rest mass which has been lost in the reaction. Q equals $q_j - q_i$, the energy liberated as kinetic energy of j (or as gamma energy) less the energy supplied as kinetic energy of i.

Reactions which possess a positive Q proceed spontaneously once the compound nucleus is formed. Such reactions are termed "exoergic"; they are observed at projectile energies below the barrier energy, show an exponential rise in probability up to this energy and are essentially independent of energy above it (except as modified by competing reactions). The only necessary and essential consideration is the formation of the compound nucleus. If energy is expended in so doing (*e.g.*, because of a high barrier), the observed energy release (q_j) will be the sum of Q and q_i. However, reactions with a negative Q will proceed only if energy equal to Q is supplied to the compound nucleus (as q_i); these are termed "endoergic" reactions. They have definite threshold energies which are quite independent of the barrier energy and, once over the threshold energy, they increase in probability with energy. The formation of the compound nucleus is not the sole criterion as to whether or not this type of reaction will proceed; surplus energy, equal to or greater than Q, must be supplied, and the energy released by the reaction will be again equal to $Q + q_i$ (but Q is negative in this case). In elements of high Z, so much energy must be expended in surmounting the barrier that endoergic reactions may actually predominate over exoergic ones. The barrier energy for H^+ or D^+ ions is only 3 Mev at $Z = 8$, 5 Mev at $Z = 17$ and 7 Mev at $Z = 29$, but rises to 9 Mev at $Z = 43$ and 14 Mev at $Z = 89$.

In making such a calculation, one must take account of the masses of the extranuclear electrons. In the reaction $P^{31}(n,\gamma)P^{32}$, there is no change in these and they may be ignored (atomic masses are used in the above calculations). The same is true for the subsequent decomposition of P^{32} to $S^{32} + \beta^-$, since the electron lost to the P^{32} nucleus has, in effect, joined the valence shell of the S^{32} atom. However, in a d,n reaction, Z increases by 1 unit and an electron must be acquired by the resultant atom. The cases of decay by positron emission and orbital electron capture have been mentioned in the section on modes of decay (p. 120).

It should be noted that the energies of reaction and of activation are given in Mev (million-electron-volts), whereas the values for their chemical analogues are on the order of a few electron-volts. This has great significance for both radioisotope production and radioisotope usage (*cf.* Recoil Phenomena, p. 144, and footnote, p. 144), for it eliminates the possibility of the labeling of compounds by the exposure of these to neutrons or other activating particles.

2. Neutron Activation (Pile Reactions) (4, 7)

(*a*) *Neutron reactions* differ fundamentally from the charged particle reactions in that there is no barrier to the approach of the neutron. Actually, the probability of a neutron entering a nucleus is related to the length of time it spends in the vicinity of that nucleus, so that a slow neutron stands a better chance of capture than a fast one. As we have seen, the most probable reaction for a slow neutron is the n,γ reaction, and this reaction can take place with every element (except He^4) when exposed to slow neutrons (see footnote, p. 134); there is no barrier and the reaction is always exoergic.

The n,γ reaction gives rise to radioelements which are isotopic with the parent substance,[8] thus

$$_ZT^{A-1}(n, \gamma)_ZR^A \qquad (n, \gamma)$$

where T and R represent the target and resultant nuclei, respectively (the redundancy of using both Z and the chemical symbol has been discussed earlier). Over 100 cases in which the n,γ product is radioactive are known and these are usually β^- emitters (*cf.* Ref. 4, Table IV). In some cases, the product nucleus decays to a radioactive daughter, the isolation of which does yield a carrier-free radioelement. The reactions are

$$_{Z-1}T^{A-1}(n, \gamma)_{Z-1}R^A(0, \beta^-)_ZR^A \qquad (n, \gamma, \beta^- \text{ chain})$$

or

$$_{Z+1}T^{A-1}(n, \gamma)_{Z+1}R^A(0, \beta^+ \text{ or } K)_ZR^A \qquad (n, \gamma, \beta^+ \text{ chain or } n, \gamma, K \text{ chain})$$

Examples of radioelements created in these ways are the isobars I^{131} (from Te^{130} *via* Te^{131}) and Cs^{131} (from Ba^{130} *via* Ba^{131}), respectively, both of which decay to stable Xe^{131} (*cf.* Ref. 4, Table VI).

If these were the only ways in which high specific activity or carrier-free radioisotopes could be obtained from the pile, this device would never have achieved its present repute as an isotope production machine. Fortunately, there do exist (1) some pile neutrons of energy measured in Mev rather than fractions of one ev, which are thus able to overcome the barrier against proton and α emission in some cases, and (2) resonances in certain nuclei which also permit n,p and n,α reactions to proceed. These two reactions (*cf.* Ref. 4, Table V), while rare in the pile, give rise to transmutations and, hence, to carrier-free material:

$$_{Z+1}T^A(n, p)_ZR^A \qquad (n, p)$$

$$_{Z+2}T^{A+3}(n, \alpha)_ZR^A \qquad (n, \alpha)$$

The n,p reaction in the pile results in good yields of C^{14}, P^{32} and S^{35}, small yields of Ca^{45} and negligible yields of Fe^{59}; the latter is an example of a neutron reaction profitable only with fast neutrons (as from a cyclotron) which alone can cause the ejection of charged particles from heavier nuclei. Only two practical examples of the n,α reaction with pile neutrons to give

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[8] **Hence inseparable from the parent except in the special cases discussed under Recoil Phenomena (p. 144).**

useful radioisotopes have been demonstrated, $Li^6(n,\alpha)H^3$ and $Ca^{40}(n,\alpha)A^{37}$; faster neutrons from cyclotrons[9] effect many reactions of these types throughout the Periodic Table.

Only one other reaction of practical significance with neutrons need be considered:

$$_ZT^{A+1}(n, 2n)_ZR^A \qquad (n, 2n)$$

This, like the n,γ reaction, yields a primary product isotopic with the target. Since two neutrons are ejected, the incoming neutron must possess considerable (~ 14 Mev except in light elements) energy; hence, this reaction is not prominent in the pile. The n,n reaction, essentially an inelastic collision, gives rise to nuclear isomers, thus

$$_ZT^A(n, n)_ZR^{A*} \qquad (n, n)$$

As a source of radioactive material for the uses discussed earlier, this reaction has no practical significance.

(*b*) *Yield and Cross-Section* (3, 7). If a beam of nuclear particles of intensity I hits a target layer of matter so thin that no part of any nucleus lies behind another when viewed in the direction of the beam (perpendicular to the surface), most of the particles will pass through the layer without collision, since the distance *between* nuclei is of the order of 10^{-8} cm. while the nuclei themselves are only about 10^{-12} cm. in diameter. The diminution in the incident beam, $-\frac{dI}{I}$, will be equal to the fraction of the total area of the target layer occupied by nuclei. In each cm.^{2} of such total area, there will be ($N\sigma dt$) cm.^{2} of nuclei, where N is the number of nuclei in 1 cm.^{3} of the target material, dt is the thickness of the target layer in cm. (Ndt thus being equal to number of nuclei/cm.^{2} of target) and σ ("cross-section") is the cm.^{2} occupied by each nucleus in this layer. Thus $N\sigma dt$ is the fraction of the total area occupied. Hence

$$-\frac{dI}{I} = N\sigma\, dt$$

whence

$$\frac{I_t}{I_0} = e^{-\sigma Nt} \qquad (16)$$

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[9] Cyclotron neutrons radiate spherically (with a distribution in energy depending on the angle to the incident beam) from the target where they are produced by the d,n reaction on Be, Li or D. Hence, the secondary target can intercept only a fraction of the neutrons produced, with a corresponding inefficiency of use of energy. A pile target, on the other hand, is immersed in a sea of neutrons which come to it from all angles.

I_t/I_o is thus the degree of "shadowing" of nuclei at depth t due to those nuclei above them. This is not the only effect of depth; modification of the energy of those particles which penetrate may also result from their collisions.[10] (It is assumed in this discussion that no scattering back into the beam takes place.)

Intensity can be expressed in this equation in any units, since it is a ratio; one unit commonly used, particularly in neutron exposure work, is "particles/cm.²/sec.", called the "flux" (nv), which is derived from the number of neutrons/cm.³ (n) times their velocity in cm./sec. (v). When I_o, the incident flux, is known and I_t can be measured, the difference ($I_o - I_t$) gives directly the number of nuclear collisions or "events" or "processes" taking place per unit of time to diminish the beam strength. Values of σ[11] can be calculated in similar fashion from experimental data for each of the different nuclear reactions which contribute to the total number of events. For example, σ_e, the "excitation cross-section" for a reaction resulting in a radioisotope, can be calculated from a measurement of the number (D^*) of radioactive nuclei disintegrating per second at a time t_2 after the end of an exposure period t_1 as follows:

$$\sigma_e = \frac{\text{No. hits/sec. producing } D^* \text{ nuclei} = \text{decay rate at saturation } (D^*_\infty)}{\begin{array}{r}\text{No. particles/cm.}^2\text{/sec.} \times \text{total target nuclei} \\ = I_o \times N \times \text{volume of target}\end{array}} \tag{17}$$

$$= \frac{D^*_{(t_1+t_2)}}{I_o \times N \times \text{volume of target } (1 - e^{-\lambda t_1})e^{-\lambda t_2}}$$

It is assumed that "shadowing" does not occur to an appreciable extent; this can be checked by transmission experiments on the material. σ_e, derived as indicated above for a specific (n,x) reaction, is termed the "excitation cross-section for the n,x reaction." It is valid only for particles of the same energy distribution as that from which it was derived, since it is markedly dependent upon neutron energies in the slow or thermal regions, and for the particular reaction observed.

The total or "transmission" cross-section, as measured by the diminution in the incident beam (Eq. 16), is designated by σ_t for the material exposed. σ_t is obviously the sum of the cross-sections of the "absorption" reactions (n,γ; n,p; n,α; n,f[12]) and the "scattering" reactions (any collisions not

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[10] Slow neutrons (thermals being among these) are produced from fast neutrons by causing them to undergo a series of elastic collisions. An elastic collision, to be efficient in slowing neutrons, requires a nucleus of nearly the same mass as the neutron and of very low capture properties. The graphite in the pile serves this purpose (7).

[11] σ is expressed in cm.² or in "barns"; 1 barn (b) = 10^{-24} cm.²

[12] n,f = neutron producing fission.

resulting in capture but only in the deflection of incident neutrons out of the beam), designated by σ_a and σ_s, respectively. Both of these are the sums of the cross-sections for the particular processes contributing to them; *e.g.*, $\sigma_a = \sigma_{n\gamma} + \sigma_{np} + \sigma_{n\alpha} + \sigma_{nf}$. Thus, σ is treated as an *empirical constant* relating numbers of observed processes of any particular kind or kinds to the numbers of particles required to produce them.

We have arrived at a point where we can derive absolute values for the constant, D_∞^*, in the growth formula (9) for the nuclear reaction ${}_{Z_1}P^{A_1}\ (n,x)\ {}_{Z_2}D^{A_2}$. D_∞^* is the decay rate at "saturation," which is defined as the point at which decay rate equals production rate; hence D_∞^* is equal to the rate at which new radioactive D nuclei are produced throughout the entire growth period (during which the flux is kept constant). Hence,

$$D_\infty^* = IP\sigma_{nx} \tag{18}$$

where D_∞^* is expressed in atoms decaying per unit time, I is the average incident flux[13] in neutrons (particles)/cm.^{2}/unit time, P is the number of ${}_{Z_1}P^{A_1}$ nuclei in the entire target and σ_{nx} is the cross-section of the reaction in cm.^{2}/atom of ${}_{Z_1}P^{A_1}$. By the use of the proper constant, D_∞^* can be calculated directly in terms of curies per mole (at saturation) for any given production device; the actual value of D^* at less than saturation (∞) exposure and after appreciable decay is given by Eqs. (2) and (9).

3. *Cyclotron Activation* (3, 9, 9a, 16, 20)

(*a*) *The Deuteron.* Although protons, deuterons and α particles can be accelerated by cyclotrons and used to induce nuclear reactions creating radioisotopes, the deuteron has been and remains the projectile of choice. Part of this preference arises from operating considerations (deuteron beams being more easily obtained than proton beams), part from yield considerations (singly-charged particles giving beams of greater intensity than doubly-charged helium ions). The major factor in choosing the deuteron over the other particles for cyclotron usage, however, is the small binding energy (2.15 Mev) between its proton and neutron. This binding energy is so small that the repulsive force between target nucleus and deuteron projectile is sufficient (throughout most of the Periodic Table) to break the *n-p* bond in the latter and absorb only the neutron, giving rise to the equivalent of the n,γ reaction (9, 18).

(*b*) σ *vs. E.* Thus the d,p reaction can proceed at much lower energies than barrier considerations indicate are needed for deuteron capture. When this factor is reduced in importance (*e.g.*, projectile energy above barrier

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[13] If no significant "shadowing" of interior atoms takes place by virtue of a high σ_t or σ_a, I is equal to I_0. When shadowing is significant, an average value of I can be calculated from Eq. (16).

energy) the *d,n* reaction competes with the *d,p* reaction since less energy is required to eject a nuclear neutron than a proton. At energies below the barrier heights, σ_a increases exponentially with energy but is relatively constant once this energy is exceeded. The particular reactions whose cross-sections contribute to the total σ_a will also exhibit the initial exponential rise, but those of lower threshold energy may drop off at high projectile energies due to competition of the more endoergic reactions. Thus $\sigma_{d,p}$ for $_{29}$Cu becomes appreciable at 2–3 Mev, $\sigma_{d,n}$ at 4–5 Mev, $\sigma_{d,2n}$ at 6 Mev; at high energies (~ 15 Mev), the last (an endoergic reaction) competes with the first two (exoergic) reactions, which drop above 10 Mev.

(*c*) *Charged-Particle Yields* (9, 9a). Total radioisotope yields can be obtained from cross-sections (or thin target yields) as indicated in the case of neutron-induced activities. However, one major difference should be noted. A conventional neutron target rarely diminishes significantly either the intensity or the energy spectrum of the neutrons incident upon it. The conventional cyclotron target, on the other hand, is usually thick enough to stop the beam entirely[14] or at least thick enough to diminish it significantly and to change its energy. Thus, the total yield of a cyclotron reaction will involve the integral of Eq. (16), relating penetration to thickness, and also the relationship of σ to energy, which will vary as indicated above. Qualitatively, it can be seen that increasing the penetration of a deuteron beam, which varies as the 3/2 power of the energy, can more than offset the constancy or slight diminution in σ with increasing energy (above barrier height) as far as total yield is concerned. Thus, for equal intensities, beams of higher energy make possible the use of thicker targets and greater total yields; against the desirability of thick targets must be set the diminution in specific activity of the non-transmutation (*d,p*) reaction. For example, the *d,p* reaction on Na is first evident with deuterons at 1–2 Mev energy. $\sigma_{d,p}$ rises to a maximum at 5.5 Mev and falls to half this value at 14 Mev, although total yield at 14 Mev is 6 times that at 5.5 Mev for the same intensity of beam.

(*d*) σ *vs.* Z. Since σ varies with energy of projectile and the latter is set by considerations of barrier heights which are properties of the charge (Z) on the target nucleus, σ for a given capture reaction at a given energy will vary with Z. The barrier height (for a H^+ or D^+ projectile) in N, for example, is about 2 Mev, in Cl about 5 Mev, rising to 14 or 15 Mev in the very heavy elements. Thus, in the lower part of the Periodic Table, where the barrier is nearly the same as the deuteron binding energy, the *d,n* reaction predominates; there is not enough repulsive energy to break the *p-n* bond before capture of the projectile. In the case of very heavy nuclei, so

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[14] This does not mean that all deuterons undergo nuclear reactions. Only a fraction of a per cent do so, the rest becoming H^2 atoms, molecules or compounds.

much energy must be put on the deuteron to pass the barrier that endoergic reactions such as $d,2n$ and $d,3n$ compete successfully with the d,p reaction.

(*e*) *Deuteron Reactions.* The most prominent reaction of deuterons, for the reasons discussed above, is the exoergic d,p reaction, the similarity of which to the n,γ reaction, both in product and in general occurrence, has been mentioned:

$$ {}_{Z}T^{A-1}(d, p){}_{Z}R^{A} \qquad (d, p) $$

With the exception of the rare endoergic d,H^3 reaction (the analogue of the $n,2n$), this is the only deuteron-induced reaction which does not lead to a transmuted (carrier-free) species except—again as in the case of the n,γ reaction—as it initiates a β^-,β^+ or K-capture decay chain.

The second most prominent reaction, which is exoergic and has the highest cross-section once the deuteron has the energy to penetrate the target nucleus as a whole, is

$$ {}_{Z-1}T^{A-1}(d, n){}_{Z}R^{A} \qquad (d, n) $$

Its endoergic analog (requiring 12–16 Mev),

$$ {}_{Z-1}T^{A}(d, 2n){}_{Z}R^{A} \qquad (d, 2n) $$

has a parallel in the p,n reaction, which is the most common proton-induced reaction. Less common[16] is the exoergic reaction

$$ {}_{Z+1}T^{A+2}(d, \alpha){}_{Z}R^{A} \qquad (d, \alpha) $$

which is the best source of the 3-year Na^{22}.

(*f*) *α Reactions.* Although α particle irradiations do not give the high yields of deuteron reactions, several unique transformations can be accomplished. The most common reactions, as might be expected from the discussion of deuteron reactions, are those leading to neutron emission:

$$ {}_{Z-2}T^{A-3}(\alpha, n){}_{Z}R^{A} \qquad (\alpha, n) $$

$$ {}_{Z-2}T^{A-2}(\alpha, 2n){}_{Z}R^{A} \qquad (\alpha, 2n) $$

There is also the reaction

$$ {}_{Z-1}T^{A-3}(\alpha, p){}_{Z}R^{A} \qquad (\alpha, p) $$

The higher the energy of the α-particle, the more particles will be emitted; thus 40 Mev α-particles produce ten $(\alpha,3n)$ reactions to each $(\alpha,2n)$ or $(\alpha,4n)$ reaction. The principal advantage of this type of reaction is the double increment in Z.

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[16] See footnote, p. 138.

III. CHEMICAL FACTORS IN RADIOISOTOPE PRODUCTION

1. Target Considerations (3)

(*a*) ***Side Reactions.*** The increase in yield obtained with higher energies in charged particle reactions is somewhat offset by the increased radiochemical contamination which is also produced. Because of the fact that reactions to produce carrier-free or high specific activity radioisotopes are usually found at high energies, a whole host of undesired nuclear reactions can appear. The bombardment of ${}_{Z}T^{A}$ with high energy deuterons can lead to the new nuclei[16] ${}_{Z}R^{A+1}$, ${}_{Z+1}R^{A-1}$, ${}_{Z+1}R^{A}$, ${}_{Z+1}R^{A+1}$ and ${}_{Z-1}R^{A-2}$. If ${}_{Z}T$ is not monoisotopic, each of its isotopes will also undergo these reactions. Any other atoms present, either as parts of the exposed compound or as impurities in it, will undergo these reactions, and any radioactive daughters of these primary products will also be present. Thus, the exposure of Ge to deuterons to produce carrier-free As^{74} (which cannot be produced in the pile) by the primary reaction $Ge^{73}(d,n)As^{74}$ gives, in addition: As^{70}, As^{71}, As^{72}, As^{73}, As^{76} and As^{77} by d,n and $d,2n$ reactions on the other Ge isotopes; Ge^{71}, Ge^{73} and Ge^{77} by d,p reactions on these; Ga^{70}, Ga^{72} and Ga^{74} by d,α reactions. Many of these are short-lived and need not be separated before use; this may also be true of the activities produced from the other elements present. An additional possible source of unwanted activities is the target plate itself and any bonding material used in mounting the sample.

The problem of radiochemical contamination of product exists in the neutron-induced reactions as well. With the slow neutrons from the pile it is much less severe, as the number of possible reactions is limited and becomes less as Z and the number of isotopes per element increase; furthermore, there is a wider choice of target material and no bonding to a target plate is needed. A fast neutron reaction is more troublesome; *e.g.*, the cyclotronic production of Fe^{59} by the reaction $Co^{59}(n,p)Fe^{59}$ leads to large quantities of Co^{60} (a 5-year γ-emitter) and to 2.6 hour Mn^{56} and 310d Mn^{54}. (*Cf.* also C^{14} production discussion, p. 140.)

(*b*) *Cyclotron Targets* (9, 9a, 14, 16, 24). The physical requirements to be met by cyclotron target materials (other than neutron targets) depend upon the mode of exposure chosen. In contrast with the requirements of neutron targets, which are set practically solely by the need for physical confinement and low non-excitation cross-sections, charged-particle targets must possess either stability to heat in a high vacuum or else the ability to be bonded to (water-cooled) metal in such a way as to dissipate heat rapidly through that metal, and also the ability to be mounted in a vertical plane

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[16] The number of new nuclei which will be produced from 200 Mev deuterons in the newest cyclotron may run into the dozens, creating in effect "fission spectra" throughout the Periodic Table.

(since charged particle trajectories are usually in a horizontal plane). The heat input arises from two facts: the complete stoppage of practically all impinging particles (whereas most neutrons are not stopped by the ordinary neutron target) and the inefficient utilization of the deuteron or α beam (only about 0.1% of the impinging particles are captured even at high energy and low Z). The heat to be dissipated on an internal target (see below) may be of the order of 300 microamperes × 16 Mev ÷ 0.5 cm.^{2} of target area = 10 Kilowatts (or 2500 calories/sec.)/cm.^{2}; on an external target, the increased target area alone may drop this by a factor of 100.

Two differing types of target are in general use. In one, known as the "probe" mounting, the target material is located inside the high-vacuum chambers ("dees") where the beam is most concentrated. Materials to be exposed internally in this fashion must be stable at high temperatures *in vacuo*, must not lose any of their substance into the vacuum chamber, must possess a high heat conductivity and be capable of soldering or plating on a suitable metal (copper) surface which is water-cooled. Metals, metallic substances or refractories are thus practically mandatory. The fact that a bonding substance is usually necessary is a complication, for each element in this material becomes radioactive by all possible reactions, thus complicating the recovery of the desired radioelement. On the other hand, the greater beam intensity available inside the cyclotron makes possible higher specific activities from d,p reactions.

The other method of cyclotron target mounting is the external or "bell-jar" target. A thin window, which will contain the vacuum but be reasonably well penetrated by the beam, is used to allow the beam to come out of the vacuum chamber and into an external enclosure which may be kept at any desired pressure of any desired gas. In addition to the factors of accessibility, choice of gas and choice of pressure, the beam on an external target is considerably broader in area and lower in total beam strength (usually less than one-fifth the inside strength), both of which factors aid in solving the cooling problem. The bonding problem may also be diminished, with resultant lessening of the subsequent radiochemical purification problems. However, the drop in beam strength and concentration act to lower the resultant specific activity as compared to that obtainable in the same time on an internal target. It should be noted that both an internal and an external target may be used at once and, if either produces neutrons, neutron targets may also be exposed.

The exposure of P to produce P^{32} may illustrate these factors. Elemental phosphorus (red or violet variety) would be the substance of choice, since every atom is capable of undergoing the $P^{31}(d,p)P^{32}$ reaction. However, this material cannot be exposed inside the vacuum chamber because of disadvantageous heat-conducting, bonding and volatility properties; hence, we are limited to external use of this material. For higher specific and total activities, metal phosphides, iron phosphide in particular, are used. Iron phosphide withstands high temperatures. However, radioactive species produced from Fe(Fe*, Co*, Mn*) and from impurities in the phosphide (*e.g.*, from As impurities in the original P would arise Ge*, As* and Ga*) as well as from the target plate (Cu will yield Zn*, Cu* and Ni*) and from any soldering or bonding material will occur.

(*c*) *Pile* (*Neutron*) *Targets* (4, 7). The physical requirements for pile target material are not as restrictive as those of a cyclotron. The latter must dissipate practically the entire power of the deuteron beam as heat and preferably do so while in a high vacuum. Target material in a pile, however, is subject practically only to that heat which is characteristic of the particular pile in question;[17] theoretically, this can be any temperature, but in practice, since the present piles are cooled by air and by water, this is somewhat higher than room temperature but not excessive. The target material thus need not be mounted for rapid conduction of heat but can be exposed in relatively simple containers as powder or granules, as a liquid or even, if desired, as a gas. The only precautions in mounting the target are those needed to prevent physical loss of material in exposure and subsequent handling. Attention must be paid to the associated parts of the molecule, if a salt or compound is being exposed, to be sure that material which exhibits excessive neutron capture properties (or which becomes excessively radioactive or decomposes under β,γ or neutron irradiation) is not included. Highly absorbing material (for example, Cd or B or Cl) not only acts as a pile "poison" but also tends to "shadow" the element which is being activated.[18] The material of which the exposure vessel itself is made must be chosen as carefully as the compound of the element in question; it must be free of both high neutron absorbers and elements which activate easily to dangerous levels and which thus complicate subsequent handling (pure aluminum, graphite, quartz, *etc.*, are generally used; glass contains too much B and Na).

As an example of pile target considerations we may consider briefly the manner in which C^{14} is produced in the graphite pile at Clinton Laboratories. $Ca(NO_3)_2$, in pellet form, is the target material; it was chosen because of (1) ready availability in ton lots, (2) stability in irradiation and chemical handling, (3) purity of available material, (4) ease of canning, (5) low absorption cross-sections in the Ca and O atoms, (6) no long-lived γ emitters are produced, and (7) ease of recovery of C^{14}. The pellets are en-

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[17] The heat of the nuclear reaction itself (recoil energy) is usually not significant compared to the heat produced by the fissions which keep the pile going.

[18] Cd absorbs only slow neutrons (below 0.3 ev); it is thus useful in diminishing unwanted n,γ reactions when n,p and n,α reactions are desired. However, since the pile runs on slow neutrons, there is a limit to the extent of this permissible.

cased in aluminum which is as free as possible of such metals as Cu or Fe (which would give rise to Cu^{64} and Fe^{59}, γ emitters). During irradiation the following reactions take place to yield measurable amounts of radioisotopes:

$N^{14}(n,p)C^{14}$
$Ca^{40}(n,\alpha)A^{37}$
$Ca^{44}(n,\gamma)Ca^{45}$
$Ca^{48}(n,\gamma)Ca^{49}$ (2.5 hr. γ-emitter)
$Al^{27}(n,\alpha)Na^{24}$ (15 hr. γ-emitter)
$Al^{27}(n,\gamma)Al^{28}$ (2.4 min. γ-emitter)

Subsequent handling must take into account the short-lived γ-emitters induced in the can. Since the C^{14} is formed primarily as CO_2 and recovered as such, the presence of active A^{37} must be recognized.

2. *Radiochemical Isolation* (1a, 3, 4, 7)

Having brought about a nuclear reaction in order to create a given radioactive species, steps must be taken to reclaim the desired radioisotope from the target material and from all the simultaneously produced activities and to put it into the desired chemical form.[19] In principle, this is analogous to the recovery of an organic compound after a synthesis, where separation from parent compounds and from the results of side reactions is required. However, since we are dealing primarily with elements in their simple inorganic forms, the chemical fundamentals involved are those of inorganic chemical separations. Two important differences from conventional chemical mixtures must be noted: the minute (sub-microgram) masses of the newly created radioelement,[20] which are in contrast to the "normal" (g.) amount of target element or compound, and the ever-present biological hazard of the radiations from the radioactive elements present, which requires the isolation procedure to be effected by more or less remotely-controlled processes. It should be noted that the maximum radioactive hazard of any radioelement is usually encountered during the isolation process.

Radiochemical isolation may be easy or difficult depending upon the iso-

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[19] *Cf.* "Specifications of a Radioisotope," p. 126.

[20] Using equation (1), $N = \frac{-dN}{dt} \times \frac{1}{\lambda}$, it can readily be shown that 1 γ of a radioisotope is equivalent to $\frac{130}{At_{\frac{1}{2}}}$ curies, where A = atomic weight and $t_{\frac{1}{2}}$ is the half-life in days. Hence, 1 γ of $I^{131} = \frac{130}{131 \times 8} = \frac{1}{8}$ curie, or 1 mc of I^{131} is contained in 0.008 γ of I^{131} atoms. On the other hand, 1 mc of C^{14} is contained in 0.2 mg. of C^{14} atoms. 5 mc/mg. is, therefore, the maximum specific activity of C^{14} attainable.

tope in question, the manner in which it was created and the form in which it is desired. Not all radioisotopes need be isolated from the target element. Those created by n,γ or d,p (non-transmutation) reactions are not separable from it;[21] the most that might be required in this case is 1) the removal of radioactive isotopes produced by transmutation reactions on the parent material, 2) the change of the chemical form of the element in question (metathesis or synthesis). Since the elements produced by transmutation, in this case, are waste products, no hesitation is experienced in adding isotopic "carrier"[22] to the mixture; from this point on, the separation may be carried out by the procedures of classical inorganic chemistry which were designed for milligram to gram amounts. Because transmutation-bred species exist in such negligible masses of their elements that they cannot interfere chemically with the subsequent uses of the materials, it is also permissible to let them decay away or to devise measuring procedures which are insensitive to the radiations they emit. For example, the active K^{42} produced in KH_2PO_4 along with the desired P^{32} can be ignored if use and measurement is delayed for several days. Similarly, S^{35} in a P^{32} experiment will be effectively eliminated by the use of thick-windowed measuring devices.

The problem is more difficult when it is desired to isolate a transmutation-born activity in the carrier-free[23] state from a mass of its parent, and still more difficult when the isolation of two or more such species from a mixture is required. The latter situation is seen in its most complex form in the "fission products" which occur as a result of nuclear fission of uranium, where long-lived radioisotopes of Cs, Sr, Ba, Y, La, Ce, Pr, Nd, 61, Ru, Tc, Te, I, Zr and Cb are created in a single mixture (18a).

(*a*) *Chemical Methods.* We may list the major chemical methods which have been used for the separation of carrier-free radioisotopes (1a).

1. *Co-precipitation* with an element non-isotopic with, hence eventually separable from, the radioisotope in question, is of several types. The most important of these are "isomorphous substitution," as in the carrying of Sr^* and Ba^* by a precipitate of $PbSO_4$ or of $Pb(NO_3)_2$, and what has come to be known as "scavenging," as in the carrying

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[21] Except in the case of Szilard-Chalmers reactions and isomeric transitions (*cf.* p. 144).

[22] "Carrier": a macro component added to carry a microcomponent with it through macrochemical procedures. The most effective carrier is, of course, material isotopic with the radioisotope itself; this is termed "isotopic" carrier. When carrier is added to prevent the precipitation of a microcomponent on a macrocomponent, it is termed "holdback" carrier.

[23] By "carrier-free" is meant that the only atoms present with the same Z as the radioisotope are the radioisotope atoms themselves. Since the addition of small amounts of isotopic material as impurities is very probable and practically not detectable, this term really indicates the goal rather than the achievement of the separation process.

of $P^{32}O_4^{=}$ by a Fe^{+++} + NH_4OH precipitate or of a nondescript mixture of trivalent ions by $La(OH)_3$, *etc.* Coprecipitation involves the addition of macro amounts of material; in this lies its greatest disadvantage, for the ordinarily negligible amounts of impurities in the substance added may include some which are isotopic with, or otherwise not easily separable from, the carrier-free material sought. The risks of isotope dilution, of chemical contamination of product and of laboratory and personal contamination give precipitation, even though it is the keystone of classical inorganic chemistry, a low rating in comparison with ion-exchange and solvent extraction methods.

2. *Ion-exchange*, similar in many practical aspects to adsorption, is really a type of precipitation. Its chief advantage is the fact that it can be quantitative even at extremely dilute ("trace") concentrations. Other advantages are that the adsorbing (precipitating) ion—the anion in the case of cation-adsorbers—is fixed in position, can be exhaustively purified before using and can be reused *in situ* indefinitely. Using the new synthetic ion-exchange resins, together with chemical agents which form complex ions with polyvalent cations, it has been possible (22a) to separate curie amounts of carrier-free radioactive fission species from their mixtures and to reduce the total solids in these solutions to a few milligrams. At the same time, the sensitivity of this method is such that neighboring rare earths, carrier-free or in macro amounts, can be isolated from each other (23a). The simplicity of use, which consists solely of passing fluids through columns, lends itself well to the requirement of remote control imposed by radioactivity.
3. *Solvent extraction*, like ion-exchange, uses as bulk materials substances which are usually very easily purified of possible isotopic diluents or contaminants. Although most of the radioelements whose isolation is difficult are not adaptable to solvent extraction *per se*, the use of complexing agents may solve this problem as well as that of specificity, as noted in the case of ion-exchange. Adaptability to remote control is also a feature of solvent extraction.
4. *Distillation* is of limited application at the present time, in contrast to the methods listed above, solely because of the lack of basic information on the high-temperature behavior of chemicals in trace amounts. Among those radioelements which are isolated by distillation without carrier is iodine.
5. *Electrolysis* is useful in those cases where the oxidation-reduction potentials are sufficiently different. However, this method has not been exploited to the extent of those listed above as far as carrier-

free separations are concerned. It possesses the advantage, common to all but precipitation processes, of depending upon single-ion reactions but has the disadvantage of the latter in susceptibility to chemical contamination of product due to its dependence upon large amounts of inorganic compounds.

(*b*) *Recoil Phenomena* (3, 20). Although chemical reactions cannot effect nuclear transformations, nuclear transformations nearly always result in chemical changes. The ejection of a particle (α,β or photon) by a nucleus imparts recoil energy to that nucleus; this is often of sufficient magnitude to eject the nucleus from its molecular setting, which means a change in chemical state of the emitting atom. This may be an advantage, a disadvantage or of no consequence, depending upon when it occurs in the life of a nucleus. Obviously, if the recoil is simultaneous with the disappearance of radioactivity (instability), the atom has, in effect, disappeared from our system and need no longer be considered. Similarly, if the atom produced by the disintegration is not measured in the detection device used, the recoil disruption is of no consequence to the experiment.

(*c*) *Szilard-Chalmers Reaction.* If the recoiling atom is one which has just been activated by n,γ reaction, there exists the possibility that it may be ejected in a chemical state which will permit its separation from the (isotopic) parent material. The classic example is neutron-activated ethyl iodide, from which most of the activated iodine can be extracted with water. From this it is concluded that it exists as iodide or iodine, free of the ethyl group, a true carrier-free radioisotope. Since this is, in effect, an isotope separation, it is inadmissible in tracer usage but is exactly the effect sought in tracer production. Such reactions, which one might list in the tabulation of neutron reactions as the (n,γ, recoil) reaction, is named after the investigators who first demonstrated it (23). Its most common use in production work has been to prepare carrier-free bromine from ethyl bromide using cyclotron neutrons. No practical reaction of this type has yet been demonstrated in the pile.

The attractiveness of this method of preparing a carrier-free activity is offset to a considerable degree by the difficulty in meeting the conditions for successful isolation. Assuming that sufficient energy is emitted to free the capturing atom from its molecule,[24] the conditions must still be such that it does not undergo chemical exchange with the inactive target atoms and that it is separable from them. Exchange with target atoms is facilitated by (1) mobility (hence solids show much less exchange than liquids), (2) the existence of equilibria between the chemical forms of parent and daughter (*e.g.*, $Fe^{++} \rightleftarrows Fe^{+++} + e^-$), (3) the large amount of energy imparted to the

[24] Since chemical bonds involve only a few electron-volts while the recoil energy of even the heaviest atoms from a several Mev γ-ray is about ten times that amount, this condition is usually met.

recoiling atom which may cause reactions to happen which otherwise would not, and (4) the existence of compounds of parent and daughter (*e.g.*, I_2 and I^- have the compound form I_3^-). Many of these conditions are also conditions of separability; for instance, Cl^- and $CHCl_3$ are separable from each other and do not exhibit exchange. It should be noted that the phenomenon of exchange is the major requirement whenever isotopic dilution, for purposes of carrying or of labeling, is desired. The term "interchange" has been coined to signify the degree to which all chemical forms of an element have reached a common specific activity (isotope ratio). A Szilard-Chalmers reaction is unprofitable as an isotope separation scheme in proportion to the rate of interchange exhibited.

(*d*) *Nuclear Isomerism* (15, 21). Several cases are known in which two energy states of a single nucleus exist, the upper state decaying to the lower which decays subsequently, with a measurable half-life, by β emission. In most of these cases, the half-life of the upper state is quite long compared to the lower but in those where the half-life of the lower state is comparable to (or longer than) the interval between experiment and measurement we have essentially the same possibility of isotope separation as we have in the Szilard-Chalmers reaction. If an isotope, in its upper energy state, is located in a particular molecule, the "isomeric transition" γ-ray emitted on decay may cause it to be ejected from this molecule by recoil. Such ejection is particularly probable if "internal conversion" of the γ-rays, with electron emission, takes place. The atom will then exist in a different chemical form than the one in which the disintegration of the upper state took place.

Although isomeric states occur widely among radioactive species, most of them have a lower state whose half-life is so short (seconds to microseconds) as to be insignificant in this matter. If there were more lower states with appreciably long half-lives, chemical isolations of carrier-free radioelements could be evolved which would take advantage of this phenomenon. As it is, the only significance of it for tracer work is negative; it is no more safe to use a lower state as an indication of the behavior of the upper one (and isomers are usually assayed by the β-rays from the lower state) than it would be to use the 40 hour La^{140} as a tracer of its Ba^{140} parent. Unless sufficient time elapses before measurement to permit daughter isotopes, isolated by recoil, to decay away, false conclusions will be derived as to the behavior of the parent in the experiment.

IV. AVAILABILITY OF RADIOISOTOPES

1. *Pile vs. Cyclotron* (4, 9a)

From the practical standpoint, there are only two sources of radioactive isotopes: the cyclotron and the pile. The cyclotron is primarily a device for accelerating charged particles, particularly deuterons and α-particles. Neu-

2. *A Practical List of Producible Radioisotopes*

		Is there a radioisotope with $t_{\frac{1}{2}} > 30$ minutes		Is such a radioisotope available in high specific activity		Carrier-free radioisotopes best made in pile
		Known?	Made in pile?	From pile? (* = yes; see last column)	From cyclotron? (* = same as pile only)	
I	H	+	+	*	(*)	H^3 (n,α)
	He	–	–	–	–	
II	Li	–	–	–	–	
	Be	+	(+)	–	Be^7	
	B	–	–	–	–	
	C	+	+	*	(*)	C^{14} (n,p)
	N	–	–	–	–	
	O	–	–	–	–	
	F	+	–	–	F^{18}	
	Ne	+	–	–	–	
III	Na	+	+	Na^{24}	Na^{22}	
	Mg	–	–	–	–	
	Al	–	–	–	–	
	Si	(+)	(+)	(Si^{31})	(*)	
	P	+	+	*	*	P^{32} (n,p)
	S	+	+	*	(*)	S^{35} (n,p)
	Cl	(+)	(+)	(*)	(*)	
	A	+	+	*	*	A^{37} (n,α)
IV	K	+	+	–	–	
	Ca	+	+	*	(*)	Ca^{45} (n,p)
	Sc	+	+	Sc^{46}	*	
	Ti	+	+	–	*	
	V	+	–	–	V^{49}	
	Cr	+	+	(Cr^{51})	*	
	Mn	+	(+)	–	$Mn^{52,54}$	
	Fe	+	(+)	–	$Fe^{55,59}$	
	Co	+	+	Co^{60}	$Co^{56,57,58}$	
	Ni	+	(+)	–	Ni^{57}	
	Cu	+	+	Cu^{64}	*	
	Zn	+	+	–	*	
	Ga	+	+	Ga^{72}	*	
	Ge	+	+	–	*	
	As	+	+	As^{76}, As^{77}	16d As^{74}, 90d $As^{73,4}$	
	Se	+	+	–	Se^{75}	
	Br	+	+	Br^{82}	*	
	Kr	+	+	(Kr^{85})	34h $Kr^{79,81}$	

2. A Practical List of Producible Radioisotopes—Continued

		Is there a radioisotope with $t_{\frac{1}{2}} > 30$ minutes		Is such a radioisotope available in high specific activity		Carrier-free radioisotopes best made in pile
		Known?	Made in pile?	From pile? (* = yes; see last column)	From cyclotron? (* = same as pile only)	
V	Rb	+	+	Rb^{86}	*	
	Sr	+	+	*	*	Sr^{89} (n,f)
	Y	+	+	*	*	Y^{91} (n,f)
	Zr	+	+	*	Zr^{89}	Zr^{95} (n,f)
	Cb	+	+	*	*	Cb^{95} (n,f)
	Mo	+	+	—	(Mo^{93})	
	Tc	+	+	Tc^{97}	(Tc^{100})	Tc^{99} (n,f)
	Ru	+	+	*	*	$\text{Ru}^{103,106}$ (n,f)
	Rh	+	+	Rh^{105}	*	
	Pd	+	+	—	$(\text{Pd}^{103,109}$	
	Ag	+	+	—	Ag^{106}	
	Cd	+	+	—	Cd^{109}	
	In	+	+	In^{114} (In^{117})	*	
	Sn	+	+	—	*	
	Sb	+	+	Sb^{122}	*	
	Te	+	+	*	Te^{121}	$\text{Te}^{127,129}$ (n,f)
	I	+	+	*	$\text{I}^{130,131}$	I^{131} (n,f) (n,γ,β^-)
	Xe	+	+	*	Xe^{127}	Xe^{133} (n,f)
VI	Cs	+	+	Cs^{131}, Cs^{134}	*	Cs^{137} (n,f)
	Ba	+	+	*	Ba^{133}	Ba^{140} (n,f)
	La	+	+	*	*	La^{140} (n,f)
	Ce	+	+	*	*	$\text{Ce}^{141,144}$ (n,f)
	Pr	+	+	Pr^{142}	*	Pr^{143} (n,f)
	Nd	+	+	*	*	Nd^{147} (n,f)
	61	+	+	*	*	61^{147} (n,f)
	Sm	+	+	?	?	
Rare earths	Eu	+	+	$\text{Eu}^{154,155}$	*	
	Gd	?	?	?	?	
	Tb	+	+	?	*	
	Dy	?	?	?	?	
	Ho	+	+	?	?	
	Er	+	+	?	?	
	Tm	+	+	?	?	
	Yb	+	+	?	?	
	Lu	?	?	?	?	
	Hf	+	+	Hf^{181}	*	
	Ta	+	+	Ta^{182}	*	
	W	+	+	W^{187}	*	

2. A Practical List of Producible Radioisotopes—Concluded

		Is there a radioisotope with $t_{\frac{1}{2}} > 30$ minutes		Is such a radioisotope available in high specific activity		Carrier-free radioiosotopes best made in pile
		Known?	Made in pile?	From pile? (* = yes; see last column)	From cyclotron? (* = same as pile only)	
VI (cont'd)	Re	+	+	Re^{186}	Re^{184}	
	Os	+	+	$Os^{191,193}$	*	
	Ir	+	+	$Ir^{192,194}$	*	
	Pt	+	+	Pt^{197}	*	
	Au	+	+	Au^{198}, Au^{199}	Au^{196}	
	Hg	+	+	–	Hg^{197}	
	Tl	+	+	–	Tl^{202}	
	Pb	+	(+)	–	Pb^{203}	
	Bi	+	+	–	Bi^{207}	
	Po	+	+	Po^{210}	*	
	At	+	+	–	At^{211}	
	Rn	N	N	N	N	

– = No
\+ = Yes
? = Uncertain

N = Natural source available
( ) = only isotopes available have $t_{\frac{1}{2}} < 12$ hr. (but > ½ hr.), a low yield or mixed isotopes

trons are made in it only as secondary products, which makes the cyclotron relatively inefficient as a neutron source; however, it is the only practical source of fast neutrons for transmutation reactions above $Z = 24$. The characteristics of operation and the physical requirements placed upon target materials restrict severely the materials which a cyclotron can bombard with charged particles and the length of exposure which can be made; competition, with many side reactions, is favored, leading to complex radiochemical isolation problems. However, a cyclotron can make many more varieties of radioisotopes and can effect many more transmutation reactions than a pile.

The pile, on the other hand, is exclusively a source of neutrons and of very slow neutrons in particular; thus, it can transmute only a few of the lighter elements. It has advantages in that the physical requirements placed upon target materials are much less severe, the length of time during which a material may be exposed is limited only by the life of the pile itself and there are fewer competing reactions. The happy fact that most of the biologically important radioisotopes (C^{14}, P^{32}, I^{131}, S^{35}) can be produced by pile neutrons in large amount and in high specific activity reduces in significance its lack of variety, but it must be remembered that many isotopes cannot be made in the pile at all (Be^7, F^{18}, Na^{22}, V^{49}, Mn^{52}, Mn^{54}, As^{74}, 85^{211}) or are presently made in too low a specific activity for effective use (*e.g.*, Fe^{55}, Fe^{59}).

The table which precedes summarizes the present situation with regard to what can be made with available machines (January, 1947).

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Fundamentals of Radioactivity and Its Instrumentation

By ROBLEY D. EVANS

CONTENTS

I. Interaction of Radiation with Matter

Man's senses of sight, touch, taste, smell, and hearing are all unresponsive to ordinary amounts of nuclear radiations. Isaac Newton and Louis Pasteur had no way of detecting the many types of ionizing radiation which, nevertheless, were present in their laboratories. Contemporary knowledge of the properties of radiation rests on delicate measurements of the effects of collisions of these radiations with the electrons and nuclei of atoms.

1. *Alpha-Rays and Other Heavy, Charged Rays*

The α-ray is simply a swiftly moving stripped atom of helium $[{}_2He^4]^{++}$. This helium nucleus is a highly stable sub-unit or building-block in atomic nuclei, and is composed of two protons and two neutrons tightly bound together. The α-rays emitted in radioactive decay of heavy elements have initial velocities of ejection in the neighborhood of $1\text{–}2 \times 10^9$ cm./sec., which is 3–6% of the velocity of light. With a rest mass of 4.0 amu[1], the initial kinetic energy of such an α-ray is some 2–8 Mev[1]. Artificial α-rays of even greater energy may now be obtained by the acceleration of helium nuclei in cyclotrons.

Protons $[{}_1H^1]^+$, and deuterons $[{}_1H^2]^+$ can also be accelerated to high energies by cyclotrons and other electronuclear machines. Protons, deuterons, and α-rays behave very similarly in their interactions with matter, and can be considered together.

All types of radiation are ultimately detected and measured by the primary and secondary ionization which they produce. On the average, the production of one ion-pair (1 free electron and 1 residual positive ion) in air requires the expenditure of 32.5 ev. Thus a typical natural α-ray will produce a total of some $0.6\text{–}2.4 \times 10^5$ ion pairs before being brought to rest by the expenditure of its kinetic energy. In dry air at 15°C. and 1 atmosphere pressure, an α-ray travels in substantially a straight path, without important deflections in direction, and produces a dense trail of ionization. Fig. 1 shows the variation of the ionization per mm. of path in air, and the velocity of the α-ray, as a function of the residual range. The residual range is the distance which the α-ray can still travel before being brought to rest and becoming simply a neutral helium atom through the capture of two atomic electrons from the air molecules.

A group of α-rays, all having the same initial velocity and energy, will actually travel slightly different total distances in the air absorber, because of statistical fluctuations in both the number of collisions suffered and in the energy lost in each individual collision. This statistical phenomenon is known as *straggling*. The ranges of individual α-rays show a normal-law distribution about the mean-range of the group. For Po α-rays in air, the standard deviation of the ranges of the individual rays is about 1% of the mean range. The ratio of standard deviation to mean-range increases slowly with the atomic number *Z* of the absorber, and with the initial velocity of

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[1] **1 atomic mass unit (amu) = $1/6.02 \times 10^{23} = 1.66 \times 10^{-24}$ g.**
1 Mev = 10^6 electron volts (ev) = 1.60×10^{-6} erg = 3.8×10^{-14} g.-cal.

the ray. For protons, deuterons, and α-rays, of the same initial velocity, the ratio of standard-deviation, σ, in range to the mean range, R, of the group can be shown to vary as $1/\sqrt{M}$, where M is the nuclear mass of the ray, and to be independent of the charge ze on the ray.

The mean-range of α-rays is shown in Fig. 2 as a function of the initial kinetic energy of the ray. Roughly, the range is proportional to V^3, where V is the initial velocity. For protons, and other nuclear rays, the mean range R can be shown to be proportional to M/z^2 for rays of the same initial velocity. Table I summarizes the variations: of energy E and, hence, total ionization; of mean range R; of specific ionization I; and of per cent straggling σ/R, for protons, deuterons, α-rays and oxygen nuclei of the same initial velocity V. Thus these quantities can be deduced for any nuclear ray from knowledge of the properties of α-rays as given in Figs. 1 and 2.

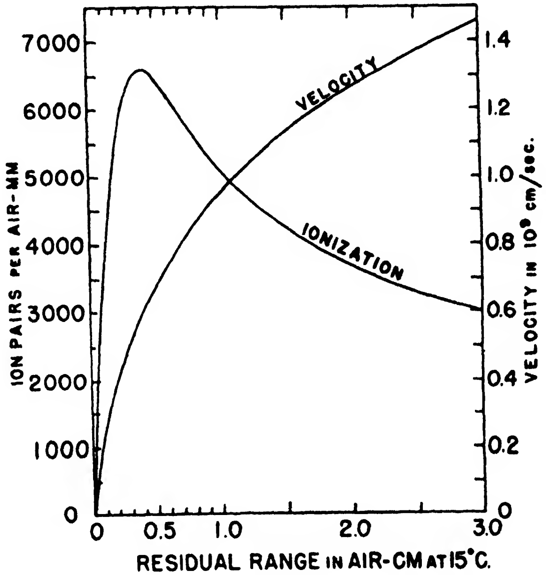

FIG. 1

The velocity and specific ionization of an average α-ray in air, at 15°C. and 1 atmosphere pressure, as a function of residual range.

The mean range L of various nuclear rays in materials other than air can be computed accurately from detailed theory (Mano, 1934). For many ordinary purposes, the Bragg-Kleeman Rule gives a sufficiently accurate (±10%) indication, in terms of the mean range R in air of the same type of ray. Thus:

$$L = 0.00032\, R(\sqrt{A}/d)$$

TABLE I

Summary of the Properties of Some Heavy Charged Particles, in Relation to the Known Properties of α-Rays, for Rays of the Same Initial Velocity V

Property	Symbol	Proportional to	Type of Particle			
			Protons	Deuterons	α-Rays	Oxygen nuclei
Charge	z		1	1	2	8
Mass	M		1	2	4	16
Energy and total ionization	E	$MV^2/2$	¼	½	1	16
Mean range	R	M/z^2	1	2	1	¼
Specific ionization	I	z^2	¼	¼	1	16
Straggling	σ/R	$1/\sqrt{M}$	2	$\sqrt{2}$	1	½

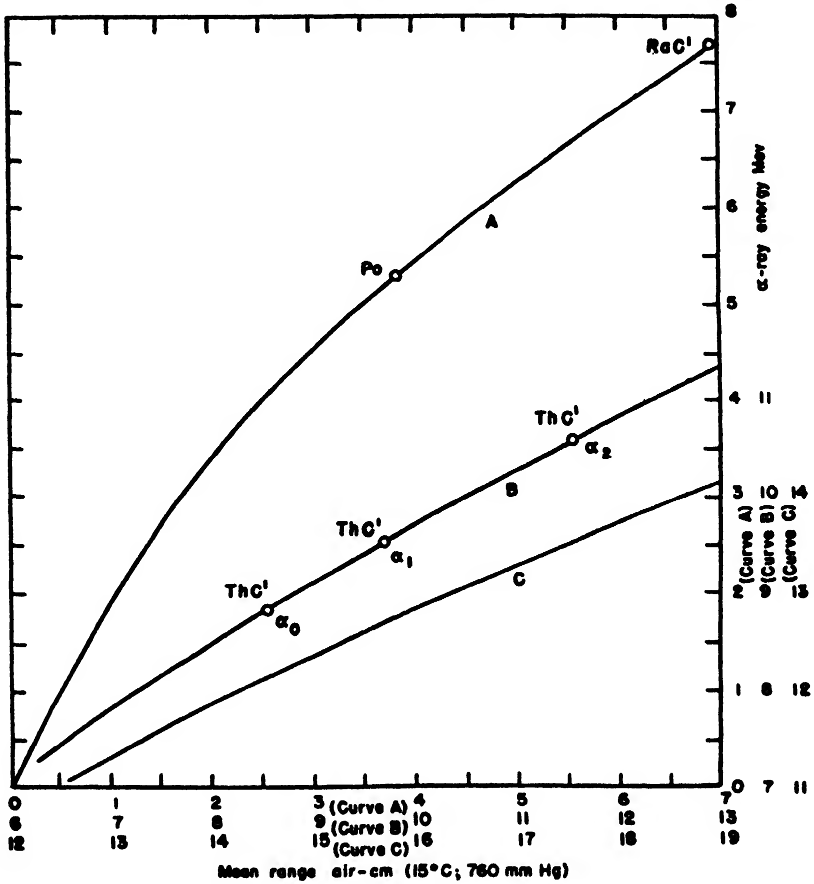

FIG. 2

The mean-range, in cm. of dry air at 15°C. and 1 atmosphere pressure, for α-rays of various initial kinetic energies between 0 and 14 Mev.

where A is the mean atomic weight, and d is the density of the absorber in g./cm.[3] It is seen that, for α-rays whose mean range R is about 6 air-cm. the mean range L in ordinary solids such as aluminum, water, or tissue is only about 30–40μ.

2. *Beta-Rays*

Beta-rays are high speed electrons produced in radioactive transformations. They may be either positrons (β^+) having a positive charge ($e = 4.80 \times 10^{-10}$ esu $= 1.60 \times 10^{-19}$ coulomb) or negatrons (β^-) having a negative charge of exactly the same magnitude but of opposite sign. The negative β-rays are identical with ordinary atomic electrons (e^-), and, in fact, become atomic electrons after they have lost their initially large kinetic energy, through ionizing collisions.

Through γ-ray interactions, and nuclear internal conversion transitions, ordinary atomic electrons (e^-) may be given very large kinetic energies, when they behave exactly like negative β-rays (β^-) in their interaction with matter.

The rest-mass of any electron (β^+, β^-, or e^-) is $m_o = 0.9 \times 10^{-29}$ g., and its relativistic rest-energy by virtue of this rest-mass is $m_oc^2 = 0.51$ Mev.

Whenever a positron collides inelastically with a negative electron, the two annihilate each other, the charges cancel and disappear, and the rest-energy of the two electrons is emitted from the scene of the collision as two exactly oppositely directed photons of *annihilation radiation*, each having an energy $h\nu = 0.51$ Mev. The annihilation of positrons ordinarily occurs only after they have reached a very low velocity and, hence, have a negligible kinetic energy compared with their rest-energy. Aside from the terminal phenomenon of annihilation, the interaction of all swift electrons (β^+, β^-, e^-) with matter is independent of the sign of the charge or the mode of origin of the particle.

The majority of the electrons dealt with in radioactive transformations have energies between about 0.05 and 5 Mev, and must be treated relativistically because the kinetic energy is comparable with the rest-energy. The relativistic momentum of an electron is most accurately determined by observing its radius of curvature, ρ cm., when deflected in a uniform magnetic field, H gauss. Then the momentum, mv, is:

$$mv = H\rho(e/c)$$

where e/c is the electron charge in electromagnetic units. If E is the corresponding kinetic energy of the electron, in Mev, and $H\rho$ is the magnetic rigidity in gauss-cm., then it can be shown that:

$$H\rho = (10^4/3)\sqrt{E(E + 1.02)}$$

Fig. 3 is a graphical representation of this relationship, for electrons of any energy between 0 and 35 Mev.

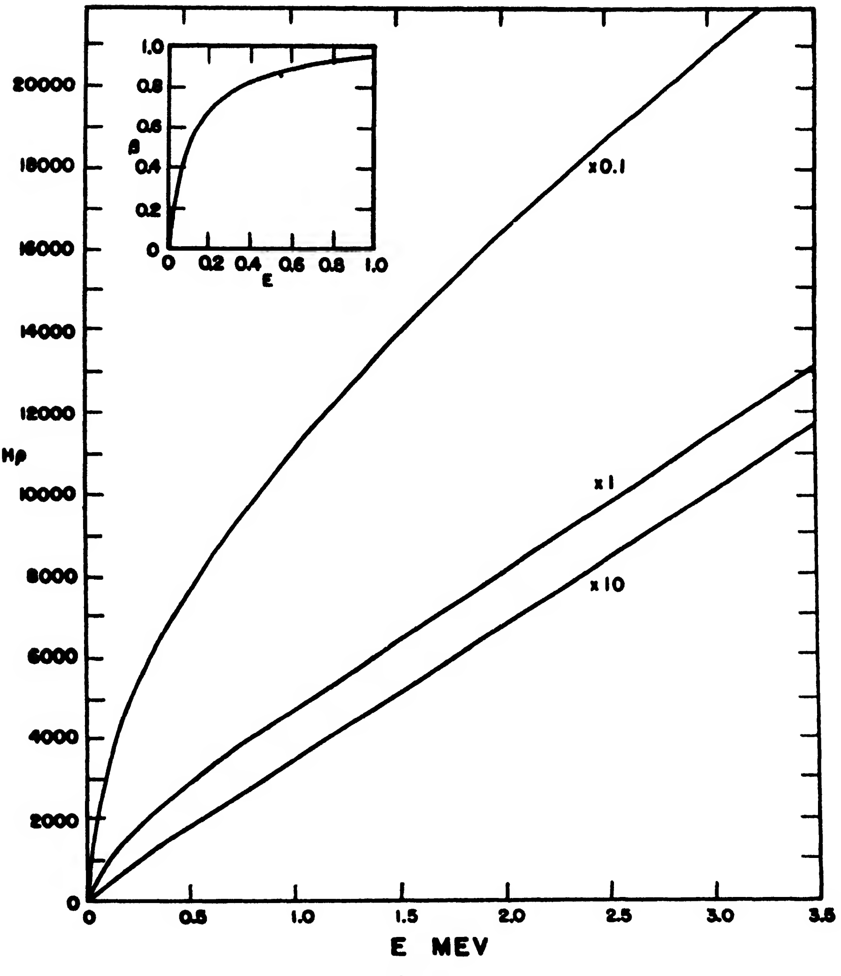

Fig. 3

The kinetic energy E in Mev of an electron for various values of the momentum, or magnetic rigidity, $H\rho$ gauss-cm. Both sets of coordinates are to be multiplied by the factor shown adjoining the appropriate curve. Thus, electrons with an energy of 3.0 Mev (3.0 × 1) have a momentum of 11,500 gauss-cm. (11,500 × 1). Electrons with an energy of 0.3 Mev (3.0 × 0.1) have a momentum of 2100 gauss-cm. (21,000 × 0.1). The inset gives the velocity, v, in terms of the velocity of light, c, expressed as $\beta = v/c$, for electrons having kinetic energy E Mev. Above 1 Mev, β continues to approach more closely to unity as the electron energy increases.

The velocity v of an energetic electron is 0.86 of the velocity of light, c, at 0.5 Mev, and is $0.94c$ at 1.0 Mev. The variation of velocity $\beta = v/c$ with energy E is shown in the inset of Fig. 3.

In traversing matter, electrons experience four principal types of interaction with the atoms of the absorber.

1. Elastic scattering by atomic electrons is especially pronounced for very soft (low-energy) electrons. At very low, non-relativistic energies, the probability of deflection of the moving electron by elastic scattering varies as $1/E^2$/atomic electron. If there are N atoms of atomic number Z/cm.^{3} of absorber, then there are NZ atomic electrons/cm.^{3}, and the probability of elastic scattering by an atomic electron, will be proportional to NZ/cm. of travel.

2. Elastic scattering by atomic nuclei is also pronounced for soft electrons, but the probability of deflection through any particular angle is proportional to Z^2/atom of absorber, or to NZ^2/cm. of path. Elastic nuclear and electronic scattering are substantially equal in hydrogen ($Z = 1$). For heavy elements, nuclear elastic scattering is about Z times ($=NZ^2/NZ$) as severe as electronic elastic scattering. Thus, in elements such as copper, silver, and palladium, which are often used as β-ray source mountings, the back-scattering of soft β-rays is primarily nuclear scattering, only 2–4% of the elastic scattering being due to atomic electrons. An appreciation of the severity of scattering is gained from the observation (Schonland, 1925) that the fraction of electrons reflected (*i.e.*, scattered by more than 90°) from a gold foil only 0.187 μ thick is about 3% at 0.1 Mev and, with diminishing electron energy, rises rapidly to 50% at about 0.035 Mev. The nuclear elastic scattering of electrons is so pronounced because of their very small rest-mass. It can be shown that the effect varies inversely with the square of the rest-mass of the moving particle, consequently nuclear scattering is about $(4 \times 1840)^2 = 5 \times 10^7$ times as important for electrons as it is for α-particles.

3. Inelastic collisions with atomic electrons produce ionization and account for the major portion of the energy lost by electrons of intermediate energy. The ionization mechanism is basically the same for electrons and for heavy charged particles. If the moving electron interacts electrostatically with an atomic electron with sufficient intensity and duration, the atomic electron will be removed leaving a positive ion and a free electron. These separated electric charges are called an ion-pair regardless of whether the struck electron remains free or becomes attached to a neutral atom forming a negative ion. As in the case of α-particle ionization, the kinetic energy lost by the moving electron depends somewhat on the velocity of the moving charged particle, but averages 32.5 ev per ion-pair produced in air. The ionization loss depends also on the absorber, and is only 25 ev per ion-pair in argon.

The specific ionization, I, as in the case of α-rays varies inversely with v^2, and, for electrons, is quite well represented between 0.06 and 1.6 Mev by the simple relation:

$$I = 45/\beta^2 \text{ ion-pairs/cm. of dry air at 20°C., 1 atm.}$$

At higher energies (above the minimum expected at $3m_0c^2 = 1.5$ Mev) theory predicts that the ionization losses should rise very slowly (by only 60% between 1.5 and 500 Mev) approximately in proportion to the logarithm of the energy.

It is to be noted that the specific ionization along the path of an α-ray (Fig. 1) is about 1000 times as great as the specific ionization along the path of an electron. The electron, since it loses energy much more gradually than the α-ray, will have a correspondingly greater range.

Except for a small correction depending logarithmetically on Z, the ionization losses are proportional to the number of atomic electrons/cm.^{3} of absorber, NZ. Thus the energy dE lost by ionization along a path of length dr, is, for any particular velocity,

$$dE = K\,NZdr$$

The number of atoms/cm.^{3} N is $(\rho n/A)$ where ρ = density in g./cm.^{3}, n = Avogadro's number, A = atomic weight, consequently:

$$dE = K\,(\rho nZ/A)dr.$$

Now the ratio (Z/A) of atomic number to atomic weight is nearly constant for all elements, decreasing from $Z/A = 0.5$ for He, C, O, to $Z/A = 0.4$ for Pb. Hydrogen alone has the anomolous value $Z/A = 1$. Consequently, for all elements, except hydrogen, Z/A may be taken as effectively constant, and the ionization energy losses of moving electrons can be expressed not in terms of distance dr cm. traversed, but in terms of ρdr g./cm.^{2} traversed. Thus, whether the absorbing material is air, water, paper, tissue, aluminum, copper, *etc.*, nearly the same thickness of material, measured in g./cm.^{2}, will be required to stop the electron whenever the principal energy losses are by ionization, as is the case in the energy domain of about 0.05 to 2 Mev met in most observations on β-rays. The range of β-rays is, therefore, usually expressed in g./cm.^{2} When the absorber is hydrogen the ranges will be only about half the expected number of g./cm.^{2}, because hydrogen has twice as many atomic electrons/g. as any other element.

The actual slight decreases in Z/A and in the logarithmetic correction term with increasing Z for the heavy elements are almost precisely compensated for by the increased effects of nuclear scattering in the heavy elements. Unlike an α-ray, the path of an electron in an absorber is not straight, but is generally tortuous because of the numerous small deflections suffered through elastic nuclear collisions. Actually the path length, measured along the actual path of the electron through the absorbing material, will be from 1.5 to 4 times the actual measured thickness of the absorber. These scattering effects increase the true path length of the electron in the absorber by almost exactly the amount required to compensate for the decrease in Z/A with increasing Z. The overall result is that β-ray ranges may be expressed, empirically, by a relationship between initial electron energy E Mev and absorber thickness in g./cm.^{2}

4. Inelastic collisions of swift electrons with atomic nuclei are important for high energy electrons. The moving electron is deflected by the Coulomb field of the nucleus, and, at the same time, all or a portion of its kinetic energy is converted to a quantum of X-radiation. These radiative collisions are the origin of the familiar continuous X-ray spectrum, or Bremstrahlung. The average energy loss, dE_{rad}, by radiation, along a path element of length dr, is given by:

$$dE_{\text{rad}} = NZ^2E\Phi dr$$

where Φ is a slowly varying logarithmic function of E and Z. Thus, as in the case of deflection by elastic nuclear scattering, the radiative or inelastic nuclear collisions are proportional to Z^2. Very importantly, the radiative losses increase with increasing electron energy E.

For energies above 1.5 Mev, the ratio of radiative to ionization losses is given closely by:

$$\frac{(dE/dr)_{\text{rad}}}{(dE/dr)_{\text{ion}}} = \frac{EZ}{820}$$

where E is in Mev. Thus, the radiative and ionization losses of a 10 Mev electron passing through lead ($Z = 82$) are equal (each about 1.6 Mev/mm. Pb).

Due to the effect of Z, it is clear that the production of secondary X-rays through the absorption of energetic β-rays is minimized by using absorbers of low Z. Thus, for example, lead ($Z = 82$) is a poor choice for shielding a very strong source of radiophosphorus. Aluminum ($Z = 13$) or graphite ($Z = 6$) would absorb the energetic electrons by ionization processes, with very little production of hard secondary X-rays.

3. Absorption of Electrons and Beta-Rays

By "absorption" we understand the combined effects of elastic and inelastic collisions between swift electrons and atomic electrons and nuclei. The effects are evidently complex. Nevertheless, careful observation of the effects of various absorbing foils on electrons of known energy and homogeneity can be used to define practical extrapolated and maximum ranges for electrons.

In spite of variations in the form of the absorption curves, the thickness of material required to reduce the ionization or counting to essentially zero is a fairly definite observable quantity. Reproducible results are obtained in the case of initially homogeneous electrons, such as conversion electron groups, by extrapolating the approximately linear middle portion of the absorption curve, until it cuts the value assigned to background effects. Thus the extrapolated range R_o for homogeneous electrons is obtained as shown in Fig. 4.

All available data on the extrapolated ranges of homogeneous electrons are presented in Fig. 5. From 0.5 to 3 Mev these data on *line spectra* may be represented within about ±5% by the linear relationship

$$R_o = 0.52\,E - 0.09$$

where E is in Mev and R_o is in g./cm.^{2}

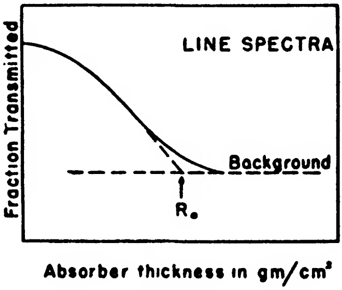

FIG. 4

Typical absorption curve for initially homogenous electrons. The "extrapolated range" is at the intersection of the extrapolated ionization or counting curve with the estimated contribution of background due to γ-rays and other causes. See Fig. 5 for R_o *vs*. E.

Extrapolated ranges have also been measured for very low energy electrons in aluminum. These results are also plotted in Fig. 5 for homogeneous electrons from 0.01 to 0.15 Mev. Because of the greater ionization and scattering losses at such low energies, these ranges are much smaller than those for higher energies but can be represented between $E = 0.03$ and 0.15 Mev by

$$R_o = 0.15\,E - 0.0028.$$

When a beam of initially homogeneous electrons passes through even a thin layer of absorber, which is much less than R_o, the emergent distribution of electron energies is greatly broadened, due to straggling. The shift in the most probable emergent energy is represented by the slope of the curve of "integrated range" Σ of Fig. 5, which is computed from the experiments of White and Millington (1928). The linear dependence of the integrated range Σ on initial energy, leads to the following useful empirical

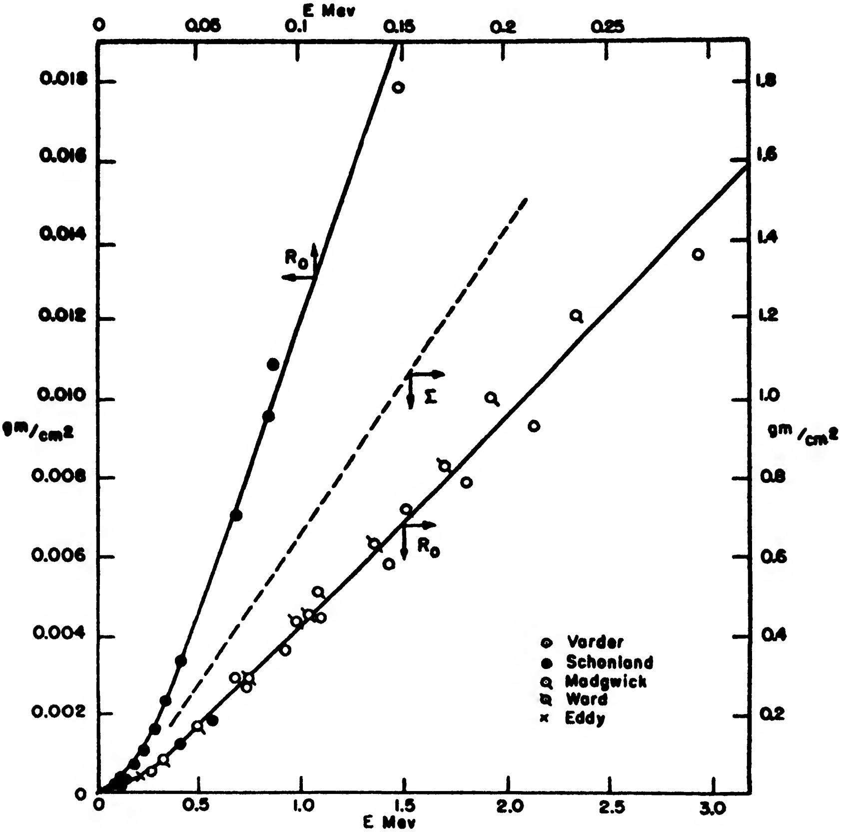

FIG. 5

The extrapolated range R_o of initially homogenous electron groups, as a function of electron energy E in Mev. Note that the upper curve of R_o uses the coordinates at the top and left of the diagram, while the lower curve of R_o uses the coordinates at the right and bottom of the diagram. The dotted curve marked Σ is the "integrated range," or amount of material required to reduce the most probable momentum (in the broadly straggled momentum distribution produced by even a thin layer of absorber) to zero (1 mm. Al = 0.271 g./cm.²).

relationship between the most probable energy loss, $E_o - E$, and the thickness σ g./cm.² of absorber:

$$E_o - E = 1.3\sigma$$

which is valid when $E_o - E$ is of the order of 10% of the initial energy E_o. Thus, a window thickness, on a β-ray counter, of 0.1 g./cm.² will reduce the initial energy E_o by 0.13 Mev if E_o is of the order of 1–2 Mev.

The majority of the electron spectra encountered in nuclear physics are *continuous*, but have a definite maximum energy E_m Mev. All β-ray spectra, both of positrons and negatrons, are continuous, with a maximum energy E_m which is usually between 3 and 4 times the average energy. The shape of two typical β-ray spectra is illustrated in Fig. 6. All the secondary electrons produced by the absorption of γ-rays have a continuous distribution in energy, with a definite maximum energy. Usually, the maximum energy holds the major physical interest, and empirical methods have been developed for estimating E_m from absorption data.

Because the lower energy electrons are most rapidly absorbed, the absorption curves for continuous spectra fall off more rapidly than those for line spectra. For radioactive β-ray spectra, the absorption curve obtained in ionization measurements is often nearly exponential over the majority of its length, and can then be represented approximately by:

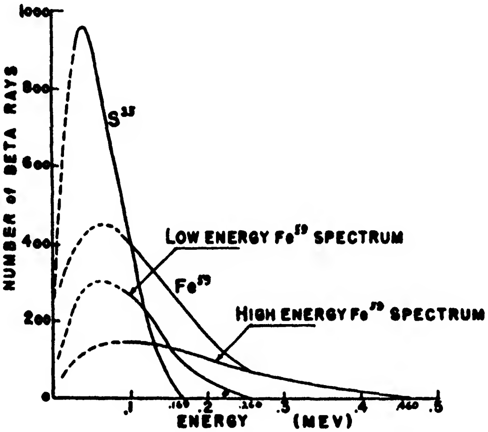

FIG. 6

The β-ray spectra of S^{35} (Osborne, 1947) and Fe^{59} (Deutsch *et al.*, 1942) illustrating the presence of β-rays of all energies from zero (probably) to a definite maximum or end-point E_m, which is characteristic of the particular radioactive isotope. Many isotopes have "simple" spectra, like S^{35}. Other isotopes have one or more internal end points, and like Fe^{59}, their β-ray spectrum is a superposition of two or more "simple" spectra, having different maximum energies and different relative abundances.

$$I/I_0 = e^{-\mu x}$$

where I/I_0 is the fraction of the initial ionization, measured after the β-rays have passed through x cm. of absorbing material, and μ cm^{-1} is the *apparent* absorption coefficient for the particular spectrum. If ρ g./cm.^{3} is the density of the absorber, it is found experimentally that *the mass absorption coefficient μ/ρ is nearly independent of the atomic weight of the absorber*, rising only slightly with increasing atomic number. A plot of all known values of μ/ρ against the maximum energy of the β-ray spectrum E_m shows that, within a probable error of about 0.2 Mev, the following empirical relationship describes these data:

$$\mu/\rho = 22/E_m^{1.33}$$

where E_m is in Mev, and μ/ρ is measured in cm.^{2}/g. of aluminum. Then the absorber thickness D required to reduce I/I_0 to 0.5 is approximately:

$$D = \frac{0.693}{(\mu/\rho)} = 0.032E_m^{1.33}$$

where the half-value thickness D is in g./cm.^{2} of aluminum, or some neighboring light element. In computing I and I_0 the background must first be deducted from all readings.

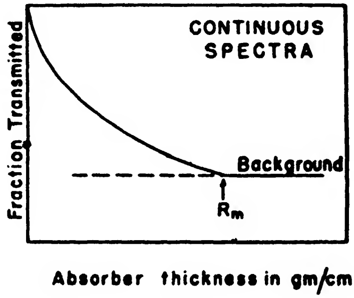

FIG. 7

Characteristic absorption curve for continuous electron spectra. The maximum range R_m is the absorber thickness required to reduce the ionization or counting to the background value. Particularly when ionization chamber detection is employed, the upper part of the absorption curve may be approximately exponential in shape. However the effective exponential absorption coefficient will depend not only on E_m, but also on the type and geometry of the source and of the detection device used, and on the presence or absence of soft β-ray components (*e.g.*, Fig. 6) and conversion electrons in the spectrum.

Because the shape of the absorption curve will depend somewhat on geometrical conditions, more reproducible results may be obtained by observing the thickness of absorber required to stop the β-rays of highest energy. This maximum thickness is called the *maximum range*, R_m. The definition of maximum range is illustrated by Fig. 7. In the case of several radioactive substances, relatively reliable measurements are available on both the maximum range, and the observed maximum energy as determined from magnetic spectrograph measurements. These data are plotted in Fig. 8. A straight line through these points is given by:

$$R_m = 0.54E_m - 0.15$$

which is valid, within the known uncertainties, for maximum energies E_m near the 1–3 Mev domain.

4. *Gamma-Rays*

Electromagnetic radiations (photons, quanta) which are emitted by atomic nuclei are called *γ-rays*. They are similar in all physical properties to *X-rays*. However, X-rays originate outside the nucleus, as in Bremstrahlung (continuous X-rays) or in transitions between the K, L, *etc.*, shell of atomic electrons (characteristic X-rays).

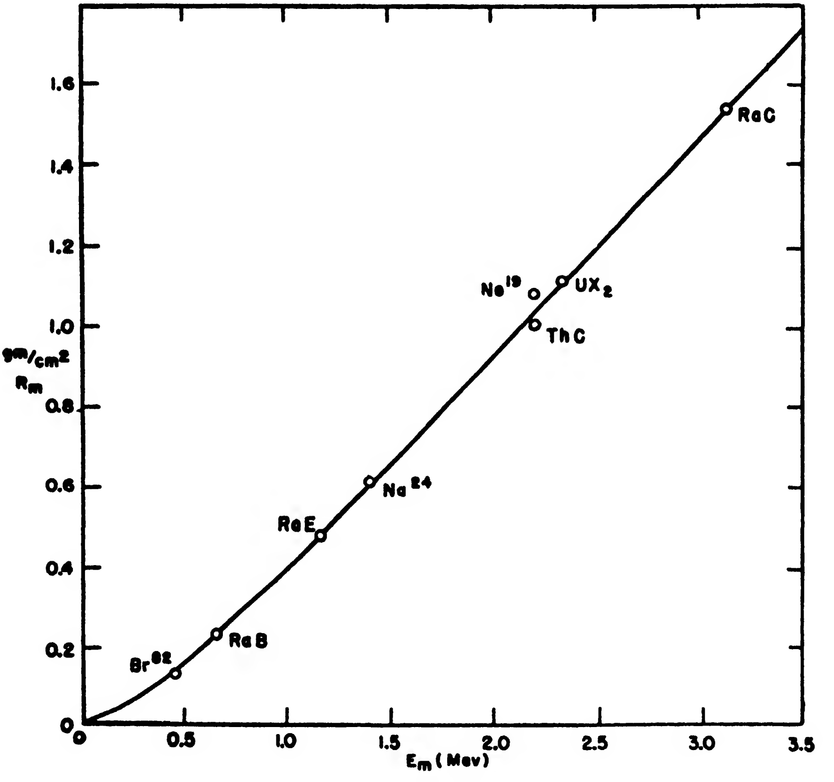

FIG. 8

The maximum range R_m in g./cm.² of Al, for continuous β-ray spectra of some radioactive isotopes. Below 0.5 Mev the curve is drawn to follow the R_o *vs*. *E* curve of Fig. 5.

Gamma-rays arise as a result of spontaneous rearrangement of the configuration of neutrons and protons in an atomic nucleus. Thus γ-rays represent the energy difference between quantized excited states in the nucleus. The photon energy involved is ordinarily of the order of 0.03–3 Mev, and the radiation from a single nucleus is emitted as a short pulse of only about 10^{-12}–10^{-13} sec. duration.

As in the case of any other type of electromagnetic radiation (X-ray, ultraviolet, visible, infrared, radio) the quantum energy is given by Planck's constant h ($= 6.624 \times 10^{-27}$ erg sec.) times the frequency ν sec^{-1} of oscillation of the electromagnetic radiation. Thus the quantum energy of the photon is written $h\nu$. The interactions of the photon with matter are understood to be independent of its mode of origin (γ-ray, X-ray, annihilation radiation, *etc.*) and dependent only on quantum energy. The wavelength λ of electromagnetic radiation is $\lambda = c/\nu$, where $c(= 3.0 \times 10^{10}$ cm./sec.) is the velocity of light. Consequently the relationship between wavelength and quantum energy $h\nu = E_\gamma$ in Mev is:

$$\lambda = hc/h\nu = hc/(10^6 eE_\gamma/300)$$

where e is the electronic charge in esu, and 300 is the number of volts/statvolt. Then:

$$\lambda = (1238./E_\gamma) \times 10^{-13} \text{ cm.}$$

All γ-ray wavelengths are seen to be much greater than the radius of a nucleus or of an electron ($\backsim 10^{-13}$ cm.), and comparable with or smaller than the radius of an atom ($\backsim 10^{-8}$ cm.).

Finally, each photon has a momentum $p = h\nu/c$ in its direction of propagation; an angular momentum $h/2\pi$ about the direction of propagation, and an oscillating electric field E and magnetic field H at right angles to the direction of propagation.

An individual photon in the energy domain of ordinary γ-rays can interact with matter in any one of several independent ways. A beam of high energy photons will generally involve all these possible modes of interaction. Some individual photons suffer one type of collision, and others another type. We shall not discuss here the coherent scattering of photons by crystal planes (Bragg reflection), nor the excitation and disintegration of nuclei by photons, because in the energy domain of most nuclear γ-rays these two modes of interaction are usually of very minor consequence in competition with the atomic interaction (photoelectric effect), electronic interaction (Compton effect), and nuclear interaction (electron pair-production).

(*a*) *Photoelectric Effect.* In the photoelectric effect, the photon interacts as a wave with the entire struck atom. The general result is that a single atomic electron somewhere in the atom (generally a K or L electron) receives all of the energy of the photon. This electron is consequently ejected from the atom with a kinetic energy

$$E_{\text{photo}} = h\nu - B$$

where B is the original binding energy of the electron in the atom. Thus, for example, the kinetic energy of a photoelectron ejected by a 0.510 Mev photon from the K shell of Pb ($B_K = 0.088$ Mev) would be $0.510 - 0.088 = 0.422$ Mev.

The photoelectrons tend to be ejected from the atom at an angle of 90° (or a little less) to the direction of the incident photon, as indicated in Fig. 9.

After the photoelectron is ejected, the emitting atom has a corresponding vacancy in its electronic structure. The subsequent readjustments of the electronic structure result in the emission of a characteristic X-ray quantum, or of Auger electrons, or both.

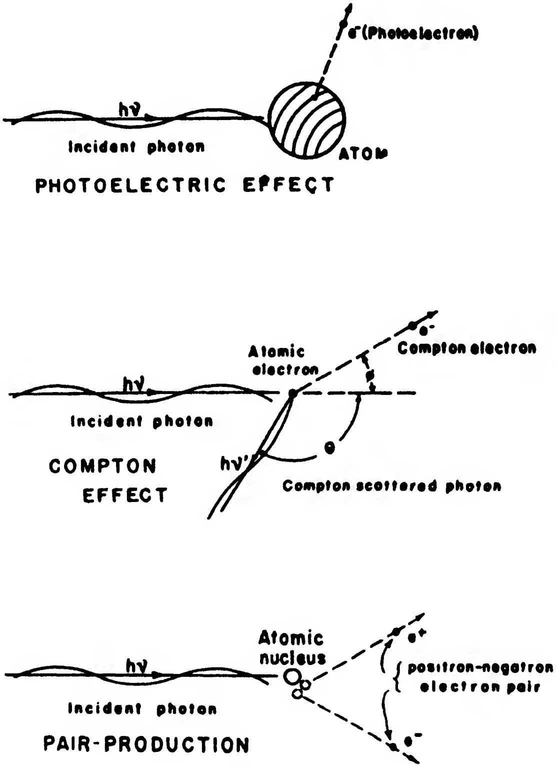

Fig. 9

Schematic diagrams to illustrate the three principal competitive types of interaction of γ-rays with matter.

When a collimated beam of photons, each of energy $h\nu$ passes through a thickness dx cm. of absorbing material of atomic number Z containing N atoms/cm.^{3}, the fraction of the photons which will be eliminated by photoelectric encounters is τdx, where τ is the linear absorption coefficient for photoelectric encounters, and is given by:

$$\tau = NZ^4 f_1$$

Here f_1 is a complicated function of photon energy and Z. Its variation with Z acts as a small correction (order of $Z^{\pm 1}$) on the Z^4 term in the expression for τ. Its variation with $h\nu$ can be expressed approximately as $(1/h\nu)^n$, with n varying from about 3 for very low energies (∽ 0.02 Mev), to about 1.6 for $h\nu$ ∽ 2 Mev. Values of f_1 for Pb(Z = 82) may be obtained from Figs. 10 and 11, which contain τ_{Pb} as a function of $h\nu$. The photoelectric absorption coefficients τ for an element of density ρ, atomic weight W, and atomic number Z, may be estimated from the values τ_{Pb} plotted for lead by setting $\tau/N = Z^4 f_1$ and $N = \rho n/W$, where n is Avogadro's number. Then:

$$\tau = \tau_{\text{Pb}} \left(\frac{\rho}{11.35}\right)\left(\frac{207.2}{W}\right)\left(\frac{Z}{82}\right)^4$$

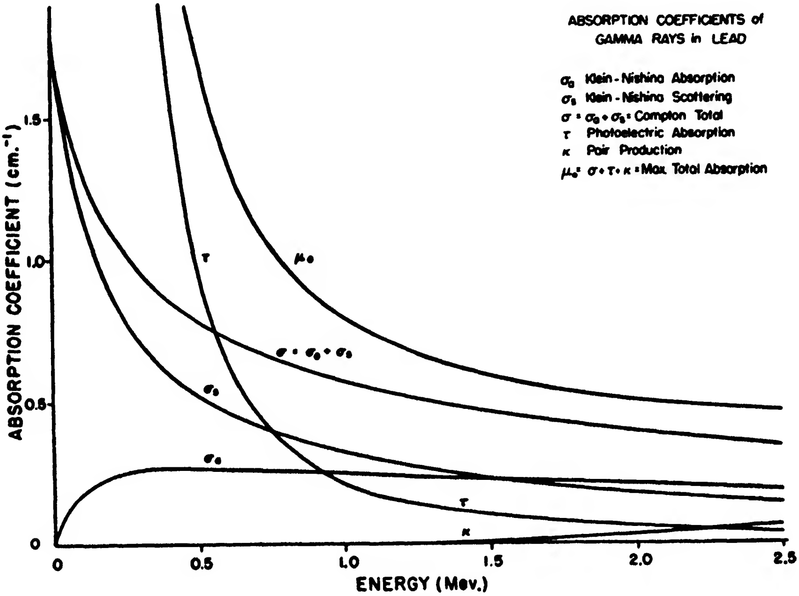

FIG. 10

Absorption coefficients for photons in lead. (Z = 82)(N = 11.35 × 6.02 × 10^{23}/207.2 = 3.30 × 10^{22} atoms/cm.^{3} Pb.) Below 0.5 Mev the photoelectric effect is the dominant mode of interaction, and τ increases rapidly while $h\nu$ decreases, as given in the following table:

$h\nu$ (Mev).........	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
τ (cm.$^{-1}$).........	0.96	1.26	1.65	2.3	3.3	5.4	9.7	21.	61.

The probability of photoelectric absorption of low energy photons in lead is so great (because of its large atomic number) that lead and other neighboring elements are often spoken of as "black" or "opaque" for photons of less than about 0.4 Mev. On the other hand, the photoelectric absorption is relatively unimportant in light elements, such as aluminum, except at very small quantum energies.

(*b*) *Compton Effect.* The Compton effect is an elastic collision of a photon with a single atomic electron. Except for basic physical studies of this interaction (DuMond, 1933), the binding energy of the struck electron can be neglected because all electrons have an equal chance of being struck by the photon, and the majority of the electrons in an atom of any material are only lightly bound compared with γ-ray energies. The more tightly bound K or L electrons are either much in the minority (in heavy elements), or have very small binding energies (in light elements).

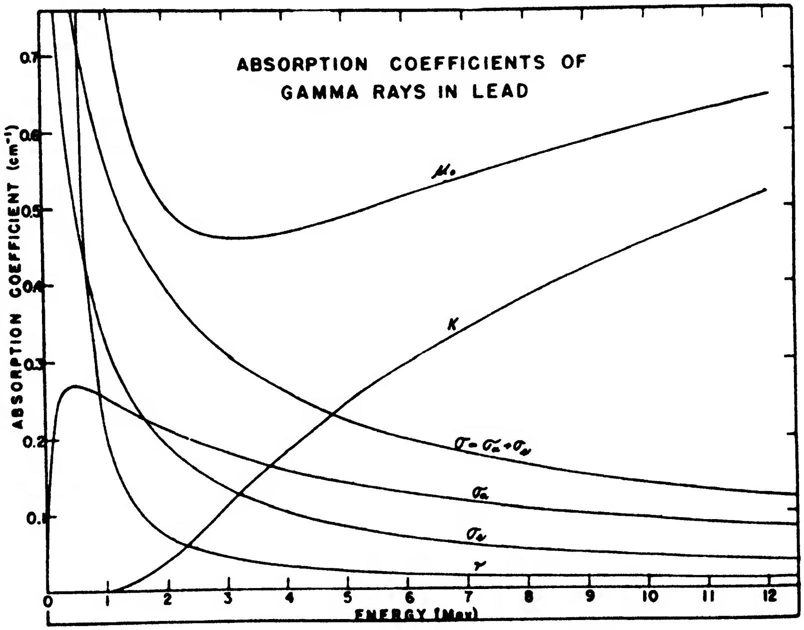

Fig. 11

Absorption coefficients for high energy photons in lead. Note that the maximum total absorption coefficient, μ_0, exhibits a minimum at about 3 Mev, the increase at high energies being due to pair-production absorption.

After its collision with an atomic electron, the momentum and energy of the incident photon are shared between the struck electron and the degraded or scattered quantum $h\nu'$, as suggested schematically in Fig. 9. The angle of scattering ϑ can have any value between 0 and 180°, and its most probable value decreases with increasing photon energy. Conservation of momentum and energy leads to the value

$$h\nu' = m_0c^2/[1 + (m_0c^2/h\nu) - \cos\vartheta]$$

for the quantum energy $h\nu'$ of a photon scattered through an angle ϑ. For incident photons of very high energy $h\nu$ compared with the rest energy $m_0c^2(= 0.51$ Mev) of an electron, the energy $h\nu'$ of the scattered photon approaches:

$$h\nu' \rightarrow 0.25 \text{ Mev at } \vartheta = 180°, \text{ for } h\nu \gg 0.51 \text{ Mev}$$

$$h\nu' \rightarrow 0.51 \text{ Mev at } \vartheta = 90°, \text{ for } h\nu \gg 0.51 \text{ Mev}.$$

The energy $h\nu - h\nu'$ lost by the electromagnetic radiation in the scattering process appears as kinetic energy E_{comp} of the Compton electron, so E_{comp} can have any value between zero and some definite upper limit dependent on $h\nu$. In general:

$$E_{\text{comp}} = h\nu - h\nu' = h\nu/[1 + (m_0c^2/h\nu)/(1 - \cos\vartheta)]$$

or its equivalent:

$$E_{\text{comp}} = h\nu[2(h\nu/m_0c^2)\cos^2\varphi]/[(1 + h\nu/m_0c^2)^2 - (h\nu/m_0c^2)^2\cos^2\varphi]$$

which has its maximum value when the Compton electron is projected straight forward in the direction of the incident photon ($\varphi = 0$), and the photon is scattered through $\vartheta = 180°$. Then

$$(E_{\text{comp}})_{\text{max}} = h\nu/[1 + m_0c^2/2h\nu] \qquad (\text{at } \varphi = 0)$$

which approaches the limiting value of:

$$(E_{\text{comp}})_{\text{max}} \rightarrow h\nu - 0.25 \text{ Mev (for } h\nu \gg 0.51 \text{ Mev)}$$

for high energy photons. The quantum energy of photons is often measured by observing the maximum energy of the Compton electrons which they can produce.

When a collimated beam of photons, each of energy $h\nu$ passes through a thickness dx cm. of absorbing material of atomic number Z containing N atoms/cm.^{3} and hence NZ electrons/cm.^{3}, the fraction of the photons which will experience a Compton encounter is σdx, where σ is the linear total attenuation coefficient for the Compton effect, and is given by:

$$\sigma = NZf_2/\text{cm}.$$

The electronic cross-section f_2 cm.^{2}/electron is dependent only on $h\nu$, and experimental measurements are in good agreement with the theoretical values deduced by Klein and Nishina (1928).

The Compton collision does not result in complete removal of the electromagnetic energy of the incident photon, as is the case in the photoelectric collision and in the electron pair-production collision. The photon $h\nu$ is simply scattered elastically through some angle ϑ, and thereby loses a part of its energy.

The effects produced by absorbers therefore depend strongly on the geometry of the source-absorber-detector system. In what is called "good geometry" the beam of primary photons is accurately collimated. Then each Compton collision removes the energy $h\nu$ completely from the beam, because the degraded quantum $h\nu'$ is deflected out of the narrow beam, and the balance $h\nu - h\nu'$ of the original photon energy is converted to kinetic energy of a secondary electron.

In what is called "poor geometry," but is often good practice, collimation may be minor or absent. The γ-radiation may be diverging in all directions, as from an isolated small source of a suitable radioactive isotope. Then, if a thin spherical absorber is placed around the source, as many photons will be scattered into a given direction as are scattered out of that direction, and the total number of photons emerging through the thin spherical absorber will be unaltered by Compton encounters in the absorber. Many of the emerging photons may have experienced Compton collisions, and will therefore have a smaller energy $h\nu'$ than the original photons $h\nu$ emitted by the source. The reduction of *energy* flux through the spherical absorber will then be dependent, so far as Compton collisions are concerned, only on the kinetic energy acquired by Compton secondary electrons at the expense of the photons.

We see that it is necessary to break down the Compton electronic cross-section f_2 into two parts, f_s representing scattering or deflection of photons, and f_a true absorption by the struck electron of energy from the photon. Thus:

$$f_2 = f_s + f_a$$

$$\sigma = \sigma_s + \sigma_a = NZf_s + NZf_a$$

where σ_s is the Compton scattering coefficient, determining the fraction of the incident photon energy (number of photons × energy per photon) which is deflected by collisions with electrons; σ_a is the Compton absorption coefficient, determining the fraction of the incident photon energy converted to kinetic energy of secondary electrons, and hence into ionization in the absorber; and σ is the total Compton coefficient, determining all losses from a strictly collimated beam, either as a fraction of energy or as a fraction of number of photons. The total Compton coefficient σ is often loosely called the Compton absorption coefficient, and caution must be exercised to avoid confusing σ with the Compton absorption coefficient σ_a, which represents only kinetic energy acquired by electrons and is thus the only true absorption of photon energy taking place, the balance σ_s being mere deflection of the photons.

The Compton coefficients σ_s, σ_a, and σ each depend only on $h\nu$, and may be deduced for any element from the values shown in Figs. 10, 11, and 12 which are based on the theory of Klein and Nishina. Thus, for any particular energy, the total Compton coefficient σ for an element of atomic weight W, atomic number Z, and density ρ, is:

$$\sigma = \sigma_{\text{Pb}} \left(\frac{\rho}{11.35}\right)\left(\frac{207.2}{W}\right)\left(\frac{Z}{82}\right)$$

where σ_{Pb} is σ for lead. Similar expressions of course hold for σ_s and σ_a.

It will be noted (*e.g.*, Fig. 11) that the true Compton absorption σ_a is small for very low energy photons, while the Compton scattering σ_s is large.

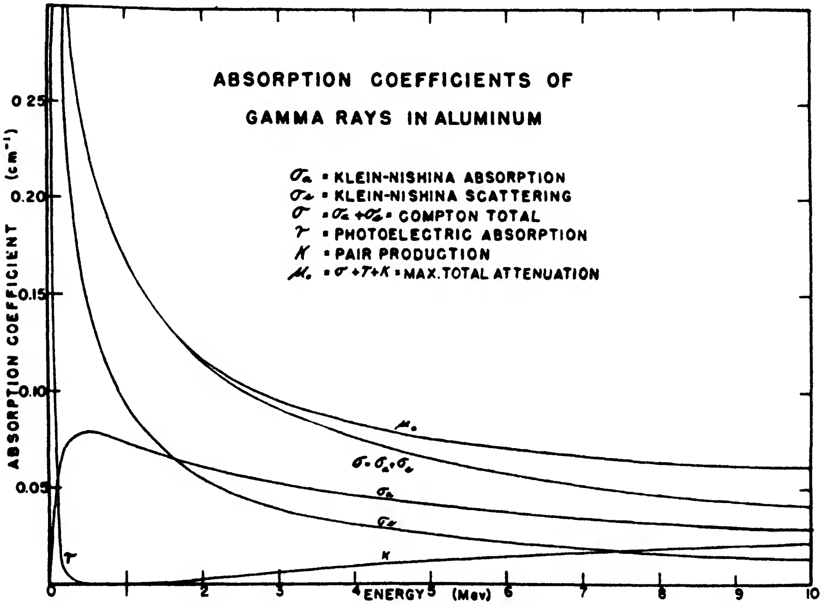

FIG. 12

Absorption coefficients for photons in aluminum ($Z = 13$. $N = 2.7 \times 6.0 \times 10^{23}/27 = 6.0 \times 10^{22}$ atoms/cm.^{3} Al. $NZ = 7.8 \times 10^{23}$ electrons/cm.^{3} Al).

Scattering and absorption are of equal effectiveness for attenuating collimated beams of 1.6 Mev photons, and above 1.6 Mev scattering is less important than true absorption.

(c) *Electron Pair-Production*

A photon of sufficiently great energy may be completely absorbed near the boundary of an atomic nucleus, but not "in" the nucleus, by an interaction with the strong electrostatic Coulomb field of the nucleus. Two new electrons, one positive and the other negative, are produced at the scene. Because their rest masses are each m_0, this requires a minimum photon energy of $2m_0c^2 = 1.02$ Mev. The original photon energy in excess of $2m_0c^2$ appears as kinetic energy, and is shared by the positron, negatron, and nucleus. Because the mass of the nucleus is very large compared with the mass of the electrons, the nucleus receives a negligible fraction of the kinetic energy available. Then if the kinetic energy of the positron is E_{e+}, and of the negatron is E_{e-}, we have:

$$E_{e+} + E_{e-} = h\nu - 2m_0c^2$$

In the most probable case, the positron and negatron share the kinetic energy available almost equally, the positron receiving only a few per cent more than the negatron. But, in general, either particle may have any kinetic energy between zero and $h\nu - 1.02$ Mev. Electron pair-production, like the Compton encounter, thus produces a continuous distribution of electron energies from zero up to a maximum determined in each case by $h\nu$. On the other hand, the pair-production interaction results in the complete absorption and removal of the incident photon $h\nu$, as is the case in the photoelectric interaction.

The probability of pair-production depends on Z^2, as well as on $h\nu$, and the pair-production absorption coefficient κ can be written:

$$\kappa = NZ^2f_3/\text{cm}.$$

where f_3 depends only on $h\nu$, is zero for $h\nu < 2m_0c^2$, and at higher photon energies rises approximately linearly with $h\nu - 2m_0c^2$. Exact values (Bethe and Heitler, 1934) of f_3 may be determined from Figs. 10, 11, and 12, which give κ cm^{-1} for Pb and Al. As with the other modes of photon interaction, absorption coefficients for other elements may be computed readily from these curves, remembering here the Z^2 variation with atomic number, so that in the previous notation we have:

$$\kappa = \kappa_{\text{Pb}}\left(\frac{\rho}{11.35}\right)\left(\frac{207.2}{W}\right)\left(\frac{Z}{82}\right)^2.$$

(*d*) *Annihilation Radiation*. Each positron eventually loses its kinetic energy by collisions, and finally combines with an atomic electron. Thus, although no *net* electric charge was created in the pair-production interaction, and total electric charge is always conserved, the positron has only a short lifetime and in its death removes also a negative electron, which stands in for the original negative electron produced with the positron. In the annihilation, the rest energy $2m_0c^2$ of the two electrons is converted to electromagnetic radiation, and *two* oppositely-directed quanta each of energy $m_0c^2 = 0.51$ Mev are created. Thus, some of the energy of the original quantum $h\nu$ which created the positron-negatron pair, reappears as 2 quanta of 0.51 Mev annihilation radiation. The annihilation radiation is randomly directed from the scene of annihilation, which is likely to be close to the original scene of the pair-production because the range of the positron (Fig. 5) is at most only a few millimeters in solids. Consequently, there are many practical cases in which the pair-production absorption process gives rise to important amounts of secondary 0.51 Mev electromagnetic radiation from the absorber.

(*e*) *Attenuation of Gamma-Rays.* The results of the preceding sections can now be drawn together. If a well collimated beam ("good geometry") of photons, all of one energy $h\nu$, pass through a thickness dx of material whose absorption coefficients have been determined from Fig. 10, the decrease, $-dI$, in intensity I should be

$$-dI = (\tau + \sigma_s + \sigma_a + \kappa)I dx = \mu_o I dx.$$

Rearranging, and integrating, we find that the ratio of emergent intensity I, to incident intensity I_o, after passing through a thickness x of material, is:

$$I/I_o = e^{-\mu_o x}$$

which is the usual exponential absorption formula.

If the incident radiation had consisted of a mixture of N_1 quanta/second having energy $h\nu_1$ and absorption coefficient μ_1, and N_2 quanta/second of energy $h\nu_2$ and absorption coefficient μ_2, then the attenuation of each component must be written separately. If ϵ_1 and ϵ_2 are the efficiencies of the detector for quanta of energy $h\nu_1$ and $h\nu_2$, then the response with zero absorber will be:

$$I_o = \epsilon_1 N_1 + \epsilon_2 N_2$$

while the response with x cm. of absorber will be:

$$I = \epsilon_1 N_1 e^{-\mu_1 x} + \epsilon_2 N_2 e^{-\mu_2 x}$$

Extension to any number of components is evident.

In most practical cases the beam is not accurately collimated. "Poor geometry" is the rule rather than the exception. Then Compton scattered quanta, annihilation radiation, and Bremstrahlung from the secondary electrons are not excluded from the detector. The effective total absorption coefficient in such cases will depend on many factors, including the relative sensitivity of the detector to the soft scattered photons and also to the secondary electrons which are produced in the absorber.

As the absorber thickness is increased from zero, the relative intensity of these secondary photons and electrons increases at first, then reaches and maintains a constant ratio to the transmitted intensity I of the primary photons. When the absorber is thick enough to produce this constant ratio of secondary to primary radiation intensity the primary radiation is said to be "in *equilibrium* with its secondary radiation." This concept of equilibrium is of special importance in absolute ionization measurements of γ-rays, and enters, for example, into the practical measurement of γ-radiation in roentgen units.

Some detectors, such as a copper cathode Geiger-Müller counter, have a sensitivity ϵ which is nearly proportional to $h\nu$. Such detectors give a response which is proportional to the total photon energy traversing the detector (summation of number of photons $\times$ $h\nu$). Then the apparent linear absorption coefficient, μ', for thin absorbers in poor geometry will be the fraction of the incident photon energy which is converted to kinetic energy of secondary electrons/cm. of absorber. In so far as Compton collisions are concerned, this involves only σ_a. Therefore:

$$\mu' \simeq \tau + \sigma_a + \kappa$$

As the absorber thickness is increased, a transition effect occurs, due to the gradual establishment of equilibrium between the primary photons and their secondary photons and electrons. A very thick absorber will not permit the escape of quanta scattered in its first few layers, *e.g.*, near the source. When the absorber is thick enough to severely attenuate scattered quanta produced in its first layers, the apparent total absorption coefficient will increase accordingly. Then μ will approach closer to its maximum limiting value for good geometry of:

$$\mu_o = \tau + \sigma_a + \sigma_s + \kappa$$

Gray (1937) has measured the ionization in small air cavities at various distances from a radium γ-ray source embedded in the center of a large (40 $\times$ 60 cm.) cylinder of aluminum. The apparent absorption coefficient within about 4 cm. of the source is actually less than $(\sigma_a + \tau)$, because of reflection (back-scattering) of photons from the outer layers of absorber. Beyond 4 cm. from the center, the apparent absorption coefficient rises, reaching a maximum value at about 24 cm. This maximum absorption coefficient is substantially equal to $(\sigma_a + \sigma_s + \tau)$ of the primary γ-rays. We see here that some 24 cm. of Al is necessary in this case to establish equilibrium between the primary photons and the secondary scattered photons. The energy losses, apparent as ionization, are all due to τ, κ, and σ_a. The scattered quanta gradually increase in relative abundance as one goes out from the source. The ionization produced by these secondary quanta, in photo-, Compton-, and pair-interactions, increases the total γ-ray energy losses/cm., that is, the total ionization, until they equal the rate of total attenuation $(\sigma_a + \sigma_s + \tau + \kappa)$ of the primary photons. The following table shows, as a function of distance x from the source, the steady increase in the proportion of the total observed ionization which is due to ionization by secondary photons, *i.e.*, Compton scattered radiation and annihilation radiation.

x cm.	0	10	20	30	40	50
P = Primary Ionization (calculated)	100	20.2	4.95	1.35	0.39	0.11
$P+S$ = Observed Total Ionization..	100	55.	20.7	7.20	2.53	0.87
S/P = Secondary Ionization/P.....	0	1.72	3.18	4.33	5.48	6.90

The absorption of γ-rays of between about 0.1 and 2 Mev energy is especially simple in light elements. Then the photoelectric absorption is small because of its dependence on Z^4, and the pair-production absorption is also small because of its energy-dependence and its variation with Z^2. Compton interactions will therefore dominate, and the attenuation will be proportional to the number of electrons/cm.^{2} of absorber, $NZdx$, or

$$\begin{aligned} dI &= \sigma I dx = NZf_2 I dx \\ &= (\rho n/W)Zf_2 I dx \\ &= n(Z/W)f_2 I \rho dx \end{aligned}$$

where n is Avogadro's number. Because Z/W is 0.45 ± 0.05 for all elements except hydrogen, the attenuation can be expressed in terms of the mass-absorption coefficient (σ/ρ), where:

$$\sigma/\rho = n(Z/W)f_2$$

is substantially independent of Z. Then the attenuation is essentially proportional to the superficial mass of absorbing material $d(\rho x)$ g./cm.^{2} For example, Fig. 12 shows that for 1 Mev photons:

$$\mu \simeq \sigma = \sigma_s + \sigma_a = 0.095 + 0.073 = 0.168 \text{ cm.}^{-1} \text{ of Al,}$$

or: $\sigma/\rho = 0.168/2.7 = 0.062$ cm.^{2}/g. Al, in good geometry,

and: $\sigma_a/\rho = 0.073/2.7 = 0.027$ cm.^{2}/g. Al, in poor geometry.

Then a thickness of 1.5 g./cm.^{2} of any low Z material, *e.g.*, air, water, paper, tissue, aluminum, or copper, will give substantially the same transmitted intensity of:

$$I = I_o e^{(\mu/\rho)(\rho x)} = I_o e^{-0.062 \times 1.5} = 0.90\, I_o$$

in "good" geometry. While in "poor" geometry the transmitted intensity may be about:

$$I = I_o e^{-(\mu/\rho)(\rho x)} = I_o e^{-0.027 \times 1.5} = 0.96\, I_o.$$

Tables of mass absorption coefficients applicable to "good" geometry, for various values of Z and for the low energy domain from about 0.25 to about 0.017 Mev, are given by Compton and Allison (1935).

Most practical γ-ray measurements must be made on samples of finite size, and some of the γ-rays are therefore absorbed and scattered within the source itself (see Evans and Evans, 1948).

II. Radioactivity Units and Standards

(*a*) ***Electron-volt.*** The electron-volt, (ev) and million electron volt (Mev) are units of energy.

$$1 \text{ ev} = 1.60 \times 10^{-12} \text{ ergs} = 3.82 \times 10^{-20} \text{ g.-cal.}$$

$$1 \text{ Mev} = 10^6 \text{ ev}$$

$$1 \text{ kev} = 10^3 \text{ ev}$$

$$1 \text{ atomic mass unit (a.m.u.)} = 931. \text{ Mev/atom}$$

$$m_oc^2 = 0.51 \text{ Mev (rest energy of the electron)}$$

$$1 \text{ ion-pair in air} = 32.5 \text{ ev (Gray, 1936, 1944; Binks, 1936)}$$

(*b*) *Curie.* A curie is the quantity of radon (0.66 mm.^{3} at 0°C. and 760 mm.) in radioactive equilibrium with 1 g. of radium. The International Radium Standard Commission in 1930 (Curie *et al.*, 1931) recommended extending the curie unit to include the equilibrium quantity of any decay product of radium, especially polonium. Thus "1 curie Po" is 2.24×10^{-4} g. of Po, or the amount which has the same rate of emission of α-particles as 1 g. of radium. The Commission expressed its opposition to extension of the curie unit to radioactive substances outside the radium family.

The absolute disintegration rate of radium has been the subject of many measurements by a number of methods, giving individual values between 3.40 and 3.72, with some indication in the more recent measurements that the true value probably lies in the neighborhood of $3.67 \pm 0.03 \times 10^{10}$ disintegrating atoms/sec./g. Ra. The Commission recommended the use of the arbitrary value 3.7×10^{10} until such time as agreement on the third figure could be reached.

Following the discovery of artificial radioactivity, the curie unit came into widespread unofficial use as a statement of the quantity of any radioactive isotope. Thus, 1 millicurie (1 mc = 0.001c) P^{32}, Na^{24}, or C^{14} meant the amount of the isotope necessary to provide 3.7×10^7 disintegrating atoms per second. Then the number of atoms and the weight of isotope required to make 1 mc is inversely proportional to the radioactive decay constant λ or directly proportional to the half-period T, or to the mean-life $\tau(= 1.44T = 1/\lambda)$. If N is the number of atoms and M the number of grams in 1 mc, n is Avagadro's number, and W is the atomic weight, then

$$N\lambda = 3.7 \times 10^7 \text{ sec}^{-1}$$

$$N = (M/W)n = 3.7 \times 10^7 \, \tau$$

$$M = 3.7 \times 10^7 \, \tau \, W/n$$

and 1 mc of P^{32} ($T = 14.30$ days $= 12.35 \times 10^5$ sec), would contain:

$$N = (3.7 \times 10^7)(1.44 \times 12.35 \times 10^5) = 6.58 \times 10^{13} \text{ atoms of } P^{32}$$

or:

$$M = (6.58 \times 10^{13})32/6.02 \times 10^{23} = 3.5 \times 10^{-9} \text{ g. of } P^{32}.$$

Actually, the absolute rate of disintegration is exceedingly difficult to measure accurately for any isotope, especially all those which do not emit α-rays. It is necessary to know the "disintegration scheme" of the isotope, that is, the β- and γ-ray spectrum, and the relative abundance and energy of all the radiations emitted. At the beginning of 1947 the disintegration schemes of only about two dozen artificially radioactive isotopes were established with certainty. Representative examples are given in Fig. 13. When it is known that each disintegrating atom gives say one β-ray, and if calibrated β-ray counters (e.g., Peacock, 1944) are available, a good estimate of the absolute activity of the sample can be made. If conversion electrons are present, their abundance and energy must be known, and suitable corrections must be made.

If some of the transitions are by electron-capture, instead of by β-ray emission, then 1 mc will emit less than 3.7×10^7 β-rays/sec. For example, the 6.5 day Mn^{52} decays by positron β-ray emission in 35% of the transitions, and by electron capture in 65% of the transitions; therefore, 1 mc of Mn^{52} emits only $0.35 \times 3.7 \times 10^7 = 1.3 \times 10^7$ β-rays/sec. The curie unit has been misused occasionally to denote 3.7×10^{10} β-rays/sec., instead of 3.7×10^{10} disintegrations/sec.

A second and much more serious misuse of the curie unit has been its widespread use as an ambiguous unit of γ-radiation. This has arisen from the radiological use of radon, whose absolute disintegration rate is properly measured in curie units. Radon gives rise to the decay products RaB and RaC whose complex γ-radiations (Fig. 14) have long been used in radiological practice. Many laboratories have misused the curie unit to describe any radioactive source which produces the same γ-ray response as 1 curie of radon. An outstanding technical objection to this practice is that the γ-ray response depends profoundly on the detection instrument used. For example the ratio of the apparent γ-ray intensity of a source of 8-day I^{131} to a source of radium (or of radon) is 4 times as great if a platinum cathode counter is used for both measurements as if a copper cathode counter is used with the same sources, geometry, and filtering. Moreover, many isotopes emit no γ-rays at all, *e.g.*, C^{14}, P^{32}, S^{35}. An educational effort should be made by everyone to eliminate the conflicting, confusing, and irrational use of the curie as a unit of γ-ray source intensity.

In the meantime, caution is advised in interpreting the meaning of curie (c), millicurie (mc), and microcurie (μc) in the past and current literature.

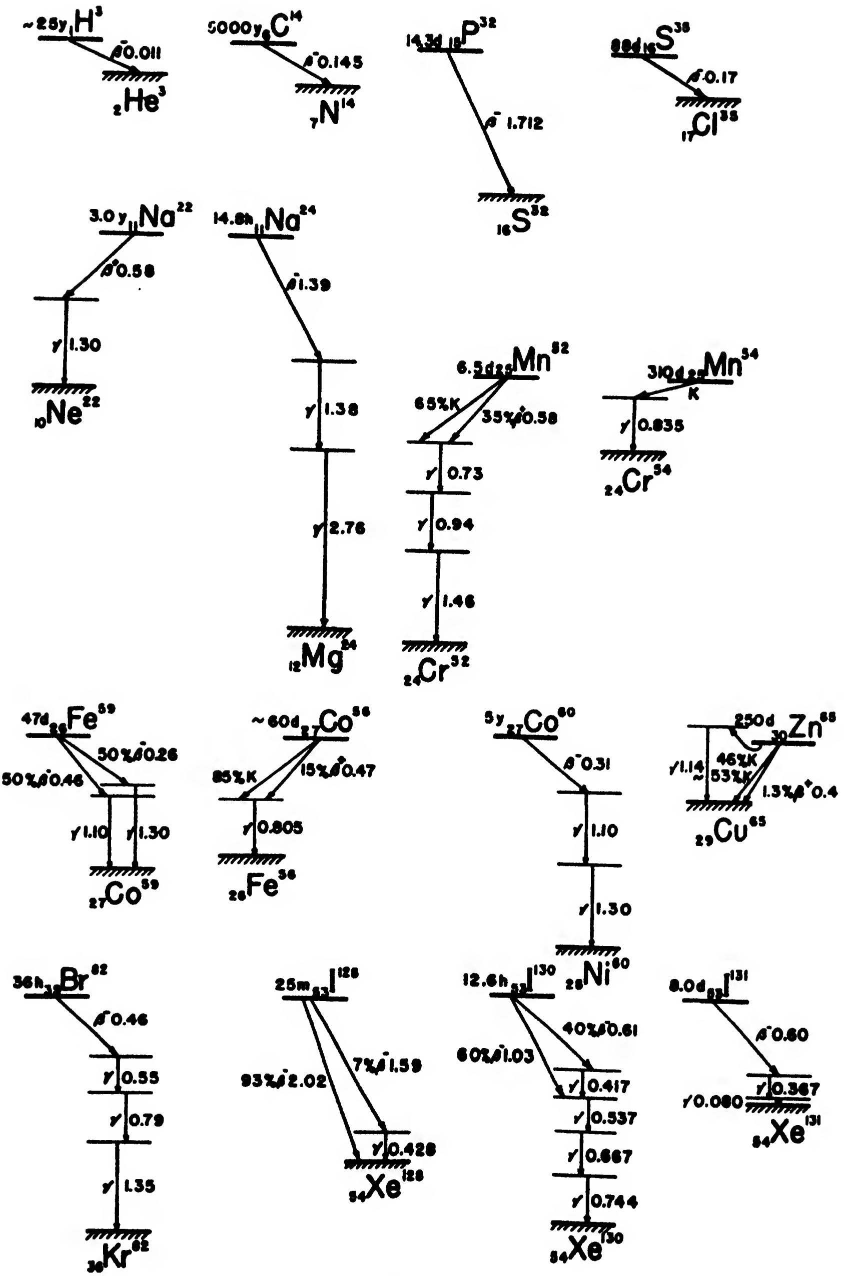

FIG. 13

Nuclear energy level diagrams and disintegration schemes for some representative isotopes. Symbols: β^- = negative β-rays; β^+ = positron β-rays; K = orbital electron

Even when the reader can assure himself that "curie" is used in the sense of disintegrating atoms per second, he must ascertain whether the number 3.7×10^{10} or some other popular contender, especially 3.47×10^{10}, has been the author's intent.

(*c*) *Rutherford.* In view of the justifiable position held by the International Radium Standard Commission that the curie unit be used only for radium (1 g. Ra = 1 curie), radon, polonium and other members of the radium series, a carefully defined new unit, the rutherford (rd), has been proposed for general use. Ambiguities due to choice of numerical values, due to failure to distinguish between β-rays /sec. and disintegrations/sec., and due to extensions to arbitrary and undefined γ-ray intensities, can then

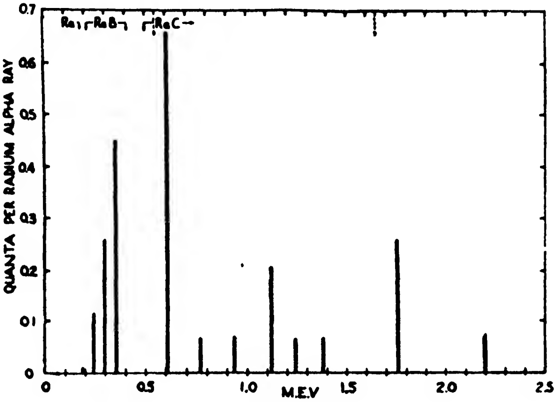

FIG. 14

The principal γ-ray lines of radium and its equilibrium decay products. Note that radium itself emits only a low energy γ-ray of 0.184 million electron volts (Mev) and then only in 1.2% of the disintegrations, 98.8% of the radium α-rays being unaccompanied by any γ-radiation (Stahel and Johner, 1934). The decay product radium B emits β-rays and three important but low energy γ-rays. Radium C emits β-rays and eight important γ-rays having energies as high as 2.2 Mev. On the average, each atom which decays through all the stages shown in Table II will have emitted 2.3 quanta of γ-radiation. References: Ellis and Aston, 1930; Gray, 1937; Ellis, 1933; Sizoo and Willemsen, 1938. Significantly different results for the relative intensity and energies of the lines in RaC at energies greater than 1.1 Mev have been reported by Latyschev *et al.*, 1940.

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capture; γ = γ-ray. Internal conversion transitions will be associated competitively with the γ-ray transitions shown. The actual order of emission of cascade γ-rays is unknown in all cases. The numbers beside each transition show the energy of the γ-rays in Mev, or the maximum energy E_m of the continuous β-ray spectrum, in Mev. The diagrams are to scale. Therefore, the nuclear energy separation plotted for each β-ray transition is the total energy of the disintegration (E_m + 0.51 Mev) = (maximum kinetic energy + rest energy) of the β-ray emitted. In electron capture transitions the final nuclear level may lie above the initial level, as in the 46% transition of Zn^{65}, because the reaction receives 0.51 Mev from the rest-energy of the captured electron. The nuclear energy change in the electron capture disintegration of Mn^{54} is unknown, but is probably less than 0.51 Mev because no competing positron β-rays can be observed.

be avoided. With the advice and concurrence of a number of interested individual physicists and laboratory groups especially in the United States, Great Britain, and France, the National Bureau of Standards and the Committee on Radioactive Standards of the National Research Council have proposed (Condon and Curtiss, 1946) that the amount of any radioactive isotope which disintegrates at the rate of 10^6 disintegrations/sec. be called 1 rutherford, abbreviated rd, to distinguish between it and the roentgen (r). Thus:

$$1 \text{ rd} = 1.00 \times 10^6 \text{ disintegrations/sec.}$$

$$1 \text{ rd} = (1/37) \text{ mc} = 27\ \mu\text{c of Ra, Rn, Po, } etc.$$

$$1 \text{ mc} = 37 \text{ rd}$$

The prefixes, "mega" (1 Mrd = 10^6 rd), "kilo" (1 krd = 10^3 rd), "milli" (1 mrd = 10^{-3} rd), and "micro" (1 μrd = 10^{-6} rd), may be used where needed, but in contrast with the curie unit the major part of the laboratory use of radioactive isotopes will need no such prefixes. Thus the therapeutic dose of P^{32} is of the order of 100 rd, while tracer doses of radioactive isotopes used in biological studies and the amounts of many isotopes used in studies in nuclear physics are normally in the domain of 0.1 to 10 rd. The weakest sources which can normally be measured conveniently with Geiger-Müller counters are in the domain of 1 μrd (= 60 disintegrations/min.).

(*d*) *Roentgen.* The roentgen unit was originally developed for use as a unit of radiological dose. Workers in physics and in radiobiology have made use of the roentgen and a number of units derived from it.

The roentgen unit (r), as redefined at the Radiological Congress at Chicago in 1937, is "that quantity of X- or γ-radiation such that the associated corpuscular emission per 0.001293 g. of air, produces, in air, ions carrying 1 electrostatic unit of quantity of electricity of either sign."

The mass of air referred to is 1 cc. of dry air at 0°C. and 760 mm. Hg. One electrostatic unit is $1/(3 \times 10^9)$ coulombs, or $1/(4.80 \times 10^{-10}) = 2.083 \times 10^9$ ion pairs. Therefore, 1 r produces $2.083 \times 10^9/0.001293 = 1.61 \times 10^{12}$ ion pairs/g. of air. If an average of 32.5 ev is expended (Gray, 1936, 1944; Binks, 1936) to form each ion pair in air, then 1 r corresponds to the absorption of 5.24×10^{13} ev of energy (or 5.24×10^{13} ev $\times 1.60 \times 10^{-12}$ erg/ev = 83.8 ergs)/g. of air. This ionization is produced by the secondary electrons (photo-(τ), Compton-(σ_a), and pair-(κ)) produced in 1 g. of standard air. Of course the relative proportion of photo-, Compton, and pair-electrons will vary with the energy of the γ-radiation.

Correct use of the roentgen as a unit of "quantity of γ-radiation" requires us to realize that the term "quantity" is used here in its common, or household, sense of "amount," and does not connote any exact physical magnitude involving the energy per photon, or the total number of photons, or the total energy of the beam of photons. Indeed, the roentgen does not involve any precise physical information at all about the quality (energy per photon) or intensity (energy flowing through unit area in unit time) of the γ-radiation. The "quantity" to which the roentgen really refers with physical exactness is the "*quantity of ionization*" produced in air by the secondary electrons formed in γ-ray collisions with air molecules. Thus, the roentgen is a unit of energy dissipation, by γ-rays or X-rays, in a standard arbitrary material, air. A schematic representation of the roentgen unit is shown in Fig. 15.

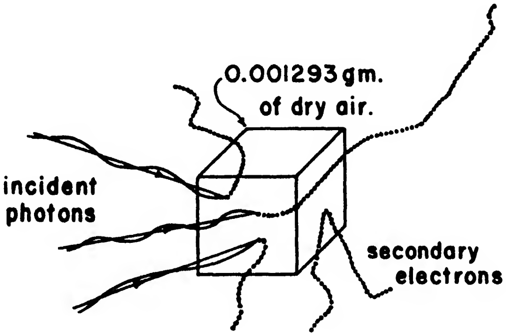

FIG. 15

A schematic representation of the roentgen unit of photon energy dissipation. Photons, of any energy, are incident from any directions, over any time interval. Even if the photons are collimated, the cross-sectional area of the beam is unspecified. The absorption of these photons in 0.001293 g. of dry air (1.000 cc. at 0°C., 760 mm.; 1.073 cc. at 20°C., 760 mm.), produces secondary electrons which, if completely absorbed in air, would produce a total ionization of 1 electrostatic unit of charge (1 statcoulomb), that is $1/4.80 \times 10^{-10}$ or 2.083×10^{9} ion pairs. This ionization would be distributed along the relatively long individual paths of the secondary electrons.

The roentgen does not depend on the *time* required for the production of the ionization. Consequently γ-ray *dosage rates* are represented in terms of roentgens per unit time. For example an unvarying dosage rate of 12.5 mr/hr. if continued for 8 hours, would give a total dose of 100 mr, or 0.1 r. The maximum permissible daily dose for laboratory personnel exposed to γ-radiation over the entire body, is commonly taken in the United States as 100 mr in a 24 hour day (Burnam, 1938; Cantril, 1946). Again, an ionization rate of 1 ion pair/cm.^{3} of standard air/sec. is $(1/2.08) \times 10^{-9}$ r/sec. or 1.73 microroentgens (μr)/hr. The cosmic radiation produces about 2.4 ion pairs/cm.^{3} of standard air/sec. at sea level, or 0.10 mr/24 hr. day. The cosmic and local γ-radiation at sea level is usually equal to or greater than 0.2 mr/24 hr.

In summary, the official 1937 definition of the roentgen, when combined with current values of physical constants, leads to the following equivalent quantities:

$$\begin{aligned} 1\ \text{r} &= 1\ \text{e.s.u./cc. std. air} \\ &= 2.083 \times 10^9\ \text{ion pairs/cc. std. air} \\ &= 1.61 \times 10^{12}\ \text{ion pairs/g. air} \\ &= 6.77 \times 10^4\ \text{Mev/cc. std. air} \\ &= 5.24 \times 10^7\ \text{Mev/g. air} \\ &= 83.8\ \text{erg/g. air.} \end{aligned}$$

(*e*) *Gamma-Ray Intensity.* The unit of dosage rate, r/sec., is often loosely used in the older literature as a unit of γ-ray intensity. Really, the r/sec. is a unit of *ionization intensity* in a particular reference medium, dry air at 0°C. and one atmosphere pressure.

True γ-ray intensity is the rate at which photon energy flows past any point (or small region). True γ-ray intensity can be expressed only in absolute units such as ergs/cm.^{2} sec., or as Mev/cm.^{2} sec. The ionizing effects depend not merely on the true intensity, but on the number and energy of the photons involved. Thus if 1000 photons/cm.^{2} sec., each of 1 Mev quantum energy, traverse an area of 1 cm.^{2}, the γ-ray intensity at this surface is 1000 Mev/cm.^{2} sec. The same intensity would be given by 500 photons/cm.^{2} sec., each of 2 Mev quantum energy. The Compton absorption coefficient σ_a for 1 Mev photons is 3.60×10^{-5} cm^{-1} in standard air. Similarly, for 2 Mev, the linear Compton absorption coefficient σ_a is 3.00×10^{-5} cm^{-1} in air. For air ($Z_{av} = 7.22$), photoelectric and pair-production absorption will be negligible at these energies. Consequently, the energy absorbed/cm. of travel in air, or /cm.^{3} of air, for these two photon beams of equal γ-ray intensity will be:

$$\begin{aligned} &\text{For 1 Mev: } 1000 \times 1 \times 3.6 \times 10^{-5} = 0.036\ \text{Mev/cm.}^3\ \text{sec.;} \\ &\text{For 2 Mev: } 500 \times 2 \times 3.0 \times 10^{-5} = 0.030\ \text{Mev/cm.}^3\ \text{sec.} \end{aligned}$$

Now, if 1 r is (2.083×10^9 ion pairs/cm.^{3}) $\times$ (32.5 ev/ion pair) $= 6.77 \times 10^4$ Mev/cm.^{3}, the dosage rates or *ionization intensities* produced by these two beams of equal *γ-ray intensity* will be:

$$\begin{aligned} &\text{For 1 Mev: } 0.036/6.77 \times 10^4 = 5.3 \times 10^{-7}\ \text{r/sec.;} \\ &\text{For 2 Mev: } 0.030/6.77 \times 10^4 = 4.4 \times 10^{-7}\ \text{r/sec.} \end{aligned}$$

We see that r/sec. is not a measure of γ-ray intensity but is, instead, proportional to the resulting *ionization intensity in standard air.* Thus r/sec. is a rate of energy dissipation, not of energy incidence.

(*f*) *"Air-wall" r Chambers.* In the example just considered, some of the Compton electrons will have initial kinetic energies of the order of 0.7–1.7 Mev, and, hence, ranges of as much as 0.3–0.8 g./cm.^{2}, or 2–6 meters of air.

Yet the definition of the roentgen requires that we measure the total ionization produced by all the secondary electrons formed per cm.^{3} of standard air. To avoid large and cumbersome apparatus, small "air-wall" ionization chambers, or "thimble chambers," (*e.g.*, Wilson, 1945) have been developed. These follow the general physical principles (Bragg, 1912; Fricke and Glasser, 1925; Gray, 1928, 1936) governing the ionization produced in a small cavity (*e.g.*, air) in a large mass of absorbing material traversed by γ-rays. When a tiny cavity, such as a small ionization chamber, is introduced into a large homogeneous absorbing medium which is uniformly irradiated, the atmosphere of secondary electrons traversing the cavity is identical in every respect with the electron atmosphere which existed in the medium before the cavity was introduced. The cavity must be small compared with the range of the secondary electrons in the gas with which the cavity is filled (Gray, 1936; Sievert, 1940).

In a series of careful experiments, Gray (1936) has verified this principle, according to which the ionization, J, in ion pairs/cm.^{3} sec. in a small air cavity is given by

$$J = \Delta E/W\rho$$

where ΔE is the γ-ray energy absorbed in the medium in ev/cm.^{3} sec.; W is the average energy expended per ion pair formed in the gas ($W = 32.5$ ev for air); and ρ is the stopping power of the medium relative to that of the gas for the secondary electrons, that is: an electron will lose the same energy in travelling Δx cm. in the medium, or $\rho\Delta x$ cm. in the gas of the cavity. The average energy per ion pair, W, varies only slightly (Binks, 1936) with the electron's velocity, because swift electrons tend to transfer an important portion (perhaps 10–60 kev) of their energy to each atomic electron which they strike in an ionizing collision, and the average energy loss per ion pair is always characteristic of the less violent ionizing collisions by these slower secondary electrons. The relative stopping power, ρ, is almost independent of the electron's velocity, but decreases slowly with atomic number Z. In agreement with theory, Gray found ρ to be proportional to the relative electron densities NZ of the medium and the gas, except for the expected small decrease in ρ with increasing Z. This decrease amounts to about 9% between $Z = 6$(C) and $Z = 13$ (Al), and an additional 8% between $Z = 13$ (Al) and $Z = 29$ (Cu). Thus, in a set of thimble chambers, the ions/cc. of air filling will increase slightly, with increasing Z of the wall materials. Taking the average atomic number of air as $Z = 7.22$, an error of less than 5% is made by regarding ρ as simply the ratio of the densities (g./cm.^{3}) of the medium and the cavity gas, provided the medium is composed of light elements between $Z = 6$ and 13.

The thickness of medium necessary to validate the cavity-ionization principle is small. It is only required that the medium be thick enough to establish equilibrium between the primary photons and their secondary electrons, that is, about as thick as the maximum range of the secondary electrons in the medium. Since this is only of the order of a few mm. in media such as graphite, bakelite, Tenite II (Eastman cellulose acetate butyrate), Aerion (bakelite and graphite composition), or aluminum, one can simply employ a small air-filled ionization chamber having walls a few mm. thick as both the cavity and the medium. Fig. 16 shows the effect of wall thickness on the ionization produced in a small graphite chamber filled with air and irradiated with γ-rays from RaB + C. Actually the wall of an "air-wall" chamber can be somewhat thinner than the maximum range of the secondary electrons, because most of the ionization is produced by electrons formed in the inner few mm. of wall material. It is to be noted, also, that the walls of the chamber should be thick enough to prevent secondary electrons from the surroundings, from filters, *etc.*, as well as any β-rays from the γ-ray source, from reaching the sensitive volume of the chamber.

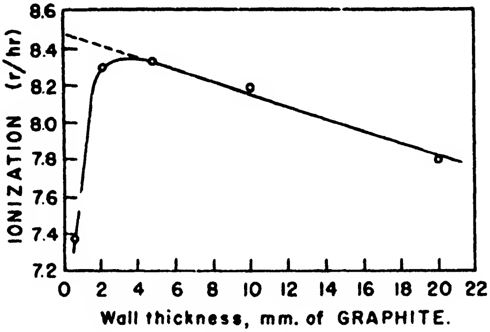

FIG. 16

Variation of ionization, due to a constant source of γ-rays, with wall thickness for a small graphite chamber filled with air. A wall thickness of *ca.* 4 mm. is required to produce effective equilibrium between the γ-rays and their secondary electrons. For thicker walls, the attenuation of the γ-rays by the walls is noticeable (Mayneord and Roberts, 1937).

The ionization (ion pairs/cm.^{3}) then, in a satisfactory "air-walled" ionization chamber should be:

(1) independent of the volume of the chamber;
(2) proportional to the density of the gas in chamber;
(3) proportional to the γ-ray energy absorbed/cc. of wall material; and
(4) inversely proportional to the stopping power of the walls for electrons.

If the chamber gas is air, and if the walls are composed of materials having an atomic number Z near that of air, then the ionization/g. of air in the chamber will be substantially the same as the γ-ray energy loss/g. of air at the point where the chamber is located.

In an ideal thimble chamber, and if all ions formed are collected, 1 r/sec = $1/(3 \times 10^9)$ coulombs/sec·cc. std. air = 3.33×10^{-10} amps/cc. and:

$$1 \text{ r/hr} = 0.926 \times 10^{-13} \text{ amps/cc. std. air.}$$

(*g*) *Roentgen-Equivalent-Physical* (rep). We have seen that the roentgen is a unit of photon energy dissipation in an arbitrary material, air, where 1 r is about 83 ergs/g. In contemporary radiobiology, ionization in tissues is often produced by radiations other than photons. There is good evidence that the biological effects of various ionizing radiations depend, among other things, on the density of ionization (specific ionization) produced along the path of the particle in tissue. Thus a different degree of tissue damage can be expected from the absorption of 1 Mev of α-ray energy, than from 1 Mev of β-ray energy. Moreover, the relative effectiveness will, in general, vary from one species of organism to another.

The roentgen has come to have a clinical interpretation because so many observations in terms of roentgens have been made of the effects of X-rays and γ-rays on tissues. When tissue ionization is produced by any primary radiation other than photons, (*e.g.*, β-rays, protons, α-rays, neutrons) the doses may not be expressed in roentgens.

Then, if the energy lost by ionization in the tissues is the same as the energy loss for one roentgen of gamma radiation absorbed in air, the dose is spoken of as one roentgen-equivalent-physical* (abbreviated rep). For example if one g. of tissue absorbs 10^8 β-rays having an average energy of 0.52 Mev, the total energy absorbed would be 52×10^6 Mev/g., or 83 ergs/g., or 1 rep. Then

$$1 \text{ rep} = 83 \text{ ergs/g. tissue.}$$

It is to be noted that 1 r of photons, which produces about 83.8 erg/g. of *air*, will produce a somewhat different energy dissipation in various tissues, because of the differences in chemical composition. The number of ergs/g. of tissue/r varies (Spiers, 1946; Lea, 1947) with tissue composition and photon energy approximately as follows:

	825 kev	124 kev	12 kev
Muscle (Spiers)	95	94	87
Fat (Spiers)	89	84	42
Bone (Spiers)	157	245	883
"Wet tissue" (Lea)	92	91	86

The somewhat conventional statement that 1 r of photons = 1 rep is therefore an approximation.

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* The rep and rem units were introduced by Dr. H. M. Parker.

Other units for the tissue doses delivered by various types of radiation include the *gram-roentgen* (Mayneord, 1940) which is the energy dissipated by γ-rays in 1 g. of air, *i.e.*,

1 gram-roentgen = 83.8 ergs in an unspecified amount of tissue,

thus:

$$1 \text{ rep} = 1 \text{ gram-roentgen/g. of tissue.}$$

Gray and associates (Gray *et al.*, 1940) in considering the energy dissipated in tissue by fast neutrons, have used an "*energy-unit*" defined as the same energy in 1 g. of tissue as is dissipated by γ-rays in 1 g. of water, which is about 93 ergs. Thus:

$$1 \text{ "energy-unit"} = 93 \text{ ergs/g. of tissue.}$$

(*h*) *n Unit, for Fast Neutrons*. The availability of commercial "air-wall" thimble chambers, designed for the measurement of γ-ray and X-ray dose, made their use convenient when it later became necessary to undertake the measurement of radiation doses due to fast neutrons. Extensive theoretical and experimental studies have been made of the response of small ionization chambers to fast neutrons (*e.g.*, Gray and Read, 1939; Gray, 1944; Aebersold and Anslow, 1946). Material which is rich in hydrogen is especially effective in absorbing energy from fast neutrons, by the production of energetic recoil protons. The ionization produced in air-filled thimble chambers, relative to the ionization produced in tissue, therefore depends strongly on the hydrogen content of the walls of the thimble chamber. There is now general agreement that less ionization is produced in the Victoreen 100 r bakelite-walled thimble chamber than in tissue by a given beam of fast neutrons. When this size thimble chamber exhibits 1 esu/cc. of standard air (*i.e.*, 1 r) of ionization due to fast neutrons, the dose is often called 1 n. That is, 1 n produces the same ionization in the Victoreen 100 r thimble chamber as would 1 r of γ-rays. However, the ionization produced in tissue by the fast neutrons is found by various workers to be between 2 and 2.5 times the ionization in the chamber. That is:

$$1 \text{ n} = 2\text{–}2.5 \text{ rep} \simeq 190 \text{ ergs/g. tissue}$$

(*i*) *Roentgen-Equivalent-Man* (*or Mammal*), (rem). The relative biological effectiveness (RBE) of ionization due to secondary recoil nuclei produced in tissue by fast neutrons, and an equal amount of tissue ionization due to gamma-rays, has been the subject of many experimental studies on various species of microorganisms, viruses, plants, and animals. Values of RBE between 0.5 and 20 have been observed among the wide variety of biological materials studied thus far. When any one particular biological reaction has been compared in several species of mammals, the reported RBE values have exhibited species variations of less than a factor of two. As measured for various radiation effects (acute mean lethal-dose, shortening of life by chronic irradiation, blood counts, *etc.*) in mice, the secondary ionization produced by fast neutrons (about 2 Mev energy) is about 4 to 10 times as effective as the same amount of energy absorbed from the secondary electrons produced by gamma-rays. That is, the RBE for neutron/gamma is 4 to 10 for these experiments (Zirkle *et al.*, 1946; Henshaw *et al.*, 1947; Gray *et al.*, 1940).

One rem is the estimated amount of energy absorbed in tissue which is biologically equivalent in man to 1 r of gamma- or X-rays. By definition:

$$1 \text{ rem} = \frac{83}{\text{RBE}} \text{ erg/g. tissue.}$$

In the absence of adequate human data, the tentative assumption of no species difference between man and other mammals is commonly made, and the RBE for fast neutrons to gamma rays is taken as about 5 for general effects on mammals. Then, for radiation protection in the case of fast neutrons:

$$1 \text{ rem} = \frac{83}{5} \text{ erg/g. tissue} = 0.2 \text{ rep} = 0.1 \text{ n}$$

(*j*) *Roentgen/Hour-at-One-Meter* (rhm). The r/hr. at 1 meter (rhm) is a physical unit of radioactive source strength. It is an unambiguous means of assigning a numerical value to the total "amount" of any radioactive substance which emits γ-rays or X-rays, whether or not the disintegration scheme is known. The rhm is not a medical, radiological, dosage nor protection unit. Thus 1 rhm of Co^{60}, for example, is that amount of Co^{60} whose unshielded γ-ray emission produces 1 r/hr. of ionization in air at a distance of 1 meter from the source. If this source were then enveloped in a spherical lead shield such that the transmitted γ radiation produced only 0.1 r/hr. at 1 meter, then the effective radioactive source strength of the shielded material would be 0.1 rhm.

The necessity of assigning a numerical value indicating the degree of radioactivity present in some sample is an everyday occurrence in many laboratories. For local use it is often sufficient to cite the response of some stable readily available counter or electroscope. The non-uniformity of detection instruments used by various laboratories makes such arbitrary readings of little use for intercomparisons between two or more laboratories. The situation is better if there are available in both laboratories β- and γ-ray counters whose absolute sensitivity as a function of "geometry" and of electron or photon energy is known. But even so, it is necessary to know the disintegration scheme of the radioactive isotope if the observations are to be reduced to absolute units. The use of curie or of rutherford units presupposes knowledge of the absolute number of disintegrating atoms per second, and this can only be determined if the disintegration scheme is known. At the beginning of 1947 the disintegration schemes of only about two dozen isotopes were known with certainty. This is only about 5% of the total number of known radioactive isotopes.

Premature attempts to express activities in curie units have already led to unnecessary confusion. For example, the 6.5-day Mn^{52}, whose disintegration scheme has been established recently (Peacock and Deutsch, 1946) gives 3 photons (0.73, 0.94, and 1.46 Mev) for each disintegrating atom, but a β-ray is emitted in only 35% of the disintegrations. In the majority (65%) of the disintegrations, orbital electron capture is the primary mode of radioactive decay, and is, of course, accompanied by X-ray and Auger electron emission. Thus, even an absolute count of the rate of emission of β-rays from a Mn^{52} sample cannot be expressed as an absolute rate of disintegration without knowing this disintegration scheme. The number of β-rays is about $\frac{1}{3}$ the number of disintegrations. Similarly the number of photons emitted is 3 times the number of disintegrations. Examples presenting other difficulties are numerous, *e.g.*, the 43-day Be^7 and 65-day Sr^{85} are examples of isotopes which decay by electron capture only, and give γ-rays and X-rays, possibly a few conversion electrons, but no β- or α-rays at all.

Reasonably reliable interlaboratory comparisons of all isotopes which emit γ-rays can be made without knowledge of the disintegration scheme by using a standard instrument, technique, and unit. The roentgen unit, and instruments developed for its measurement, meets these requirements, and also has the advantage of not depending on the photon energy of the γ-rays.

Thus, the source strength of any γ-ray emitter can be expressed in terms of the number of roentgens per unit time produced at some arbitrary distance. Portable and reasonably inexpensive instruments reading in r/hr. or mr/hr. have been developed and are generally available as a result of recent advances in radiobiology and health physics. These usually have ionization chambers whose minimum linear dimensions are of the order of 10 cm., hence a convenient source-to-detector distance (center-of-source to center-of-chamber) is 1 meter. Accordingly, the roentgen/hr.-at-one-meter (abbreviated rhm) has been introduced recently as an arbitrary but unambiguous expression of the strength of any radioactive source which emits γ-rays.

A number of careful experimental studies have been made of the gamma radiation from radium and its short-lived decay products. The number-energy distribution of these photons is complex. The disintegration of radium itself gives rise to a 0.184 Mev photon in 1.2% of the disintegrations.

There are 3 principal lines following the β-ray transition RaB → RaC, and 8 important lines following the RaC → RaC′ β-ray transition. The photon spectrum is shown in Table II, and in Fig. 14.

Several of the more recent direct measurements of the γ-ray source strength of 1 g. Ra and its short-lived products, in terms of rhm are shown in Table III. In general, these measurements are made on radium contained in a small capsule or "needle" having 0.5 mm. platinum walls. The Pt walls absorb the α- and β-rays completely, but they also attenuate the γ-radiation by approximately 10 ± 2% (Wilson, 1945; p. 34). The 0.5 mm. Pt filtration is standard radiological practice in the therapeutic use of radium, hence is usually not corrected for in the determination of the rhm output. We can then take, for radium in equilibrium with its short-lived products, and with 0.5 mm. Pt filtration:

TABLE II

The Gamma-Ray Spectrum of Radium and Its Equilibrium Decay Products

Transition	Average No. of quanta/α-ray of Ra	Energy/photon: Mev	Total photon energy Mev	Compton absorption coefficient	$n \cdot h\nu \cdot \sigma_a$
	n	$h\nu$	$n \cdot h\nu$	σ_a in 10^{-5} cm^{-1} std. air	10^{-5} Mev/cm. air
Ra → Rn	0.012	0.184	0.0022	3.35	0.007
RaB → RaC	0.115	0.241	0.0277	3.56	0.098
	0.258	0.294	0.0758	3.68	0.279
	0.450	0.350	0.1575	3.77	0.594
RaC → RaC′	0.658	0.607	0.4000	3.81	1.523
	0.065	0.766	0.0498	3.74	0.186
	0.067	0.933	0.0625	3.66	0.228
	0.206	1.120	0.2310	3.51	0.810
	0.063	1.238	0.0780	3.44	0.268
	0.064	1.379	0.0882	3.37	0.297
	0.258	1.761	0.4540	3.13	1.42
	0.074	2.198	0.1626	2.93	0.476
Total	2.290		1.7893 Mev		6.19

$$\text{Then: } 1\text{ g. Ra} = \left(\frac{3.7 \times 10^{10} \times 3600}{4\pi \times 100 \times 100\text{ cm.}^2\text{ hr.}}\right)\left(\frac{6.19 \times 10^{-5}\text{ Mev/cm.}}{6.77 \times 10^{4}\text{ Mev/cm.}^3\cdot\text{r}}\right) = 0.969\text{ rhm}$$

Note that this numerical result is proportional to the value assumed for the absolute disintegration rate of radium. If the true value is found later to be less than 3.7×10^{10} disintegrations/sec./g. of radium element, the expected value of rhm/g. Ra will be reduced in proportion.

$$1\text{ g. Ra} = 0.84 \pm 0.02\text{ rhm.}$$

The Co^{60} isotope emits one 1.10 and one 1.30 Mev photon per disintegration, which have Compton absorption coefficients (excluding scattering σ_s) of $\sigma_a = 3.53 \times 10^{-5}$ and 3.41×10^{-5}/cm. of air at 0°C. and 760 mm. Hg. Photoelectric and pair-production absorption are negligible for these energies in air. We can, therefore, anticipate that 1 rd (10^6 disintegrations/sec.) of Co^{60} will produce at 1 meter (neglecting the absorption of about 0.3% in 100 cm. of air path):

TABLE III

Some Experimental Values for rhm/g. of Radium, in Equilibrium with Its Short-Lived Decay Products

The theoretical value, calculated from the data of Table II, is 0.969 rhm, without any filtering. This is in satisfactory agreement with direct measurements of the effect of platinum absorption (Wilson, 1945, p. 35) which give about 0.94 rhm for zero platinum.

Authors	*Filtration* mm. Pt	*rhm/g. Ra*
Taylor and Singer (1940)	0.5	0.816
Gray (1937a)	0.5	0.84
Mayneord and Roberts (1937)	0.25	0.88
	0.5	0.83
	2.5	0.63
	5.0	0.46
White, Marinelli and Failla (1940)	0.5	0.847

$$1 \text{ rd } Co^{60} = \frac{10^6 \times 3600}{4\pi \times 10000} \frac{(1.10 \times 3.53 \times 10^{-5} + 1.30 \times 3.41 \times 10^{-5})}{6.77 \times 10^4 \text{ Mev/cm.}^3 \cdot \text{r}}$$

$$= 0.352 \times 10^{-4} \text{ rhm, or } 0.0352 \text{ mrhm.}$$

while 3.7×10^{10} disintegrating atoms/sec. would produce $3.7 \times 10^4 \times 0.352 \times 10^{-4} = 1.30$ rhm.

Similar calculations for a number of other isotopes which emit γ-rays are summarized in Table IV.

III. BASIC INSTRUMENTS FOR THE DETECTION OF IONIZING RADIATIONS

All types of charged particle radiation ionize gases through which they travel. This includes α-rays, β-rays, and high velocity ions such as protons, deuterons, or heavier recoil nuclei, such as fission fragments. This ionization is due to the electrostatic forces between the atomic electrons in the gaseous absorber and the charge on the moving particle. The magnitude of the ionization per unit length of particle path, therefore, depends primarily on the velocity of the moving particle, since this determines the duration of the electrostatic interaction, and on the charge on the moving particle, because this determines the magnitude of the interaction forces. To first approximation the ionization/cm. of path varies as $(ze/v)^2$ where ze is the charge and v the velocity of the charged particle.

TABLE IV

Milliroentgens per Hour Produced at One Meter (mrhm) by the Nuclear Gamma-Rays and the Annihilation Radiation from the Isotopes Whose Disintegration Schemes are Shown in Fig. 13

Small effects such as the Bremstrahlung from inelastic collisions of β-rays with nuclei are neglected, as are also the soft characteristic X-rays emitted following orbital electron capture transitions, the small deficit of γ-rays in some transitions where internal conversion has been measured (*e.g.*, *ca.* 0.5% in I^{130}), and the absorption of the γ-rays and annihilation radiation in 1 meter of standard air (∼0.3%). The accuracy of the values for mrhm is the same as our knowledge of the nuclear γ-ray energies, which is about ±1–3%. Values of mrhm are given both for 10^8 disintegrations/sec. (100 rd), and for 3.7×10^7 disintegrations/sec. (37 rd) which in the case of RaB + C is 1 mc, or 1 mg. Ra in radioactive equilibrium with its short-lived decay products. Isotopes such as H^3, C^{14}, P^{32}, S^{35} emit no γ radiation, hence have zero mrhm values.

Isotope	*mrhm*/100 rd	*mrhm*/37 rd
Na^{22}	3.52	1.30
Na^{24}	5.20	1.92
Mn^{52}	5.23	1.93
Mn^{54}	1.31	0.485
Fe^{59}	1.76	0.651
Co^{58}	1.51	0.560
Co^{60}	3.52	1.30
Zn^{65}	0.80	0.30
Br^{82}	4.06	1.50
I^{128}	0.049	0.018
I^{130}	3.39	1.25
I^{131}	0.59 to 0.71[a]	0.22 to 0.26[a]
RaB + C	2.27[b]	0.84[b]

[a] The degree of internal conversion of the 80 kev level in I^{131} is unknown. The lower limiting value of mrhm corresponds to complete internal conversion (*i.e.*, no 80 kev γ-rays); the upper limiting value corresponds to zero internal conversion (*i. e.*, one 80 kev γ-ray/β disintegration). A 0.65 Mev γ-ray transition following about 10–15% of the β-rays of I^{131} has just been observed by C. L. Meaker and tentatively confirmed by M. Deutsch. If, after further work, these numerical values are found to be substantially correct, then the mrhm values for I^{131} should be increased by about 6%.

[b] With 0.5 mm. platinum filtration.

Uncharged radiations (such as γ-rays and neutrons) produce charged secondary particles by their collisions with absorbing materials, and these charged secondaries are capable of ionizing gases.

The study of nuclear radiations is, therefore, essentially the problem of the quantitative detection of the ionization produced by charged particles. Many types of instruments have been developed for such investigations, and this section will describe the operating principles of the basic instruments. An almost infinite variety of detection equipment exists, but, in general, nearly all instruments are variations or combinations of the fundamental types described.

1. *Ionization Measurements*

The work necessary to produce one ion pair (*i.e.*, one positive and one negative ion, each carrying unit charge $e = 4.80 \times 10^{-10}$ esu, due to the ionization of a neutral molecule) is about 32.5 electron volts for air, and about 25.1 electron volts for argon, which are the most common filling gases for detection apparatus. When the average ionization due to a moderately strong source of radiation is to be measured, say more than 10^4 ion pairs per second, the simplest apparatus is usually an *ionization chamber* containing two oppositely charged electrodes and fitted with a window or other opening for the entrance of the radiation which is to ionize the gas confined in the chamber. The ionization chamber is basically only an insulated condenser, and obeys the ordinary laws of electrostatics as regards charge, capacity, and voltage. The positive ions produced by ionization between the electrodes of the condenser are drawn by its electrostatic field to the negative terminal, while the negative ions migrate to the positive electrode. The collection of one positive and one negative ion (an ion pair) is therefore equivalent to transporting one unit negative charge from the negative electrode to the positive electrode, and thus serves to decrease the charge Q, and hence the potential difference V on the condenser whose capacity is C. Differentiating the well-known expression for condensers

$$Q = CV \tag{1}$$

gives us the relation between the changes dQ in charge, dV in potential, and dC in capacity, in case the latter exists

$$dQ = CdV + VdC \tag{2}$$

Whereas dQ is the quantity desired in the ionization measurement, it is usually more convenient to observe dV by means of an electrometer connected permanently to the ionization chamber. It is assumed that the reader is acquainted with several forms of electrometer, such as the quartz fiber, duant, quadrant, Compton, string, Wilson tilted gold leaf, or other varieties. All of these indicate voltage changes by the deflection of a moving electrode under the compensating action of electrostatic forces and mechanical restoring forces. They are, therefore, electrically equivalent to condensers, and when connected to an ionization chamber their electrostatic capacity must be included in C of Eqs. (1) and (2). The movement of the fiber across the electrometer scale, therefore, involves a change dC in the electrometer's electrostatic capacity, and this is the origin of the last term VdC in Eq. (2). Some types of electrometer, the quartz piezo-electric crystal, the vibrating reed, and the various vacuum tube electrometer circuits, give readings without mechanical deflection and are therefore free from the correction term VdC. For these, and others in which VdC has been made small by suitable mechanical design, *Eq.* (2) shows that the ionization current dQ/dt is proportional to the observed time rate of change of voltage, dV/dt.

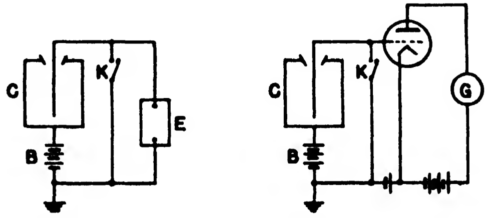

FIG. 17

Schematic representation of an ionization chamber C, operating on rate-of-drift method with an external electrometer *E*, or vacuum-tube electrometer and galvanometer G. The ion-collecting potential is supplied by B. When the grounding key K is closed, then reopened, the initial potential difference across the ionization chamber is B, while the potential difference across the electrometer is zero. As ions are collected, the potential difference decreases across the ionization chamber and increases across the electrometer. The sum of these two potential differences is always equal to B. The ionization current is then proportional to the rate of change of the potential difference readings across the electrometer.

What has just been described is known as the "*rate-of-drif* method" of measuring an ionization current, and is indicated schematically in Fig. 17.

In operation, the grounding key K is first closed, thus charging the ionization chamber to a potential B and discharging the electrometer. Floating the system by opening K then allows the electrometer to accumulate a deflection of dV volts, due to the collection of ions representing a charge dQ, as given by Eq. (2).

If the ionization currents are sufficiently large (say about 10^{-13} amperes or greater), it is possible to replace the electrometer by a high resistance, R, of the order of 10^{10} to 10^{12} ohms. Then the ionization current, i, will produce a potential drop, iR, across the resistor. This potential drop can be read with a sensitive vacuum tube voltmeter circuit. The output due to a constant ionization current is then steady in value. Therefore this mode of operation is known as the "*steady-deflection method.*"

A number of satisfactory electronic circuits have been developed, both for stationary installations and for battery-operated portable ionization meters (*e.g.*, Shonka, 1946a). When small ionization currents are to be measured by the steady-deflection method, the time constant CR of the ionization chamber may easily be as large as 10 seconds (*e.g.*, $C = 10$ $\mu\mu$farads and $R = 10^{12}$ ohms, for measurements in the domain of 10^{-13} amperes). When a faster time response is needed, the output of the electronic circuit can be coupled back to induce additional charge on the collecting electrode of the ionization chamber. This has the overall result of degenerating the capacitance of the ionization chamber, and hence, of speeding up the response by materially decreasing the effective time constant of the ionization chamber and its series resistor (Shonka, 1946b).

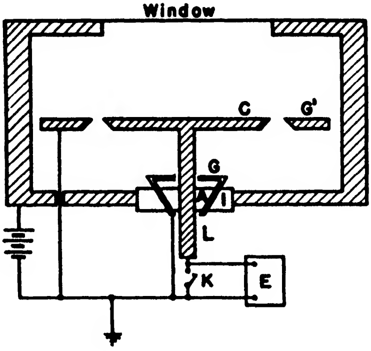

Fig. 18

Schematic illustration of a parallel-plate ionization chamber and its associated components and auxiliaries.

(*a*) *Ionization Chambers.* A simple *parallel-plate ionization chamber* is shown schematically in Fig. 18, to illustrate the essential elements of a complete ionization chamber and electrometer system. The collecting electrode C is usually a brass disc mounted through a polystyrene, lucite, or amber insulator A. Surrounding the main insulator A is a brass guard-ring G, which maintains a low potential difference across the insulator and thus prevents excessive surface leakage and electrical "soaking" due to dielectric changes in the insulator. Outside this guard-ring a low-grade insulator I, of material such as bakelite, separates the guard-ring from the ionization chamber wall. The potential difference across this insulator is maintained by the collecting-potential B, hence superior insulating quality is not required. The guard-ring G may be shaped so that the collecting electrode cannot "see" the high voltage insulator, thus preventing surface charges on the insulator from affecting the collecting electrode by electrostatic induction. The lead L from the collecting electrode goes to the insulated terminal of any suitable electrometer E. Surrounding the collecting electrode, a field-defining guard-ring *G′* is often used to straighten the electrostatic lines of force and thus to define more accurately the active gas volume from which ions are drawn to the collecting electrode.

Clearly, the geometry of the electrode system in an ionization chamber should be adapted to the type of radiation to be studied, and to the complexity of construction which is feasible. The *cylindrical ionization-chamber* is often favored because of its ease of construction. In this type the collecting electrode usually is simply a wire or rod running up the axis of a cylindrical outer electrode. The inner guard-ring G′ is either absent or is replaced by a coarse wire netting lining the chamber about 2 cm. inside its outer walls. Such a "Hoffmann net" is very effective in reducing that part of the *background ionization* which is due to α-rays from the unavoidable radioactive contamination of all materials.

If the center electrode is made larger, and hollow, then compact sources of radiation can be placed within it, and the chamber can be made to subtend nearly 4π steradians of solid angle at the source.

Spherical ionization-chambers are particularly suitable when a small source of radiation is to be placed in the center of the chamber on a small spherical electrode and symmetry is desired, such as in some α-ray measurements. This geometry also has a mechanical advantage when the chamber is to be operated under high pressure.

High-pressure ionization chambers may be used with sensitive rate-of-drift electrometer circuits to reach ultimate sensitivities of the order of 1–2 β-rays/sec. (Barnes, 1939) if the β-rays have energies of the order of 0.5 Mev or greater. A duralumin window of 0.001 inch thickness and 1 inch diameter will stand pressures of about 4 atmospheres, and is thin enough to transmit all but the softest β-rays with reasonably good efficiency.

(*b*) ***Electroscopes.*** When a simple electrometer movement is mounted directly inside the ionization chamber, the combination is known as an *electroscope*. The once familiar gold-leaf electroscope has been vastly improved in recent years by employing the same electrostatic repulsion principle, but replacing the gold-leaf by a gold-covered single fiber of quartz, Fig. 19. The mechanical restoring force then becomes the tension in the quartz fiber, and the instrument is substantially insensitive to gravity and can be mounted in any position. The quartz fiber also offers a more definite object on which to focus a microscope for observing its motion. It can also be made so small that the contribution of the VdC term in Eq. (2) is negligible compared with the larger term CdV due to the total capacity of the collecting electrode.

The main disadvantage of the electroscope is that when fully charged the deflecting system must read its full scale value V, and a small change dV in this voltage is only represented by a small change in this total deflection. This makes the instrument inherently less sensitive than the external electrometer and ionization-chamber systems, where any desired voltage sensitivity can be selected and the total deflection corresponds to dV instead of to V. This disadvantage has been partly circumvented in special electroscope suspensions whose geometrical design gives them a non-linear scale which is insensitive at low voltages and attains maximum sensitivity at full voltage (Neher, 1938).

The most sensitive quartz-fiber electroscopes are capable of detecting of the order of 10 β-rays/sec. within the sensitive volume of the chamber, provided these β-rays have initial energies of say 0.1 Mev or greater within a chamber filled with air or argon at atmospheric pressure. High sensitivity electroscopes are often very sensitive to temperature variations, and precautions must be taken to maintain constant temperature conditions. A lamp used to illuminate the scale should usually be left on for at least several hours before taking readings.

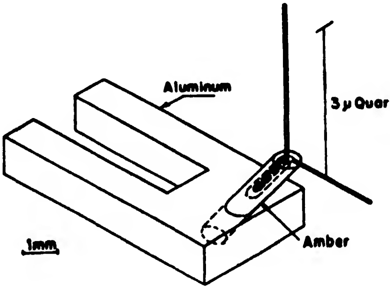

FIG. 19

The form of quartz fiber electrometer or electroscope element introduced by Lauritsen. A number of other types of quartz fiber elements have been developed by Neher, by Landswerk, and others. (LAURITSEN, C. C., and LAURITSEN, T., *Rev. Sci. Instruments*, 8, 438 (1937).)

2. Particle Counting Instruments

During the last decade rapid strides have been made in the development of vacuum-tube amplifiers and special low-capacity ionization chambers (Lewis, 1942). These permit the detection of individual α-rays, β-rays and some other particles, and therefore are approaching an ultimate limit by dealing with radiation one particle at a time. The three principal types of particle counters depend upon: (1) direct, vacuum-tube amplification (linear-amplifier) of the original ionization produced by an α-particle or proton; (2) preliminary amplification in the ionization-chamber (proportional-counter) through ionization by collision, followed by vacuum tube amplification; (3) the original ionization triggers a short-lived corona discharge in the ionization-chamber (discharge-counter), which is detected by a simple vacuum-tube amplifier.

Any of these particle counters can be connected to *coincidence amplifiers* (*e.g.*, Rossi, 1930; Johnson, 1938), which register only when the same ionizing particle, *e.g.*, a β-ray, passes through both counters, or when two or more particles of any types separately excite two counters at about ($\sim 10^{-6}$ sec.) the same instant. Also *anti-coincidence amplifiers* may be arranged so as to register only when coincidences are not present.

When the individual particles strike the counter at such a rapid rate that accurate counting is impossible because of the time lag of the recorder, it is convenient to use a "*scaling circuit*" (Wynn-Williams, 1931; Reich, 1938; DeVault, 1941) which reduces the output counting rate by any predetermined factor, usually some power of two, *e.g.*, 4, 8, 16, 32, · · · .

The counting-rate meter is an alternate type of counter-amplifier which indicates the average counting rate directly (Gingrich, Evans and Edgerton, 1936; Kip *et al.*, 1946). It consists fundamentally of a pulse-leveling circuit which, for each count, delivers a small charge q to a condenser of capacitance C. This tank condenser is shunted by a resistor R, the current in which is therefore proportional to the counting rate. The time-constant CR of the tank circuit determines the speed of response of the circuit to variations in the true counting rate. The standard deviation of a single reading of the counting-rate meter output, due to random statistical fluctuations in the activity of a source of constant average intensity, is the same as would be obtained by direct counting over a time interval equal to $2CR$. As in the case of direct counting, the statistical uncertainty of an average reading over a time T is proportional to $1/\sqrt{T}$. The statistical analysis of counting-rate meter data (Schiff and Evans, 1936; Kip *et al.*, 1946) is somewhat different from the analysis of either direct-counting data or scaling-circuit data.

A number of types of well stabilized voltage supplies have been developed, so that all d.c. power supplies and high voltage supplies can be obtained readily (Hunt and Hickman, 1939).

(*a*) *Linear Amplifier*. Until a decade ago the Hoffmann vacuum duant electrometer was the only instrument with sufficient voltage sensitivity to register directly the ionization of about 10^5 ion pairs from a single α-ray. With the improved thermionic vacuum tubes recently developed for radio, and other purposes, it is now possible to attain useful voltage amplifications of several million through the use of negative feed-back, resistance-capacity coupled, multi-stage amplifiers (Waddel, 1939). The present limitation in amplification is imposed by tube noises arising in the first stage of the amplifier, which is coupled directly to the collecting electrode of any ionization chamber having a reasonably low electrostatic capacity. The Western Electric 310-A tube has been used extensively as the first-stage in such low-noise linear amplifiers.

The output from the amplifier is a very short voltage pulse, whose duration is determined by the time-constants of the amplifier circuit and whose peak voltage is proportional to the number of ion pairs produced in the ionization chamber by a single ionizing particle.

It has been shown recently (Allen and Rossi, 1947) that electron attachment can be prevented, and that electrons can be collected directly in suitable ionization chambers. Because of the high mobility of electrons, their collection time can be reduced to the order of 10^{-6} second, while their associated positive ions have a collection time of the order of 10^{-2} to 10^{-3} second. This permits the use of an amplifier with a very small time constant, and a low noise-level, designed to respond only to the electron collection. The positive ions remain nearly stationary during the electron collection time, and thus provide a space charge which determines the height of the voltage pulse on the amplifier. This pulse height is dependent, then, not only on the total number of ions formed but also on their position in the chamber. Pure electron collection can be accomplished in ionization chambers constructed of baked-out metal and glass or porcelain, with a filling of pure argon plus 5–10% of carbon dioxide.

With an ordinary air-filled parallel-plate ionization chamber of about 2 mm. depth and 20 mm. diameter, and using a carefully adjusted amplifier, the pulses from 2 mm. of the path of a single α-ray in air at atmospheric pressure (about 10^4 ion pairs) can be amplified to 10 volts or more, with a noise level of the order of 0.1–0.5 of this due to microphonics and random electrical fluctuations in the tubes, resistors, batteries and circuit. These output pulses may be observed visually or recorded photographically with oscillographs, but usually they are fed into a pulse-height selector, or "discriminator" circuit to operate a mechanical message register which totalizes the number of pulses received in a measured time interval. By adjusting the input grid bias on the discriminator circuit only pulses which are larger than any preselected voltage may be recorded.

The linear-amplifier involves the most sensitive amplifying circuit used in particle counting apparatus, and hence offers practical operating obstacles to the uninitiated. It is suitable for direct ionization measurements on α-rays, protons, and other heavy ionizing particles having a comparably high specific ionization, whether they are primary particles or secondaries produced by neutron collisions, artificial nuclear disintegrations, fission, *etc.* Due to its characteristic of linear amplification of the original ionization, it does not respond to electrons or positrons and is, therefore, insensitive to

β-rays and to γ-rays unless they are intense enough to raise the noise-level to a value where it interferes with α-ray observations. As the linear-amplifier can be operated with atmospheric pressure in the ionization chamber, it is possible to use a wire gauze as an entrance "window" for the radiation.

(*b*) *Vacuum Tube Electrometers.* Recently special thermionic electrometer tubes have been developed (*e.g.*, Lafferty and Kingdon, 1946) which have exceptionally fine grid insulation, low residual gas pressure, and other features which give them a useful current amplifying factor of about 500,000 for a single stage. This is sufficient to amplify the ionization current from a single α-ray to a value which will give a throw of several centimeters on a high sensitivity galvanometer, with a noise-level which is negligible. The time-constant of such an amplifier is usually determined by the period of the recording galvanometer, and is therefore several seconds. The amplifier (DuBridge and Brown, 1933; Penick, 1935) can be made very stable and simple, and is admirably suited to counting α-ray pulses at an average rate of less than 5/min. (Finney and Evans, 1935).

The vacuum tube electrometer has a significant advantage over electrostatically operated electrometers because of the absence of mechanical motion, hence $VdC = 0$ in Eq. (2). Moreover, it is essentially a *current amplifier*, not a voltage amplifier, and hence it can also be used advantageously without a grid resistor (rate-of-drift method) for the direct measurement of ionization currents dQ/dt.

(*c*) *Proportional Counters.* The "linear-amplifiers" are limited in their sensitivity by the ratio of amplifier noise to ionization. This limitation can be made less important by increasing the number of original ion pairs produced in the ionization chamber for each α-ray or other ionizing particle. In the ordinary ionization chamber the potential gradient in the ion-collecting field is low enough and the gas pressure is high enough so that while moving one mean free path the ion being collected never acquires enough energy to ionize the gas molecules which it strikes. The number of ions collected is then the same as the number formed by the radiation, except for those which are lost due to the formation of neutral molecules by recombination of some ion pairs.

Now if the gas pressure is lowered to about 0.1 atmosphere the mean free path is proportionately increased, as is the average kinetic energy acquired by an ion between collisions. If the potential gradient of the ion-collecting field is also increased a condition can be realized in which *ionization by collision* will result in an average of n ions reaching the collector for every primary ion produced in the gas by the original radiation. Ionization chambers in which the pressure and voltage gradient are adjusted so that $n > 1$ are called *proportional counters.* Gas amplifications of $n = 10$–100 can be rather readily obtained with a wide variety of electrode geometries. The vacuum tube amplifier and the output recording devices for a proportional counter are similar to those used with a linear amplifier, except that the amplifier can have fewer stages, is not nearly as critical in its adjustment, and is much more rugged (*e.g.*, Brubaker and Pollard, 1937; Elliott, Brown and Evans, 1942; Korff, 1942; Benson, 1946). Like the linear amplifier, the proportional counter responds to α-rays, protons, and energetic positive ions from neutron collisions or nuclear disintegrations, and is usually insensitive to β-rays, γ-rays or cosmic ray mesons. Because it is ordinarily operated at a pressure of a fraction of one atmosphere, the source must be mounted inside the low pressure enclosure, or a thin window provided through which the ionising rays can pass.

Three geometrical arrangements of the electrodes have received the most attention. (a) The *cylindrical proportional counter* was the first electrical method of counting individual rays (Rutherford and Geiger, 1908). It is identical with the Geiger-Müller tube-counter except that the anode wire is usually 1 mm. or more in diameter. (b) The *ball-counter* offers a smaller effective volume, which is sometimes an advantage (Geiger and Klemperer, 1926). A disc-shaped effective volume can be produced by arranging 7 or more similar spherical anodes in a plane, within the same cathode (Duncanson and Miller, 1934). (c) Careful specification of the sensitive volume of the counter has been realized in the *parallel-plate proportional counter* (Zipprich, 1933). Zipprich has reported gas amplification ratios of as high as $n = 10^4$ with this arrangement of the electrodes. The α-rays or other ionizing rays pass between an upper pair of electrodes, A and B, producing their appropriate number of primary ions. These ions are then drawn toward and through the wire-mesh electrode B by a low voltage collecting potential. After passing B at a low drift velocity, these primary ions are accelerated toward the final collecting electrode C by a high potential between B and C. The collision ionization is entirely confined to the region between B and C, and every primary ion utilizes the full path from B to C for producing ionization. This uniformity gives the parallel-plate instrument a greater useful amplification than that of other forms of proportional counter.

(*d*) *Discharge Counters.* The Geiger (1913) point-counter, and the Geiger-Müller (1928) cylindrical-counter are the most familiar examples of discharge counters. We can best understand the operation of these discharge counters by considering the special case of the cylindrical discharge counter, that is, a Geiger-Müller counter (*e.g.*, Good, Kip and Brown, 1946), and its relationship to cylindrical proportional counters.

In a proportional counter having a cylindrical cathode and an axial wire anode, the gas amplification ratio n increases as the collecting voltage across the counter is increased. Fig. 20 shows schematically how the magnitude of the output pulse increases, as the ion-collecting voltage applied to the counter is increased (Montgomery and Montgomery, 1941). In the proportional region n may be of the order of 1–100, and seldom as great as 1000. At higher voltages, even a single free electron within the counting volume, can trigger a discharge. The magnitude of the output pulse becomes substantially constant, and is determined by the geometry, filling gas, and operating voltage of the counter, rather than on the amount of ionization produced by the initiating ray. Each pulse is very much larger than the pulses in the proportional region, and in the discharge region the average effective gas amplification factor is of the order of 10^7.

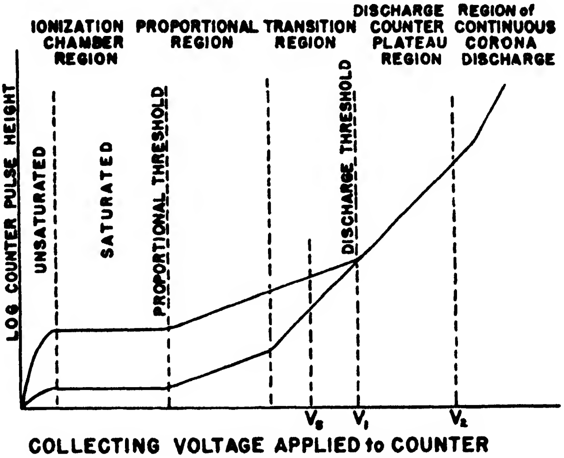

FIG. 20

Schematic representation of the magnitude of the pulse produced in a cylindrical counter by a single ionizing ray, as a function of collecting voltage applied to the counter. (a) At very low voltages, some of the ions will be lost by recombination, but substantially all the ions formed will be collected at somewhat higher voltages, still within the ionization chamber region. (b) As the collecting voltage is raised, a region of proportional gas amplification is found; here each original ion results in the collection of n ions. As the ion multiplication factor n is increased, say above $n \simeq 100$, strict proportionality is no longer observed, and a transition zone to the discharge (Geiger-Müller) region occurs. (c) In the discharge region, the pulse size is independent of the number of ions in the original ionizing ray. All output pulses are substantially equal in size, regardless of whether the initiating ray produced a large number of original ions (such as would an α-ray or a fission fragment), or a small number of original ions (as would a β-ray electron, a secondary electron produced by a γ-ray, or a cosmic ray meson). In the discharge region, even a single free electron produced near the cathode will produce a pulse. (d) At very high voltages, above the discharge region, the pulse-quenching mechanisms fail and a single ionizing event causes either a series of pulses or initiates a continuous corona discharge in the counter.

Properly constructed discharge counters can be made to count more than 99% of the ionizing rays which enter the sensitive volume. If the number of counts per minute due to a radioactive source of constant intensity if plotted against the operating voltage, a curve similar to Fig. 21 should be obtained. An operating voltage V_o is chosen somewhere in the plateau region, where the dependence of counting rate on voltage is smallest.

If a total voltage V is applied across a cylindrical counter whose anode and cathode radii are a and c, the radial potential gradient E within the counter will be inversely proportional to the radial distance r from the axis of the anode, and is given by:

$$E = V/r \ln (c/a) \tag{3}$$

We see at once that the electric field strength E is greatest for small r, that is, near the center wire. Also the relatively large field strengths required in discharge counters are most easily obtained by using relatively fine wire (usually 3–6 mil in diameter) for discharge counter anodes.

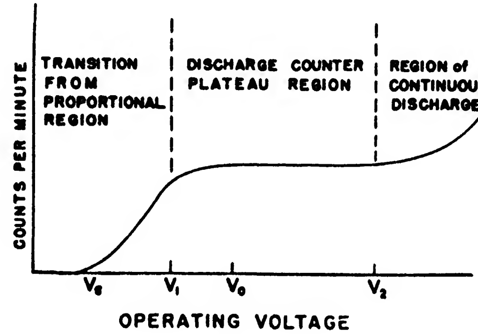

FIG. 21

Characteristic curve of a discharge counter. The starting potential V_S depends on the sensitivity of the amplifier used and is actually in the proportional region, or in the transition region of Fig. 20. Between V_1 and V_2 the counting rate will rise at most only a few per cent/100 volts. The optimum operating voltage V_o should be in the low end of the plateau, to afford maximum lifetime for self-quenched discharge counters. The "threshold" potential is V_1, although the term "threshold" is often loosely applied to the starting potential V_S.

(1) Non-Self-Quenching Counters. The mechanism of the gas discharge in counters is complicated, but involves in an idealized case the following principal phenomena (Montgomery and Montgomery, 1941; Brown, 1942; Korff, 1946). Consider first a so-called non-self-quenching discharge counter, containing only a monatomic gas such as helium or argon, or a diatomic gas such as hydrogen or nitrogen. When an ionizing ray passes through this gas, one or more pairs of positive ions and electrons will be produced. Consider one electron, originally set free near the cathode either by gas ionization, or as a secondary electron knocked out of the cathode by a primary γ-ray photon.

This electron will be accelerated toward the center-wire by the collecting field E. In an average counter the mean free path will be of the order of

10^{-3} cm., and consequently the electron will have many collisions with the filling gas. At each collision, most of the kinetic energy gained since the last collision will be lost. As the electron approaches the center wire, the field E increases and so does the kinetic energy gained between successive collisions. At some critical distance the electron first acquires enough kinetic energy between collisions to permit it to cause ionization by electron impact in its next collision. There are now two free electrons in the counter. These continue toward the center wire, and each may produce ionization by collision at its next impact. Thus a Townsend avalanche is initiated. The electrons so produced also cause further ionization, and as a result an avalanche of electrons reaches the center wire. Most of the electrons in this first avalanche were produced near the center wire, and at the scene of production of each electron in the avalanche there will remain a positive ion. The positive ions have a very much smaller mobility (about 1 cm./sec. velocity at atmospheric pressure, per volt/cm. of field strength) than the electrons, and begin to move very slowly out toward the cathode.

Among the collisions of electrons with atoms in the formation of the first avalanche, there will have been many collisions which resulted not in ionization, but in optical excitation of the atoms. The lifetime of these excited atoms against radiation is of the order of 10^{-8} sec., which is usually considerably shorter than the mean time between atomic collisions, so most of the excited atoms will give up their excitation energy by radiation. Many of these radiation quanta will be in the ultraviolet region, and will cause photoelectric emission from the cathode surface, and to a smaller extent from other atoms in the gas. Thus, during the first avalanche, additional electrons are produced which also form Townsend avalanches. The current flowing in the counter as a result may be of the order of 10^{-6} amp. Within about 0.1–1 microsecond the aggregate avalanche will have reached its peak value, because the presence of a large number of relatively slow moving positive ions near the anode produces a space charge which increases the effective anode radius, and decreases the potential gradient near the center wire. Within this space charge sheath the original $1/r$ potential gradient is replaced by a substantially constant field (Brown, 1942). The large potential gradients near the anode, which accounted for the electron avalanche, are thus removed by the space-charge of the sluggish positive ions. The electron pulse is "quenched" by space-charge.

Meanwhile, the positive ions have begun their relatively slow journey to the cathode, where they arrive after some 100–500 microseconds. When a positive ion approaches to within a few ionic diameters of the cathode, the electric field of the ion is strong enough to extract an electron from the metallic cathode surface. This electron joins the ion, and forms a neutral but probably excited gas atom. When this excited atom (11.5 ev in the case of argon ions and a copper cathode) strikes the cathode, its excitation energy may liberate an electron from the cathode. This mechanism (Oliphant and Moon, 1930; Korff and Present, 1944) for the liberation of an electron by a positive-ion bombardment of the cathode, would result in a new and unwanted avalanche if the full operating voltage V_0 had been allowed to remain on the counter.

In the conventional high-resistance-quenching circuit, Fig. 22, the voltage is maintained below the counting threshold (*i.e.*, below that necessary to produce an electron avalanche) during the entire collection time for the positive-ions. This is accomplished by the potential drop due to the counter discharge-current passing through a high resistance R connected in series with the counter and its voltage supply. Because the capacitance to ground of the counter anode, its leads, and the input capacitance of the amplifier is usually of the order of 10^{-11} farads, the series resistance R must be of the order of 10^7 to 10^8 ohms to provide a time constant CR greater than 10^{-4} seconds. The voltage pulse passed on through the coupling condenser to the control grid of the preamplifier vacuum tube has a shape which is due to the combined action of the arrival of the electron avalanche at the counter anode, the slow motion of the positive ion sheath to the cathode, and the flow of the resulting currents through the high resistance.

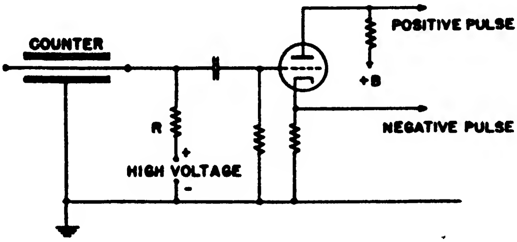

FIG. 22

Fundamental amplifier circuit for discharge counters. (a) When used with non-self-quenching counters, the resistor R must be about 100–1000 megohms. Then a positive pulse of the order of 10–100 volts is available. When so used the circuit is sometimes referred to as a "classical preamplifier," or a "conventional high-resistance-quenching" circuit. (b) When self-quenching counters are used, R may be a very low resistance, although the most useful values usually lie between 0.1 and 10 megohms. The output pulses are then small, and the circuit is commonly used as a cathode-follower to drive a transmission cable to the main amplifier by taking a negative pulse from the cathode resistor.

Actually, it can be shown (Werner, 1934; Lewis, p. 105, 1942; Korff, p. 63, 1946) that the length of the plateau $V_2 - V_1$ in non-self-quenching counters is approximately proportional to R, which must be of the order of 10^8–10^9 ohms to produce a satisfactory plateau of about 100 volts length. Such high resistance values are undesirable because of the large time-constant (~ 0.001–0.01 sec.) which they cause, thus preventing the amplifier from responding to two ionizing events which are closely spaced in time.

To increase the speed of counting, various *quenching circuits* have been introduced (*e.g.*, Neher and Pickering, 1938; Getting, 1938). These circuits respond to the electron avalanche by sharply reducing the high voltage applied to the counter, and by subsequently reestablishing the full operating voltage to the counter only after a delay of 10^{-3}–10^{-4} sec., during which the positive ions have been collected. The quenching circuits, such as Fig. 23, generally use counter resistances R of less than 1–10 megohms, but their action is such that the plateau is not unduly shortened, as it would be in a classical circuit using such small resistances. This is achieved by taking advantage of the amplification factor μ of the vacuum tube (Johnson, 1938), so that, while current is flowing in the counter, the effective value of the series resistance becomes nearly μR. This provides resistance-quenching against multiples due to positive ions striking the cathode. However, when current ceases to flow in the counter, the effective counter resistance returns to its low ohmic value R, thus permitting rapid restoration of full counting voltage. Thus the quenching circuits permit faster counting rates when used with non-self-quenching counters, without sacrificing plateau width.

A second important advantage of quenching circuits is that they permit the successful use of counters having unconventional geometrical arrangements of the cathode and anode (Brown and Evans, 1939). It is no longer necessary to provide for internal quenching of the electron avalanches by positive ion space charge through the use of a fine-wire anode. The anode and cathode may be cylinders of comparable radii, or concentric spheres, or parallel planes, or a wire loop and a plane, or even a table-fork and spoon. Thus counters may be designed to have optimum geometry for any particular experimental arrangement.

Both the conventional high-resistance preamplifier and the quenching circuits maintain the counter at a potential below the counting threshold during the collection time of the positive ions in a non-self-quenching counter. The counter is, therefore, unaffected by successive ionizing rays which enter it during the recovery period of the amplifier, and the minimum time τ between countable ionizing rays is determined by the amplifier, not by the counter. In the presence of radiation of very high intensity, the counter should respond periodically, at time intervals τ, and therefore at a counting rate of $1/\tau$.

(2) Self-Quenching Counters. If a polyatomic vapor (such as ethyl alcohol, ammonia, or amyl acetate) is added to the fixed gas (such as helium, argon, or nitrogen) in a counter, a number of important changes in the discharge characteristics take place, and the counter becomes truly self-

quenching. A common filling mixture is about 90% argon and about 10% ethyl alcohol vapor.

The polyatomic vapor will, in general, have a continuous ultraviolet absorption spectrum and a lower ionization potential than the fixed gas. The polyatomic molecules, therefore, absorb the ultraviolet quanta emitted by the excited atoms of the fixed gas. Ultraviolet quanta from the first avalanche close to the center wire cannot reach the cathode. Only the initial avalanche can form fully, because additional electrons are not liberated from the cathode.

A polyatomic molecule, after absorbing an ultraviolet quantum, will either become a positive ion by photoelectric emission, or will dissociate, or will radiate its excitation energy as several quanta in the vibrational and rotational infra-red spectrum. Those which emit a photoelectron provide additional electrons near or in the avalanche region, and these electrons produce additional ionization by collision as they are drawn to the center wire. Thus, the discharge spreads laterally along the center wire, at a speed of about 10^7 cm./sec. (Hill and Dunworth, 1946). Within the order of a microsecond or less, the discharge and the thin positive ion sheath will be fully formed along the entire length of the center wire. The positive ion space charge will have reduced the potential gradient near the wire to below the minimum field necessary for counting. The pulse size will be of the order of 10^9 ions, or 10^{-10} coulombs, and will be directly proportional to the length of the center wire (Stever, 1940).

The discharge having been quenched, the positive ions begin their slow journey to the cathode. En route, an argon ion will have some 10^5 collisions with neutral polyatomic molecules. Because the ionization potential of the polyatomic molecule (*e.g.*, 11.3 ev for ethyl alcohol) is less than that for the fixed gas (*e.g.*, 15.7 ev for argon), there will be in these collisions a high probability of the argon ion becoming neutralized by capturing an electron from an alcohol molecule, leaving the alcohol molecule ionized or dissociated, and radiating the final energy difference of $\sim$ 4.4 ev. Thus the probability that a positive ion of argon will succeed in reaching the cathode and initiating a second electron avalanche is extremely small, though finite.

Upon arrival within about an ionic diameter from the cathode, each polyatomic molecular ion will become neutralized by drawing off and capturing an electron from the cathode. Upon reaching the cathode, the neutral but excited molecule will then dissociate, and will not liberate an avalanche-producing electron from the cathode. In general, this second electron cannot be liberated from the cathode if the ionization potential of the polyatomic molecular ion is less than twice the electronic work function of the cathode surface (Present, 1947).

Thus, the polyatomic molecule performs three functions in the discharge: (a) it absorbs ultraviolet radiation from the fixed gas atoms and prevents the photoemission of secondary electrons from the cathode, (b) it neutralizes the positive ions of the fixed gas, becoming ionized or dissociated itself, and (c) its positive ions upon reaching the cathode, do not produce secondary electrons.

It is evident that some 10^9–10^{10} molecules of polyatomic vapor are destroyed by dissociation every time the counter discharges. An average counter will contain of the order of 10^{20} polyatomic molecules, and therefore has a finite life of less than 10^{10} counts. In practice, the order of 10^8 counts is a creditable lifetime for a self-quenching counter, after which it must be pumped, cleaned, and refilled.

The useful life of a self-quenched counter can be extended by using a very heavy vapor, such as amyl acetate. Such complex molecules break down into smaller molecules which are also capable of quenching the counter discharge.

It has been found recently (Farmer, 1947) that the discharge in a methane counter also causes the formation of heavy organic molecules, which accumulate as a deposit on the cathode, and prevent the counter from functioning.

Finally the vapor fragments will consist mainly of hydrogen gas and other light molecules incapable of quenching action, and the counter will require pumping, cleaning, and refilling.

The possibility of continuously reconstituting the quenching vapor by providing some internal means for recombining the molecular fragments, and for preventing the formation of heavy organic molecules, offers an interesting challenge for future experimentation. Probably a "permanent" filling could be devised by suitable choice of a vapor whose fragments would not polymerize into a solid material, and whose fragments would have a strong tendency to recombine, through a "back-reaction" to reconstitute the original polyatomic vapor.

If self-quenching counters are to be used at low temperature, such as winter field-use, the character and partial pressure of the vapor must be chosen so as to avoid internal condensation.

As the partial pressure of the quenching vapor constituent decreases, due to continued use of the counter, fixed gas ions have a higher probability of reaching the cathode, and of liberating an electron which will cause a second and spurious pulse. Such spurious discharges occur occasionally (say 5–15%) even in new self-quenched counters, especially when the counter is operated at a voltage considerably greater than the counting threshold. Then the pulses are large, and the very small but constant probability that a fixed gas ion can reach the cathode before being neutralized by the quenching vapor results in a larger fraction of the true counts being closely followed by a spurious count. These spurious counts are responsible for the gradual increase of the counting rate with operating voltage, even in the plateau region of a self-quenched counter. The upper end of the useful operating voltage range is reached when a significant fraction (say one-fifth) of the true pulses are accompanied by a spurious pulse.

A self-quenching counter will exhibit with continued use: (1) an increasing threshold voltage, due to the increase in·gas pressure caused by the accumulation of degradation products of the quenching vapor, and (2) an increase in slope of the plateau, because of the increased occurrence of multiple counts due to incomplete quenching of the positive ions.

It is seen that the lifetime of a self-quenched counter is increased by: (1) using a complex polyatomic vapor, whose breakdown products will also exert a quenching action; (2) using as large a proportion of the quenching vapor as is possible without unduly increasing the starting voltage; and (3) operating the counter in the low voltage end of its operating range, so as to minimize the discharge currents and to destroy as few molecules of vapor per pulse as possible.

Every discharge in the self-quenched counter is self-extinguishing, without the aid of a series resistance or an external electronic circuit. (Even those pulses which result in spurious doublets, have a negligible chance of forming spurious triplets or higher order multiples.) The fundamental requirement on the external circuit is that it provide a means of recording the discharge in the counter. This is accomplished most easily with the fundamental circuit of Fig. 22, using a counter resistance R of between 0.1 and 10 megohms. Then the discharge current of the counter produces a small negative voltage pulse, which can be amplified easily. The capacitance of the counter and its connections may be of the order of 10^{-11} farads or more, so the time constant RC with 10 megohms will be of the order of $10^{-11} \times 10^{7} = 10^{-4}$ second or longer. The natural dead-time of a self-quenched counter is about 2×10^{-4} seconds, as will be discussed later, so there is nothing to be gained by reducing R below say 1 megohm except when two or more counters are operated in coincidence circuits. Then R may usefully be reduced to as little as 0.1 megohms, and the amplifiers can be designed to be actuated only by the steeply rising front-face of the voltage-time discharge curve (Lewis, p. 123, 1942).

Of course, the discharge current is determined by the geometry and filling of the counter, so the magnitude of the voltage pulse is determined by the input capacitance of the amplifier and by the resistance R used. With 1 megohm, the voltage pulses from an average self-quenched counter will be of the order of 1 volt.

If a quenching-circuit, *e.g.*, Fig. 23, is used with a self-quenching counter the minimum time between recordable true pulses will be somewhat longer than the recovery time of the counter (Roberts, 1941).

In self-quenching counters, the time required for the full development of the electron avalanche and the positive ion sheath along the entire length of the anode is of the order of 10^{-6} seconds. The time required for the positive ion sheath to migrate far enough toward the cathode to restore minimum counting potential gradients near the anode is called the *dead-time*.

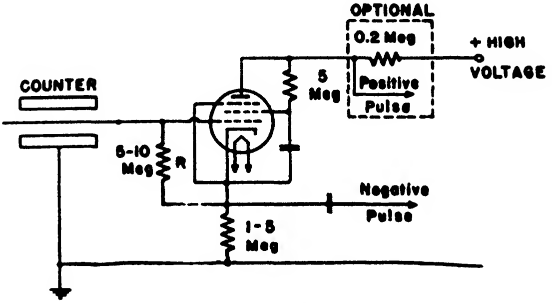

FIG. 23

Neher-Pickering quenching circuit, modified for screen-grid self-bias. The tube is normally conducting, the plate current of about 0.2–1 milliamp being determined by the cathode resistor and the high voltage supply. Because the tube drop is small, substantially the entire high voltage is across the cathode resistor and the counter. A discharge in the counter drives the control grid negative, and reduces the plate current so that the potential drop across the cathode resistor is slightly less than the counter threshold voltage. A negative pulse equal to the counter overvoltage can be taken from the cathode, or a positive pulse can be taken from the plate by inserting 0.2 megohms in the high voltage supply line. The dynamic characteristics of the vacuum tube make the circuit behave like a non-linear counter resistance. While counter discharge-current is flowing through R, its effective resistance approaches the limiting value of μR, where μ is the control-grid-plate amplification factor of the vacuum tube. When the discharge ceases, the vacuum tube becomes ineffective and the counter voltage is returned with a recovery time-constant appropriate to the small value of R. With self-quenching counters, R may be even further reduced since a large effective resistance μR is not required then for resistance-quenching of the positive ion phases of the discharge. Then the circuit becomes essentially only a modified cathode-follower for driving a cable to the main amplifier.

The dead-time is about $1\text{–}2 \times 10^{-4}$ seconds, the shorter values corresponding to light quenching vapors like alcohol, and the longer values corresponding to more complex vapors such as amyl acetate. During the dead-time, any new electrons produced in the counter by external radiation (*e.g.*, γ-rays) cannot produce a new discharge. Such potential counts are therefore lost, and the results of experiments at high counting rates must be corrected for these unobserved counts.

Following the dead-time, the counter requires an additional *recovery-time* of about $2\text{–}5 \times 10^{-4}$ seconds for the positive ion sheath to reach the cathode. Any new electrons produced in the counter during the recovery-time will initiate a new discharge, but the pulse will be smaller than the normal pulse (Fig. 24) because the full normal potential gradients are not reestablished in the counter until after the positive ion sheath reaches the cathode. If the resolving time of the amplifier is short enough (say $<10^{-4}$ sec.) and if its sensitivity is great enough, pulses arriving during the recovery-time will be recorded. Otherwise, electrons arriving during the recovery-time of the counter and the resolving time of the amplifier will not be recorded. However, these ionizing events will cause discharges in the counter and will render it again insensitive during its new dead-time plus the recovery-time of the counter or the resolving-time of the amplifier, whichever is longer. A very great source strength will, therefore, paralyze the apparatus and, in the limiting case of an infinitely strong source of radiation, no counts at all will be recorded.

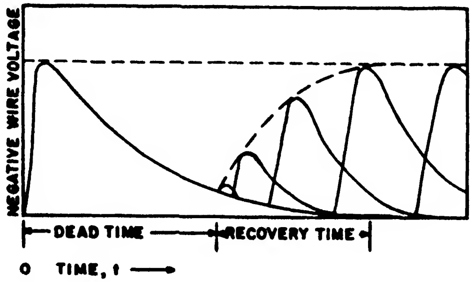

FIG. 24

In a vapor-filled self-quenching counter the collection on the anode wire of the avalanche electrons causes a sudden change, in the negative direction, of the potential of the wire. The gradual return to normal is determined by the current-equivalent of the positive ion sheath as it moves toward the counter cathode, and by the capacitance and resistance of the counter circuit. The counter is insensitive during a *dead-time* of about 1–2 × 10^{-4} seconds, during which the positive ions move sufficiently far toward the cathode to restore the field strength near the anode to the minimum value required for counting. After this dead-time the counter can again respond to ionizing rays, although the pulse produced in the counter will be small. The *recovery-time* of 2–5 × 10^{-4} seconds is required for the positive ions to reach the cathode, after which a new pulse will be full-size. The curves at the right indicate the height and shape expected for a single pulse, occurring at various times t after the first pulse at the left of the figure (Stever, 1940).

(*e*) *Correction of Counter Data for Counts Lost Due to Inadequate Resolution.* It is seen that the conditions under which ionizing events fail to be recorded depend strongly on the characteristics of the counter and of the amplifier. Two limiting cases or types may be identified easily (Beers, 1942).

To make the examples definite, suppose the sensitive volume of the counter is traversed by an average of about 1000 β-rays/min. from a radioactive source, and that the background due to cosmic rays and local radiation can be neglected. The time of occurrence of individual β-rays will be random, and will therefore obey the Poisson distribution, and the Interval

Law. Accordingly, if m is the average number of β-rays expected in a particular time interval, the probability P_x that x β-rays will actually occur is (Bateman, 1910):

$$P_x = m^x e^{-m}/x! \tag{4}$$

Then the Interval Law (Marsden and Barratt, 1911) which can be derived from this Poisson distribution, shows that the probability $P(\tau, \infty)$ that the interval between any two successive β-rays will be longer than τ is:

$$P(\tau, \infty) = e^{-N\tau} \tag{5}$$

where $N\tau$ is the average number of β-rays expected to occur in the time τ.

Type I. Suppose the counter and its amplifier and recorder respond to a particular β-ray, and that thereafter the amplifier is unresponsive to successive pulses during a resolving time τ. Suppose also that any successive β-rays entering the counter during the time interval τ may produce a new discharge in the counter, and cause the amplifier to remain insensitive until it shall have had a time interval of at least τ for recovery between two successive β-rays. All this is equivalent to saying that the amplifier is unable to resolve the counts unless there is a time interval of at least τ between them. The situation is illustrated in Fig. 25. Such a situation exists, for example, when a quenching-circuit of relaxation time τ is connected to a self-quenching counter whose dead-time is greater than τ. Then any β-rays occurring during the recovery time of the counter will cause new discharges and will keep the quenching circuit from recovering. The same situation will be found in a non-self-quenched counter connected to a classical preamplifier and then to an amplifying and recording circuit whose resolving time τ exceeds the positive ion collection time of the counter and the recovery time of the preamplifier.

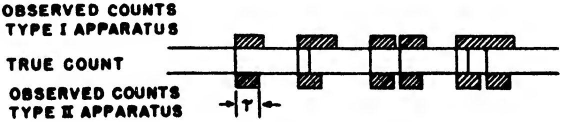

FIG. 25

Schematic illustration of the behavior of counting systems having a resolving time τ. The time axis is from left to right. True counts occur at the times shown by vertical lines along the center section. Apparatus of Type I responds only to intervals longer than τ. The number of observed counts and the time during which the Type I apparatus is insensitive are shown by shaded blocks. Apparatus of Type II is insensitive for a time τ after one pulse, but then can respond again even if the interval between successive true counts is less than τ, as illustrated by the triplet on the right. In the hypothetical example shown, there are 8 true counts, of which 6 are recorded by a Type II apparatus, and only 5 are recorded by a Type I apparatus.

Counter systems really do not count β-rays, but rather the intervals between β-rays. Systems of Type I count only those intervals which are longer than τ. Accordingly, if N is the true counting rate, the observed counting rate n will be:

$$n = Ne^{-N\tau} \tag{6}$$

It is seen that if the true counting rate is very large, the observed rate will approach zero, that is, the apparatus becomes paralyzed. If the true counting rate N is small, so that only a few counts are missed, then the observed counting rate is given approximately by:

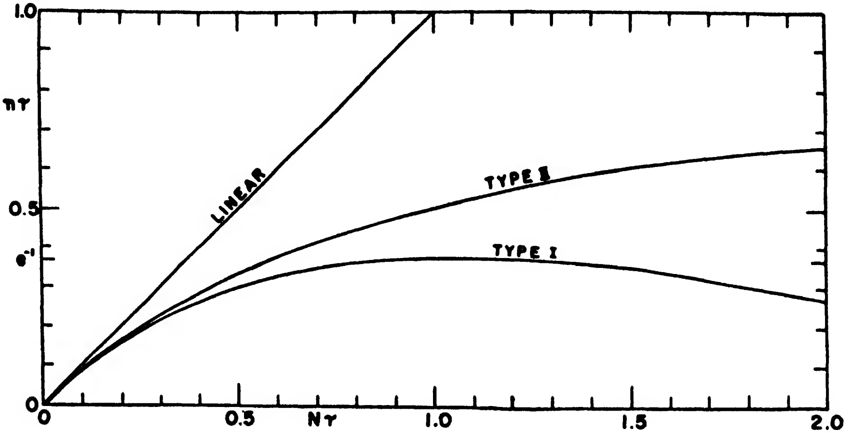

FIG. 26

Due to the finite resolving time τ, of the apparatus the observed counting rate n is always less than the true counting rate N. Here $n\tau$ is plotted against $N\tau$ for two limiting cases. Type I apparatus counts all intervals longer than τ; note that the maximum observed counting rate corresponds to $n\tau = 1/e$, and occurs when $N\tau = 1$. Type II apparatus is completely insensitive for a time τ after each true count, then regains full sensitivity. An apparatus with zero resolving time, would follow the straight line marked "linear."

$$\begin{aligned} n &\simeq N(1 - N\tau), \\ \text{or:} \quad N &\simeq n(1 + n\tau), \end{aligned} \tag{7}$$

where $N\tau \ll 1$. As the true counting rate is increased further, differentiation of Eq. (6) with respect to N shows that when $N\tau = 1$ the observed counting rate n passes through a maximum given by:

$$n_{\max} = N/e = 1/e\tau \qquad (e = 2.7183) \tag{8}$$

If N is increased still further, the observed rate actually decreases, as paralysis of the apparatus becomes increasingly worse due to the scarcity of intervals longer than the resolving time τ (Fig. 26).

Type II. The opposite statistical extreme is found in apparatus which, after responding to one β-ray, is completely unresponsive for an interval τ, but is not affected in any way by β-rays occurring during τ.

Equipment of this type is illustrated by a non-self-quenching counter connected to a quenching-circuit which maintains the applied voltage below the counting threshold for a time τ, during which both the counter and the recording circuit are able to undergo complete recovery. The same characteristics would be exhibited by a self-quenching counter connected to a very sensitive preamplifier capable of responding to all pulses received after the dead-time, but during the recovery-time of the counter, provided the resolving time of the entire amplifier and recorder is less than the dead-time, τ, of the counter.

Under these circumstances the apparatus is dead for a time τ after each recorded count. If the observed counting rate is n, then the fraction of the total time during which the apparatus is dead is $n\tau$, and the fraction of the time during which the apparatus is sensitive is $1 - n\tau$. The fraction of the total true counts which can be recorded is then:

$$n/N = 1 - n\tau \tag{9}$$

At relatively low counting rates, when $N\tau \ll 1$, we can write:

$$\begin{aligned} N &= n/(1 - n\tau) \\ &\simeq n(1 + n\tau) \end{aligned} \tag{10}$$

which is the same as for Type I apparatus provided $N\tau$, and consequently $n\tau$, is small compared with unity.

Type II apparatus does not exhibit complete paralysis, but as N is increased, the observed counting rate n rises uniformly, asymptotically approaching the value

$$n_{\max} = 1/\tau, \qquad \text{for } N = \infty \tag{11}$$

To an infinitely strong source of radiation the counter responds periodically, with a frequency of $1/\tau$, all traces of statistical randomicity having been erased.

All that has been said above assumes that the resolving time is independent of the counting rate. There is some evidence (*e.g.*, Muehlhause and Friedman, 1946) that the dead-time in self-quenching counters may decrease when the counting rate is elevated to very high values. But, at the lower counting rates met in most measurements, the assumption of constant resolving time agrees well with the observations. Many apparatuses, however, do not conform perfectly to either of the limiting cases treated above, but show resolution characteristics intermediate between Type I and II. Happily, the expressions for both limiting types converge at low counting rates. If $N\tau \leq 0.05$ the exact expressions for n/N differ from each other by less than 0.1% (Reid, 1946) and the relation:

$$N = n(1 + n\tau) \tag{12}$$

may be used for any counting apparatus.

The resolving time of a reasonably well designed counter and amplifier will usually be found to be between 3 and 6×10^{-4} seconds, which is equivalent to between 5 and 10×10^{-6} minutes. Then, at an observed counting rate of 1000 counts/min. the fraction of the counts which are lost is $n\tau = 5\text{–}10 \times 10^{-3}$, or 0.5–1%. At 2000 counts/min., the same apparatus loses 1–2% of the counts. Based on Eq. (12), counting losses are often cited simply as "per cent loss per thousand counts/min." Observed counting rates can be corrected easily and accurately for counting losses if $N\tau < 0.05$, that is, up to observed rates of 5000–10,000 counts/min. for most counters.

The simplest satisfactory method for measuring the resolving time is to compare the response of the apparatus to the radiation from two approximately equal sources, taken separately and then taken simultaneously. Let N_A and N_B be the true counting rates for two sources, while n_A and n_B are the observed counting rates after correction for background. Then, when both sources are measured simultaneously, the sum of their radiation will give a true rate of $N_S = N_A + N_B$, but the observed rate n_S will be slightly less than $n_A + n_B$. It follows from Eq. (12) that:

$$\begin{aligned} N_A &= n_A(1 + n_A\tau) \\ N_B &= n_B(1 + n_B\tau) \\ N_S &= n_S(1 + n_S\tau) \\ N_S &= N_A + N_B \end{aligned}$$

Solving these relationships simultaneously, gives:

$$\tau = (n_A + n_B - n_S)/(n_S^2 - n_A^2 - n_B^2)$$

or to a satisfactory approximation:

$$\tau = (n_A + n_B - n_S)/2n_An_B \tag{13}$$

Then, for example, if $n_A = n_B = 1000$ counts per minute (cpm), while $n_S = 1984$ cpm, we have $n_A + n_B - n_S = 16$ cpm, and $\tau = 16/2 \times 10^6 = 8 \times 10^{-6}$ minutes. Then the true rates would be $N_A = N_B = 1008$ cpm, $N_S = 2016$ cpm.

We have assumed thus far that the apparatus has only one controlling time-constant, which is independent of counting rate. Certain scaling-circuits, connected to slowly operating mechanical registers or recorders, may exhibit two important time-constants. At low counting rates, the losses may be determined only by the resolving time of the counter and the first stage of the amplifier, as described in the preceding paragraphs. As the counting rates are elevated a situation will occur in which, for example, a scale-of-four will occasionally receive 5 or more pulses in less than the time interval required for action of the mechanical register which it should be driving each time 4 counts are received. Then the resolving time of the mechanical register becomes important. A scale-of-n reduces the standard deviation of the statistical fluctuations by $\sqrt{n}$, tending always to produce an output which is less random and more nearly periodic than the input distribution. Consequently the mathematical analysis of the scaling losses in such cases is more complicated (Lifschutz and Duffendach, 1938; Lewis, 1942), and will not be discussed here because it can be avoided easily by proper design of apparatus.

3. *The Cloud Chamber*

The Wilson cloud chamber (Wilson, 1911) probably has contributed more to our detailed understanding of nuclear processes than any other single instrument. This is because it allows us to "see" actually the ionization along the path of any individual ray or particle, and, hence, to observe directly its entire trajectory, and the phenomena attending its individual collisions with atoms and nuclei. Each ionizing ray might be thought of as analogous to a cyclone, in the study of which much more can be gained from direct and detailed observations of the path of wreckage than from telegraphed reports on the number of houses wrecked.

The cloud chamber is an instrument which condenses water or other vapor on each ion formed within its sensitive volume. These liquid droplets are large enough to scatter light, and, hence, may be observed visually, or photographed for detailed study and permanent record. In general, the cloud chamber gives a picture of the distribution of ions at the moment of its operation, and it is sensitive for only the order of 0.01–0.1 second. Much work is in progress to increase the duration of sensitivity, but at present a few seconds is the maximum which has proved practical. The cycle of operations can be repeated a few times a minute at most.

The detailed theory of the condensation process in cloud chambers is still incomplete, but the basis of the operation is fairly clear (Thomson, 1928; Becker and Doring, 1935; Das Gupta and Ghosh, 1946). The essential parts of a simple expansion cloud chamber, consist of a gas-tight cylindrical enclosure fitted with a movable piston. The piston is originally at the top of its motion, and the volume of the enclosed space is V_1. For definiteness, we shall have V_1 filled with dust-free air and saturated water vapor at 20 C. and a total pressure of one atmosphere. By means of a battery and two internal electrodes, all the ions have been swept out of the active volume. Suppose now an α-ray traverses this gas, producing a trail of ionized molecules and atoms, and immediately the piston is rapidly withdrawn the maximum distance allowed by the stops. The volume of the gas and vapor mixture will be increased to V_2, and, due to the rapidity of the expansion, the change may be regarded as adiabatic. For the adiabatic expansion of an ideal gas we have:

$$T_1/T_2 = (V_2/V_1)^{\gamma-1} \tag{14}$$

where T_1 and T_2 are the absolute temperatures before and after the expansion, and γ is the ratio of the specific heats of the gas at constant pressure and volume. ($\gamma = 1.67$ for ideal monatomic gases, 1.40 for diatomic gases, such as air.) The expansion thus cools the air in the chamber and it becomes *supersaturated* with water vapor. Small molecular aggregates of water vapor are believed to be present in this supersaturated state, and these will form centers of possible condensation for the growth of visible droplets. Suppose such an incipient droplet has a radius a cm, and the surface tension of the liquid is S ergs/cm. Then the surface tension energy of the droplet is $4\pi a^2S$, and the droplet will tend to evaporate in order to reduce its surface area and its surface tension energy. With a moderately high degree of supersaturation in the chamber, this droplet will not grow in size. If, however, the droplet is formed on an ion having a charge e, then its electrostatic energy is $e^2/2a$, and this portion of its total energy becomes smaller if the droplet grows in size. The surface tension and electrostatic energy W of a charged droplet is, therefore,

$$W = 4\pi a^2S + e^2/2a \tag{15}$$

and the rate of change of this energy with drop radius is

$$dW/da = 8\pi aS - e^2/2a^2 \tag{16}$$

Thus, a charged droplet can increase its radius with less difficulty than one which is uncharged. A degree of supersaturation can therefore be found (by careful adjustment of the *expansion ratio* V_2/V_1) which will cause charged droplets to increase in size until they become visible, while uncharged droplets will still tend to evaporate. By this mechanism every ion formed by the original α-ray will become encased in a visible droplet, and the trail of these ions through the gas can be observed readily.

Under suitably controlled conditions, the ionization density along the α-ray path can be determined from the intensity of the light scattered by these droplets (Feather and Nimmo, 1928). If the chamber is operated at reduced pressure and the ion pairs are allowed to drift apart or are pulled apart by an electric "sweeping field" before the expansion, the individual ions can be counted on the photograph of the track (Anderson, 1933; Wilson and Wilson, 1935).

In the operation of cloud chambers, it is important to avoid turbulence of the gas, since this will distort the shape of the tracks. For this purpose, gauze baffles (Wilson, 1933) are commonly used, and the advantage of a small expansion ratio becomes obvious. Since the monatomic gases have the highest ratio of specific heats, and hence the lowest expansion ratio by Eq. (14), argon is favored over air for some cloud chamber purposes. Also, the thermodynamic properties of ethyl and propyl alcohol are such that lower expansion ratios than those obtaining for water vapor may be used. Argon and propyl alcohol is therefore a favored mixture. Argon also has a higher electron density than air, and therefore offers more intense ionization and easier photographic recording procedures. It is unsuitable, however, for some purposes, such as energy measurements on electrons, based on the curvature of their paths in a magnetic field, because of the much higher probability of nuclear scattering, which rises with the square of the atomic number. Here hydrogen is distinctly the preferred filling gas, as the deflections due to elastic collisions with nuclei are less than 1% of their values for argon or air.

Elaborate and rugged automatic control apparatus has been developed for cloud chambers, so that they can be operated entirely unattended if desired. The expansions may be made according to a regular cycle of operation with a periodicity which maintains a uniform ambient temperature, or the chamber can be set in operation by the passage through it and neighboring coincidence counters of a particular ionizing ray whose photograph is desired, as is often done in cosmic ray studies.

The detailed design of an individual cloud chamber should depend upon the purpose for which the chamber is intended, and there are almost as many individual varieties as there are workers in the field. Usually cloud chambers are the order of 6–12 inches in diameter, but, as can be seen from a consideration of their operating principles, very small and very large chambers are equally possible. If the ionization to be studied is very dense, as in the case of recoil nuclei, pressures as low as a few cm. of Hg may be used (Joliot, 1934). For studies of processes giving very diffuse ionization, pressures up to several atmospheres have been used (*e.g.*, Brubaker and Bonner, 1935).

4. Autoradiographs

All ionizing radiations affect a photographic emulsion, producing a latent image which is developable by the usual photographic techniques. The track of a single ionizing particle, such as an electron or an α-ray, is seen as a series of developed grains, spaced about 2μ apart in the case of α-rays. The detailed mode of action of ionizing rays on a photographic emulsion is imperfectly understood, but by empirical methods a number of special emulsions have been perfected which are sensitive to heavy ionizing rays such as α-rays and fission fragments, but are almost insensitive to visible light. For β-rays, either X-ray film or medium contrast lantern slide plates are usually preferred.

It was the action of the radiations from uranium on a photographic emulsion which constituted Becquerel's experimental discovery of radioactivity itself. Mügge (1909) first employed autoradiography extensively to disclose the distribution of radioactivity in minerals. Many recent workers have used the photographic emulsion to obtain a visible representation of the distribution of artificially radioactive isotopes in a variety of substances, including plants, insects, animal and human tissues, and ores in which radioactivity has been induced in tungsten, gold, manganese, *etc.*, by neutron irradiation.

The biological and medical studies employing autoradiography have been reviewed recently by Gross and Leblond (1946). In general, the specimen is either laid directly on a photographic emulsion, or a sheet of mica, cellophane, or thin paper is laid between the specimen and the emulsion in order to minimize the chance of a false result due to uneven mechanical pressure or to chemical action on the emulsion. Recently Belanger and Leblond (1946) have perfected a technique of fixing histological slides which permits the addition of melted photographic emulsion directly to the histological specimen. This gives much more intimate contact and better resolution. After development of the autoradiographs so obtained, it is still possible to apply biological stains to the combined tissue specimen and autoradiograph. Thus, the autoradiograph can be studied in perfect register with the stained tissue.

The time of exposure required depends on the energy of the β-rays, the specific activity and thickness of the tissue, and the type and thickness of emulsion used. For ordinary lantern slide plates, something of the order of 2000–10,000 β-rays/mm.^{2} are required to produce a good degree of blackening.

For high resolution autoradiography, tissue slices of 2–10μ thickness should be used. The resulting autoradiographs can be magnified satisfactorily by 60–100 diameters for examination. The limit of resolution, due to graininess of the emulson and to spreading of the β-radiations, is usually of the order of 25μ (Hamilton, Soley and Eichorn, 1940). Thus, the visualization of the distribution of a radioactive isotope within a single cell of ordinary size is not yet possible. However, much valuable information can be obtained on the distribution of groups of various types of cells, such as the iodine-collecting cells of the thyroid gland. Recently Pelc (1947) has obtained greatly improved resolution, of about 4μ with I^{131}, by using stripping emulsions and generally improved techniques.

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Health-Physics, Instrumentation, and Radiation Protection

By H. M. PARKER

CONTENTS

I. Scope of Health-Physics

1. Introduction

Shortly after the discovery of X-rays and radium, the damaging properties of penetrating radiation were observed. The earliest injuries affected the surface of the body, notably the fingers and hands, and steps were taken to adjust the exposure of the individual in such a manner that these injuries would not arise in future cases. It was some time before more insidious forms of damage, frequently not accompanied by easily recognized early signs of injury, were observed. This period was one in which many people exposed to radiation were subsequently afflicted with anemia. Throughout the history of the use of penetrating radiation, steps have been taken to organize the work program in such a manner that observed injuries would not be reproduced in the future. In practically all cases, protection has been a *corrective procedure* following a series of misadventures, and in some cases there was a situation approximating a public scandal before suitable remedial measures were established. Only within the last few years has there been attention to prophylactic action against new hazards before injury experience was developed. In a general sense, Health-Physics has existed for 50 years, of which the last 25 have seen steady improvement in the techniques involved. The particular title, Health-Physics, was a product of the war-time organization within the Plutonium Project. There has been no absolute definition of the scope of the proposed new subject of Health-Physics, but in general it is concerned with the physics and biophysics involved in the interaction of radiation with the human body, with special emphasis on the protection of radiation workers against the potential hazards of their occupation. Health-Physics is, thus, a restricted aspect of Medical Physics in which the basic intention can be seen from a study of proposed alternative titles.

2. Nomenclature

While Health-Physics was used as the descriptive title at the Metallurgical Laboratory in Chicago and at the Clinton Laboratories in Oak Ridge, the equivalent organization at Hanford Engineer Works was called the "Health Instrument Section," (commonly abbreviated H. I.) for security reasons. The title "Radiation Hazard Control" has also been proposed, and this is probably the closest short description of the scope of the program. A less ambitious title, "Hazards Evaluation" has recently been introduced at the Argonne National Laboratory. The intention here is similar except that a measurment or evaluation of the hazards without any promise of effective control is implied

The pre-war title "Radiation Protection," which is understood to mean the protection of workers against radiation, is certainly not out of place, and would be more directly in line with previous experience outside the Manhattan District Project. Perhaps the only justification for regarding Health-Physics as a new subject is that the recent large-scale expansion in the use of radiation and radioactive materials has apparently established the need for a group of men engaged full-time in the considerations of radiation protection. In previous experience, medical physicists were required to give only part-time attention to the protection problems involved in their occupation. Whether Health-Physics will become a permanent branch of Medical Physics of sufficient magnitude to justify separate definition is a matter for debate; it is entirely conceivable that the subject approximates its maximum status at the present time. Despite the fact that utilization of nuclear machines and other uses involving radiation and radioactive materials will undoubtedly expand during the next few decades, it is to be expected that the methods of hazard control will become sufficiently stabilized that specialists may no longer be required to regulate safety aspects, with the exception of the consultant services of a small group of qualified experts. This would restore the relative importance of the subject to its prewar status.

3. *Nature of Advances*

Regardless of nomenclature, *fundamental* advances in the protection aspects of Medical Physics have been few or nonexistent. This by no means belittles the advances that have been made. It does imply that they are not of fundamental research character, but fall more into the development field or are practical problems of group organization and protection policy. This chapter will describe these phases, and will at the same time specify indirectly what is meant to be included in the expected proper field of Health-Physics.

Health-Physics is a borderline subject surrounded by Industrial Medicine, Radiobiology, Industrial Safety, Public Health, Physics, Engineering and Chemistry. The area between these boundaries is ill-defined. Whether any area at all is to remain will be largely determined by the experience of laboratories and industrial organizations with the present Health-Physics groups. The immediate prospect appears to be that such groups have thoroughly demonstrated their value and adequacy in the case of industry, whereas laboratory groups may cover the same territory by suitable extensions of the various surrounding subjects.

II. Past Experience in Protection

1. *Luminous Paint Industry*

Of the various modes in which radioactive materials have been used in the past, that concerned with the handling of radioactive luminous compounds has received the most careful consideration. Acceptable standards for the handling of such materials have been published in the National Bureau of Standards Handbook H-27 (1941). The known hazards, in the order of their importance, are, (1) ingestion or inhalation of solid radioactive luminous compounds; (2) inhalation of radon liberated from compounds into the air; and (3) exposure of the whole body to γ radiation from compounds.

A. RADIUM POISONING

Accumulation of small quantities of radium in the body may result in eventual damage to the blood-forming organs, or to the bone-forming cells, with malignant change as the end-result. Such injuries have been known to result fatally when the fixed radium content of the body was approximately 1 μg (Evans, 1943). The accepted limit for radium deposition in the body is 0.1 μg. The inhalation of radium dust is more hazardous than its ingestion. Approximately 100 μg administered orally, or 20 μg intravenously or by inhalation results in the deposition of 1 μg of 'fixed' radium (Rajewsky, 1939). In the past there has been inadequate attention to the possible concentration of radium dust in the air, whereas ingestion, which now must come largely from transfer from the fingers, is rather easily and routinely controlled. Emphasis on the ingestion hazard is possibly a legacy from the early catastrophe in the widely-known New Jersey poisoning case in which the material was primarily ingested by the pointing of brushes at the lips. In current plant practice, the greatest body radium content frequently is found not in dial painters, but in inspectors and assembly workers who remove small chips of the dried compounds in the course of their work. The protection of radium-dial painters against the accumulation of radium in the body depends largely on good housekeeping, which includes the provision and maintenance of a clean work location, and of suitable protective clothing. Power ventilation is used to remove radium dust as well as escaping radon. Routine breath radon analyses are used as the most sensitive index of radium accumulation. A radon concentration of 1 $\mu\mu$c/l. of air is said to correspond to a body content of about 0.1 μg Ra. However, there is no general agreement that the approximately 50% escape of radon represented by this figure always occurs. For radium of long fixation, emission as low as 7% has been quoted (Jones and Day, 1945). Despite the precautions taken, substantially all dial painters, inspectors and assemblers ingest or inhale a sizeable fraction of the tolerance amount of radium, and about 15% exceed the tolerable amount (Evans, 1943). In another report, 30% of workers of less than one year's standing and 60% of all others showed breath samples above tolerance (Morse and Kronenberg, 1943). The worker removed from radium exposure usually excretes enough radium to restore the content to less than the tolerance amount. Experience of overexposure is reported from locations in which considerable care is taken to offer proper working conditions to employees. It represents the results of a philosophy in which temporary excursions above the tolerable amounts are considered proper. The efficacy of this approach depends on the frequency of the breath tests, the numerical accuracy of the results, and the extent to which temporary overexposures are harmless. In one published series, a maximum of 850% of the tolerance amount was observed. With breath analyses, every 4–6 months, a substantial portion of the radium in the body at the time of test may be permanently fixed, and accumulations of this order of magnitude would not readily be excreted down to the tolerance level (Hoecker, 1944). Conservative practice should lead to a work system in which the deposited amount *at no time* exceeds the stated tolerance value. Routine measurements of active dust concentration in the air seem to be uncommon, despite the ease with which they could be made, and the stimulus they might give to the maintenance of improved conditions.

B. RADON EXPOSURE

Evidence on the exposure of humans to radon in the atmosphere is less definite than that arising from deposits of radium in the body, but it is generally accepted that a tolerance value of 10 $\mu\mu$c Rn/l. is safe. In European practice, 100 $\mu\mu$c Rn/l. has been frequently used as an acceptable standard (Russ, 1943; Jones and Day, 1945). Maintenance of an acceptable air concentration in factories has been achieved by suitable design of a ventilating system. An intake-rate of about 50 cubic feet per minute per hood is the customary standard. The accepted standards of ventilation appear to insure that the room air content does not exceed 30% of the tolerable concentration. However, in a survey of 10 plants, Evans showed about 15% of the samples above tolerance. These values occurred mainly in storage or packing rooms and in offices not provided with suitable ventilation. Morse and Kronenberg reported excessive concentrations in 60% of the tests. Wartime experience in England has shown normal concentrations in luminizing rooms and radium laboratories, in the range of 20–100 $\mu\mu$c/l., with some values as high as 8000 $\mu\mu$c/l. There has been no apparent ill effect up to the present time, but the experience should ultimately be of interest in determining whether the American standards are unnecessarily stringent.

C. GAMMA RADIATION

The third hazard, namely, that arising from the external irradiation by the γ-rays from radium and its decay products, is no longer a serious consequence in any location in which a legitimate attempt is made to meet the recommended standards of protection. Control is achieved by limitation of the working amount of compound, and by housekeeping to reduce general irradiation in a room. The normal exposure appears to be about 20 mr/day.

D. GENERAL POLICY

The provision of the necessary protective mechanisms in this industry is expensive, and its continued proper operation requires careful examination. In modern plants it appears that the proper safeguards lead to conditions close to the supposed tolerance standards, an acceptable condition if those standards have been written with a reasonable margin of safety. However, there is not equal diligence in all parts of the country in maintaining such standards. Relocation of luminous paint industry in regions which have not previously had occasion to regulate such operations may lead to a relaxation of standards. Education of radium workers in the hazards of their occupation is one of the best mechanisms for promoting the preservation of good conditions. The trained worker can assist the conscientious employer in the preservation of safety and can suitably guide himself in other cases.

2. X-Radiation in Hospitals

X-ray protection has received detailed consideration, especially by the Advisory Committee on X-Ray and Radium Protection, which has published and revised at suitable intervals a unified set of safety recommendations (National Bureau of Standards Handbooks HB-15 and HB-20). Valuable additional details have been published by many authors (Braestrup, 1938, 1942; Taylor, 1944; Glasser *et al.*, 1944). For protection considerations, the field of X-rays is subdivided into five classifications: (1) diagnostic, (2) superficial therapy, (3) deep therapy, (4) super-voltage therapy, and (5) multimillion-volt therapy. In each case, there are two primary types of possible protection failure; the one being accidental exposure to a single damaging dose or excessive dosage in a short series, and the other the eventual damage arising from prolonged exposure to low intensity radiation. Accidental exposures to physicians and technicians arise primarily from carelessness in entering the radiation beam, while accidents to patients come from omission of the proper filter, treatment at the wrong distance, treatment for an excessive period, or by an outright electrical defect in the equipment. Few, if any, of these occurrences fall outside the category of negligence on the part of physicians or technicians; their occurrence in modern usage is rare, and many are beyond the scope of Health-Physics. The hazard of principal concern is that arising from the small exposures which occur daily, and in this field there is a rather sharp differentiation between the diagnostic and therapy cases.

A. FLUOROSCOPY

In fluoroscopy, the physician must necessarily observe the fluorescent screen. The screen itself has to be backed by glass of sufficient thickness to reduce the transmission of radiaton to a safe level, and be surrounded by wings of protective material of adequate thickness. Protective aprons and gloves may be required by the physician or technician where the equivalent protection cannot be built into the equipment itself. The requirements are well known, but not always well respected. Possibly the greatest hazard in fluoroscopy arises in those cases where the physician is required to introduce his hands into the direct beam for palpation. This can be done safely when lead rubber gloves are worn. In the practice of some institutions, however, these examinations are made without protective gloves, the exposure received being held to a minimum by selection of the smallest possible irradiated field, and by fast operation. There is no unanimity of opinion on the permissible daily dose to the fingers under such conditions. The Mayo Clinic group has made careful attempts to measure the exposure which in their experience had proved to be safe (Cilley, Kirklin, and Leddy, 1934, 1935a, b). This was about 250 mr per day and as much as 700 mr per day to the finger tips. On the other hand, it has been suggested that radium exposures as low as 4 r per week have been known to produce injury (Parker, 1943). It seems evident that the restriction to 100 mr per day as required for whole body radiation is unnecessarily severe for this particular case, but whether 200, 500 or 1000 mr per day should be substituted has not been established. The continued widespread occurrence of finger damage among fluoroscopists should be sufficient to encourage the maintenance of a conservative standard. The risk of injury in fluoroscopy is greatest among those whose utilization of the equipment is sporadic. Orthopedic surgeons and veterinarians are particularly vulnerable in this sense, and reminders of the hazard (Harding, 1945) are much in order.

B. PHOTOFLUOROGRAPHY

The recent extension of photofluorography introduces additional hazards for both patient and operator in comparison with the previous routine chest film technique. The average entrance dose in photofluorography is about 1 r (Birnkrant and Henshaw, 1945), and as high as 2.5 r in some cases (Gamertsfelder, 1943). This exposure is approximately twenty times that required by direct radiography. It is preferable that radiation workers be given the advantage of the low exposure method. Repetition of a photofluorographic exposure, which appears to be required with significant frequency in some clinics, is especially out of place for a radiation worker. The increased exposure results in a corresponding 20-fold increase in the room scatter and may also lead to overexposure of the operator, if suitable restrictions on permitted approach to the patient are not maintained.

C. RADIOGRAPHY

Properly conducted radiography has none of the hazards peculiar to fluoroscopy. It can be conducted like superficial therapy. That the fingers of dentists and their assistants continue to show damage as a result of holding films in position is directly ascribable to inadequate training. The customary practice of having the patient hold his own films is presumably sound, but in one community of radiation workers a simple wooden device has been used to avoid this unnecessary exposure, and it is surprising that the same method is not universally employed.[1]

D. THERAPY

In the various branches of therapy, it is the exception to find injuries to physicians or technicians working under modern conditions of shielding with up-to-date equipment. The protection of the room in which the tube is housed is usually such that the normal work conditions give negligible exposure to persons outside the treatment room. The increasing use of electrical interlocks and similar safety devices helps to prevent such errors as operation of the tube with the lead protective doors open. Training of the operator to keep all except the patient outside the therapy room during treatment also seems to be successful. In one series of film tests (Clark and Jones, 1943), 84% of the films from a hospital read less than 100 mr per day, and 56% from a hospital where some diagnostic work was done. Two other locations, doing mainly diagnostic work showed 99% less than 100 mr per day. It should be noted that 200 mr was the accepted daily dose, and 93% of all cases came within this limit.

Protection of super-voltage therapy equipment has been for the most part at an acceptable standard, although greater possibility has existed in these cases for casual overexposure. This has sometimes arisen from the combination of the therapy tube with facilities for physical measurements, and is therefore not properly chargeable to X-ray therapy.

Multimillion-Volt Therapy, which is intended to cover the range from approximately 10 million volts up to perhaps 300 million volts, has not yet been extensively developed, but in this case an attempt is being made to establish proper safety standards before the equipment is used (Failla, 1945).

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[1] In the same location, the "avoidance of unnecessary exposure" principle has led to a critical attitude toward the use of radiation in shoe stores, *etc.*

3. Radium in Hospitals

The protection of workers against exposure to radium, particularly in the applications to hospital practices, has been formulated by the Advisory Committee on Radium and Radium Protection, and published as National Bureau of Standards Handbook H-23 (which superseded the earlier H-18). Protection is required against, (1) local overexposure to radiation, especially upon the hands, and (2) overexposure of the entire body. Frequently overlooked in the manipulation of radium sources is the possibility of leakage which may permit the emission of radon and its accumulation in dangerous quantities in confined spaces such as radium safes (Read and Mottram, 1939). In the past, there has been a record of injury produced by rather short-time exposure to γ radiation from radium, particularly at the time when the so-called radium bombs were first developed. In present practice, the hazards are essentially confined to those which arise from long-continued exposure at low radiation intensities. The planned application of radium to patients for therapeutic reasons can easily result in damage to the patient; this is the physician's responsibility. However, it is possible for the patient to be unnecessarily injured by the introduction of leaking radium needles, or by the superficial application of radium sources with improper filtration. These in part would be considered Health-Physics problems, and methods of avoiding such injuries should logically be included in the general rules for radium protection. More generally, the injuries from hospital handling of radium occur to (a) technicians assigned to the preparation of treating units, (b) therapists and nursing staff in the operating theatre, and (c) nurses when patients are hospitalized in wards.

A. HAND EXPOSURE OF TECHNICIANS

It is especially prevalent to find that technicians tolerate a fairly close approach of the hands to radium during manipulations. In the past, there has been no specific attempt to maintain the hand exposure at the limiting value for general exposure. In fact, as recently as 1934, 5 r per day was described as the permissible exposure for the fingers (Handbook H-18). At the present time, by far the greatest amount of injury to radium technicians occurs on the hands, and there are certainly cases in which hand exposure of closer to 5 r per week than 5 r per day have resulted in skin changes which may later assume a dangerous character. The conception that a certain degree of skin injury can be tolerated and that the technician may terminate his handling of radium sources at such a stage without the expectation of subsequent deterioration in his condition has been widely sponsored (Failla, 1932; Nuttall, 1943). Although true as applied to the primary injury, it appears that radiation effects skin changes in such a way that subsequent trauma, inconsequential in a normal hand, may lead to breakdown in the irradiated skin up to several years after the termination of significant exposure. Even the professional worker finds it difficult to avoid the minor trauma that is sufficient to initiate degeneration. Precise measurements of the actual exposure of fingers of radium technicians have been infrequent. The contribution due to γ radiation is fairly well known, both by measurement with thimble chambers and by calculation from time and distance of exposure, but the additions due to β radiation are quite variable. Where sources with a minimum filtration of 0.5 mm. Pt are employed, it is rare for the secondary β-ray dose to exceed 25% of the γ-ray dose. With other sources, especially glass capillary tubing containing radon, the β-ray contribution may be extremely high.

B. GENERAL EXPOSURE OF THERAPISTS

Whereas the risk of overexposure to the radium technician may be greatest with respect to the fingers, it appears that radium therapists should consider the general body radiation as the determining factor. Such men will usually receive more radiation to the hands than to the rest of the body. It is, therefore, tacitly assumed that the limiting tolerance is higher for the hands. It has been by no means uncommon in the past decade for therapists to retire from active radium work as a result of a progressive change in the blood count, used as an index of the welfare of the individual. It has been shown (Paterson, 1943) that the average worker in the Holt Radium Institute, Manchester, shows a permanent reduction in white-cell count in comparison with other persons with similar general surroundings without radium exposure. The stage at which white-cell changes can be considered harmful is not yet known, and modern tendency has been to regulate the exposure in such a way that the average white-cell count more closely approximates the normal value. It is of interest that the best evidence for a sustained and possibly non-injurious fall and reduction in the white-cell count comes from this Institute, where considerable attention has been given to proper protection (Nuttall, 1939). This Institute has operated with 200 mr per day as the tolerable exposure, and has in fact condoned exposures closely approximating 100 mr per day. Common practice in radium institutions has permitted daily general body exposure of about 70% of the tolerance value adopted. Experience in a typical teleradium installation has shown average daily exposures between 130 and 250 mr (Wilson, 1940). This experience is extremely useful in its applications here by providing a body of evidence of exposure at the supposed present-day tolerance limit. It is not known to what extent additional exposure was tolerated in England during the war period when staff reduction was necessary.

4. *Industrial Preparation of Radium Sources*

Preparation of radium sources may be considered to begin with the mining and refining of uranium ore. The health conditions under which this has been done have not been clearly defined except in the experience of the Canadian Great Bear Lakes deposits (McClelland, 1933; Leitch, 1935). The methods of protection in the purification stages are similar to the requirements in the luminous compound industry, with the exception that as the matter is further purified the hazard is increasingly that of general body exposure to γ radiation. The further stage of subdivision of the radium into capsules for hospital use has not been governed by general regulations of the type applied to the luminous paint problem. Such work is performed by relatively few companies, and the standards of protection accepted by them appears to be variable, and in some cases less desirable than the standards proposed by the Advisory Committee on X-Ray and Radium Protection. The average radon concentration in the general laboratory of one of the most reputable companies has been quoted as 2200 $\mu\mu c/l$. (Read and Mottram, 1939). The operation of radon plants, either commercially or in hospitals, can be one of the most hazardous modes of handling active materials since it may lead to radon contamination of the air as well as hand exposure to β radiation and general body exposure. Such installations as that designed and built by J. E. Rose in the U. S. Marine Hospital, Baltimore, Md., however, demonstrate many of the best protection practices. In general, a radon plant should be located in a separate building to localize contamination.

5. *Industrial Radiography*

Radiography has had to be guided by the recommendations of the Bureau of Standards that were specifically made for hospital application. The wartime publication of rules by the American Standards Association (1945) has been a valuable advance. More could be achieved by future closer liaison between all organizations using radiation methods. Industry has developed considerable experience with supervoltage X-radiation, and the use of betatrons at 20 MV and even up to 100 MV may be common practice within a year or two. Radium experience, especially in naval ordnance, has also been extensive. The wholesale production of fission products has opened up the portentous possibility that sources of 100 or 1000 curies may be used in the future for radiography where sources of small dimensions are required.

6. *Transportation of Radioactive Sources*

The shipment of radium, radon or similar radioactive substances through the mails has been prohibited by postal regulations in the United States.

Shipment of radium up to 100 mg. in a single shipment is permitted by Railway Express under a well-established code of protection (Curtiss, 1941). These regulations were designed to prevent damage to photographic films, an immediate effect subject to damage claims. Possible damage to individuals by the transportation of radioactive sources may not become evident for a considerable length of time, and the possibility of claim and subsequent correction of malpractice becomes poor. In this instance, the public has been protected against exposure hazards by the greater sensitivity of film than of the individual. Shipment of larger quantities of radium has been performed either by special license or in some instances by courier, in which case persons in the vicinity of the source have not always been satisfactorily protected. The existing regulations have been rendered inadequate by the recent increase in shipment of active materials and by unexpected changes in its character. The establishment of revised regulations is one of the most important functions of any organization concerned with radiation protection. These will have to include special consideration of neutron sources, active materials with parent-daughter relationships, where the hazard increases during shipment, liquid samples and α-emitters where the hazard is not detectable by such simple means as Geiger counters.*

7. *Universities and Similar Institutions*

With the development of atomic and nuclear physics, particle accelerating equipment—such as high voltage X-ray machines, positive ion tubes, cyclotrons and linear accelerators—have become familiar features of the average University laboratory.

Pioneer work has been done with this equipment, and it is unreasonable to expect the highest standards of protection at all times. The radiation physicist was required to protect himself against neutron irradiation before the neutron beam could be used to irradiate patients or even animals. The radiochemist prepared new radioisotopes before the biochemist could determine their metabolism. That very few men have been permanently disabled by radiations from these machines and their by-products is much to the credit of the scientists responsible for these installations. Nevertheless, when the pioneer stage has been passed, there has been some reluctance to install safeguards comparable with industrial safety practice.

A. RADIOCHEMICAL MANIPULATION HAZARDS

At the low level of activation produced by exposure to most nuclear machines, no particular damage has in general occurred as a result of sam-

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* *Note added in proof:* At the request of the Interstate Commerce Commission, the Bureau of Explosives, Association of American Railroads, assisted by the Subcommittee on Shipment of Radioactive Substances of the Committee on Nuclear Sciences of the National Research Council, formulated such improved regulations, effective January, 1948.

ple manipulation. This has been helped too by the fact that much of the handling has been done by graduate students not assigned to such work for prolonged periods. This has fostered the belief on the part of many of the leading radiochemists in the country that the hazards from hand exposure in the range of 200 mr per day to 2 r per day have been greatly exaggerated by the health physicists. At the present time, this is one of the major potential weaknesses in radiation protection in the country. Even when an attempt is made to maintain safe levels, the measurement of contact exposure with an instrument suitable only for remote measurements frequently gives results low by one or two orders of magnitude.

Future experience will be with sources which produce activated materials of greatly higher intensity. A change in work habit as a result of increase in source strength is one of the more difficult developments for the scientists to make, since the chemical and other associated properties remain unchanged, while only the relatively intangible radiation levels go up. The advantages of proper shielding and remote handling in future radiochemical operations cannot be too widely publicized (Levy, 1946).

B. GENERAL RADIATION INJURIES

In addition to the hazards of radiochemical manipulation of laboratory materials, the generating sources themselves are a potent source of injury, and it is recognized that they have, in many cases, been operated under conditions which depart from the ideal. Again, the number of eventual injuries which may arise from long-continued exposure at low intensity is not known, and is probably held to a minimum by the frequent turnover of personnel under University laboratory conditions. Attempts have been made to specify proper protection against new types of radiation (Aebersold, 1941; Warren, 1941). In the more immediate field of visible radiation injury occurring either in a single accident or as a fairly rapid result of overexposure, more can be stated about the customary standards achieved. There appears to be no comprehensive survey of the frequency rate of such palpable injuries in radiation laboratories, but a private survey led the writer to propose the figure of one palpable injury per 30-man-years of active employment in radiation work. Subsequent discussion has indicated that many health physicists would accept a higher rate such as one injury per 20-man-years as a representative value. Whether the rate is 1:20 or 1:50 years, it compares very unfavorably with a record such as that at the Hanford Engineer Works where over 3,000-man-years of active exposure have been compiled without a single palpable radiation injury. Original research work carried on in laboratories is of such a character that safety in handling cannot be expected to equal that in a well-regulated, established industry. However, the differences are sometimes over-emphasized, and, in the particular example cited, the first operation of Power Piles and the wholesale production of plutonium can hardly be described as well-established industries. The postwar interest in all the major radiation laboratories in maintaining radiation safety comparable with Project standards may be a deciding factor in the permanent establishment of Health-Physics.

8. Manhattan Project Experience

A. DEVELOPMENT OF THE HEALTH DIVISION

The anticipated scale of the radiation quantities involved in those parts of the Manhattan Project which were to be concerned with the development of chain-reacting units led to a fairly early recognition of the need for the establishment of an extensive Medicophysical organization for the protection of workers against deleterious effects of radiation. The Health Division, under the direction of Dr. R. S. Stone, worked along three major lines:

"(1) Adoption of pre-employment physical examinations and frequent re-examinations, particularly of those exposed to radiation.

"(2) Setting of tolerance standards for radiation doses and development of instruments measuring exposure of personnel; giving advice on shielding, *etc.*; continually measuring radiation intensities at various locations in the plants; measuring contamination of clothes, laboratory desks, waste water, the atmosphere, *etc.*

"(3) Carrying out research on the effects of direct exposure of persons and animals to various types of radiation, and on the effects of ingestion and inhalation of the various radioactive or toxic materials such as fission products, plutonium and uranium. (From Smyth, H. D., 1945)."

Paragraph (2) comes close to a definition of the Health-Physics activities on the Project. The purely medical activity of Paragraph (1) developed more and more along normal industrial lines, as the incidence of significant radiological findings proved to be essentially zero. Radiobiological research as in Paragraph (3) provided invaluable data for the progressive improvement of tolerance dose data, and in some cases there was a satisfactory integration between Radiobiology and Health-Physics.

The early work in the Metallurgical Laboratory differed neither in principle nor in magnitude from that previously practiced in several University laboratories. Since it developed from the putting together of the work of several such groups, the initial attention to radiation protection followed the standards of the parent institutions. In this period, also, the available staff for improvement in the hazard control and the necessary instrumentation for this purpose were both in short supply. However, after the successful operation of the first chain-reacting unit in December, 1942, there was, throughout the Project, a steadily increasing interest in radiation hazards. The introduction of industrial organizations of the regular kind helped to stimulate the extension of protection rules and their more rigid observation. Those branches of the Project under such industrial control rather naturally became leaders in the establishment of functioning Health-Physics service organizations. The parent organization in the Metallurgical Laboratory continued to make valuable contributions, especially in the design and fabrication of new instruments.

B. TYPICAL EXPOSURE PROBLEMS

(*a*) *Shielding.* In principle, the Project program posed no new problems with regard to the irradiation of personnel by penetrating radiation. The required shielding of Pile units and of the chemical equipment involved in the Separations Process was deduced by a logical extension of existing modes of calculations of the absorption of radiation in such materials as concrete, steel and lead. Uncertainties in the calculation of the transmission through unusually large thicknesses[2] of these materials did not exceed a factor of perhaps 3, and if an entire structure was designed for an intended safety factor of 10, no great harm would have been done by finding a residual safety factor of 3 or 30. For Pile units themselves, the calculations of neutron absorption in the proposed shields represented a greater extrapolation from previous information. The early work of the theoretical and experimental physicists in this field was substantiated when the Pile structures were actually in operation. One interesting factor of the protective measures required in the new designs was the necessity for protection of workers from "sky-shine," *i.e.*, radiation scattered by free air from a primary beam which itself would not irradiate the observer. This had not been of practical consequence in earlier experience unless one considers a limited case such as the exposure of an observer standing just outside a primary X-ray beam. No new phenomenon was involved in the sky-shine calculations, which were essentially an exercise in the application of the Klein-Nishina formula. As a corrollary to shielding, the development of remote-control handling, both for laboratory tools and on the grand scale, became a fine art. Although steered by health physics, credit for this phase belonged to design engineers.

(*b*) β *Radiation.* The effects of β-ray exposure had to be closely considered, although, in fact, this experience was no different from that intrinsically available in the earlier radium knowledge, when the use of β-ray sources was a common practice. Reliable dosage calculations of these cases

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[2] Greater than 3 feet of concrete or equivalent. The maximum thickness in standard tables was 10 cm. Pb, or 54 cm. concrete (Taylor, 1940).

proved to be so infrequent that the subject was essentially a new one. These β-ray exposures were expected to arise primarily on the hand, forearm and face; and typical sources of the activity were the natural uranium itself, materials activated in Piles, and the fission products. It was late in the Project history before biological data on controlled β exposures was available (Life, 1947).

(*c*) *Metabolism.* The metabolism of the anticipated fission products, and of uranium and plutonium, became of prime importance in the consideration of how much of each particular element could be allowed to come in contact with the individual by such methods as skin absorption, ingestion, inhalation, and introduction through open wounds. With the exception of uranium, this metabolism had to be evaluated by animal experience with meager amounts of the active materials, prior to the time that the operating Piles made them available in quantity. It was obviously desirable to have at least provisional answers on the toxicity of all these materials before this stage of plentiful supply was reached. The work of the biological organizations at the Metallurgical Laboratory, and later at Clinton Laboratories (together with the invaluable contributions of many associated laboratories, notably the Radiation Laboratory, University of California, at Berkeley, and the University of Rochester, Rochester, New York), cannot be evaluated too highly in the final assessment of the merit of the health protection of the Plutonium Project. Despite these efforts, the development of precise information on toxicity of radioactive materials at low levels over long periods of time could not be expedited by accelerated experiments, nor could the findings in animals be translated into the human case in terms of numerical tolerable dose. Fortunately, the relative deposition of active material within the animal was a good index of the corresponding deposition in the human case, and, where the toxicity was a function of the radiation effect, the health physicist could calculate the limiting intake of the noxious material to produce a specified dose in the organ most affected. This was the mode of approach to the establishment of tolerable dosage for the various fission products before any extensive body of exposure information had been accumulated.

(*d*) *Waste Disposal of Fluids.* As indicated in the Smyth Report, the operation of the facilities at Clinton Laboratories involved the emission of active gases, particularly radioxenon and radioiodine, into the atmosphere. It was the responsibility of the Clinton Laboratories to determine conditions of operation under which such emission would cause no hazard, not only to the operating personnel in the laboratories but to the large public area over which these gases might have been disseminated (Parker, 1944). This was successfully accomplished, and the method of approach established a satisfactory standard for the consideration of allied problems at future locations faced with the problem of large-scale disposal of active gas. The second problem was the related one of disposal of active liquid wastes. This was solved by the retention in underground storage of all very active wastes and the dilution of other materials to such an extent that no possible interference with the safety of public water supplies was effected. The required standards for waste disposal of this type constitute one of the most pressing problems for future Health-Physics considerations. This is particularly true in connection with the operation of radiochemical laboratories in urban areas, where the disposal of certain types of waste to the sewer is complicated by the precipitation or absorption of the active material in the sewer pipe or its soil contents. Under unfavorable conditions this could produce a hazard at a much later date to unsuspecting persons repairing or otherwise handling such pipes. In large bodies of water, the concentration of activity in algae or in colloidal materials with its possible utilization by fish, later used for food, presents a chain of events of great consequence to the public health. Up to the present time, these problems have been bypassed by ultraconservative policy in waste disposal, but future pressure for economical disposal facilities greatly point the need for extensive research on these problems.

(*e*) *Waste Disposal of Solids*. All future radiation organizations will be faced with the disposal of solid contaminated items which may range from collections of waste paper used to clean contaminated equipment to large pieces of equipment which are so contaminated that their restoration to service would be more expensive than their replacement. As regards its basic nature, this problem is no new one, since it is well-known that the photographic industry has had to institute routine tests for activity of the paper used to package photographic film, as a result of the uncontrolled return for reprocessing of paper contaminated in luminous compound operations. If this source is enough to contaminate the paper stock of the country, it is easy to visualize the condition set up by the indiscriminate use of similar pieces of paper from operations which dwarf the activity of the entire radium industry. Obvious means of preventing the return of such material to the public domain are storage in special vaults, burial in underground pits, or dumping in the sea, which are currently employed. The implications of contamination of the stocks of pure metal, for example, which might occur by careless release of contaminated materials or scientific equipment require careful consideration by some Advisory Board of high caliber.

(*f*) *Protective Clothing and Decontamination*. Although special clothing is provided in such industries as luminous compound operations, the requirements of the Manhattan Project for deconatmination of clothing, equipment and personnel, constituted almost a new subject. There appears to be no absolute guide to the preferred method of decontamination, but information of general interest is that fission products can be most suitably removed from clothing by laundering in dilute acetic acid, and that, whenever the condition of the equipment warrants its use, nitric acid is a preferred cleanser for practically any radioactive material. For the removal of radioactive contamination from the hands or other parts of the body, a titanium dioxide paste has been found effective against fission products, while an application of potassium permanganate solution, followed by sodium bisulfite, is effective against Plutonium contamination. The use of nitric acid and hydrochloric acid on the hands has been condoned under carefully regulated conditions.

(*g*) *Safety Performance.* The overall safety record of the Project enterprise has been remarkable. At the Hanford Engineer Works, the largest organization concerned with virtually all the forms of activity of practical interest, the exposure record for 1946 shows, for example, that no single individual received as much as 200 mr in any one day; that only 4 individuals received more than 100 mr in any one day; and that the average daily exposure of all radiation workers was less than 5 mr per day. There has been no known instance of the ingestion, inhalation, or other mode of introduction of a damaging amount of radioactive material into any individual. This standard was achieved with a working force of less than 3% of the total payroll. The financial considerations involved have, therefore, not been out of proportion, although it has been suggested (Bale, 1946) that industrial economies might lead to a reduction in the present coverage. It is felt, rather, that in the future expansion of radiation work, the laboratories and similar institutions might be the ones which would apply somewhat less than the present rate of 3% to the protection program. It is just these institutions which most need to maintain a technical force engaged in hazard control, because of the variable nature of the basic hazards encountered in a research program. In the industrial field, it is evident that stabilization of a given operation, additions to the protective equipment, and the provision of improved instrumentation, especially in the field of automatic recording, will lead to a logical and safe reduction in the full-time protection force. However, the foreseeable change is not such as to reduce the percentage representation appreciably below 2%.

III. EXPOSURE STANDARDS

1. Dose Units

Discussion of tolerance dose has been obscured in some cases by ambiguity or misinterpretation of the meaning of "dose" as applied to radiation exposure. "Dose," as intended in radiobiology, is a measure of the energy absorption of radiation per unit volume or mass of tissue. In practical usage, dose is measured in terms of the energy absorption in air which closely parallels the absorption in tissue for the particular range of X-radiation and γ radiation for which the principles of dosimetry were established (Gray, 1937a,b). The practical unit of dose, the 'roentgen,' corresponds to the absorption of 83 ergs/g. of air. This is usually considered equivalent to the absorption of 83 ergs/g. or /cc. of tissue.[3] It is convenient to use subsidiary dose units based on the energy absorption/cc. of tissue. That "dose" of any ionizing radiation which produces energy absorption of 83 ergs/cc. of tissue is defined as 1 rep. This is an abbreviation of "Roentgen Equivalent Physical," an expression which replaces such other titles as tissue roentgen, roentgen equivalent, or equivalent roentgen, which have become familiar in the literature. This unit may now be applied to X-radiation, γ radiation, α, β, proton, or neutron irradiation. It is well known that equal energy absorption arising from exposure to these various radiations will not produce the same biological effects. When the additivity of different types of radiation is to be considered, an additional unit—the "rem"— is introduced. One rem is that dose of any radiation which produces a biological effect equal to that produced by one roentgen of high-voltage X-radiation. The title "rem" is derived by abbreviation of "Roentgen Equivalent Man" or, optionally, mammal. It was selected in preference to the more obvious "roentgen equivalent biological" to avoid the confusion between "rep" and "reb" in speech. It is obvious that biological equivalence may depend on treatment conditions, protraction, fractionation, tissue exposed, and so on. In some cases, there can be no true equivalence since one type of radiation may produce damage of a character never observed with other modes of exposure. Moreover, the mere introduction of the unit "rem" does not solve the problem of writing down the numerical value of the equivalence, which remains a complex problem in experimental biology. Despite these disadvantages, the use of the expressions "rep" and "rem" has been found to simplify discussions of exposure to radiations other than X or γ radiation, and especially to the mixed radiations that have been common in the experience of the Plutonium Project. For tolerance purposes, only the relative values of "rep" and "rem" for long-continued exposure to low intensity radiation need be considered. An accepted scale of relation is as follows:

X-rays and γ-rays	1 r = 1 rep = 1 rem
β-Rays	1 rep = 1 rem
Protons and fast neutrons	1 rep = 4 rem
Slow neutrons	1 rep = 4 rem
γ Particles	1 rep = 10 rem

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[3] The actual energy absorption/cc. of tissue varies between 42 and 89 ergs/cc/r in fat, and between 87 and 95 ergs/cc/r in muscle in the energy range 12 KEV to 830 KEV (Spiers, 1946).

2. *Tolerance Dose*

For the present purposes, tolerance dose or permissible exposure will be assumed to be that dose to which the body can be subjected without the production of harmful effects. When the exposure considered is given on a single occasion, the tolerance dose or permissible exposure is clearly defined. Health-Physics is commonly concerned with the case in which repeated small exposures occur, and it is not entirely clear whether dose in this connection should refer to the total dose or to the element of dose in a given time. A suitable convention follows the latter view, with the given time as one day.

3. *Tolerance Dosage-Rate*

"Tolerance dosage-rate" has to be interpreted as that dosage-rate which is continuously tolerable. It will be maintained that the daily tolerance dose is 100 mr (in the general case). If one writes that the tolerance dose is 100 mr/day, it is argued that this is dimensionally a dosage-rate, and that one should write "tolerance dosage-rate is 100 mr/day." This statement has an entirely different meaning, and implies the existence of a maximum permissible rate of about 3×10^{-6} r/sec.

4. *Tolerance Dose Versus Tolerance Dosage-Rate*

The question of interpretation between "dose" and "dosage-rate" as applied to the tolerance problem is fundamental in the consideration of the permissible exposure of the body. The tolerance dosage-rate of 10^{-5} r/sec. was apparently introduced by Wintz and Rump (1931), who very clearly were equally content to quote a weekly or monthly total *dose*. The same election of convenient choice between tolerable exposure of 0.2 r/day or 1 r/week and tolerance dosage-rate of 10^{-5} r/sec. is contained in the current International Recommendations. It is entirely erroneous to suppose that these sources intended a *rate* in excess of 10^{-5} r/sec. to be considered hazardous. The utility of the rate was restricted to survey measurements around fixed installations, which would be safe if all readings fell below this limit.

The practical background on permissible exposure has, of course, come from exposures at much higher rates, *viz.*,

Occupation	Approximate Dosage-Rate of Exposure r/sec
Fluoroscopists: Hands	10^{-2}–10^{-1}
Body	10^{-4}–10^{-3}
X-ray Therapy Technicians	10^{-4}
X-ray Patients: Scattered radiation	10^{-3}
Radium therapists and technicians	10^{-4}–10^{-3}

The main body of experience has been in the range 10^{-4}–10^{-3} r/sec., and the rate of 10^{-5} r/sec. should receive no special significance. On the other hand, there is no sound information relative to high rates, except for erythema and therapeutic doses (McWhirter, 1935) where no significant rate dependence exists. In the tolerance field, daily short exposures at high rates *may* be more damaging than equal doses at normal rates. Simple control policy makes it impractical to condone receipt of a daily quota of 100 mr in less than 30 secs. This is a rate of 3×10^{-3} r/sec., only a factor of 10 from the ordinary case, and significant changes in this range are unlikely. For a single major exposure, of course, the damage produced appears to be a rapid function of the rate. Most, if not all, of the variation is due to the necessary change in total exposure time (protraction) in this single shot case.

The proposed restriction to a *daily* tolerance dose of 100 mr is arbitrary and occasionally unnecessary. One cannot prove (without a long animal experiment) that 200 mr and zero on alternate days or 300 mr followed by two clear days are unacceptable. For *control purposes only* the restriction to 100 mr per day is proper in almost all cases. Deliberate exposure of 100 N mr balanced by a radiation-free period of $(N - 1)$ work days is foolhardy if N exceeds 10, and unwise if N exceeds 3.

5. Foundations of Tolerance Experience

The development of any extensive experience of tolerance is restricted to exposure to X-radiation and γ radiation. The following account is included primarily to emphasize the scanty and inadequate data on which tolerance figures have had to be based even in these cases. Inadequate dose measurement and statistically insignificant groups have been common.

Although the first case of X-radiation injury was described in July, 1896, the first published tolerance dose appears to be due to Rollins in 1902. His photographic plate-fogging limit was perhaps 10 r/day. Early radiation injuries were primarily confined to the skin,—but the demonstrations of the radiosensitivity of the blood-forming organs (1904–1905) and of the reproductive organs of animals (1903–1904) carried fair warning that more dangerous damage than dermatitis could be anticipated. It is important and instructive, however, to note that the first *organized* step to insure protection from X-radiation was taken in 1915 (Russ). It has been pointed out by Henshaw (1941) that the war activity at that time resulted in delay on protective measures, and undoubtedly contributed to the large group of radiation injuries, especially aplastic anemia, manifest in 1919–1921. The American Roentgen Ray Society formed a Committee in 1920 to recommend protection measures, which were formulated and published in 1922. The British X-ray and Radium Protection Committee presented its first recommendation in 1921. The two sets of recommendations were similar and dealt largely with protective materials recommended for use in building X-ray and radium laboratories and apparatus.

At the second International Radiological Congress, 1928, protection proposals were carefully considered, and subsequently an International Committee on X-ray and Radium Protection was formed. The recommendations of this Committee contained no specific reference to a tolerance dose until the reports of 1934 and 1937 which described the tolerance dose as being 0.2 roentgen per day.

Mutscheller (1925) had published a tolerance figure of 0.01 of the erythema dose per month, based on measurements in several installations in which no apparent injury to the operators was being occasioned. This figure was later substantiated (Mutscheller, 1928) and subsequently extended (Mutscheller, 1934) for rays of higher penetration. Erythema dose for this quality was 340 r and the tolerance dose, therefore, 3.4 r per month, or about 150 mr per work day. The German Committee on X-ray and Radiation Protection (Glocker and Kaupp, 1935) accepted the same figure. Laboratory and hospital measurements by Sievert (1925) led to the same statement of safe dose. Typical of the necessity to extrapolate from insufficient data was the publication by Barclay and Cox (1928) of a safe daily exposure of 0.00028 of an erythema dose[4] on the basis of determinations on two individuals. This permissible exposure, equivalent to about 170 mr per day, was believed at that time to include a safety factor of 25-fold. A reconsideration of the early data (Kaye, 1928) led to a proposal of 0.001 of an erythema dose in 5 days, closely equivalent to 100 mr per day. The first comprehensive report on tolerance exposure to γ radiation (Failla, 1932) led to a value of 0.001 of a threshold erythema dose (radium) per month, or of the order of 60 mr per work day.

An important step in the improvement of protective practice in the United States was the formation of an Advisory Committee on X-ray and Radium Protection which published proposals in 1931 in the Bureau of Standards Handbook H-15. This and subsequent handbooks have been a model of sound approach to radiation hazard control. This first handbook recommended a tolerance dose of 200 mr per day, but the revised handbook (No. 20, 1936) quoted a value of 100 mr per day with no specific explanation

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[4] The translation of an early result expressed in terms of erythema dose to the equivalent roentgen dose is by no means clear-cut, but the following table includes representative values:

Quality	To Produce Erythema
Grenz rays	100 r
100 kv	350 r
200 kv	600 r
1000 kv	1000 r
γ-rays (radium)	1500 r (threshold erythema = $\backsim$1000 r)

of the reduction. In an independent publication, the chairman of the Advisory Committee referred to the safety value as 20 mr per day (Taylor, 1941). A League of Nations publication (Wintz and Rump, 1931) reviewed the various statements of tolerance dose, and concluded that the permissible exposure is 10^{-5} r per second, assuming a 7-hour working day, and 300 working days per year. This is equivalent to 250 mr/day. The exposure was qualified for persons "remaining in proximity to sources of radiation giving off rays without intermission," (*i.e.*, radioactive preparations) by reducing it by a factor of 3, or in round figures, to 100 mr per day. The limit of 100 mr per day has been widely established in American practice for both X-radiation and γ radiation although the higher value of 200 mr per day still remains in the International Recommendations.

6. *Recent Considerations*

Recently proposed changes in tolerable exposure for X or γ radiation are a fairly general desire on the part of health physicists to establish a lower rate for multimillion-volt radiation (Failla, 1945), as a precautionary measure until more is known about this field, and a suggestion that the exposure of women employees should be less than that of men (Lorenz, 1946), in consideration of the possible irreversible effects on the reproductive organs. In both cases, provisional values of 20 mr per day have been suggested.

The stimulus of the Manhattan Project has initiated much valuable experimental work in animals exposed over long periods of time to low intensity radiations. Not much is known of the results of these experiments beyond the fact that there is a general tendency to feel that the present limit of 100 mr per day is well chosen, although the safety factor involved is by no means as large as the earlier observers intended.

7. *Biological Aspects of Tolerance Dose*

For completeness in developing the current approach of Health-Physics to the tolerance problem, some considerations of the biological effects of radiation on the body is required; this is not the place for an extensive description or discussion of these effects. Radiosensitivity of tissues and its dependence on differentiation, rate of growth, and cellular environment are of basic importance in the study of the effects of radiation on the tissues. Also paramount is the distinction between threshold and non-threshold effects (Henshaw, 1944). Fortunately, the majority of radiation effects are thought to be of threshold type, which simplifies the practical problems of protection at the expense of introducing many variables into the manner in which radiation exposure can be legitimately received. The reversibility of radiation effects is of particular consequence in occupational exposure. This depends upon the reparative properties of the tissue, and where recovery is large the *total* radiation dose can be materially increased. When the potential damage in question is reversible, the protraction and fractionation of the given dose are major determinants of the end result. Repeated exposure initially followed by repair may eventually exhaust the regenerative reserve and result in permanent damage. Exhaustion of bone marrow reserve is typical. The dramatic decline in the welfare of the individual following a long period of apparent normalcy is one of the most disturbing problems for the physician and health physicist to face in the investigation of permissible exposure to any type of radiation. The damaging effects of protracted exposure are customarily divided up as follows:

A. GENERAL BODY EFFECTS

The early toxic signs in man resulting from external irradiation of essentially the whole body are (a) lassitude and fatigue (Nuttall, 1943), and (b) demonstrable effects upon the leucocytes of the blood (Goodfellow, 1935; Paterson, 1943; and many others). Either diminution in the total number of white cells or an altered ratio of neutrophils to lymphocytes are considered as possible indexes of early radiation effects, although it is well known that either may be caused by many other agents. Significant changes in the red blood cell picture are said to be late effects of continued overexposure. The coagulogram has been reported to be another critical index (Kaufmann, 1946). The consensus is that regular blood counts, carefully done, serve as an adequate early index of overexposure. Technicians can arrive independently at the same answer on a blood count within 10% (Cantril, 1943). The observation of the Manchester group (Paterson, 1943) that the normal white cell count of radiation workers is maintained at a level statistically demonstrated to be significantly lower than the general norm is of prime importance. If this can be accepted, then the white count index is certainly sufficiently sensitive, provided that the subjects of the Manchester experience are found not to suffer any eventual ill effects. Unfortunately, if shortening of life is one of the residual effects, then even the large Manchester group is too small to establish this difference, nor would the results be available soon enough to be of interest to the present generation of medical physicists.

B. INTERNAL EMITTERS

General body effects arising from internal radiation have been extensively studied in the special cases arising from the ingestion or inhalation of radium, mesothorium and their decay products. The effect on the blood-producing organs has been correlated with the level of radioactivity of the expired air (McClelland, 1933; Schlundt *et al.*, 1931).

Widely discussed, too, is the effect of radon inhalation in the lungs and its possible contribution to lung cancer in the Schneeberg miners (Peller, 1939; Dohnert, 1938). If lung cancer in man is essentially carcinoma of the bronchus, it can be shown that the expected daily dose to the bronchus for men in these mines is 300 mrep per day, approximately 20 times that derived as an average dose throughout the lung. Since the exposure is almost entirely due to α radiation, the equivalent daily dose would be 3 rem. By the same calculation, the tolerable concentration of radon in the air would be 10^{-13} curie/cc. to produce a bronchial irradiation of the permitted 100 mrem per day. It is of interest that this is the concentration considered tolerable in the British and Continental philosophy, and 10-times higher than the recommended value in the United States. Specific damage to the lung or bronchus is perhaps not properly included as a general body effect, but the inhalation of radioactive gases or dust has become a prominent hazard in recent experience, and whether the effect is limited to lung damage or becomes a general body effect is determined only by the rate of absorption of the toxic material through the lung surfaces. The general effects produced by the ingestion of radioactive material are only too well known, specifically with reference to radium (Martland, 1931). Careful studies by Evans and Rajewsky and their respective co-workers have shown that damage to the individual can occur as the result of the permanent deposition of approximately 1 μg Ra in the body, and the accepted safety limit is invariably taken as 0.1 μg Ra. This represents a daily irradiation of the average bone of about 16 mrep per day, or 160 mrem per day.[5]

C. SKIN EFFECTS

(*a*) *Erythema and the Layer of Passive Absorption.* The characteristic effect of large doses of X-rays and γ-rays upon the skin is the production of an erythema, the result of dilation of the fine capillaries, venules and arterioles supplying the skin. The outermost layer of the epidermis, the *stratum corneum*, consists of dead, hornified, flattened cells. The thickness of this outer layer may be 0.4 mm. on the palm, 0.8 mm. on the sole, and approximately 0.05–0.1 mm. elsewhere. This layer of passive absorption (Wilhelmy, 1936) may be considered as an *inert filter* which serves to protect the sensitive levels of the skin from injury by α-particles, low-energy β radiation, and extremely soft X-radiation. Prolonged exposure of the skin to natural α-radiation, therefore, produces no erythema and probably no

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[5] The average daily dose of the bone when the whole body receives the tolerable X-ray dose is:

500 mrep at 100 KEV (150–200 KV therapy)
250 mrep at 150 KEV (200–300 KV therapy)

where a volume rather than mass basis for the statement of 'rep' is used.

significant change in the skin condition.[6] On the other hand, skin injury can occur with accelerated α-particles where the rays can penetrate the horny layer (Larkin, 1941). In the average case, β radiation is well able to penetrate the passive layer and to produce severe skin injury. In very special cases, the additional thickness of the horny layer is of value in reducing the effective β-ray exposure. This might be the case, for example, if the palm only were involved. In laboratory manipulation of β sources, the exposure of the dorsal surface of the fingers usually exceeds that of the palmar surface, and here the passive layer is relatively thin.

(*b*) *Late Skin Damage*. The more insidious forms of damage to the skin occur in occupational exposure when the administered dose extends over one or more years. There may be at no time an erythema, and the first injury sign may be ridge changes on the finger tips, epilation, polishing around the nail beds, fissuring or ridging of the nails, and skin dryness. Later evidence of injury includes telangiectasis, pigmentation, atrophy, and thickening with the appearance of wart-like growths. Ulceration may follow minor abrasions, which heal reluctantly. This may progress to cancer of the skin which, in some 25% of the cases (Colwell and Russ, 1934), may spread beyond the hands. The widespread occurrence of skin injury has led to a diligent search for improved methods of early recognition. Dental compound impressions of the skin ridges of the finger tips have been used in one case, and have shown promising results where the picture is not obscured by ridge flattening due to other causes. Another promising approach has been the microscopic study of the capillaries of the nail bed region (Nickson and Nickson, 1946). As indicated already, uncertainties as to the permissible skin dose exists. A limit of 200 mrep per day on the surface of the skin would be generally considered acceptable.

D. EFFECTS ON REPRODUCTIVE ORGANS

This most dreaded hazard in the lay mind is usually considered subordinate to the effects on skin or on the blood-forming organs (Nuttall, 1943). Formal attempts to estimate fertility reduction in terms of the family-size of radiation workers have been obscured by the influence of other social conditions on this factor. There is no clear-cut evidence of such a reduction, and there is, in fact, some suggestion of an anxiety-stimulated overcompensation. For accidental short-term overexposure, permanent sterilization is produced by 400–600 r to the ovary, or 800–1000 r to the testes. There is a threshold dose which must be exceeded before any effect becomes evident. Early experiments in rats (Russ and Scott, 1937, 1939) show that continued exposure to γ radiation at 2 r/day led to a reduction

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[6] Presumably α emitters applied to skin could be absorbed and so penetrate the natural filter

in fertility. Extensive work at the National Cancer Institute stimulated by the potentialities of the Manhattan Project showed damage to the ovaries of mice at levels not far above 100 mr per day. The effect on the female mouse is significantly different from that on the male, as it proves to be an irreversible change, and therefore a function of the total dose and not the manner in which it is distributed in time (Lorenz, 1946). This has formed the basis for the recommendation of reduced radiation exposure of female workers.

E. RADIOGENETIC EFFECTS

An excellent discussion of the potentialities of the genetic effect in its relation to radiation protection has been given by Henshaw (1941). Extensive experimental work has been restricted to exposure of the fruit-fly in which it has been shown that the genetic effect has no threshold, and exposure is not only cumulative in the individual but in succeeding generations. On this basis, there would be no true tolerance dose, but rather an acceptable injury-limit. Similar considerations led to doubt concerning the safety of repeated fluoroscopic exposures of children (Buschke and Parker, 1942). Fortunately, several factors indicate that fear of the genetic effect may have been over-emphasized. In the fruit-fly, single exposure of 30–40 r doubles the low natural mutation rate, whereas doses of 500 r are required to produce recognizable mutations in the mouse. Dose-effect in the human case is entirely unknown. Speculations have been made regarding the contribution of cosmic radiation to such mutations; present information appears to exclude this possibility. It is also clear that the transmission of recessive characteristics would require the irradiation of substantial portions of the population, which is not anticipated. Of fundamental importance is a recent suggestion that the genetic effect in the fruit-fly when examined closely at low dosage is not truly of the non-threshold type.* If this were substantiated, and applied also in the human case, there might be no fear whatever of genetic injury arising from prolonged exposure at very low intensity, which is a necessary corollary of radiation work. This has far-reaching consequences in the present communities of exposed persons, where, for the first time, there is a significant percentage of intermarriage of exposed persons.

8. Dependence of Tolerance Dose on Nature of the Radiation

A. SPECIFIC IONIZATION

Although the mechanism of the interaction of radiation with living matter may not be fully understood, it is certain that one determining factor is the specific ionization of the radiation in the irradiated material (Zirkle 1940; Gray and Read, 1943). This has led to the acceptance on the Project

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* *Note added in proof:* Later developments in the experiments that prompted this statement appear to deny such a convenient escape from the problem.

of the ratio of 1 rep = 4 rem for neutron radiation, and the arbitrary extrapolation of 1 rep = 10 rem for α-particle ionization.

B. X-RAYS

The original choice of the general tolerance dose was, of course, founded on experience with low energy radiation with an effective wavelength of the order of 0.3 A. For 100 mr measured at the body surface (without backscatter), the tissue dose can be taken as 150 mr at 1 cm. deep, and 50 mr at 10 cm. deep. 400 KV radiation at the present time is as common as was 150 KV radiation when the tolerance was first established. The comparable depth doses with this radiation are 120 mr at 1 cm. and 50 mr at 10 cm. The systemic effect on the body is surprisingly constant over this range, assuming biological equality of the radiation. This is only true when the defining dose of 100 mr is measured "in air."

Little has been written about the damaging effects of radiation generated between 400 and 2000 KV. In this range the increase in percentage depth dose is not very large. It is reasonable to suppose that the tolerance dose established for 150 KV radiation with a margin of safety of 5 or better would still be entirely safe for X-radiation up to 2000 KV. Experience with teleradium sources should also be applicable in this case.

Multimillion volt X-rays show no striking increase in tissue ionization, because the increased penetration is offset by the fact that an increasing portion of the body falls in the transitional layer where equilibrium with the secondary radiation has not been established (Koch *et al.*, 1943). The relative integral dose at different radiation energies is plotted in Fig. 1, where the incident dose is supposed to be measured in a Bragg-Gray air walled cavity. Concern has been expressed over the excessive ionization in bone at high voltage, due to the pair-production effect in calcium and phosphorus. The figure shows that up to 100 MV at least, the energy absorption in bone, is comparable with that at 150 KV. At lower energies (*e.g.* normal 200 KV therapy), the bone dose caused by photoelectric absorption exceeds any corresponding increase due to pair-production at high energies. The calculations are made with allowance for absorption in tissue and with a simplified picture of the body distribution of bone. On this basis, it appears that a reduction of tolerance dose with deference to bone ionization is unnecessary. Preliminary biological experience at 20 MV shows no striking difference from 200 KV experience (Quastler and Clark, 1945).

C. GAMMA-RAYS

Adequate experience with γ radiation prior to the Project was largely confined to the use of radium and its products. It has been accepted that the danger of γ radiation in practice exceeds that of "traditional" X-radiation for two reasons:

(1) X-ray equipment can be de-energized when not in use,
(2) γ-ray penetration is higher.

On the basis of 98 mr at 1 cm. and 67 mr at 10 cm. one might have a factor of 2- or 3-fold to represent the additional total body ionization, compared with the X-ray case. The expression of tolerance dose in terms of total body ionization rather than in terms of the incident dose has recently gained favor (Clarkson, 1945). The unit employed for this purpose is the gramme-roentgen. It is worthy of note that the total body ionization changes much more rapidly as a result of the position of the exposed person relative to the source than as a result of quality change from soft X-radiation to radium γ-rays.

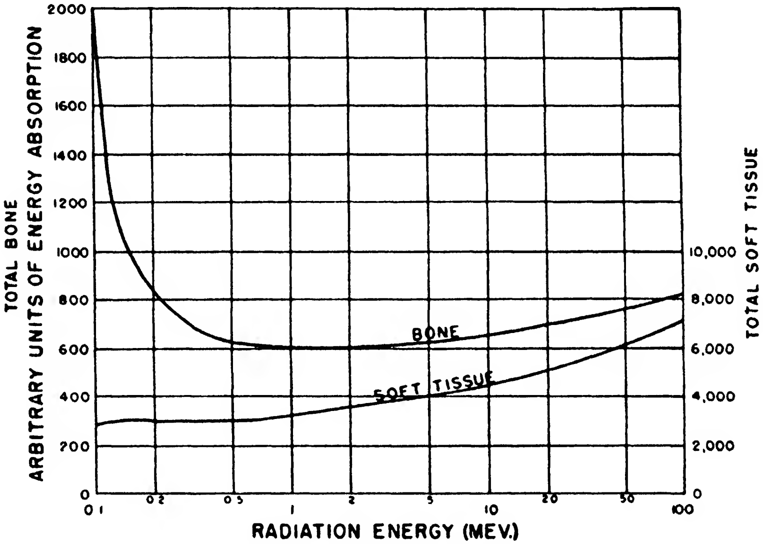

FIG. 1

Relative energy absorption in the whole skeleton and the whole soft tissue as a function of radiation energy, when the body is exposed to a remote source of radiation.

	Relative Total Body Ionization[7]
Soft X-rays from large distance	1
Gamma-rays " " "	2.5
" " " point source at 100 cm	1.9
" " " " " at 10 cm	0.6

Although the integral dose point of view may have significance in measuring the relative potential injury for different qualities of radiation and

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[7] Clarkson quotes a ratio of 5 in integral dose for exposure to stray radiation from deep therapy and diagnostic irradiations.

different geometrical depositions, it can hardly supplant the existing method of limiting the incident daily dose. This would be especially true if the damaging dose were not significantly different for blood changes, skin damage, or effects on the reproductive organs. If the existing tolerance dose does not have a large safety margin for the second and third of these effects little is gained by an elaboration of the gramme-roentgen aspect of exposure.

Recent experience in the administration of radioisotopes for therapeutic purposes has introduced the problems of permissible exposure from this source. When the distribution of the material is known, the effective dose can be readily calculated; in general, it is overshadowed by the contribution of the accompanying β radiation.

D. BETA-RAYS

There is no extensive body of evidence on the permissible exposure of the body to β radiation despite the extensive use of potent β-ray sources in the past, and related sources such as cathode ray tubes and Lenard tubes. With such installations, it is easy to maintain adequate shielding at all times, and the damage from their use has been essentially restricted to short exposure to the direct beams, with insufficient knowledge of the dose received.[8] It is assumed for the present that, for equal ionization in tissue, β radiation will produce the same effect as X-radiation of average energy. The tolerable exposure to β radiation has, therefore, been taken as 100 mrep per day. This should be conservative since the damaging effects of external β radiation must be confined to the skin or to tissue within a few mm. of that surface. The protection of the horny layer is introduced to set a limit to the lower energy of the β radiation that need be included in measurements of the skin exposure. Alternatively, one can conveniently elect to use β-ray chambers with wall thickness equivalent to that of the layer of passive absorption (approximately 7 mg./cm.^{2}).

E. NEUTRONS

(*a*) *Fast Neutrons.* The effects of prolonged exposure of the body to low intensity beams of fast neutrons are unknown. At higher intensity, there have been many comparisons of the radiation effects on biological materials when irradiated respectively by neutrons and X or γ radiation. The neutron dose has been frequently stated in "*n*" units, representing the scale deflection of a Victoreen Condenser R Chamber, which is unsuited to fast neutron dosimetry. In more recent practice, attempts have been made either to measure or to calculate the ionizing effect of the radiation in a "tissue"

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[8] This applied also in a recent accidental exposure to scattered β-rays.

chamber (Gray and Read, 1939). For cyclotron neutrons, it is considered that 1 *n* is equal to 2.5 rep. Biological effectiveness ratios between 5 and 9 in terms of roentgen to "*n*" unit are quite common, and led to the proposed tolerance value of 0.01 *n* per day. This is the basis of the currently accepted relation 1 rep = 4 rem for fast neutrons.

A more refined study would make allowance for biological effectiveness dependence on energy of the incident neutrons. The reliable evidence of neutron exposure in humans rests on the erythema observations by Stone and Larkin (1942) mainly with neutrons from the 60″ cyclotron. Erythema dose was 110 *n* for conditions approximately comparable with a 675 r erythema dose for 200 KV X-radiation or about 850 r of 400 KV radiation, the proposed standard for rep comparisons.[9] The biological equivalence ratio is, therefore, 1 rep = 3.4 rem. Gray's specific ionization calculations would give—

37″ cyclotron neutrons	1 rep = 5.5 rem[10]
d,d neutrons	1 rep = 8.7 rem

For deeper effects, the neutrons will be slowed down in tissue with a consequent increase in biological effectiveness. On this basis, the usual value 1 rep = 4 rem is far from conservative. Moreover, there is a possibility that the effectiveness ratio may increase sharply under the extended low intensity bombardment of interest in tolerance.*

(*b*) *Slow Neutrons.* The present estimate of tolerance to fast neutrons has been deduced from some experimental evidence, but at the beginning of the Manhattan Project there was no experimental foundation for a slow neutron tolerance exposure. Three factors were considered:

(1) Production of γ-rays in the body by the interaction of neutrons with hydrogen nuclei,
(2) Production of fast protons by the neutron reaction on some constituent atoms, and
(3) Production of new atomic nuclei.

Although it seemed at first that the exposures would be primarily controlled by the first effect (in which case one would have 1 rep = 1 rem), it appeared later, both by revised calculations of the nuclear reactions and by animal experimentation, that the reaction N^{14} (*np*) C^{14} played a considerable part in the total effect. This led to a provisional effectiveness ratio of 1 rep

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[9] Lower voltage radiation introduces a photoelectric contribution at the sensitive depth in skin. Appreciably higher voltage radiation has a surface transitional layer thicker than the layer of passive absorption.

[10] Stone and Larkin observed 90 *n* as the erythema dose on a short series. This makes 1 rep = 4.2 rem. Their instrumentation was admittedly somewhat open to question.

* *Note added in proof:* This may now be considered proved, and a relation 1 rep = 10 rem for pile neutrons is desirable.

= 4 rem. In numerical value, the ratio must be a function of the size of the irradiated body, because the production of fast protons is a purely local phenomenon, whereas the conversion to γ radiation will be larger in the larger animal. Further refinement is hardly necessary until there is a greater body of biological observation of the slow neutron damage. The actual slow neutron flux that will produce the daily tolerable exposure of 100 mrem is said to be 1500 $n/\text{cm.}^2$ sec. This is probably a lower limit of a value which may be as much as 3 times higher.[11] The precise value depends on measurements of body ionization arising from slow neutron incidence, which have not been satisfactorily performed. Slow neutron fluxes around nuclear machines appear to be low in comparison with other hazards, and there has been no critical urgency to perform detailed physical experiments required to specify the tolerable flux in greater detail. The data on epithermal neutrons, those in an energy range between that required to produce proton tracks and the approximately thermal energy significant for the $N^{14}(n,p)C^{14}$ reaction, is incomplete. There may be some molecular disturbance in the tissue by such neutron bombardment. For measurement purposes, the epithermal flux is indicated qualitatively by surrounding slow neutron counters with hydrogenous moderators. Large counts under these conditions are corrected by alterations on the primary shielding.

F. ALPHA-RAYS

As stated before, there is no danger from the external radiation by natural α particles under normal conditions. The tolerance to accelerated particles is based on the tissue ionization with the biological effectiveness ratio of 1 rep = 10 rem. The ratio is the best guess of α-ray exposure in small organisms (Zirkle, 1946). Inside the body, the same tolerance is allowed; the practical problems are confined to the calculation of energy absorption arising from a given amount of the α emitter in the tissue or organ of interest.

G. PROTONS AND OTHER PARTICLES

Bombardment of the body by fast neutrons results in a fast proton effect in the body. Consequently, for a primary fast proton bombardment, the same biological ratio of 1 rep to 4 rem is assumed to apply. Radiological use of fast protons is anticipated when new machines yielding 125 MEV protons or better are completed (Wilson, 1946). More detailed consideration of proton effects may then be required.

H. COMBINED RADIATIONS

In the absence of evidence to the contrary, it is assumed that small contributions of different types of radiation produce additive effects when

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[11] J. S. Mitchell (1947) has recently published a value of 1250 $n/\text{cm.}^2$ sec.

measured in the biological equivalence units of rem.[12] There is a wide field of biological experiment in the examination of this proposition. From the physical point of view, the heavy weighting of neutron, proton, and α particle bombardment in terms of biological damage makes it important to apportion the total ionization as accurately as possible to the respective causative agents. In particular, measuring devices which record mixed radiations in terms of rem are to be avoided. The rep/rem ratios are provisional, and such devices exclude the possibility of back-correction of data.

9. *Geometrical Considerations*

A. CONDITIONS OF MEASUREMENT OF TOLERANCE DOSE

In the field of therapeutic irradiation, there is one school which normally quotes all surface dose in terms of the measurement in an air chamber without the presence of the human body as a scattering medium; and another school which quotes the alleged skin dose which includes the air measurement supplemented by whatever scattered radiation would arrive at the surface from the patient's body. With the exception of the recent industrial regulations (American Standards Association, 1945), there has been no clear definition of the method intended in the tolerance literature. If one supposes that the European and International systems of 200 mr per day exposure include all scattered radiation, while the American system of 100 mr per day *specifically excludes it,* there would be fairly close agreement for low energy X-radiation on which the tolerance was originally founded. At higher energy, the American system becomes relatively more conservative. On this system, the readings of suitably designed monitoring instruments in exposure-areas would apply directly in tolerance considerations. On the other hand, an ionization chamber worn on the body would give a reading greater than that involved in the tolerance calculation. This difference is rarely taken into account, but it can amount to as much as 40 or 50%.

B. DIRECTIONAL AND NARROW BEAM IRRADIATION

Other principal factors which make the estimation of the dose received by the body in terms of a pocket-meter reading unreliable are: (1) directional radiation, and (2) limited or subdivided beams. With X-radiation of normal penetration, the error can be by a factor of about 10 if the meter is worn on the chest and the irradiation incident on the back. This extreme case would be evidence of considerable stupidity on the part of those responsible for protection, but similar cases of lesser degree necessarily occur.

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[12] Although the definition of rem does not necessarily imply such additivity, the concept would be virtually useless without it.

Under item (2), one has the familiar overexposure of hands and head while assembling radium sources behind a lead shield. Another aspect arises in the shielding of equipment which requires controls to be brought to the outside, and again in the use of shielding boxes or vessels with removable lids or plugs. Proper design of such boxes calls for the elimination of intense narrow beams. Much attention has been given to this in the protection of X-ray treatment rooms. It was an important consideration in Pile design where a regular pattern of holes through the principal shield was required. Although it is recognized that a considerably higher dose can be given safely to a small field than to a large one, the difference is hardly great enough to justify change in tolerance dose. Also, if the exposure is measured on the person rather than by monitoring equipment, the recording pocket-meter may happen to spend much of its time in a shielded zone while parts of the body are in fact being irradiated. For these reasons, it is considered good policy to restrict the permissible dosage-rate of emergent beams to a necessarily safe level.

IV. Organization and Functions of a Typical Health-Physics Group

1. General

A general account of the responsibilities and functions of a Health-Physics organization has been given by Stone (1946) and by Morgan (1946a); these references should be consulted. Activities are divided into two parts: (1) Operational or Service, and (2) Control and Development.

2. Operational Division

By convention, the Operational Division has been further subdivided into (a) Personnel Monitoring, and (b) Area Monitoring or Survey.

A. PERSONNEL MONITORING

Personnel Monitoring has included routine records of the daily γ-ray exposure of each individual as recorded by pocket ionization chambers. Early equipment for this purpose was unsatisfactory; the policy of wearing 2 meters to decrease the percentage of readings totally lost by instrument defects was adopted and maintained. Mass observation of pocket meter readings has shown that well-prepared meters do not give the same reading when uniformly exposed to 100 mr (say). The readings fall on a probability curve of standard deviation corresponding to 6 mr. The chance of reading a tolerance exposure as 80 mr or less is, therefore, about 1 in 1000. In large well-protected plants this might occur about once in four years. For weekly totals, the random daily fluctuations become inconsequential. Mass preparation of data essentially accurate to within 25 mr per week is practicable.

Pocket-meter readings have been supplemented by the observation of the blackening of special dental films arranged to record β and γ radiation separately. Fast neutron exposures were recorded by the measurement of proton recoil tracks produced in the Eastman Kodak special fine grain-particle film. Slow neutron exposures have been observed by means of boron-lined pocket meters. Exposures of special parts of the body, notably the fingers, have been made either by microionization chambers or by small film packs contained in special rings. These operations have been conducted on a scale approximately 100 times that of similar pre-Project experience, and the records probably constitute the best available collection of personnel exposure data of reasonable technical accuracy.

B. AREA MONITORING

The responsibility of area monitoring has been discharged by (a) fixed instruments with recorder charts, (b) technical graduates trained in the measurement and interpretation of radiation data, and (c) technicians trained in radiation measurement. Contrary to some reports, this area monitoring was entirely similar to prewar practice. Differences were in degree only and included greater frequency, more varied instrumentation, and improved reporting. For the latter, various room or area maps have been used for data plotting. While suitable for special locations, these lack the three-dimensional feature frequently required, and well planned tabulations have proved more adaptable. Exposure standards for all radiation types were established. Special interpretation was needed for surface contamination. For α emitters, the hazard was confined to possible transfer to the body. β-Contamination required consideration both in this manner and as a direct contact hazard. Hazard limits in either case came close to the minimum conveniently detectable contamination. With the area monitoring was combined a written control system for access to a potentially hazardous area. The cooperation between operating, maintenance, technical and health groups prior to, and throughout, any maneuver involving radiation, utilized the monitor results to best advantage.

The war-time use of technical graduates for survey, when scientific talent was at a premium, was indicative that the task was more involved than would appear at first sight. Qualifications for area monitoring are given in Paragraph IV, 4, D.

As available instruments were steadily improved and made more suitable for specific applications, and as many phases of laboratory and industrial operations became stereotyped, the amount of monitoring executed competently by specially trained technicians increased. It is anticipated this procedure will be further extended in the future, but successful elimination of the graduate surveyor is improbable.

3. *Control and Development Division*

In the Control and Development Division is included such control items as measurement of activity on protective clothing and radio-analysis of samples of many kinds submitted by the separate monitoring groups, or collected over a wide area to maintain surveillance of the contamination conditions of air, ground, or water, at all relevant locations. Bioassay of the amount of activity excreted or exhaled by laboratory personnel is another control function. One vital responsibility is the calibration of all instruments, which, experience has taught, requires to be done with great frequency. The development field is subdivided into groups whose function is to improve methods of solution of such exposure problems as the body content of radiotoxic materials, absorption through skin, *etc.* Although the Health-Physics departments have been provided with instruments through the very successful efforts of general instrument departments, it has been found essential that they should themselves continue research and development into specific forms of instrumentation. The special biophysical requirements of radiation measurement instruments have not been well known to those most familiar with the general instrument field, and it has required a particular combination of the skills of both groups to achieve adequate results. In some cases, required radiobiological investigations have been profitably integrated with the Health-Physics activities, and form a natural part of the responsibility for the improvement in health hazards control.

4. *Factors Contributing to a Successful Health-Physics Organization*

A. THE OBJECTIVE

The primary difference between radiation protection in general, and that offered where this is an established Health-Physics organization, rests in the *completeness of control.* The luminous compound industry has no unsolved protection problem, yet violations of safe practice are commonplace (U. S. Dept. of Labor, 1942). In the hospital field, one clinic alone saw 80 cases of possible radiodermatitis in physicians between 1934 and 1939 (Leddy and Rigos, 1941). Another clinic saw 70 radiation injuries in 3 years (Uhlmann, 1942); 30 injuries followed treatment, and the remainder occurred in diagnostic or technical work. In a survey of 45 leading radiation hospitals, hand exposures over 20 r per occasion were noted, and skin changes in one-quarter of the radiologists observed (Cowie and Scheele, 1941). Four physicians died in 1946, as a result of X-ray accidents or complications (Editor, *J. Am. Med. Assoc.*, 1947). The Plutonium Project locations,[13] entering new levels of potential exposure for the first time, have a

[13] Metallurgical Laboratory, Argonne National Laboratory, Clinton Laboratories, and Hanford Engineer Works.

record of *no radiation injury*. What factors have permitted this advance, and can it be perpetuated in future laboratory and industrial practice? It is essential to appreciate that the success of a protection program comes only in part from the health-physics unit. Some of the factors which influence the overall program are described below:

B. ATTITUDE OF LABORATORY OR PLANT PERSONNEL

Management support is a prerequisite for success in any safety program of which radiation safety is one component. Also, full cooperation of each and every employee in protecting himself and his colleagues is required. It is common to find a junior scientist who believes he has reasonable regard for the safety of co-workers, but is willing to take chances, or, if a senior man, "calculated risks," in his own exposure. Industrial experience has shown that this fails as a safety policy; the radiation problem is no exception. Reaction of senior staff personnel to the protection program frequently conditions the standards for a whole laboratory. Where there is passive acceptance of the program, or even covert resistance, the probability of effecting detailed safety control is low. Radiation accidents befall men who know how to conduct themselves and feel that rules are for the uninitiated.

C. APTITUDE OF THE HEALTH DIVISION LEADERSHIP

In the control of a radiation project scaled up by orders of magnitude beyond previous experience, it is generally conceded that the primary health responsibility is preferably divided between a competent radiotherapist and a medical physicist. In normal laboratory or industrial practice, the special clinical skills of the radiotherapist are not required. The protection program is then largely in the hands of medical physicists, supplemented by very necessary industrial-medical examination of the health of the personnel.

D. APTITUDE OF THE HEALTH-PHYSICS FORCE

The key members of the organization are the H.I. engineers, health-physics surveyors, or radiological monitors, on whom rests the day-to-day contact between protector and protected. Requirements for a successful engineer or surveyor are:

(*a*) *Personality and Diplomacy.* The engineer must be able to "sell" the employee on the advantages of close hazard control. Law enforcement or policeman tactics impede the program.

(*b*) *Technical Skill.* He must have a sound, not necessarily profound, knowledge of the health hazards, with a knowledge of tolerance and protective policies. He needs some electronics background to standardize instruments, and critically examine their fields of performance. He must also have a general knowledge of laboratory or industrial processes involved. Where the program includes research or development, he must be competent to appreciate the objectives. He need not, and in fact should not, be a research-type himself.

(*c*) *Appreciation of His Responsibilities.* The engineer's occupation has a negative character, the prevention of overexposure, and he has nothing tangible to show for his day's effort. Unless he learns to appreciate the confidence that other employees develop in his recommendations, he will fail to realize the contribution that he has made. Whenever this happens, his morale and that of his contacts will suffer.

(*d*) *Training Ability.* The engineer is required to advise diverse members of the force—research physicists and chemists, electricians, pipe fitters, chemical operators, *etc.*—on how to execute their work safely. Special ability to indicate the necessary maneuvers simply, under these conditions, is required.

E. TRAINING PROGRAMS

Health-Physics organizations have trained all their engineers and technicians because there was no available pool with prior experience. The large industrial units have set as their objective, the training of all other senior employees in Health-Physics. This is accomplished either by formal training of personnel already assigned to other duties, or by apprenticeship to the health-physics organization. Whenever circumstances permit, assignment to health instrumentation for a period of between 6 months and 1 year has proved profitable.

F. LIAISON WITH OPERATING OR TECHNICAL GROUPS

A system of written instructions for the execution of all hazardous jobs has been formulated, and has become an important feature of radiation hazard control. At the Hanford Engineer Works, for example, a Special Work Permit is completed by all the organizations involved in a proposed job prior to its inception. This method of control was developed not by the Health-Physics group but by a general committee representing all groups. Such agreement on method of approach ensures a high standard of cooperation in the planning of each specific, hazardous operation. Where the nature of the work is technical, and especially as it borders on original research, the operation of similar formal work permits is considered less applicable. However, this difference has been overrated, and considerable success achieved in some cases with the application of permits to technical work.

G. INSTRUMENTATION

Particular stress must be laid on the use of instruments in good condition and on their frequent calibration. The manner in which instrument readings are permanently recorded can also greatly affect the overall response. For health instrumentation, printing registers are frequently superior to rate meters, although the technical reliability may be identical.

H. TOLERANCE LIMITS

With the exception of the nationally accepted value of permissible dose of X or γ radiation, there may be legitimate debate on other exposure limits. A competent Health-Physics Section must keep in touch with the main biological developments that could lead to a better understanding of such limits. Flexibility of interpretation has to be maintained, and there should be no reluctance to change exposure standards. Conservatism is essential when dealing with incompletely evaluated hazards. If X μg is the tolerable deposition of a toxic element deduced from early animal experimentation, and it later turns out that $X/10$ μg is the limit, then the health-physicist who has a number of colleagues with a body content of this element between $X/10$ and X μg occupies an infelicitous position. Final exposure limits have generally been lower than those originally proposed, and there can be little criticism of the physicist who elects to preserve an additional safety factor (up to 10) in permissible exposures. A corollary to the proper statement of standards is the necessity to measure the exposures competently. Where this hinges on a bioassay, as it does for many problems of internal deposition, the measurement of the eliminated amounts may prove to be an extremely difficult technical procedure. It is imperative that health groups establish the highest possible technical standards in these cases.

I. NEW PROTECTION SERVICES

There is danger that the protection policy becomes stereotyped perhaps with good control of general body radiation (especially γ). Method development must be continued in order to put protection against other radiations, and especially against various forms of body exposure such as inhalation of active gases, inhalation or ingestion of α-emitting dusts, β-emitting dusts, exposures to neutrons, *etc.*, on a routine basis as soon as proper procedures can be established.

J. REPORTS AND RECORDS

Good records are required to make long-range studies of small exposures. To correct any condition which may be substandard, and for legitimate protection against fraudulent claims of overexposure, the primary record should be as complete as possible. Any instance of apparent overexposure should be thoroughly investigated to ascertain its cause and prevent its repetition. Wide publicity of incidents involving imperfect control, regardless of actual exposure, is desirable.

K. DETAILED CONTROL

In the last analysis, hazard control, especially where surface contamination is the issue, depends upon attention to minute details—invariable wearing of gloves, covering of work surfaces with paper, segregation of clean from contaminated tools, *etc.* Success in this field depends largely on the personal attitude of the individual concerned. In laboratories, in particular, it may represent the determining feature between good and fair hazard control.

L. TRIPLE SAFEGUARD PHILOSOPHY

Misadventures occur when several things go wrong in sequence. All important steps should, therefore, be protected by 3 safety devices so that all would have to fail before exposure occurred. The general policy is applicable to all phases of radiation-handling from the operation of a major Power Pile down to laboratory manipulation of active solutions. Safeguards should not be substituted for vigilance—the chemist careless while wearing gloves, and the driver reckless because he has good brakes have much in common.

M. CONSISTENCY OF RULES AND ENFORCEMENT

Protection rules must be consistent, and should observe the unities of time and place. Housekeeping rules, including contamination clean-up, respirator and glove wearing, eating and smoking are particularly vulnerable in this respect. Hazard control which allows smoking in laboratories on some days, and forbids it on others, is incompatible with the best practice. Rules should *never* be enforced by a Health-Physics organization. An advisory capacity is the objective, and the degree to which advice is accepted is a measure of the group's success.

N. DESIGN

Radiation protection is relatively simple in any building initially provided with properly planned hazard control facilities. This includes suitable shielding of fixed apparatus, portable shielding for temporary sources, test stations for hands, shoes and clothing, facilities for the provision of protective clothing, and change-house facilities where needed. When the basis of operation has been properly prepared, there is a saving of time in any subsequent operation performed. Economically, this partially offsets the initial higher cost of protection planning.

V. INSTRUMENTS

1. *Status*

One surprising feature of Health-Physics experience since 1942 has been the realization that, prior to that time, equipment needed for radiation monitoring at the levels of interest in protection was generally unreliable, improperly calibrated, or not available at all. There has been no extensive release of information on the improvements effected during the war years. From some publications (Morgan, 1946a; Jesse, 1946a), it can be deduced that considerable effort was devoted to this subject. Also, certain specific instruments have been released for general use. Brief accounts of such are included.

2. Calibration and Maintenance

No quicker way of destroying confidence in protective schemes exists than the use of instruments which do not appear to be functioning perfectly. This applies sometimes to devices such as counting rate meters which show a proper statistical fluctuation but are not as convincing to the average observer as a scaler and register that gives a definite count in the same time. More important is the elimination of loose connections, sticking needles, poor switches, tired batteries, defective tubes and like defects that arise in sensitive electronic equipment subjected to fairly rough treatment. Preventive maintenance, which could be practiced more extensively in laboratories and institutions, is required. Calibration of health instruments should be done at least once a week. This includes a direct test of the instrument response to known amounts of radiation at different scale deflections and for all ranges. Whenever possible, calibration at more than one radiation energy is advocated. Standardization, which by custom means a spot check of response at one scale-position on each range, should be practised every time a portable instrument is to be used. The provision of safe portable test sources for high intensity taxes the physicist's ingenuity. For such instruments as pocket meters, it is generally conceded that daily standardization is unnecessary, and monthly calibration adequate.

3. Basic Health Instrumentation

The primary specialty that differentiates health instrumentation from process or control instrumentation is the behavior of the radiation receptor. This has to be made to give a response related in the simplest possible manner to the pertinent biological exposure. In the simplest case one designs an ion chamber to read in roentgens; this is done empirically over the required range of wavelength and is an exercise in the construction of an electrically conducting shell with an insulated electrode, the whole having an effective atómic number equal to that of air. For more refined measurements, tissue-wall vessels with "tissue-gas" are required. As applied to fast neutron irradiation, the close imitation of the hydrogen content of tissue is indicated; for slow neutron irradiation the precise amount of all elements present has to be regulated to govern the activation. More frequently, secondary methods have to be utilized, such as the physical measurement of slow neutron flux, and its theoretical conversion to tissue dose.

The physicist is normally interested in the number of particles or photons emitted by a source, or crossing unit surface. The health-physicist searches for the energy absorption in tissue-like materials. Typical of the consequences are the difficulties of interpretation of G.M. Counter readings, especially for γ-ray counting. Very few health-physicists will accept portable counters as more than qualitative tools to locate areas of activity or contamination. Quantitative survey results come almost exclusively from ionization readings. In control work, counters, especially for α-particles, are invaluable and have been brought to standards of refinement normally unnecessary in analytical work.

4. Fixed Instruments

A. AREA MONITORS

Beta-gamma monitoring of selected work locations is accomplished by custom-built combinations of ion chambers with commercial microammeters and recorders. Where there is no size restriction, any desired sensitivity may be reached by using large chambers filled with air at atmospheric pressure. These are normally more reliable than pressure vessels and a suitable "air-wall" more easily provided. Sensitivity is approximately 1 $\mu\mu$amp = $\frac{10}{V}$ mrep/hr, where V = chamber volume (l.). It is optional whether the circuit be made to indicate the instantaneous dosage-rate or to integrate the exposure over a work period. The ideal monitor accomplishes both. Alarm systems may be coupled in, actuated either by high rate or high integrated dose.

B. PERSONNEL MONITORS

(*a*) *Alpha Hand Counters.* The detection of α-particle contamination on the hands is accomplished by adaptations of the standard pulse-type counters or of proportional counters with counting surface as large as the hand. A convenient modification is the two-fold counter which has flat multiwire proportional counters arranged to make contact with both sides of the hand. The collecting electrode system consists of a series of fine tungsten wires, to which approximately 2500 volts are applied, producing proportional counter-action in the flat air-filled chamber. The electronic circuit consists of an A.C. amplifier, pulse leveler, integrating circuit and vacuum tube voltmeter. Registration in this case is by counting-rate meter with a useful range from 200 to 10,000 dis/min. None of these devices records the presence of α-particle contamination on the medial and lateral surfaces of the fingers. Where some contamination on the hands is found, sound policy calls for a detailed inspection with a small proportional counter probe. The objective in this type of counting is to determine the *total* amount of toxic material present. Its local concentration is unimportant except as an aid to localized removal treatment.

(*b*) *Beta-Gamma Hand Counters.* Combinations of thin-walled counter tubes are normally disposed for this purpose to cover adequately all four principal hand surfaces simultaneously. As the objective is to locate contamination and remove it immediately, there is no need for quantitative measurements. The calibration of these instruments is made on the basis that contamination is spread over 3 sq. in. of the hand, to give a surface dosage-rate of 4 mrep/hr. Where the contamination can be limited to a small spot, the local surface dose may be quite high. Only if this occurred repeatedly on the same skin area would hazard arise. Protection against this is maintained by special checks for small spots. Preferred operation of the counter tubes is through a scaler and register system, with a permanent record of the results. In some cases, spot checks can be taken more conveniently on a system using 4 counting rate meters.

(*c*) *Foot Counters.* Groups of counters in parallel can be arranged to check for contamination on the soles of the shoes, the early detection of which reduces the tracking of active material from one location to another. These units may be coupled to the hand counters for simultaneous registration.

(*d*) *Thyroid Counters.* Typical of a check depending on the metabolism of a specific active material is the use of γ counters for the measurement of radioiodine in the thyroid in laboratories or other areas where this could be present. The method has been widely used in therapy with radioiodine. For Health-Physics, a suitable calibration is obtained by putting a standardized radioiodine solution in a glass model thyroid in a neck phantom. Practical sensitivity limit is about 4% of the tolerable value.

5. *Equipment, Atmospheric and Miscellaneous Monitoring*

A. STANDARD ALPHA COUNTER

The standard parallel plate chamber is used for the precision-measurement of the α-particle emission of samples of low mass, such as come from the evaporation of water samples, scrapings from contaminated surfaces, ashed tissue, *etc.* With reasonable care, the background may be reduced to 1 dis/min., and the useful range is then from 1 dis/min. to about 40,000 dis/min. Resolution of α particles occurs in the presence of up to 10^6 β counts/min.

B. SIMPSON PROPORTIONAL COUNTER

The Simpson proportional counter (Simpson, 1946) avoids the microphonic and electric disturbances of the standard type; it permits very fast counting (up to 5×10^5 dis/min.), in the presence of strong β counts (up to

5×10^9 dis/min.). It is occasionally of value in Health-Physics work, but the limitation on sample size to 2 cm. diameter, and the difficulty of decontamination, make it troublesome unless the high speed and resolution are essential.

C. LOW BACKGROUND COUNTER

Of special interest is the low background counter designed to expedite the detection of minimal amounts of α emitters in the urine, tissue, *etc.* If the daily elimination of 'fixed' radium were 0.001% of the body content, as little as 0.025 μg Ra in the skeleton could be detected with ease. The background of these counters is easily maintained at 6 counts/hr, although values as low as 0.1 count/hr have been claimed. With normally pure metals for the electrodes, the natural emission would amount to 3 counts/hr (Rajewsky, 1939). Surfaces of electrolytically pure metal could eliminate most of this. Then radon and thoron and their products would introduce 0.5 counts/hr, unless inactive gas (*e.g.*, old air) were used to sweep out the counting enclosure. Radon concentration can also be appreciably reduced in a perfectly air-conditioned room.

D. BETA-GAMMA COUNTERS

(*a*) *General.* Innumerable variations on the mounting of single or multiple G.M. tubes to facilitate the contamination test of laboratory glassware, tools and other items can be postulated. Other obvious applications are:

(*b*) *Gas Counters.* Representative gas samples are collected in prepared evacuated cans, and then introduced into a previously evacuated vessel containing a counter. In some cases, introduction into an ion chamber is preferred, as in radon breath sampling. In either case, subsequent contamination of the equipment may be troublesome.

(*c*) *Water Counters.* Counters immersed in vessels of active water may be used to measure the activity, especially if readings in different sized vessels are obtained. In all such applications, contamination of the parts, and the peculiar wavelength response of some counters makes quantitative work difficult.

(*d*) *Dust Monitors.* The collection of active dust from the air on filter paper surrounding a counter tube permits the evaluation of the air contamination in terms of the rate of increase of deposited activity. A natural limit of sensitivity is set by the simultaneous deposition of decay products of radon and thoron.

E. SPECIAL INSTRUMENTS

Extrapolation chambers (Failla, 1937) are invaluable for the determination of contact dosage-rates of active sheets. Especially useful is an inverted type to measure surface activity of a liquid. The customary equipment is not portable, and the source has to be brought to it. Where this is impracticable, subsidiary standard sheets are calibrated and taken to the field. Extrapolation chambers require the best available electrometers. Both the project-improved Lindemann electrometers and the Vibrating Reed electrometer appear to have features superior to the traditional FP54 electrometer circuit. Triple coincidence counters in the standard arrangements are a valuable asset in the health instrumentation list.

6. Portable Survey Instruments.

A. AREA MONITORING. ALPHA TYPES

(*a*) *Poppy.* For the rapid detection of α particle contamination, a proportional counter system is preferred. The typical probe operates with about 2500 volts on a fine tungsten wire collector. Its output operates an A.C. amplifier integrating circuit and vacuum tube voltmeter indicating circuit. An audio-oscillator triggered by each pulse makes the audible "pops" responsible for the popular name "Poppy." The Poppy is sensitive to mechanical and electrical disturbances, and is affected by moisture. The "geometry" is also sensitive to the operating conditions and should be tested frequently during operation. Inasmuch as the primary function of the equipment is to locate contamination that has to be removed, these variable features cause little concern. Sensitivity down to 200 dis/min. over the probe face is the usual limit.

(*b*) *Zeuto.* The Zeuto (Jesse, 1946b) is a convenient unit including an ion chamber with thin screen window transparent to α particles, and a circuit employing a balanced pair of miniature tubes for amplifying the ion current with a microammeter in a bridge circuit for measurement. A third tube controls positive feed back, which reduces the time constant sufficiently to permit use of a 10^{12} ohm input resistor. The instrument is sensitive to about 200 dis/min., and high readings can be accommodated by scale changing. As applied to large flat surfaces, Zeuto is at least competitive with Poppy. The latter is effective where curved surfaces and narrow strips are concerned.

B. BETA-GAMMA TYPES

(*a*) *Zeus.* Many successful β-γ monitors have been constructed, but apparently few are available for description. One such is the Zeus (Jesse, 1946c) a survey meter for α, β or γ work, but rather insensitive and nonuniform for α quantification. Built-in α and β shields provide a convenient method for estimating the relative intensity of β and γ components, and indicating the presence of α contamination. Zero stability is good and the time constant short. The useful range of the Zeus is from 1 to 2500 mrep/hr in three ranges, *viz.*, 1–25, 5–100 and 100–2500. Separate calibration of each range is needed. Ion chamber current is amplified by a balanced pair of miniature triodes and measured by a microammeter in a bridge circuit. The range is selected by changing the value of the input resistor, which, at maximum sensitivity is 10^{11} ohms. The wire chamber screen and metal chamber wall on some instruments will cause energy dependence with soft γ radiation.

(*b*) *Lauritsen Electroscope*. The Lauritsen electroscope (Lauritsen and Lauritsen, 1937) modified by the provision of a thin window with screens to eliminate α particles or α and β at will is an excellent tool for the small laboratory. It has to be calibrated over a wide range, because there is a peculiar intensity response due to the inadequate collection field around the fine quartz fiber system. Limiting factors in the use of the instrument are energy dependence for γ radiation, fixed sensitivity, and the inconvenience of obtaining measurements with the aid of a microscope while timing the observation with a stopwatch. Reliable determinations of dosage-rate can be made in the range from 0.1 mr/hr to 1 r/hr, provided sufficient time is available for low intensity measurements. The instrument is unsurpassed for reproducibility.

(*c*) *Landsverck-Wollan Electrometer*. Improvements on the Lauritsen electroscope have been incorporated in the Landsverck-Wollan electrometer. The new device is superior in linearity and sensitivity. A condenser-resistor circuit is used to flash a neon lamp at the beginning and end of one of two predetermined time periods, permitting the fiber to be observed at those instants, effectively making it a dual-range instrument. The timing system eliminates the need for a stopwatch to time fiber drift, when high precision is not required. The useful ranges of the instrument are 0–200 mr/hr, and 0–2 r/hr.

(*d*) *Victoreen Radiation Meter*. The Victoreen Radiation Meter[14] was primarily designed for measurements of stray radiation from X-ray installations, but can be put to wider use in the protection field. Obvious circuit changes could make the meter suitable for higher ranges, by a combination of chamber size and resistor variation. The cautious observer would wish to mount the chamber on the end of an extension rod for high level operation. It is advantageous to prepare special scales calibrated over the whole range, and to standardize frequently.

(*e*) *Betty Snoop*. The Betty Snoop is a lightweight portable meter for measuring high intensity radiation fields. Its probe contains a small plastic ion chamber, range switch, input resistors and the electrometer tube. An extension cable permits using the probe at some distance from the meter

[14] Victoreen Instrument Co., Cleveland, Ohio.

circuit. Four sensitivities of nominal maxima, 0.2, 2, 20, and 200 rep/hr are provided. The calibration curve for the three lower ranges is nearly linear, but the high range curves with a negative slope. The circuit employs a balanced pair of triodes and a microammeter in a bridge circuit, with a third tube providing positive feedback to permit use of a 10^{12} ohm input resistor on the most sensitive range. A long time constant results on the lower ranges, and attempts to reduce it by increased feedback may cause oscillation. The chamber has an easily removable cap, which permits optional γ or β registration. As the chamber is only 3 cm. in diameter, it is well suited to measurements in narrow beams or close to small sources.

(*f*) *Condenser Chambers*. Detachable condenser chambers of all sizes can be used as portable monitors where only an integrated dose is required. The method is cheap, reliable and versatile. Ideal construction is still similar to the Sievert chambers (1932). A series of such chambers with different wall composition can be used to calculate energy absorption in tissue. Ranges from 0.04 mr to 1000 r are easily obtained on a versatile recorder such as a Lindemann electrometer. The familiar Victoreen Condenser-R-Meter is one suitable rugged commercial application of the principle. All such meters require calibration with the specific types of radiation to be used.

(*g*) *Portable G. M. Tube Sets*. Since the first portable G. M. tube circuits were made, they have been used for the detection of lost radium and for health measurements. Low voltage counters (Chalmers, 1934) were particularly suited for systems weighing less than two pounds (Pallister, 1937). Innumerable circuit modifications have been used by commercial manufacturers and by private laboratories including registration by audio-signal, neon tube flashes, or integrating circuits. For health instrumentation, utility is restricted to qualitative detection rather than measurement. Good design features are, therefore, stability, light weight, and freedom from unsuspected blocking. Modern circuits use mainly self-quenching tubes, but these exhibit a temperature coefficient (Korff *et al.*, 1942), which can lead to racing counts in cold locations and no response in hot places. In survey applications, one uses a special filling of low temperature coefficient or restricts the operation to a certain temperature range.

(*h*) *Sigmion*. A useful Project innovation was the Sigmion, a simple chamber and D.C. amplifier set to integrate up to 20 mr and then reset. The total exposure is tallied on a register. Successful operation depends on balancing the normal reverse leak by a subsidiary active source.

(*i*) *Victoreen Proteximeter*. Somewhat similar in general design and function is the Victoreen Proteximeter. Dose up to 200 mr is indicated on the instrument meter. These instruments are convenient for location close to a technician. When the instrument is found to be fully discharged, there is no reset mechanism and no record of the total dose as provided by the Sigmion.

C. NEUTRON METERS

(*a*) *Differential Chambers.* Quantitative measurements of fast neutron exposures are made in differential ion chambers, one of which is made sensitive to neutrons by the use of a hydrogenous gas or paraffin-lined wall, while the other provides a γ-ray balance by ionization in argon. For high sensitivity, pressure vessels are required and the final equipment is cumbersome. Where rates above 50 $n/\text{cm.}^2$ sec. are involved, truly portable systems can be used. In all cases, special precautions are needed to compensate for asymmetrical γ fields. Radium-beryllium sources are widely used for calibration.

(*b*) *BF_3 Counters.* Slow neutrons are counted in conventional BF_3 Counters, or in boron-lined counters or ion chambers. All such slow neutron counters can be made into convenient detectors of fast neutrons by surrounding them with a 4″ thick sleeve of paraffin, to moderate incident fast neutrons. Calibration is effected by comparison with the twin chamber apparatus, and is valid only for similar neutron spectra.

7. *Portable Dust Monitors*

Sampling for radioactive dust is easily accomplished by filtration or electrostatic precipitation. Standard formulae allow for the elimination of the Ra-Th contributions after two subsequent measurements. A convenient nomogram for the elimination of thoron decay product counts when α contamination is to be measured, is shown (Fig. 2). It is customary to delay the initial count until 4 hours (or better, 6) after the sample collection to reduce the radon decay product contribution to a negligible value.

8. *Personnel Meters. Beta-gamma*

A. FILM BADGES

A permanent record of integrated weekly dose is given by film badges. A typical badge contains two pieces of dental film in a silver or cadmium holder 1 mm. thick, with a window to admit β radiation. Blackening is measured on scales separately calibrated for β and γ radiation. One piece of film is the sensitive industrial radiography type, for the normal exposure, while the other is chosen with a range up to 40 rep to cover a possible gross exposure. The metal filter approximately compensates for wavelength dependence of film above the K limit of silver. However, soft quantum radiation on the window produces intense blackening which masks β contributions.

B. POCKET METERS

Pocket ionization chambers in the general form of "pencils" have been used in many forms. Typical of the commercial species is that produced by

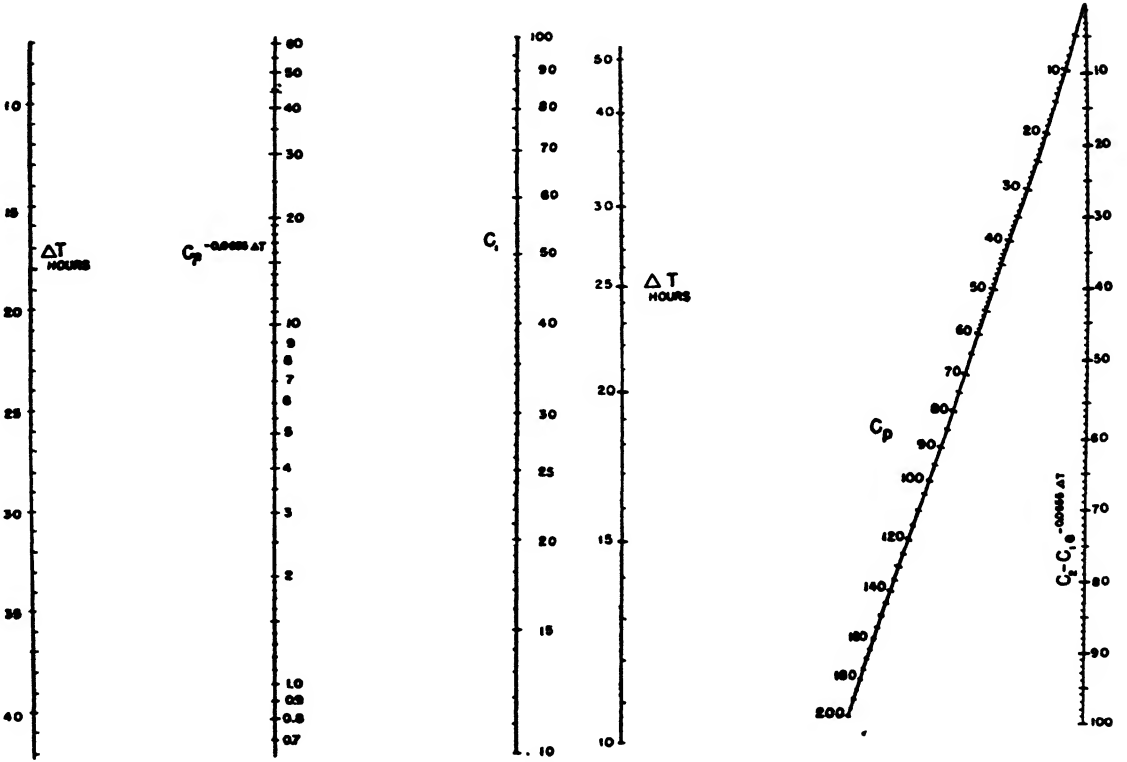

FIG. 2

Calculation of α counting rate, Cp, due to contaminant in presence of thoron decay products. C_1 = counts/min after 4 to 6 hours. C_2 = counts/min ΔT hours later. On left hand figure, straight edge through ΔT and C_1 defines $C_1e^{-0.0655\,\Delta T}$ on center scale. Subtract this value from C_2. Straight edge between $C_2-C_1e^{-0.0655\,\Delta T}$ and ΔT on right hand figure defines Cp on sloping scale.

the Victoreen Instrument Co., Cleveland, Ohio, and used in conjunction with a simple string electrometer—the Minometer. Such pocket meters should be rugged, wavelength-independent, free from insulator leaks, and unaffected by humidity. Currently available meters fall short of these ideals. With good maintenance, reliable service can be effected, and the use of duplicate meters reduces 'lost' readings to an insignificant score. Technical improvements of the meters, especially protection against dust and moisture, and simplified manufacturing methods require study. Pocket meters with thin walls for β registration can be used, or small models can be worn on the finger.

C. NEUTRON METERS

Slow neutrons can be recorded in boron-lined chambers (Landsverck, 1947). Slow neutron doses up to 200 mrem can be read on the standard model with only 1% interference by an incident γ tolerance dose. Neutron-sensitive film can be worn for fast neutron checks. A cadmium filter over regular film is an alternate meter for slow neutrons.

VI. Some Elementary Formulae and Calculation Methods

1. General

Refined calculations of shielding, maximum permissible exposure, *etc.*, are subjects for detailed treatises on the Health-Physics specialty. Much can be done by simplified methods to indicate the order of magnitude of exposure and some typical examples are given.

2. Shielding

X-ray shielding can be calculated from standard absorption data. It is customary to quote tables for lead absorption and to refer to other materials by their "lead equivalent" (Taylor, 1944; Glasser *et al.*, 1944). The Graphic Calculator (Failla, 1945) is a convenient chart for protection calculations. Similar methods serve for the laboratory manipulation of radioactive sources. One convenient tabulation of the required shielding is shown in Table I.[15] The addition of three quantities, with due regard to sign, and multiplication by one factor permit calculation of shield thickness for all normal values of source strength, quantum energy, handling distance, daily exposure time, and customary shield material. Interpolation is unnecessary because precision in temporary shielding need not be high.

3. Emission from Complex Sources

The emission of primary γ radiation from a large radiating mass, in which the space variation of activity follows a simple power law, and which is

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[15] Prepared by C. C. Gamertsfelder, 1943.

covered by a uniform filter or shield, is formally identical with the radiation problems considered by Gold (1909). The intensity can be expressed as a series of integrals of the form

$$\int_0^{\pi/2} e^{-x \sec \theta} \sin \theta . \cos \theta^{(n-2)} . \, d\theta$$

which transforms to $\int_1^{\infty} \frac{e^{-xu}}{u^n} \, du$

Tables of this function have been published. Approximations to the radiation from large nuclear machines, tanks of active materials, reservoirs of waste material, *etc.*, can be made on this basis. Where neutron radiation is involved, applications of diffusion theory may be needed. Many simpler cases, such as the γ radiation from thin active discs or plates are familiar in the literature (*e.g.*, Mayneord, 1932). Special cases of interest are the emission from active sheets or blocks and the emission inside extended masses of active material.

4. *Emission from Sheets or Blocks*

Consider a sheet emitting n particles/cc./sec., each of range R cm. in the material. The emergent integrated flux is

$$\int_0^R \frac{2\pi n r^2 \, dr}{4\pi r^2} = \frac{nR}{2} \text{ particles/cm.}^2\text{sec.}$$

If k = number of ion pairs/cm. air produced by each particle, the surface dosage-rate is: $n\ R\ k\ 4.8 \times 10^{-10} \times 3600$ rep/hr. $= 8.7 \times 10^{-7}\ n\ R\ k$ rep/hr.

Radiation	k	R
α	$\backsim$50,000	From tables: allow for relative stopping power
β	$\backsim$ 75 for fast particles[a] $\backsim$100 for average particles	Range for $\frac{\text{maximum energy } (E)}{3}$
γ	$\frac{\sigma E}{W} = \frac{3.5 \times 10^{-5} \times E \times 10^{6}}{32}$ approx. $= 1.1E$ E = energy per disinteg. in MV	Reciprocal of linear absorption coefficient in medium

[a] $k = 45$, in the physicists measure, follows the electron on its tortuous path. Our measure is a 1 cm. translation.

TABLE I

Shield Thickness for Laboratory Sources

Select column for energy required (use next higher if exact value is not given). Entry gives thickness in cm. Pb for different source strengths at 1 m. for 8 hrs./day to give 100 mr. *Add algebraically,* correction terms for other working ranges or times, and *multiply* by factor for shield material.

E.g., 500 mc of 1.8 MEV source at 50 cm. for 4 hr./day = (7.21 + 2.77 − 1.39) × 1.43 cm. Fe = 12.3 cm. Fe

Activity	0.2 Mev	0.5 Mev	0.8 Mev	1.0 Mev	1.5 Mev	2.0 Mev	2.5 Mev	3.0 Mev	4.0 Mev
10 mc	− .20	− .71	− .95	− .98	− .83	− .61	− .33	− .11	+ .19
20 mc	− .14	− .36	− .27	− .11	+ .37	+ .77	+ 1.15	+ 1.40	+ 1.70
50 mc	− .07	+ .11	+ .63	+ 1.03	+ 1.95	+ 2.61	+ 3.10	+ 3.39	+ 3.69
100 mc	− .01	+ .46	+ 1.31	+ 1.90	+ 3.14	+ 3.99	+ 4.57	+ 4.90	+ 5.20
200 mc	+ .04	+ .82	+ 1.99	+ 2.77	+ 4.34	+ 5.38	+ 6.05	+ 6.40	+ 6.70
500 mc	+ .12	+ 1.28	+ 2.89	+ 3.91	+ 5.92	+ 7.21	+ 7.99	+ 8.40	+ 8.69
1 c	+ .17	+ 1.64	+ 3.57	+ 4.78	+ 7.11	+ 8.60	+ 9.47	+ 9.90	+10.20
2 c	+ .23	+ 1.99	+ 4.25	+ 5.64	+ 8.31	+ 9.98	+10.94	+11.41	+11.71
5 c	+ .30	+ 2.46	+ 5.14	+ 6.79	+ 9.89	+11.82	+12.89	+13.40	+13.70
10 c	+ .36	+ 2.81	+ 5.82	+ 7.66	+11.08	+13.20	+14.37	+14.91	+15.21
20 c	+ .41	+ 3.17	+ 6.50	+ 8.52	+12.28	+14.59	+15.84	+16.42	+16.71
50 c	+ .49	+ 3.63	+ 7.40	+ 9.67	+13.86	+16.42	+17.79	+18.41	+18.71
100 c	+ .54	+ 3.99	+ 8.08	+10.53	+15.05	+17.81	+19.27	+19.91	+20.21
Danger Range	plus	plus	plus	plus	plus	plus	plus	plus	plus
20 cm	+ .26	+ 1.64	+ 3.16	+ 4.02	+ 5.55	+ 6.44	+ 6.85	+ 7.00	+ 7.00
50 cm	+ .11	+ .71	+ 1.36	+ 1.73	+ 2.39	+ 2.77	+ 2.95	+ 3.01	+ 3.01
1 m	.00	.00	.00	.00	.00	.00	.00	.00	.00
2 m	− .11	− .71	− 1.36	− 1.73	− 2.39	− 2.77	− 2.95	− 3.01	− 3.01
5 m	− .26	− 1.64	− 3.16	− 4.02	− 5.55	− 6.44	− 6.85	− 7.00	− 7.00
10 m	− .37	− 2.35	− 4.52	− 5.76	− 7.94	− 9.21	− 9.80	−10.01	−10.01

Working Time	plus	plus	plus	plus	plus	plus	plus	plus	plus
1 hr. day	− .17	− 1.06	− 2.04	− 2.60	− 3.59	− 4.16	− 4.42	− 4.52	− 4.52
2	− .11	− .71	− 1.36	− 1.73	− 2.39	− 2.77	− 2.95	− 3.01	− 3.01
4	− .06	− .35	− .68	− .87	− 1.20	− 1.39	− 1.47	− 1.51	− 1.51
8	.00	.00	.00	.00	.00	.00	.00	.00	.00
24	+ .09	+ .56	+ 1.08	+ 1.37	+ 1.89	+ 2.20	+ 2.34	+ 2.39	+ 2.39
Absorber	**times**	**times**	**times**	**times**	**times**	**times**	**times**	**times**	**times**
Pb	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fe	8.80	2.88	1.96	1.74	1.49	1.43	1.47	1.48	1.59
Al*	41.67	9.80	6.18	5.33	4.83	5.00	5.28	5.68	6.39
H_2O	106.84	21.54	13.42	11.59	10.36	11.11	11.19	12.11	12.78

* Or concrete.

Note added in proof: Source activity is quoted in millicuries or curies, where one curie is that amount of radioactive material that disintegrates at the rate of 3.4×10^{10} dis./sec. The table is computed on the further (erroneous) assumption that each disintegration yields one γ photon of the selected energy. This leads to inaccuracies whenever the disintegration scheme is complex. More accurate calculations can be made when the disintegration scheme is known. Ignored also is the increased effective transmission of shields under wide beam irradiation. The table is a useful guide for erection of *temporary* laboratory shielding.

Examples

1. A uranium sheet emits 1.27×10^4 α particles/sec./g. from U^{238} or 2.38×10^5/sec./cc. There will be an equal number from the isotopic U^{234}. $\therefore n_\alpha = 4.76 \times 10^5$. Average range = 3 cm. air = 6×10^{-4} cm. U $\therefore$ α contact dosage-rate = 12.6 rep/hr.
   Only the UX_2 β-rays will be worth consideration. $n_\beta = 2.38 \times 10^5$. Range for average energy 2.32/3 is 0.25 g. Al/cm.² = 0.34 g. U/cm.² = 0.018 cm. U
   β Dosage-rate = 280 mrep/hr.
   γ-Ray energy is 0.8 MEV, R = 0.4 cm. U
   γ Dosage-rate = 7.2 mr/hr.

2. A thick plastic sheet, s.g. 1.2, contains 1 mc P^{32}/cc. Range for average energy 1.7/3 is 0.14 g. Al/cm.² = 0.12 cm. in the sheet.
   Contact dosage-rate = $8.7 \times 10^{-7} \times 3.7 \times 10^7 \times 100 \times 0.12 =$ 385 rep/hr.

Such calculated values can be relied upon to about ±30%. For very thin sheets, an approximate value is obtained by inserting thickness t cm. instead of R in the formula.

5. Emission Inside Large Active Masses

The energy absorption per cc. of the mass is evidently equal to the energy emission. When the energy absorption in the mass can be simply related to the energy absorption/cc. of air, water or tissue, the exposure in the mass can be written down immediately.
If C = concentration in μc/l. and
E = (γ energy + average β energy) in MEV per disintegration[16] the following formulae result:

$$\text{In air, dosage-rate, D.R.} = 2000\,CE \text{ mrep/hr.}$$
$$\text{In water, dosage-rate} = 2.6\,CE \text{ mrep/hr.}$$

or in any medium, density ρ, dosage-rate $= 2.6 \dfrac{CE}{\rho}$ mrep/hr

Persons exposed in such masses may usually be supposed to receive the β component from one hemisphere only. Also, on the ground for an active air case and at the surface of a water mass, the γ rate is usually taken as one-half the above. Tolerable concentrations can readily be established on this basis. Formally, the surface case is identical with the already considered thick sheet problem, when transitional equilibrium effects are negligible.

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[16] Usual application is to β-γ emitters. Same formula applies for α emission (*e.g.*, in bone).

Examples

1. An air mass containing 1 μc Xe^{133}/l. contacts the ground.
   Average β energy $= \frac{0.33}{3}$ MEV. γ energy $= 0.084$ MEV [Siegel, 1946].
   A man in an airplane receives $2000\ (0.055 + 0.084) = 280$ mrep/hr.
   On the ground he receives $2000\ (0.055 + 0.042) = \sim 200$ mrep/hr.
   Tolerance concentration (24 hrs. daily) is: 0.021 μc/l.
2. A river contains 0.02 $\mu c Na^{24}$/l. Average β energy $= \frac{1.39}{3}$ [Siegbahn 1946], γ energy $= (2.76 + 1.38)$. A small organism in the river receives $2.6\ (0.46 + 4.14) \times 0.02 = 0.24$ mrep/hr.
   A man in a boat receives $1.3 \times 4.14 \times 0.02 = 0.11$ mr/hr.
   Immersion tolerance concentration (man, 8 hrs./day) = 1.1 μc/l.

6. *Tolerance for Internal Emitters*

A. SIMPLE CASE

In almost all cases, the local energy absorption is governed by the particle radiation, α or β. Even a small organ is then an effective large mass and the relation D. R. $= 2.6 \frac{CE}{\rho}$ mrep/hr. holds. This has been more frequently used in the form D. R. $= 62 \frac{EQ}{W}$ rep/day where E = av. en. in MEV, $Q = \mu c$ deposited, W = grams of tissue containing the Q μc (Cohn, 1946). Let f be the fraction of the administered dose Qa deposited in the relevant tissue. Then for a single short exposure $Q = fQa$.

For prolonged exposure $Q = f \frac{Qa}{\lambda}$ where Qa = daily dose and λ = decay constant in days^{-1}. Where the deposition is eliminated, this is assumed to be exponential with a biological decay constant λ_b. The effective decay constant is then $(\lambda + \lambda_b)$. It is also necessary to distinguish between cases of prolonged exposure to a continuously maintained concentration (the main interest in tolerance dose), and to a decaying concentration, such as might follow accidental dispersal or the Bikini tests.

B. OTHER VARIABLES

Recognized formulae of radioactive transformation can be applied to the case where one active material is taken in and produces a damaging daughter. Typical of the manipulation of such formulae and their application to tolerance are the data by Morgan (1946b); some provisional tolerance values are listed in Table II. These depend on the values of f. This is usually a composite, *e.g.*, for ingestion, f = fraction absorbed from gut into blood,

x = fraction deposited from blood into tissue. For inhalation, one considers (1) lung retention from air, (2) absorption from lung to blood, and (3) de-

TABLE II

Representative Tolerance Values Calculated by Morgan (1946b)

1	2	3	4	5	6	7
Element	Grams per Curie	Assumed effective half life	Method of body intake	Organ affected and fraction of amt. in body that is in organ	Fraction taken into body that reaches organ	μc in organ to produce tolerance rate when $t = 0$
Ra^{226} pdts	1	~2 wks.	Breathing	Lungs (.5)	0.25	.013
Ra^{226} pdts	1	~10 yr.	Ingestion	Bone (.6)	0.05	.155
Pu^{239}	16	2 mo.	Breathing	Lungs (.3)	0.25	.035
Pu^{239}	16	10 yr.	Ingestion	Bone (.6)	0.0003	.42
Pu^{239}	16	10 yr.	Breathing	Bone (.6)	0.0375	.42
Natural U	1.47×10^{6}	2 mo.	Breathing	Lungs (.3)	0.25	.041
Enriched U	2.7×10^{4}	2 mo.	Breathing	Lungs (.3)	0.25	.039
U^{233}		2 mo.	Breathing	Lungs (.3)	0.25	.037
Po^{210}	2.24×10^{-4}	82 d.	Breathing	Kidneys (.05)	0.011	.010
Po^{210}	2.24×10^{-4}	82 d.	Ingestion	Kidneys (.05)	0.001	.010
Sr^{89}	3.7×10^{-5}	43 d.	Ingestion	Bone (.5)	0.075	32
$Sr^{90} \rightarrow Y^{90}$	7.74×10^{-3}	Sr—197 d. Y—2.49 d.	Ingestion	Bone (.5)	0.075	88 (20)[a]
C^{14} (graphite)	0.23	2 mo.	Breathing	Lungs (.3)	0.25	32
C^{14} (CO_2)	0.23	10 d.	Breathing	Total body (1)	0.25	2260
H^3 (water)	2.59×10^{-4}	2 d.	Breathing	Lungs (.02)	0.25	320
I^{131}	8×10^{-6}	6.3 d.	Ing. or br.	Thyroid (.2)	0.20	2.0
Na^{24}	1.13×10^{-7}	14.8 hr.	Submersion	Body		
Na^{24}	1.13×10^{-7}	14 hr.	Ing. or br.	Blood (.25)	0.25	2.2
Na^{24}	1.13×10^{-7}	11.5 hr.	Ing. or br.	Lungs (.037)	0.037	0.5
P^{32}	3.48×10^{-6}	14.3 d.	Submersion	Body		
P^{32}	3.48×10^{-6}	13 d.	Ing. or br.	Bone (.9)	0.09	39
$Ba^{140} \rightarrow La^{140}$	1.33×10^{-5}	Ba—11.75 d. La—1.51 d.	Ingestion	Bone (.6)	0.06	48
S^{35}	2.3×10^{-5}	25 d.	Ing. and br.	Skin (.2)	.05 (.1)[b]	150
Ca^{45}	6.15×10^{-5}	150 d.	Ing. or br.	Bone (.99)	.15 (.4)[b]	190

The tolerance values in columns 9, 10, 11, 12 and 13 are for continuous exposure. If the exposure is for a 40 hour week, multiply these values by 4.2.

Column 9 is the tolerance concentration rate, P, in μc/sec, to the body organ that will produce a tolerance rate of exposure after 365 days of consumption.

It should be noted that values given in column 6 depend on the chemical form and in the case of inhalation they depend upon the size particles. Until the most likely forms of these elements in a given laboratory are known, it is difficult to assign typical values of tolerance concentration in columns 10, 11, 12 and 13.

Values in column 9 can be obtained directly from equation 14 or by dividing values in column 8 by the seconds in a year.

8	9	10	11	12	13	14
µc in organ to produce an av. tolerance during year	One year tolerance concentrations rate (µc/sec)	One year tolerance concentration in air		One year tolerance concentration in water		Effective energy
		µc/cc	µg/cc	µc/cc	µg/cc	MV
.23	7.5×10^{-9}	2.0×10^{-10}	2.0×10^{-10}			$14\alpha\gamma$
.16	5.1×10^{-9}			4.4×10^{-8}	4.4×10^{-8}	$14\alpha\gamma$
.15	4.7×10^{-9}	1.3×10^{-10}	2×10^{-9}			5.16α
.43	1.4×10^{-8}			2.0×10^{-6}	3.1×10^{-2}	5.16α
.43	1.4×10^{-8}	2.5×10^{-9}	4.0×10^{-8}			5.16α
.17	5.5×10^{-9}	1.5×10^{-10}	2.1×10^{-4}			4.43α
.16	5.1×10^{-9}	1.4×10^{-10}	3.8×10^{-8}			4.7α
.156	5.0×10^{-9}	1.3×10^{-10}				
.033	1.0×10^{-9}	6.4×10^{-10}	1.4×10^{-13}			5.3α
.033	1.0×10^{-9}			4.5×10^{-5}	1.0×10^{-8}	5.3α
190	6.0×10^{-6}			3.4×10^{-3}	1.3×10^{-7}	0.6β
34	1.1×10^{-6}			6.2×10^{-4}	4.8×10^{-6}	$.22, .8\beta$
130	4.3×10^{-6}	1.2×10^{-7}	2.6×10^{-8}			$.05\beta$
5.7×10^{4}	1.8×10^{-3}	4.8×10^{-5}	1.1×10^{-5}			$.05\beta$
4×10^{4}	1.3×10^{-2}	3.5×10^{-5}	9×10^{-9}			$.005\beta$
81	2.6×10^{-6}	8.5×10^{-8}	6.8×10^{-13}	5.5×10^{-4}	4.4×10^{-9}	$.2\beta\gamma$
		6.3×10^{-7}	7.1×10^{-14}	4.9×10^{-4}	5.5×10^{-11}	$3.3\beta\gamma$
960	3.0×10^{-5}	8.1×10^{-7}	9.1×10^{-16}	5.2×10^{-8}	6.0×10^{-10}	$3.3\beta\gamma$
258	8.2×10^{-6}	1.5×10^{-6}	1.7×10^{-13}	9.5×10^{-8}	1.1×10^{-9}	$3.3\beta\gamma$
		4.2×10^{-6}	1.5×10^{-11}	3.2×10^{-3}	1.1×10^{-6}	0.5β
750	2.4×10^{-5}	1.8×10^{-6}	6.2×10^{-12}	.011	4.0×10^{-6}	0.5β
170	5.3×10^{-6}			3.8×10^{-3}	5.1×10^{-8}	$.4, 2.3\beta\gamma$
1500	4.7×10^{-5}	3.1×10^{-6}	7.3×10^{-11}	.041	9.4×10^{-7}	$.05\beta$
400	1.3×10^{-5}	2.1×10^{-7}	1.3×10^{-11}	3.6×10^{-3}	2.2×10^{-7}	0.1β

Column 8 is the µc in the lung, bone, kidney or blood required to irradiate the organ with 3.65 roentgens of α or 36.5 roentgens of beta-gamma in a year. It is the µc in the thyroid required to irradiate it with 365 roentgens of beta-gamma in a year.

[a] The Sr-Y activity reaches a maximum after 15 days. The 88 µc is required to produce tolerance exposure rate soon after Sr reaches the bone. Only 20 µc is required to produce tolerance exposure rate on the 15th day. The 34 µc produces an average yearly tolerance dose. (See Fig. 4.)

[b] It is assumed that the fraction reaching the skin by way of the gut is 0.05 and by way of the lungs is 0.1 in the case of S^{35}. For Ca^{45} it is assumed that 0.15 reaches the bone by way of the gut and 0.4 by way of the lung.

position from blood. These factors vary for each radioactive material, its valence state, and some features of the physical and chemical form. Determination of these variables rests on prolonged radiobiological and biochemical studies, initially in animals, but in part on humans (especially for inhalation). For this reason there will continue to be discrepancies in the published tolerance values. With the termination of the war effort and the consequent restoration of channels of scientific information, the current multiplicity of values can be screened by a national committee to stabilize the best values.

C. STANDARD MAN

Computers of tolerance concentrations have used a wide range of values for body organ weights, respiratory rate, water intake, *etc.* Uncertainties in the metabolism of isotopes and ultimately the idiosyncrasies of each exposed person will always exceed the range of variation introduced in this manner. Consequently, the continued publication of tolerance values founded on irregular basic values only further confuses the issue. Agreement on uniform figures should be possible as a first step toward complete standardization. The following values adjusted* from those proposed by R. S. Stone form a logical basis for this:

Total body weight	70 kg.
Muscle	30 kg.
Skin and subcutaneous tissues	8.5 kg.
Skeleton	7 kg.
Blood	5400 g.
Heart	350 g.
Liver	1700 g.
Lungs (pair)	950 g.
Bone marrow (red)	1500 g.
Bone marrow (yellow)	1500 g.
G.I. tract (empty)	2300 g.
Kidneys (pair)	300 g.
Pancreas	65 g.
Spleen	200 g.
Testes (pair)	40 g.
Thyroid	30 g.
Respiratory rate	1 m.^{3}/hr.
Water intake	3 l./day
Primary lung retention of small particles	25%

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The Use of Radioactive Isotopes in the Study of Iron and Hemoglobin Metabolism and the Physiology of the Erythrocyte

By P. F. HAHN

CONTENTS

I. INTRODUCTION.

Reviews of the field of iron metabolism ten years ago revealed many gaps in the state of our knowledge of the relationship of this important element to body processes (Heath and Patek, 1937; Hahn, 1937). Some of these gaps have been partially closed. In many instances we lack much desired information but the direction of future efforts is much better indicated.

The importance of iron in human and animal economy is poorly appreciated. It would probably be fair to say that a lack of sufficient iron represents one of the greatest nutritional deficiencies in the world as a whole today. This is less apparent in the relatively well fed and nourished United States than elsewhere. In China, India and northern Brazil, as well as many other large centers of population, the incidence of iron deficiency is very high. The reasons for this we shall discuss in detail below.

As a vital constituent of every mammalian body cell iron plays a role in the oxidation of metabolites. Here its need is so great that under the conditions of greatest stress the cell holds on to its cytochrome and catalase iron. There is also a considerable amount of iron in the form of muscle hemoglobin in striated muscle which similarly is not depleted regardless of the other demands of the organism. Iron in these forms is more or less "fixed" in the body and does not usually concern the clinician, nutritionist or dietitian. It carries such a high priority that the need for it will be filled first. However, with a limited supply available, such a priority is frequently reflected in a diminution of iron in other important body tissue fractions and it is then that we begin to see the manifestations of deficiency, whether shown by elaborate chemical or physical tests or by frank signs of clinical deficiency symptoms. The more important of these other fractions of body iron are the red cell hemoglobin iron, the labile reserve stores, and the plasma iron.

Much of our recently acquired information as to the path taken by iron in the metabolic processes of the body has been obtained through the use of the artificial radioactive isotopes of iron. The future of this field is made much brighter in its prospects due to the existence of these valuable tools. The employment of tagged atoms in studying the fate of an element or compound in the body indicates very clearly the necessity for collaboration and a high degree of cooperation among various categories of scientists. Without the cyclotron or chain-reacting pile for the production of isotopes in the hands of the physicists, without the biological or medical acumen of the physiologist and clinician, without the aid of the atomic chemist in the separation and purification of materials, and without the aid again of the physicist in detecting and quantitating the radioactivity, little could be accomplished in any reasonable length of time. Too many fields of highly specialized activity are involved for any one individual to cope with the multitude of problems which arise. Such is likely to be the usual pattern for much of the future research in medicine and related sciences.

II. ABSORPTION OF IRON

1. *Site of Iron Absorpion*

At what part of the gastro-intestinal tract the greater part of iron absorption occurs is not too clearly evident at the present time. Using a dog with an isolated stomach and an anastomosis of the esophagus to the duodenum we were able to show by means of tagged iron that as much absorption took place through the isolated gastric mucosa as would have been taken up in this same animal had there been an intact gastro-intestinal tract (Hahn, Bale, Ross, Balfour and Whipple, 1943). Such an experiment may be criticised as being "unphysiological" and it is true that the high degree of absorption may well have been related to the long continued contact with the gastric mucosa. It is worthy of note however that in this experiment it was found that the plasma radio-iron level was dropping before the material was washed out of the pouch. In the light of our present concept of the mechanism of iron absorption, which will be stated below, these findings take on added significance regarding the importance of the stomach in iron absorption.

Using the Fe^{59} isotope we were also able to demonstrate qualitatively the uptake of some iron from duodenal and jejunal pouches. Taking advantage of the high specific activities obtainable with the use of Fe^{55} Copp and Greenberg, 1946, found evidence of absorption of 0.05 mg. doses of iron in both the small and large intestine of the rat.

It is of considerable interest to note that Granick, 1946, found larger amounts of ferritin in the duodenum than in any other part of the gastro-intestinal tract. This work was done with guinea pigs and there might conceivably be species differences involved.

Pouch experiments only throw light on the *possibility* of iron absorption at various sites in the gut and afford little evidence of what transpires under normal conditions in the intact animal. As we stated ten years ago (Hahn, 1937) pH is almost certainly a major factor in determining the extent of iron absorption. The alkalinity of the bile and pancreatic juices must certainly militate against any considerable amount of absorption of this element beyond the ampulla of Vater since the insolubility of iron would become of limiting importance.

2. *Mechanism of Iron Absorption*

Very early in the course of conducting tracer experiments with Fe^{59} it was found that considerably more iron was absorbed from the intestinal tract of animals which had been depleted of their reserve stores of this element than from the G-I tract of normal animals (Hahn, Bale, Lawrence and Whipple, 1939). At first we were at a loss to explain the unusual manner in which the body handled iron as contrasted with other mineral elements. The ability of the body to excrete iron has been shown to be extremely limited (Welch, Wakefield and Adams, 1936; McCance and Widdowson, 1937; Hahn, Bale, Hettig, Kamen and Whipple, 1939). It was therefore suggested that absorption must play a limiting role in the total body iron level unless the latter were to increase indefinitely. Simple diffusion as determined by intestinal lumen-plasma gradients did not offer a satisfactory explanation. We were therefore led to postulate the existence of a compound in the gastric and intestinal mucosa which was capable of combining reversibly with iron. It was suggested that this compound might be apoferritin or ferritin, a protein which is capable of combining stoichiometrically with 23% of its weight of iron (Hahn, Bale, Ross, Balfour and Whipple, 1943). The existence of such an acceptor mechanism would explain the limited ability of the body to absorb iron, the relatively greater efficiency of absorption of small doses of iron, and the differential absorption as determined by the level of the reserve stores. That ferritin is actually present in the gastro-intestinal mucosa has been ably demonstrated by Granick, 1946, and he has made further studies to show its distribution there. In Fig. 1 we reproduce Granick's modification of our hypothesis of the mechanism of iron absorption.

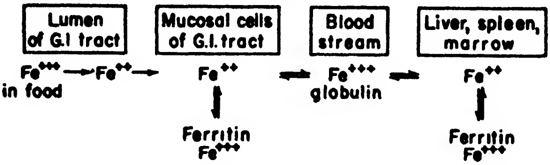

FIG. 1

Mechanism of iron absorption (After Granick, *Chem. Revs.* **38**, 379, 1946).

According to this theory, in the normal adult male the ferritin in the mucosa cell would have its full stoichiometric quota of iron and therefore could not take up more iron from the lumen of the G-I tract. During conditions of growth, pregnancy, or hemorrhage the labile stores of the body, largely from the liver, would be utilized. Such labile stores are in turn in equilibrium with the plasma globulin-bound iron, and when the latter is diminished iron would be released from the mucosa cell ferritin. The resultant apoferritin in the mucosa would then be capable of taking up more iron from the lumen of the G-I tract. That the reverse of this process does not take place as a mechanism of iron excretion is considered due to the fact that the plasma iron bound by globulin is too firmly attached to be taken up by ferritin partly saturated with iron or apoferritin. Recently the nature of an iron-binding component of human blood plasma has been identified as fraction 1V-3,4 (Cohn), which is rich in α- and β-globulins, (Schade and Caroline, 1946).

Excessive amounts of iron in the body can conceivably be deleterious, especially if one were to associate the fibrosis occurring in hemachromatosis to the iron deposits in the liver and pancreas. Therefore, it is probably more than fortuitous that the body is provided with an efficient means of protecting itself against an undesirable accumulation of this metal.

3. *Efficiency of Iron Absorption*

Fed at therapeutically practical levels the efficiency of iron absorption is notoriously poor. This fact is seldom given the proper consideration it deserves, and ignorance of it is probably responsible for many disappointing results of treatment. When iron is fed humans or experimental animals at low levels of 0.2–1.0 mg. as much as 50–80% absorption may take place. Even higher percentage uptakes have been reported in rats when the dosage level was reduced to as little as 0.05 mg. (Copp and Greenberg, 1946). As the dosage level is increased to levels corresponding to amounts in the normal dietary the efficiency of uptake is sharply reduced, and at the 2.0–5.0 mg. level may be only about 12% even in the growing child (Darby, Hahn, Steinkamp and Kaser, 1947), or 30% in the latter part of pregnancy (Hahn, Carothers, Cannon, Sheppard, Darby, Kaser, McClellan and Densen, 1947). We have as yet to determine the efficiency of absorption in humans in any large series at levels corresponding to what is customarily given therapeutically. Some years ago it was shown that, in anemic iron-depleted dogs, iron fed at a dosage level of 40 mg./day over a two-week period was absorbed and utilized to the extent of about 35% (Whipple and Robscheit-Robbins, 1936). When this dose was increased by a factor of ten to 400 mg./day the percentage uptake dropped to 5 or 6%. Under the two experimental dosage levels the total iron accumulated by the systemic circulation was, respectively, of the order of 12 and 20 mg./day. Thus, increasing the dose by a factor of ten times did not double the actual uptake. These findings are in accord with those we have encountered in the use of tagged iron administered in single doses to iron-deficient dogs. There must obviously be some optimum level at which iron should be fed for maximum efficiency. It is very likely that this optimum will be in an exceedingly low range of dosage level and will necessitate the administration of frequently repeated small doses.

Common clinical practice is to administer iron at dosage levels of about 325 mg. It is quite likely that the efficiency of uptake is only about 5% at such levels of intake and, therefore, only about 16 mg. of iron, equivalent to about 5 g. of hemoglobin, are actually absorbed and utilized. This must be taken into consideration in judging the response of a patient to treatment.

We know little as to the effects of other factors on iron absorption. There are a number of reports indicating that phytic acid and its derivatives, as well as phosphates, inhibit the absorption of the iron. Therefore, therapy with iron would probably best be directed to administration between meals.

4. Effect of Valence State on Iron Absorption

Much controversy existed for many years as to the effect of the form of combination and state of oxidation of iron salts on uptake. By means of tagged iron we were able to show that in dogs and in humans ferrous chloride was much more readily absorbed than was the corresponding ferric salt (Hahn, Lowe, Meneely and Bale, 1944; Hahn, Jones, Lowe, Meneely and Peacock, 1945). Moore, Dubach, Minnich and Roberts, 1944, reported similar observations in humans, although, like Whipple and Robscheit-Robbins, 1936, they found little difference in the uptake by dogs. It would appear at this time that ferrous chloride or sulphate would be the salt of choice in iron therapy or prophylaxis.

Some patients tolerate iron in any form so poorly when administered orally that it becomes difficult to treat them adequately. In such cases either parenteral iron or whole blood transfusions are indicated, and since there is not at present a satisfactory parenteral agent available one must rely on transfusion.

III. DISTRIBUTION AND TRANSPORT OF IRON

1. Distribution of Iron in the Body

No adequate studies of the true distribution of iron in the human body have been made to date. By analysis of perfused tissues of dogs and rats it has been possible to arrive at fair approximations as to the distribution of the various iron fractions (Hahn and Whipple, 1936; Hahn, 1937; Austoni, Rabinovitch and Greenberg, 1940). This is indicated in Table I below.

Thus there are 270 g. of hemoglobin in circulation and available iron equivalent to 90 g. of hemoglobin in reserve which could be supplied as needed. Therefore, the dog has about a 33% reserve potential hemoglobin reserve. It is probable that the distribution of iron in the human is similar. The term parenchyma iron refers to cytochrome, catalase, *etc.*, iron of the tissues which is necessary for the functioning of the cells and which is not depleted regardless of the demands placed on the system by anemia, malnutrition, pregnancy, growth, *etc.*

2. *Transport of Iron*

It is now generally agreed that following absorption of iron in the ferrous state it is oxidized on reaching the blood where it is transported in firm combination with the plasma globulin (Starkenstein and Harvalik, 1933). This transport function of plasma has been studied extensively by Moore *et al.*, 1937, 1939; Waldenström, 1944, and by the use of the radioactive isotope by Hahn, Bale, Lawrence and Whipple, 1939 and Yoshikawa, Hahn and Bale, 1942). Depletion of the labile stores has been shown to be reflected in a lowered value for serum iron (Moore *et al.*, 1939; Skouge, 1939; Heilmeyer and Plotner, 1937; and Waldenström, 1944). Normal values range from 100–200 γ/100 ml. In iron-deficiency anemia or pernicious anemia in remission they may be markedly lowered, while in pernicious anemia in relapse the level may be elevated. Attempts have been made to quantitate the amount of iron absorbed following oral administration by changes in the level of plasma iron, but it should be remembered that two simultaneous processes are taking place whose rates may be entirely different, namely, absorption and removal from the circulation by the storage depots and the hematopoietic system. As shown by tracer studies large amounts of ferric iron may be removed from the blood stream by the liver in a few hours (Granick and Hahn, 1944). As yet no clear-cut demonstration of transport of iron from the stores to the bone marrow has been made either with or without the use of isotopes.

TABLE 1[a]

Distribution of Iron in the Perfused Tissues of the Dog[b]

	Mg.	Per cent of total body iron
Blood hemoglobin iron	900	57
Muscle hemoglobin iron	110	7
Total hemoglobin iron	1010	64
Parenchyma iron (muscle and other tissues)	240	16
Available visceral storage (liver, spleen, and marrow)	225	15
Available iron (other tissues, estimated)	75	5
	1550	100

[a] Hahn, P. F., *Medicine* **16**, 249 (1937).

[b] These values are calculated for a dog of about 20 kg. body weight having a circulating blood volume of 1500 ml. and a bulk of striated muscle of 6.5 kg.

In spite of the relatively small amounts of iron in circulation in the plasma at any one time, the importance of this fraction of the body iron should not be underestimated. It represents the most *labile* part of the metabolic iron and is in equilibrium with the ferritin iron of the gastrointestinal mucosa and the reserve stores as well as the site of destruction and site of origin of red cell hemoglobin. It should be mentioned that the level of serum iron is lowered in infectious processes but the significance of this has not been established at this time. Use of tracers should help clarify many of our ideas relative to the transport and internal metabolism of iron.

3. Muscle Hemoglobin Iron

When one considers the importance of muscle hemoglobin we must admit to an appalling state of ignorance concerning this fraction of the body iron. If there is no proliferation of muscle tissue during growth and the iron necessary for the later development of this tissue is already present preceding growth, one might expect to find higher iron content of muscle in children than in adults. Such is not demonstrable. Very little work has been done with reference to the development and destruction of muscle hemoglobin. Although we know that when red blood cells undergo destruction the porphyrin entity of the contained hemoglobin can be accounted for quantitatively by the elimination of bilirubin in the bile, we do not know whether the catabolism of muscle hemoglobin contributes to the biliary output of pigment (Hahn, P.F., 1947). In other words, we do not know whether muscle hemoglobin iron is concerned with the give and take between storage depots, exogenous sources, and blood hemoglobin iron or, if once incorporated into the muscle cell, the iron remains to be used over and over as the cell undergoes destruction and rebuilding. Here again is a very promising field of study using the iron isotopes. It is of fundamental interest to determine whether the muscle cell is capable of forming the hemoglobin molecule and the tracer technique lends itself readily to the solution of such a problem.

4. Ferritin and Its Relation to Hemosiderin

Through the Prussian blue reaction pathologists for many years have recognized the presence of iron in tissues associated with the presence of the pigment hemosiderin. This pigment is found in large quantities in the macrophage-lymphoid system in diseases involving chronic or massive acute blood destruction, following repeated transfusions, especially in patients with a decreased hematopoietic function, in aplastic and pernicious anemia, as well as in certain more rare conditions in which there is arrest of red cell formation at various stages, such as Mediterranean anemia. Not much deduction was necessary to see the relationship between the end products of hemoglobin disintegration and hemosiderin, the latter representing either the iron or iron-globin residue.

Using experimental hemolytic anemia, induced by subcutaneous administration of acetylphenylhydrazine, it is possible to show that the iron liberated from the destroyed red cells is nearly quantitatively reutilized for hemoglobin formation (Cruz, Hahn and Bale, 1942) and, when proper correction is made for the circulating red cell mass (Hahn *et al.* 1943) and the small amount of iron lost through the biliary tract during the period of maximal blood breakdown (Hawkins and Hahn, 1944), the quantitative relationship is nearly complete. Since the porphyrin can be accounted for by the extra bile pigment eliminated, many investigators felt that the globin was swept into the metabolic protein pool and the iron remaining represented the hemosiderin, perhaps in the form of the hydroxide of iron.

About five years ago Granick began an exhaustive study of the chemical and physical properties of ferritin. This iron protein compound had been isolated from the spleens of horses by Laufberger, 1937. It contained 23% of iron and could be obtained readily in crystalline form. The iron-free compound apoferritin could be obtained with identical crystalline form by treatment of ferritin with dipyridyl in a reducing buffer solution. Granick (1943) was able to crystallize ferritin from liver, spleen, bone marrow and some other tissues, as well as demonstrating its presence (Granick, 1946) immunologically in a wide variety of tissues. In a collaborative experiment Granick and Hahn (1944) were able to show that ferritin was rapidly formed in the liver following the intravenous administration of tagged ferric ammonium citrate. By means of both radioactive iron and magnetic moment measurements it was further shown that red blood cells tagged with the isotope and transfused into recipient dogs could be broken down by hydrazine administration with the resultant formation of ferritin (Hahn, Granick, Bale and Michaelis, 1943).

It would seem then that in hemolytic processes the iron fraction of the hemoglobin molecule is deposited in the macrophage-lymphoid system in the form of ferritin. One is tempted to suggest that ferritin is actually an iron-globin complex derived directly from hemoglobin. The molecular weight of apoferritin has been shown to be 460,000 whereas that of globin is close to 68,000 (Granick, 1946), but possibly the former is some aggregate of the latter. If the identity of ferritin and hemosiderin could be established and shown to be closely related to the iron-globin derived from hemoglobin by the simple loss of porphyrin we should be much further along in our understanding of the construction and disintegration of hemoglobin. Perhaps we have been misled in the past by the artificial classifications of proteins by salting-out techniques and have consequently often missed the relationships exisiting among proteins from apparently different sources. In this connection it is interesting to recall the electrophoretic similarity between globin and serum albumin (Pederson and Waldenström, 1937).

At least we can say at this time that ferritin and hemosiderin are deposited in certain tissues of the body under the same circumstances and they can have a common origin in red cell hemoglobin.

5. Iron in Cellular Enzyme Systems

We are in a position to say practically nothing concerning the metabolism of certain of the essential iron compounds of the body. The heme molecule is known to be a part of the enzyme catalase as well as cytochromes a, b, and c. Their vital part in the metabolism of cells is just beginning to be properly appreciated (Lemberg, Legge and Lockwood, 1938, 1939). Peroxidase may also be found to be involved in the body economy although its function here (Barkan and Schales, 1937, 1938) is not well understood at present. Very little is known about the role of iron in these compounds and such was the case ten years ago. Unless some capable group of investigators assumes responsibility for the study of these compounds in relationship to iron metabolism as a whole we shall not likely be any wiser ten years hence.

IV. EXCRETION OF IRON

1. Excretion of Iron by the Kidney

It has been realized for many years that the renal excretion of iron was negligible. Except in the cases of gross hematuria or hemoglobinuria there is no appreciable loss of iron by this route. Even the small amounts which escape through the kidney can easily be measured, however, if the isotope technique is used, and it was shown that shortly after venous administration of soluble iron salts to dogs, small amounts of the tagged iron were to be found in the urine (Hahn *et al.* 1939). Even under conditions of massive blood destruction only slightly larger amounts than normal are excreted in the urine. The same findings were made in the case of the rat (Greenberg, Copp and Cuthbertson, 1943) in spite of the fact that cobalt salts are primarily excreted by this route.

2. Biliary Excretion of Iron

Some loss of iron takes place from the biliary tract although this amounts only to the order of 0.2 mg./day in the dog (Hahn, Bale, Hettig, Kamen and Whipple, 1939; Hawkins and Hahn, 1944) and also in the rat (Greenberg, Copp and Cuthbertson, 1943), both species of animals being studied by means of biliary fistulae. Thus, we do not know whether any of this iron might normally participate in the "entero-hepatic" circulation and therefore be reabsorbed, but it seems unlikely. When large numbers of red cells are undergoing destruction due to the administration of acetylphenylhydrazine the biliary iron may be increased by a factor of ten times. Its loss through this channel parallels very closely the excretion of bile pigment by dogs. Furthermore, the iron and bile pigment were lost at rates which were independent of the volume of the bile secreted (Hawkins and Hahn, 1944).

3. *Fecal Excretion of Iron*

Normally there is very little iron excreted by way of the gastro-intestinal tract as mentioned earlier (Widdowson and McCance, 1937; Welch *et al.*, 1936; Hahn *et al.*, 1939; Maddock and Heath, 1939; Austoni and Greenberg, 1940). Fecal iron derived from red cells tagged with the radioactive isotope ranged from 0.05 to 0.4 mg./day (Hahn *et al.*, 1939), and it has been suggested that since the total fecal iron was so little in quantity it might derive from desquamated epithelial cells of the intestinal mucosa. The recent work of Granick, 1946, in which he found ferritin in the mucosa of the gastro-intestinal tract would lend credence to this suggestion.

In the intact animal some of the fecal iron could derive from that secreted by the biliary tract as discussed above in the event the latter was not reabsorbed. It is doubtful that the loss by this route would ever be very considerable except when massive blood destruction is taking place.

V. MISCELLANEOUS EXPERIMENTAL PROCEDURES USING TAGGED IRON

1. *Rate of Utilization of Iron*

The rate of iron utilization is probably limited by the number of red cell precursors which are available in the hematopoietic tissue at that stage at which iron is taken up. This is in all probability then a function of the degree of normoblastosis. Some suggestive evidence is shown in Fig. 2 below. Three animal experiments are represented in which a single dose of iron tagged with Fe^{59} was administered by gavage. These dogs had been maintained at a chronic anemia level by blood withdrawal for many months and were fed a diet low in iron during this whole period. The first (Experiment 6) was given 84 mg. of iron as the ferric salt. Against the time in days, as the abscissa, is plotted the per cent of the maximum whole blood isotope level in the whole blood, 100% being complete utilization of the iron absorbed. It can be seen that it took about 9 days for complete utilization. In the same animal after the circulating isotope had been removed by repeated bleeding, a dose of only 1.2 mg. of iron was administered (Experiment 7) and it can be seen that the maximum level of whole blood isotope was reached in about 7 days. Again after bleeding the isotope from the circulation, the same dog was given a single dose of only 0.2 mg. of iron (Experiment 8) and, as can be seen, the maximum was reached in about 5 days. Such data cannot be trusted too much from a quantitative standpoint because of the variation in levels obtained but they are suggestive. If one scans the percentage of maximum uptake for the three experiments at the end of one day the results are more striking. In such experiments it must be kept in mind that the maturation time of the erythrocyte precursor from the time of uptake of iron to its deliverance to the peripheral circulation is a factor which must be considered in such studies. The experiments offer room for speculation in this regard since, if one assumes that the iron is acquired at the normoblast stage, and further assumes that these normoblasts are not present in the circulating blood, a limit for the maturation time of the normoblast to the reticulocyte could be conceivable based on the results of carefully conducted experiments of a similar nature.

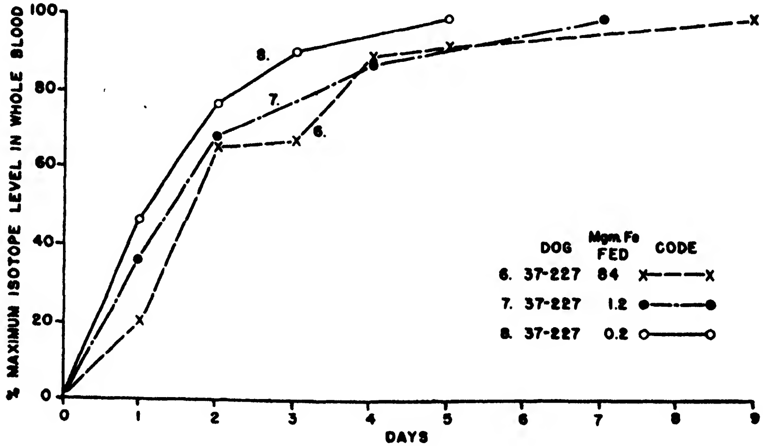

FIG. 2

Rate of hematopoiesis as limited by dosage level.

Another set of experiments demonstrating the effect of the degree of activity of the hematopoietic system on the rate of utilization of iron is shown in Fig. 3 below. In the first three experiments a dog was given single doses of iron tagged with Fe^{59} superimposed on a diet low in iron. In each case, on the first day following feeding, less than 20% of the maximum ultimate whole blood isotope level was reached. In experiment 4 the same animal, after the circulating isotope was as usual removed from the red cells by repeated bleeding, was given a supplementary feeding of 400 mg. of non-radioactive iron for a two week period. Finally, a single dose of tagged iron was administered and it can be seen that, on the first day after this, 50% of the maximum ultimate whole blood isotope level had been reached. Another dog was given a two week pre-feeding of raw lean beef and then a single dose of tagged iron administered. A similar effect was noted as with the pre-feeding of iron.

It should be noted that the fraction of the dose actually absorbed is not involved in these two sets of experiments because of the manner in which the data are plotted. We are concerned with the *rate* at which the hematopoietic system can utilize the iron which is presented to it (Hahn, Ross, Bale and Whipple, 1940). In the experiments shown in Fig. 2 it would appear that very few cells were immediately prepared to accept the iron since there is such a noticeable difference in the rate with which the 1.2 mg. dose was handled as compared with the 0.2 mg. dose. The conditions of the experiment must be kept in mind, namely that the animals, although being anemic and iron-depleted, had extremely low iron intakes for long periods preceding the experiments and this undoubtedly resulted in a different morphological state of affairs in the bone marrow than would obtain in a normal animal a week after a single acute massive hemorrhage.

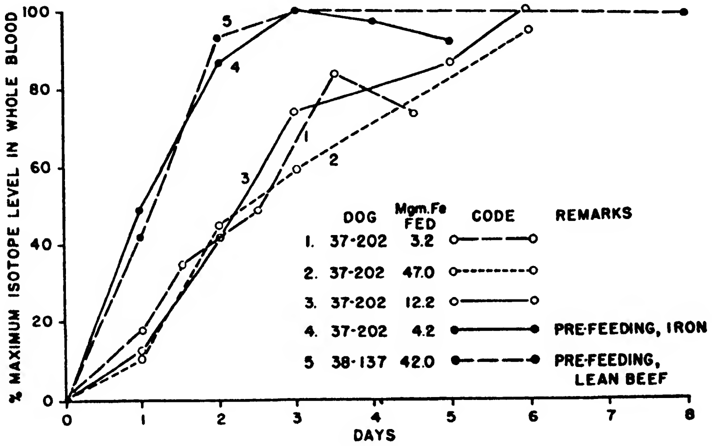

FIG. 3

Rate of hematopoiesis as influenced by prefeeding.

In the experiments included in Fig. 3 it is apparent that the pre-feeding of lean beef (a moderately good source of hemoglobin building materials) or iron resulted in the establishment of a considerable excess of centers of hematopoiesis than existed in the control experiments (Hahn and Whipple, 1939). Such data taken with the recent findings of Granick, 1946, in which the actual amount of ferritin in the gastro-intestinal tract was found to increase in response to iron feeding cause one to suspect that, aside from a simple replacement action, iron also may well exert a stimulative effect on the hematopoietic system. We shall consider this possibility in more detail elsewhere (Hahn, 1947; Ross, 1942, 1943).

2. Interpretation of Blood Isotope Curves

Certain types of experiments concerned with iron metabolism, as well as hemoglobin and red cell metabolism, can be carried out by means of employment of the radioactive isotopes which require an appreciation of some fundamental changes in the blood isotope picture. Much information can be obtained in the experimental animal and human by the employment of a few simple manipulations such as controlled bleeding, hydrazine anemia induction, *etc.* Quite characteristic responses are obtained with regard to the red cell and whole blood isotope concentrations under such conditions and we shall consider a few typical experiments below.

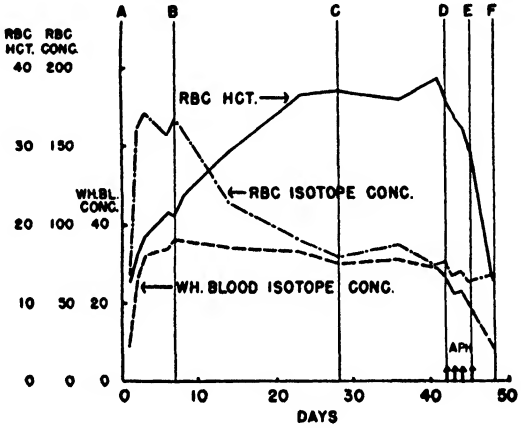

FIG. 4

Red cell and whole blood isotope levels after single feeding.

In Fig. 4 is shown the result of administration of a single dose of tagged iron to an anemic, iron-depleted dog fed an unrestricted diet of table scraps. At point A, with a red blood cell hematocrit of 13%, the animal was given the iron by gavage. There was a prompt rise in the concentration of the isotope in the whole blood reaching a maximum at B indicating complete utilization of all the tagged iron absorbed in 7 days. The red cell isotope concentration, however, had reached its peak on the third day and subsequently dropped somewhat, indicating that the regeneration of red cells and hemoglobin as a whole was proceeding at a rapid rate and suggesting that after the third day the utilization of untagged iron either absorbed previous to or after the single tagged dose was proceeding at a more rapid rate. Thus, although the actual total circulating isotope (as shown by the whole blood curve) was still increasing, nevertheless the concentration of isotope in the red cells was falling due to dilution by exogenous untagged iron. From B, and continuing through C, the hematocrit is gradually seen to rise to a low normal value of 37%. The whole blood isotope level remains essentially unchanged while the red cell isotope level drops off in indirect proportion to the hematocrit values. Period C through D represents a period of stability of the whole blood picture and would be the ideal time for study of the effect of any experimental procedure. At D the subcutaneous injection of 300 mg. of acetylphenylhydrazine was begun daily for 4 days. During the course of these injections, and for 3 days following, the hematocrit dropped precipitously from 36% to 13% and there was an expected corresponding drop in the whole blood isotope concentration. It is to be noted that the red cell isotope concentration remained constant and we interpret this to mean that all the red cells reacted similarly to the action of the drug, there being no preferential destruction of isotope-containing cells or of non-isotope-containing cells. This is in accord with the fact that there are no very new or very old cells in circulation, all being adult cells of at least 30 days of age (see period BD). Later we shall see how this picture may vary.

3. *Constancy of the Total Blood Volume*

The experiment described in Section 2 above provides us with other information than merely the behavior of the blood isotope levels under conditions of hemorrhagic and experimental hemolytic anemias. The fact that, with a gradually increasing red cell hematocrit, the whole blood isotope level remains constant while the red cell isotope level falls may be interpreted as indicating that the total blood volume of this dog remained constant (Hahn, 1944) in spite of the fact that there was a change of 100% in the hematocrit value. This interesting fact can be arrived at from the data by a different approach as shown below.

When the isotope is incorporated in the red cell it remains there at least for the life of the red cell as shown elsewhere. Therefore, since there is no exchange of the isotope with other iron of the body, or loss by excretion, we may say that during the life span of the red cell the total amount of isotope in circulation remains constant after it has all been used after absorption. This may be expressed as follows:

$$\text{Total RBC mass} \times \text{conc. isotope in RBC} = K_1 \qquad (1)$$

Since it has been shown that the circulating red cell mass of the dog is equal to the total red cell mass, *i.e.*, all the red cells are in active state of circulation (Hahn, Ross, Bale, Balfour and Whipple, 1942), and since it has been shown that the circulating cell mass is about 75% of the value obtained indirectly by dye methods for determing blood volume:

$$\text{Blood vol. (dye)} \times \text{Jug. Hct.} \times 0.75 \times \text{conc. isotope in RBC} = K_2 \quad (2)$$

In Chart C it is apparent from the shape of the curves for red cell isotope concentration and jugular hematocrit that their products are a constant:

$$\text{Conc. isotope in RBC} \times \text{Jugular Hct.} = K_3 \qquad (3)$$

Dividing expression (2) by (3) we obtain the simple relationship:

$$\text{Blood vol.} = K_4$$

Therefore, as the number of red cells in the circulation is increased due to hematopoiesis the volume of plasma will diminish in order to maintain the total volume of blood at a constant value. It is to be noted that the experiment cited above covers a sufficient period of time so that there is ample time for equilibrium of the plasma volume to take place between sampling. This is important since the relationship Total Blood Volume = K will not hold under pathological conditions such as in cardiac failure, shock, inanition, and acute hemorrhage, *etc.*, as will be pointed out in more detail in another experiment below.

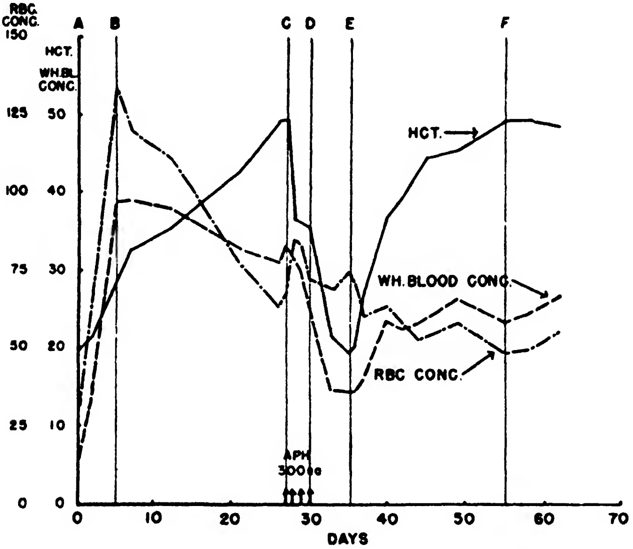

FIG. 5

Increased susceptibility of young red cells to acetylphenylhydrazine.

4. Increased Susceptibility of Young Red Cells to Acetylphenylhydrazine

At point A in Fig. 5 this anemic, iron-depleted dog with an hematocrit of 20% was given a single feeding of tagged iron. The hematocrit, red cell and whole blood isotope levels rise through B indicating a complete utilization of the absorbed radio-iron in 5 days. From B to C the hematocrit rises to a normal level of 49% and there is little change in the whole blood isotope level. However, as seen earlier (Fig. 4), the red cell isotope level drops in indirect proportion to the hematocrit rise. At C, no period of stabilization being allowed, acetylhydrazine was administered daily in doses of 300 mg. During the period of drug administration the hematocrit begins to drop and here we note a definite temporary increase in the red cell isotope concentration. This we feel is ascribable to the fact that some of the most recently formed red cells containing no radio-iron were of a young age group (3–5 days) and were probably more susceptible to the action of the drug (Cruz, Hahn and Bale, 1942) and, therefore, there is left an increased proportion of tagged cells in the circulation until they also begin to succumb. From D to E, while the hematocrit reaches its lowest level, there is a drop in the whole blood isotope, as might be expected, while the concentration of radio-iron in the red cells remains substantially what it was before drug injection. This indicates no preferential breakdown of tagged or untagged cells during this period but shows an absolute loss of isotope from the circulation. During spontaneous recovery, from E to F, the hematocrit resumes its normal level. The concentration in whole blood rises to nearly the predrug level. There is a slight drop in red cell radio iron, due possibly to some dilution by utilization of exogenous iron. However, the absence of any marked decrease in red cell isotope level shows that reutilization of the Fe^{59} from the destroyed red cells is nearly complete. It has been pointed out elsewhere that this latter observation is to be noted even if a considerable amount of untagged iron is in the storage depots and indicates a preferential use of iron from recently destroyed cells.

5. Utilization of Iron in Acute and Chronic Anemia

At point A (Fig. 6) an apparently normal dog *having adequate reserve iron stores* whose hematocrit was 49% was made acutely anemic by the removal of 400 ml. of blood. At B (24 hours) the hematocrit had fallen to 28% due to dilution of the circulating blood by fluid thrown into the circulation in an attempt on the part of the body to maintain a normal or near normal blood volume. At this time a single dose of tagged iron was administered by gavage. Over a period of 6 days, until we reach C, the hematocrit gradually rises as do the whole blood and red cell isotope levels. From C to D the level in the whole blood remains essentially constant and, at the same time, the hematocrit continues to rise resulting in some dilution of the red cell isotope. At D another single dose of tagged iron is given as before. By this time the hematocrit has reached 38% and it must be remembered that a considerable amount of the available iron reserve stores have been withdrawn. Thus we see a much more marked response to the feeding of radio-iron than before, and from D^1 to E the whole blood and isotope iron levels are rising. At E a series of four bleedings were begun which reduced the hematocrit from 43% to 25%. The loss of isotope from the circulation is indicated by the drop in whole blood concentration. In an uncomplicated case one might expect the red cell isotope level to remain constant, at least temporarily, but it drops rapidly in parallel to the whole blood level. We believe this is to be explained on a basis of the immediate response to the bleeding. Greatly increased rate of red cell and hemoglobin formation involving utilization of more untagged storage iron and dietary iron is made possible by the hyperplasia of the bone marrow which undoubtedly exists as a result of the previous acute bleeding three weeks earlier. At F the hematocrit has reached its low level as has the whole blood radio-iron and from this point on there is spontaneous regeneration of red cells and hemoglobin involving the use of only untagged iron. As a result there is no change in the isotope concentration of the whole blood but there is a marked inversely proportional drop in red cell iron as the hematocrit increases to its normal level indicating tagged cell dilution.

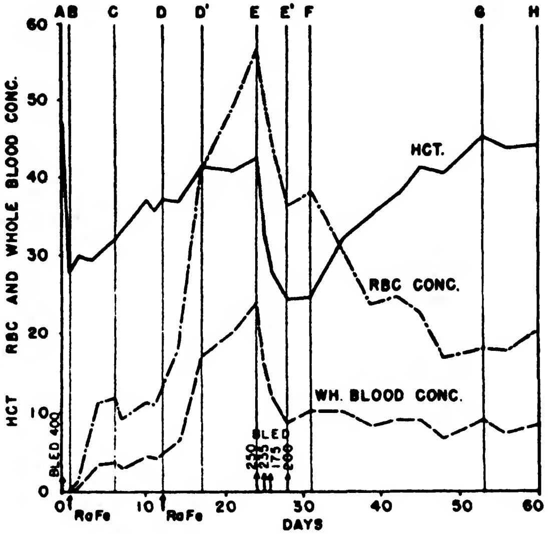

Fig. 6

Iron absorption in acute and chronic anemia.

VI. Effect of Various Conditions on Iron Uptake

1. *Influence of Inflammation on Iron Absorption*

It is well recognized that, in disease conditions involving inflammatory processes of long standing as well as in infectious processes, there is a high frequency of accompanying anemia. Several experiments were carried out using the sterile turpentine abscess as a controlled type of inflammatory process to study the effect of this condition on the uptake of iron. The dogs were healthy, anemic, iron-depleted animals. They were given single doses of radio-iron and the uptake in the circulation determined. After the isotope had been bled out, the animals were then given 1 ml. injections of turpentine subcutaneously in the axillary region and later again fed single doses of the labeled iron. The subsequent utilization by the red cells was again determined. The results can be seen in Table II. The question might arise as to the possibility of absorption having taken place but lack of utilization of the iron being due to the inflammatory process. Therefore, as indicated in Table II, one of these animals was systematically bled and the radioactivity of the blood removed was determined and compared with the loss of isotope from the total circulation. The agreement was well within the limits of the experimental methods employed. Therefore, we are probably justified in feeling that the abscess in the axillary area had a systemic effect which resulted in impairment of iron absorption (Hahn, Bale and Whipple, 1946).

TABLE II

Iron Absorption by Dogs with Subcutaneous Turpentine Abscesses

Dog	Date 1943	Hct. *per cent*	Dose *mg.*	Form	Per cent absorption	Experiment type
40–149	June 17	13.4	9	$FeCl_2$	41.0	Control
"	July 20	23.0	10	"	4.1	Turpentine 1 day prev.
"	September 13	18.3	10	"	18.1	Control
41–164	January 9	26.9	38	FeAmCit	4.1	Turpentine 2 days prev.
"	February 8	28.6	34	"	17.6	Control
37–196	January 9	22.4	38	"	5.3	Turpentine 2 days prev.
"	February 8	23.4	34	"	6.9[a]	Control

[a] This animal had been splenectomised and was, therefore, liable to *Bartonella* infection. That this infection was present and may have interfered with iron uptake at this time as suspected seemed later indicated by later reaction to Mapharsen.

Balance Sheet on January 9, 1943 feeding of dog 37–196

January 19 Hct. 35.2%, estimated C.V. 265 ml.	Total circ. radio-iron	4720 cpm.
February 3 Hct. 28.7	" " "	635
	Radioactivity lost to circulation	4085
Radioactivity found in 1900 ml. of blood removed in period January 19 to February 3		4045

This balance shows there was no additional absorbed and stored iron to be used under the stimulation of blood loss.

Gibson and Finch (1947) have been studying the effect of infection on the *utilization* of iron tagged with the radioactive isotope and injected intravenously. "The results obtained showed that the utilization of iron for hemoglobin synthesis was impaired to a degree roughly proportionate to the severity of the infection as judged by clinical evidence." The test dose used was less than 1 mg. of iron, injected in the form of ferric ammonium citrate. They found that normal individuals would have about 80% of the injected iron in the circulation within 2–3 weeks time. Infectious cases showed the following utilization percentages: one case of pulmonary tbc., active, 46%; two cases of subacute bacterial endocarditis, 36% and 60%; one case of lung abscess with carcinoma, 30%; one case of psittacosis (with pneumonia), 24%; one case of lobar pneumonia, 14%; one case of atypical pneumonia, 72%; and one case of trichinosis, 40%.

Thus, it appears that inflammation and infection both interfere with the body mechanisms for the handling of iron. Clinical experience also shows the futility of attempting to treat anemias while such processes are actively present.

2. Iron Uptake During Growth

As has been pointed out, the normal adult male has been shown to absorb and utilize only small amounts of tagged iron when it is fed. Since the actual need for iron as reflected in the depletion of the reserve stores is a determining factor in uptake, we expect and find increased absorption during growth, pregnancy, and after chronic hemorrhage.

Uptake of tagged iron may be studied in a fairly satisfactory manner from a quantitative standpoint in standard iron-depleted animals. For the most part reproducible results can be obtained in the same animal although the individual variations may be very appreciable (Hahn, Jones, Lowe, Meneely and Peacock, 1945). Therefore, if one is to determine the response of humans to iron feeding the results must be interpreted with a great deal of caution or a large series must be used. In what was probably the first use of isotopes on a large scale survey, a study of the uptake of tagged iron (Fe^{59}) in some 200 schoolchildren was undertaken in two Nashville schools (Darby, Hahn, Steinkamp and Kaser, 1947). The children were in the 8–11 age groups. They were fed single doses of tagged iron at levels of 2–6 mg. Samples of venous blood were drawn 10–15 days later and the radioactivity of the iron in the red cells determined. It was assumed that all the iron absorbed was combined as red cell hemoglobin and, on a basis of this concentration as well as an estimate of the red cell circulating mass based on body weight and hematocrit, the total iron uptake was calculated. Simultaneous determinations were made of red cell counts, hemoglobin levels, hematocrit values, vitamin levels and a record of a week's dietary intake was made. The mean percentage of iron absorbed was 13.5%, 25% of the children showing less than 7% uptake and 75% less than 17% uptake. For the dosage levels studied, these uptakes are considerably higher than one would find in the normal adult male. At the same time they are much lower than the absorptions found in a subsequent study of pregnant women (Hahn, 1947b). It is interesting to note that there was no correlation between the hemoglobin levels and the iron uptake in the children referred to above. There was, however, a marked correlation with the rate of growth as determined from the tables published by Heath and Patek (1937). Because of the narrow age group studied we cannot draw any sweeping conclusions as to the growth requirements for iron. However, calculations based on the data obtained would indicate that boys and girls of this age group require between 2 and 4. mg. of *absorbable* iron per day. It is to be noted that this is a considerably lower daily intake than recommended by the Food and Nutrition Board of the National Research Council. However, the latter estimates must have been based on supposition, since no data existed at the time they were made on which a good approximation could be based.

TABLE III

Uptake of Radioactive Iron in School Children of Seven- to Ten-Year Age Group

Age	7		8		9		10	
Sex	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Mean absorption (*per cent*)	9.26	7.75	10.38	15.84	16.14	16.89	16.68	14.5
	±1.41	±0.78	±1.22	±2.15	±1.01	±2.03	±1.92	±2.4
Median absorption (*per cent*)	7.75	7.00	8.25	12.00	15.00	13.75	17.75	12.75
Estimated total year requirement, mg.[a].	70	67	72	108	152	120	130	163
Number in group	17	24	26	34	26	23	17	9

[a] From data of Heath and Patek (1937).

3. *Iron Uptake During Pregnancy*

A long term survey of the nutritional requirements during pregnancy has been underway at Vanderbilt University Hospital as a collaborative study of the Departments of Obstetrics, Biochemistry, Medicine, and Preventive Medicine. As part of this survey all white women seen in the obstetrics out-patient clinic were routinely fed single tracer doses of iron labeled with the Fe^{59} isotope over a period of 15 months. Over 800 women were studied, no selection being made. Iron was administered on the second visit whenever that happened to occur in the gestational period, (Hahn, Carothers, Cannon, Sheppard, Darby, Kaser, McClellan and Densen, 1947). In a preliminary evaluation of the first 380 subjects, it was found that iron at any given dosage level was absorbed much more efficiently in the latter half of gestation. A brief summary of these preliminary findings can be seen in Table IV.

4. Hemorrhagic Iron-Deficiency Anemia

The etiology of iron-deficiency anemia can be roughly classified as follows: *a*, Nutritional; *b*, Hemorrhagic; *c*, Parasitic; *d*, Gestational. Of these, hemorrhagic, either acute or chronic, is the commonest type of anemia found in the United States. It has been stated repeatedly elsewhere, and probably with ample justification, that microcytic, hypochromic anemia does not occur in the normal adult male unless there has been blood loss. The common causes of acute hemorrhage would include gastric and duodenal ulcers, ruptured esophageal varices, uterine fibromata, tumors of the gastro-intestinal tract, *etc*. These nearly all also contribute largely to the causes of chronic hemorrhage, to which should be added hemorrhoids, menorrhagia, metrorrhagia, and a few other conditions. There is still considerable disagreement in the medical profession as to the use of transfusion in certain cases of acute hemorrhage, some clinicians feeling that, in patients who have bled copiously to an extent short of shock, there is danger of further hemorrhage if transfusion is too quickly resorted to. There is no argument as to the advisability of iron therapy in these cases as a whole. Due to the inefficient absorption of iron a considerable time element is encountered in oral therapy and, unless iron is administered at frequent intervals for many weeks, enough of the element will not be absorbed to satisfactorily represent replacement therapy and the results will be disappointing. There being no suitable form of iron for parenteral use at the present time, the only resort by which large quantities may be effectively administered in a

TABLE IV

Uptake of Radioactive Iron at Various Stages of Gestation in 329 Subjects

No. patients in group	Uptake at weeks of gestational period			
	0–10	11–20	21–30	31–40
22	18.2			
122		21.4		
108			31.5	
77				38.5
329 Total				

brief period is by transfusion of whole blood or suspended red cells. Such therapy should be augmented by iron feeding, however, since it is imperative that the reserve stores be again filled in all cases of acute hemorrhage because of the possibility and probability of impending repetition of the bleeding episode. Where there is chronic low grade blood loss it is a comparatively simple matter to maintain the patient in equilibrium as long as the replacement therapy is vigorously and faithfully applied. For such treatment it is recommended that small doses of the order of 30–60 mg. of iron in the form of the ferrous salt be administered at frequent intervals, preferably between meals, and over prolonged periods of many weeks.

5. *Gestational Iron-Deficiency Anemia*

Among the commonest causes of iron-deficiency anemia in this country is that induced by multiple pregnancy. Provided the dietary intake of iron is adequate, such anemia is manifest usually only for a few months following delivery. Our knowledge of the iron "balance sheet" in pregnancy is very incomplete since, on the one side, we have the extremely variable individual catamenic blood loss saved over 10 lunar months. On the negative side we have the fetal iron needs which are made up of red cell hemoglobin, muscle hemoglobin, tissue parenchyma iron and a considerable amount of reserve store iron used to tide the infant over the nursing period. Also to be considered are the iron in the adnexa and an unpredictable amount associated with parturition and post-partum hemorrhage, these latter values having never been adequately measured. It might be said that here is represented an exceedingly useful field of study which lends itself to use of the isotopes of iron in a particularly valuable way. Some of these problems are under study at the present time (Hahn, Carothers, Darby, Sheppard, Cannon and Densen, 1947). The use of tagged red cells in which either Fe^{55} or Fe^{59} is incorporated in the hemoglobin (see Section VIII, *1* and *3*) should assist us greatly in the investigation of these problems. We have been able to show thus far that the fetus takes up about 10% of the amount of iron absorbed by the maternal organism following administration of single doses of labeled iron. How this uptake varies at different stages of gestation, and what the effects of other factors might be, must await analysis of the whole series under study.

6. *Iron Deficiency Due to Parasitism*

Infestation with ancylostoma (uncinaria) is uncommon in the United States and relatively rare even in the South. In northern Brazil, however, this disease is endemic, and it is also very common in China, Japan, and the East Indies. It is quite well accepted that the anemia related to this infestation is due to the blood-sucking activities of the hookworm in the intestinal tract (Wells, 1931) where there is a concomitant lack of iron in the diet. Much has been learned about this disease through the work of Cruz in Brazil and that of Rhoads and Castle in Porto Rico. Before anthelminthics are administered it is advisable to begin a course of iron administration for a short period. In other words, most investigators feel that the parasitism should not be treated until the anti-anemia therapy is well under way. Ferrous iron is probably the agent of choice followed later by use of trichloroethane or carbon tetrachloride.

Parasitic diseases such as malaria in which there is an associated anemia but where the latter is due to an hemolytic process do not respond to iron therapy as might be expected, since the end products of hemoglobin disintegration are retained by the body and no iron deficiency exists (see Section on hemolytic anemias).

7. *Nutritional Iron-Deficiency Anemia*

The incidence of simple nutritional iron deficiency anemia in the United States is not very high. Where it exists it may be a result of food fadism or, in some instances, local peculiarities of dietary intake. This condition is commonly encountered in China and India and in connection with parasitic diseases mentioned above where the load of hemoglobin production is greatly augmented. Most dietaries in western countries contain adequate amounts of iron for maintainence purposes. In a recent survey (Darby, Hahn, Steinkamp and Kaser, 1947) conducted on school children divided nearly equally into two groups at opposite ends of the economic scale it was found that there were not only no differences of significance in the hemoglobin levels of the peripheral blood, but that there was also no difference in the need for iron as reflected by the uptake of single doses of tagged iron. In the normal individual, even during the period of rapid growth, the requirement for iron is quite low, probably of the order of 5 mg./day.

The addition of iron to certain foodstuffs such as bread on a national scale is unwarranted. Well over half the population would be unaffected thereby and economically such iron supplements would not be feasible. However, in certain localities, especially in some foreign countries, some means of combating iron-deficiency anemia of nutritional origin must be found and should be undertaken as a public health measure.

In the general population the chief danger associated with a mild degree of iron-deficiency anemia would be the lack of hemoglobin reserve to tide the individual over a period of acute hemorrhage due to any one of a number of causes. Otherwise, it has never been shown that there are any ill effects associated with a sub-clinical anemia which might be incompatible with long life. In fact, one must keep in mind the possibility that such a low grade anemia results in a maintenance of a hyperplastic marrow. Low grade anemia in women certainly does not unfavorably influence their longevity. Following such an argument one might say that the individual is as old as his or her bone marrow.

8. Replacement Requirements of Iron Therapy

The replacement requirements for iron can be defined simply. The chief difficulty is in estimating the true approximation of blood loss. When it can be estimated, one may conveniently apply the .figure of 0.5 mg. of iron needed to be taken up by the systemic circulation for each ml. of blood of normal hematocrit lost.

VII. Iron Metabolism in Pathologic States

1. Iron Metabolism in Pernicious Anemia

As in the hemolytic anemias, iron is of no benefit in the treatment of uncomplicated pernicious anemia. Arrest at an early stage of development in the red cell results in iron from previously destroyed cells being deposited in the usual depots in lieu of being reutilized. Therefore, all the iron which would normally be in circulation in the peripheral erythrocytes is stored in the body and is available for use if and when the necessary material specific for cell maturation is supplied. As stated earlier the plasma iron is elevated in pernicious anemia in relapse but drops to normal levels on administration of parenteral liver and presumably under treatment with folic acid, although the latter observation has as yet to be put on record.

In the rare instance where pernicious anemia occurs early in life during the growing period, or where coincident bleeding has taken place due to extraneous causes, pernicious anemia may be found in association with iron-deficiency anemia. Under such conditions, when the patient receives specific therapy for the pernicious anemia, the iron deficiency manifestations become more pronounced and the subject will respond secondarily to iron therapy. Diagnosis of such individuals is not usually simple. However, there is usually considerably more anisocytosis than would ordinarily be found in pernicious anemia and the Price-Jones curve would be broadened.

Ordinarily patients with pernicious anemia show little absorption of test doses of radioactive iron as compared with those having iron deficiency. Therefore, if the facilities are available and such a test is carried out and a high uptake noted, in pernicious anemia it is good indication that a concomitant iron deficiency exists and a second reticulocyte response to the iron may be expected after liver or folic acid has been used. Such a case is cited below (Peterson and Dunn, 1946):

A. A., a five year old white female had been found to have pernicious anemia at the age of 18 months at the time of her first admission to Vanderbilt Hospital. She was shown to have achlorhydria and arrest of the red cell maturation at the megaloblastic level. During the next few years she was regularly shown to respond to intramuscular liver extract and when it was discontinued underwent relapse on a number of occasions. The clinical response to specific therapy was consistently good. Fed a single dose of 10 mg. of iron labeled with Fe^{59} she was found to take up 25% of the metal, as shown by the red cell isotope concentration two weeks later. This amount of absorption was much greater than would ordinarily be found in a case of pernicious anemia and would be representative of the uptake in iron-deficiency anemia. On a basis of this subject's response to liver therapy and the uptake of radio-iron it was predicted that she would show a second reticulocyte response to iron adminstration after remission from liver. Such was found to be the case. In retrospect we can explain the etiology of the iron deficiency in this child as follows. During a considerable part of her first 5 years of life she had pernicious anemia in relapse and this militated against the absorption of adequate amounts of iron for purposes of growth and normal increase in blood volume. Hence, when she was given sufficient liver extract to elicit a normal response there was not enough iron present in the tissues to take care of the demands.

2. *Iron Metabolism in Hemolytic Anemia*

Just as in pernicious anemia, there is no iron deficiency involved in either the familial or acquired forms of hemolytic anemia. This is also true of such anemias resulting from red cell destruction due to drugs or other agents. Since no appreciable amounts of iron are lost, even in repeated bouts of cell destruction, the liberated iron is stored for use as soon as the hematopoietic system can keep up with the destruction phase. For the breakdown of each g. of hemoglobin there will be 3.4 mg. of iron deposited and 35 mg. of bile pigment secreted by the biliary tract.

3. *Iron Metabolism in Polycythemia Vera*

Treatment of polycythemia is accomplished by one of three methods. 1. By phlebotomy. 2. By administration of radioactive isotopes such as P^{32} or "spray" X-radiation. 3. By administration of hydrazine derivatives or similar agents which produce hemolysis of red blood cells. Of these procedures the simple removal of blood by venesection is the one most easily controlled and the only one free of danger to the patient. Lack of success in handling patients by phlebotomy is for the most part due to inability to appreciate the quantity of blood which should be removed to obtain the desired result. This disease is characterised by a marked shift of the distribution of red cells and plasma in the vascular bed, and using the ordinary clinical laboratory criteria one is likely to obtain a false impression as to the relative amount of excess red cells with which he is dealing. It is necessary, therefore, to remove a quantity of *red cells* which are present *in excess* of the normal and not merely to reduce the hematocrit of the blood from the cubital vein. The discrepancies which exist between the mass of red cells in the vascular bed as estimated by ordinary laboratory data and that which can be shown to exist by use of direct measurement of the cell mass can best be appreciated by study of the findings in two cases in Table V below. Also, it must be kept in mind that the reserve iron stores must be depleted if one is not to have an excess of hemoglobin "building blocks" available for new red cell formation. This is important only insofar as the relapse rate will in some measure be determined by such reserves.

The column labeled "Normal" in Table V shows the red cell mass an individual of this body weight and having a normal (45%) red cell hematocrit would be estimated to have. The column labeled "Estimated" indicates the cell mass calculated in the same manner but using the venous large vessel hematocrit found. The column labeled "Determined" indicates the red cell mass found by actual measurement using cells tagged with the Fe^{59} isotope according to the method described in detail elsewhere (Hahn, Ross, Bale, Balfour and Whipple, 1942) and applied to study of human subjects (Gibson and associates, 1947). It is obvious that the red cell mass is not a direct function of the large vessel hematocrit under these pathologic conditions. It is of interest to point out also that in patient J.W., Table V, on admission there was, besides the usual clinical signs of plethora, also a greatly enlarged spleen. After having been bled a total of 4500 ml. of whole blood over a period of 5 months there was no noticeable change in either the plethora or the size of the spleen, in spite of the fact that the venous hematocrit had dropped from an original level of 67% to 42%. At a later date we shall describe in more detail the successive findings in such patients as treatment progresses.

TABLE V

Circulating Red Blood Cell Mass in Polycythemia Vera as Determined by Donor Tagged Cells

Patient	Body wgt. kg.	RBC's *M/mm.*^{3}	Hct. *per cent*	Red blood cell mass in milliliters			
				Normal	Estimated	Determined	Excess over Estimated
J. W.	85	10.8	67	2300	3400	6200	2800
C. Y.	70	7.0	61	1890	2560	3960	1400

4. *Iron Metabolism in Hemachromatosis*

Hemachromatosis is the outstanding though rare disease in which an iron dyscrasia manifests itself as of primary significance, (Sheldon, 1935).

Whereas a normal individual has a total body iron content approximating 4.5 g. (of which 2.5 g. is in circulation as red cell hemoglobin iron) a patient with a full blown hemachromatosis may have from 30–50 g. of iron in his tissues. Since the circulating hemoglobin is within normal limits the excess is accounted for as iron deposited in the liver, pancreas, lymph nodes, *etc.* It is obvious that sometime during the life of the individual upwards of 30 g. of iron was accumulated by some absorption phenomenon. Very few individuals have been found in which the disease had progressed to the point where it was possible to make a clinical diagnosis under the age of 40 years, and, in most instances, diagnosis under this age was only made at autopsy.

There is reason to suspect that the disease represents some inborn error in metabolism. If the iron were to be picked up over the life span of the patient, whose age incidence of presenting symptoms is around 50 years, it would represent only a positive balance of 1 g. /year or about 3 mg./day. Fowler and Barger (1937) have reported finding a high positive iron balance in one individual whose disease they felt was at an early stage. However, in a classical case of the disease in a 49 year old man we (Balfour, Hahn, Bale, Pommerenke and Whipple, 1942) were unable to demonstrate any absorption of iron tagged with the radioactive isotope on either of two occasions. That this finding did not represent absorption with lack of utilization of the iron was indicated in that this subject did not show radioactivity in the circulating red cells following several 500 ml. bleedings.

Pigment deposits characteristic of hemachromatosis are due in part to a non-iron-containing pigment hemofuscin and also an iron-containing one, hemosiderin. The hemosiderin is not confined to cells of the reticulo-endothelial system as in such diseases as pernicious anemia and hemolytic anemia, but is found in the sweat glands and epithelial cells of the liver. In collaboration with Granick and Michaelis (1943) several years ago we found evidence to suggest that ferritin and hemosiderin are probably related closely since they are deposited in the lymphoid-macrophage system under the same conditions and from similar starting materials. Hemosiderin has been shown to be available for utilization for hemoglobin production in dogs with experimental anemia and therefore does not represent an irreversible deposition of iron. If this is also the case in hemachromatosis one might be justified in treating this disease by repeated bleeding such as to induce an anemia. The rationale of such therapy would of course be based on the assumption that the fibrotic changes taking place in hemachromatosis are caused by the deposition of the iron pigment. Cases of this rare disorder are difficult to find. When some are available for study a great deal can be learned about iron metabolism by use of the isotopes.

VIII. Red Cells Tagged with Radio-Iron

1. Red Cell Mass by Donor Tagged Cell Method

Some years ago we described a direct method for the determination of the circulating mass of red blood cells in dogs (Hahn, Ross, Bale, Balfour and Whipple, 1942) and it was pointed out from evidence obtained by this method that all the red cells of the dog were in active circulation normally. It was also shown that the cell mass, as determined directly, was about 25% lower than that obtained by the indirect means of dye estimation of plasma volume and application of the venous hematocrit relationship to arrive at a value for red cell mass. It was possible to show that the average body hematocrit was lower than that of the large blood vessels and that the hematocrit of the small vessels (arterioles, capillaries and venules) was still lower. The concept of the "rapidly circulating plasma volume" and some of its implications were discussed. Twenty per cent of the plasma volume was found to be in sluggish circulation, this fraction including the amount of plasma necessary to wet the walls of the blood vessels.

This method has been applied to the study of human red cell circulating mass (Meneely, Wells and Hahn, 1947) and some of the results have been mentioned briefly under the discussion of polycythemia vera. Gibson and his associates (1946, 1947) have used the procedure extensively in the study of shock in dogs. Ross and Chapin (1943) applied the method of tagging red cells with the Fe^{59} isotope to the study of red cell preservatives, and this procedure was of military value during the war in determining the best materials for the preservation and transportation of red cells to the battle front. Similar studies were carried out by Gibson and his associates (1946, 1947).

2. Red Cell Mass and Hematocrit Relationship

Using the tagged donor cell technique it was found that, under conditions of simple anemia, in dogs there was a linear relationship existing between the red cell mass and the jugular hematocrit (Hahn and Bale, 1942; Hahn, 1944). It was stressed that such a relationship held only under conditions in which equilibrium between plasma and red cells could take place. Obviously in conditions of inanition, cardiac failure, shock, *etc.*, no such proportionality would be expected to exist. Recent studies, in which considerable amounts of blood or suspended red cells were infused rapidly, also indicate that under these conditions an extremely false evaluation of the circulation might be obtained from large vessel hematocrit values (Hahn, 1947). For example, in Fig. 7 we see on the 46th day of an experiment, where a dog whose red cell mass determination showed 245 ml. in circulation was transfused with an equal amount of suspended red cells. Theoretically there would be a 50% dilution. By radioactivity measurements this was found to be 46%, well within the limits of overall error of the methods used. However, the red cell hematocrit of blood from the jugular vein showed only an increase of 14%. As shown by the isotope dilution there had been no actual destruction of red cells and the clinical behavior and appearance of the plasma bore this out. Therefore, under these conditions there was a marked change in the distribution of red cells and plasma in the vascular tree. Such alterations very likely exist in a wide variety of clinical conditions, such as extreme anemia, shock, polycythemia, and following the administration of many drugs. Therefore, it cannot be sufficiently stressed that the laboratory studies of blood of the large blood vessels can be very misleading in the interpretation of the condition of a patient's circulatory dynamics and formed elements. A typical case in point came to the writer's attention several years ago. An anemic patient showed a red cell count of 750,000 rbcs./mm.³ and, following transfusion of 800 ml. of blood, showed no change in the peripheral cell count. The house officer in charge was greatly perturbed and looked diligently for signs of hemolysis. He found none, as

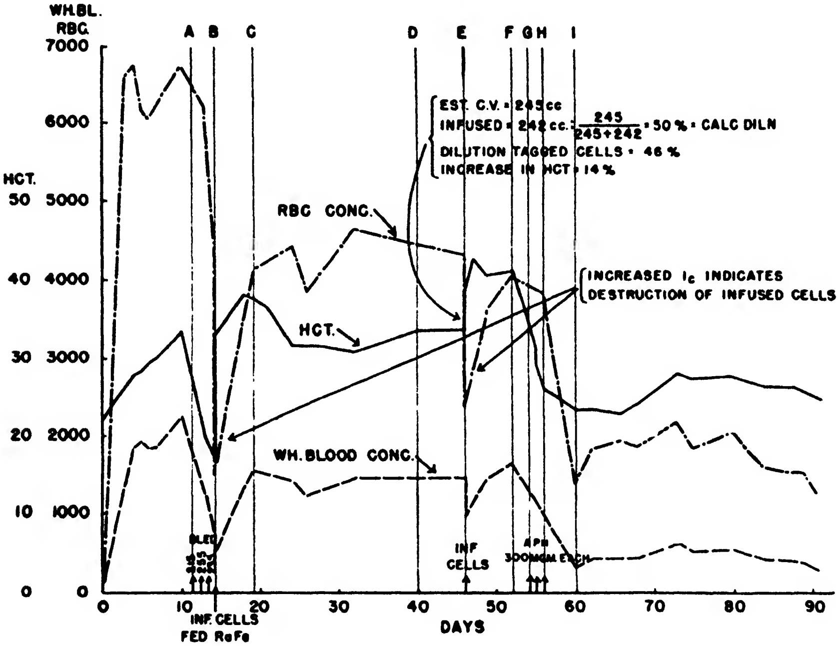

Fig. 7

Acute red cell mass changes not quantitatively reflected in hematocrit.

might be expected. However, on questioning he readily admitted that the condition of the patient had improved markedly from a clinical if not a laboratory standpoint. In such extreme degrees of anemia it is likely that there is considerable vasoconstriction of blood vessels in the body with a shunting of most of the available red cells to the vital centers. Administration of additional but sub-optimal amounts of blood only serves to take care of some of the local deficits, and this is not always reflected immediately in an improved peripheral blood picture.

3. *Use of the Double Isotope Technique*

Most studies carried out during the past 8 years using the iron isotopes have involved the use of Fe^{59} only. This is usually prepared from the lowly abundant Fe^{58} by deuteron bombardment in a cyclotron. There are, therefore, limits of specific activity obtainable, and where the course of small amounts of total iron must be followed, the Fe^{55}, made either by the cyclotron or pile by a transmutation process, may be used. Gibson, Weiss, Evans, Peacock, Irvine and Kip (1946) and Peacock *et al.*, 1946), have studied both isotopes simultaneously in various experiments in shock in which it was possible to measure the "tissue hematocrit." They have also demonstrated the feasibility of studying the survival of red cells of various ages by use of the double isotope approach using donors tagged respectively with the Fe^{55} and Fe^{59} isotopes. Many problems which were time consuming and prevented the study of patients under the same conditions, such as the determination of relative efficacy of ferrous and ferric salts could have been expedited with the use of the double isotope technique.

Eventually it may be possible to utilize some of the four stable isotopes of iron by use of mass spectrography and, in conjunction, as many as 5 or 6 compartments may by this means be followed simultaneously. Iron occurs in so many forms in the body that it may eventually be necessary to marshal all such resources for a complete understanding of this vital element.

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Radioactive Phosphorus: Its Application to the Study of Phospholipid Metabolism

BY I. L. CHAIKOFF AND D. B. ZILVERSMIT

CONTENTS

I. Introduction

The widespread occurrence of phospholipids in animal and plant cells and their presence in large amounts in such tissues as liver, kidney, and brain, and in the yolk of the egg account for the biochemist's interest in these compounds. Certain areas of the brain contain as much as 10% fatty acids, most of which are components of phospholipid molecules, whereas as much as 8% phospholipids have been found in the yolk of the hen's egg. The observation that about $\frac{2}{3}$ of circulating or plasma fatty acids are not free but exist as part of phospholipid molecules is suggestive of their importance in intermediary metabolism of fats.

The 3 well-known types of phospholipids found in animal tissues are the lecithins, the cephalins, and the sphingomyelins. In addition to phosphorus, these compounds contain fatty acids and nitrogenous bases. Other types of phospholipids that have been isolated from animal tissues are distinguished by their content of inositol or by their lack of a nitrogenous base, as for example cardiolipin. For the purpose of this discussion, the structure of a phospholipid molecule may be exemplified by lecithin, which is shown in Fig. 1. It may be regarded as formed from 5 primary components: 2 fatty acids, glycerol, choline, and phosphoric acid. These are joined by 2 general types of bonds: a fatty acid-glycerol linkage and a phosphate-alcohol ester bond. Phosphate is bonded in the lecithin molecule by 2 ester linkages, one with glycerol, the other with the base choline.

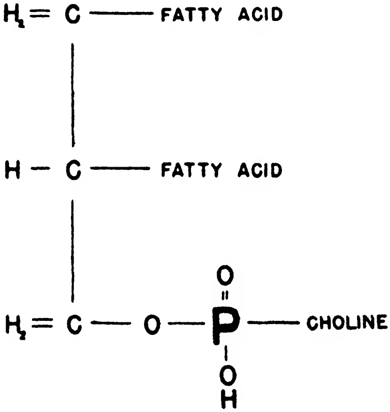

Fig. 1

Structure of a lecithin.

The formation of the bonds in phospholipid molecules has been studied with various labeling agents. Since but a single stable isotope of phosphorus exists, its radioactive isotope P^{32} provides the only means of investigating the formation of phosphate bonds. The first to use it in the study of phospholipid metabolism was Hevesy in Denmark; this was soon followed by extensive work in Berkeley and elsewhere in this country.

The present review illustrates many applications of P^{32} as a tracer in intermediary metabolism of fat. These have provided for the first time information on such problems as the rate and site of formation of phospholipids in the animal body, the relation of their synthesis to energy-producing enzyme systems, the rapidity of their utilization, and the tissues in which it occurs.

II. Some General Considerations on the Use of Radioactive Phosphorus as a Labeling Agent in the Animal Body

1. *The Principle of the Steady State*

When one considers the complexity of the animal organism, *viz.*, the multitude of reactions occurring simultaneously, the chemical dissociations and recombinations, the syntheses and destructions incessantly taking place, the shifting of metabolites from one compartment or tissue to another, it is amazing that body fluids and tissues are relatively constant in their chemical composition. In view of this homeostasis, the dynamic movement of metabolites can not be followed by a chemical determination of tissue constituents. Before the introduction of labeling agents it was not possible to measure dynamic aspects of biological systems without effecting drastic changes in the steady state, a procedure that at best provided a distorted picture of normal metabolic processes. A new approach to this problem has been made available by the use of molecular labeling agents, particularly radioactive isotopes, since they can be prepared with exceedingly large amounts of radiation in association with negligible, even chemically non-detectable, mass. With such isotopes we can now "date" a molecule, thus making it possible to distinguish between newly formed molecules and those present before the introduction of the isotope into the body.

2. *The Amounts of Labeled Phosphate Introduced into the Body*

It is possible to prepare samples of radioisotopes that contain atoms numerous enough for detection by their radioactivity but too few for chemical measurement. By the administration of such a tracer dose of a radioisotope, it is possible to label the naturally occurring element in the body without altering measurably its total amount in the body. This feature is particularly valuable when the naturally occurring element is present in the body in very minute amounts. By this means flooding of the animal body with unphysiological amounts of the element is avoided and the steady state of the system is maintained. In the case of phosphate, however, which is present in appreciable amounts in the body, it has been shown that relatively large amounts of labeled phosphate can be administered without interfering with its value as a labeling agent for endogenous metabolism.

Fries and Chaikoff (1941a) found no difference in the P^{32} recovery in brain and blood when such widely differing amounts of phosphate (Na_2HPO_4 containing P^{32}) as 27.5 and 1.4 mg. were injected into 200 g. rats. It has also been shown that the fraction of the administered P^{32} recovered in the phospholipid of liver, muscle, or blood of rats after a 12-hour interval was the same even though the amounts of labeled phosphate injected varied from 6 to 48 mg. The finding that such large variations in the amount of Na_2HPO_4 injected produced no change in the percentage of the administered labeled phosphorus recovered in brain and blood is not surprising. Although 48 mg. of phosphate is an appreciable amount, it represents but a small fraction of the total phosphate available for labeling in the adult rat; hence, the introduction of this amount into the rat does not markedly increase the animal's phosphate content.

3. The Tracer Dose for Various Tissues

The amount of P^{32} used in the Berkeley laboratories as tracers in phospholipid studies is not kept constant; the dose injected is determined by the tissue studied. Thus, in the case of the brain, which is relatively inactive in its uptake of P^{32}, a dose corresponding to about 0.05 microcurie[1]/g. body weight is employed for the rat. In the case of a more active tissue like the liver, amounts of P^{32} yielding a radioactivity of 0.001 microcurie/g. body weight are administered. Jones (1942) has shown that within very wide limits the percentage recovery of total P^{32} and phospholipid P^{32} in tissues of the mouse is not influenced by variations in the radioactivity of P^{32}. Five groups of tumor-bearing mice were injected with solutions of Na_2HPO_4 containing the same amount of P^{31} but differing in their P^{32} content. The radioactivity varied from 1 to 70 microcuries. In spite of the 70-fold difference in radioactivity of the dose employed, no difference was observed in the percentage of the administered dose recovered as phospholipid P^{32} or as total P^{32} in liver or tumor. Apparently there is a large factor of safety in the radiational dosage of P^{32} when used as a tracer.

4. Expression of Radioactive Data

Percentage Recovery. The percentage of the administered P^{32} incorporated into phospholipid has been widely used as an index of phospholipid synthesis. The limitations of this measure are dealt with below.

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[1] A curie of any radioactive material undergoes the same number of disintegrations per unit time as 1 g. of radium, namely 3.7×10^{10} disintegrations /sec. In the Berkeley laboratories, P^{32} is calibrated in terms of disintegrations against a uranium (UX_2) standard. One microcurie of P^{32} /g. of tissue in a case where P^{32} is uniformly distributed in a large tissue mass produces an amount of ionization equivalent to 42 r /day. (Low-Beer *et al.*, 1942.)

Specific Activity. This term is commonly used in investigations with radioactive isotopes; it denotes the number of radioactive units of an element in a given compound per unit weight of the non-radioactive isotope of the element in that compound. In other words, the specific activity of phospholipid phosphorus is the ratio $\frac{\text{phospholipid } P^{32}}{\text{phospholipid } P}$. The numerator is expressed either as radioactive units or as a percentage of the administered P^{32}. The denominator is usually expressed in milligrams or in millimols. For the use of *relative specific activity* see page 336.

III. The Amounts of the Administered P^{32} Incorporated into Phospholipids by Tissues of the Animal Body

After having administered radioactive phosphate to animals, the early investigators were concerned primarily with the determination of the percentage recovery of the P^{32} as phospholipid in various organs and tissues. The presence of radioactive phosphorus in phospholipid molecules some time after the administration could only mean that such molecules were newly synthesized with respect to their phosphorus component. Typical results of such experiments are presented in Table I.

Although the values shown in Table I for the percentages of the injected P^{32} incorporated into phospholipid have been used to compare the rates of lipid phosphorylation in various tissues, such values do not afford a good measure of the actual amounts of phospholipid formed. To obtain such a measure, information on the amount of phosphate that is labeled by the administered P^{32} is required. Let us assume that 1000 radioactive units of P^{32} are injected into a system in which it becomes and remains intimately mixed with 100 mg. of phosphate; then the recovery of 100 radioactive units as phospholipid means that 10 mg. of phosphate have been converted to phospholipid. Unfortunately such a simple calculation does not apply to the animal body. Although only the phosphate of plasma is labeled immediately after the introduction of P^{32} into the blood stream, the amount of phosphate with which the P^{32} becomes intimately mixed increases rapidly thereafter. This is illustrated by Hevesy's data (1938) on the distribution of P^{32} among the various tissues of a rat 4 hours after its administration (Table II). Hence, in order to derive quantitative significance from the data of Table I as a measure of phospholipid formation, it is necessary to know the actual amounts of body phosphate that are being labeled during the interval studied.

During the past 10 years a considerable amount of data on the conversion of administered P^{32} to phospholipids in various tissues has accumulated. These data are discussed in some detail in the following sections.

TABLE I

Percentages of Administered P^{32} Incorporated into Phospholipid by Normal Tissues of Various Animals

Animal and its weight	Route of administration of P^{32}[a]	Hours after P^{32} administration	Tissue	Per cent of administered P^{32} incorporated into phospholipid	
				Per g. tissue	Per whole organ
Mouse 25 g.	Intraperitoneally	24	Liver	1.9	2.3
	"	48	Muscle	0.2	1.3
	"	24	Brain	0.15	0.03
Rat 200 g.	Stomach	24	Liver	0.3	1.8
	"	24	Kidney	....	0.35
	"	24	Gastrointestinal tract	....	1.0
	Subcutaneously	48	Muscle	0.007	0.8
	"	24	Brain	0.01	0.02
Bird (domestic fowl) 2 kg.	Subcutaneously	12	Liver	0.06	1.4
	"	12	Kidney	0.04	0.32
	"	12	Small intestine	0.02	0.46
	"	12	Colon	0.01	0.02
	"	12	Leg muscle	0.001	
	"	12	Heart	0.008	0.30
Dog 19 kg.	Intraperitoneally	18	Liver	0.0089	2.8
	"	18	Kidney	0.0046	0.23
	"	18	Small intestine	0.0023	0.62
	"	18	Muscle	0.00011	
	"	18	Plasma	0.0011	

[a] In all experiments P^{32} was administered as an isotonic solution of Na_2HPO_4.

TABLE II

Distribution of P^{32} in a Rat 4 Hours after its Administration as Phosphate by Mouth

(From Hevesy, 1938)

Tissue	Per Cent of Administered P^{32} Recovered
Bones	48
Muscles	25
Digestive tract	10.8
Liver	9.8
Blood	2.4
Kidney	2.3
Skin and Hair	1.2
Spleen	0.3
Brain	0.08

1. Liver

Inorganic phosphorus is rapidly incorporated into phospholipid of the liver (Artom *et al.*, 1937b, 1938; Perlman *et al.*, 1937; Entenman *et al.*, 1938; Hevesy and Hahn, 1940). After the injection of a single dose of P^{32} in the form of Na_2HPO_4, a sharp rise and fall in the content of phospholipid P^{32} are observed in the liver; maximum concentrations of radiophospholipid occur in the liver of the rat and mouse (Jones *et al.*, 1939) at about 8–10 hours after the injection. This deposition of radiophospholipid in the liver is unimpaired in rats deprived of both kidneys and gastrointestinal tract (Fries *et al.*, 1938). Chargaff (1939, 1940) states that, in the liver, lecithin is more rapidly formed than cephalin. Hevesy and Hahn (1940) also point out that the renewal of liver cephalin differs from that of lecithin. According to Hunter (1941), sphingomyelin P^{32} accounts for only a small part of the phospholipid P^{32} deposited in the liver.

2. Kidney

In the intact animal, administered phosphate is readily incorporated into kidney phospholipid. Following a single injection of P^{32}, the rate of deposition and of disappearance of labeled phospholipid is slower in kidney than in liver or small intestine (Artom *et al.*, 1937b; Perlman *et al.*, 1937). Kidney has also been compared with other tissues with respect to the specific activity of phospholipid phosphorus (*i.e.*, the ratio of phospholipid P^{32} to phospholipid P^{31}). According to Artom *et al.* (1937a, 1937b, 1938), the specific activity of kidney phospholipid is lower than that of small intestine and liver phospholipid at the early intervals after P^{32} administration in the rat. Fishler *et al.* (1943) measured the deposition of phospholipid P^{32} per gram of phospholipid in kidney, liver, and small intestine of the dog at 6, 18, 36, and 98 hours after the intraperitoneal injection of P^{32}. At the first time-interval the values for kidney were lower than those for liver but equal to or higher than those for the small intestine. At 18 and 36 hours the specific activities of the kidney phospholipids remained lower than those of the liver and about equal to those of the small intestine. At 98 hours the specific activities of phospholipid in all 3 tissues were roughly the same.

That kidney *can* phosphorylate fat has been established by *in vitro* experiments with surviving slices of this tissue (page 348). That lipide phosphorylation does take place *within* the intact kidney has been demonstrated by injecting P^{32} into the hepatectomized dog (Fishler, 1943). Despite the fact that no phospholipid P^{32} was found in the plasma of the liverless dog, its kidneys contained radiophospholipid in amounts similar to those found in normal dogs.

Weissberger (1940) found that acidosis induced by the ingestion of NH_4Cl

increases the specific activity of kidney phospholipid. A relationship between phospholipid metabolism of kidney and acid-base regulation is suggested.

3. *Intestine*

Radiophospholipid is rapidly deposited in the gastrointestinal tract after the administration of P^{32} (Perlman *et al.*, 1937; Artom *et al.*, 1938; Entenman *et al.*, 1938; Fries *et al.*, 1938; Chargaff, 1939; Hevesy and Hahn, 1940). The phospholipid activity of its various parts is not the same; the small intestine is very much more active in lipide phosphorylation than stomach or large intestine (Fries *et al.*, 1938). In the bird the deposition of injected inorganic P^{32} as phospholipid is greater in the small intestine than in the gizzard, proventriculus, ceca or colon (Entenman *et al.*, 1938). Artom *et al.* (1938) state that the specific activity of phospholipid phosphorus in the intestine exceeds that in the liver when the P^{32} is administered orally. Fries *et al.* (1938) have also observed that the recovery of phospholipid P^{32} in the small intestine is greater when P^{32} is administered *per os* than when it is administered subcutaneously.

4. *Blood*

Administered P^{32} appears rapidly in phospholipid of plasma. This has been shown by Hevesy and Hahn (1940) in experiments in which they maintained a constant level of labeled inorganic phosphorus in plasma of rabbits for 9 days and by Fishler (1943) in experiments with single injections of P^{32} in dogs. As early as 3 hours after a single injection of P^{32}, appreciable amounts of radiophospholipid are already present in plasma of the dog. By 40 hours, 0.5–1% of the administered P^{32} is incorporated into phospholipids of the dog's total plasma.

The incorporation of administered P^{32} into phospholipid is a much slower process in corpuscles than in plasma (Hevesy and Aten, 1939; Hevesy and Hahn, 1940; Fishler *et al.*, 1943). Following the injection of a single dose of radiophosphorus in the dog, there occurs a sharp rise in the phospholipid P^{32} content of the plasma, the maximum being attained by 36 hours; it requires more than 2 days for measurable amounts of phospholipid P^{32} to make their appearance in the dog's cells (Fishler *et al.*, 1943). At no time do cells attain a phospholipid P^{32} content greater than 25% of the maximum found in plasma. The highest amount of radiophospholipids in cells appeared between 200 and 300 hours; at 500 hours about one-half of that amount was still present in the cells.

According to Hevesy and Aten (1939), the percentage renewal of corpuscle phospholipid in a period of 28 hours is only one-third of that in the plasma of the laying bird. In man, the specific activity of phospholipid phosphorus in corpuscles reaches a value about half of that of plasma as late as 8 days.

Plasma phospholipids do not penetrate corpuscles readily (Hahn and Hevesy, 1939; Hevesy and Aten, 1939; Hevesy and Hahn, 1940; Fishler, 1942; Zilversmit *et al.*, 1943b). Hahn and Hevesy injected P^{32} into a rabbit and separated its plasma; this plasma, containing radiophospholipid, was then mixed with corpuscles removed from another rabbit. At the end of 4.5 hours of incubation only 5% of the phospholipid present in corpuscles had exchanged. The results obtained *in vivo* are equally striking; 37 hours after the injection of P^{32} into a dog, Fishler (1942) found that the phospholipid P^{32} content/cm.^{3} of plasma was more than 10 times that of the corpuscles. Although large amounts of radiophospholipid were present in plasma at a 12-hour interval, practically no phospholipid P^{32} was observed in corpuscles at this time despite the fact that they already contained considerable amounts of P^{32}. Even as late as 60 hours, a marked difference between phospholipid P^{32} content of corpuscles and plasma was still observed. These observations are not incompatible with the view that lipide phosphorylation within the corpuscle occurs mainly at the time it is formed.

5. *Muscle*

Administered P^{32} makes its appearance as phospholipid P^{32} slowly in skeletal muscle (Entenman *et al.*, 1938; Jones *et al.*, 1939; Hevesy, 1940; Hevesy and Hahn, 1940). This may be related to the rate of entrance of inorganic phosphorus into the muscle cell (Hevesy and Hahn, 1940; Hevesy and Rebbe, 1940; Manery and Bale, 1941). No difference was observed in the phospholipid P^{32} contents of leg and breast muscle of the bird at 6 and 12 hours after the injection of labeled phosphate (Entenman *et al.*, 1938). Cardiac muscle incorporates more of the injected P^{32} into phospholipid than does skeletal muscle (Entenman *et al.*, 1938).

The activity of phospholipid phosphorus is increased in the muscle of rats maintained on a diet deficient in fat (Hevesy and Smedley-Maclean, 1940). Denervation increased the rate at which administered P^{32} is deposited in muscle phospholipid (Artom, 1941; Friedlander *et al.*, 1941). When compared with the corresponding muscle of the intact side, the denervated muscle may show increases of over 200% in the amount of phospholipid P^{32} incorporated. This change appears before any appreciable atrophy of the muscle.

6. *Brain*

The incorporation of P^{32} into brain phospholipid of the *intact* animal has been shown by many workers to be a slow process (Hahn and Hevesy, 1937; Artom *et al.*, 1938; Changus *et al.*, 1938; Chargaff, 1939; Chargaff *et* *al.*, 1940; Fries *et al.*, 1940; Hevesy and Hahn, 1940; Fries and Chaikoff, 1941b). This may be explained by the slow penetration of labeled phosphate into the brain as a whole as well as into its extracellular spaces. A progressive increase in the content of radiophospholipid was observed in the rat for as long as 200 hours after a single administration of inorganic P^{32} (Changus *et al.*, 1938). Once the maximum amount of labeled phospholipid has been deposited, its loss from the brain occurs very slowly. Even as late as 800 hours after P^{32} administration the brain still contained 70% of the amount of labeled phospholipid present at the 200-hour interval. Brain cephalin and sphingomyelin take up P^{32} more rapidly than lecithin (Chargaff *et al.*, 1940; Hevesy and Hahn, 1940).

A larger fraction of the administered P^{32} is recovered as radiophospholipid in the various brain divisions of the young than in those of the old animal (Fries *et al.*, 1940; Fries and Chaikoff, 1941b). An interesting correlation was observed between these recoveries of P^{32} as phospholipid and the rate of deposition of total phospholipid in all brain divisions of the growing rat. Maximum deposition of total phospholipid in all parts of the brain (Fries *et al.*, 1941) occurs in rats between the ages of 1 week (15 g.) and a little over 2 weeks (30 g.). This latter interval represents the period of intense myelination in the brain (Watson, 1903).

Within the same animal the recoveries of P^{32} as phospholipid/g. of tissue are not uniform in the various divisions of the brain (Fries *et al.*, 1940). The highest recovery of phospholipid P^{32} was found per g. of spinal cord from birth until the time the rat attained a weight of 50 g. During this time the recovery of P^{32} as phospholipid in spinal cord is 2 or more times that in forebrain.

The slow incorporation of P^{32} into brain phospholipid as compared with that into liver and small intestine raised the interesting question whether nervous tissue can phosphorylate fat independently or whether it derives its phospholipid from plasma. To answer this question a study was made of the formation of phospholipid by surviving brain slices kept in a Ringer-bicarbonate solution containing radioactive phosphorus (Fries *et al.*, 1942). The ability of brain of both old and young rats to form phospholipid independently was demonstrated. The excised nerve of a dog was also shown to be active in converting phosphate to phospholipid. The *in vitro* formation of phospholipid is dealt with on page 346.

7. *Neoplastic Tissues*

Phospholipid metabolism of tumors as measured with P^{32} presents several unique characteristics. Their activity in converting injected inorganic P^{32} into phospholipid is relatively high as compared with that of such tissues as liver, kidney, and intestine (Jones *et al.*, 1939; Haven, 1940; Hevesy, 1940). In contrast to the latter tissues, however, maximum deposition of phospholipid P^{32} in neoplastic tissues may last from 10 to 50 hours (Jones *et al.*, 1939); the highest concentration of phospholipid P^{32} in liver was observed about 10 hours after P^{32} administration. The turnover of lecithin, cephalin, and sphingomyelin in carcinoma 256 has been measured (Haven, 1940; Haven and Levy, 1941); lecithins were found to be renewed somewhat more rapidly than cephalins.

The rate at which injected P^{32} is converted to phospholipid P^{32} is not the same in all neoplastic tissues. Thus, lymphosarcoma and mammary carcinoma were found distinctly more active in this respect than sarcoma 180 and lymphoma (Jones *et al.*, 1939). This individuality among tumors was demonstrated by a procedure in which 2 or 3 types of tumor transplants were grown side by side in the same animal (Jones *et al.*, 1940). No relation was observed between the phospholipid P^{32} content/g. of tumor and its preceding growth rate as measured by increase in mass (Jones *et al.*, 1939, 1940).

8. The Laying Bird and the Egg

The laying bird is characterized by a high phosphorus turnover. A single 60 g. (chicken) egg may contain as much as 0.5 g. of phosphorus combined in various compounds. Egg-laying increases the rate at which injected inorganic P^{32} is deposited as phospholipid in 3 tissues of the bird: blood, ovary, and oviduct (Entenman *et al.*, 1938).

Administered P^{32} makes its appearance rapidly in the phospholipid and other phosphorus compounds of the yolk (Entenman *et al.*, 1938; Hevesy and Hahn, 1938). The amount of P^{32} deposited in the yolk can be accounted for as a function of 2 variables: yolk growth and P^{32} availability during the period of new yolk formation (Lorenz *et al.*, 1943). From a comparison of the specific activities of various tissues in the laying bird, Hevesy and Hahn (1938) have concluded that the bulk of yolk phospholipid is derived from plasma but formed in the liver. According to Hevesy *et al.* (1938), the phospholipid molecules of the chick embryo are not derived from the yolk but are synthesized by the embryo itself.

Although the concentration of lipides in the blood and liver of the male and immature female bird does not differ greatly from that found in other species, such as man and dog, avian lipid metabolism differs from mammalian in being under the control of ovarian hormones (Lorenz *et al.*, 1938a and b; Zondek and Marx, 1939; Entenman *et al.*, 1940; Riddle, 1942). Thus, in the bird actively engaged in egg-laying, there occurs a rise in the levels of fat, phospholipids, and cholesterol of the blood; in this state phospholipids of the blood may rise as high as 900 mg./100 cc. as compared with about 300 mg. in the non-laying state. It has since been shown that this rise in lipides of the blood could be reproduced by the injection of natural estrogens and diethylstilbestrol. The mechanism responsible for the rise of plasma phospholipids produced by the injection of diethylstilbestrol has been investigated with the aid of P^{32}. In stilbestrol-treated birds, Flock and Bollman (1944) found that the rates of formation and utilization of plasma phospholipids are increased. Taurog *et al.* (1944) compared the rates of incorporation of inorganic radioactive phosphorus into phospholipid by surviving slices of liver obtained from 1) normal birds and 2) birds that had received a single injection of diethylstilbestrol. A significant increase in the *in vitro* formation of radioactive phospholipids of the liver of the stilbestrol-treated bird was observed. This observation suggests that the increase in plasma phospholipids produced by the injection of diethylstilbestrol results from an increase in the rate of formation of phospholipids by the liver.

IV. How P^{32} Can Be Used to Measure the Rate of Turnover of Phospholipids in the Animal Body

Artom *et al.* (1938) and Hevesy and Hahn (1940) were the first to calculate the turnover rate of phospholipid by means of P^{32}. The latter employed a slow and continuous infusion of radioactive phosphate into the blood stream of a rabbit in order to maintain the specific activity of plasma phosphate relatively constant during the course of the experiment. When the specific activity of phospholipid phosphorus of a given organ was $\frac{1}{10}$ that of the plasma phosphate, they argued that $\frac{1}{10}$ of the organ's phospholipid had been renewed—on the assumption that extracellular phosphate is a direct source of that organ's phospholipid. They were aware, however, that this assumption may not hold; they recognized that, if the organ's phospholipid is formed inside the cell, the rate at which labeled plasma phosphate penetrates the intracellular spaces is an important factor in determining the specific activity of the newly formed phospholipid. For example, since inorganic phosphorus enters kidney cells rapidly, it is *not* safe to infer merely from the rate at which injected P^{32} is converted to phospholipid P^{32} that the turnover of phospholipid by the kidney is a rapid process. Their calculations, which are shown in Table III, demonstrate quite clearly that it makes considerable difference whether intracellular or extracellular phosphate is assumed to be the immediate precursor of phospholipid phosphorus.

It becomes obvious from the above considerations that, if we are to succeed in measuring phospholipid turnover with isotopic phosphorus, we must know the specific activity of the immediate precursor of phospholipid; this implies, of course, that we must know the identity of this precursor.

Methods by which it can be identified and its specific activity measured are dealt with below.

1. *Determination of the Specific Activity of the Immediate Precursor of Phospholipid*

Let us first consider what happens to labeled phosphate after it is injected into the blood stream. The P^{32} becomes uniformly mixed with the inorganic phosphate of plasma and, since this is a rapid process, complete mixing may be said to occur immediately. The initial specific activity of inorganic phosphate of plasma then decreases rapidly. Labeled plasma phosphate mixes with the non-radioactive phosphates of extracellular and intracellular fluids of tissues, with bone phosphate and organically bound phosphates of tissues (see Table II). This process, which diminishes the concentration of the radioactive phosphate of plasma (*i.e.*, its specific activity), proceeds at a rate which can be expressed as an exponential function of time.

TABLE III

The Per Cent Renewal of Phospholipids in Various Organs of a 2.6 Kg. Rabbit
(P^{32} was injected continuously for 215 minutes, after Hevesy and Hahn, 1940)

Organ	A	B
Liver	19.0	3.86
Kidney	4.8	3.7
Small intestine	7.1	1.61
Stomach	7.7	.91
Heart	2.7	.31
Lungs	6.1	.82
Spleen	5.2	.74
Marrow	4.0	.33

A. Calculated on the assumption that the formation of the phospholipids took place at the expense of cellular inorganic phosphate.

B. Calculated on the assumption that the formation of phospholipids took place at the expense of extracellular inorganic phosphate.

Let us next consider how a phospholipid molecule becomes labeled in a given tissue. Let us assume for the present that plasma phosphate is the *immediate* precursor of this phospholipid. At the earliest intervals, the phosphorus that is incorporated into the phospholipid molecules has a very high specific activity because the specific activity of plasma phosphate is high at this time. In other words, a relatively minute amount of phospholipid formed at this early interval contains a great deal of P^{32}. As time progresses, however, less and less P^{32} is contained in similar portions of newly formed phospholipid, and the amount of phospholipid P^{32} lost by breakdown increases steadily as the specific activity of the phospholipid increases.[2]

Now let us follow this process of formation and destruction until the specific activity of the newly formed phospholipid is equal to that of the phospholipid breaking down. At this point the tissue phospholipid has reached its maximum specific activity. Since, at the time of its formation, the specific activity of phospholipid is equal to that of its precursor, and since the specific activity of the phospholipid breaking down is the same as that of the phospholipid present (see "random breakdown"[2]), it is valid to conclude that *at the time when the specific activity of the phospholipid in a tissue has reached its maximum value, the specific activity of the precursor is equal to the specific activity of the total phospholipid in that tissue.*

The above considerations are subject to mathematical analysis, as has been pointed out by Zilversmit *et al.* (1943a), who showed that there exists at any time a proportionality between the rate of increase of the specific activity of the phospholipid of a tissue and the difference between the specific activity of precursor and phospholipid at that time.

The same considerations will hold, of course, whether plasma phosphate or some other phosphorus-containing compound in a given tissue is the immediate precursor of its phospholipid. Fig. 2 illustrates the relation between the specific activities of phospholipid and its precursor. In this case the precursor is not plasma phosphate but some intermediate which reaches a maximum specific activity rather slowly.

Let us illustrate the above reasoning in the case of the liver, an organ in which we wish to measure the turnover rate of phospholipid phosphorus. To do this we require, as already pointed out, 2 types of measurements: (1) the "specific activity-time" relations of phospholipid phosphorus and (2) the "specific activity-time" relations of its immediate precursor. The first presents no difficulty: P^{32} is injected and samples of the liver are removed for determination of phospholipid P^{32} at various intervals thereafter. To identify the precursor, it is necessary to obtain "specific activity-time" curves of all phosphorus-containing compounds in the liver other than phospholipid. The one that fits the conditions outlined in Fig. 2 repre-

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[2] It is assumed that "random breakdown" occurs, *i.e.*, that the newly formed phospholipid molecules break down in the same proportion as the "older" phospholipid molecules. In other words, the body does not distinguish between old and new molecules. It follows directly from this assumption that the specific activity of the phospholipids that break down is the same as that of the entire mass of phospholipids present. The only tissue for which, so far as we know at present, this assumption does not hold is the red blood cells, since the older cells break down before the new ones.

sents the curve of the immediate precursor. Should the phosphate radicals of all phosphorus-containing compounds other than those of phospholipids be in rapid equilibrium with one another, then the "specific activity-time" relations of any one of them can be used for the calculation of turnover rate as described in the following section.

2. Determination of the Phospholipid Turnover Rate from Specific Activity Data

When the specific activity of the phospholipid precursor has been determined by the method described above, it is relatively simple to calculate the turnover rate of phospholipid.

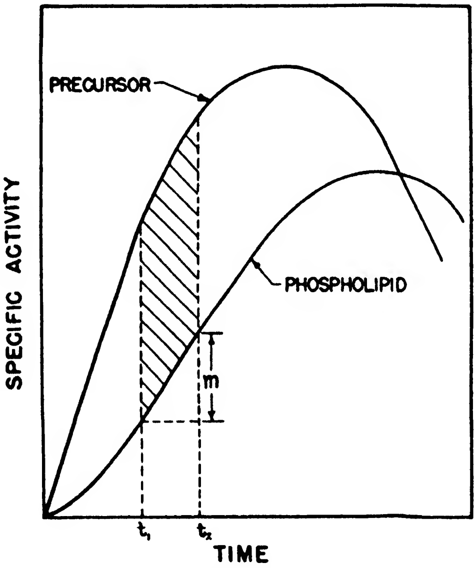

FIG. 2

The "specific activity-time" relations between phospholipid and its immediate precursor.

It is assumed in these calculations that (1) a steady state exists, *i.e.*, the amount of phospholipid present in a tissue is constant during the interval of the experiment, (2) random breakdown as described above occurs, and (3) the rate of phospholipid formation is constant during the time of the experiment.

The above assumptions can be expressed by

$$\frac{dx}{dt} = pf(t) - p\frac{x}{r} \qquad (1)$$

where p = the rate of phospholipid formation (phospholipid turnover rate),
r = the amount of phospholipid present in the tissue,
x = the amount of radioactive phospholipid present in the tissue,
and $f(t)$ = the specific activity of the immediate precursor of phospholipid (as determined above).

All the variables in this equation can be measured experimentally and thus the turnover rate p can be obtained. For a more exact determination of p the above equation should be integrated. A graphical solution is illustrated in Fig. 2.

$$t_t = \frac{\text{shaded area}}{m} \tag{2}$$

where t_t = turnover time of phospholipid, *i.e.*, the time in which an amount of phospholipid equal to that present in the tissue is turned over. The turnover rate can then be determined directly from the turnover time by the relation $p = \frac{r}{t_t}$.

The above equation can be integrated quite easily in cases where the specific activity of the precursor is (1) constant, or (2) a linear function of time, or (3) an exponential function of time—the three cases that occur most frequently.

As an example we may take the case presented in Fig. 2. In the initial phase of the experiment we can represent the specific activity of the precursor as a linear function of time. By taking the specific activity of the phospholipid and its precursor at any one time during this interval and by substituting these values in the solution of Eq. (1) we can determine the turnover rate of the phospholipid.

It is not always possible to establish the "specific activity-time" relationship as shown in Fig. 2 in a single animal. Attempts have been made to modify the above methods so as to make them applicable to measurements on different animals at various time intervals after the injection of P^{32}. As was shown above, the specific activity of the phospholipid, other conditions being equal, depends ultimately on the specific activity of the initially labeled phosphate of the body. It is found experimentally, however, that the specific activities of plasma phosphate at a given time may differ considerably in 2 dogs of the same weight, even though the same amount of P^{32} has been injected. Consequently, the specific activities of phospholipid in these two dogs will also differ, even if the turnover rates of phospholipid were the same. Therefore, in comparing different animals an expression more useful than specific activity is the *relative specific activity* (Hevesy, 1938), which is the specific activity of the phospholipid divided by the specific activity of plasma phosphate, or better yet, divided by the specific activity of the immediate phospholipid precursor. As must be evident by now, this latter ratio is related to the phospholipid turnover rate in the two animals.

3. *The Rate of Disappearance of Plasma Phospholipids as a Measure of Their Turnover Rate*

A simplified method for the determination of the turnover of plasma phospholipids has been developed by Zilversmit *et al.* (1943b). In this method the disappearance of labeled plasma phospholipids is measured and, since a steady state exists in the animal, its disappearance rate is equal to the rate of turnover. This procedure makes unnecessary the measurement of the specific activity of the immediate precursor, and so the path of formation of phospholipid need not be known.. An example of this procedure is described on page 339.

TABLE IV

A Comparison of the Specific Activities of Phospholipid Phosphorus of Various Tissues of the Dog

Dog weight, *kg.*	Time after P^{32} injection, *hr.*	Specific activity[a]				
		Plasma	Liver	Kidney	Small intestine	Muscle
9.6	6	3.8	8.0	4.8	3.8	0.15
9.8	18	8.3	8.5	5.3	5.0	0.38
10.2	36	10.5	10.3	7.3	9.5	0.30
7.0	98	7.0	6.5	6.8	5.3	2.5

[a] The specific activity is the per cent of the injected P^{32} recovered/g. of phospholipid phosphorus. The P^{32} was injected intraperitoneally.

V. PROBLEMS IN PHOSPHOLIPID METABOLISM TO WHICH P^{32} HAS BEEN SUCCESSFULLY APPLIED

1. *The Site of Formation of Plasma Phospholipids*

From studies on the incorporation of labeled inorganic phosphate into phospholipids it has been inferred that plasma phospholipids are formed largely in the liver (Hevesy and Aten, 1939; Hevesy and Hahn, 1940; Artom, 1941). This inference is based on a comparison of the specific activities of phospholipid phosphorus of plasma with those of other tissues. Similar observations have been made by Fishler *et al.* (1943). Phospholipid specific activities of several tissues of the dog were determined at 6, 18, 36, and 98 hours after the intraperitoneal injection of inorganic P^{32} (Table IV). At the first interval the phospholipid P^{32}/g. of liver phospholipid was higher than that of any other tissue. At the 18 hour-interval the values for liver and plasma were about the same and higher than those for small intestine, kidney, and muscle. At 36 hours the values for plasma and liver were still about the same, but higher than those for other tissues. At 98 hours the values for kidney and small intestine approximated those for liver and plasma.

Although the above observations do not contradict the view that the liver is the source of plasma phospholipids, interpretations based on comparisons of specific activities of phospholipid phosphorus are open to the criticism that they do not deal with a single substance but with a mixture of several different compounds. The following limitation should be noted even in the case of a single substance: the finding that the specific activity of an organ's lecithin phosphorus is lower than that of plasma serves to exclude that organ as the principal source of plasma lecithin, but the observation that the specific activity of an organ's lecithin phosphorus is higher than that of plasma means only that the organ *may* be the source of plasma lecithin.

TABLE V

The Specific Activities of Plasma Phospholipid Phosphorus of the Normal and the Liverless Dog[a]

Dog	Weight, *kg.*	Specific activity[b]
Normal—Control	19.5	2.8
Normal—Control	19.5	1.8
Liverless	22.2	0.050
Liverless	19.8	0.075
Liverless	20.0	0.050

[a] The specific activities were measured 6 hours after the intravenous injection of P^{32}.

[b] The specific activity is the per cent of the injected P^{32} recovered/g. of phospholipid phosphorus.

To provide a direct attack on the problem of the liver's role in the production of plasma phospholipids, the capacity of the liverless dog to synthsize phospholipids was tested with the aid of radioactive phosphorus (Fishler *et al.*, 1943).

In Table V the specific activities of *plasma phospholipids* in normal and liverless dogs are compared. Negligible amounts of phospholipid P^{32} were recovered in the plasma of the latter as late as 6 hours after the excision of the liver. These results lead to one conculsion, namely that plasma phospholipids are synthesized mainly in the liver.

2. The Rate of Utilization of Plasma Phospholipids

It was pointed out above that, in order to measure the turnover of phospholipid in an organ, it is necessary to know the "specific activity-time" relations of the phospholipid itself and of its precursor. For the determination of the turnover rate of phospholipid of plasma, however, the procedure can be greatly simplified, since it is feasible to introduce into the circulation a small amount of plasma containing the labeled phospholipid and measure its disappearance from the circulating fluid. The phospholipid in its labeled form can be obtained by synthesis *in vitro* or from plasma of an animal that synthesized the labeled phospholipid. The latter procedure has the advantage, in that the labeled phospholipid introduced is of the same type as that present in plasma, and its physicochemical state is not altered by chemical treatment. In other words, the radiophospholipid whose disappearance is being measured is chemically indistinguishable from the animal's own plasma phospholipid. It is shown below how the above principle has been applied to the determination of the rate at which phospholipids are turned over in plasma of the dog.

Twenty-four hours after it received P^{32}, dog A is exsanguinated and its plasma separated. This plasma, which contains phospholipids labeled with P^{32}, is then injected into dog B, and the rate at which the labeled phospholipids disappear from the circulation is measured. The results are shown in Fig. 3.

The following equation was derived by Zilversmit *et al.* (1943b) for the disappearance of phospholipid phosphorus from the circulating fluid:

$$\frac{x}{r} = ce^{-(p/r)t} \tag{1}$$

or

$$\frac{x}{f} = \left(\frac{r}{f}\right)ce^{-(p/r)t} \tag{2}$$

in which p = rate of disappearance of phospholipid from the plasma,
x = the amount of phospholipid P^{32} present in the plasma at any time,
r = the total amount of phospholipid phosphorus present in the plasma,
and f = the amount of plasma present in the body.

Therefore, $\frac{x}{f}$ gives the radioactive units of phospholipid P^{32}/ml. of plasma at any time, and $\frac{r}{p}$ is equal to the turnover time[3] (t_t) of phospholipid phos-

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[3] Plasma phospholipid turnover time was defined in an earlier section as the time in which an amount of phospholipid equal to that in plasma is turned over.

phorus in the plasma, while $\frac{r}{f}$ is a measure of the plasma phospholipid phosphorus level.

Since

$$\log \frac{x}{f} = \log\left(c\frac{r}{f}\right) - \frac{t}{t_t}\log e, \tag{3}$$

a plot of the logarithm of the amount of phospholipid P^{32}/ml. of plasma against time should give a straight line. That this is indeed the case can be seen from Fig. 3, where the phospholipid P^{32}/ml. is plotted against time on semi-log paper.

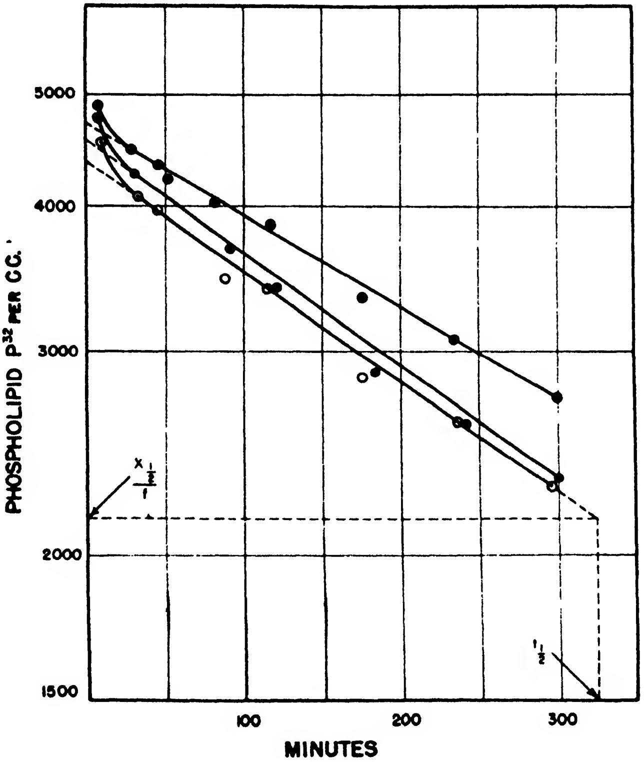

Fig. 3

The disappearance of injected radioactive phospholipids from plasma of the dog. The ordinate scale is logarithmic. For explanation see text.

To obtain turnover time it is useful to write Eq. (3) for 2 time intervals, namely zero time (t_0) and the time ($t_{\frac{1}{2}}$) required for phospholipid P^{32} to reach a concentration equal to half of that present at zero time.

At t_0,

$$\log \frac{x_0}{f} = \log c \frac{r}{f};$$

at $t_{\frac{1}{2}}$,

$$\log \frac{x_{\frac{1}{2}}}{f} = \log c \frac{r}{f} - \frac{t_{\frac{1}{2}}}{t_t} \log e.$$

Then, by subtraction

$$\log \frac{x_0/f}{x_{\frac{1}{2}}/f} = \log 2 = \frac{t_{\frac{1}{2}}}{t_t} \log e,$$

or

$$t_t = 1.44 t_{\frac{1}{2}}.$$

The half time ($t_{\frac{1}{2}}$) can be readily obtained from the curves in Fig. 3. Experimental values for t_t obtained by this method on 3 dogs are recorded in Table VI. The results show that, in dogs weighing from 6.6 to 8.5 kg., an amount of plasma phospholipid equal to that contained in their plasma is completely turned over in 8–9 hours.

TABLE VI

The Utilization Rate of Plasma Phospholipids by Dogs

Weight, *kg.*	Turnover Time,[a] *hr.*	Turnover rate mg. phospholipid/hr.
8.8	9.3	175
6.6	7.2	130
5.7	7.8	200

[a] Turnover time is the interval in which complete turnover of an amount of plasma phospholipid equal to that contained in plasma occurs.

To determine the turnover rate of phospholipid (*i.e.*, the amount of phospholipid turned over per hour) in plasma it is necessary to know the turnover time (t_t) and the total amount of phospholipid present in plasma.

The total amount of phospholipid in plasma can be obtained by the isotope dilution procedure. The concentration of radiophospholipid at "zero-time," *i.e.*, at a time when none of it has been metabolized, can be found by extrapolation of the experimental curve. This is shown by the dotted lines in Fig. 3.[4] The total amount of phospholipid phosphorus in plasma is obtained by dividing the amount of injected phospholipid P^{32} by the specific activity of phospholipid phosphorus at zero time.

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[4] It should be pointed out that the "zero-time" concentration of radiophospholipid cannot be determined experimentally by taking a plasma sample just after the injection of the radiophospholipid, since it takes some time for the labeled phospholipid to become uniformly distributed throughout the circulating fluid. This is illustrated by the finding that the first experimentally determined points on the three curves lie above the extrapolated parts of these curves.

Let us illustrate this procedure by an example. If 1,000,000 radioactive units of phospholipid P^{32} are injected intravenously and if it is found that each milligram of plasma phospholipid phosphorus contains 20,000 radioactive units, it follows that the total amount of plasma phospholipid phosphorus is 50 mg.

The turnover rate of plasma phospholipid (p) is now obtained from the total amount of phospholipid in the plasma (r) and its turnover time (t_t) as follows:

$$p = \frac{r}{t_t}.$$

Values for p are recorded in Table VI. The results show that dogs weighing 6.6–8.5 kg. turn over from 130–200 mg. of phospholipid/hr.

TABLE VII

The Distribution of Radioactive Plasma Phospholipids 5 Hours after Their Intravenous Injection into Normal Dogs

Tissue	Per cent of the injected phospholipid P^{32} per whole organ		
	Dog 1, 8.8 kg.	Dog 2, 6.6 kg.	Dog 3, 5.7 kg.
Plasma	57.7	51.4	53.0
Liver	16.2	15.2	11.1
Kidney	0.76	1.27	1.13
Small intestine	....	2.59	2.44
Spleen	....	0.30	0.35
Red blood cells	0.36	0.35	1.15

3. The Fate of Plasma Phospholipids in the Animal Body; Their Role in Fat Transport

The rapidity with which phospholipids are turned over in plasma raised the question as to which tissues are concerned with their utilization. To answer this question a study was made of the distribution throughout the body of labeled (radioactive) plasma phospholipids that had been injected intravenously into dogs (Zilversmit *et al.*, 1943b). The experiment was similar in design to that described in the preceding section. The results are shown in Table VII.

The results demonstrate that the largest amounts of plasma phospholipids are transported to the liver. These findings are in agreement with those reported by Hahn and Hevesy (1939) and by Haven and Bale (1939). Here, then, is an interesting situation: not only is the liver the site of formation of plasma phospholipids, but the liver also appears to be the organ most concerned with their fate. In view of this close relation between liver and plasma, it became of interest to know what would happen to labeled phospholipid introduced into the circulation of a dog deprived of its liver (Entenman *et al.*, 1946b). The results of such an experiment are presented in Table VIII. The "turnover time" of plasma phospholipids was compared in the *same* dog before and after hepatectomy. Before the liver had been removed from the circulation, turnover times of the 4 dogs studied were 380–580 minutes; after hepatectomy the values ranged from 2250 to 9500 minutes. *It is evident that in the liverless dog the turnover of plasma phospholipids practically stops.*

These results are illustrated in Fig. 4, in which a plot has been made of the specific activities of plasma phospholipid phosphorus on a logarithmic scale against time. The disappearance of phospholipids from plasma of the normal dog proceeds at a fairly rapid rate. When, however, the liver is removed from the circulation, there occurs an abrupt decrease in the rate at which the injected radioactive phospholipids disappear from plasma.

TABLE VIII

Turnover of Plasma Phospholipids in Dogs before and after Removal of Their Livers

Dog	Turnover time (minutes)			
	1	2	3	4
Normal, *i.e.*, before removal of liver	380	410	420	580
After removal of liver	2,250	4,750	3,400	9,500

These results leave no doubt that the liver is the principle tissue in the body concerned with the utilization of plasma phospholipids. They raise the interesting question as to what function plasma phospholipids serve in the animal body. The finding that the liver alone is so much more important than the total mass of extrahepatic tissues in their utilization lends little support to the view that the main function of plasma phospholipids is to transport fatty acids from liver to other tissues.

4. The Adrenal Gland And Phospholipid Formation

Verzar (1939) proposed that adrenalectomy interferes with the process of phosphorylation of fat. The role of the adrenal gland in lipide phosphorylation remained a moot question, however, until labeling agents were applied to the solution of this problem by Barnes *et al.* (1941) and by Stillman *et al.* (1942). The latter measured phosphorylation of fat directly by means of the incorporation of P^{32} into phospholipids of liver and small intestine of rats completely deprived of both adrenal glands. No interference in the conversion of administered P^{32} to phospholipid was observed in adrenalectomized rats, no matter whether they were in good condition while maintained on a high NaCl intake or whether they were showing manifestations of adrenal insufficiency.

There are 3 types of bonds that must be formed in order to build a lecithin or cephalin molecule from its 5 components: 1, a fatty acid-glycerol ester linkage; 2, a bond between phosphate and glycerol, and 3, a bond between phosphate and choline. Barnes *et al.* (1941) found no interference in the rates of incorporation of administered conjugated fatty acid into the phospholipid molecule after adrenalectomy; hence the adrenal gland is not necessary for the formation of the first of these bonds. As noted above, studies with P^{32} show that the 2 phosphate bonds can be formed in the absence of the adrenal glands. It can no longer be doubted that the adrenalectomized animal can synthesize new phospholipid molecules.

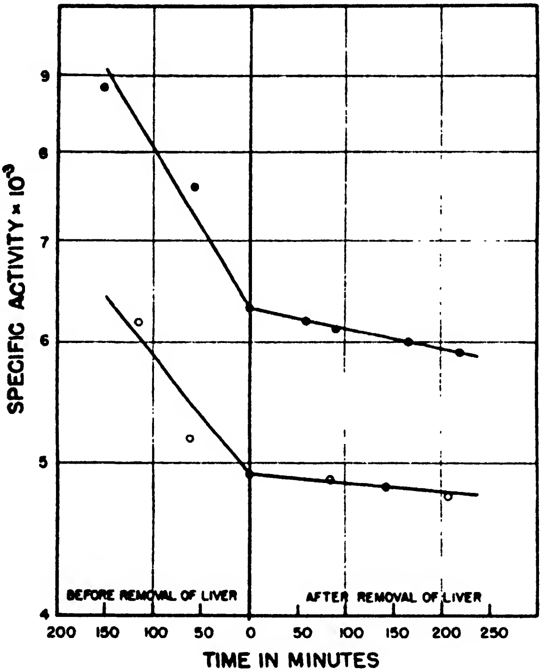


FIG. 4

The disappearance of injected radioactive phospholipids from plasma of the dog before and after removal of the liver. The ordinate scale is logarithmic.

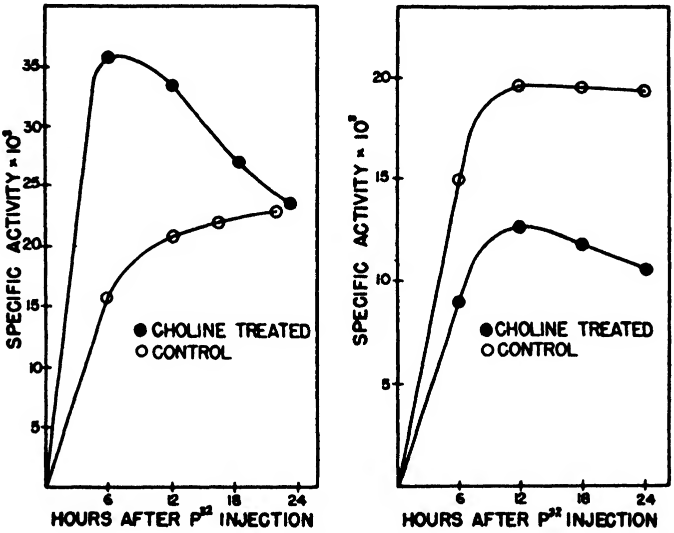
5. *The Action of Choline on Phospholipid Turnover*

One of the most interesting observations in lipid metabolism is the action of choline on fatty livers. Hershey (1930) discovered that lecithin had a striking effect upon the liver of the depancreatized dog maintained with insulin, but it remained for Best and his coworkers (1932, 1933) to show that its action in reducing the fat content of the liver in this preparation as well as in the rat fed a high-fat diet was due to its choline content. Although several mechanisms had been proposed to explain this action of choline, the first evidence to establish clearly an association between ingested choline and lipide phosphorylation was obtained by the use of P^{32}. The initial studies of Perlman and Chaikoff (1939) demonstrated that under the influence of ingested choline the rate of incorporation of P^{32} into liver phospholipids is stimulated. The stimulating effect of a single dose of choline on lipide phosphorylation is of short duration. In the rat the additional amounts of radioactive phospholipids make their appearance in the liver as early as 3 hours after a single feeding of choline, but are no longer detectable 10 hours later. This study again shows the value of the radioactive indicator in metabolism, since the accelerated synthesis of radiophospholipid induced by a single dose of choline is not reflected in a change in the phospholipid level of the liver.

In later studies by Entenman *et al.* (1946a), the effects of ingested choline upon the specific activity-time relations of (1) the choline-containing phospholipids (lecithin and sphingomyelin) of liver, (2) the non-choline-containing phospholipids (cephalin) of liver, and (3) plasma phospholipids (nearly all of which are of the choline-containing types) were examined. The results are shown in Figs. 5, 6, and 7. Surprisingly enough, ingested choline influenced the specific activities of only those phospholipids that contain choline; indeed the activities of the non-choline-containing phospholipids of the liver were depressed.

The fact that choline increased the specific activities of the choline-containing phospholipids of the liver is not sufficient in itself to permit the conclusion that an increase in the turnover of these phospholipids had occurred. It was pointed out on page 332 that, in order to measure the turnover rate of a given substance, it was necessary to provide data on the "specific activity-time" relations not only of the given substance but also of its immediate precursor. Unfortunately, the immediate precursor of the choline-containing phospholipids of the liver has not yet been identified. Although the liver data presented in Fig. 5 could be merely a reflection of an increased turnover of a phosphorus-containing precursor of the choline-containing phospholipids of the liver, a preliminary examination of the behavior of various phosphorus-containing fractions of the liver suggests that ingested choline does produce a true stimulation of the turnover rate of the choline-containing phospholipids of the liver.

Plasma phospholipids, on the other hand, provide an example of a case in which the specific activity-time relations of both a compound and its precursor are known. Plasma phospholipids are of the choline-containing types and, since they are synthesized in the liver, there can be no doubt that their immediate precursors are the choline-containing phospholipids of the liver. Hence, a good measure of the turnover rate of plasma phospholipids can be obtained from the data provided in Figs. 5 and 7. Calculations based on these data show that the increased specific activities of plasma phospholipids observed under the influence of choline are not due to an increase in their turnover rate but reflect similar changes in the specific activities of their precursor, namely, the choline-containing phospholipids of the liver.



FIGS. 5 AND 6

The effects of ingested choline on the "specific activity-time" relations of choline-containing (Fig. 5) and noncholine-containing (Fig. 6) phospholipids of the dog's liver.

6. *The in vitro Formation of Phospholipids*

The demonstration of the incorporation of phosphate into the phospholipid molecule by surviving excised tissue slices was made possible by the application of radioactive phosphorus as a labeling device. The sensitivity of the radioactive procedure is such as to permit the accurate measurement of traces of newly synthesized phospholipid. Of equal importance is the fact that detection of this type of phosphorylation could only have been made with a tagged phosphate, since formation of new phospholipid molecules in the slice proceeds in the presence of a net decrease in total phospholipid in the system. Although this tissue-slice technique provided an entirely new approach to the study of phospholipid metabolism, yielding, in particular, information on the mechanism of synthesis of these molecules by such tissues as liver, brain, and kidney, data obtained by the isolated-tissue technique, because of its inherent limitations, must always be interpreted in conjunction with results obtained from the intact animal.

(*a*) *What Tissues Are Capable of Synthesizing Phospholipids?* It has already been pointed out that in the intact animal the highest amounts of

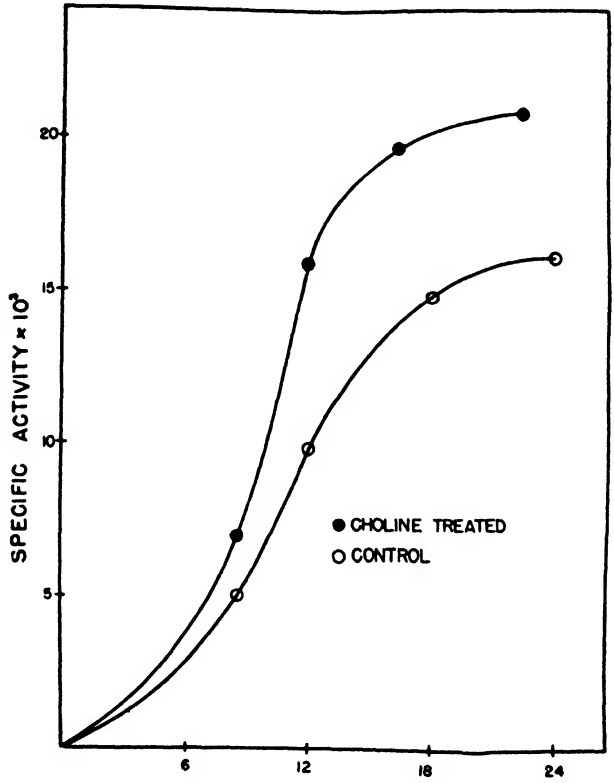


FIG. 7

The effect of ingested choline on the "specific activity-time" relations of plasma phospholipids of the dog.

administered P^{32} are incorporated into phospholipids of liver, kidney and small intestine, whereas brain incorporates very small amounts of P^{32} into phospholipids. In a complex system such as the intact animal, however, it is not always possible to distinguish between phospholipids that are formed within an organ and phospholipids removed by an organ from the blood. This difficulty has been overcome by employing the tissue-slice technique. Thus, the *in vitro* procedure has demonstrated that the following tissues can incorporate P^{32} into phospholipid molecules: liver (Fishler *et al.*, 1941), kidney (Taurog *et al.*, 1942), small intestine, muscle, brain and nerve (Fries *et al.*, 1942). Data for certain of these tissues are recorded in Table IX. No synthesis was observed in isolated plasma. As noted on page 338, plasma phospholipids are synthesized in the liver. In the liverless dog, even though none of the administered phosphate is converted to plasma phospholipid, there is no interference in the incorporation of P^{32} into phospholipid of kidney and small intestine. Apparently phospholipid molecules formed *within* the kidney and small intestine are not available to plasma.

(*b*) *Tissue Organization and Phospholipid Synthesis.* The extent of the *in vitro* formation of phospholipids by liver, kidney and brain is related to the state of tissue organization. Homogenized liver completely loses its ability to incorporate P^{32} into phospholipid molecules (Fishler *et al.*, 1941). Much less phospholipid formation was observed for homogenates of kidney and brain than for slices of these tissues (Fries *et al.*, 1942).

TABLE IX

In Vitro Synthesis of Radioactive Phospholipid by Surviving Slices of Liver and Kidney

(300 mg. of tissue slices were incubated in 5 c.c. of a bicarbonate-Ringer solution containing 0.80 mg. of phosphate (KH_2PO_4) labeled with P^{32}.)

Tissue	Period of incubation	Per cent of medium's P^{32} recovered as phospholipid/g of tissue				
		Oxygen present		Anaerobic conditions		
		On the basis of wet weight	On the basis of dry weight	On the basis of wet weight	On the basis of dry weight	Per cent inhibition
	hours					
Liver	2	3.8	17	0.2	0.9	94
Liver	4	6.5	29	0.5	2.3	92
Kidney	2	3.3	18	0.2	1.0	95
Kidney	4	4.7	26	0.06	0.3	99

(*c*) *Relation of Cellular Oxidations to Phospholipid Formation.* The dependence of lipide phosphorylation upon oxygen consumption was shown by measuring the incorporation of P^{32} into phospholipids by surviving slices of liver and kidney under anaerobic conditions (Taurog *et al.*, 1942). In a nitrogen atmosphere, phospholipid formation by kidney and liver was reduced by 90%. Apparently, lipide phosphorylation occurs only in the presence of aerobic oxidations. Further support for this view came from a study of the effects of the respiratory inhibitors, cyanide, hydrogen sulfide, azide, and carbon monoxide, upon lipide phosphorylation. In the presence of cyanide and sulfide, phospholipid formation by slices of liver and kidney was inhibited to the extent of 90% or more. Concentrations of cyanide as low as 0.005 *M* almost completely inhibited phospholipid formation in both tissues. Carbon monoxide in high enough concentrations and azide (NaN_3) as low as 0.005 *M* also severely reduced lipide phosphorylation. These respiratory inhibitors are believed to interfere with the activity of cytochrome oxidase. This evidence suggests that the energy required for lipide phosphorylation is provided by aerobic oxidations through the cytochrome system.

(*d*) *The effect of Hexoses and Pentoses on Phospholipid Formation by the Brain.* It is well known that the oxygen consumption by brain preparations remains nearly constant for long periods of time when placed in a Ringer's medium containing glucose, whereas in a glucose-free Ringer's medium the oxygen uptake decreases rapidly. In keeping with the above view of the dependence of phospholipid synthesis upon aerobic oxidations, it was found that glucose produced a 5-fold increase in the conversion of P^{32} to phospholipid by brain slices (Schachner *et al.*, 1942). Similar increases in radiophospholipid formation were observed in the presence of other hexoses: fructose, mannose, and galactose. The pentoses were without influence in this respect.

(*e*) *Is the Incorporation of P^{32} into Phospholipid by Tissues an Exchange Reaction?* Although inorganic phosphorus is readily incorporated into phospholipids, the intermediary steps in this conversion are not known. It is possible, however, to rule out certain mechanisms designated here "exchange reactions."[5]

Inorganic phosphate does not exchange with the phosphate radical of phospholipid when sodium phosphate is simply shaken with a phospholipid solution. Although the situation in the animal body is not analogous to the above example because of the presence of enzymes, there is, nevertheless, ample evidence to indicate that inorganic phosphorus is not converted to phospholipid phosphorus in animal tissues by means of an exchange reaction.

Convincing evidence that exchange through collision does not explain lipide phosphorylation in animal tissues is provided by the failure of homogenized liver to form radioactive phospholipid from inorganic P^{32} and by the inhibitory effects upon this formation observed in the presence of such agents as cyanide, azide, and carbon monoxide, substances that are known to interfere with the cytochrome oxidase system.

[5] Exchange may be defined as the formation of radioactive phospholipid by simple collision of radioactive phosphate radicals of the medium with preformed non-radioactive phospholipid molecules of the tissue, a process that results in the replacement of the phosphate components of the latter by the radioactive phosphate radicals. Exchange reactions occur without the aid of energy from an external source and without the expense of energy-producing reactions.

VI. SUMMARY

The application of P^{32} as a labeling agent to the study of intermediary metabolism has greatly facilitated and furthered research in the field of lipide phosphorylation:

A. It has made possible investigations into the dynamics of phospholipid metabolism of various tissues without disturbing the steady state of the animal.

B. It has provided methods for the study of turnover rates of phospholipids in plasma and tissues.

C. The *in vitro* synthesis of phospholipids by surviving slices of liver, kidney, brain, *etc.*, was established by the use of P^{32} as a labeling agent, thus providing a new method of value in the study of certain problems in phospholipid formation, such as the determination of what tissues in the body are capable of phospholipid formation independently.

D. The following contributions to our knowledge in phospholipid metabolism can be ascribed to the use of the radioactive isotope of phosphorus:

1. Plasma phospholipids are synthesized only by the liver.
2. Plasma phospholipids are completely turned over in the dog every 8–9 hours.
3. The liver is the only tissue concerned with the utilization and destruction of plasma phospholipids.
4. The formation of phospholipid molecules proceeds in the absence of the adrenal gland.
5. Choline, a lipotropic substance, stimulates phospholipid turnover in the liver.
6. Liver, brain, kidney, small intestine, and muscle can synthesize phospholipids *independently*.
7. The energy required for phospholipid formation is provided by aerobic oxidations through the cytochrome-cytochrome oxidase system.

We are indebted to Dr. C. Entenman and Dr. Alvin Taurog for many helpful suggestions in the preparation of this manuscript.

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Iodine Metabolism

By C. P. LEBLOND

CONTENTS

I. INTRODUCTION

The concentration of iodine in the thyroid gland is 10,000 times greater than that found in any other organ. This is an unusual phenomenon in biology, since the concentration of other elements in the body tissues usually varies within narrow limits.

This singular behavior of iodine might be accounted for by its atomic configuration. First, the atomic weight of iodine is high, 127, whereas other elements of biological significance have atomic weights below 56. Furthermore, iodine belongs to the halogen group of elements, which seem to localize preferentially in the thyroid gland, as is well demonstrated in the case of bromine (Perlman *et al.*, 1941). The entry of fluorine in the thyroid has not as yet been investigated, but its effect on the gland suggests a similar mechanism (v. Fellenberg, 1934; Wilson and De Eds, 1940; Wilson, 1941). The heaviest member of the halogen series, element 85 (Astatine), which is known only in its radioactive unstable form, shows almost as high an affinity for thyroid tissue as iodine itself (Hamilton and Soley, 1940) It may also be noted that manganese, an element with an atomic structure resembling that of the halogens, is fixed in greater amount in the thyroid than in other organs (Ray and Deysach, 1942).

However, iodine may be sharply differentiated from the other halogens, not only because it is fixed in greater amounts by the thyroid, but also because it is utilized by this gland to make its highly active hormone, thyroxine. If in thyroxine the iodine atoms are replaced by other halogens, such as bromine or chlorine, the substances obtained—tetrabromothyronine and tetrachlorothyronine—are about 200 times less active (Abderhalden and Wertheimer, 1928; Leblond and Grad, unpublished results).

Iodine is not abundant in nature. Only minute amounts are found in foodstuffs and water, the amount being influenced by the iodine content of the soil in which the food was produced. This variation in the soil of various regions explains why there are considerable differences in the consumption of iodine among geographically separated communities. Modern methods of food distribution have tended to reduce these local differences. However, isolated rural districts, especially in mountainous areas, may still be subjected to a very low iodine intake.

The body requires little of this element, since about 100 γ daily appear to be adequate. When the intake is below this level, derangements of thyroid function occur. These are rather vaguely grouped under the term "goiter." Although there are undoubtedly other contributing factors, the present tendency is to consider iodine deficiency as the predominant cause of a goitrous thyroid, whether it be a simple increase in the size of the gland with only mechanical disturbances, or a complex change resulting in degeneration of the gland with loss of function, or on the other hand, exacerbation of thyroid activity resulting in a dangerously high basal metabolism, or even malignancy. It is, therefore, evident that a lack of iodine disturbs the functional balance of the thyroid.

At any rate, iodine plays a dominant role in thyroid physiology. An understanding of this role demands an exact knowledge of the entry and fate of iodine in the body. Extensive reviews, published on this subject by Elmer (1938), Curtis and Fertman(1943), and Salter (1940), show that the enthusiasm aroused two decades ago by the isolation and synthesis of thyroxine had gradually decreased to such an extent that, by 1938, iodine research was nearly at a standstill. Recently, the availability of radioactive iodine (Livingood and Seaborg, 1938; Tape, 1939) stimulated a renewed interest in the subject. Indeed, by the use of radio-iodine, many new possibilities have arisen. It is possible to trace in the body minute doses of iodine, comparable to those which normally circulate in the blood. Furthermore, the administered labeled element may be distinguished from the iodine previously present in the body. Finally, the addition of various amounts of carrier iodine (non-radioactive iodine) to the radioactive element makes it possible to trace, not only the physiological behavior of this element, but the fate of any dose of exogenous iodine up to and above pharmacological amounts. This review will be an attempt to present some of the recent data concerning the entry, utilization and excretion of iodine in the body, with special emphasis on the formation and fate of the thyroid hormone.

The techniques used to trace a radioactive element in the body are of two different kinds. The first, and most popular, method consists in measuring the amount of radioactive element in an organ or chemical fraction of an organ by means of the electroscope or Geiger counter. The other or "autographic" method makes use of the ability of radioactive elements to affect photographic emulsions. Tissue sections from a radio-iodine treated animal are placed in contact with a photographic emulsion, which is then affected by each accumulation of radioactive material in the section, thus making possible a precise localization of the radio-element (Gross and Leblond, 1946).

Three radio-iodine isotopes have been commonly used. The first investigations made use of the isotope 128 with a half life of 25 minutes (Hertz, Roberts and Evans, 1938; Leblond and Süe, 1940). The discovery of the cyclotron resulted in two longer-lived isotopes, so that thyroid investigations were carried out with I^{130} whose half life is 12 hours (Mann *et al.*, 1942) and I^{131} whose half life is 8 days (Hamilton and Soley, 1939; Perlman *et al.*, 1941). Today, because of improvements in the cyclotron and the availability of material from the uranium pile, the long-life isotope I^{131} is used almost exclusively. This isotope has proved satisfactory for both its autographic localization in tissues and its measurement with Geiger counters or electroscopes.

One of the fundamental findings obtained with isotopes is the ability of the thyroid to incorporate almost all of a physiological dose of radioiodine, thus providing the ideal condition for a radioactive bombardment limited to thyroid tissue. Extensive investigations are being carried out on the possible application of these facts in the treatment of hyperthyroidism (Hertz and Roberts, 1946; Chapman and Evans, 1946) and thyroid malignancies (Seidlin *et al.*, 1946). A cautious optimism, relative to the possibilities of partial or complete destruction of the thyroid gland, may be in order.

II. The Entry and Distribution of Iodine in the Body

Except for the highly specific concentration of iodine in the thyroid, its distribution in the organs of the body is not remarkable. It is somewhat similar to that of the other halogens, chlorine, bromine, *etc.*, insofar as the general distribution in the extracellular spaces and the excretion in the urine and the gastrointestinal tract are concerned. However, the halogen behavior of iodine is apparent only when fairly large doses are administered. With minute doses, the removal of iodine from the blood by the thyroid is so intense that the iodine in blood and tissues decreases rapidly to a very low level. It is, therefore, necessary to consider the effect of large, or "pharmacological", and of minute, or "physiological", doses separately.

The entry of iodine into the body may theoretically take place through the skin, the inhaled air, or the mucous membranes of the mouth (Elmer, 1938). However, normally iodine is obtained through the food and fluids entering the gastrointestinal tract.

Since most of the iodine in foodstuffs is present in the ionic form (iodide), most tracer work has involved the use of radioactive iodide. Administration of the radio-element through various routes did not markedly affect the behavior of iodine. For instance, fairly similar results have been obtained by giving the same dose of radioactive iodide to guinea pigs by gavage or by intravenous injection; in both cases, the same radioactive level was reached in the blood in about an hour (Leblond, 1942).

1. The Fate of Pharmacological Doses of Iodine

A number of authors have described the distribution of large doses of iodine in the various organs (Loeb, 1907; v. Fellenberg, 1926; Ariel *et al.*, 1941; see also below, and Fig. 1).

To account for the variations observed, several general laws of distribution have been proposed. For instance, the German school claimed that, besides the specific fixation in the thyroid, a large amount of iodine occurred in the *endocrine glands*, especially the hypophysis, ovaries and adrenals (Sturm and Buchholz, 1928; Salter, 1940). However, King (1941), using a more precise method for iodine titration than the German authors, did not confirm the presence of large amounts of iodine in the endocrines. Neither did the distribution of large doses of radio-iodine show a greater deposition in the endocrine glands than in the other organs (Leblond, 1942).

On the other hand, Wallace and Brodie (1937) showed that a large dose of iodine distributes itself throughout the *extracellular spaces*. This was fully confirmed by a study of the distribution of pharmacological doses of radio-iodine soon after injection. One of the methods for proving the distribution in the extracellular spaces was to show that radio-iodine had the same general distribution in the various tissues, whether or not the animals were pretreated with several doses of ordinary iodine. This finding implied a free interchange of iodine ions from the blood to the extracellular fluids and back (Leblond, 1942).

There are, however, two important exceptions to this rule, *i.e.*, the excretory organs and the thyroid. In the *excretory organs*, some of the radio-iodine progresses beyond the extracellular spaces and enters the cells of the organ. Thus, there was more radio-iodine in the liver than may be accounted for by the known capacity of the extracellular space. Apparently some of the iodine enters the hepatic cells to reach the bile, where its presence has been recognized (Ibuki, 1927; Elmer, 1938). Similar results were obtained with kidneys, intestine, and especially stomach.

Large quantities of iodine are excreted by the kidney after iodide administration (Elmer, 1938; Hamilton and Soley, 1940). Indeed, within a week, close to 100% of a large dose of iodine was found in the *urine*, most of the excretion taking place within the first few days after administration, with a maximum output during the first 5 hours (Abderhalden and Slavu, 1909). This rapid elimination is reflected in the rapidly decreasing level of radio-iodine in the blood and the extracellular spaces of the various organs (Perlman *et al.*, 1941).

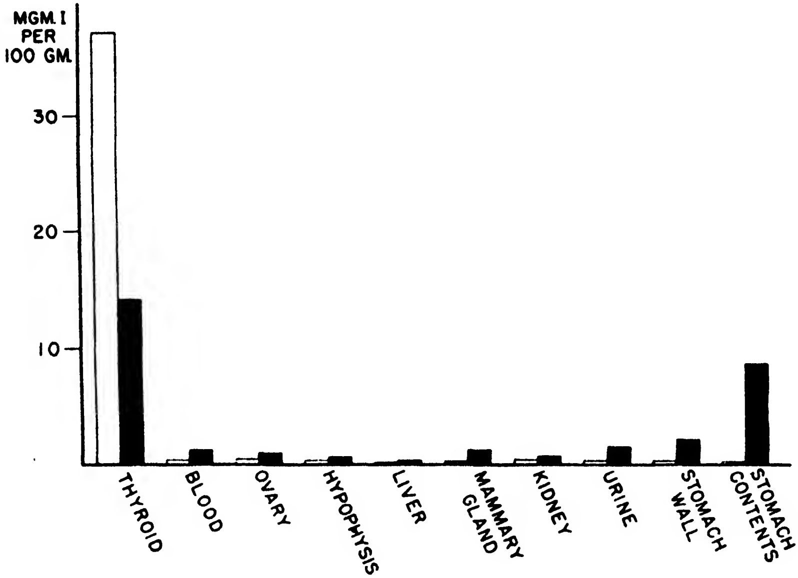

FIG. 1

The solid columns represent how much of a pharmacological dose of radio-iodide enters the various organs. The light columns represent the total iodine (values taken from the literature).

In contrast to the large amounts of iodine excreted in the urine, only a small quantity may be detected in *feces* (Elmer, 1938). This is all the more surprising in view of the large quantities of radio-iodine passing into the intestine, the bile and especially the stomach (Lipschitz, 1929; Berger, 1929). The amount found in the stomach varied according to the activity of the organ. Thus, the gastric juice of animals that were digesting food or had received histamine contained much more radio-iodine than that of fasted controls, up to 30% of the dose being found in the active stomach 30 minutes after intravenous injection. The radio-iodide behaved like the chloride ion, since it was largely present as hydroiodic acid (while chloride occurs as hydrochloric acid). Finally, the amount of iodine passing into the stomach was proportional to the injected dose, as shown in a series of animals given radio-iodine 2 hours after a meal (Leblond, 1942).

It has been known for a long time that iodide may be absorbed from the isolated stomach, as well as from various other isolated parts of the gastrointestinal tract (Cohn, 1932). This indicates a constant interplay of excretion and resorption taking place in the stomach and also at the lower levels of the gastrointestinal tract. After injection of radio-iodine, its release into the lumen of the stomach is considerably greater than its resorption, while the small amount of iodine in the feces indicates that the opposite must be true in the case of the rest of the alimentary tract. Indeed, the enormous amounts released in the upper portions of the gastrointestinal tract must be resorbed lower down. This fact insures some degree of storage and thus prevents a too rapid elimination of iodide in the urine.

The thyroid gland of animals given a dose of 5 mg. of radio-iodide per kg. fixes from 20 to 50 times more iodine than would be expected if it went only into the extracellular spaces (Leblond, 1942). This type of experiment illustrates the specific ability of the thyroid to fix iodine.

Baumann showed, in 1896, that the thyroid contains a considerable amount of iodine, the total content of the human thyroid varying around 10 mg., that is, a concentration of about 40 mg./100 g. of fresh tissue. It was only in 1915 that Marine, using pharmacological doses of iodine (about 5 mg./kg.), actually proved that iodide injected into the blood stream finds its way into the thyroid (Marine, 1915; Marine and Rogoff, 1917). He even showed that a thyroid gland perfused *in vitro* with fluid containing iodide, extracted the iodide from the fluid (Marine and Feiss, 1915). Similar experiments, carried out by other workers, generally confirmed that the thyroid takes up much more iodine than other organs (Sturm, 1930; van Dyke, 1921). The failure of Toxopeus (1939) to find this specific behavior of the thyroid in regard to iodine fixation can be explained on the basis of the enormous doses which he injected and which flooded the extracellular spaces throughout the body, including those of the thyroid gland, thus hiding the specific behavior of the thyroid shown with the usual pharmacological dose. This will be treated in more detail below.

In Boston, Hertz, Roberts and Evans used radio-iodine for the first time and confirmed Marine's results on the fixation of iodine in the thyroid (1938). The dose used by these authors was of the same order of magnitude as the doses injected by Marine; but the use of the radio-element enabled much more clear-cut conclusions: The uptake of iodine by the thyroid was found to reach a maximum within 10 minutes. Furthermore, they calculated that the normal thyroid collected up to 80 times as much as did the other body tissues on the average (Hertz *et al.*, 1938). Other studies on the effect of such pharmacological doses were carried out in Paris (Leblond and Süe, 1940, 1941) and in California (Perlman *et al.*, 1941).

There is little doubt that iodine is made available to the thyroid when it is in the ionic form. The main argument in favor of this point of view was the demonstration by Marine that iodide is extracted from the perfusion fluid by the surviving thyroid gland *in vitro*. When iodine is not administered as iodide, but is in such chemical forms as iodate or diiodotyrosine, it does not immediately enter the thyroid. Indeed, such compounds were found to break down to iodide in the blood stream and then enter the gland as iodide (Leblond and Süe, 1941). Thyroxine was not believed to enter the gland as such, since no apparent modification of the iodine content of the thyroid was obtained after thyroxine administration (van Dyke, 1925; Sturm, 1930). However, recent work with radioactive thyroxine (Gross and Leblond, 1947) shows that some of the iodine from the radiothyroxine finds its way into the thyroid gland, but rather slowly and in small amounts, presumably originating from the breakdown of thyroxine to iodide. The radioactivity appeared in the thyroid more slowly and in smaller quantities after injection of radio-thyroxine than after a similar dose of radio-diidotyrosine, suggesting that the breakdown of thyroxine is much slower than that of diidotyrosine.

In contrast to complex iodine compounds, intravenously injected iodide entered the thyroid so rapidly that the maximum fixation was reached in less than 5 minutes. It must be emphasized that such an almost instantaneous entry of iodine is observed only if large doses of iodine, such as 5 mg./kg., are administered. The percentage of the injected dose entering the gland is about 0.5% immediately after the injection. This is a maximum. Later, the radio-iodine content of the gland changes little over a long period of time (Hertz *et al.*, 1938; Leblond and Süe, 1941; Perlman *et al.*, 1941).

These authors also showed that a second dose of iodine given after the first one, enters the thyroid to a much more limited extent than the first one (Fig. 2). Large amounts of iodine are thus prevented from entering the gland, which is considered to be "saturated" in view of the fact that it no longer accepts large amounts of iodine. In general, the thyroid gland in dogs, guinea pigs, rabbits or rats, under normal conditions, may fix only 10–20 mg. of iodine/100 g. of fresh tissue. The cause of the decrease in the fixation of the second dose is twofold, since, besides the saturation, there is in the blood a dilution of the second dose of iodine by the first one.

Saturation, without dilution effects, was apparent in other experiments in which the doses were increased well above 5 mg./kg. The iodine content of the thyroid following such a dose represented the maximum uptake, since injecting doses up to 10 times as great did not significantly raise the amount of iodine entering the gland (Hertz *et al.*, 1938; Leblond and Süe, 1941). This result, incidentally, explains why Toxopeus was unable to find a marked difference between the thyroid and other organs when injecting very large amounts of iodine, since the saturation prevents further addition of iodine in the thyroid tissue and most of the apparent increase in the thyroid iodine when the administered dose goes over 5 mg./kg. is due to a flooding of the extracellular spaces.

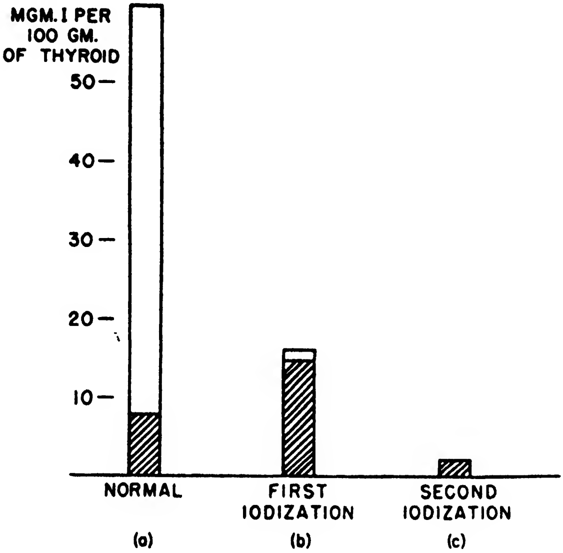

Fig. 2

From left to right, note columns representing:

(a) Amount of iodide (cross-hatched) and organic iodine (light) in the normal thyroid.

(b) Amount of radio-iodide (cross-hatched) and organic radio-iodine in the thyroid of animals treated with a pharmacological dose of radio-iodide ½ hour previously.

(c) Amount of radio-iodide (cross-hatched) with no organic radio-iodine in the thyroid of animals treated with pharmacological doses of, first, inactive iodide and, ½ hour later, radio-iodide.

Conversely, minute doses of iodine cannot saturate the gland, but they may slightly reduce the amount of iodine taken up (unpublished results). The word saturation has been used by Lein (1943) for the partial inhibition of the iodide-combining mechanism of the thyroid gland produced with rather small doses of iodine. It is probable that the same mechanism may be at work in this partial inhibition as in complete saturation.

Finally, a complete lack of iodine, such as is obtained in animals fed on an iodine-deficient diet, will remove all inhibition, and thus iodine will be absorbed rapidly and in maximal amounts.

In conclusion, it may be stated that pharmacological doses of the iodine ion are taken up by the thyroid gland in rather large amounts and nearly instantaneously. However, the thyroid is endowed with a mechanism which prevents the addition of more than 10–20 mg.-% of iodine to its preexisting store. At this point, the gland is said to be "saturated". In the other organs, a pharmacological dose of iodine is at first distributed in the extracellular spaces. Then gradually the iodine leaves the extracellular spaces to become concentrated in the excretory organs, so that the iodine level in the blood and tissues falls off after the initial high values. The excretion mechanism functions in proportion to the magnitude of the dose. The kidneys, as well as the stomach, the intestine and the liver, rapidly accumulate most of the iodine in the body. The large quantity in the lumen of the gastrointestinal tract is eventually resorbed, while the urine gradually eliminates an amount nearly equal to the injected dose.

2. The Fate of Physiological Doses of Iodine

Before radio-iodine became available, the entry of iodine into the thyroid gland could not be examined under physiological conditions. The modern technical advances which enable us to trace the physiological behavior of iodine result from the fact that radio-iodine is now prepared from the transmutation of tellurium bombarded in either a cyclotron or an uranium pile. With the help of pure reagents it is possible to extract the radio-iodine from the tellurium and avoid contamination with ordinary iodine to the extent that the weight of iodine in the sample will be below that detectable by the method of Sandell and Kolthoff (1937) which is sensitive to about 0.01 γ. With such a minute dose, the behavior of radio-iodine is quite comparable to that of the small amounts of iodine supplied with the food, and therefore provides a direct method for investigation into the physiological transformation of iodine in the body (Hamilton and Soley, 1939). Such a minute, physiological quantity of radio-iodine is known as a "tracer" dose. In contrast, a pharmacological dose is obtained by mixing such iodine with ordinary iodine in the proportion desired.

Hamilton and Soley (1940) found that guinea pigs fixed a large amount of a physiological dose of iodine—about 20% of the injected dose—and that the maximum level in the gland was reached between 20 and 65 hours after injection. These results were extended to the rat, in which over 60% of the injected dose found its way into the thyroid in about 24 hours (Perlman *et al.*, 1941). In the dog, maximum values were observed 48 hours after the injection and were in the neighborhood of 35% of the injected dose (Mann *et al.*, 1942). Doses of 50% or more have been found regularly in human patients not pretreated with iodine (Leblond *et al.*, 1946; Hertz and Roberts, 1946; Chapman and Evans, 1946).

The amount of radio-iodine found in the thyroid at any given time is obviously smaller than the total amount going through the gland, since some radio-iodine is continuously excreted out of the gland. Indeed, after injection of radio-iodine, radioactive organic compounds behaving like diiodotyrosine and thyroxine are found in the blood stream, presumably coming from the thyroid gland (Morton *et al.*, 1941). On the other hand, it has been mentioned previously that diiodotyrosine and thyroxine are gradually hydrolyzed in the body, thus yielding ionic iodine to the blood stream. Obviously this ionic iodine, like the original dose of radio-iodide, will go to the thyroid, with the exception of a small fraction entering the excretory channels. This repeated use of iodine by the thyroid plays an important part in iodine metabolism as shown by the following considerations.

When thyroxine is administered to a thyroidectomized or myxedematous patient, it takes 250–350 γ daily of thyroxine to maintain a normal metabolism (Thompson *et al.*, 1935; Means, 1937); that is to say, about 200 γ of iodine. This means that, if the iodine available from food was completely used up once by the thyroid, the daily requirement of iodine in man would have to be about 200 γ. Evaluation of the daily intake in many different countries showed that in most places the intake was far below this figure, being commonly less that 50 γ (Elmer, 1938, page 128). The conclusion to be drawn from such a comparison is that the available iodine must go through the thyroid a number of times to supply the required amount of thyroxine to the normal individual.

It may be recalled here that, if a tracer dose of iodine is administered to animals or patients given an iodine-rich diet, and consequently having a high level of inorganic iodine in the blood, the behavior of the thyroid is altogether different. The thyroid is apparently in a chronic condition of saturation, resulting in the uptake of a very small percentage of the injected dose (Leblond *et al.*, 1946). In other words, the same result was obtained as by injection of a large dose of iodine.

It is clear that the thyroid which receives only the small amount of iodine present in the average human diet is not saturated with iodine and therefore is in a position to absorb an extremely high proportion of the physiological supply. This is a unique example of the adaptation of the human body to use economically an element which is relatively rare in nature.

When doses intermediary between the physiological and pharmacological doses are administered, the saturation level is rapidly reached (See Table I).

The distribution of tracer doses of iodine in tissues other than the thyroid showed quite a different picture from that obtained with pharmacological doses (Perlman *et al.*, 1941). Very soon after the administration, the uptake in various organs was relatively high and decreased within a few hours to low levels. Indeed, the decrease in the level of blood iodide depends on the thyroid activity, since Lein (1941) found that the blood iodide fell to a much lower level in rabbits with thyrotrophin-induced hyperplasia of the thyroid than in normal controls. The parallelism in the decrease of labeled iodine in blood and tissues suggests that iodine diffuses in and out of the extracellular spaces, rather than being selectively retained.

The excretion of a physiological dose of radio-iodine was first examined by Hamilton and Soley (1940) who found that 4 hours after injection 12% of the radio-iodine was in the urine but less than 1% was excreted in the feces. Eighteen hours after the injection, 37% had been passed into the urine, but now 17% had appeared in the feces. Unfortunately the chemical nature of the radio-iodine thus found in urine and feces was not investigated.

TABLE I

Influence of Blood Iodine Level on the Fixation of Iodine by the Thyroid

Amount of radio-I injected/kg. of body weight (*mg.*)	Radio-I in blood (*mg /100 cc.*)	Concentration of radio-I in thyroid (*mg./100 g.*)	Ratio: $\frac{\text{Thyroid I}}{\text{Blood I}}$
50	7.31	23	3
5	1.25	16	13
0.1	0.032	7	219
0.005	0.003	1.6	533

All values were taken at the time of maximum concentration of the radio-iodine in the thyroid.

As a general rule, it is felt that the amount of radio-iodine found in the urine may be used as an index of the activity of the thyroid (Hertz and Roberts, 1946; Seidlin *et al.*, 1946; Chapman and Evans, 1946). More precisely, the greater the amount taken up by the gland, the less will be found in the blood and urine. It is the practice of some of the hospitals where patients are treated with radio-iodine to evaluate the thyroid activity by checking on the urinary radio-iodine of the patients.

In conclusion, it may be stated first that under physiological conditions, the thyroid gland possesses an extremely efficient mechanism to extract from the circulation an amount of iodine which, in view of the possibility of repeated reentry of the same atoms of iodine into the gland, may appear to be well over 100% of the amount injected and secondly, the greater the fixation of iodine in thyroid, the smaller the amount excreted in the urine soon after administration.

III. THE FATE OF IODINE IN THE THYROID GLAND

The preceding section was devoted to the entry of iodine into the thyroid. The present one deals with the formation of the thyroid hormone from iodine. This distinction may appear somewhat arbitrary since iodine entering in physiological doses is swiftly incorporated into organic compounds. However, by making use of such anti-thyroid compounds as thiourea and its derivatives, it is now possible to separate the phenomenon of the entry of iodine from that of its chemical transformation. These anti-thyroid substances interfere little with the collection of iodine from the blood by the thyroid (McGinty and Sharp, 1946), but they prevent its incorporation into what is to be the thyroid hormone.

The formation of the thyroid hormone from iodine proceeds rather differently depending upon whether a pharmacological or a physiological dose of iodine is used.

1. The Fate of Pharmacological Doses of Iodine in the Thyroid

Since the thyroid gland normally contains small amounts of iodine in the ionized form (iodide) (Gutman *et al.*, 1932; Mann *et al.*, 1942), the question arises as to whether the entry of radio-iodine into the gland is due to an interchange of atoms between the iodide in the gland and the iodide in the blood or whether it is due to a true addition of iodine. Marine and Rogoff (1916) had already shown, by chemical titration methods, that there was a real increase in the iodine content of the thyroid after the injection of iodide. Actually, with radio-iodine, it is possible to show that some of the iodine entering the gland is chemically fixed in the gland, but some of it is exchangeable.

The amount of this exchange in the thyroid may be roughly estimated by measuring the radio-iodine entering the glands in animals pretreated with ordinary non-radioactive iodine (Fig. 2). In this case, the uptake of radio-iodine by the thyroid is much reduced, being only 14–25% of the normal at one-half hour after injection (Hertz *et al.*, 1940; Leblond and Süe, 1941). However, before assuming that these figures truly indicate the rate of iodide exchange between thyroid and blood, it was necessary to examine whether some of the iodine had been trapped into a non-exchangeable combination.

It is known that, in normal thyroids, there is usually less than 5% ionized iodine, the rest being present in the form of thyroglobulin, a protein molecule which contains two iodized amino-acids, diiodotyrosine and thyroxine. It should be noted that at pH 7 *in vitro*, the iodine of these compounds is not exchangeable with iodide (unpublished results). The problem consists, therefore, in deciding whether the iodine which had not been exchanged had actually been incorporated into the gland as thyroglobulin.

The separation of the iodine fractions of the thyroid may be accomplished by several methods based on the preferential solubility of thyroxine in *n*-butyl alcohol (Leland and Foster, 1932; Gutman *et al.*, 1932; Leblond *et al.*, 1946). Such methods applied to the thyroid 30 minutes after injection of a pharmacological dose of radio-iodine showed that only approximately 10% of the radioactivity was incorporated into thyroglobulin, all the rest being present in the iodide fraction (Leblond, 1942). It may, therefore, be concluded that the iodide enters the gland as such; and, the figures above may indicate the rate of exchange of the thyroid iodide, namely, about 20% per half hour under these conditions.

Results similar to those reported here were obtained when a minute dose of radio-iodine was injected into dogs with a high blood iodine level. In this case, about 75% of the radioactivity in the gland was found in the iodide fraction of the gland 30 minutes after injection (Leblond *et al.*, 1946).[1] It must be emphasized that these results obtained in animals with a high blood iodine are quite different from those described below, in which minute doses of iodine are used in animals with a normal blood iodine. The explanation of this oddity seems to be that apparently, because of the high blood iodine, a minute dose becomes "diluted" with the large amount of circulating iodide and acts as a pharmacological dose.

While 30 minutes after injection of radio-iodine to animals with a high blood iodine level, most of the radioactivity in the thyroid is present as iodide, in contrast, the results obtained 24 hours later showed that the radioactivity had decreased in the iodide fraction, apparently to be supplied to the diiodotyrosine and thyroxine fractions. This is shown in Fig. 3, where the radioactivity of the various iodine fractions, calculated per unit weight of iodine (specific activity), may be compared at various time intervals.[2] It may be seen that the specific activities in the iodide fraction of the thyroid are high 30 minutes after injection and decrease rapidly over a period of 24 hours, while the specific activities of diiodotyrosine and thyroxine increase.

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[1] The blood values in Table II of the publication by Leblond *et al.* (1946) are expressed in mg.-% and not in γ-% as erroneously indicated.

[2] A convenient method for expressing specific activity is to calculate the so-called relative specific activity of each thyroid fraction, that is to say, the percentage of thyroid radioactivity found in the fraction under investigation, over the percentage of total thyroid iodine found in the fraction. This method reduces the effect of individual variations. Furthermore, the values obtained in all fractions, if the transformations of iodine were instantaneous, would be equal to one. Therefore, soon after injection, values higher than one are found in the fractions where the radioactivity is deposited at first ("mother" compounds), while the relative specific activity in the "daughter" compounds is low at first and rises later. Finally, when the value for the mother compound falls below that of the daughter compound, no further increase in the relative specific activity of the latter may take place.

The relative specific activity is most useful with physiological doses.

In conclusion, soon after administration of fairly large doses of radio-iodine, almost all of it is found in the gland in ionized form. This ionized iodine will be the source from which the other iodine compounds originate.

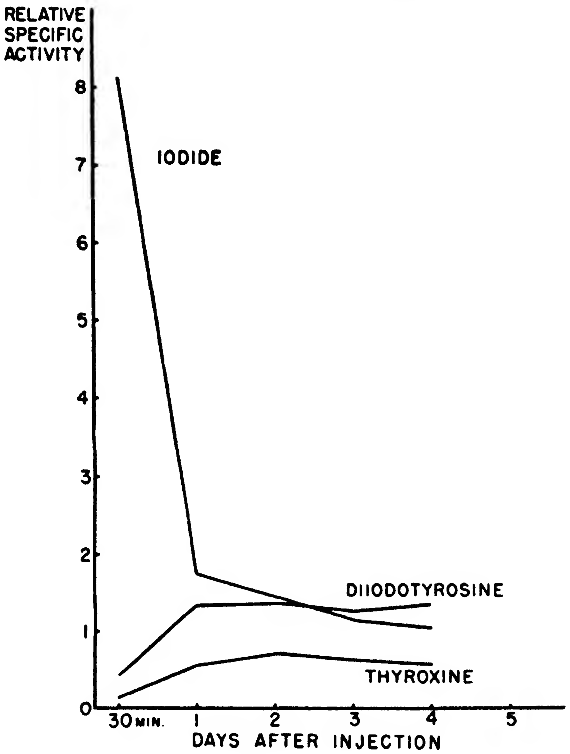


FIG. 3

Specific activity of the iodine fractions of the thyroids after administration of a small dose of radio-iodide to dogs fed a high-iodine diet.

2. The Fate of a Physiological Amount of Iodine in the Thyroid

Using minute doses of iodine, the physiological behavior of iodine in the thyroid may be assessed by examination of the three fractions of the gland. The data available on the subject are, however, rather limited and some of the conclusions will have to be speculative.

(*a*) *The Origin of Iodide.* The amount of iodide in the normal thyroid is rather small, usually below 5% of the total iodine (Leblond *et al.*, 1946). It has generally been assumed that this iodine had been collected from the blood. Indeed, it has been shown above that iodine, administered as a pharmacological dose of iodide, entered the gland as such (see also Leblond *et al.*, 1944). Similarly, in rabbits treated with a rather small dose of radio-iodine, the entry of iodine into the thyroid as iodide was demonstrated by showing that, soon after injection of radio-iodine, the iodide fraction contained most of the radioactivity in the gland (Lein, 1943). However, in dogs receiving a tracer dose of iodine, the amount of radioactivity per γ of substance (or specific activity) was smaller in the iodide than in the diiodotyrosine fraction at 30 minutes after the injection (Mann *et al.*, 1942). This suggests that part of the iodine entering the iodide fraction is not labeled and therefore does not come from the blood; presumably it has its origin in the breakdown of the organic forms of iodine in the gland.

(*b*) *The Formation of Diiodotyrosine.* It has been known for a long time that the treatment of proteins with free iodine (I_2) substitutes iodine in tyrosine groups, yielding diiodotyrosine groups (Oswald, 1910). When Harington (1933) discovered the presence of diiodotyrosine in the thyroid gland, he suggested that an oxidation reaction had transformed iodide into free iodine, enabling it to be fixed on tyrosine groups. The mechanism of this reaction has been examined *in vitro* (Li, 1942).

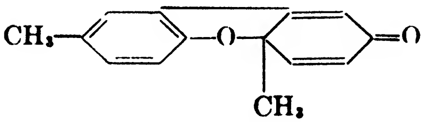
In the thyroid, it appears likely that the diiodotyrosine iodine finds its source in the iodide fraction. Indeed, it could not come from the thyroxine fraction because in the dog, for 2 days after injection of radioactive iodine, the specific activity of thyroxine was consistently lower than that of diiodotyrosine (Mann *et al.*, 1942). It is clear, therefore, that diiodotyrosine must be formed from iodide, as suggested by Harington (1933). This conclusion appears to conflict with the finding of a low specific activity in the iodide fraction of the dog by Mann *et al.* (1942). However, the dilemma may be solved if one speculates on the basis of a dual origin to the thyroid iodide, namely (1) the blood iodide, which has a high specific activity and should be transformed into diiodotyrosine very soon after entering the thyroid cell and (2) the iodide originating from organic iodine compounds which has a low specific activity and should be less apt to be transformed into diiodotyrosine, possibly because of its location where organic compounds are stored, that is to say in the colloid of the thyroid follicle (see below). The rough rules set by Hevesy for measuring the rate of formation of a substance suggest in man (Leblond *et al.*, 1946) that the renewal of diiodotyrosine from iodide takes place in about a day.

(*c*) *The Formation of Thyroxine.* For some time following the injection of radio-iodine, the specific activity of the thyroxine fraction usually remains smaller than that of either iodide or diiodotyrosine. Either one or both of these two substances could be the precursor of thyroxine.

A direct transformation of iodide into thyroxine would imply the fixation of the iodine directly onto a molecule of thyronine, a compound identical with thyroxine except for the lack of the iodine atoms. This hypothesis is favored by some authors (Marenzi and Villallonga, 1944), but it lacks a solid chemical basis, since no thyronine has ever been found in the thyroid gland and, furthermore, it has not been possible to achieve thyroxine synthesis by the addition of iodine directly onto the molecule of thyronine (Harington, 1944).

On the other hand, in the dog (Mann *et al.*, 1942), the specific activity of iodide remained at a low level, while the specific activity gradually increased in the thyroxine fraction. In this case, only the diiodotyrosine fraction had a specific activity steadily higher than that réached by thyroxine 48 hours after injection, and thus was presumably the precursor to thyroxine. Since the level of specific activity in the diiodotyrosine fraction was fairly constant, the regular increase in the specific activity of thyroxine during the first two days after injection was satisfactorily explained by the formation of thyroxine from diiodotyrosine (Mann *et al.*, 1942).

In support of the theory that thyroxine comes from diiodotyrosine in the thyroid, experiments should be mentioned in which this transformation was obtained *in vitro* (Ludwig and von Mutzenbecher, 1939; Block, 1940). This reaction is slow, but may be accelerated by the addition of hydrogen peroxide and by the removal of thyroxine as it is formed (Harington, 1944). The mechanism of this transformation of diiodotyrosine into thyroxine has been elucidated by comparison with results obtained in studies on phenol oxidation. Westerfeld and Lowe (1942) obtained three types of compounds by the oxidation of *p*-cresol with peroxidase and hydrogen peroxide: a tetrahydrobenzofurane derivative:



the 2,2-dihydroxy-5,5-dimethylbiphenyl; and analogous terphenyl derivatives. By comparing the behavior of diiodotyrosine with that of cresol, it was concluded that the presence of iodine atoms on the benzene ring would prevent the formation of the second and third types of compounds, thus making possible the formation of only the first type. Furthermore, in the first type of compound, the iodine would block the secondary ring closure of the quinol ether occurring in the formation of the dibenzofurane derivative. It is apparent that thyroxine may be obtained in a similar fashion (Fig. 4). The study of this reaction also showed that the oxidative coupling of two molecules of diiodotyrosine is accompanied by the elimination of one side chain from which pyruvic acid and ammonia have been obtained (Johnson and Tewkesbury, 1942).

Harington (1944) believes that the formation of diiodotyrosine from iodide and that of thyroxine from diiodotyrosine may take place under the

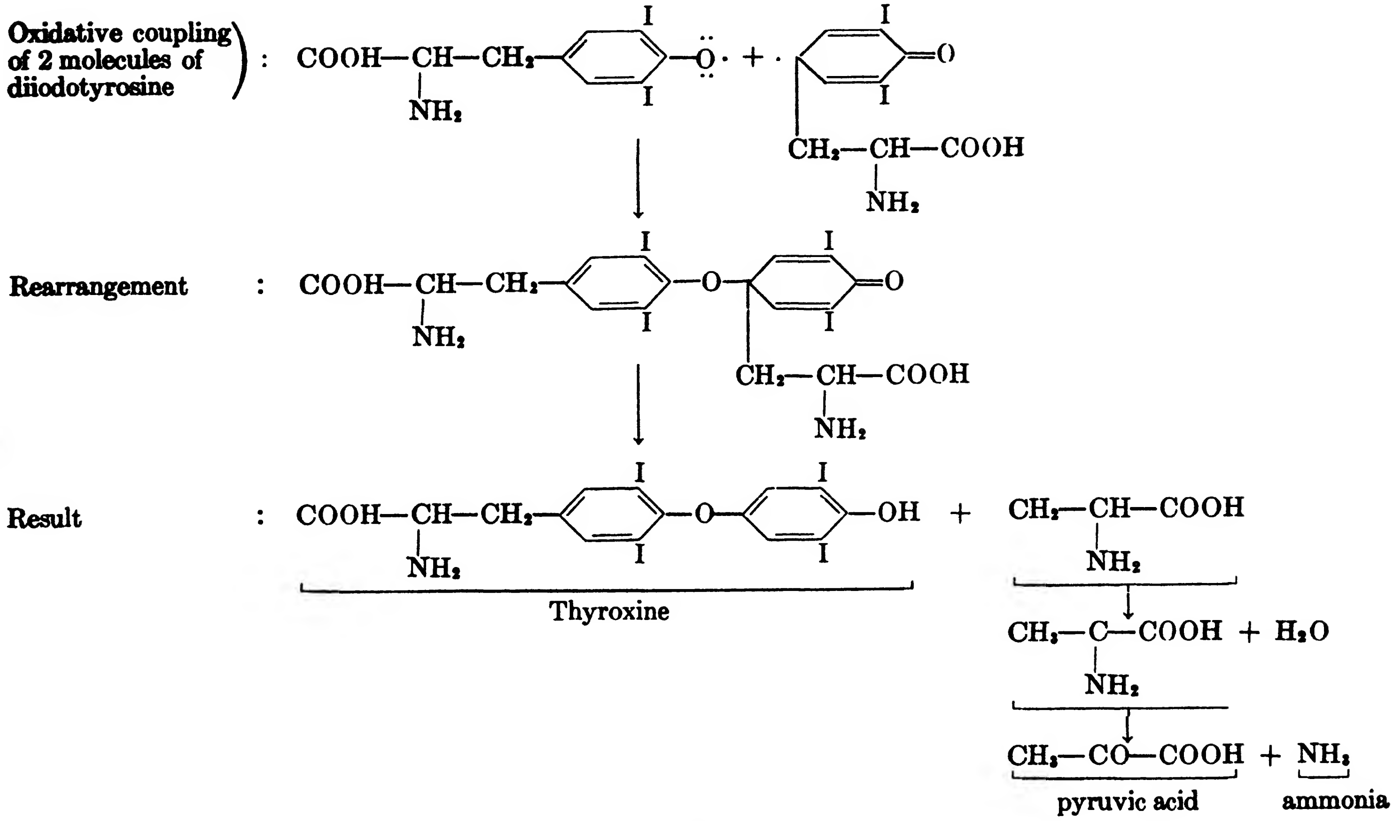


FIG. 4
Mechanism of thyroxine synthesis from diiodotyrosine.

action of the same oxidizing agents. This conclusion is derived in part from the fact that treatment of proteins with free iodine (I_2) produces both diiodotyrosine and thyroxine groups in the protein molecule. While it has been known for a long time that free iodine may react with the tyrosine chains of proteins to produce diiodotyrosine (Oswald, 1910), Abelin and Florkin (1933) discovered that I_2 treatment also endowed proteins with thyroxine-like activity. Such iodo-proteins have been shown to relieve myxedema (Lerman and Salter, 1939). Indeed, crystalline DL-thyroxine (Ludwig and v. Mutzenbecher, 1939; Harington and Pitt-Rivers, 1939) could be isolated from iodized casein by alkaline hydrolysis. These results were confirmed by Reineke and Turner (1946) who obtained the natural L-isomer of thyroxine. Apparently, the most efficient method for thyroxine production consists in iodizing casein or soy bean proteins at about 38°C. with 6 atoms of iodine per mole of tyrosine and then incubating with vigorous agitation at 70°C. in presence of air. It was suggested that diiodotyrosine is formed during the iodine treatment at 38°C. and thyroxine during the subsequent incubation.

The formation of thyroxine in iodized protein, as well as the formation of thyroxine from pure diiodotyrosine, is catalyzed by atmospheric oxygen, manganese dioxide and hydrogen peroxide (Reineke and Turner, 1946; Harington, 1944). Oxidation mechanisms, therefore, play an important role in thyroxine synthesis. In the thyroid gland, Johnson and Tewkesbury assume that the active agent for these reactions is hypoiodite (1942). Harington (1944) believes that, at the physiological pH, hypoiodite cannot exist except in an infinitesimal concentration. Free iodine, on the other hand, could be formed from iodide and would act as its own catalyst to react with tyrosine in producing diiodotyrosine and to oxidize the latter to thyroxine.

Whether free iodine or hypoiodite or other intermediaries occur, an oxidative enzyme system must be active in the thyroid. The nature of this enzyme system has been the object of numerous discussions. It seems clear that the cytochrome oxidase which is believed to take part in the reaction could not play a direct role; since this enzyme is not affected by thiouracil and other thyroid inhibitors (Glock, 1946). On the other hand, basing his conclusions on histochemical evidence, Dempsey (1944) suggested that a peroxidase was the effective agent. It may be recalled that Westerfeld and Lowe stated that since the oxidative condensation of *p*-cresol could be easily obtained with hydrogen peroxide and a peroxidase, the quinol ether linkage in thyroxine might be formed through a peroxidase oxidation. Keston (1944) proposed the idea that the xanthine oxidase system might play a part in biological iodinations through furnishing the peroxide essential for peroxidase action. Using milk as a source of xanthine oxidase, peroxidase and caseinogen, and adding xanthine as a substrate, he was able to transform radio-iodide into radio-thyroxine and radio-diiodotyrosine, this presumably taking place through iodination of the caseinogen. The same reaction without xanthine oxidase produced radioactive diiodotyrosine with little radioactive thyroxine. The addition of xanthine oxidase accelerated the formation of both substances, but mostly that of thyroxine. Keston's results provide an attractive working hypothesis for the mechanism of thyroxine formation in the gland. However, Glock claimed that neither peroxidase, nor xanthine oxidase could be found in the thyroid and that the apparent peroxidase activity of the gland was due to the hemoglobin from the blood (1944). Further criticism of the peroxidase theory will be found in Randall's paper (1946). Very recently, however, the presence of peroxidase has been demonstrated in thyroid cells by a number of methods, such as the oxidation of benzidine, Nadi reagent, *o*-phenylenediamine, guaiacol and pyrogallol, as well as by the liberation of iodine from iodide. This peroxidase activity of the thyroid disappears when the tissue is heated to 120°C. De Robertis and Grasso claim that this action is specific and is not due to hemoglobin (1946).

While both the formation of diiodotyrosine from iodide and thyroxine from diiodotyrosine are believed to be produced by the same oxidative mechanism, results obtained with autographic methods suggest that the two reactions may not always take place in the same locations. It is known that radio-iodine like other radio-elements may affect photographic emulsions. This property was utilized to locate radio-iodine in the thyroid tissue, by placing a mounted section against a photographic plate. A blackening appeared on the emulsion wherever it was in contact with a zone of the section containing radioiodine. Only organically bound radio-iodine could be located by this method, since any iodide present was dissolved out during the mounting of the section (Hamilton *et al.*, 1940; Leblond, 1943). Recent unpublished work (Leblond, Findlay and Gross) showed that an hour after administration of a physiological dose of radio-iodine to the rat, organically bound radio-iodine may be visualized by the autographic method in the thyroid follicle and is located in the cells or in the colloid or in both. Actually, its predominance in the cells is seen very exceptionally and only with diets fairly high in iodine. However, the possibility of this occurrence is proof enough of the fact that the cells are capable of incorporating the iodide coming from the blood into organic molecules. Gersh and Caspersson (1940) proved that the cells play a role in iodine metabolism by showing the absorption spectrum of iodo-proteins in the cytoplasm of thyroid cells. On the other hand, in animals fed on normal or low iodine diets, the transfer of organic radio-iodine through the cells into the colloid is so rapid that, at no time after radio-iodine injection, is there enough of it in the cells to make it visible by the autographic method, while it can be plainly detected in the colloid.

The presence of organic radio-iodine in the colloid as soon as 30 minutes after injection, that is to say, when most of the radioactivity is in the diiodotyrosine fraction and little as yet in the thyroxine fraction, indicates that thyroxine formation takes place in the colloid, where the thyroxine precursor, diiodotyrosine, has been deposited.

However, a positive oxidation-reduction potential, which is assumed to be necessary for thyroxine synthesis, while being always present in the cells of the rat thyroid, appears in the colloid only after stimulation of the thyroid (De Robertis and Grasso, 1946).

It is rather difficult to reconcile the results reported for the rat thyroid in the last two paragraphs. It will be shown later that, in a normal rat thyroid there are marked differences in the activity of the individual follicles. Possibly thyroxine synthesis takes place in the colloid only in the more active follicles.

In conclusion, under normal physiological conditions, two oxidative reactions transform the iodine in the thyroid: a faster one insuring the iodination of tyrosine groups, and a slower one coupling the diiodotyrosine groups thus formed to produce thyroxine. A comparison of chemical and autographic results indicated that these two reactions may take place in the thyroid cells, but that at least the formation of thyroxine is also taking place in the colloid of the more active follicles.

IV. THE MECHANISM OF THE EXCRETION OF IODIZED COMPOUNDS FROM THE THYROID

It is surprising how little is known about the excretion of the iodized compounds which are synthesized in the thyroid. On the basis that iodized compounds are included in the molecule of the thyroglobulin in the thyroid, many efforts have been made to find thyroglobulin itself in the circulation. However, Lerman, using immunological techniques, was unable to detect thyroglobulin either in the peripheral blood or in the blood of the thyroid vein (1940, 1942). Harington drew attention to the fact that thyroglobulin *per os* is active in the thyroidectomized animal; and, therefore, since thyroglobulin, like any other protein molecule, is broken down in the intestine into its component amino acids and is not likely to be resynthesized in the absence of the thyroid, the thyroglobulin fragments must be active *per se* (1944). Furthermore, Trevorrow showed that the organic iodine of the blood may be subdivided into two fractions, neither one of which is a protein, but which resemble diiodotyrosine and thyroxine, respectively (1939). The possibility that the thyroxine present in the circulation is bound into a small peptide must be taken into consideration, but is not supported by satisfactory evidence (Harington, 1944). It may be concluded that diiodotyrosine and thyroxine in the blood may be bound in small non-protein molecules, but are more likely to occur in their free form.

After the administration of tracer iodine, radio-diiodotyrosine and radiothyroxine appear not only in the thyroid, where they are bound in the large thyroglobulin molecules, as shown previously, but also in the blood plasma, as demonstrated by Morton *et al.* (1941, 1942). From these authors' data, it was calculated that soon after radio-iodine is given, the proportion of labeled diiodotyrosine to thyroxine is similar in both the blood and the thyroid gland. This result implies that there is not a preferential secretion of either one of these two compounds, but that both are simultaneously released from the thyroid to the blood, in the same proportion as they exist in thyroglobulin. The question that arises is how this release takes place.

Formerly, it was thought that the "colloid" material, in which thyroglobulin is stored, reached the blood vessels by infiltration between the thyroid cells (Zimmermann, 1898; Klein, 1931). This concept, even in its modern form (Williams, 1937), has been more or less abandoned. The theories based on the assumption that thyroid cells secreted alternately toward the colloid and toward the blood vessels (Cowdry, 1922; Ma, 1925; Feyel and Varangot, 1937, 1938), and the theories of direct secretion into the blood vessels (Bensley, 1916), are not in keeping with other data. The polarity of the thyroid cell in the direction of the colloid, as suggested by the presence of terminal bars, centrosome, colloid globules and Golgi apparatus on the side of the nucleus facing the colloid, is proved by autographic studies showing the rapid deposition of tracer radio-iodine in the colloid of thyroid follicles soon after injection (section III). These experiments showed further that the colloid contains the thyroglobulin from which diiodotyrosine and thyroxine are to be released into the blood.

De Robertis investigated the colloid extracted from the follicles of the rat thyroid and discovered in it the presence of a proteolytic enzyme which was active under physiological conditions (1941). This result was confirmed, using more accurate chemical methods (Dziemian, 1943; De Robertis and Nowinski, 1946). Furthermore, the amount of proteolytic enzyme was found to be greater in the actively secreting thyroids of animals treated with thyrotrophic hormone than in normal controls. The proteolysis of the thyroglobulin in the colloid provides the mechanism required to obtain a release of diiodotyrosine and thyroxine into the blood in the same proportion as is found in the gland. On the other hand, the breaking down of the large thyroglobulin molecule into small amino acid fragments would enable their diffusion out of the follicle.

It may be possible to relate the histological appearance of the colloid to its proteolytic properties. It is known that the use of Mallory and Masson trichrome stains in histological sections of the thyroid enable the identification of basophilic,[3] acidophilic and mixed follicles. In normal animals, 30 minutes after administration, tracer radio-iodine was found in the basophilic, and to a lesser extent in the acidophilic colloid. Twenty-four hours after the injection, radio-iodine predominated in the acidophilic follicle. This suggested that the material in basophilic follicles is rapidly renewed, while in acidophilic follicles the turnover is slower. Indeed, basophilia predominates in thyroids induced to secrete actively by treatment with hypophyseal extract, while acidophilia was characteristic of the resting glands in hypophysectomized animals (Leblond, 1944).

On the other hand, treatment of acidophilic colloid *in vitro* with the proteolytic enzyme, trypsin, converted acidophilic into basophilic colloid (unpublished results). This finding associates the presence of a proteolytic enzyme with basophilia, which, as seen above, is indicative of an active thyroid, while the scarcity of proteolytic enzyme appears to characterize the less active acidophilic follicles. In normal animals, the mixed staining of most of the follicles would indicate an intermediary stage with a limited amount of proteolytic enzyme.

It may be concluded from the above data that the thyroglobulin present in the colloid of thyroid follicles is believed to be hydrolyzed by a proteolytic enzyme system, and thus transformed into smaller molecules of peptides or amino acids capable of diffusion through the thyroid cells in order to reach the blood stream.[4]

V. THE FATE OF THE IODIZED COMPOUNDS EXCRETED BY THE THYROID

The investigation of thyroxine and diiodotyrosine present in blood and tissue offers a method of approach for the study of this problem. However, such an approach is complicated by the fact that diiodotyrosine and thyroxine appear to be synthesized outside the thyroid gland to some extent. Chapman has shown that thyroidectomized rats receiving a diet high in iodine had a higher basal metabolic rate than similar animals fed an iodine-deficient diet (1941). Morton *et al.* (1943), after administration of radio-iodine to thyroidectomized rats, were able to demonstrate the presence of both radio-diiodotyrosine and radio-thyroxine in their tissues. They concluded that conversion of iodide to complex organic compounds had taken place outside the thyroid gland. The implications of this finding are of rather fundamental importance, not only for the mechanism of thyroxine formation, but also for the understanding of the general metabolism of thyroidecto-

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[3] Dempsey (1944) and Dempsey and Singer (1946) have shown that the ribonucleic acid in the colloid is responsible for the basophilia. According to these authors, some degree of basophilic staining is present in all follicles.

[4] The presence of free thyroxine and free diiodotyrosine in thyroid tissue has been observed by Leblond and Gross (unpublished).

mized animals and of lower athyroid species. It is necessary to confirm and extend these results; for the percentage of the injected dose found by Morton *et al.* (1943) in the blood thyroxine and diiodotyrosine fractions of the thyroidectomized animal is surprisingly close to the percentage values found in the intact animal. However, a lower absolute amount of both these compounds might result from a possible depression of the blood iodide level in the thyroidectomized animal. At any rate, the possibility of an extrathyroidal origin of diiodotyrosine and thyroxine must be kept in mind in any investigation of the blood iodine fractions.

The tracing of radio-iodine fractions in the blood of normal animals was carried out by Chaikoff and his co-workers (Perlman *et al.*, 1941; Morton *et al.*, 1941; Morton *et al.*, 1942). Within 2 hours after injection of labeled iodine, the amount of labeled diiodotyrosine in the blood is greater than that of thyroxine. Indeed, as mentioned in the previous section, the proportion of radio-diiodotyrosine to radio-thyroxine is similar in thyroid and plasma at that time interval. Later on, the amount of radio-thyroxine in the blood increased well over that of radio-diiodotyrosine. In the meantime, the amount of labeled diiodotyrosine in the thyroid remains about equal to, or greater than, that of labeled thyroxine. Since, as stated above, diiodotyrosine and thyroxine appear to be released in the same proportion as they are formed, the low values for the blood diiodotyrosine at the later intervals suggest that this substance is more rapidly broken down or excreted than thyroxine. It will be seen now that both substances are rapidly withdrawn from the blood.

1. The Fate of Diiodotyrosine in the Body

The metabolism of diiodotyrosine has been investigated by Oswald (1909) and Foster and Gutman (1930). These workers using enormous doses of diiodotyrosine (several g./kg. of body weight) observed that part of the dose was destroyed, while a large amount was excreted in the urine either without modification or after transformation into a new substance. Study of the fate of doses a thousand times smaller indicated that an extremely rapid destruction of diiodotyrosine to form iodide takes place in the blood (Leblond and Süe, 1941). Such a rapid destruction fits in with previous results of Foster (1934), Gutman *et al.* (1933), and Snapper and Grunbaum (1937). This rapid breakdown of diiodotyrosine accounts for the small amount of labeled diiodotyrosine observed in the blood at the later intervals after radio-iodine administration.

2. The Fate of Thyroxine in the Body

In the past, many workers have followed the fate of both thyroid preparations and synthetic thyroxine in the body by using either chemical titrations of iodine or biological assays. It must be understood that the biological activity of tissues after the administration of thyroid preparations provides a rough measure of the amount of thyroxine in them, whereas chemical titrations of iodine reveal, not only thyroxine, but other iodized compounds.

Thyroglobulin, when injected into the blood, was found to be rapidly broken down to iodide (Blum and Grutzner, 1920). Furthermore, a number of authors found that the blood iodine level returned to normal within 24 hours after administration of large doses of either a thyroid preparation (Abelin and Scheinfinkel, 1925; Veil and Sturm, 1925; Zawadowsky and Asimoff, 1927) or thyroxine (Asimoff and Estrin, 1931; Müller and v. Fellenberg, 1932; Gaebler and Strohmaier, 1942). Therefore, it is apparent that neither thyroglobulin, thyroxine, nor the iodide derived from their breakdown remains in the blood for a long time.

An even more rapid disappearance from the blood was obtained with nearly physiological doses (2 mg.) in man, when the rise in blood iodine disappeared within 2 hours after injection (Boe and Elmer, 1931). Using radioactive thyroxine in either pharmacological—mg.—or physiological —γ— doses in the rat, the decrease in the amount remaining in the plasma was quite rapid, since 2 hours after administration, there remained 1–2% of either the pharmacological or the physiological dose (Gross and Leblond, 1947).

It is interesting to note that thyroxine lost none of its activity when added to blood and incubated *in vitro* for 48 hours (Müller and v. Fellenberg, 1932), while it vanished from the blood fairly rapidly in experiments carried out *in vivo*. It may, therefore, be assumed that the blood itself does not cause the disappearance of thyroxine, but that apparently some other organ withdraws this substance from the circulation.

The *liver* was recognized early as fixing a large amount of iodine after injection of thyroglobulin (Blum and Grutzner, 1920). In fact, perfusion of the liver with a fluid containing thyroid extract resulted in a marked destruction of the thyroglobulin in this extract with formation of iodide (Blum and Grutzner, 1920). Later, it was more accurately proven that, with large doses of thyroxine, the liver takes up thyroxine from the blood and releases it into the bile (Kendall, 1919; Abelin and Scheinfinkel, 1925; Zawadowsky and Asimoff, 1927; Krayer, 1928; Asimoff *et al.*, 1931). Chemical fractionation of the bile after thyroxine injection showed the presence of both thyroxine and iodide in about equal amounts (Elmer and Luczinski, 1933; Barnes, 1932 and 1933).

In this connection, it is of interest to note that large doses of thyroxine produce a greater increase of metabolism in hepatectomized than in normal animals (Kellaway *et al.*, 1945). This observation suggested that some inactivation of the larger doses of thyroxine takes place in the liver.

In the *endocrine glands*, it has not been possible with radio-thyroxine

(Gross and Leblond) to confirm the claims of a specific fixation of thyroxine in the hypophysis (Sturm and Schneeberg, 1933; Joliot *et al.*, 1944), but a remarkable accumulation was found in the adrenals in confirmation of the results obtained by Müller and von Fellenberg (1932). Soon after the administration of radio-thyroxine little radioactivity is found in the thyroid; but later, presumably because thyroxine is broken down to iodide, more radioactivity accumulates in this gland.

Krayer (1928) and Müller and von Fellenberg (1932) described large amounts of thyroxine in the skin. Recently, it was found, however, that the concentration of radio-thyroxine was not high in the skin, although the total amount present in the whole skin of the animal is rather large (Gross and Leblond, 1947).

After the administration of thyroxine or thyroid preparations, the excretory organs may show an important uptake (Kendall, 1919; Asimoff and Estrin, 1931). The iodine in the *kidney and urine* was predominantly in the form of iodide (Veil and Sturm, 1925; Zawadowsky and Asimoff, 1927). In our experience, thyroxine may be found in the kidney, but is eliminated into the urine only in the inorganic form.

Very little work has been done on the relations between the thyroid hormone and the *gastrointestinal system.* However, with large doses of radiothyroxine, a high proportion of the injected doses was found in the stomach and the jejuno-ileum (Gross and Leblond). Most of this material comes from the bile, but some material is secreted by the intestine throughout its length. Since, on the other hand, it is known that thyroxine deposited in a ligated intestinal loop is rapidly absorbed (Schittenhelm and Eisler, 1932), it appears likely that some of the thyroxine finding its way into the intestinal lumen is resorbed and then passes through the liver. However, the fecal excretion plays an important role, since, 24 hours after injection of a large dose of thyroxine, about 75% of it may be found in the feces.

It may be concluded that the liver and gastrointestinal system play an important role in the metabolism of thyroxine.

VI. Influence of Various Factors on Iodine Metabolism

Histological examination and chemical determinations reveal wide variations in the normal human thyroid. These are probably due to the cumulative action of factors such as outside temperature, physical work, fasting, *etc.* (Elmer, 1938), any one of which may modify the iodine content as well as the size and staining characteristics of the follicle.

1. Pituitary Secretions

There is little doubt that the hypophysis, exerting its effects through the secretion of the proteic thyrotrophic hormone, is the most important factor controlling thyroid activity.

Following the pioneer investigations of Aron (1929) and Smith (1930), it is now an established fact that the thyrotrophic hormone controls the thyroid gland by inducing a mitotic proliferation and an increase in size of the cells.

Using pharmacological doses (0.5 mg. of radio-iodine/kg. of body weight), it was found that thyrotrophic hormone increased, and pituitary extirpation decreased the ability of the thyroid to accumulate iodine. It is interesting to note, however, that several months after hypophysectomy the thyroid was still able to fix radio-iodine to a much greater extent than any other tissue in the body. This indicates that the specific behavior of the thyroid toward iodine is not dependent on the hypophysis, but is an intrinsic property of thyroid tissue itself (Leblond, Süe and Chamorro, 1940; Leblond and Süe, 1941). Furthermore, by calculating the cellular volume in normal, hypophysectomized and thyrotrophin-treated animals, it was found that a rough parallel existed between the total cellular volume and the amount of radio-iodine entering the thyroid, thus again suggesting that the thyrotrophic hormone affected the size of the cell rather than its iodine-fixing ability.

On the other hand, the transformation of iodine to thyroxine in the gland seems to be directly influenced by the pituitary hormone, as shown with tracer physiological doses of radio-iodine (Morton *et al.*, 1941, 1942). In the intact animal, the formation and excretion of thyroxine was considerably enhanced by thyrotrophic hormone. In the hypophysectomized animal, because of the absence of thyrotrophic hormone, the transformation of iodine hardly proceeded beyond the formation of diiodotyrosine.

This suggests that, in addition to affecting the size of the thyroid cell, the thyrotrophic hormone may act in some way as a catalyst for the formation of thyroxine. Recently, Rawson *et al.* (1946) showed that the thyrotrophic hormone is inactivated by treatment *in vitro* with free iodine and may be reactivated by thiourea and its derivatives, the latter apparently removing the iodine fixed in the protein molecule. Rawson believes that such an inactivation might take place to some extent in the hypophysis of the normal animal. This possibility is suggested by the finding that thiourea treatment of normal hypophyseal extract *in vitro* increases its thyrotrophic activity, even though the extract had not been pretreated with iodine. Correlating these data with the finding that thyrotrophin is incorporated into the thyroid cell and loses its activity there (Rawson, Graham and Riddell, 1943), it is interesting to speculate on the possibility of the hypophyseal hormone acting as an acceptor for the transport of the iodine ion to organic combination in thyroglobulin.

It is likely that cold (0°C.), which increases, and heat (35°C.), which decreases, radio-iodine fixation by the thyroid (Leblond *et al.*, 1944) produce their effect through the pituitary gland, since cold does not stimulate the thyroid of the hypophysectomized animal as it does in normals (Uotila, 1939). It is probable that most other factors affecting iodine metabolism also act through the pituitary.

2. Iodine Level in Blood

The iodine level of the blood influences the thyroid by both a direct effect on the gland and indirectly through the hypophysis.

In the case of *overdosage* with iodine, it has been seen in Section I that the thyroid becomes saturated and thus prevents the entry of iodine to some extent. Furthermore, a "saturated" thyroid contains more iodide than normally. It has been shown that an excess of iodide slows down thyroxine synthesis by a direct effect on the thyroid, as demonstrated in surviving thyroid slices by a decrease in thyroxine formation after addition of iodide (Morton *et al.*, 1944). The slowing down of thyroxine synthesis was apparent in dogs fed a high iodine diet (Fig. 3), in which the rise in the specific activity of thyroxine seems more than twice as slow as in normals. On the other hand, an excess of iodide may possibly slow down the release of thyroxine since there may be a reduction in proteolytic enzyme after iodine treatment *in vivo* (De Robertis, 1941, 1946a; Dziemian, 1943). At any rate, all these results explain why pharmacological doses of iodine produce a drop in metabolism, a fact which is used clinically in the treatment of hyperthyroid patients (Means, 1937).

In the case of *iodine deficiency*, the gland has an increased avidity for iodine, as shown by radioactive experiments (Leblond and Mann, 1942). Since the blood and thyroid become depleted of iodine, there is no local inhibition in the thyroid, reducing iodine entry. Furthermore, a deficiency of thyroid hormone results from the deficiency of its necessary component, iodine, and gradually induces a situation comparable to that of thyroid extirpation. In fact the hypophyses of iodine-deficient rats, like those of thyroidectomized rats, exhibit the so-called thyroidectomy cells, a feature which is taken to indicate an increased secretion of thyrotrophic hormone. Indeed, histological signs of stimulation, similar to those obtained after thyrotrophic hormone administration, are found in the thyroids of iodine-deficient rats. This thyrotrophic stimulation accentuates the avidity of the deficient gland for iodine. Hence, the marked fixation of radio-iodine.

3. Anti-Thyroid Drugs

It is well known that thiourea, sulfonamides and related substances produce a functional thyroidectomy in experimental animals, as evidenced by their low basal metabolic rate (MacKenzie and MacKenzie, 1943; Astwood *et al.*, 1943) and heart rate (Leblond and Hoff, 1944). These results suggested the absence of thyroxine secretion by the thyroids of such animals, although the histology of the thyroids indicated an intense stimulation from the animals' own thyrotrophic hormone.

The state of the thyroid in such cases was investigated with radio-iodine. *In vitro*, the addition of thiourea and related thiol compounds (Franklin *et al.*, 1944) or of sulfonamides (Franklin and Chaikoff, 1944) to thyroid slices prevented the usual transformation of radio-iodide to radio-thyroxine; the radio-iodide, however, was fixed into the thyroid tissue. *In vivo*, the radio-iodide, injected into animals treated with these various compounds, is taken up to a smaller extent by the thyroid and is not transformed into bound iodine (Keston *et al.*, 1944; Couceiro *et al.*, 1944; Larson *et al.*, 1945; VanderLaan and Bissell, 1946). Recently, it was shown that the thiouracil effect is different for pharmacological and physiological doses of iodine. This drug inhibited the entry of iodine in the thyroid when physiological doses were administered. However, when larger amounts of iodine were given, the iodine accumulated in the thyroid in proportion to the injected dose, but remained there as iodide (McGinty and Sharp, 1946).

The mechanism of the inhibition of thyroxine formation was examined by Calvo and Goemine (1946) who showed that thiourea-type drugs prevent the iodination of casein by free iodine *in vitro;* they hinted that, *in vivo*, these drugs might compete with the oxidized form of iodine which assumedly occurs in the thyroid, and thus prevent the formation of thyroxine. A similar mechanism was suggested by Miller *et al.* (1945). According to De Robertis and Grasso (1946) this hypothesis accounts for the action of sulfonamides, but thiourea derivatives, on the other hand are believed to directly inactivate the oxidative enzyme system in the thyroid.

Another type of anti-thyroid drug, thiocyanate, seems to act exclusively by preventing the entry of iodide into the gland, as shown with radio-iodine *in vivo* (Rawson *et al.*, 1944) and *in vitro* (Franklin *et al.*, 1944).

4. *Pathology*

There is only meager information in the literature regarding the behavior of iodine in the pathological thyroid gland. So far, it seems that myxedematous or hyperplastic thyroids show functional deviations similar to those observed in hypophysectomy or thyrotrophic stimulation, respectively. For instance, a study by Hamilton *et al.* (1943) carried out with children suffering from *hypothyroidism* revealed that those cases in which there was no palpable enlargement of the gland concentrated little radio-iodine in their thyroid. In two children, however, with enlarged thyroids, the uptake of radio-iodine was comparable to that of normal glands, but the autographs showed the frequent presence of iodine in the follicular epithelium, an observation which might be interpreted as a sign of reduced activity.

Little is known of what occurs in *hyperthyroidism*. The entry of iodine and its transformations are rapid (Hamilton and Soley, 1940; Hertz *et al.*, 1942).

In *adenomas* of the thyroid, a study of 5 cases established that a considerably smaller amount of radio-iodine was fixed in the adenomatous tissue than in the surrounding normal thyroid tissue. The difference was greatest in the so-called fetal adenomas and smallest in colloid nodules. However, the rate of iodine turnover was rather similar in the nodules and the normal thyroid tissue (Leblond *et al.*, 1946). Since the supply of thyrotrophic hormone must have been similar in the nodules and the normal thyroid tissue, the different iodine fixation must be attributed to local factors, namely, a decrease in the thyroid-like property of the adenomatous tissue.

In the case of *thyroid carcinomata*, the results varied a great deal according to the structure of the tumor. The published reports suggest that the less differentiated the tumors are, the less radio-iodine they fix, and *vice versa*. Hamilton *et al.* (1940) reported 2 cases that did not fix radio-iodine. Several other reports, referring to adenocarcinomata of low malignancy, have shown the entry of radio-iodine in both tumors and metastases (Keston *et al.*, 1942; Leiter *et al.*, 1946; Seidlin *et al.*, 1946). These authors observed that the hyperthyroidism and the spread of the malignant tissue may be checked by the therapeutic use of radio-iodine with high activity.

VII. Conclusions

A minute amount of iodide, approximately 0.1 mg., is absorbed daily by man in his food. Since it is not possible to investigate the fate of such small amounts of iodine by ordinary chemical methods, research work on the subject was limited until the advent of radioactive isotopes. With radioactive iodine, it is easy to trace doses as small as a fraction of a microgram and thus obtain an insight into the physiological behavior of iodine. It has been shown that, 24–48 hours after the administration of such a *minute dose* of radio-iodine, 50% may be found in the thyroid. The high efficiency revealed by this figure is further enhanced by the existence of the following mechanism for the conservation of the iodine supply. The iodized products, diiodotyrosine and thyroxine, synthesized by the gland are excreted into the blood, broken down to iodide further on in the body, returned as such to the thyroid, resynthesized and reexcreted. The repetition of this cycle over and over again insures an extremely efficient use of iodine.

The available evidence indicates that the iodide entering the thyroid is swiftly fixed to the tyrosine groups of protein molecules (thyroglobulin). The resulting diiodotyrosine groups are gradually coupled to yield thyroxine. These two reactions appear to be catalyzed by the action of an enzyme system in which a peroxidase seems to play the major role.

In contrast to what occurs under physiological conditions, a different situation is obtained with *large* (pharmacological) *doses* of iodine, such as are used in medical practice. Such doses are not used efficiently, since, within a few minutes after administration, the thyroid becomes "saturated" and does not accept more iodine. Only 0.5% of the dose may enter the gland. Chemical studies indicate that the entering iodine is stored as iodide in the gland. The excess of iodide thus obtained inhibits thyroxine synthesis and thus causes the well-known depressing action of iodine on basal metabolism.

The *excretion* of thyroxine and diiodotyrosine from the thyroid gland is best explained as an enzymatic proteolysis of thyroglobulin, which takes place in the colloid of the thyroid follicle. The amino acid fragments resulting from such proteolysis readily diffuse through the thyroid cells into the blood.

The thyroxine which enters the circulation is rapidly withdrawn from the blood, especially by deposition of more than half of it into the gastrointestinal tract and liver. The stomach and duodenum excrete large amounts of thyroxine; some may be resorbed lower down in the small and large intestine, but most of it is excreted in the feces.

A balance is established between the amount of thyroxine released by the thyroid and the amount of thyrotrophic hormone released by the hypophysis, resulting in what is the normal level of thyroid activity and iodine turnover of each individual. This equilibrium is rather stable and easily adjusted to changes in outside temperature, physiological variations, *etc.*

The control of the thyroid by the *thyrotrophic hormone* is shown by radio-iodine to consist of an increase in cell size and an accentuation of the synthesis of thyroxine from diiodotyrosine rather than a direct action on other phases of iodine metabolism.

While most factors involving the thyroid act through modifying the release of thyrotrophic hormone, a series of drugs known as *anti-thyroid drugs* act directly on the thyroid tissue. The thiocyanate salts prevent the entry of iodine into the gland. Thiourea derivatives and sulfonamides inhibit the transformation of iodine into thyroxine by inhibiting in various ways the oxidative system which is responsible for this transformation.

The use of radio-iodine in *pathological conditions* of the thyroid indicates that hyperplastic thyroids behave as if there were an excess of thyrotrophic hormones, while myxedematous thyroids react as if there were a deficiency of this hormone. The adenomas of the thyroid fix less iodine than normal thyroid tissue, but this decrease is marked only in the less differentiated types, *e.g.*, fetal adenomas. In general, the non-differentiated malignant tumors of the thyroid do not fix radio-iodine, but the differentiated adenocarcinoma is able to incorporate this iodine.

It is obvious that a tremendous improvement in thyroid knowledge resulted from the use of radio-iodine. The ripening of this knowledge into clinical applications is also apparent, since the therapeutic use of highly active radio-iodine may check the toxic and even the malignant thyroid.

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The Effects of the Atomic Bomb Irradiation on the Japanese

By JOE W. HOWLAND AND STAFFORD L. WARREN

CONTENTS

I. INTRODUCTION

With the atomic bombing of Hiroshima and Nagasaki, what might be called the first, and, it is hoped, the last observations on the effect of acute whole body irradiation on the human subject were made, and on a mass scale. Prior to that time certain experiments had been conducted on a chronic exposure basis with dosages administered from apparatus of relatively low capacity and over an extended period of time. Sublethal amounts were used. Accordingly, it becomes essential to report the following findings in as brief a manner as possible, with statements as to the method of procurement of the data as well as its relative validity.

The sudden widespread physical destruction of the cities, the dislocation of practically all survivors, the death or complete disorganization of even the surviving authorities and medical personnel, together with the loss of all records, made an accurate medical and casualty survey almost impossible. Survey personnel were, however, mobilized rather quickly from the surrounding areas by the Japanese, and sufficient data were accumulated to enable a general analysis of the catastrophe. It must be stated that such studies are incomplete but undoubtedly characterize the typical picture of the clinical and pathological findings resulting from whole body irradiation by atomic explosion. The data lack objective information on the first week following the explosion, during which time no medical survey groups from the surrounding areas were in residence. The material gained in the next five to six weeks is almost completely furnished by the reports of small and uncoordinated Japanese study parties from various parts of the empire, or by casualties transported from the stricken areas to undamaged hospitals for study. The information obtained during the next four weeks was obtained by the Manhattan District Medical Survey group who pooled their findings with that body detailed from the Army Surgeon General's office, which extended the study for the next three months. The detailed findings are to appear in the official joint report of these two groups. The information pertaining to the one year follow-up was obtained by a Manhattan District survey party in September, 1946.

The information contained in this summary is presented in brief form and deals (1) with the damaging physical agents produced by atomic explosion, (2) with the type of injury produced by these agents, (3) with a brief description of the physiological and pathological changes following whole body irradiation in animals for comparison, (4) with a survey of the clinical picture produced, and, finally, (5) with a review of the general pathological findings.

In a short review of this character it is obvious that many details and specific tabulations must be omitted, yet sufficient is given to cover the overall picture in general terms. Most of the percentages and calculations are given in relative amounts only and must be interpreted as qualitative only. Disputed or debatable facts are entirely omitted. Although much information of varying degrees of validity has been published during the past year no review of the relative merits of such articles will be included.

II. NATURE OF THE PHYSICAL AGENTS

The chain reaction of atomic explosion produces a spectral range of energies similar to those emanated by a small sun in the absence of a protective atmosphere. Ample evidences of radiation were produced, ranging from the extremes of the long heat bands beyond the infrared through the visible spectrum down to the short penetrating wavelengths of the γ-rays.

With such a spectral distribution of energies corresponding effects were observed on the human subject. Flash burns were noted resulting from the long heat rays and infrared. The black hair and dark clothing were particularly susceptible. Pigmentation followed a primary erythema associated with intense infrared and ultraviolet irradiation. Marked biological effects were observed as due to the intense penetrating short radiations including the γ-rays and, in those individuals near the explosion center, possibly neutrons. In addition to all of these, the powerful cyclonic blast wave produced by the explosion, followed by an equally strong succession wave, produced traumatic injuries involving all parts of the body. The nature of these injuries will be discussed in detail in a later section.

The relative natures of those physical agents which produce the burns of various types and the blast injuries are comparable with those arising from any high explosive detonation. In general, they differ here only in their geographic extent and widespread severity. The possible natures of the penetrating radiations demand, however, some further discussion, for heretofore they have not been seen in their entirety except in experimental animals.

Three possible effects from radiation and radioactivity occur with the explosion of an atomic bomb. Evidences of these effects will be discussed in further detail.

1. Evidence of the liberation of high energy γ-rays and neutrons by the nearly instantaneous chain reaction (acting in a short interval of time) are definite. The total amount of penetrating energy released can be stated relatively to be in the order of tons of radium equivalent (measured in roentgens delivered). With such energies available, it is obvious that biological effects will be produced even when detonation occurred at a considerable elevation over the area (as in the case of both Japanese cities).

2. Secondary effects resulting from neutron-induced reactions with matter on the ground beneath the bomb were observed by both Japanese and American investigators. Some neutron-induced radiophosphorus, radiosulphur and radiocalcium, and traces of other radioactive materials were found in areas around the ground zero-point of the explosion and along the fall-out trail for a considerable distance from these two cities. Some activity was likewise found in the dental fillings of casualties in that area. The amount of induced radioactivity was, however, of such small amount and of such type (short lived β emitters for the most part) that no known biological damages were produced in individuals (Japanese) entering any of the slightly contaminated areas immediately, or at any time, after the catastrophe.

3. The third potential possibility is that of the deposition of fission products formed in the chain reaction, as well as the unreacted radioactive com-

ponents of the bomb. This was not observed for the following reason. The height of the explosion and the intensity of the following updraft of the hot gases produced, carried such materials high up into the air where they were dispersed and ultimately scattered over a wide area of the globe in almost undetectable amounts.

From the above discussion it is obvious that the biological reactions observed in the humans were due to the burns of various types, trauma from the blast, and, most important, to the direct action of the γ-rays, and possibly neutrons, released at the time of the explosion.

FIG. 1. Epilation.

III. TYPES OF INJURY (GENERAL)

The injuries produced were of mechanical, thermal and radiation types. In their general features, the types of injury were similar in both cities. For clarity in the interpretation of those injuries due solely to radiation, brief statements will be made concerning the complicating mechanical and thermal injuries.

The mechanical injuries were caused either directly by blast or indirectly by falling debris and materials thrown by the blast. Blast injury, similar to that found following high explosions, was almost unrecognized. Ruptured eardrums were observed in less than 1% of individuals in each city, although in comparable towns bombed by high explosives, such ruptures were very common. No lesions in the lungs or the intestines and viscera could be ascribed to the blast, probably because such casualties died within the first day and were either consumed by fire or overlooked in the general confusion. Lacerations, cuts, *etc.*, were produced largely by flying glass and fragments of buildings. These injuries were exceedingly frequent.

FIG. 2. Epilation.

FIG. 3. Petechiae.

The burns were largely of "flash" type and resulted from the tremendous radiant heat produced by the bomb, probably of only a fraction of a second's duration. The burns observed involved only that side or part of the body exposed directly to the path of the heat rays and were consequently sharply outlined. Within 1500 meter zone, little pigmentation was found, except on the periphery of the burns. In areas beyond that distance, pigmentation from heating of the skin was quite prominent. Clothes, particularly when light in color, offered considerable protection by reflection at distances beyond 1500 meters. Flash burns were observed at distances up to 4500 meters.

The effects of the ionizing radiation resemble those previously produced by X-rays or γ-rays in animals and man, and were similar in both cities. Characteristic lesions were noted in the skin, gastrointestinal tract, gonads, lymphatic system, and bone marrow.

Most of the observations, especially during the initial acute period, were made by the Japanese, most of whom were unfamiliar with the nature of the biological damage from whole body irradiation. Accordingly, gaps exist in the data which prevent a detailed chronological analysis as to the progression of the clinical and pathological picture following this type of insult. Hence, a brief summary of the clinical and pathological picture of the sequence of events following whole body irradiation of animals (rats and dogs) by high energy X-rays is included. Clinically and pathologically, the effects produced by such X-rays, the γ-rays and neutrons by atomic detonation, or from emission by radioactive materials, are identical.

FIG. 4. Gingivitis.

IV. CLINICAL AND PATHOLOGICAL EFFECTS OF WHOLE BODY IRRADIATION IN ANIMALS

1. *Clinical Effects*

After normal animals are exposed to a lethal dose of X-rays, there is a latent period of 1–2 hours before the development of symptoms. Usually, the initial finding is a preliminary period of prostration associated with a primary fall in blood pressure. Some salivation, diarrhea, nausea, and vomiting may follow at this time. The diarrhea is seldom of a bloody nature. The peripheral blood may show an elevation in the circulating polymorphonuclear leucocytes up to 20–30% over control values. This is associated with a sharp decline in the circulating lymphocytes to as much as $\frac{1}{10}$ of the normal values. The above symptoms progress through the second day with a continuation of the accelerated heart rate, lowered blood pressure, and occasional vomiting. Very little food is taken and some weight is lost.


Fig. 5. Flash burns.

On the third day (approximate) the appetite returns and some weight is regained. The polymorphonuclear leucocyte count has now fallen to a new low level, amounting at times to as much as 0.2–0.1 of the normal value. The lymphocyte depression persists. This general state continues up to approximately the tenth day, at which time an abrupt rise in the temperature of the animal occurs. There is a concentration of the blood with a reduction in the plasma volume. Further concentration occurs by the onset of diarrhea which is often of bloody nature. Copious salivation may be noted. Hemorrhages occur in the skin, mucosal areas and other body organs, and are associated with a decrease in circulating thrombocytes. Signs of sepsis and localized infection then appear. Death occurs, with the onset of shock, usually between the tenth and twentieth day after irradiation. Animals surviving this period usually have a fair chance of recovery.

FIG. 6. Flash burns.

2. *Pathological Effects*

As in the human, pathological changes are noted in those tissues which are most sensitive to the action of irradiation. The sensitive tissues include the lymphatic system, bone marrow, gastrointestinal canal, and gonads. Little or no damage is observed in the pancreas, kidney, lung, heart, adrenal, nervous tissue, skin, or muscle. This latter statement refers to direct X-ray damage and not the secondary effects of bone marrow depression, such as hemorrhage and infection. The effects on the various sensitive tissues will be discussed briefly.

FIG. 7. Flash burns.

Following a killing dose of X-rays, the bone marrow shows conspicuous signs of degeneration of a progressive nature. The changes are first seen in the myelocyte series followed shortly thereafter by similar changes in the red blood cell series. This occurs within several days of the insult. The degenerative process is progressive through the first two weeks, shorter or longer times being noted which vary with the animal. At the end of this time the marrow appears to be a reticulum with occasional islets of cellular debris. At first, there is a definite engorgement of the marrow with mature erythrocytes, but this later disappears. A period of marrow depression is noted, during which time it remains relatively acellular. This is followed (particularly in animals which survive) by the reappearance of small islands of cells in the marrow reticulum. In general, erythropoesis is initiated before myelopoesis. This reformation may occur as early as the fifth to seventh

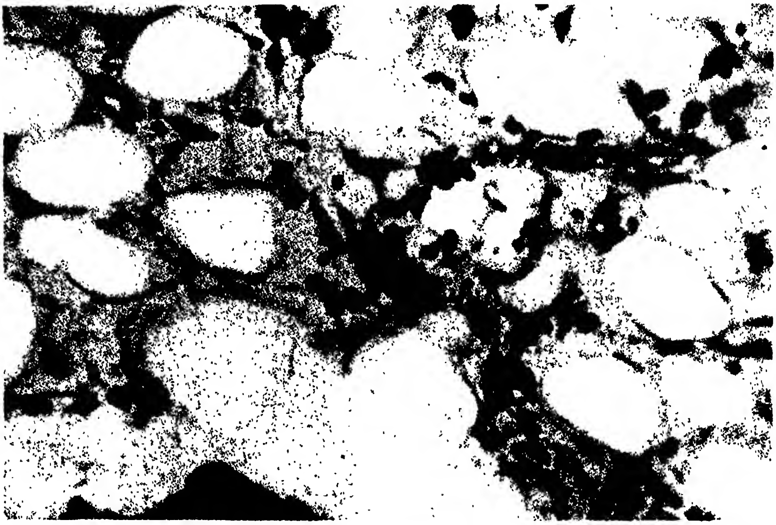


Fig. 8. Femoral bone marrow 16 days after irradiation (Hiroshima).

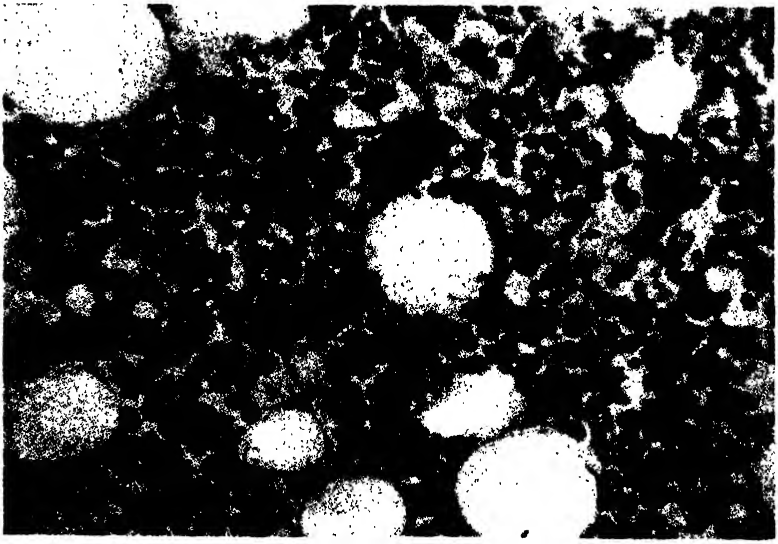


Fig. 9. Gelatinous femoral bone marrow 60 days after irradiation (Hiroshima).

day, or may be considerably later, again varying with the species and the individual animal studied. The new cells appear to be relatively normal, although peripheral blood smears may show an increase in monocyte-like forms. Sufficient regeneration, however, to cause definite changes in the peripheral counts does not occur until approximately the third to fourth week following the insult. The marrow at this time shows a gelatinous appearance due to the tremendous numbers of formative white cells in various stages. The regenerative period continues usually up to the point (in surviving animals) where marrow counts will reveal more than twice the normal number of cellular elements. Following this increase it reverts to the normal state.

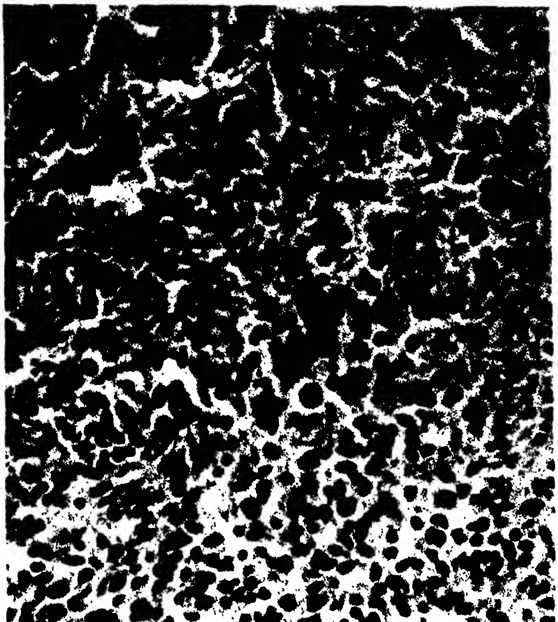


FIG. 10. Lymph node (mesenteric) 28 days after irradiation (Hiroshima).

The lymphatic system (spleen and lymph nodes) is composed of that group of cells in the entire body which is most sensitive to irradiation. The degenerative changes following irradiation to both spleen and lymph nodes appear in a matter of hours and are of such a rapidly progressing nature that, within 6–10 days, the lymphatic elements have almost entirely disappeared leaving behind a reticulum filled with cellular debris. This is associated with a very early fall in the circulating lymphocytes of the blood to an extremely low level. The reparative process again follows a period of depression, and, like the bone marrow, consists in the establishment of new islands of cells around which follicles are formed. The reparative process is rather more rapid than that noted in the marrow, with a return to normal appearance in times as early as 4–5 weeks.

The effects of irradiation on the epithelium of the gastrointestinal canal are well known as are those physiological phenomena associated with it. The damage appears to be proportional to the total amount of irradiation and occurs rapidly in a matter of hours, developing to a maximum extent in a period of 2–3 days. The degeneration is in the form of a hyaline necrosis. Reparative processes are quite rapid in their onset and are fully in progress within a few days after the initial damage. Repair proceeds to complete healing in surviving animals. Those which die may show ulceration and hemorrhage. Certain animals show hemorrhage and ulceration into the bowel at the 2–3 week period, this, however, being associated with bone marrow depression.

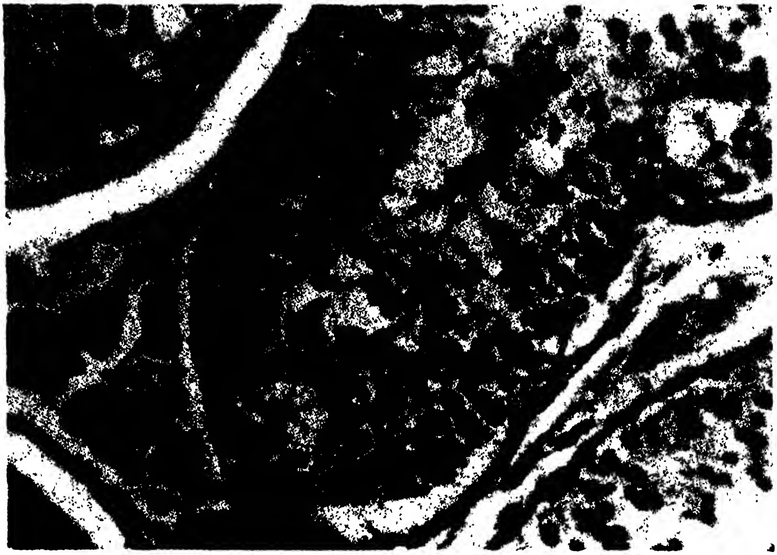


FIG. 11. Testis 23 days after irradiation (Nagasaki).

Of the gonadal tissues, the changes in the testis are perhaps best known. Following exposure there is a gradual depression in sperm formation. The first cells involved are the primary sperm cells adjacent to the walls of the tubule. These become swollen, lose their identity and disappear, followed, in turn, by the spermatogonia, spermatids, *etc.* The sperm disappear by a process of continuous maturation, so that for 20–30 days there is a production of mature sperm in steadily decreasing amounts. The Sertoli cells and stroma remain as the only remnants of the original active tissue. Regeneration occurs in reverse order by the establishing of the primary cell followed in turn by the maturation stages. Changes in the ovary are less well understood and consist mainly in the absence of developing follicles in the presence of primary follicles.

V. Clinical Manifestations

The clinical appearance of the fatal cases of radiation divides itself into those showing very severe injury, those showing severe injury, and those showing mild injury. The first group usually had a fatal outcome within the first two weeks following the bombing. The second group within the third to sixth week, and the third group after the sixth week. The clinical, as well as the pathological, picture as observed in irradiated individuals was drawn as nearly as possible from those cases uncomplicated by burns or mechanical injuries. The symptomatology in these three types is shown in the accompanying tables.

TABLE I

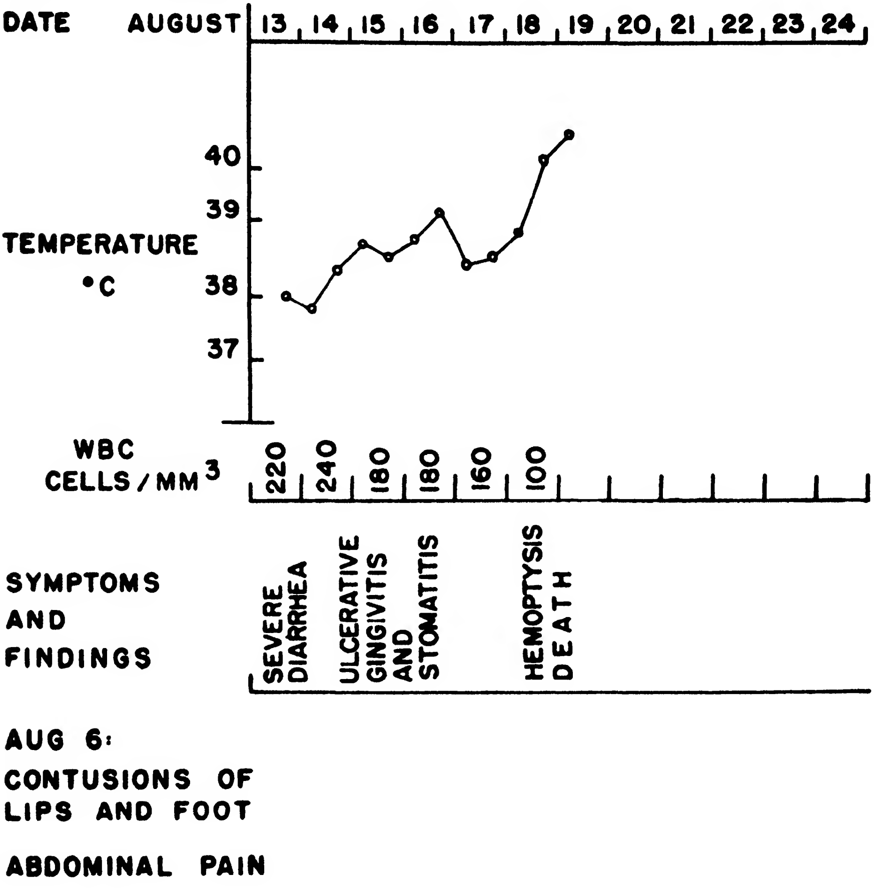
Most Severe Cases

Day	Symptoms
1	Nausea and vomiting after 1–2 hours
2–4	No definite symptoms (latent period)
5–9	Bloody diarrhea Vomiting Oropharyngeal lesions Fever Rapid emaciation Death

Mortality—probably 100%.

The very severe cases showed definite evidence of injury to the skin, gastrointestinal tract, lymphoid tissue, gonads, and bone marrow. A tabular representation of symptoms is given in Table I. Sufficient time did not elapse for the development of epilation. The thrombocytopenic purpura associated with the severe cases was rarely seen except in an occasional case. Patients complained of nausea and vomiting on the day of the bombing and then, almost immediately, developed progressive fever, anorexia, severe diarrhea, and thirst. Death usually occurred within the first two weeks.

Leucopenia was noted in most patients by the fifth day. Mortality rate for such individuals was probably 100%. (Illustration of the progress of a typical case is shown in Chart I.)



27 YEAR OLD FEMALE HOUSEWIFE. AT TIME OF BOMBING OF HIROSHIMA WAS 800 METERS S.W. OF GROUND ZERO INDOORS.

DIAGNOSES: RADIATION INJURY, SEVERE
CONTUSIONS, MILD

CHART I

In the severe cases observed during the third to sixth weeks after the bombing the following characteristic symptoms due to radiation occurred.

Table II gives a tabular representation. Nausea and vomiting were frequent on or about the day of the bombing. This was followed by a latent period of from a few days to as long as four weeks, at the end of which time epilation, thrombocytopenic purpura or inflammatory lesions of the mucous membrane of mouth and throat developed. Fever and diarrhea occurred with the development of the latter two symptoms. Examination of the blood at the time when the symptoms were most marked showed an extreme leucopenia, thrombopenia, and progressive anemia. Hemorrhagic and necrotizing lesions are noted during this period and resemble those seen in aplastic anemia and agranulocytosis. The cause of death appeared in practically all instances to be directly ascribable to the hypoplasia of the bone marrow and concurrent infection of various types, including pneumonia, dysentery, aphthous stomatitis, and the like. Hemorrhage from the bowel with profound blood loss undoubtedly contributed to an early death in many individuals. Alopecia (epilation) was noted as early as the 11–14th day. Chart II is representative of a typical case.

TABLE II

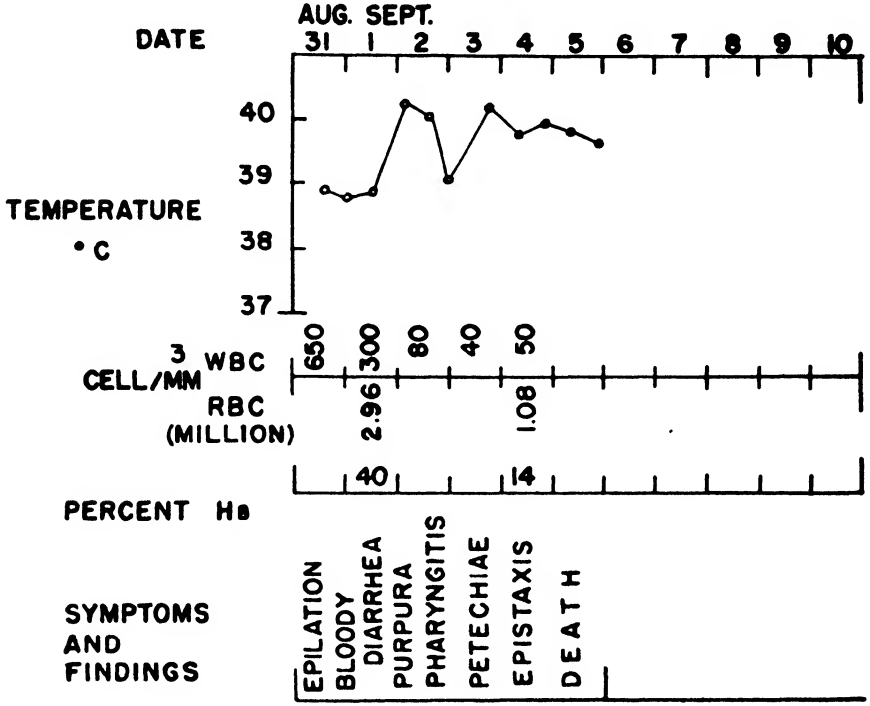
Moderately Severe Cases

Day	Symptoms
1	Nausea and vomiting after 1–2 hours
2–11	No definite symptoms (latent period)
11–14	Epilation progressing until death
18–30	Anorexia and general malaise Fever Progressive stomatitis, necrotic and hemorrhagic gingivitis Pallor Petechiae, bloody diarrhea, epistaxis and hematemesis Rapid emaciation Death

Mortality—approximately 50%.

Observations over the first 3 months indicated that approximately 50% of the individuals showing all or several of the above symptoms after four weeks survived. Few of those showing the leucopenia and epilation after six weeks died. Recovery was invariably associated with the return of the leucocyte count to normal levels. As noted previously, the reappearance of the red blood cells and resulting correction of anemia was delayed beyond this point.

In the mild cases, certain patients showed some or all of the above symptoms but to a considerably lesser extent. Table III illustrates the symptom complex of the individuals. Death was usually associated with some com-



AUG 6:
UNCONSCIOUS 3 HRS.
MULTIPLE LACERATIONS

16 YEAR OLD FEMALE. AT TIME OF BOMBING OF HIROSHIMA WAS 800 METERS SOUTHWEST OF GROUND ZERO OUTDOORS.

DIAGNOSES: RADIATION INJURY, SEVERE.
CEREBRAL CONCUSSION
LACERATIONS, MULTIPLE

CHART II

plicating illness. Following a period of 2–3 months, most individuals with mild symptoms recovered completely in so far as clinical data would indicate. However, others maintained a persistent debility even three months after the bombing had occurred. There is some evidence that a considerable number of the mild cases have died within the year but the data on this are poor.

TABLE III

Mild Cases

Day	Symptoms
1–18	No definite symptoms (latent period)
19–60 plus	Epilation Anorexia and malaise Sore throat Pallor (anemia) Petechiae Diarrhea Moderate emaciation

Recovery unless complicated by other disease.

Prognosis

a. The closer the individual was to the center and the less protected by heavy construction in the form of concrete, steel, *etc.*, the more severe the damage.

b. The speed at which the symptoms appeared following irradiation relates directly to the severity of the effect and its outcome. In those individuals in which a definite prolonged latent period preceded the first appearance of symptoms, the prognosis of recovery was quite good.

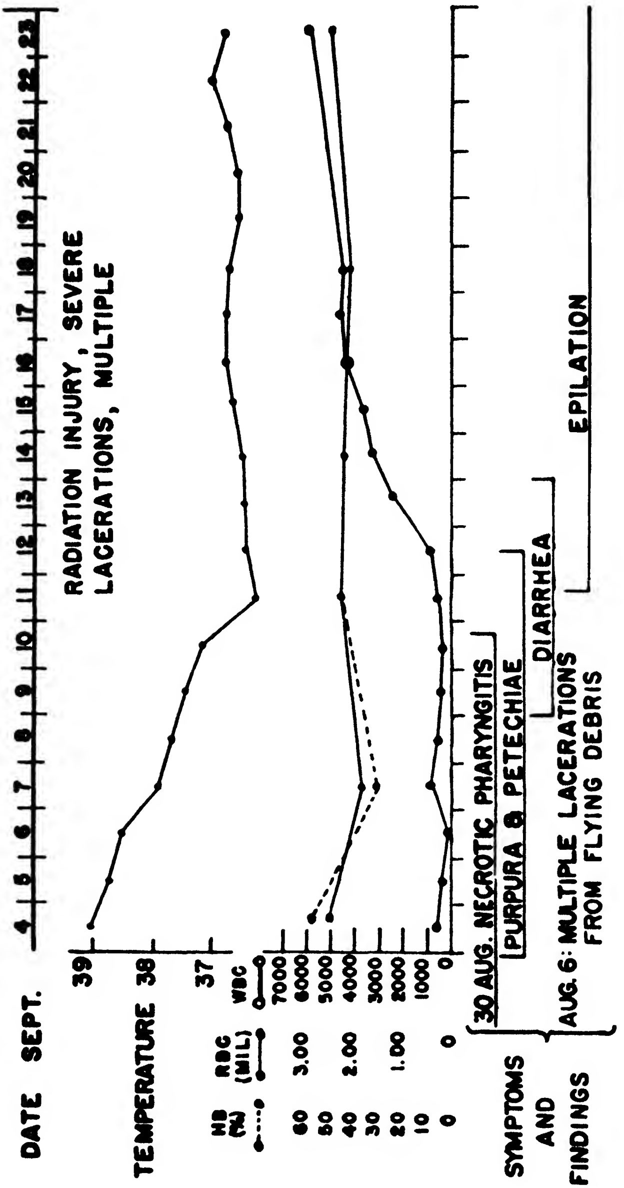
c. Those patients demonstrating all the symptoms of irradiation damage, namely, epilation, bleeding, and inflammation of the mouth and pharynx, exhibited less chance of survival.

d. Laboratory information that showed a leucocyte count of less than 1000/mm.^{3} and certainly less than 500 at any time within the first 6–8 weeks after exposure, carried a very poor prognosis.

Effects of the Atomic Bomb—One Year Later

In *general* the victims of the atomic bombs were in satisfactory physical condition one year later, and do not show changes which can be attributed specifically to the radiation effect, except for the few instances mentioned. Other types of health problems accounted for the greater part of their medical problems.

The most noticeable abnormality was the presence of large numbers of keloids in those burned by the bombs, approximately one-third of those burned, later developing such lesions. As far as is known the Japanese do not exhibit a characteristic tendency toward keloid formation as noted in



25 YEAR OLD MALE. AT TIME OF BOMBING OF HIROSHIMA WAS AT 700 METERS FROM GROUND ZERO INSIDE A JAPANESE TYPE BUILDING____

CHART III

the negro. Malignant degeneration of keloids was not seen, although sufficient time has not yet elapsed for this change to be manifest. Burns otherwise were well healed. A large percentage of the scars showed hyperpigmentation, expecially at the periphery. A number also showed central depigmentation in the scars. Scar contractures had occurred but were of expected types. A few ulcers were present in previously burned areas largely located at points of strain in skin contractures or in areas subject to frequent trauma.

A large percentage of women apparently developed amenorrhea following the bombing and recovered in varying periods of time. A few who previously were normal complained of amenorrhea one year after the radiation was received. No definite conclusions can be drawn from this observation.

The rate of pregnancies and births has been steadily increasing during 1946. This is thought to be related to the rehabilitation of life and remarriages among those families broken up by the bombings. No reliable information regarding the abortion rate could be obtained, but apparently no unusual incidence of abortions has occurred. Babies delivered by mothers who were victims of the bombs have been normal as far as can be detected. No abnormalities of growth in children who were in the bombed areas have been observed.

Very meager data have been obtained on follow-up studies of sperm counts. Early studies revealed a large percentage of men with relative or complete sterility. One reported study (Japanese) was made in October, 1945, with a follow-up of the same men in February, 1946. Twelve of fourteen remained sterile, one showed reduced sperm counts and only one was normal. The last study was made in May, 1946, when ten of twelve were sterile, one with reduced sperm counts and only one fertile. Eight of the men were married and six had previously had children. Many men complained of impotency after the bombing; a few still complained of this one year later.

No permanent epilation was observed except in one woman who had a small area on her forehead where the hair had not yet returned. The hair which grew in epilated areas was, in most cases, more sparsely distributed and, in some cases, more curly and of finer texture. The color was the same as before, with previously gray hair usually returning in the same position. Some claimed increased amounts of gray hair.

No definite late blood abnormalities were detected. A few records still showed some low red counts and hemoglobin values but these were inconclusive. Most differential white counts are marked by an eosinophilia, but the incidence of parasitic infections in Japanese is so high as to make this seem of little significance as an effect of the atomic bomb. Only one case of leukemia (acute monocytic leukemia) has been detected.

The information collected was obtained for the most part from the Japanese and is subject to inaccuracies and limitations arising from the lack of recent exhaustive well controlled studies. Also contributing are the many personal and domestic difficulties which the Japanese people are experiencing.

VI. PATHOLOGICAL FINDINGS

(*a*) *Bone Marrow*

The bone marrow showed marked damage to ionizing radiation. Cells were either reduced in number or completely destroyed, the damage probably varying with the intensity of the exposure and the time at which the observation was made. In the majority of cases where patients died within six weeks after the bombing, the marrow was hyperplastic. However, a few individuals showed, toward the end of the first month, areas of focal myeloid differentiation with large numbers of myelocycles. Occasional cases showed tremendous hyperplasia of the marrow in the presence of a leucopenia in the peripheral blood. This may be interpreted as evidence of maturation defect. The formative cells of both the red and white cell series were involved. The clinical effect of the reduction in white cell formation appeared almost immediately while the anemia following red cell precursor destruction required some weeks for its development. In an occasional case, observed in the terminal state two months after irradiation, almost complete disappearance of the red cell series was noted, while the regrowth to almost complete regeneration, of the white cell series, had already occurred. The regeneration of the thrombocytes in general paralleled that of the white cell series. As is observed in animals, recovering individuals usually produced an overabundance of myelopoietic tissue which later decreased to the normal resting state (Figs. 8, 9, p. 396).

(*b*) *Lymphoid Tissue*

Even as early as three days after exposure a remarkable atrophy of lymphoid tissue was noted. Mature lymphycytes had almost completely disappeared from the reticulum of the spleen and lymph nodes, leaving a network covered with pycnotic debris. This was manifested clinically by a decrease in the peripheral lymphocyte count. On or about the 5th day, typical mononuclear cells were noted in spleen and lymph nodes, in some instances resembling lymphoblasts or pleomorphic cells (like Sternberg cells). Occasionally, secondary follicles reappeared quite early in the regeneration of both spleen and lymph nodes (Fig. 10, p. 397).

(*c*) *Gonads*

As early as the fourth day definite changes were noted in the testes. A definite sloughing of the germinal epithelium from the basal membrane of the tubules was accompanied by an apparent increase in the Sertoli cells. Changes from this time on were progressive up to complete loss of the germinal elements, which were either discharged from the lumen of the tubules or became necrotic *in situ*. On or around the fifth to sixth week, some thickening of the basal membrane of the tubules was noted with a slight hyperplasia of the interstitial tissue. A definite decrease in the sperm count of patients in areas adjacent to the bomb explosion was found to be apparently a result of exposure to low dosages of radiation (Fig. 11).

Less striking changes were noted in the ovaries. Occasionally, it was possible to demonstrate primary follicles in the process of atresia, with resulting decrease in the number of developing ova. What appeared to be most constant was the absence of developing follicles in the presence of primary follicles. Those observed showed absence of corpus luteum effect, appearing solely in the resting phase. Amenorrhea was quite common in women close to the center of explosion and its frequency and duration seemed to be inversely proportional to the distance from the ground zero point. The incidence of amenorrhea in war-time Japan was noted to be quite high throughout the country and this may color the above findings.

(*d*) *Gastrointestinal Tract*

In the most heavily exposed individuals, a severe bloody diarrhea commenced soon after the bombing and persisted until death, within the period of three days to two weeks. The amount of the melena varied considerably. Even in surviving individuals diarrhea was a frequent symptom. As early as the fourth day following the bombing, marked histological changes were observed in the gastrointestinal tract, consisting of the appearance of extremely bizarre cells—some with enormous nuclei possessing coarse chromatic network and cytoplasmic abundance. Atypical mitoses were noted in other cells and tripolar figures were occasionally found. Cases dying within the first two weeks, at times showed a definitely ulcerative process with a necrotic mucosa and edematous submucosa. Great masses of bacteria were found in the necrotic tissue but no leucocyte infiltration was noted. In portions of the gastrointestinal canal lined by stratified epithelium, definite swelling of the cells were noted, involving both cytoplasm and nuclei. No leucocytes were found in this area, although occasionally plasma cells were observed.

(*e*) *Skin and Hair*

In the skin some epithelial changes were noted at the margins of ulcerative lesions in patients dying during the third week. Occasional epithelial lesions occurred resembling those of X-ray dermatitis. In Hiroshima, epilation occurred approximately two weeks following the bombing, while in Nagasaki, the maximum number of cases were noted around the twentieth day. Some cases were reported by the Japanese as occurring as early as one week, but all these were said to have died shortly thereafter. The distribution of such epilation resembled that of ordinary baldness in man, in some instances extending to the nape of the neck. A few scattered hairs were practically always noted in such areas, even when epilation was almost total. There were no sex differences. The histological process appears to be similar to that observed in ordinary loss and regrowth of hair, although some atrophy of the sebaceous glands paralleled the hair loss. Sweat glands histologically remained almost unchanged, although in extreme instances, definite signs of atrophic degeneration were noted. The epithelium of the scalp is noticeably thinner and is accompanied by hyperpigmentation of basal layers and the flattening of the skin surface. Noticeable changes of regenerative type were present within six weeks to two months of the bombing and were followed by gradual regrowth of black hair in a normal manner. The new hair seemed to be somewhat finer in texture.

VII. Summary

A brief description is given of the medical effects following the atomic explosions at Hiroshima and Nagasaki. A discussion of the immediate clinical human pathology resulting from blast and burns is given. The effects of acute whole body irradiation on the human are considered in detail as well as pertinent observations on living individuals observed one year later. The comparative clinical and pathological effects of whole body irradiation on animals are given briefly. The pathological findings on tissues sensitive to irradiation, namely, lymphatic system, bone marrow, gastrointestinal canal, and gonads, are discussed.

Nucleic Acid Metabolism

By G. HEVESY

CONTENTS

I. INTRODUCTION

Both types of nucleic acids, the ribose (pentose) and the desoxyribose type, are substances of great biological importance. Just to mention two among the numerous examples of the importance of these substances, we may point out that the substance inducing transformation of an attenuated pneumococcal type into the fully encapsulated and virulent organism was a nucleic acid of the desoxyribose type, as demonstrated by Avery and his colleagues (1944), and that the milk factor, which plays such an important part in the etiology of spontaneous mammary cancer in mice, has been found to contain ribosenucleoprotein (1). The existence of these two types of nucleic acids was recognized at an early date by the pioneer work of Miescher (1897), Kossel (1882), Hammarsten (1920), W. Jones (1920), Levene (1931), and others. It was originally thought that ribosenucleic acid was characteristic of yeast cells and plant tissues and that desoxyribosenucleic acid characterized animal cells; this idea, however, had to be abandoned later.

II. STRUCTURE OF THE NUCLEIC ACIDS

The basic constituents of nucleic acids, the nucleotides, are formed by condensation of a purine or pyrimidine base with a sugar radical and phosphoric acid. Four such nucleotides, each containing a different base, are condensed together, the resulting tetranucleotide presumably forming the unit from which the nucleic acids are formed by a process of polymerization. If the sugar radical is desoxyribose, the product is a desoxyribosenucleic acid (or thymonucleic acid, since this type of nucleic acid is strongly represented in the thymus gland); if it is ribose (pentose), the product is a ribosenucleic acid (or yeast nucleic acid, this type of nucleic acid being strongly represented in yeast).

Desoxyribosenucleic acid (desoxyribosepolynucleotide) is produced from the two purine bases, adenine and guanine, the two pyrimidine bases, cytosine and thymine, phosphoric acid and the carbohydrate D-2-desoxyribosefuranose, the bases being condensed each with one molecule of sugar and one molecule of phosphoric acid in the form of nucleotides.

Ribosenucleic acid is made up of similar nucleotide units in which the bases are adenine, guanine, cytosine, and uracil, and the carbohydrate is mainly D-ribosefuranose.

The desoxyribosepolynucleotides are highly polymerized. By measurements of viscosity and double refraction of flow Signer and his associates (1938), and by X-ray measurements Astbury and Bell (1938) and Astbury (1947), have shown that the polymerization of desoxyribosenucleotides results in the formation of long fibrous molecules. The molecular weight of these molecules is found to have values of up to 1 million (*cf.* also Caspersson, 1938).

The ribosepolynucleotides are less polymerized than the desoxyribosenucleotides. For the molecular weight of these nucleic acids isolated from yeast, values up to 10,000 were obtained by Fischer *et al.* (1941a and b; 1942), the molecular weight and degree of polymerization varying considerably according to the method of preparation.

The nucleic acids isolated from the pancreas as early as 1894 by O. Hammarsten (1894) were shown by E. Hammarsten and Jorpes (1922), Jorpes (1924), and Feulgen (1919–20), to be pentosepolynucleotides. Pentosepolynucleotides were later found in all animal tissues. The successive steps in this development are reported in detail by Davidson and Waymouth (1944a) in their review of nucleic acids and tissue growth and by Gulland (1938, 1947). An important source of pentosepolynucleotides is found in tobacco mosaic virus and ring spot virus, as shown by Loring (1939), and Stanley (1939), respectively, and in some other viruses. Cohen and Stanley (1942), by extraction with sodium chloride from heat-denaturated virus protein, obtained a highly asymmetric nucleic acid with a particle weight up to

300,000. Recently (1947) both ribosenucleic and desoxyribosenucleic acids have been obtained by Knight from purified particles of PR8 influenza virus.

Levene (1931) advanced in 1921 his theory of the tetranucleotide structure of ribose- and desoxyribosenucleic acids. This theory was based fundamentally on the fact that, under proper conditions, 4 mononucleotides, and only 4, could be isolated from nucleic acids. It was also stated by Levene and Bass (1931) that the tetranucleotide theory gives the minimum molecular weight and the nucleic acid may as well be a multiple of it. That this was the case became evident from investigations made on the ribosenucleic acid of the tobacco mosaic virus in comparison with that from yeast. Loring *et al.* (1947) offer as a working hypothesis that the composition of a particular ribosenucleic acid may not be characteristic of the organism or tissue from which it is isolated but may reflect rather the stage of growth involved. Thus the nucleic acid of an actively growing organism, such as yeast, might be expected to differ in composition from that present in the wheat seed. A review of the structure and synthesis of nucleotides was recently given by Lythgoe and Todd (1947).

A survey of the enzymatic degradation of nucleic acids is given in recent papers of Greenstein (1947), Brachet (1947), and Catcheside and Holmes (1947), while hydrolytic effect of phosphoesterase from calf intestinal mucosa is discussed by Zittle (1947) [*cf.* also Klein and Thannhauser (1933, 1934, 1935); Lehman-Echternacht (1941); Allen and Eiler (1941)].

III. NUCLEIC ACID CONTENT OF TISSUES

All tissue contains appreciable amounts of nucleic acids. Embryonic tissue contains more than adult tissue, as seen in Tables I and II, which show the results obtained by Davidson and Waymouth (1944c). In adult sheep, lymph nodes, spleen, and small intestine have the highest nucleic acid content; in the embryo, thymus, small intestine, the cortex of the kidney, and the spleen. The total nucleic acid has been estimated in 15–25 mg. portions on the basis of nucleoprotein phosphorus (NPP) determinations by the method of Javillier and Allaire (1926; 1931) and Berenblum and Chain (1938), respectively, in which lipoid P is extracted with an ethanol-chloroform mixture and the acid-insoluble P with trichloroacetic acid. The residual insoluble P, which is mainly composed of nucleic acid phosphorus, includes an unknown P fraction which is, however, low in all tissues (Davidson and Waymouth, 1944b, c; Euler and Schmidt, 1934; Schmidt and Thannhauser, 1945). The residual fraction also includes such well-defined compounds as the phosphoglucoseproteins and glucosenucleoproteins (Wells, 1925).

The mechanism of the Feulgen reaction, frequently applied in identifying desoxyribosenucleic acid, is still a matter for discussion. By comparing the observations made by different authors as to the specificity of the Feulgen test, Brachet (1947) arrives at the result that the observations are in favor of the classical opinion that the Feulgen test is specific for desoxyribosenucleic acid (*cf.* also Danielli, 1947).

Ribosenucleic acid and desoxyribosenucleic acid have been estimated by methods involving the determination of pentose and desoxypentose after extraction and precipitation of nucleic acids.

In extracting the nucleic acids from the tissue powders the use of alkali is often avoided. Most methods for the isolation of desoxyribosenucleic acid are sufficiently drastic to cause at least partial destruction of ribosenucleic acid, but a suitable reagent for the reaction of both nucleic acids has been found to be 10% NaCl (Jorpes, 1928, 1934). From the NaCl extract the nucleic acids are precipitated as lanthanum salts, which have been shown by Hammarsten *et al.* (1935) to be very insoluble. In the work of Mirsky and Pollister (1942, 1943, 1946), use is made of the remarkable differences in solubility of nucleoproteins in sodium chloride solutions of different strength (*cf.* also Gulland and associates, 1947). Mirsky and Pollister (1946) isolated a desoxyribose nucleoprotein complex with concentrated saline solutions from a great variety of cells, mainly animal but also plant and bacterial. This substance, termed chromosin, was found not to contain detectable quantities of ribosenucleic acid (*cf.* also Jenner 1944).

Davidson and Waymouth's method for extraction and purification of tissue nucleic acids has been recently modified by Euler and Hahn (1946a) with the intention of eliminating losses of ribosenucleic acid during the procedure. For the determination of ribosenucleic acids these authors make use of the coloration produced by pentoses when heated with phloroglucinol (*cf.* Tollens (1896), Salkowski (1892), Pinoff (1905)), and hydrochloric acid in the presence of acetic acid. Desoxyribosenucleic acid does not interfere with the coloring obtained, nor is the reaction disturbed by the presence of moderate amounts of proteins, histones, or protamines; pectin and pectinlike substances were however found to interfere. Ribonucleoproteins can be isolated (*cf.* Brachet 1947) with the ultracentrifuge as minute, spherical particles when tissues are extracted with dilute phosphate solutions.

Methods for the determination of desoxyribose- and ribosenucleic acids have also been proposed by Schneider (1945), Stowell (1942), and by Schmidt and Thannhauser (1945), and Hammarsten (1947). The last two mentioned methods, which have found application in tracer experiments, are described below.

Except in a few cases the ribosenucleic acid content of tissue is larger than the desoxyribosenucleic acid content. In the lung and the spleen, and especially in the thymus, desoxyribosenucleic acid preponderates. Of

TABLE I

Nucleoprotein Phosphorus (NPP) and Water Contents of Adult and Embryonic Tissues (Davidson and Waymouth, 1944c)

Sheep embryos, 9–10 weeks. Chick embryos, 16 days.

Each figure represents the mean value of several determinations from different animals.

NPP on dry-weight basis calculated from NPP on wet-weight basis and water content.

Tissue	Adult			Embryo			Ratio Embryo NPP/Adult NPP	
	Water content	NPP content (mg./100 g.)		Water content	NPP content (mg./100 g.)			
		Fresh wt.	Dry wt.		Fresh wt.	Dry wt.	Fresh tissue	Dry tissue
	Per cent			*Per cent*				
				Sheep				
Liver	69.7	70	231	82.6	103	592	1.5	2.6
Lung	79.0	88	419	89.0	84	763	1.0	1.8
Small intestine	80.3	110	559	88.4	78	673	0.7	1.2
Heart	77.7	29	129	87.0	64	492	2.2	3.8
Spleen	77.2	129	566	83.0	109	642	0.8	1.1
Kidney (cortex)	81.0	80	421	87.0	85	654	1.2	1.6
Kidney (medulla)	85.5	47	324	90.4	34	354	0.7	1.1
Brain (grey matter)	84.2	18	114	92.6	24	324	1.3	2.8
Brain (white matter)	74.2	53	206	90.5	24	253	0.5	1.2
Muscle	74.8	19	75	87.4	47	373	2.5	5.0
Cartilage	60.1	21	53	78.4	43	199	2.0	3.8
Testis	86.4	58	427	....	....	....	....	....
Ovary	81.9	101	558	....	....	....	....	....
Thyroid	75.4	25	102	80.2	78	394	3.1	3.9
Thymus*	....	....	....	84.2	170	1080	....	....
Skin	63.5	38	107	89.3	56	524	1.4	4.9
Placenta	87.8	59	484	....	....	....	....	....
Lymph node (calf)	79.6	185	925	....	....	....	....	....
				Fowl				
Heart	80.1	32	161	91.0	75	835	2.3	5.2
Liver	72.3	113	408	80.0	95	475	0.8	1.2
Brain	79.1	25	119	89.1	50	459	2.0	3.9
Nerve	71.2	59	205	....	....	....	....	....
Muscle	75.9	30	125	81.4	7.4	398	2.5	3.1
Rous sarcoma	88.2	61	516	....	....	....	....	....

* 15-week embryo.

lymphoid organs in the rat the lymph nodes have been found to have the largest nucleic acid P (residual P) content, as is seen in Fig. 1. The residual P content of the lymphoid organs changes markedly with age, the maximum

TABLE II

Analyses of Tissue Powders Remaining after Extraction of Acid-Soluble and Lipoid P, and of Lanthanum Salts Precipitated from the NaCl Extract of These Powders (Davidson and Waymouth, 1944c)

All tissues from the adult or 9–10 weeks' embryo sheep unless otherwise stated. Nucleic acid P = purine N × 0.886 (assuming purine N: P atomic ratio of 10:4). DNAP = desoxyribosenucleic acid P. RNAP = ribosenucleic acid P.

Tissue	Extracted tissue powder				P in NaCl extract as percentage of P in extracted powder	Precipitate of La salts			
	Total P	Purine N	Nucleic acid P calculated from purine content	Nucleic acid P as percentage of total P		P in La ppt. as percentage of P in NaCl extract	DNAP as percentage of P in La ppt.	RNAP as percentage of P in La ppt.	Ratio $\frac{\text{RNAP}}{\text{DNAP}}$
	mg./ 100 g.	*mg./ 100 g.*	*mg./ 100 g.*						
Adult liver	384	353	313	82	84	74	19	65	3.5
Embryo liver	1175	1241	1101	94	75	60	21	61	2.6
Adult lung	565	397	352	62	94	73	50	43	0.9
Embryo lung	1225	866	767	63	93	69	50	35	0.7
Adult gut	636	622	551	87	88	66	41	39	1.0
Embryo gut	958	782	693	72	82	83	31	35	1.1
Adult heart	219	174	154	70	56	65	19	70	3.6
Embryo heart	616	546	484	79	81	79	25	59	2.4
Adult spleen	914	974	863	95	78	67	50	27	0.5
Embryo spleen	1380	1448	1284	93	97	70	47	26	0.6
Adult kidney	442	400	355	80	74	74	29	52	1.8
Embryo kidney	923	949	840	91	79	72	49	41	0.8
Adult brain	369	215	190	52	40	67	31	65	2.1
Embryo brain	556	340	301	54	82	79	23	55	2.3
Adult muscle	147	145	129	88	55	71	19*	66	3.6*
Embryo muscle	650	572	507	78	95	70	37	53	1.4
Adult cartilage	84	85	75	89	63	40	41	....	....
Embryo cartilage	382	343	304	80	92	53	40	60	1.5
Adult thyroid	166	167	148	89	63	68	24	48	2.0
Embryo thyroid	682	732	649	95	83	69	39	41	1.1
Adult skin	135	141	125	93	68	48	32	60	1.9
Embryo skin	842	824	730	87	94	68	43	41	1.0

TABLE II—*Continued*

Tissue	Extracted tissue powder				P in NaCl extract as percentage of P in extracted powder	Precipitate of La salts			
	Total P	Purine N	Nucleic acid P calculated from purine content	Nucleic acid P as percentage of total P		P in La ppt. as percentage of P in NaCl extract	DNAP as percentage of P in La ppt.	RNAP as percentage of P in La ppt.	Ratio $\frac{\text{RNAP}}{\text{DNAP}}$
	mg./ 100 g.	*mg./ 100 g.*	*mg./ 100 g.*						
Adult testis	660	624	553	84	70	66	26	67	2.6
Adult ovary	484	444	394	81	87	42	39	52	1.3
Sheep placenta	655	613	543	83	83	74	33	65	2.0
Thymus (15-week sheep embryo)	2760	2696	2390	87	97	72	66	13	0.2
Whole blood (embryo sheep)	152	....	....	....	63	73	32	63	2.0
Nuclei (embryo sheep liver)	2630	2382	2110	80	86	82	75	14	0.2
Dried sheep embryo extract	192	175	155	81	82	93	11	83	7.4
Nucleoprotein fraction from embryo extract	658	746	661	101	82	80	81	10	8.0
Whole chick embryo (9 days)	1040	1017	901	87	86	74	29	58	2.0
Whole human embryo (3 months)	590	613	544	92	80	75	29	59	2.1
Ox pancreas	1670	1782	1580	95	79	87	10	82	8.0

* The total nucleic acid content of muscle is so low that the figures quoted should be regarded as provisional.

content being observed by Andreasen and Ottesen (1945), at the age of 3–4 months.

The ratios of the ribose- and desoxyribosenucleic acid content of the liver given by various authors are stated in Table III.

The highest concentration of ribosenucleic acid was found in the liver by Norikoff and Potter (1947) 1.25—4 days after partial hepatectomy of the rat. The maximum increase in concentration expressed in terms of concentration in the original liver was 62%.

In fresh explants of the 12-day chick embryo heart cultivated in plasma clot in roller tubes Davidson and associates (1947) found the RNA P content to be 2–3 times greater than the DNA P content.

Dounce states that 22.6–25.3% of the dry weight of liver cell nuclei in the rat (1943b) and 38–42% of the dry weight of erythrocyte nuclei in the chicken (1943c) are composed of desoxyribosenucleic acid.

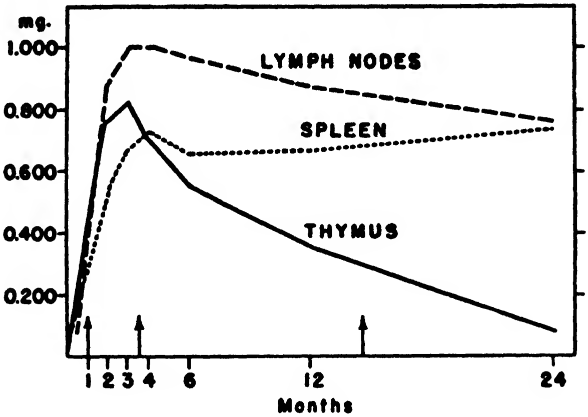


FIG. 1

Graphs showing the residual phosphorus in the lymphoid organs of female rats (Andreasen and Ottesen, 1945). The arrows indicate the stages of development at which the organs were examined.

TABLE III

Ratio of Ribosenucleic and Desoxyribosenucleic Acid P of the Liver

Animal	Ratio
Sheep	3.5 (Davidson and Waymouth, 1944c)
Rat	3.0 (Hammarsten and Hevesy, 1946)
Rat	3.9 (Schneider, 1945)
Rat	3.9 (Schmidt and Thannhauser, 1945)
Rat	3.1 (Euler, Hevesy and Solodowska, (1948)

In Jensen sarcoma of the rat Euler and associates (1948) report finding 50 and 70 mg-% desoxyribose- and ribosenucleic acid P, respectively; thus a higher ribosenucleic- than desoxyribosenucleic acid content. In the human, and also in Rous sarcoma, Davidson and Waymouth (1944c) have observed a higher ribosenucleic acid content.

High nucleic acid content of tumors was reported in early papers (*cf.* Davidson and Waymouth, 1944a). Greenstein and associates (1941) concluded, on the basis of chemical analyses, that the corresponding nucleoprotein fractions from rat liver and from transplanted hepatic tumors were nearly identical. Dounce (1943a) extracted the desoxyribosenucleic acid from normal rat liver, rat hepatoma 31, and Walker rat carcinosarcoma 256 by chemical methods. He concluded that the nuclei of Walker tumors have nearly the same concentration of desoxyribosenucleic acid as the nuclei of normal rat liver, while the nuclei of hepatoma 31 have a much lower desoxyribosenucleic acid content, calculated per unit dry weight of nucleus.

When using ultraviolet absorption for the study of nucleic acids, Mitchell (1942) did not observe any significant difference among normal tissues, hyperplastic tissues, and malignant cells.

Cowdry (1928) has compared tissue cultures of rat sarcoma and of normal fibroblasts and found an increased amount of Feulgen-stained material in the nuclei of sarcoma cells; by applying a photometric histochemical method Stowell (1945b, 1947), in investigating the human epidermoid carcinoma, has observed that the desoxyribosenucleic acid per unit volume of tissue and per cell decreases in the following order: carcinoma—normal—hyperplastic epidermis (*cf.* Santesson and Caspersson, 1942). In the study of human carcinoma Caspersson and associates (1940, 1941, 1944, 1946) have found the cytoplasm of tumor cells to contain larger amounts of ribosenucleic acid than the corresponding normal cells. By carrying out a cytological analysis of 565 neoplasms Koller (1943a) reports that the nucleoli in cells of the same tissue as well as in separate tumors differ in their chemical content and size. The larger nucleoli, which varied greatly in size, contain large amounts of ribosenucleic acid.

Yeast contains about 5–10% and some bacteria contain 15–20% of ribosenucleic acid on a dry weight basis (Mirsky, 1943). Of the total nucleic acid content in streptococci, 10–30% are of the desoxyribose type and the remainder of the ribose type. Two to 6% of the dry weight of bacteria are stated by Sevag and associates (1940) to be desoxyribosenucleic acid. A general review by Greenstein (1944) on nucleoproteins gives further data on the nucleic acid content of tissues.

A very low desoxyribosenucleic acid content is found in the nuclei of yeast, comprising 0.03% only of the wet weight of the yeast. Ribosenucleic acid is, in contrast, abundant in the cytoplasm of yeast cells and makes up 3% of their wet weight (Jenner and Brachet, 1944). In *Cl. Welchii* and streptococci the ratio of ribosenucleic acid and desoxyribosenucleic acid content is greater than 8 (Stacey, 1947).

The highest nucleic acid content reported is that of the sperm heads. In the dry, lipoid-free, sperm heads of the salmon, Stedman and Stedman (1947) report a nucleic acid content of 48.5%.

While, as shown by Kosterlitz (1944) and by Davidson and Waymouth (1944b), fasting does not influence the desoxyribosenucleic acid content of the liver, the ribosenucleic acid content of the liver of fasting animals is found to be lower than that of the controls. That fasting decreases the ribosenucleic acid content of the liver was also shown by Brachet and associates (1946). A similar result had been obtained in an earlier investigation of the pentosenucleic acid content of the pancreas by Caspersson and his colleagues (1941), who found the effect of fasting greatly enhanced when administering pilocarpine which strongly stimulates secretion.

Euler and Rönnestam-Säberg (1946) found that the ribosenucleic acid content of the rat liver was increased after feeding ribosenucleic acid.

Metabolic processes involving extensive quantitative changes in the content of protein and nucleotides of nerve cells were found by Hyden (1943, 1947) to take place in connection with motor and sensory function (*cf.* also Bodian, 1947).

a. Separation Method of Schmidt and Thannhauser

This method (1945) is based on the following assumptions. The only phosphorus fractions present in tissue thoroughly extracted first with trichloroacetic acid, then with ether, alcohol, chloroform and methanol, are nucleic acid P and possibly some phosphoprotein P. When dissolving the extracted tissue in 1 *N* potassium hydroxide the desoxyribosenucleic acid is present as such in the solution, while ribosenucleic acid splits into mononucleotides (Schmidt *et al.*, 1947).

The desoxyribosenucleic acid is precipitated by addition of 0.2 volume (of the aliquot) of 6 *N* hydrochloric acid and 1 volume (of the aliquot) of 5% trichloroacetic acid. An aliquot of the filtrate serves for the determination of the total P according to Fiske and SubbaRow. In a second aliquot, the inorganic phosphate is precipitated according to Delory. The acid filtrate contains, beside mononucleotide phosphorus, some inorganic phosphorus, possibly representing the phosphorus of phosphoproteins. The difference between the total phosphorus of the alkaline hydrolyzate and the total phosphorus of the acid supernatant represents the phosphorus of desoxyribosenucleic acid. The difference between the total phosphorus of the acid supernatant and the inorganic phosphorus represents the phosphorus of ribosenucleic acid.

In tracer experiments (Euler *et al.*, 1948), the specific activity of the P of the precipitated desoxyribosenucleic acid is determined according to Schmidt and Thannhauser, while the specific activity of the ribosenucleic acid P is computed from the specific activity data of the total phosphorus (ribosenucleic P + inorganic P) supplied by colorimetric and radioactive analyses of an aliquot of acid supernatant after wet ashing, and from the specific activity data of inorganic P precipitated from another aliquot of the acid supernatant. The radioactivity of the inorganic P fraction of the acid supernatant of animal tissues treated as described above is much smaller than the radioactivity of the ribosenucleic P fraction.

b. Hammarsten's Method

While the above-described method is based on the determination of the nucleic acid phosphorus only, Hammarsten (1947) determines the phosphorus and nitrogen content of the nucleotides as well. His procedure was worked out chiefly with a view to application in tracer work.

Tissues and cells are fragmented in the dried state by mechanical vibration and extracted with concentrated salt solution (ammonium sulfate + sodium chloride) containing 2.5 moles urea/l. Vibration with glass beads gives the best results, especially with yeast, bacteria and isolated cell nuclei. The extract is precipitated with Cu^{++}, and the precipitate is then decomposed in concentrated potassium acetate at pH 5. The potassium salts of the nucleotides are precipitated with alcohol, and the acid nucleotides with acid alcohol at -15°C. Immediately after dissolving in 0.01 N NaOH at 0°C., the nucleotides are reprecipitated with acid alcohol. This fraction contains polydesoxyribose- and polyribosenucleotides. They are termed *pd* and *pr*, respectively.

Extraction at 0°C. gives very low values, and at 20°C. the variation among the results of separate extractions on any one organ is too great for an analytical method. Extraction at 20°C., therefore, is used only for preparation. The results of the extraction are more reproducible when the suspensions are boiled (110°C.). The copper precipitation is carried out within 6 to 8 hours.

Some organs give protein-free preparations, and in other cases the polynucleotides can be freed from proteins by a second extraction of the fraction *pd* + *pr*. These protein-free preparations have the same low buffer capacity as the standard substances.

Because of the salt effect, it is necessary to remove the buffer when most of the copper has been extracted, and to treat the undissolved residue with a solution of urea in water. A negligible residue is usually left after urea treatment.

A method of separation of the fraction *pd* + *pr* is based on the fact that the sodium salt of *pd* is soluble but that of *pr* is insoluble in liquid phenol. Nevertheless, only *pd* can be separated.

In view of the fact that this separation method involves heavy losses, the fraction *pd* + *pr* is subjected to alkaline hydrolysis for the purpose of estimation. The high solubility of the low molecular weight ribonucleotides permits the precipitation at acid reaction of the lanthanum salt of the unchanged desoxyribosenucleotide.

The low molecular weight nucleotides dialyze easily and are precipitated by lanthanum ions at neutral, but not at acid, reaction. They are estimated by measurement of the light absorption in the band around 265 $m\mu$ and the amounts can be expressed as mg. nucleotide phosphorus/100 g. dry organ.

Some results obtained by Hammarsten are seen in Table III A.

TABLE IIIA

Distribution of Nitrogen and Phosphorus in Nucleotide Fractions (Hammarsten, 1947)

Mean values and standard errors are given for four separate extractions at 110°C. of each preparation of vibrated cells. Phosphorus (P) and nitrogen (N) in mg./100 g. dry organ. Light absorption coefficient (ε)/100 mg. dry organ, 10 ml. volume and 3,174 mm. *pd* = desoxyribosenucleotides, *pr* = ribosenucleotides.

Preparation of tissue of cells	Phosphorus in fractions:			Nitrogen in fractions:		Light absorption coefficient in fractions:	
	pd + *pr* (P_1)	*pr* (P_2)	*pd* (P_{pd})	*pd* + *pr* (N_1)	*pr* (N_2)	*pd* + *pr* (ε_1)	*pr* (ε_2/Q)
Rat liver, adult............2	318 ±4	239 ±6	81 ±2	540 ±12	430 ±19	2.39 ±.06	1.82 ±.05
Rat intestine, adult........3	599 ±23	304 ±25	293 ±26	1210 ±340	—	4.50 ±.17	2.13 ±.20
Rat spleen, adult...........4	480 ±27	171 ±7	300 ±15	981 ±60	—	3.69 ±.18	1.26 ±.26
Total rat, 9 days old.......5	240 ±10	136 ±4	108 ±1	650 ±60	—	1.95 ±.03	1.00 ±.04
Bakers' yeast, multiplying..6	471 ±4	459 ±2	18 ±4	1027 ±50	—	3.58 ±.12	3.28 ±.11
Torulopsis, multiplying.....7	518 ±18	511 ±18	30 ±2	1690 ±70	—	4.32 ±.17	4.15 ±.15

IV. NUCLEAR AND CYTOPLASMIC NUCLEIC ACIDS

Desoxyribosenucleic acid was first found by Miescher (1897) to be one of the main constituents of cell nuclei. The location of the nucleic acids in different regions of the nucleus and cytoplasm was made available to observation by Caspersson's method of ultraviolet absorption (1936, 1937, 1939a and b, 1944). Nucleic acids show a characteristic, high absorption maximum at 2600 Å by virtue of the conjugated double bonds of the constituent pyrimidine and purine ring. The absorption spectra themselves do not differentiate between the two types of nucleic acid; however, in conjunction with other tests, such as the Feulgen (1919–20) reaction for desoxyribosenucleic acid or the test for ribosenucleic acid proposed by Brachet

(1940), they have been used to indicate the site of each type of nucleic acid. Important knowledge has thus been obtained on the role of nucleic acids in cell division. A direct correlation has been found between cell division and nucleic acid content. The desoxyribosenucleic acid content has been found to increase in prophase and to decrease in telophase.

The ultraviolet method has also supplied observations on the nucleic acid content of the chromosome and the gene. The only known case of the presence of desoxyribosenucleic acid outside the nucleus is that of Purkinje cells. Loo (1937) reported that desoxyribosenucleoprotein may pass from nucleus to cytoplasm as age advances.

Ribosenucleic acid was first considered by Brachet (1933) to be a cytoplasmic constituent. By separating the cytoplasm from the cell nuclei Behrens (1938) has shown the presence of ribosenucleic acid in the former, this result being substantiated by several experimenters (Loo, 1937; Delaporte, 1939; Delaporte and Roukhelman, 1938; Painter and Taylor, 1942).

Schultz and Caspersson (1938) have shown that ribosenucleotides are abundant in the cytoplasm of rapidly growing plant and animal tissues, but not in the homologous mature tissues. Tumor cells have also been found by Caspersson and his associates (1941), and by Mitchell (1942), to contain abundant amounts of ribosenucleotides. Davidson and Waymouth (1943) have found evidence suggesting that rapidly growing embryonic tissues are characterized by both a high nuclear desoxyribosepolynucleotide content and a high cytoplasmic ribosepolynucleotide content. Hammarsten *et al.* were also able to confirm (1935) the presence of ribosenucleic acid in the cytoplasmic masses of the rat liver by their failure to absorb ultraviolet light after treatment with crystalline ribosenuclease.

Ribosenucleic acid is, however, not entirely confined to the cytoplasm. Schultz and Caspersson (1940) have found that the nucleolus of the salivary gland of *Drosophila melanogaster*, for example, is composed of ribosenucleoproteins containing varying percentages of ribosenucleic acid; the presence of ribosenucleic acid in the nucleolus was simultaneously reported by Brachet (1940) and later by other authors (Mirsky and Pollister, 1943; Davidson and Waymouth, 1946). According to Schultz and Caspersson (1940) the ribosenucleic acids are formed near the nuclear membrane where the nuclear and the cytoplasmic surfaces meet; they emphasize that in view of the interrelationship between desoxyribose- and ribosenucleic acids we cannot expect to find ribosenucleic acid exclusively in the cytoplasm. In the cell nuclei isolated from the intestine of frogs Brachet (1941) has found ribosenucleic acid corresponding to 9.8% of the total nucleic acid present. Koller's (1943a) investigation, in which Fuelgen-negative nucleoli of the neoplasm were found to contain amounts of ribosenucleic acid increased in proportion to other cells, is cited below.

Recently, Feulgen-positive nucleoli and nucleoli that absorb ultraviolet light after treatment with ribosenuclease have been found in rat liver cells by Davidson and Waymouth (1946). They conclude that although desoxyribosenucleoprotein appears to be the major constituent of the nucleoli, the probable presence of small amounts of ribosenucleic acid is not excluded. The presence of ribosenucleic acid in the nucleoli may be partly or wholly responsible for ribosenucleic acid found in the separated nuclei by means of the centrifugation method. Euler and Hahn (1946b) report 7% of the total nucleic acid content of the nuclei of the calf thymus and 8.1% of those of the Jensen sarcoma nuclei to be composed of ribosenucleic acid, and Thomas (1945) emphasizes that malignant cells have a greatly enlarged nucleolus which makes a higher ribosenucleic acid content of the cell nuclei of the sarcoma understandable. In the nuclei of the red corpuscles of the hen, Hammarsten (1946) found ribosenucleic acid to make up less than 1% of the nucleic acid content.

By centrifugation, Schneider (1946) has separated rat liver and kidney homogenates into a nuclear fraction, a mitochondrial or large granule fraction, and an unfractionated residue. In these experiments, desoxypentosenucleic acid was found only in the nuclear fraction, while pentosenucleic acid was found in all three fractions, but most of it in the unfractionated residue. Of 65 mg.-% pentosenucleic acid P present in the fresh liver tissue 4.9% was found in the nuclear, 11.4% in the mitochondrial fraction, and 47% in the unfractionated residue.

Evidence brought forth by different authors that pentosenucleic acid is an agent in the synthesis of protein has been supplemented and summarized by Greenstein (1944).

Koller (1943b, 1947), in collaboration with the Holt Radium Institute, Manchester, has determined the cytological characteristics of tumor cells in a great number of different tumors. Fig. 2 illustrates the cytological characteristics, properties and metabolic activity of the various cell types in tumorous tissue. In the cell population of tumors different types were distinguished. Type A cells are characterized by a high division rate. Cytological studies of X-rayed squamous-cell carcinoma of the cervix suggested that the intermitotic period is not more than four days. The proportion of type A cells is very high in the actively growing tumor regions. Type B cells are characterized by the easily observable large nucleolus or nucleoli. The rate of division in these cells is low. They undergo a great increase in cytoplasmic volume either directly or through "endomitosis", *i.e.*, chromosome multiplication without nuclear division. A variable proportion of tumor cells is in a process of degeneration, which ultimately leads to necrosis. The numerous intergrades indicate a close relationship in development between these types.

These extensive quantitative and qualitative data have been analyzed

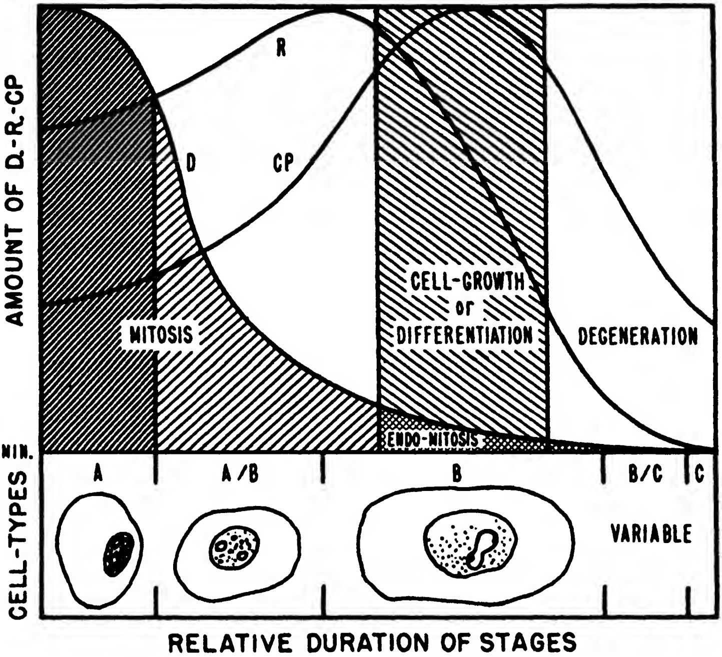


Fig. 2

Diagram illustrating the characteristic properties and the relationship of the various cell types in tumor tissue. A = proliferating, B = differentiating, C = degenerating cell types, D = desoxyribosenucleic acid, R = ribosenucleic acid, CP = cytoplasmic protein. The frequency of mitosis or division rate is determined by the amount of desoxyribosenucleic acid present in the nucleus (Koller, 1947a).

Microchemical tests, ultraviolet spectroscopy and cytogenetic investigations (Koller, 1943b) provide evidence that heterochromatin (the strictly localized regions in the chromosomes) is the central organ in the protein-forming system of the cell. In tumor cells either the regulation in the activity of heterochromatin is disturbed or heterochromatin itself has undergone a mutational change which is quantitative rather than qualitative in nature. The cytological investigation draws attention to the fact that the particular heterochromatic regions in the chromosomes that determine and regulate protein metabolism within the cell, play an important part in carcinogenesis and supports the conclusion of Santesson and Caspersson (1942) arrived at by ultraviolet spectroscopy. Their investigations show that malignant growth is conditioned by disturbances in the heterochromatic system.

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and interpreted in the light of recent knowledge on chromosome chemistry (Caspersson *et al.*, 1941; Landström-Hyden *et al.*, 1941; and Darlington, 1942, 1947). The analysis of nuclear constituents, chromosome behavior, and organization by means of Schiff's aldehyde reaction (Feulgen's method), has shown that type A is rich in desoxyribosenucleic acid; the concentration is equal to that observed in embryonic cells. Desoxyribosenucleic acid is almost absent in type B, whereas ribosenucleic acid is increased. The morphological and functional characteristics of the various tumor cell types are illustrated graphically in Fig. 2. The interrelationship among desoxyribose- and ribosenucleic acid, cytoplasmic protein, mitosis, and cell growth is shown in the various stages of development. The cytological analysis clearly indicates that protein metabolism in tumor cells is extremely active.

V. Biological Synthesis of Purine Compounds

The steps involved in the enzymatic replacement of the phosphate group of the nucleic acid molecule by labeled phosphate are not yet known. But some indications of the processes possibly involved were recently supplied by the work of Kalckar (1946) and of Colowick and Price (1946). The enzymes which catalyze the fission of purine and pyrimidine nucleosides into free purines or pyrimidines and ribose (the so-called nucleosidases) were shown by Kalckar to be phosphorolytic enzymes. He brought conclusive evidence that the ribosidic linkage was split by inorganic phosphate and that the latter was esterified either to the nitrogenous base or to the ribose component. When, in the presence of inorganic phosphate and the phosphorolytic enzyme, hypoxanthine is liberated from inosine, for each mol of hypoxanthine liberated 1 mol. of phosphate is found to be taken up in organic linkage; this can be shown to be due to the formation of ribose-1-phosphate. Colowick and Price have shown that, in the presence of a phosphorolytic enzyme, guanine is liberated from ribosenucleic acid. This reaction seems to be reversible and has been formulated:

Ribose-1-guaninenucleic acid (ordinary nucleic acid) + phosphate
= ribose-1-phosphatenucleic acid (deguaninenucleic acid) + guanine.

If an enzyme prepared from muscle is added to ribosenucleic acid in the presence of inorganic phosphate, guanine is gradually released and the acid-soluble residue contains less guanine and an equivalent amount of highly labile phosphate. If inorganic phosphate is removed, the guanine decreases in the acid filtrate and increases in the acid-insoluble residue. Nucleic acid can thus undergo a phosphorolytic cycle by which phosphate is exchanged for a nitrogenous base.

VI. Application of Isotopic Indicators in the Study of the Turnover of Nucleic Acids

The application of labeled nitrogen, carbon, hydrogen, oxygen, and phosphorus can be expected *a priori* to supply information on the mechanism and rate of formation of the constituents of nucleic acids. Barnes and

Schoenheimer (1943) and Plentl and Schoenheimer (1944) have applied stable isotopes, *viz.*, heavy nitrogen, in the study of nucleic acid metabolism. They found that neither purines nor pyrimidines supplied in the diet were utilized by the body for the synthesis of nucleoproteins. According to their views, nucleoproteins are synthesized within the nucleus from smaller molecules or are utilized for such synthesis if they are supplied as nucleosides, nucleotides, or nucleic acid.

Brown and associates (1947) found recently that adenine present in the diet, in contrast to guanine, is utilized for the synthesis of nucleic acids. In addition, as seen in Table IIIB, they have shown that adenine, labeled in the pyrimidine nitrogens, is converted to guanine with labeled nitrogen still in the same positions in the purine ring. Adenine is the first organic compound demonstrated to be incorporated as such into the nucleic acids and provides a tool with which to label specifically the carbon-nitrogen skeleton of nucleic acid.

TABLE IIIB

Incorporation of N^{15} into Nucleic Acids and Other Compounds Following the Administration of Labeled Adenine (Brown et al., 1947)

	Adenine (200 mg./kg./day)		Guanine (227 mg./kg./day)	
	Atom per cent N^{15} excess	Calcd. on basis of 100% in adenine fed	Atom per cent N^{15} excess	Calcd. on basis of 100% in guanine fed
Adenine (dietary)	6.29	100	. .	.
Guanine (dietary)	. . .	. . . .	6.40	100
Sodium nucleic acids	0.386	6.1	0.009	0.14
Adenine	0.857	13.7		.
Guanine	0.513	8.2	. .	. .
Adenosinetriphosphate	0.161	2.6	2.02	31.6
Allantoin	1.70	27	0.115	1.80
Urea	0.018	0.29	. . .	. . . .

Recent studies with isotopic nitrogen carried out by Kalckar and Rittenberg (1947) have shown that the 6-amino group of adenosine triphosphate from muscle is rejuvenated with great rapidity. Seven to eight hours after administration of isotopically labeled ammonium citrate to rats, the adenosine triphosphate analyzed as crystalline adenylic acid gave the following analysis. The 6-amino-N (obtained by means of the specific adenylic acid deaminase) contained 0.093% N^{15}, the ring N (hypoxanthine) only 0.007% N^{15}, and the 6-amino plus ring N, *i.e.*, the adenine, contained 0.021% N^{15}. The ring N is thus not rejuvenated to an appreciable extent during the period of 8 hours, whereas the 6-amino-N was rejuvenated to a very great extent. The glutamic acid of the protein only attained a N^{15}

concentration of 0.02% during the same period of time. The amide-N of the protein had an isotope concentration as high as 0.100% N^{15}* and could, therefore, be considered one of the possible ammonia donors of the inosinic acid in muscle. Kalckar and Rittenberg found, furthermore, that the purine of the nucleic acid from muscle had a comparatively high isotope concentration, 0.03% N^{15}.

When ammonium citrate containing 4.18 atom-% excess N^{15} was fed by Davidson and Raymond (1947) to pigeons and rats in doses of 50 mg./day for 3 days, the ribonucleic acid isolated from the pigeon liver contained 0.048 atom-% excess N^{15} and the desoxyribonucleic acid only 0.003 atom-% excess. The figure for the uric acid excreted in the last 24 hr. was 0.204 atom-% excess N^{15}. The ribonucleic acid isolated from rat liver contained 0.053 atom-% excess N^{15}. While the amount of excess N^{15} in the desoxyribonucleic acid was negligible, the ratio of the rate of incorporation of N^{15} in ribose- and desoxyribosenucleic acids in the rat liver is thus greater than 20.

Radioactive phosphorus has had extensive application in the study of nucleic acid metabolism.

VII. P^{32} CONTENT OF RESIDUAL PHOSPHORUS

One approach to the study of the metabolism of nucleoproteins is to administer labeled phosphate to a mouse, for example, sacrifice the animal after the lapse of some hours or days, and extract both the acid-soluble and the phosphatide phosphorus from the tissues. The P^{32} remaining in the tissue after extraction is then assumed to be nucleoprotein phosphorus. By applying this procedure, Tuttle and his colleagues (1941) have found all tissues investigated to contain radioactive nucleoproteins. They also compared the uptake of P^{32} by the organs of normal and leukemic mice. As seen in Fig. 3 the nucleoprotein isolated from tumor cells and leukemic tissues has an appreciably larger P^{32} content than normal nucleoprotein. If nucleic acid is formed in a medium containing labeled phosphate, it will acquire a P^{32} content. In some cases this formation of new, labeled nucleic acid molecules can be accounted for by the disappearance of an equal or similar amount of old, unlabeled molecules. This is, to a large extent, the case in the liver of a full-grown animal, for example. In other cases, however, the nucleic acid content of the organ increases, additional nucleic acid being formed, and this inevitably becomes labeled. Such additional nucleic acid can either remain in the organs or can be secreted. We meet with the first alternative in all growing tissue, the second in organs which are secreting nucleic acid-containing products, as, for example, lymphocytes or nucleated erythrocytes. While the percentage rate of renewal of desoxyribosenucleic

* These figures indicate an excess above the N^{15} content of normal nitrogen.

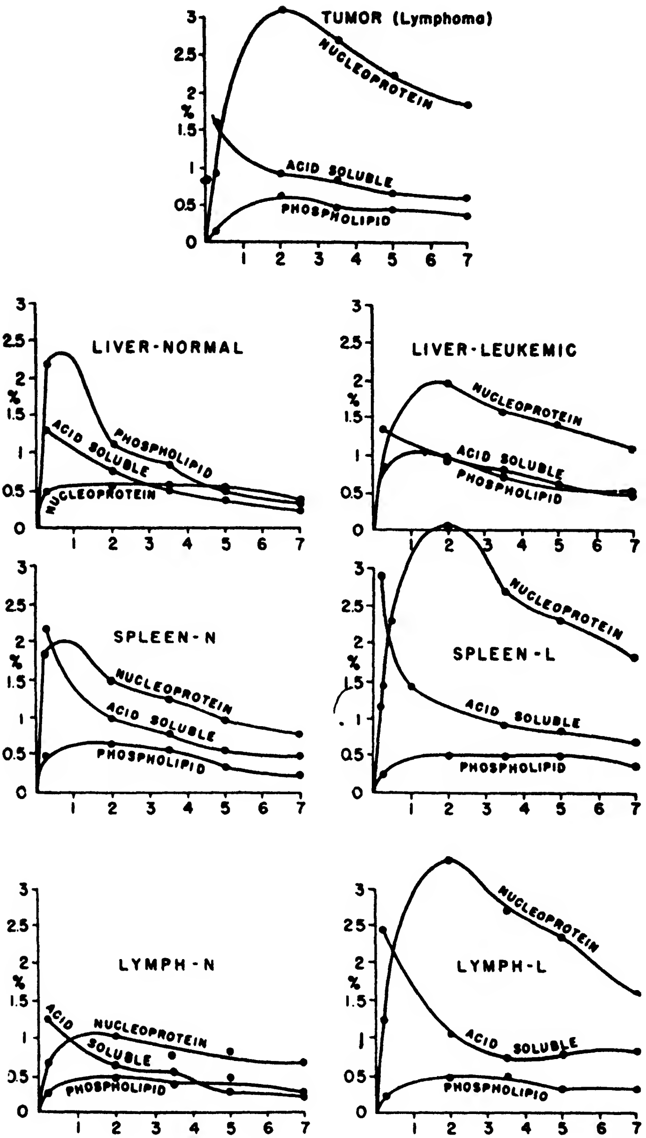

FIG. 3

The comparative uptake of labeled phosphorus in normal and leukemic mice (Tuttle *et al.*, 1941).

acid and, thus, the formation of labeled desoxyribosenucleic acid that we witness in the normal full-grown liver, for example, is very low, the formation of labeled desoxyribosenucleic acid in the growing liver is very appreciable and we expect an appreciable rate of formation of desoxyribosenucleic acid in organs manufacturing lymphocytes, as do the organs of leukemic mice.

Both the P^{31} (stable phosphorus) and the P^{32} content of the residual fractions of the liver and lymphoma cell nuclei in the mouse were determined by Marshak (1941). Some of the results obtained by him are seen in Table IV. The data given are mean values. The figures obtained indicate a high rate of renewal of the residual phosphorus in the cell nuclei of the organs investigated, which besides desoxyribosenucleic acid P contain presumably unknown P fractions of high specific activity.

The approach to the study of nucleic acid metabolism based on the investigation of the P^{32} content of the residual phosphorus—that remaining after removal of the acid-soluble fractions with trichloroacetic acid and of the phosphatides by extraction with ether, alcohol, chloroform, methanol and similar agents—has various drawbacks. The residual fraction contains desoxyribose- and some ribosenucleic acids and unknown P fractions, possibly phosphoproteins, though the amount of the latter is minute, as shown for example by Euler and Schmidt (1934) and by Schmidt and Thannhauser (1945). The rate of renewal of desoxyribosenucleic acid in the liver, for example, being very much smaller than that of ribosenucleic acid, we cannot draw any conclusions in regard to the rate of renewal of desoxyribosenucleic acid from the P^{32} content of the residual phosphorus.

TABLE IV

P^{31} and P^{32} of the Isolated Nuclei of the Organs of the Mouse (Marshak, 1941)

Organ	Residual P^{31} as percentage of total P^{31}		Residual P^{32} as percentage of total P^{32}
Liver	80.5	After 1 hr.	71.0
		After 1 day	73.4
		After 7 days	72.0
Lymphoma	92.0	After 1 hr.	....
		After 1 day	96.3
		After 7 days	66.3

VIII. INCORPORATION OF P^{32} INTO DESOXYRIBOSENUCLEIC ACID

Hahn and Hevesy (1940) isolated desoxypolynucleotides from the liver and other organs of the rabbit, making use of Hammarsten's method. After the lapse of 11.5 hours the specific activity (activity per mg. phosphorus) amounted to 4% of the activity of the inorganic phosphorus of the liver. This figure indicates a low rate of renewal of the desoxyribosenucleic acid

P of the liver, a very much lower one than shown by the activity figures found for the residual P of cell nuclei in the investigations described above. In view of the low specific activity of the desoxyribosenucleic acid of the liver, the presence of small impurities in the form of highly active phosphorus compounds may influence the results obtained very appreciably, and thus a very effective purification of the desoxyribosenucleic acid fraction was found to be imperative. This is especially the case in experiments of short duration, which are often of great interest. A very effective removal of traces of contaminating radioactive phosphorus can be obtained by repeated alternative acid precipitation (HCl dissolved in methanol) of the nucleic acid and subsequent dissolution in 1 *N* NaOH (Klein and Beck, 1935). By repeating these procedures 7–8 times, nucleic acid P^{32} having active impurities amounting to less than 1 part in 10,000 can be obtained. During the purification process the polynucleotides have depolymerized very appreciably, their molecular weight amounting, on the average, only to about 16,000. Using this method of purification, Hevesy and Ottesen (1943) obtained, for fully grown rats, the results shown in Fig. 4, which illustrate the very low rate of renewal of the desoxyribosenucleic acid of the liver and kidney, and the still lower rate of renewal of the brain

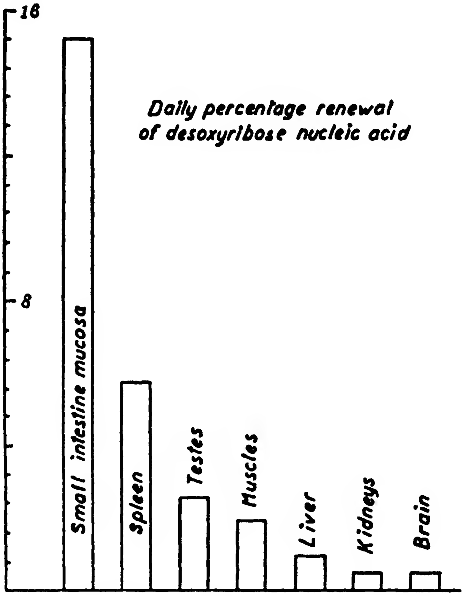

FIG. 4

Daily percentage rate of formation of labeled desoxyribosenucleic acid in the organs of the rat. (Hevesy and Ottesen, 1943) in contrast to high values found for the desoxyribosenucleic acid extracted from the intestinal mucosa and the spleen.

The data in Fig. 4 are obtained by comparing the specific activity of the desoxyribosenucleic acid P at the end of the experiment with the average value of the specific activity of the intracellular inorganic P of the organ prevailing throughout the experiment. It is thus assumed that the inorganic phosphate, or a phosphorus-containing precursor that comes relatively quickly into equilibrium with the inorganic phosphate, is incorporated in the newly formed desoxyribosenucleic acid molecules.

In the calculation of the above figures no regard was paid to the repeated renewal of some of the molecules during the experiment. The values stated for daily percentage renewal in the intestinal mucosa and, to a still more restricted extent, also those in some other organs may correspondingly be somewhat low.

TABLE V

Percentage Ratios of the Specific Activities of Desoxyribosenucleic Acid P and Inorganic P of the Organs and Plasma, Respectively, 2 Hours after Administration of Labeled Phosphate to the Mature Rat (Ahlström et al., 1945)

Organ	Percentage ratio of the specific activities of desoxyribosenucleic acid and	
	Inorganic P	Plasma P
Liver	0.14	0.20
Spleen	2.50	1.79
Kidneys	0.16	0.15
Intestinal mucosa	4.81	2.11
Jensen sarcoma	2.10	1.82

The low turnover rate of desoxyribosenucleic acid in the liver and the kidneys, and the high value in the spleen and intestinal mucosa, are also shown by the results of experiments taking 2 hours only, as seen in Table V.

In Table V the percentage ratios of the specific activities of the desoxyribosenucleic acid P and that of inorganic P, both at the end of experiments taking 2 hours only, are recorded. The figures represent the average of numerous values obtained. The end value of the specific activity of the inorganic liver P hardly differs from the mean value in experiments taking 2 hours. For the spleen the end value is about 25% higher than the mean value.

It can be assumed that much of the turnover of the desoxyribosenucleic acid takes place in the dividing or secreting cells and it is quite possible that such cells are more permeable to phosphate than the average cell of the tissue in question. Such an enhanced permeability would not greatly influence the renewal figures obtained for the liver desoxyribosenucleic acid, as within a short time the specific activity of the inorganic P of the liver reaches the corresponding value of the plasma. In the case of those organs that show a restricted cell permeability, as for example the brain, testes, and muscles, in experiments taking 2 hours, the tissue inorganic P has, however, an appreciably lower specific activity than the plasma inorganic P. In the case of such organs it is possible that the desoxyribosenucleic acid is built up from inorganic P having a higher specific activity than the inorganic P extracted from the tissue, thus the renewal values arrived at are too high. The correct renewal figures can, however, not be lower than the figures obtained by comparing the specific activity of the desoxyribosenucleic acid P of the organ with that of the inorganic P of the plasma. The latter figures are also given in Table V.

TABLE VI

Activity of 1 mg. Desoxyribosenucleic Acid P of Retrogressing Jensen Sarcoma, Expressed in Percentage of the Activity of 1 mg. Inorganic P from the Sarcoma or Plasma (Ahlström et al., 1947)

	Sarcoma	Plasma
(A)	1.92	1.34
(B)	2.14	1.42

(A) indicating the mean average for each separate experiment involving several animals, (B) the mean figure, when the sarcomata are considered collectively.

Experiments by Brues *et al.* (1944), in which the specific activity of the desoxyribosenucleic acid of the liver was investigated several days after administration of labeled phosphate are discussed on p. 433.

An investigation of the desoxyribosenucleic acid turnover in the spontaneously regressing Jensen sarcoma (Ahlström *et al.*, 1947) has shown that the turnover rate in such tumors differs little from that found in the growing tumors, as seen in Table VI, in which the average values obtained for 26 spontaneously retrogressing sarcomata are given.

In the regressing sarcoma the tissue is undergoing autolysis and the nucleic acid along with the other compounds is decomposing, but nevertheless the nucleic acid still present is renewed at almost the same rate as the nucleic acid in the growing sarcoma.

A minor diminution of the desoxyribosenucleic acid turnover of the sarcoma is obtained by administration of colchicin (Ahlström *et al.*, 1947).

Andreasen and Ottesen (1945) determined, in 3-hour experiments with new-born, young, mature, and old rats, the percentage ratio of injected P^{32} in 1 mg. of the desoxyribosenucleic acid P of lymphoid organs to that in 1 mg. of inorganic plasma P. Their results (average of 3 experiments), given in Table VII, indicate a decrease in the rate of formation of labeled desoxyribosenucleic acid in the thymus, lymph nodes, and spleen with the age of the animal, the decrease being due partly to a decreasing rate of penetration of the radioactive tracer into the tissues. The latter was found to be appreciably lower for the lymph nodes and the spleen than for the thymus. Large values were found for the specific activity of the thymus desoxyribosenucleic acid P and very large values in the case of the bone marrow.

Brachet (1947) emphasizes the striking parallelism between the intensity of the nuclear alkaline phosphatase reaction and the turnover rate of desoxyribosenucleic acid phosphorus in various organs. Organs with a high turnover of desoxyribosenucleic acid P give a strong reaction (intestinal mucosa, testis, spleen), while the reaction is very faint in the nuclei of the organs where the turnover is low (muscle, kidney, brain, avian red blood cells). Moreover, the intensity of the nuclear alkaline phosphatase reaction is increased in rats' liver after partial hepatectomy; so is also the desoxyribosenucleic acid P turnover, as shown by Brues and associates (1944).

TABLE VII

Desoxyribosenucleic Acid Turnover in the Lymphoid Organs in Rats of Different Ages in Experiments Taking 3 Hours (Andreasen and Ottesen, 1945)

Organ	Percentage ratio of the specific activity of the nucleic acid P of the organ and the inorganic P of the plasma		
	Age in Days		
	30	110	430
Thymus	7.6	5.3	5.6
Lymph nodes of intestines	6.1	1.5	1.0
Lymph nodes of skin	2.9	1.4	1.1
Spleen	2.4	1.6	0.8
Bone marrow	....	10	13

These findings make it very likely that the nuclear alkaline phosphatase is responsible for the replacement of phosphorus in the desoxyribosenucleic acid molecule. The fact that, according to Danielli and Catcheside (1945), the different bands of the giant chromosomes give phosphatase reactions of unequal intensities might well mean that the speed of the P turnover is different in the various genes.

Moog (1946), and Jenner (1946), have recently shown that the desoxyribosenucleic acid of the cell nucleus and ribosenucleic acid of the cytoplasmic granules are both included in very similar complexes.

Treated with 0.6 *M* solution of KCl these complexes separate into two fractions, one comprising desoxyribose- or ribosenucleoproteide, the other characterized by its insolubility and its high content of alkaline phosphatase.

IX. INCORPORATION OF P^{32} INTO RIBOSENUCLEIC ACID

The rate of incorporation of labeled phosphate into the ribosenucleic acid and also of desoxyribosenucleic acid of the liver and a hepatoma of the rat was determined by Brues *et al.* (1944) in experiments taking some days, while E. Hammarsten and Hevesy (1946) determined the rate of renewal of ribosenucleic acid present in the liver and some other organs of the rat in experiments lasting only 2 hours.

The specific activity of the desoxyribosenucleic acid P in the first mentioned experiments was found to be 10.6% of the specific activity of the inorganic phosphorus determined at the end of experiments taking 3 days. If we assume the average value of this specific activity to amount to $\frac{1}{6}$ of its end value (*cf.* Hevesy and Ottesen, 1943), the percentage renewal of the desoxyribosenucleic acid of the resting liver becomes 21.2% in the course of a day, thus being much greater than found in the experiments described on pages 429 and 430.

TABLE VIII

Specific Activities of Nucleic Acid Phosphorus Expressed as Per Cent of Inorganic Phosphate Specific Activities (Brues et al., 1944)

Organ	Days after injection	Ribosenucleic Acid	Desoxyribosenucleic Acid
Resting liver	3	54.9	10.6
	8	123	20.8
Regenerating liver	3	230	180
	13	314	
Hepatoma	3	171	64.0

The value found by Brues and his associates for the rate of renewal of ribosenucleic acid is about 5 times as large as that found for the rate of renewal of desoxyribosenucleic acid (*cf.* Table VII); this demonstrates the larger turnover rate of ribosenucleic acid. The turnover figures for the regenerating liver and the hepatoma are appreciably larger than those found for the resting liver (*cf.* Table VIII). Davidson and Raymond (1947) found, in experiments taking 2–4 hr., the ratio of the specific activities of the phosphorus of ribosenucleic acid and desoxyribosenucleic acid to be about 7:1. In regenerating rat livers the activity of both types of nucleic acid was increased but especially that of the desoxyribonucleic acid, so that the ratio of the specific activities fell to between 2 and 3. In fetal liver the specific activity of the desoxyribosenucleic acid frequently exceeded that of the ribosenucleic acid.

In the experiments of Hammarsten and Hevesy (1946), as recorded in Table IX, the rate of renewal of ribosenucleic acid in the liver was found to be as much as 33 times larger than the rate of renewal of desoxyribosenucleic acid. In spite of the finding that ribosenucleic acid is renewed at an even greater rate in the spleen and the intestinal mucosa than in the liver, the ratio of the rate of renewal of ribose- and desoxyribosenucleic acids in these organs is only 3 and 2, respectively. This lower ratio is due to the comparatively high rate of renewal of desoxyribosenucleic acid in these organs. From the above figures it follows that the rate of renewal of both types of nucleic acid in the organs investigated is highest in the intestinal mucosa and in the spleen.

The very conspicuous difference shown in the rate of renewal of desoxyribosenucleic acid of the liver or the kidneys and of the intestine or spleen, respectively, is not found in the rate of renewal of ribosenucleic acid.

TABLE IX

Ratio of the Rate of Renewal of the Ribosenucleic Acid and Desoxyribosenucleic Acid in the Organs of the Rat in the Course of 2 hours. (Hammarsten and Hevesy, 1946)

Organ	Percentage ratio of the specific activity of the nucleic acid P and that of the inorganic P		Ratio of the rate of renewal of ribose- and desoxyribosenucleic acid
	Ribosenucleic Acid	Desoxyribosenucleic Acid	
Liver	3.3; 3.6	0.12; 0.09	33
Spleen	3.1; 10.2	2.2 ; 2.2	3
Intestine	7.1; 4.1	3.4 ; 2.3	2

In the last mentioned experiments the specific activity of both the total desoxyribosenucleic acid P and the total ribosenucleic acid P extracted from a rat weighing 194 g. was also determined. The activity of labeled sodium phosphate amounted to 8.1 microcuries/100 g. of animal weight. The time of the experiment was 2 hours. The results of this experiment are seen in Table X.

As shown in Table X, the specific activity of the average nucleic acid P of the rat is almost identical with the corresponding value of the ribose- and desoxyribosenucleic acid, respectively, extracted from the intestine.

The interpretation of the significance of the specific activity figures obtained for the total rat encounters some difficulties, as the specific activity of the inorganic P utilized in the formation of the labeled nucleic acid molecules is unknown. If the specific activity of the inorganic P utilized in building up the average body nucleic acid corresponded to the specific activity of the inorganic liver P, the percentage "rate of renewal" of the body ribose- and desoxyribosenucleic acids would be 3.7 and 1.9, respectively. If the specific activity of the inorganic P utilized in building up the average nucleic acid of the organism corresponded to the specific activity of the inorganic intestinal P, larger values, *i.e.*, 5.0 and 2.7, respectively, would be obtained.

When calculating the last mentioned figures, we compared the specific activity of the nucleic acid P at the end of the experiment with the specific activity of the inorganic intestinal P at the end of the experiment. Correctly we should have considered the mean value of the specific activity of the inorganic intestinal P prevailing during the experiment. The magnitude of this mean value is about two-thirds of its end value. We have, therefore, to multiply the figures mentioned above (5.0 and 2.7, respectively) by about 1.5 to obtain an approximate value of the percentage renewal of the ribose- and desoxyribosenucleic acids, respectively, in the course of 2 hours.

TABLE X

Specific Activity of the Nucleic Acid P Extracted from the Total Rat Compared with the Corresponding Values for Liver, Spleen, and Intestinal Mucosa. The Value for the Specific Activity of Ribose P of the Total Rat is Assumed to be 100 (Hammarsten and Hevesy, 1946)

Sample	Specific activity		
	Ribosenucleic acid	Desoxyribosenucleic acid	Free P
Total rat	100	60	
Liver	164	4.4	5100
Spleen	292	63	2850
Intestine	112	63	2770

It is improbable that such a highly active inorganic phosphate as that found in the liver in 2-hour experiments is utilized in the building up of the average nucleic acid molecules of the body. Liver and kidneys have a privileged position in regard to the rate of penetration of phosphate. It is much more probable that free P of specific activity similar to that found in the intestine is used in the building up of these labeled nucleic acid molecules. In fact, the amount of nucleic acid present in the mucosa of the digestive tract makes up an appreciable percentage of the body nucleic acid. While the body nucleic acid also contains slightly radioactive fractions, *viz.*, those originating from the liver, the kidneys, and the brain, and fractions of restricted radioactivity originating from the muscles (*cf.* Hevesy and Ottesen, 1943), it also contains fractions of higher activity than that found in the intestinal mucosa, *viz.*, those in the bone marrow, thymus, and lymph nodes (*cf.* Andreasen and Ottesen, 1945). The lymphocytes secreted into the organism can also be expected to contain strongly active nucleic acid. This makes it understandable that the rate of renewal of the average body nucleic acid corresponds to about the rate of renewal of the intestinal nucleic acid, and it is, thus, quite pronounced for both types of nucleic acid in contradistinction to the rate of renewal found in the liver, which is very low in the case of desoxyribosenucleic acid and appreciably higher in the case of ribosenucleic acid.

The rate of turnover of desoxyribosenucleic acid of the organs of the rat does not differ significantly from the turnover rate observed in non-fasting rats (Ahlström *et al.*, 1944). Nor is the desoxyribosenucleic acid P content influenced markedly by the diet administered, as discussed on p. 417. To what extent the rate of turnover of ribosenucleic acid is influenced by the diet is not yet elucidated.

If we accept the view put forward by Brachet, Caspersson and others on the role of ribosenucleotides in the synthesis of proteins (*cf.* also Greenstein, 1944; and Dustin, 1947), the high rate of renewal of ribosenucleic acid is in no way surprising. The high figures found for the rate of renewal of ribosenucleic acid in the intestine, the spleen, and the liver is just what we should expect in view of the importance of these organs in protein metabolism. It may be of interest to recall that the incorporation of labeled sulfur into protein sulfur is found to be higher in the intestine than in any other organ (Tarver and Schmidt, 1942) and the N^{15} content of the proteins isolated from the intestinal wall of the rat after administration of isotopic L(−)-leucine is larger than the corresponding value for any other organ investigated (Schoenheimer, Ratner and Rittenberg, 1939). Somewhat smaller values for the N^{15} content of the proteins isolated from the spleen were found, and still smaller values for the N^{15} of the proteins isolated from the liver. The rate of formation of ribosenucleic acid in these three organs diminishes in the same order.

If we wish to state, not the percentage of nucleic acid that was labeled during the experiment, but the amount of labeled nucleic acid formed during the experiment, we must know the nucleic acid content of the organs of the rat and of the total rat.

Some figures obtained by Hammarsten for the total nucleotide P of the liver, spleen, intestine, and total rat, and also some preliminary figures for the share of desoxyribose- and ribosenucleotides in the total nucleotides are seen in Table XI.

Assuming the percentage renewal of the desoxyribosenucleic acid of the total rat in the course of 2 hours to be 4 (*cf.* 435), and the fresh weight of the rat to amount to three times its dry weight, in a 200 g. rat in the course of 2 hours about 3 mg. of desoxyribosenucleotide P will be renewed. The corresponding figure for the ribosenucleotide P works out to be 6. In the total rat the turnover rate of the 2 types of nucleotides thus does not differ very appreciably.

A very different result was obtained when the amounts of desoxyribose- and ribosenucleotide phosphorus renewed in the liver were compared. The figures work out, if we assume the liver to weigh 10 g., to be 0.004 mg. and 0.2 mg. respectively. Fifty times more ribosenucleotides than desoxyribosenucleotides are thus renewed in the liver during the same time.

To what extent the rate of enzymic replacement of other constituents of the nucleic acid molecule, for example the pyridine and pyrimidine groups, is similar to the rate of the enzymic replacement of phosphorus, is not yet elucidated.

The rate of formation of nucleic acid (especially of desoxyribosenucleic acid in full-grown liver and some other organs) is slow, compared with the rate of formation of other phosphorus compounds. Correspondingly, P^{32} incorporated in the early phases of the experiment is lost in the later phases of the experiment by the nucleic acid fractions at a slower rate than from other phosphorus compounds. If the labeled phosphate is administered at the start of the experiment, which is the usual case, the specific activity of the inorganic P of the plasma and organs successively decreases and in the later phases of the experiment the renewal of organic phosphorus compounds takes place with participation of slightly active inorganic P. In these phases of the experiment, the stream of P^{32} will be directed from the organic compounds into the inorganic phosphate. Because of this fact a prolongation of the time of the experiment over a certain time— over two days, for example, in the case of the Jensen sarcoma— will be disadvantageous for the yield of labeled desoxyribosenucleic acid extracted from the organs.

TABLE XI

Desoxyribosenucleotide Phosphorus and Ribosenucleotide Phosphorus Content of Some Organs and of the Total Rat (Hammarsten and Hevesy, 1946)

Sample	Approximate share of desoxyribosenucleotides in the total nucleotides	g. Nucleotide P/100 g. dry weight	g. Desoxyribosenucleotide P/100 g. dry weight	g. Ribosenucleotide P/100 g. dry weight
	Per cent			
Total rat	45–50	0.232	0.11	0.12
Liver	35	0.350	0.12	0.23
Spleen	75	9.643	0.43	0.16
Intestine	57	0.669	0.38	0.29

A loss of P^{32} from an organic compound in an intact organ can only take place when the compound is not inert, but is being renewed. For example, the avian red corpuscles formed in the marrow of a labeled organism and released into the circulation are, with increasing time, showing a decreasing P^{32} content. Since no mitotic process is taking place in the nuclei of red corpuscles and their desoxyribosenucleic acid is not being renewed, the desoxyribosenucleic acid, in contrast to the other organic phosphorus compounds, retains its P^{32} content throughout the life cycle of the corpuscle. In resting yeast the turnover of ribosenucleic acid in the absence of nitrogen was found by Spiegelman and Kamen (1946) to be quite low and much less than in other phosphate fractions. In the presence of nitrogen in sufficient amount (as ammonium sulfate) to induce assimilation but not growth, the turnover in ribosenucleic acid was increased 8-fold which was much more than the increase of almost any other fraction examined, with the possible exception of protein-bound metaphosphate.

X. Desoxyribosenucleic Acid Turnover and Cell Division

As the nucleic acid content of the cell diminishes during certain phases of the mitotic cycle, and during other phases increases (*cf.* p. 418), cellular division occurring in a medium containing labeled phosphate is bound to lead to the formation of labeled nucleic acid. This conclusion is fully borne out by experimental results. How far the formation of labeled nucleic acid takes place exclusively in connection with mitotic or similar processes is not easy to decide, however.

TABLE XII

Turnover Rate of Desoxyribosenucleic Acid in Organs of Rats of Different Age. Time of experiment, 2 hours. (Ahlström et al., 1944)

Age of rats	Organ	Activity of 1 mg. desoxyribosenucleic acid in percentage of the activity of 1 mg. inorganic P of the organ
3.5 days	Liver	2.0
	Spleen	9.86
5 weeks	Liver	0.15
	Spleen	3.8
3–15 months	Liver	0.14
	Spleen	2.5

The very low rate of desoxyribosenucleic acid turnover observed in the liver of full-grown animals can be interpreted as favoring a very close connection between mitotic activity and turnover rate of this type of nucleic acid. In the growing liver and spleen of 3.5 days old rats, which show a high percentage of mitotic figures, a much larger (14 and 4 times, respectively) desoxyribosenucleic acid turnover is observed than in the liver and spleen of full-grown rats, as was shown by Ahlström and her associates (1944) (*cf.* Table XII).

Brues and associates (1944), in experiments taking one day, found, both in the regenerating liver and in hepatoma, appreciably higher renewal figures than in the normal liver.

Kohman and Rusch (1941) administered labeled phosphate to rats and mice and determined the P^{32} content of nucleoproteins (containing both desoxyribose and ribose compounds) of the normal liver and in liver in which cancer was produced by feeding azo dyes. The tumorous liver, in which a rapid formation of new cells takes place, was found to exhibit an uptake of P^{32} increased by 45% as compared with the controls. They found after the lapse of 3 days the turnover rate of desoxyribosenucleic acid of the regenerating liver to be 18 times that of the resting liver. For ribosenucleic acid the corresponding figure was found to be only 4.

The great difference in the rate of uptake of P^{32} by the cell nuclei of tumors and normal liver found by Marshak (1941) is presumably also due to a large extent to an increased formation of labeled desoxyribosenucleic acid in the nuclei of the growing tissue.

The specific activity of the residual P of liver cell nuclei isolated by Dounce's method, expressed in percentage of the specific activity of the tissue inorganic P was found, in experiments taking 2 hours, (Hevesy 1947) to be much higher (2%) than the corresponding value of desoxyribose P, indicating that the residual phosphorus present in liver cell nuclei cannot be composed solely of desoxyribosenucleic acid.

Recently Marshak (1947) found that, when isolated liver cell nuclei were treated with desoxyribosenuclease, the specific activity of phosphorus which appeared together with purines and pyrimidines in the supernatant fluid had only ⅓ of the specific activity which was found after treatment of the cell nuclei with ribosenuclease or when no enzyme was added. This observation falls in line with the statement made above that, while the specific activity of the desoxyribosenucleic acid P of the liver is very low, the residual P of isolated liver nuclei has a comparatively high P^{32} content. Marshak suggests that the substrate, although nucleic acid, differs from ribosenucleic acid as well as desoxyribosenucleic acid.

In the experiments mentioned above (Ahlström *et al.*, 1944), in the course of 2 hours the increase in the desoxyribosenucleic acid content of the liver of 3.5 days old rats amounted to about 0.9%, while about 2% of the desoxyribosenucleic acid present was found to be labeled. About half of the desoxyribosenucleic acid molecules formed in the course of the experiment is thus due to additional formation of nucleic acid, while the other half is due to the replacement of old molecules by new ones. How far this replacement is confined to a renewal of the phosphate groups only, or also is due to the renewal of other constituents of the nucleic acid molecule, is not yet known.

A similar result to that stated above was obtained by Euler and Hevesy

(1942) in their studies on the formation of labeled desoxyribosenucleic acid in the growing Jensen sarcoma of the rat, where also about half of the labeled nucleic acid formed in the course of 2 hours (about 1.5% of that present) was found to be due to additional formation. These findings suggest that the formation of labeled desoxyribosenucleic acid to a large extent takes place in the course of cell division, the nucleic acid content of both cells formed in the course of the mitotic process from one cell being labeled. A possible interpretation of the above mentioned finding is that the formation of one of these two cells is compensated by disappearance of the mother cell and the number of labeled molecules becomes twice as high as that of the additionally formed molecules.

The comparatively high turnover rate of desoxyribosenucleic acid in the full-grown spleen can be interpreted as being due to the formation by that organ of nucleic acid-containing lymphocytes and other products, and the same applies to the high turnover rate observed in the thymus, lymph glands, and marrow. It is more difficult to interpret the high rate of renewal of the desoxyribosenucleic acid molecules of the intestinal mucosa, but Koller (1947a) found recently a surprisingly high mitotic figure in the intestinal mucosa.

The organ in which cell division is supposed to be absent is the nervous system. As was to be expected, the formation of labeled desoxyribosenucleic acid in the brain tissue is slower than in any other organ (*cf.* Fig. 4). The determination of the turnover rate in histologically controlled material, which would be of great interest in view of the point raised above, is still awaiting investigation.

A conspicuous example of the close relationship between mitosis and turnover rate of desoxyribosenucleic acid is shown by the nucleated red corpuscle. The nuclei of the hen corpuscles do not divide, nor does—in contrast to all other phosphorus compounds—formation of labeled desoxyribosenucleic acid take place in the circulation of a hen containing radiophosphate. We obtain, however, red corpuscles containing labeled desoxyribosenucleic acid if these are formed in a labeled medium, *i.e.*, if the red marrow of the hen contains radiophosphate. On this observation is based a method of determination of the life-cycle of the red corpuscle in the hen.

XI. APPLICATION OF LABELED DESOXYRIBOSENUCLEIC ACID IN THE DETERMINATION OF THE LIFE-CYCLE OF THE RED CORPUSCLE OF THE HEN

Desoxyribosenucleic acid was found by Hevesy and Ottesen (1945) to be a suitable indicator for the determination of the life-cycle of nucleated corpuscles. In experiments *in vitro*, in which hen blood was shaken in an oxygen atmosphere in the presence of labeled sodium phosphate, no active desoxyribosenucleic acid was found to be formed, in contradistinction to other active phosphorus compounds. Furthermore, activity was almost absent in the desoxyribosenucleic acid present in the circulating red corpuscles of the hen up to 5 days after administration of radioactive phosphate.

Labeled phosphate is administered twice a day to the hen in such quantities that the plasma phosphate is kept at a constant, or almost constant, level of activity. The active phosphate penetrates into the marrow and participates in the formation of the nucleic acid of the corpuscles, which thus become labeled. The percentage of labeled corpuscles increases with time, and finally the circulation will contain labeled corpuscles only. Thus, the activity of 1 mg. corpuscle desoxyribosenucleic acid phosphorus will be equal to the activity of 1 mg. marrow inorganic phosphorus and 1 mg. plasma inorganic phosphorus, respectively.

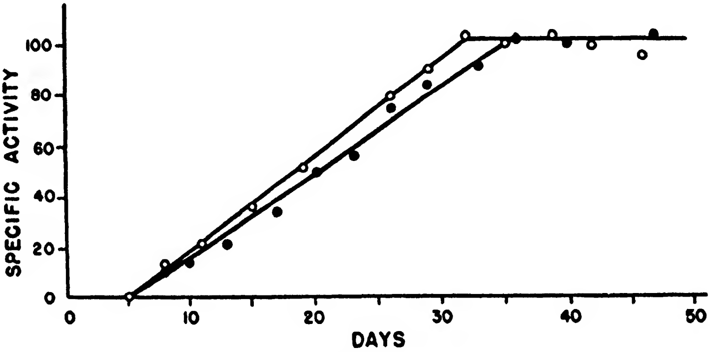

Fig. 5

Life-cycle of the red corpuscles of two hens. Abscissae: days after start of experiment; ordinates: specific activity of desoxyribosenucleic acid phosphorus extracted from the corpuscles secured at different dates. (Hevesy and Ottesen, 1945).

The results of such experiments are shown in Fig. 5, which makes it clear that in the first 5 days the nucleic acid present in the corpuscles is inactive. This may be interpreted by assuming that, in the first phase of the experiment, corpuscles containing inactive nucleic acid reach the circulation, and that it is about 5 days before corpuscles containing labeled nucleic acid are given off by the sinusoids to the circulation. The maturing of the corpuscles in the marrow thus takes about 5 days. The figure also shows that after the lapse of about 33 days the maximum value of the activity of the desoxyribosenucleic acid is reached. If we take into account that in the first 5 days no labeled corpuscles intrude into the circulation, the lifetime of the red corpuscles will be 28 days. The results obtained indicate that all, or almost all, corpuscles present in the circulation have a similar lifetime.

Ottesen (1946) obtained lifetime values almost identical with those stated above by the following simplified method. Labeled sodium phosphate is administered to the hen at the start of the experiment only. In such experiments the initially high activity of the plasma is rapidly declining. Consequently, the erythrocytes formed during the first days of the experiment contain highly active desoxyribosenucleic acid, while this compound present in the later formed corpuscles has only a restricted activity. The radioactive desoxyribosenucleic acid content of the circulation is increasing during the first 3 weeks, remains almost constant in the following few days, and then declines markedly. This marked decline is due to the fact that the first formed, highly active corpuscles are now decaying. From the data of rapid decline the lifetime of the red corpuscles can be computed.

TABLE XIII

P^{32} Uptake by Nuclei (Marshak and Walker, 1945)

Substance injected	Per cent of dose/ g. of nuclei	Per cent of liver P^{32} in nuclei
Inorganic phosphate	1.54	2.1
Rat chromatin	5.08	1.2
Rabbit chromatin	5.06	1.3
Rabbit chromatin soluble in 1 *M* NaCl	4.23	1.6
Fat-free chromatin (rat)	5.45	1.6
Lipide (rat chromatin)	5.15	1.0

XII. INTRAVENOUS TRANSFER OF P^{32} FROM CHROMATIN TO HEPATIC TISSUE

Marshak and Walker (1945) have injected labeled chromatin into the circulation of rats weighing 50 g. after partial hepatectomy and found the liver nuclei to retain much more P^{32} than after the injection of labeled free phosphate of the same activity; this may be seen in Table XIII. Another result is obtained if the P^{32} content of the nuclei is compared with the P^{32} content of the liver tissue. This comparison is also seen in Table XIII. This is another example of the enhanced uptake of phosphorus compounds foreign to plasma by the liver.

If free labeled phosphate is split off in the liver by enzymatic action from labeled chromatin, we should expect to find an enhanced formation of labeled compounds in the nuclei corresponding to the high P^{32} level maintained in the liver.

That labeled desoxyribosenucleic acid, intravenously injected into rats weighing 100-200 g., rapidly splits off labeled phosphate in the liver has been shown by Ahlström and her associates (1946b). Some of their results are seen in Table XIV.

After the lapse of 2 hours an appreciable part of the labeled phosphorus is found to be present in the liver, more than ¾ being located in the acid-soluble fraction.

In experiments in which liver slices were incubated in bicarbonate Ringer solution containing labeled desoxyribosenucleic acid the phosphate group of more than ⅔ of the nucleic acid added was found to be split off in the course of 4 hours, ⅘ of the total acid-soluble P^{32} being present in the inorganic phosphate fraction of the Ringer solution.

While it is probable that most of the labeled phosphate present in the nuclei after intravenous injection of labeled chromatin is carried into the nuclei as inorganic phosphate, the possibility of the incorporation of some nucleoprotein into the nuclei cannot be entirely excluded. McCarty and his colleagues (1946) have shown that pneumococci which in artificial culture have lost the capsules endowing them with virulence and containing the specific polysaccharides, have reverted to avirulent non-specific types..Such degenerate, non-specific pneumococcus, from whatever specific type it had its origin, could be induced by cultivation in a medium prepared from a quite virulent type to reacquire a capsule which conferred the corresponding specificity.

TABLE XIV

Percentage P^{32} Introduced as Labeled Desoxyribosenucleic Acid into the Circulation Present in Plasma and Different Organs after the Lapse of 2 Hours (Ahlström et al., 1946b)

Rat	Plasma	Liver free P	Liver acid soluble P	Liver total P	Spleen total P	Kidney total P	Plasma (present as inorganic P)
I	2.2	11.0	14.5	18.0	0.79		0.81
II	8.8	5.6	7.4	9.4	0.48		0.34
III	1.7	8.2	10.6	14.3	0.92		0.57
IV	1.9	8.6	12.1	16.0	0.65		0.58
V	1.1	6.8	9.2	13.8	0.66	2.80	0.56
VI	1.0	3.7	5.5	8.7	0.38	1.46	0.30
VII	0.32	4.3	6.2	9.0	0.44	1.39	0.25
VIII	0.78	6.1	8.7	10.4	0.77	2.04	0.37
IX (blood	3.5	10.2	13.4	20.7	0.85	4.7	
X (blood)							
XI (blood)	2.2	9.5	11.9	18.8	0.75	3.7	
XII (blood)							

Recently, Avery and his colleagues (1944) were able to isolate and to characterize a chemical principle acting in minute dosage as the specific stimulus to such a transformation. The substance inducing the transformation was found to be a nucleic acid of the desoxyribose type. It is possible that this substance enters the nucleus as such and that we meet here with one of the very few cases in which the organism avails itself of phosphorus compounds of high molecular weight and exogenous origin in fundamental synthetic processes.

XIII. Studies of Virus Reproduction with P^{32} as a Tracer

Despite the fact that the introduction of one unit of a virus within a living cell of a susceptible host is followed by the production of millions of virus units, practically nothing is known concerning the reproductive process. It seemed possible that, with the preparation and isolation of tobacco mosaic virus containing radioactive phosphorus, the inoculation of plants with virus marked by radioactivity would provide, following extensive multiplication of virus in the diseased plants, some measure of information concerning the process of virus reproduction. This line of thought induced Stanley (1942) to investigate the radioactivity of Turkish tobacco plants inoculated with labeled tobacco virus. Similar investigations were also carried out by Born and his associates (1941).

When mosaic-diseased Turkish tobacco plants were fed a nutrient solution containing radioactive phosphorus in the form of disodium phosphate over a period of several weeks, about 30% taken up by the plants was isolated by Stanley in the form of purified tobacco mosaic virus. The tobacco mosaic virus, growing at a rapid rate in contrast to the plant, is bound to take up a large percentage of the labeled phosphate that reaches the plant. While an organ or a substance grown in a labeled medium becomes labeled throughout, parts of an already grown organism only become labeled by enzymatic interchange, the rate of which varies greatly with the organ and the compound in question.

The virus containing radioactive phosphorus, to a large extent present as a constituent of the ribosenucleic acid molecule, was rubbed into the lower leaves of Turkish tobacco plants. After the lapse of 12 days the lower inoculated and the upper uninoculated leaves were investigated. The results obtained are seen in Table XV.

In Stanley's experiments (as seen in Table XV) most of the radioactivity was found to be associated with non-virus components in both inoculated and uninoculated portions of the plants. Hence, it was impossible to be certain that the small amount of radioactive virus found in the uninoculated portions of the plants arose as a result of actual movement of inoculated virus. In view of these results it is exceedingly difficult to distinguish between P^{32} taken up by the plant in the form of virus and that taken up in the form of virus disintegration products.

XIV. ENZYMATIC STUDIES WITH LABELED DESOXYRIBOSENUCLEIC ACID AS A SUBSTRATE

Ahlström *et al.* (1947) have applied labeled desoxyribosenucleic acid as a substrate in enzymatic studies. The labeled desoxyribosenucleic acid was extracted from a Jensen sarcoma or the organs of the rat to which labeled radiophosphorus had been administered a few days previously. The substrate was added to blood plasma, intestinal mucosa extract, or sarcoma extract, and the percentage of P^{32} split off at different times as inorganic P and as acid-soluble phosphorus, respectively, was determined.

In the usual phosphatase studies the increment of the inorganic phosphate content of a system under enzyme action is measured. When one makes use of a labeled substrate and determines the radioactivity of the inorganic phosphate split off, the presence of inorganic (non-radioactive) P in the system previous to the addition of the substrate—as is often the case— does not interfere with the measurement of the inorganic P split off. Apart from this advantage, by using a highly active substrate a very great sensitivity in the determination of the inorganic P split off can be obtained. In the above mentioned experiments, in which about 0.5 millicurie was administered to the rat, a substrate containing about 100γ of P was added to the system. By administering stronger P^{32} samples, the sensitivity of the method can be increased correspondingly.

TABLE XV

Distribution of P^{32} in Turkish Tobacco Plants 12 Days Following Inoculation of Lower Leaves with 58 mg. of Labeled Tobacco Mosaic Virus (Stanley, 1942)

Fraction	Relative Activity Figures
Virus isolated from inoculated leaves	8.3
All material of inoculated leaves except virus	33.4
Virus isolated from uninoculated leaves	5.8
All material of uninoculated leaves except virus	52.5

XV. EFFECT OF ROENTGEN RAYS ON THE FORMATION OF LABELED DESOXYRIBOSENUCLEIC ACID

Euler and Hevesy (1942) have found the percentage of labeled desoxyribosenucleic acid formed in the growing Jensen sarcoma to be diminished by irradiation with Roentgen rays. When, following irradiation with 2000 r, a tracer dose of labeled phosphate was administered by subcutaneous injection to the rat, the percentage ratio of the specific activity of the desoxyribosenucleic acid P and the inorganic acid P of the sarcoma was found to be only 0.65, while in the controls a value of 2.05 was found. These figures are mean values obtained in a great number of experiments. Were the permeability of the cell to phosphate influenced by this irradiation, we should expect corresponding differences between the ratio of the specific activity of the desoxyribosenucleic acid P to the inorganic P of the sarcoma tissue and the corresponding ratio for desoxyribosenucleic acid and plasma. No appreciable difference is found, as seen in Table XVI, which leads one to the conclusion that the diminished formation of labeled desoxyribosenucleic acid following irradiation is not at all, or not mainly, due to a decreased phosphate permeability of the irradiated cells, but rather to a decreased rate of formation of desoxyribosenucleic acid molecules.

Correctly, one should compare the specific activities of the desoxyribosenucleic acid P with that of the intracellular inorganic P, but in experiments taking 2 hours, when organs of such great phosphate permeability as the sarcoma are considered, the specific activity of the intracellular inorganic P does not differ essentially from the specific activity of the total tissue inorganic P. In view of the high phosphate permeability of the sarcoma a possibly higher permeability of the dividing cells than that shown by the average cell would not greatly influence the results obtained.

TABLE XVI

Effect of Roentgen Rays on the Formation of Labeled Desoxyribosenucleic Acid in the Jensen Sarcoma of the Rat. Labeled Phosphate Administered a Few Minutes after Irradiation. The Rats were killed 2 Hours Later. (Hevesy, 1945)

	Specific activity of the desoxyribosenucleic acid P as a percentage of the specific activity of the inorganic P			
	sarcoma	plasma	ratio control : irradiated	
			sarcoma	plasma
Control and (H E)	2.05[a]	1.93	3.16	3.06
Irradiated with 2000 r	0.65	0.63		
Control (A. *et al.*)[b]	1.89	1.44	2.83	2.84
Irradiated with 450–1500 r	0.67	0.68		

[a] In later experiments 2.17 was found.

[b] Rats with 2 sarcomata.

When calculating the percentage formation of nucleic acid during the experiment taking 2 hours from the percentage ratio of the specific activity of the nucleic acid P to that of the cellular inorganic P in the sarcoma, we must take into account that the activity of the inorganic P changes during the experiment, the mean value which we have to consider in our calculations being about ⅓ higher than the experimentally determined end value. The percentage formation of desoxyribosenucleic acid is thus about ⅓ higher than 2.05 and 1.89, respectively, as indicated in Table XVI, and works out to be about 2.7% in the course of 2 hours. As the desoxyribosenucleic acid concentration of the sarcoma has been found to be about proportional to the weight or volume of the latter, the percentage formation of additional desoxyribosenucleic acid in the sarcoma can be calculated from the percentage increase in the volume of the growing sarcoma in the course of 2 hours. The figure works out to be 1.5, almost half of the percentage value for the labeled nucleic acid formed. Since that number of newly formed (labeled) nucleic acid molecules is thus larger than the number to be accounted for by the absolute increase in the weight of the sarcoma, a simultaneous disappearance of a corresponding number of "old" nucleic acid molecules must be taking place. It is tempting to interpret the above result as due to the fact that the dividing mother cell gives rise to the formation of two labeled cells, as the mother cell is disappearing simultaneously. The increase in the number of nucleic acid molecules present makes up only half the number of labeled nucleic acid molecules formed. This interpretation assumes much of the nucleic acid turnover to take place in the dividing cells.

In experiments in which two sarcomata were inoculated into a rat and one was unshielded while the other was protected from the direct action of Roentgen radiation, the turnover rate of nucleic acid in the protected sarcoma was found by Ahlström *et al.* (1945) to be diminished as well, though to a lesser extent than observed in the non-protected tumor. This result suggested an investigation as to whether transfusion of blood from a strongly irradiated animal influences the turnover rate of desoxyribosenucleic acid in another animal (Ahlström *et al.*, 1946a). In experiments carried out on rabbits the turnover rate of the desoxyribosenucleic acid extracted from the kidneys was found to be slightly lower after transfusion of blood from an irradiated sister rabbit than when blood of a non-irradiated rabbit was transfused. An indirect effect of radiation on the formation of labeled desoxyribosenucleic acid in the mammary carcinoma of the mouse is described in Section XVI.

Roentgen radiation was also found to diminish the rate of formation of labeled desoxyribosenucleic acid in the liver, kidney, spleen, and intestinal mucosa to about the same extent as in the Jensen sarcoma, as shown by Ahlström and associates (1944) and as seen in Table XVII.

The percentage effect of irradiation on the formation of desoxyribosenucleic acid molecules in the normal organs of adult rats is thus similar in magnitude to the effect of Roentgen rays on the growing Jensen sarcoma listed in Table XV.

When irradiating the whole body of the mouse with 1670 r during 105 minutes, injecting labeled sodium phosphate, and sacrificing the animal 15 minutes later, H. Jones (1946) found a decrease in the formation of labeled nucleic acid in the liver of about 50%.

Roentgen rays were found to block the formation of nucleic acid in the organs of rapidly growing rats also. The percentage inhibition of the desoxyribosenucleic acid turnover was found to be similar to that found in normal organs of adult animals and in their Jensen sarcomata.

The ratio of newly formed desoxyribosenucleic acid in the organs of controls to that in the organs of irradiated rats was found to be 2.3 in experiments taking 2 hours, in which 3–4-day old rats were irradiated with doses of 2000–2250 r previous to administration of labeled phosphate. (Ahlström and associates, 1944).

In experiments in which a tracer dose of P^{32} was administered to hens after irradiation with a few hundred r, the desoxyribosenucleic acid extracted from the erythrocytes after the lapse of a few days has been found to contain much less radiophosphorus than the desoxyribosenucleic acid extracted from the red corpuscles of the controls. With increasing time this difference gradually diminishes (Hevesy and Ottesen, 1946). Thus, we can follow the action of X-rays on animals with nucleated red corpuscles by making use of the tracer technique.

TABLE XVII

Ratio of Newly Formed Desoxyribosenucleic Acid Molecules Present Before and After Irradiation in the Organs of Adult Rats (Ahlström et al., 1944)

Dose applied 1480–3000 r. Rat irradiated *in toto*. P^{32} injected after the irradiation. Rat killed 2 hours after administration of P^{32}.

Organ	Ratio of newly formed nucleic acid in the organs of controls and of irradiated rats
Liver	3.3
Spleen	2.4
Intestinal mucosa	2.3

It is the rate of formation of desoxyribosenucleic acid that Roentgen rays influence, while the disintegration of desoxyribosenucleic acid by irradiation of Roentgen rays is negligible. Nucleoproteids isolated from thymus cell nuclei when irradiated with up to 65,000 r were found (Euler and Hahn, 1946) to have the same composition and the viscosity as shown by nucleoproteids isolated from non-irradiated nuclei.

In the experiments of Sparrow and Rosenfeld (1946) solutions of 0.2% sodium thymonucleate in water and 0.4% thymonucleohistone in 1 *M* NaCl were irradiated. Viscosities of the solutions were found to be diminished under the action of 7500–120,000 r. Equal dosages cause a greater drop in viscosity of the nucleate than of the thymonucleohistone. This result indicates a greater resistance of nucleohistone to Roentgen rays, although it is not impossible that the presence of sodium chloride in the solution of thymonucleohistone exerts a protective effect. From the work of Dale (1940, 1942, 1943) and Forssberg (1946) we know that the effect of Roentgen rays on a dissolved substance may be appreciably diminished by the presence of other substances.

Errera (1946) irradiated with a few 1000 r viscous solutions containing nucleoproteins obtained by extracting cell nuclei and found their viscosity to decrease under the effect of irradiation. A similar effect was observed when avian red corpuscles were irradiated previously to extraction.

The observed diminution of viscosity and of the intensity of flow birefringence indicates a degradation or partial depolymerization of high molecular particles initially present into shorter, more symmetrical chains or segments.

A slight decrease in the desoxyribosenucleic acid content of rat and mouse tumors following irradiation with 4000 r was observed by Stowell (1945a). The relative desoxyribosenucleic acid content of transplantable mammary carcinomata in rats and mice was measured by means of the Feulgen reaction and a special microphotometric apparatus. The tumors on one side of the animal were irradiated, while those of the other side were protected by lead shielding and used as controls. The desoxyribosenucleic acid content of the irradiated rat tumors was 13% lower than that of the non-irradiated tissue. For the mouse tumor a decrease of 5% was observed.

After Roentgen ray irradiation *in vivo* of carcinoma tissue Mitchell (1942) observed a significant increase in the ultraviolet absorption of the cytoplasm. The absorbing material appearing after irradiation is not desoxyribosenucleic acid, as shown by the Feulgen reaction. Mitchell interprets the result as indicating an increased formation of ribosenucleic acid. His view is (*cf.* Davidson and Waymouth, 1944a) that ribosenucleotides are formed in the cell from unknown precursors and are reduced to desoxyribosenucleotides which finally are polymerized and deposited as desoxyribosenucleic acid. The reduction of ribosenucleotide to desoxyribosenucleotide is assumed to be inhibited by Roentgen rays with consequent accumulation of ribosenucleotide, which may polymerize to ribosenucleic acid. The application of labeled nitrogen and carbon will, it is hoped, lead to an elucidation of the mechanism of the formation of nucleoproteides and also make possible a test of the above mentioned hypothesis.

XVI. EFFECT OF BETA-RAYS EMITTED BY RADIOACTIVE COLLOIDS TAKEN UP BY LIVER ON THE FORMATION OF LABELED DESOXYRIBOSENUCLEIC ACID

As described on p. 447, in experiments in which two sarcomata were inoculated into a rat, one was shielded while the other was protected from the direct action of Roentgen radiation. The turnover rate of nucleic acid in the latter sarcoma was found to be diminished as well. This result induced H. Jones (1946) to investigate the existence of an indirect effect of irradiation on the formation of desoxyribosenucleic acid in the mammary carcinoma of the mouse. When radioactive chromic phosphate is injected into the mouse, approximately 90% of the material is found by H. Jones *et al.* (1944) to be taken up by the liver, the bulk of the remaining fraction being found in the spleen and in the lungs. The accumulation of the active chromic phosphate brings about an intensity of β-radiation in the liver and spleen which is of the order of 100 times the concentration of other tissue.

Chromic phosphate suspensions varying from 0.1 to 1.0 millicuries per mouse of radioactive phosphorus were injected intravenously. The animals were sacrificed when approximately 3400 r had accumulated in the livers during a period of 4 to 48 hours. Two hours prior to the time of sacrifice, the animals were administered a small amount of radioactive sodium phosphate (10 microcuries) intravenously. In some of the animals, the total duration of the experiment was four hours where the dose of radioactive chromic phosphate was high. In all cases, independent of the time allowed for the accumulation of radiation to 48 hours, the results were the same, showing in all cases a depression of desoxyribosenucleic acid synthesis in the tumor coincident with the β irradiation of the liver.

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Author Index

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