Biochemical Evolution

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FOREWORD

It is the author's object to show that evolution and the classification of animals can be considered from a biochemical viewpoint; in other words, that systematic biochemical characteristics exist. The idea that it is possible to superimpose upon the zoological classification, established by morphologists, a biochemical classification is by no means acceptable a priori. If demonstrated, it will support those who see in the variety of animal forms nothing but a reflection of their biochemical differences. It will also aid in fitting the study of animal evolution into a biochemical framework and in ridding it of vague and more or less theological concepts by which it is encumbered. Having demonstrated the fundamental unity of the biochemical plan, it will be necessary to establish the principles for erecting a taxonomy of biochemical characteristics which differentiate the metazoa, a new argument in favor of organic unity and, therefore, of the theory of evolution. The last chapter sums up some inferences which are still largely hypothetical but which give a clue to the task yet to be accomplished, and which each can judge according to his own taste.

M. F.

TRANSLATOR'S PREFACE

Since Darwin's time "evolution" has attained great prominence as the basic idea in practically every field of intellectual endeavor. Indeed, evolution had become the guiding concept in man's thinking during the nineteenth century and thereafter. But the juxtaposition "biochemical evolution" is a relatively young offshoot of the venerable tree, the theory of evolution. As a matter of fact, very few biologists and still fewer biochemists or physiologists are probably alive to the existence or the deep meaning of this relationship, and all will discover a wealth of information in this little volume that will delight them and materially enlarge their respective horizons.

Some may object that the title is overambitious, if not actually presumptuous, and beyond the true measure of the book's scope, inasmuch as it deals with only half of the biological world. One can easily concede this point but only in so far as the criticism pertains to the context of this book; it does not affect the broad and valid idea embodied here. It is, of course, unfortunate that the factual material is drawn entirely from the animal kingdom, but we hope that the idea will inspire a competent botanist to write a companion volume based upon factual material furnished by the plant kingdom, which may tell an even more fascinating story.

Personally, I owe a debt to Professor Florkin for generously leaving to my discretion what material should be omitted from or added to the text. And I am also glad to say that he read the manuscript and approved the English version wholeheartedly.

The most persistent difficulty encountered in the preparation of this volume was the nomenclature. Those who very kindly helped me are too numerous to mention individually, and I will merely say that I thank each and all of them very sincerely. I hope that no serious errors have crept into the text but if some are discovered I can only beg the reader's indulgence.

S. MORGULIS

November 6, 1948
Omaha, Nebraska

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ERRATA

p. 5, line 23, for *polygases* read *polyases*

p. 34, in table, for *Aplisia* read *Aplysia*

p. 34, in table, for *Cepia* read *Sepia*

p. 35, in table, for *lumpus* read *lupus*

p. 38, line 26, for *cannot* read *can*

p. 47, in table, for *Lamperta* read *Lampetra*

p. 52, in table, for *Calorcaris* read *Calocaris*

p. 75, line 27, for *Paliedina* read *Paludina*

p. 101, legend to Fig. 20,

for "solid circles" read "open circles"

for "open circles" read "solid circles"

p. 102, line 26, for *mammillata* read *mamillata*

p. 106, line 37, for *Lamperta* read *Lampetra*

p. 154, for *Callinectis* read *Callinectes*

for *Calorcaris* read *Calocaris*

for *Gosterosteus* read *Gasterosteus*

p. 155, for *Prodentia* read *Prodenia*

for *Protopterus acthiopicus* read *Protopterus aethiopicus*

p. 156, for *Vermetur* read *Vermetus*

p. 156, under crustacea, for *Portunus perber* read *Portunus puber*

p. 157, under vertebrata, for *Petromyzon fluratilis* read *Petromyzom fluviatilis*

"Our final theory of evolution will see"
it largely as a biochemical process.

J. B. S. HALDANE

CHAPTER I

Unity of the Biochemical Plan of Animals

Among animals, no matter what their position may be in the zoological classification, one finds indubitable biochemical similarities that express a unity of biochemical design in the animal organization. It would be indeed surprising if it were otherwise, since all animals consist of cells sharing biochemical properties, which constitute the field of general biochemistry. For instance, it is a common property of living matter that it consists, to a large extent, of water. Water makes up a large proportion of the mass of the body: 88% of the meat of the oyster, 77% of the weight of the frog, 60–74% of that of the mouse. Analyses of the bodies of most diverse animals always reveal the presence of a certain proportion of mineral matter. The presence of inorganic substance seems to be a general condition of life. This pertains not only to rare or exceptional constituents but to the very common substances which are widely distributed in the biosphere. In all animals by far the largest portion of the mineral components of protoplasm consists of chlorides, sulfates, phosphates, and bicarbonates of potassium, calcium, magnesium, and sodium. One also finds small quantities of interstitial fluid or tissue lymph between the cells of Metazoa representing, so to speak, an aquatic medium in which they live. But there are other biological fluids as well. Thus, in echinoderms there is a fluid in the coelomic cavity surrounding the viscera; another fluid in the system of the sinus and lacunes, and still another fluid in the ambulacral apparatus. The cavity of the body of worms contains coelomic fluid. In the higher representatives of this group one also finds a closed system containing a real circulating fluid, the blood. In arthropods the generally large body cavity consists of a primary and a secondary cavity (coelom) merged into a single hemocoel through the fusion of the mesenteries. In this hemocoel the blood, which bathes the tissues directly, is propelled by a heart. In mollusks, too, the circulation is partly vascular and partly lacunal, and the blood bathes the tissues directly, except in the case of the cephalopods, in which the blood passes from the arteries into a closed system of capillaries and then into the veins. In the vertebrates, besides the closed circulatory system containing the blood there is also the lymphatic system, a closed system of canals containing lymph. The latter originates in the interstitial fluid, which itself is formed from the contents of the blood capillaries. The lymph circulating in the lymphatic system finally returns to the blood system. No matter how complicated the ana-

tomical structure containing the biological fluid is, the true *milieu intérieur* (internal environment or medium) is always represented by the interstitial fluid. However, the relative composition of the different biological fluids of an animal is so similar that we may consider these fluids, as Claude Bernard does, as representing the *millieu intérieur* of the cells. No matter which *milieu intérieur* fluids we take, they are invariably aqueous solutions of inorganic substances and, no matter what zoological group we consider, the most common inorganic constituents found in these fluids are invariably the cations sodium, potassium, calcium, and magnesium and the anions chloride, sulfate, phosphate and bicarbonate. The same can be said about sea water. It is obvious that much fault can be found with the suggested similarity between the biological fluids of animals and of such an important constituent of the biosphere as the ocean, since the oceans cover three-quarters of the globe's surface. Nevertheless, the residue, remaining after the water surrounding the animal tissues has been evaporated, consists only to a small extent of inorganic molecules which are remarkably similar throughout animal life. The same applies also to the organic substances which are the most important constituents of the dry residue. In fact, no matter to what zoological group the animal under consideration belongs or in what stage of its ontogenetic development it is, the large mass of material recovered from these animals belongs to a small number of organic categories, viz., lipides, glucides, and proteins. As the few data in Table I

TABLE I

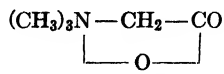
Per cent of Dry Residue

Animal	Protein a	Lipide b	Glucide c	Total a + b + c	References
Paracentrotus lividus (sea urchin)					Ephrussi and
Unfertilized egg	66.9	21.2	5.4	93.5	Rapkine (74)
12-Hour larva	63.7	19.5	5.4	88.6	Idem
40-Hour larva	60.6	17.4	3.4	81.4	Idem
Ostrea edulis	51.2	11.1	28.2	90.5	Atwater (cf. 57)
Bombyx mori (silk worm)					
Egg	56.0	19.2	7.0	80.2	Kawaze (cf. 135)
Larva (newly emerged)	55.5	13.3	1.8	70.7	Idem
Nymph, first day	52.3	31.1	8.6	92.0	Yonezawa and Yamafuji (286)
Imago	63.4	24.3	6.5	94.2	Idem

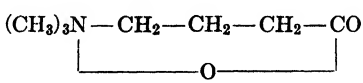
show, a strikingly similar condition is revealed in the composition of animals therein described, regardless of the stage of their development or the zoological group which they represent.

In summing up, we observe throughout the whole animal kingdom a fundamental biochemical plan common to all its members; the organism always contains water as its predominant component. In the dry residue the largest proportion of constituents is always made up of a mixture of proteins, lipides, and glucides, while the inorganic moiety always consists predominantly of chlorides, sulfates, phosphates, and bicarbonates of sodium, potassium, calcium, and magnesium.

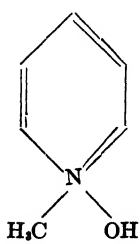
In the study of simple nitrogenous bases, which represent a very small part of the constituents of the organism and which are not included among the substances to be considered below, one recognizes molecules which at first were thought to throw light on the position of organisms in the animal series. The progress of biochemical study of these substances (Kutscher and Ackermann 156, 157) shows, however, that they are not at all characteristic of any one class and that their distribution is indeed very wide. It is true that glycine-betaine was first discovered in echinoderms, but it was rediscovered again in worms, crustaceans, cyclostomes, and fishes.



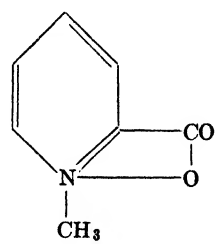
Glycine-betaine



γ-Butyrobetaine



N-Methylpyridine



Homarine

γ-Butyrobetaine was discovered in the tissues of coelenterates, mollusks, crustaceans, fishes, reptiles, and mammals. *N*-Methylpyridine was found in coelenterates, mollusks, crustaceans, and mammals. And as for homarine, which at first was considered a typical component of the lobster, it, too, was found later in the lamellibranch *Arca* and in the sea urchin *Arbatia pustulosa*.

In the preceding we confined ourselves to a consideration of the static chemical composition of animals determined at a given moment. But in the flow of time innumerable processes unroll themselves within each animal which furnish energy for the work of the cell, for the formation of its protoplasm, and for the other phenomena of life. For instance, there is a continuous exchange between the animal and its environment whereby it secures its nourishment. It is frequently difficult to establish within living matter a clear distinction between the plastic components, which are the wheels of the machine, and those which are the depots of reserve material. Neither from the morphological nor from the biochemical point of view are the engine and its fuel sharply demarcated from each other. Herbert Spencer in his *Principles of Biology* had already proclaimed this remarkable property of living matter and the capacity of its constituents of being employed in nature in two different ways. The molecules of living matter should possess a certain *stability*, because they must play the part of construction material, but they must also have a certain instability or lability to be subject to mechanisms of degradation, so that their chemical energy may be transformed into work. This, as well as the unity of the organic composition previously defined, determines that the plastic material of one animal can serve as the nutrient of another. This condition is very essential to the economy of the world and is also an expression of its organic unity. In the living world the current of matter is possible because, at the cellular level, all organisms are nourished by the same foods which we may designate as *cellular nutrients*, namely, the amino acids, glucides, and fatty acids. Here, the cells in the course of catabolic reactions utilize these packages of chemical energy for cellular work.

The cells utilize the same molecules in their *anabolism*, and construct, by assimilative processes, their characteristic cellular fabric as well as the molecular associations which enter the protoplasmic structure. As Claude Bernard said, "Animals and plants live identically, but function differently." The cells of both animals and plants utilize the same categories of organic molecules for nourishment. But in plants chlorophyll, thanks to the activity of green organs, permits the synthesis of reserves of complex molecules from water, inorganic substances, and solar energy. The sap, which is a saline solution, reaches the organs where the syntheses by chlorophyll of polymerized glucides as well as of proteins occur. Liberated from these depots with the aid of hydrolyzing enzymes, sugars and amino acids are then transported by the sap to the cells as nourishment. Contrary to green plants which are autotrophic, animals are generally heterotrophic. The food of animals consists of the substance of other organisms, plant or animal, in which the completely fabricated complex molecules are already found (especially proteins, glycerides, and polymerized glucides).

The activity of their digestive enzymes liberates the cellular nutrients, which are again transported to the cells. The preparation of cellular nutrients from the food obtained from the outside environment results from the action of a system of digestive enzymes that possesses remarkable uniformity throughout the animal kingdom. This enzymatic system consists of hydrolases, or agents of hydrolytic cleavage, which fall into groups corresponding to the principal foods.

Generally speaking, the digestion of *proteins* in animals is accomplished by a series of enzymes each of which is specific for a particular linkage in the protein molecule. These enzymes (proteases) are represented at least by the following four categories:

1. *Proteinase*, an enzyme which catalyzes the hydrolysis of proteins to polypeptides;
2. *Carboxypeptidase*, an enzyme which cleaves a polypeptide by splitting off amino acids with a free carboxyl group, and dipeptides;
3. *Aminopolypeptidase*, an enzyme which cleaves a polypeptide by splitting off an amino acid with a free amino group, and dipeptides;
4. *Dipeptidase*, which splits dipeptides into the corresponding amino acids.

The existence of these four substances with different functional specificities is generally revealed in the digestive tube of Metazoa. The hydrolysis of sugar polymers is accomplished by the glucosidases represented by *oligases* which act on glucosides, and *polygases* which act on highly polymerized compounds such as starch and glycogen. It is possible to demonstrate in the system of digestive enzymes of the most diverse animals that oligases (α- and β-oligases) are widely distributed in the Metazoa. This is also true for α-amylase. The system of digestive enzymes of animals very generally also contains *esterases*, one lipase accomplishing the hydrolysis of esters of alcohols and fatty acids, particularly of glycerides.

Once they are delivered to the cells by the fluids of the internal medium, the constituents of cellular nutriment, which have been prepared from foods by the enzymatic system common to different animals, are now subjected to metabolic transformations. The degradation of the chemical energy of the nutrient accompanying its transformation into work progresses according to the same plan in the different groups of animals. The metabolism of glucides is directed to the acceptance of oxygen whereby they are transformed into water and carbon dioxide. In the course of metabolism, trioses resulting from the action of an aldolase on phosphorylated hexoses are dehydrogenated, giving rise to pyruvic acid and hydrogen. If oxygen is present in the cells, the latter passes through a series of transmitters starting with oxaloacetic acid and finally combines

with the oxygen to form water. The pyruvic acid undergoes an oxidative decarboxylation, in which pyruvic dehydrogenase intervenes and oxygen acts as the acceptor. Pyruvic acid is thus transformed to acetic acid which in turn is degraded to water and carbon dioxide. But in the absence of oxygen the dehydrogenation of triose operates with pyruvic acid as the hydrogen acceptor, the pyruvic being reduced to lactic acid, as is the case in lactic acid fermentation. The fatty acids are metabolized in the cells principally by a mechanism of β-oxidation, consisting of a series of dehydrogenations and hydrations which, in so far as the natural fatty acids are of even carbon numbers, leads to the formation of acetic acid, the latter again being degraded to water and carbon dioxide. Under normal conditions of aerobiosis these different metabolic processes are accomplished with the intervention of oxygen which functions as a hydrogen acceptor. The function of oxygen as an acceptor is accomplished in the animal cell by means of a system of hydrogen transmitters composed of a series of four carbon acids. It is accomplished also through a series of electron transmitters, terminating with an oxidase which transfers the electrons to oxygen. The oxygen then combines with H-ions to yield water. This system contains cytochrome oxidase and can be represented schematically as follows:

$O_2 \rightarrow$ Oxidase $\rightarrow$ Electron carriers $\rightarrow$ Hydrogen carriers $\rightarrow$ Dehydrogenase $\rightarrow$ Substrate.

This process can be inhibited by cyanide. The study of the toxic action of cyanide on the respiration of various animal cells shows that, besides this system of functional substances concerned especially with glucide metabolism, there is a second system which accomplishes a less important but very constant respiratory action in the most diverse animal cells. This second system involves the participation of the yellow enzyme and, according to different authors, takes care of fatty acid metabolism. The fact that the quantity of oxygen consumed per milligram of dry tissue per hour in the presence of cyanide is of the same order of magnitude for animals of the most diverse origin (rat kidney, 0.8–2.5; rat liver, 0.2–1.7; oyster gills, 0.5; *Tenebrio molitor* larvae, 1.1) constitutes a new general property of different groups of animals.

Molecular oxygen, utilized as a hydrogen acceptor in respiration, enters the cells by simple diffusion from the external environment and this mechanism is efficient so long as we are dealing with sufficiently small organisms. But, as the volume of the animals increases, the general mechanism of transport becomes more complicated. Oxygen now enters the interior through certain specialized surfaces (gills, lungs, trachea, etc.) which are in contact with the external environment, and the oxygen is transported from the surface to the cells by means of special molecules, the *carriers of oxygen.*

In the course of functional differentiation of the Metazoa tissue cells frequently appear which are specialized for contraction (the muscle cells). Whatever the nature of these cells, the contraction of striated or of smooth muscles of vertebrates and invertebrates is always the result of the function of the particular protein actomyosin, and the supply of energy always comes from the degradation of glycogen. The anaerobic degradation of muscle glycogen (phosphorolysis) follows everywhere the same path. The glycogen is first transformed into hexosemonophosphate by means of inorganic phosphate. The hexosemonophosphate undergoes a second phosphorylation with the aid of a phosphate donator, the resulting hexosediphosphate splitting into triosephosphates which are again subjected to different reactions finally resulting in lactic acid formation. The phosphate donator, which made the transformation of hexosemonophosphate possible, is again restored to its original state by a subsidiary phosphorylation system. The general scheme of the first stages of phosphorolysis of muscle glycogen in animals is as follows:

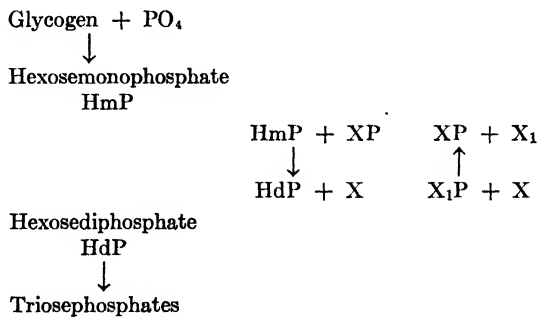


FIG. 1

General scheme of the phenomenon of phosphate transmission in the early stages of phosphorylation of muscle glycogen.

During the growth of an organism its different parts do not grow at the same rhythm as the whole. The proportions of the different organs undergo changes (relative growth) which cause the ontogenetic variations in body form. From the numerous observations in this field it has been concluded that a definite type of relationship exists which makes it possible to describe the most diverse cases of relative growth. In animals belonging to different groups the relation between weights (or dimensions) of various organs and the weight (or dimension) of the entire body (or of some particular organ selected for reference) can be expressed by an equation of the type:

$$y = bx^{\alpha}$$

where α and b are constants, y represents the weight of the organs, and x represents the weight of the whole organism. Plotting the data logarithmically one obtains curves of the angle α. When α is greater than 1, the organ grows much faster than the rest of the organism; when α is less than 1, the organ grows much slower; and when α equals 1, its growth is proportional to the total growth of the organism. If α is not equal to 1, the growth is said to be allometric, either positively ($\alpha>1$) or negatively ($\alpha<1$). The angle α of the logarithmic curve is the constant of growth while b, which represents the value of y when x is equal to 1, is the *index of origin*. To Teissier (226) belongs the credit for applying similar considerations to biochemical growth. Comparing, for instance, the increase in the various chemical constituents (water, dry weight, proteins, lipide, ash, total N, total C, total P, lipide P, nucleic P, protein N) with the increase in fresh or dry weight of an animal, the points of relative growth are

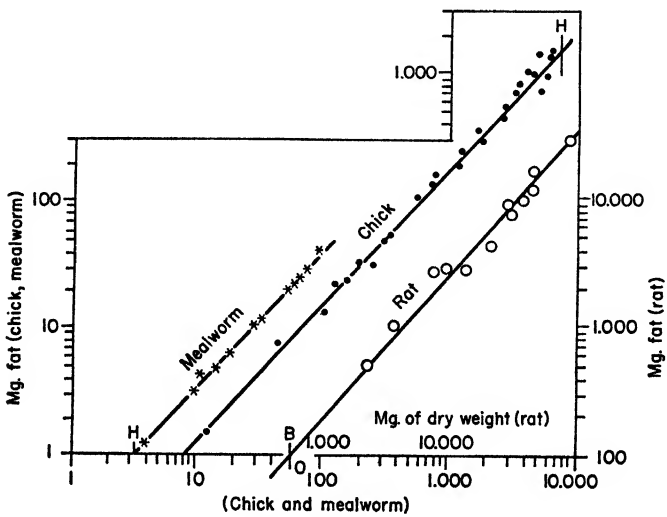


FIG. 2

(Needham, 190). Glycerides in the course of growth of the mealworm (Teissier), chick (Murray, Cahn, Romanov) and rat (Chanutin).

obtained which range themselves along one straight line in the more simple, and along two or three straight lines in the more complex instances. Furthermore, as was shown by Needham (190), the curves of relative growth of a given chemical constituent represent a remarkable uniformity even in very different animals. It may be said, therefore, that there is a unique plan of *biochemical growth* of animals.

We could cite other examples confirming the presence of a unified biochemical plan of animal life, a plan manifested in a common design according to which evolution embroidered the multiplicity of animal forms. The existence of this plan testifies once again to the organic unity of the animal kingdom, a unity which, far from contradicting the concept of evolution, is a necessary condition and a decisive argument for it.

CHAPTER II

Dissimilarities

Although it is true that animals manifest a remarkable unity of composition, further examination of their constituents or of their relative proportions allows one to discern certain biochemical differences. The body fluids (the internal environment), as was said before, represent salt solutions the composition of which is everywhere essentially the same. But if we consider the concentration of the mineral constituents, we may discover very marked differences which are manifested, for instance, in differences of electrical conductivity. The conductivity values ($K \times 10^4$),

TABLE II

The Inorganic Substances of the "milieu intérieur" in mg. %

Animal	Habitat		Na	K	Ca	Mg	Cl	References
Anodonta cygnaea	FW	I	44	1	28	0.05	42	Florkin (91)
Hydrophilus piceus	FW	I	276	54	46	54.0	142	Idem
Astacus fluviatilis	FW	I	349	11	48	6	621	Bogucki (27)
Rana	FW	V	218	39	9	4	218	Urano (268)
Helix pomatia	T	I	137	18	17	2	198	Lustig *et al.* (170)
Caudina chilensis	M	I	1010	44	39	131	1876	Koizumi (145)
Echinus esculentus	M	I	1022	38	40	122	1859	Robertson (222)
Cancer pagurus	M	I	1155	47	55	66	1808	Idem
Homarus vulgaris	M	I	1177	55	59	17	1826	Idem
Lophius piscatorius	M	V	462	27	14	2	677	Smith (249)

RELATIVE COMPOSITION

Animal	Na	K	Ca	Mg	Cl
Anodonta	104.8	2.4	66.7	0.12	100
Hydrophilus	194.4	38.0	32.4	38.00	100
Astacus	56.2	1.8	7.8	0.97	100
Rana	89.0	15.9	3.7	1.63	100
Helix	70.0	9.1	8.6	1.01	100
Caudina	53.8	2.4	2.1	7.00	100
Echinus	55.0	2.0	2.2	6.58	100
Cancer	63.9	2.6	3.1	3.64	100
Homarus	64.1	3.0	3.2	0.95	100
Lophius	68.3	4.0	2.1	0.30	100

FW — Fresh water; T — Terrestrial; M — Marine. I — Invertebrate; V — Vertebrate.

indeed, differ very greatly: 503 for the serum of the eel *Conger vulgaris;* 69 for the serum of the turtle *Emys europea* (Bottazzi, 32). On the other hand, if we measure the concentration of different ions in the fluid phase of the internal environment we also observe differences, as is shown in Table II for a series of animals consisting of several marine invertebrates (the holothurian *Caudina chilensis*, the sea urchin *Echinus*, the crab *Cancer pagurus*, and the lobster); one fresh-water crustacean, the crayfish; two mollusks, a fresh-water mussel, and a terrestrial snail; one aquatic insect, *Hydrophilus;* one marine teleost, *Lophius piscatorius;* and a frog.

Although the circulating internal fluids contain proteins, the concentration of the proteins is not the same in every case. In marine lamellibranchs the concentration is about 0.1%, while in the decapod crustaceans it is of the order of 4% (Florkin and Blum, 94). The arginine to lysine ratio of the proteins in these internal fluids is 10 to 18 in mammals (Block *et al.*, 25) but 10 to 26 in the fresh-water mussel *Anodonta* (Florkin and Duchâteau, 103). The internal circulating fluid always contains nonprotein nitrogen, but the total amount and the proportion of different nitrogenous components vary greatly, as is shown in Table III.

Furthermore, glucose is always found in the circulating medium but its concentration (true plasma glucemia) may be only 10 mg.% in a marine invertebrate like the sipuncle (Florkin, 82) or 20 mg.% in an elasmobranch like *Scylium canicula* (Florkin, 83) but 60–90 mg.% in man, and 160 mg.% in the hen (Erlenbach, 75).

TABLE III

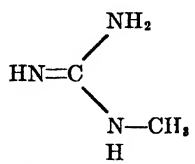
The Different Constituents of Nonprotein N in the "milieu intérieur" in mg. %

Animal	Total NPN	Urea N	Uric acid N	Amino N	Purine N	References
Asterias rubens (perivisceral fluid)	1.95	0.09	....	0.8	....	Delaunay (54)
Paracentrotus lividus (perivisceral fluid)	3.74	0.12	0.07	2.4	0.23	Idem
Palinurus vulgaris	26.5	5.5	0.3	8.0	2.1	Idem
Bombyx mori (day of emergence)	496.0	....	4.8	250.0	....	Florkin (86)
Carcharias littoralis	1125.0	1080.0	....	7.5	....	Smith (248)
Conger vulgaris	33.0	20.0	....	6.0	....	Delaunay (53)

The lipide content of the organism is represented by variable amounts. For instance, it makes up 11% of the dry substance of the meat of an oyster, 25% of the dry weight of *Bombyx mori*, and 40% of the dry weight of an average man in good health and normally nourished. But if we con-

sider the lipide portion of an animal organism from the qualitative point of view (Hilditch, 127; Lovern, 166) it may be said that each species has its own particular fat, and moreover that the lipide constituents vary from organ to organ even in the same individual. The animal fats can be divided into two main categories: triglycerides of aquatic animals containing a complex mixture of fatty acids varying greatly in molecular weight but mostly unsaturated, and the triglycerides of the terrestrial animals containing generally saturated fatty acids. The latter category can be further differentiated into two groups, the fats of rodents and birds containing 25–30% palmitic acid, and those of the pig or herbivores like cattle and sheep, in which the predominant component is stearic acid. The composition of fish fat can likewise be differentiated according to the marine or fresh-water habitat. Fresh-water fishes have a lipide with a high proportion of unsaturated acids with 16 or 18 carbons, and a low proportion of unsaturated acids with 20 or 22 carbons.

Biochemical differences between animals can thus be either quantitative or qualitative. It is true that some of the simple nitrogenous bases are widely distributed among animals, but this is not invariably so. Betaines are widely distributed but they are never found in insects, no matter what their habitat or mode of existence. Likewise, methylguanidine is found in vertebrates but not among invertebrates (Kutscher and Ackermann, 155). And trimethylamine is found in marine but not in fresh-water fishes.



Methylguanidine

The transportation of oxygen from the external environment to the cells is secured by the intervention of molecules capable of combining loosely with oxygen, but the nature of the transporter or carrier varies; it is copper containing hemocyanin in crustaceans or iron containing hemoglobin in vertebrates and in some annelids. Besides, the hemoglobin of vertebrates does not have the same composition as the annelid hemoglobin.

The mechanism of the first stages of phosphorolysis of glycogen during the anaerobic contraction of muscle is the same in all muscle tissue (illustrated in Fig. 1, p. 7), but X_1P in the vertebrates is phosphocreatine while in mollusks or in crustaceans this is represented by a different phosphagen, the phosphoarginine.

We called attention to the wonderful uniformity of the hydrolytic system of digestion in animals. However, if we compare a sponge and an annelid, we note that in the former digestion is exclusively intracellular whereas in the latter digestion proceeds in the lumen of a digestive tube by means of hydrolases discharged into the secreted juices. This reveals a characteristic difference in the location of enzymatic systems. Another instance of such differences can be demonstrated in variations of a process which result from bringing into play functional substances. Thus, for instance, in the crayfish the metabolism of the purine nucleus extends to the ultimate stage of formation of ammonia, but in the frog the metabolism does not go beyond the formation of urea, while in man this proceeds only to uric acid. Thus, one finds biochemical substances which are peculiar to some group or to some species. Such is the case as regards the presence of allantoic acid in the coelomic cells of Sipunculida (Florkin and Duchâteau, 98) or the presence in the same cells of hemerythrin which is an oxygen carrier characteristic for this particular group.

These few examples indicate the necessity for a classification of biochemical differences in animals, and for comparisons of biochemical constituents on the one hand, and of biochemical systems on the other. Certain characteristics are correlated with peculiar anatomical, physiological, and ecological conditions, while others appear as characteristics belonging only to certain groups. It is important, therefore, to establish a taxonomy of biochemical characteristics.

CHAPTER III

Evolution of Biochemical Constituents

Since we are undertaking to study comparative biochemistry of animals from the point of view of their chemical constituents, we must establish comparisons of a different order. We could consider the chemical nature of these substances regardless of whether they play a structural or functional part in the organism. We shall call substances *homologous* when they have a common chemical lineage. Biochemical homology can be complete as, for instance, in the case of inorganic molecules which make up the composition of the "milieu intérieur" (internal environment) of any animal. But where much larger molecules are concerned the homology may not be so complete. Thus, the serum proteins of different mammals are not identical. As a matter of fact, various degrees of biochemical homology may exist, from complete identity to merely a common chemical origin.

On the other hand, we shall call biochemical substances *analogous* when they exercise the same function in different organisms.

A. Biochemical Analogs

1. *Oxygen Carriers*

The transfer of oxygen from the external environment into the tissues is accomplished in most diverse animals (echinoderms, worms, crustaceans, mollusks, vertebrates) by specialized molecules capable of forming a loose combination with oxygen when its partial pressure is raised, and of giving it up again when the pressure is lowered. These are the so-called *oxygen carriers*, and they are represented by four different types:

Hemoglobins, the red respiratory pigments of vertebrates (true hemoglobins) and of numerous invertebrates (erythrocruorins), contain iron and protoheme derivatives.

Chlorocruorins, the green blood pigments of certain annelids, contain iron and derivatives of chlorocruoroheme.

Hemocyanins, the blue blood pigments of many crustaceans and mollusks, contain copper but are not heme derivatives.

Hemerythrins, the red-violet pigments in the coelom of the sipunculid, contain iron but no heme.

These different biochemical species possess in common the property of being oxygenated by combining with molecular oxygen without changing

the valence of the metal, as would be the case in an actual oxidation. This common property confers upon all four types of molecules their biochemical analogy and accounts for their physiological functions.

Oxygen combines with these carriers in definite proportions of 1 molecule/atom of iron of hemoglobin (Peters, 200) or chlorocruorin (Fox, 110), of 1 molecule/2 atoms of copper of hemocyanin (Dhéré, 57) and of 1 molecule/3 atoms of iron of hemerythrin (Florkin, 78). Hemoglobin exists in the dissolved state in the blood of most polychete and oligochete annelids, of *Chironomus* larvae, of some leeches, and of *Planorbis*. It is found enclosed in blood cells in the blood of certain nemertineans and turbellarians, of phoronids, certain lamellibranchs, and of vertebrates, as well as in the coelomic fluid of some echinoderms, echiurids, polychete annelids, and of certain mollusks (Amphineura).

Chlorocruorin is dissolved in the blood of certain annelids (Chloreminea, Sabellinea and Serpulinea).

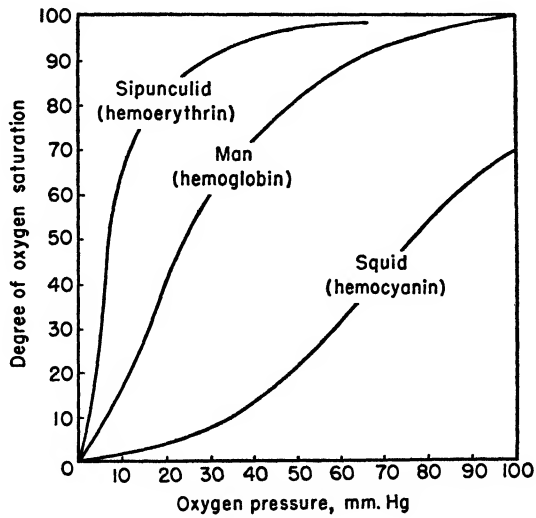


FIG. 3

Dissociation curves of various oxygen transporters under physiological conditions. Hemoerythrin (*Sipunculus*), temp. 19° and pH 7.7 (Florkin, 78); hemoglobin (human), temp. 38° and pH 7.47 (Henderson, 124); hemocyanine (squid), temp. 25° and pCO_2 3 mm. (Redfield and Ingalls, 219).

Hemocyanin is dissolved in the blood of decapod crustaceans, cephalopod mollusks, most of the gastropod mollusks and of arachnomorphs.

Hemerythrin exists enclosed in cells in the coelomic fluid of sipunculids.

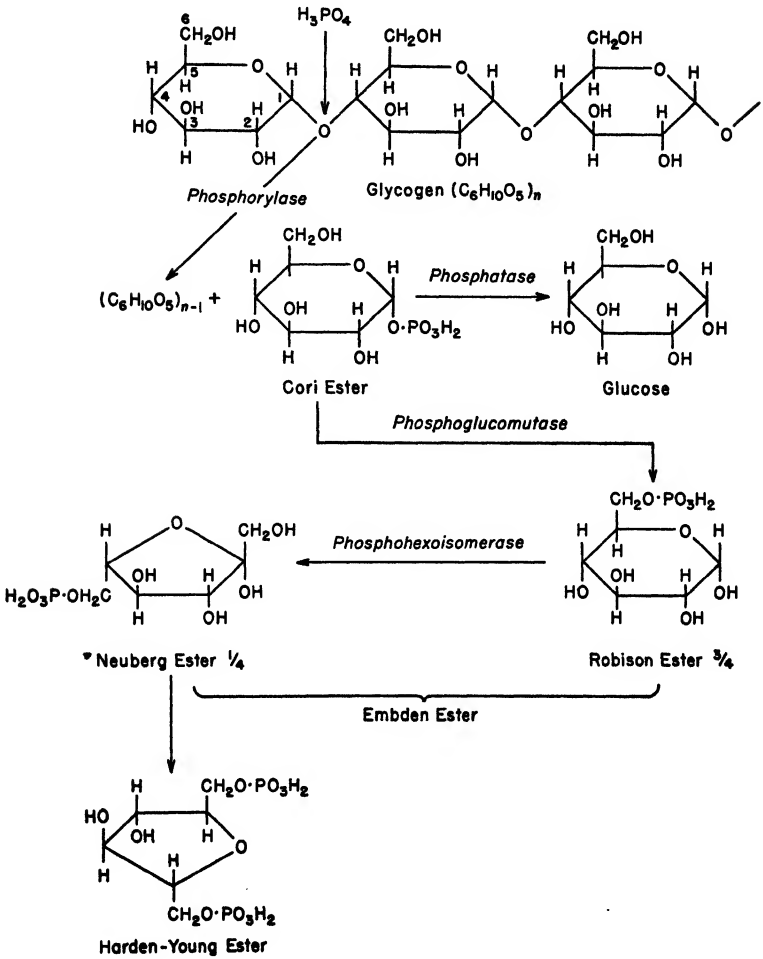
Thus, while hemocyanin is always dissolved in the biological fluids, hemerythrin is invariably contained in cells. Hemoglobin may be in either condition, but in a coelomic fluid it is always enclosed in cells. The analogy which these four categories of pigment present is their common property of functioning as oxygen carriers. To accomplish such transport, the blood must be able to become charged with oxygen at the surface of contact with the environment (lung, gill, etc.) and to discharge its oxygen load at the tissue level. This is made possible by the presence of an oxygen carrier which becomes charged with gas when its partial pressure is increased and loses it when this is decreased. Pyrogallic acid, for instance, also combines readily with oxygen but does not yield it up again; it cannot, therefore, play the part of a carrier as do the respiratory pigments by virtue of their dissociation curves. Fig. 3 represents such typically sigmoid dissociation curves of fluids containing different carriers. The concept of oxygen carriers, expressing the analogy of a series of biochemical substances, is thus a physiological concept, implying the possession of a particular physiological property of reversible oxygenation. This property justifies the classification in the same category of substances so markedly apart chemically as hemoglobin and hemocyanin.

2. *Phosphate Carriers*

In Chapter I it was pointed out that a mechanism common to all animal muscle tissue exists for the phosphorylation of glycogen. In the course of phosphorolysis glycogen is at first transformed to hexosemonophosphate which in turn is changed to hexosediphosphate, the second phosphate molecule being transmitted to it by a primary phosphate donator. The hexosediphosphate is then transformed into two triose phosphates. The donator, which furnished the phosphate for the second phosphorylation, is again restored by the intervention of a subsidiary system of phosphorylation, including a second donator which, in restoring the molecule of the first, itself becomes dephosphorylated. These general phenomena of transmission of phosphate during the early stages of phosphorylation of glycogen are summarized in Fig. 1.

In the striated muscles of vertebrates the role of the primary donator XP is fulfilled by adenosinetriphosphate (ATP) and that of the secondary donator X_1P by phosphocreatine, and the early steps of phosphorolysis are as follows:

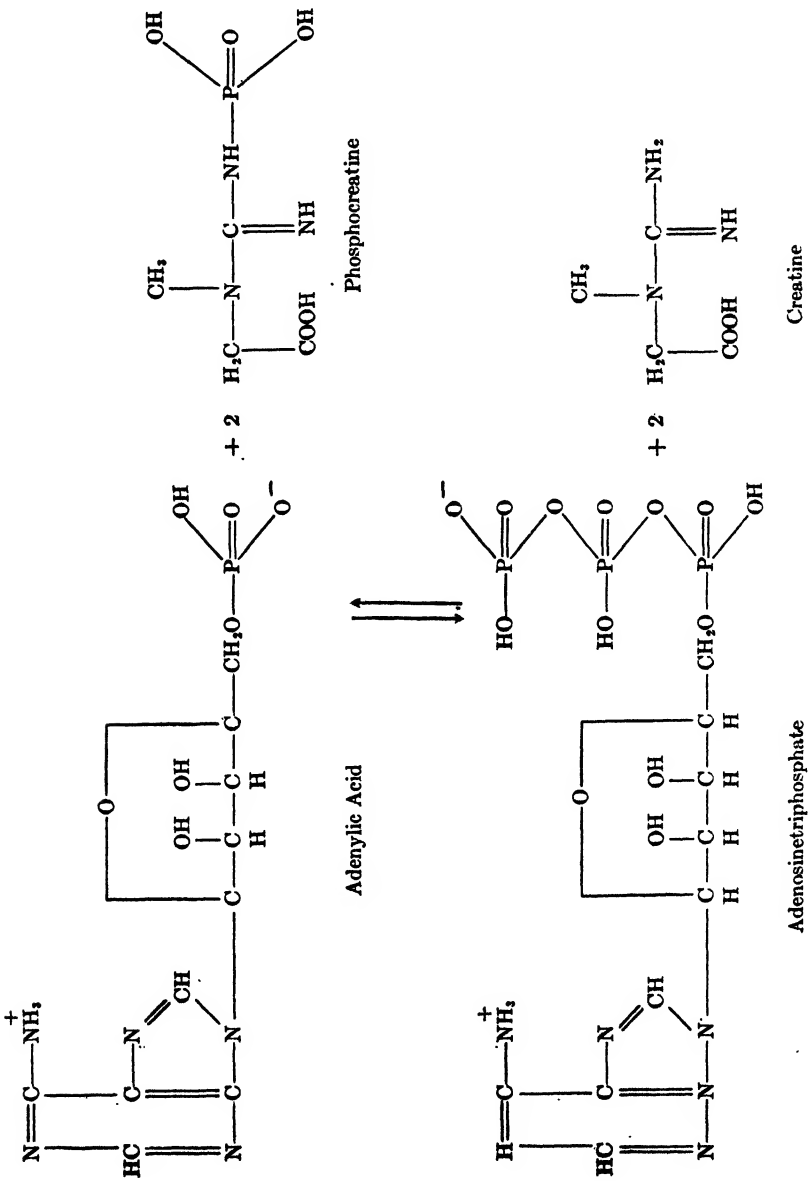
1. Glycogen + Inorganic PO_4 → Hexosemonophosphate



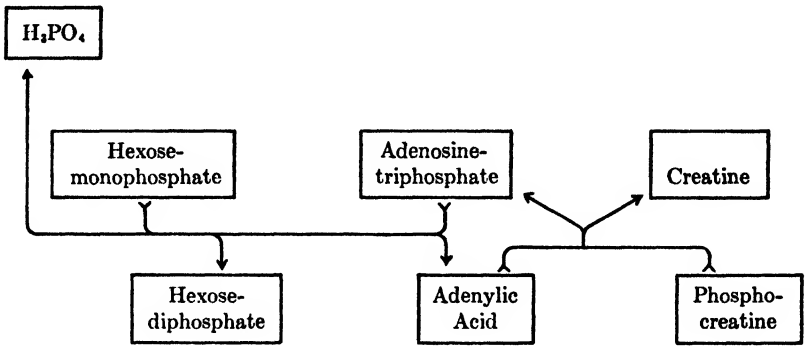
2. Hexosemonophosphate + H_2O + ATP → Hexosediphosphate + adenylic acid + PO_4

The restitution of the ATP is accomplished by means of phosphocreatine.

3. Adenylic acid + 2 Phosphocreatine → ATP + 2 Creatine



The entire process of PO_4 transmission in vertebrates, elucidated by the researches of Meyerhoff and of Parnas, may be summed up by this scheme.



So far as the vertebrates are concerned XP in Fig. 1 is thus represented by ATP and X_1P (phosphagen) by phosphocreatine. As a first approximation, the latter may be considered characteristic for vertebrates. The great majority of invertebrates possess a different phosphagen; namely, phosphoarginine.

Still, as is shown in Table IV, there are instances where both types of phosphagen are present. The latter substances are biochemical analogs and either one or the other plays the part of a subsidiary system of phosphorylation in those animals that contain them.

TABLE IV

Distribution of Phosphagens in Animals

(Needham *et al.*, 186; Baldwin and Needham, 10)

	Phosphoarginine	Phosphocreatine
Protozoa	−	−
Porifera	−	−
Coelenterata	+	−
Platyhelminthes	+	−
Annelida	+	−
Arthropoda	+	−
Mollusca	+	−
Echinodermata		
Crinoidea	+	−
Asteroidea	+	−
Holothuroidea	+	−
Echinoidea	+	+
Ophiuroidea	+	+
Protochordata		
Tunicata	+	−
Enteropneusta	+	+
Cephalochorda	−	+

TABLE IV (*Continued*)

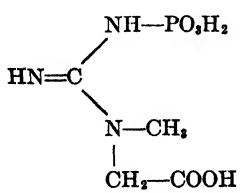
	Phosphoarginine	Phosphocreatine
Vertebrata		
Pisces	−	+
Amphibia	−	+
Reptilia	−	+
Aves	−	+
Mammalia	−	+

3. *Electron Carriers*

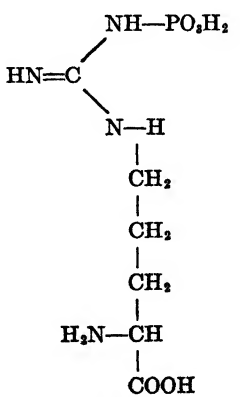
The uniformity of the system for transmitting hydrogen to oxygen from cellular donors by means of hydrogen and electron carriers has already been mentioned. In a very large number of animal cells this is the system cytochrome-cytochrome oxidase. Cytochrome consists of a variety of heteroproteins containing a heme prosthetic group, and cytochrome oxidase is also a heme derivative. In most animal cells the electron transfer operates at the cytochrome-cytochrome oxidase level according to the following scheme, in which the last link in the chain of hydrogen transmitters is succinic acid.

1. Succinic acid + $2Fe^{+++}$ (Cytochrome b) → fumaric acid + $2Fe^{++}$ (Cytochrome b) + $2H^+$
2. $2Fe^{++}$ (Cytochrome b) + $2Fe^{+++}$ (Cytochrome c) → $2Fe^{+++}$ (Cytochrome b) + $2Fe^{++}$ (Cytochrome c)
3. $2Fe^{++}$ (Cytochrome c) + $2Fe^{+++}$ (Cytochrome a) → $2Fe^{+++}$ (Cytochrome c) + $2Fe^{++}$ (Cytochrome a)
4. $2Fe^{++}$ (Cytochrome a) + $2Fe^{+++}$ (Cytochrome oxidase) → $2Fe^{+++}$ (Cytochrome a) + $2Fe^{++}$ (Cytochrome oxidase)
5. $2Fe^{++}$ (Cytochrome oxidase) + $1/2\ O_2$ → $2Fe^{+++}$ (Cytochrome oxidase) + $O^=$

$2H^+ + O^= \longrightarrow H_2O$



Phosphocreatine



Phosphoarginine

However, there are animal tissues in which the cytochromes are replaced by other heme derivatives as electron carriers. Thus, in cells of certain gastropods (Keilin) and in cells of some actinia, cytochromes are replaced by actiniohematine (Roche, 225). There is evidence to show (Baldwin, 9) that in the hepatopancreas of the snail the function of cytochrome is fulfilled by helicorubin. The pigment helicorubin is found also in the snail's intestine and, like hemoglobin, is a heme derivative. In the cellular respiratory system either cytochromes, actiniohematine, or helicorubin appear to be equivalent functionally and are, therefore, biochemical analogs.

4. *Osmotic Crystalloids*

The fluids of the internal environment of animals manifest a certain osmotic pressure which characterizes its function and exchanges. Unlike the colloidal osmotic pressure, which depends upon proteins in the circulating fluid, the crystalloid osmotic pressure depends principally upon the presence of inorganic constituents, which are the same in all the internal fluids, and are generally represented by the cations sodium, potassium, calcium, and magnesium and by the anions chloride, sulfate, phosphate, and bicarbonate. However, this is not invariably so and in the internal fluids of insects the crystalloid osmotic pressure is maintained to a very large extent by organic substances. Contrary to the general rule, in insects the osmotic pressure exerted by the diffusible organic constituents surpasses that of the inorganic constituents. While the lowering of the freezing point (Δ) of human serum is 0.56°, that corresponding to its salt content is 0.53°, to amino acid 0.03° and to glucose about 0.01°. The blood of the bee larva lowers the freezing point (Δ) 0.86°, but of this only 0.22° is due to its salts, 0.39° to amino acids and 0.067° to glucose (Bishop, *et al.*, 23). Thus, in the bee larva the amino acids play the predominant role in crystalloid osmotic pressure.

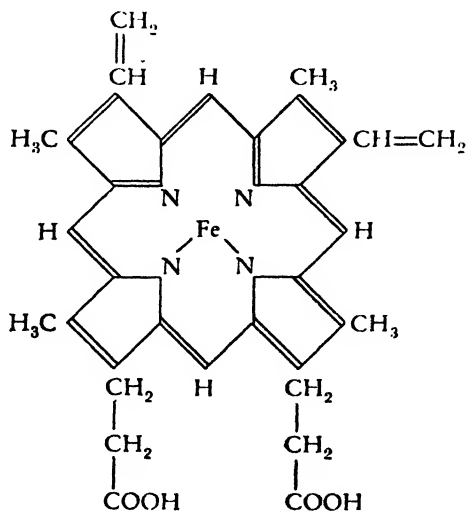
Although the amino acids play such an important part in the osmotic pressure of insect blood, other organic substances, like urea and trimethylamine oxide, exercise an analogous function in the blood plasma of fishes belonging to different families of the subclass Elasmobranchi (Selachii, Batioïdei, Chimeroïdei). The bodies of these cartilagenous fishes contain considerable quantities of urea (Städeler and Frerichs, 259), which is a peculiar characteristic of this subclass and is found among all its members. Urea accounts for 44% (on the average) of the plasma osmotic pressure in the elasmobranchs, having a concentration of 330 to 440 mM./l. (Duval, 70). The plasma of these fishes contains also 100 to 120 mM./l. of trimethylamine oxide beside the urea, so that both substances together are responsible for the largest part of the osmotic pressure (130a).

It can be seen that inorganic salts, amino acids, urea, or trimethylamine oxide exercise the same function in these different animal groups and determine the magnitude of the osmotic pressure of their internal environment (fluids). These different substances are, therefore, analogous in this respect.

B. Biochemical Homologs

It was pointed out previously that substances manifesting more or less direct relationship determined by chemical origin which binds them into a single family are considered biochemical homologs. If we consider, for instance, the group of analogous substances functioning as oxygen carriers (hemoglobins, chlorocruorins, hemocyanins, hemerythrins) we can detect among them certain homologies also.

Let us consider first the hemoglobins of the vertebrates. In every instance they are formed by the union of four protoheme molecules (resulting from the combination of protoporphyrin with iron) with the protein globin. The molecular weight of the vertebrate hemoglobin is about 70,000; its isoelectric point is near the point of neutrality, pH 6.7 for human hemoglobin, pH 6.78 for horse hemoglobin, and pH 7.2 for that of the



Protoheme

pigeon. Even this slight variation already reveals significant differences between the hemoglobins of different species of vertebrates. The hemoglobin of every species is peculiar in so far as certain of its properties are concerned. But there are also less pronounced individual differences,

which nevertheless indicate the existence of individual characteristics. The specific characteristics of the hemoglobins of vertebrates impart to them different properties. Thus, the crystal form is not the same in different species, the sulfur content varies from species to species, the composition of the globin, so far as the sulfur-containing amino acids (methionine and cystine) are concerned (22), differs while the content of arginine, lysine, histidine, and tryprophan is very nearly the same in all.

The amino acid composition recorded in Table V shows how similar the globins are of man and monkey, on the one hand, and of the dog, fox, and jackal, which all belong to the same family, on the other.

TABLE V

Globin	Per cent	
	Methionine	Cystine
Human	1.35–1.48	1.05–1.35
Monkey (*Rhesus*)	1.34–1.43	1.15–1.21
Cow	1.71–1.79	0.45–0.67
Horse	0.89–1.03	0.65–0.94
Dog	0.54–0.58	1.56–1.89
Fox	0.53–0.55	1.69–1.71
Jackal	0.59	1.62

Reduced hemoglobin presents one absorption band and oxyhemoglobin two bands in the visible spectrum. The curve in Fig. 4, which shows this absorption quantitatively, makes it possible to localize the α and β bands of oxyhemoglobin. The maximum absorption varies slightly from one hemoglobin to another; for man the α band is at 5769 A., for the horse at 5767 A., for the dog at 5770 A., for the rat at 5768 A., for the snake at 5771 A., while the maximum β band varies similarly around 5400 A.

The hemoglobins from different vertebrates vary also with respect to other properties: resistance to NaOH, solubility, antigenic properties, the value of the *span* (the distance between the peaks of the α absorption band of oxyhemoglobin and carboxyhemoglobin), etc.

The specificity of the different hemoglobins of vertebrates cannot be due to the protoheme which is always the same since the different hemoglobins yield identical hemochromogens (Anson *et al.*, 2). Furthermore, the protohemes prepared from different hemoglobins give the same hemoglobin when united with the same globin (Roche, 224). On the other hand, globins obtained from different sources, if united with the same protoheme, yield hemoglobins corresponding to the one from which the particular globin was obtained (Anson and Mirsky, 3). It must, therefore, be con-

cluded that it is the globin moiety which imparts to the hemoglobins their specific characters.

Hemoglobin of vertebrates, or true hemoglobin, has a molecular weight of approximately 70,000. Svedberg (263), who determined the molecular weights of many proteins, noted that these correspond always to the multiple of 17,600, as if the proteins were polymers of a fundamental unit of this molecular weight. True hemoglobin contains four such units. Among the various hemoglobins this combination is peculiar to the partic-

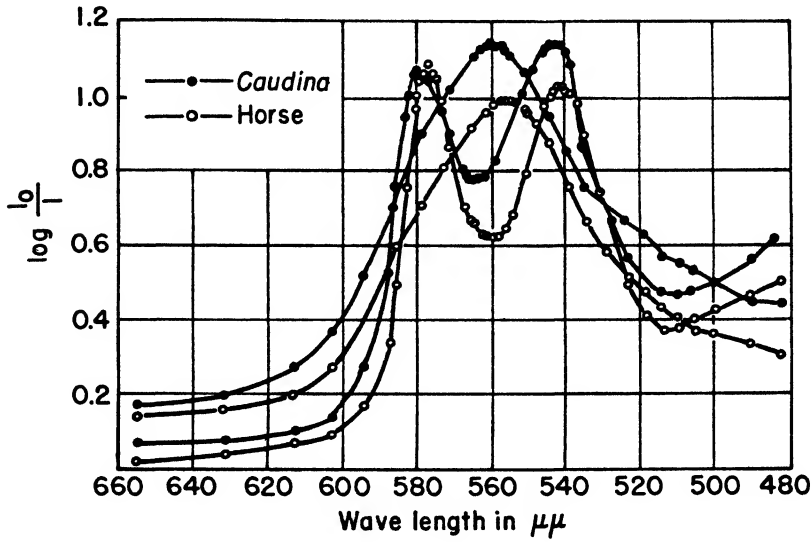


FIG. 4

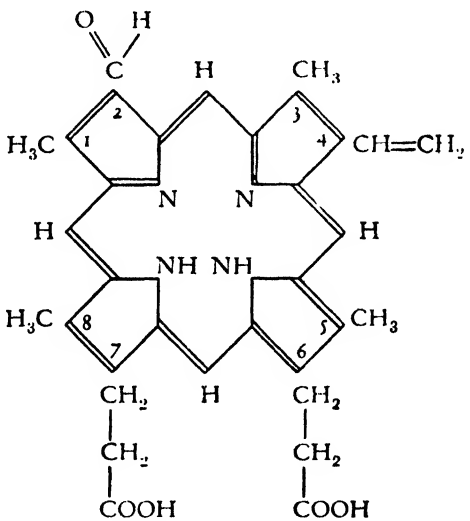
(Kobayashi, 138). Absorption curves of oxygenated (double peak) and reduced (single peak) hemoglobin of the horse and erythrocruorine of the holothurian *Caudina chilensis*. —— • —— *Caudina*; —— o —— horse.

ular hemoglobin of vertebrates. In fact, the hemoglobins of invertebrates (*Arca*, 33,500, *Chironomus*, 31,500, *Daphnia*, 422,000, *Planorbis*, 1,690,000, *Arenicola*, 3,380,000) never have a molecular weight corresponding to four units, but to different numbers of units which vary with each hemoglobin. That is the reason why the hemoglobins of invertebrates, or the erythrocruorins, are sometimes differentiated from the hemoglobins of the vertebrates. Besides, while the isoelectric points of the latter lie about the neutral point, those of the erythrocruorins (Svedberg, 264) are distinctly more acid (*Planorbis*, 4.77, *Chironomus*, 5.4, *Arenicola*, 4.76). Furthermore, on comparing the amino acid composition of hemoglobins and of erythrocruorins, one finds that the latter contain very much larger

amounts of arginine and cystine but less histidine and lysine (Roche, 225). The erythrocruorins constitute a much less homogeneous group than the vertebrate hemoglobins, although both are derived from the same protoheme, as has been demonstrated many times. The more one concentrates upon a limited group in the animal classification the smaller do the differences become which are due to the properties of the protein moiety of the carrier molecule.

It is thus obvious that the hemoglobins do not represent identical substances throughout the entire animal kingdom, as do the molecules of sodium chloride or of urea. Among the many existing hemoglobins a new biochemical species can be distinguished to which the name erythrocruorin is given. We are dealing, therefore, not with identical molecules but with molecules which are more or less distinctly homologous.

The homology between hemoglobins and chlorocruorins is less clearcut than between the different varieties of hemoglobin, which are all derivatives of protoheme. The chlorocruorins actually contain chlorocruoroheme, a



Chlorocruoroporphyrin

derivative of chlorocruoroporphyrin, which is different from protoheme. But whereas the oxygen carriers hemoglobin and chlorocruorin present various degrees of homology, hemocyanin and hemerythrin are very different chemically from these respiratory pigments since neither of the latter contains heme.

There are substances in which the biochemical homology may be even much less obvious than among the hemoglobins. It may be simply a case

of chemical relationship, very much like that between cytochromes, biliary pigments, chlorophyll, catalase and peroxidase which are all homologous with hemoglobin and chlorocruorin because they are all porphyrin derivatives. In the same sense we can say that sterols, bile acids, vitamins D, sex hormones, adrenal cortex hormones, cardiac glucosides, etc. are homologous substances because they are all pentocyclophenanthrene derivatives.

C. Evolution of Biochemical Constituents

Is evolution within a group of biochemical constituents possible? We have seen, in the case of large molecules like those of hemoglobin, that they do vary from one animal group to another. Are these variations associated with repercussions in the functions of the organism or are they purely contingent and without particular significance?

The common property of oxygen carriers, as we have seen, is their dissociation curve in relation to the partial pressure of oxygen (Fig. 3). The dissociation curve most frequently has the sigmoid form. To express approximately the form of a similar curve, we may use Hill's equation:

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n}$$

in which y represents the per cent of saturation corresponding to a partial oxygen tension x. It is possible to assume that in this equation the constant n represents, though very vaguely, the degree of interdependence between the different groups at the time of their oxygenation. This interdependence manifests itself in the existence of the sigmoid shape of the dissociation curve, because if the molecule has only one group that combines with oxygen or if the oxygenation of one group does not modify the oxygenation of other groups, n will equal 1 and the curve will then be a hyperbola. The constant K of Hill's equation is an index of the affinity of the hemoglobin for oxygen and upon its value depends the position of the dissociation curve. Let us take human blood, for instance, and compare the curve of its real oxygen absorption under physiological conditions with a curve when n equals 1 and the curve becomes hyperbolic. Both of these curves are shown in Fig. 5. It can be seen very clearly that the hyperbola would not suit the physiological conditions occurring in man. In fact, the partial oxygen tension in man is 100 mm. of mercury in the arterial and 40 mm. in the venous blood. As one passes from the arterial to the venous oxygen partial pressure, as is the case at the time the tissue level is reached, it can be seen that the sigmoid curve furnishes more oxygen than the hyperbolic, and, on the other hand, it accounts for a considerably greater uptake at the time the venous oxygen tension changes to the arterial oxygen tension in the

lung. The sigmoid shape of the dissociation curve appears to favor the fulfillment of the role of oxygen carrier. A carrier having the sigmoid type of curve can, therefore, be considered as more highly evolved than a carrier conforming to the hyperbolic curve. The latter condition is observed in hemoglobins whose molecular weight corresponds to that of a single unit of 17,600 and which consequently have only one group for combining with oxygen. This is the case of the hemoglobin of muscles (myoglobin) and of the erythrocruorin of the lampreys, both of which seem to be primitive hemoglobins.

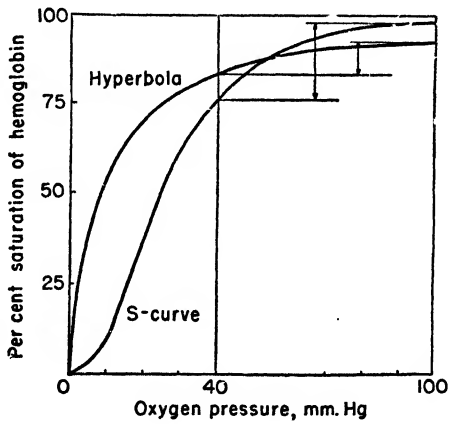


FIG. 5

(Litarczek and Dinischiotu, 164)

Relation between saturation of hemoglobin and the partial pressure of oxygen (mm. Hg).

From the viewpoint of biochemical evolution the recent contribution of Keilin *et al.* (136) on the hemoglobin of the parasitic larvae of the horse fly *Gastrophilus* assumes great significance. The larvae live in the horse's stomach for more than eight months, i.e., for the longest part of the insect's total life span. Neither the pupa, the adult, nor the first stage larva possess hemoglobin, which is present in the insect only during its second and third larval stages. This hemoglobin is very distinct from the hemoglobin of the horse. In fact the hemoglobin is synthesized by the *Gastrophilus* larva, and in its properties it resembles somewhat the myoglobin of mammals. It has a molecular weight of 34,000, which is about half the molecular weight of horse hemoglobin, and contains two instead of four heme nuclei. The absorption peak of this hemoglobin as well as the α- and β-bands of its oxy- and carboxy-combinations are very similar to those of the hemoglobin and myoglobin of the horse. However, the hemoglobin of the horse possesses the specialized sigmoid oxygen dissociation curve and undergoes

autoxidation slowly, whereas the myoglobin of the horse and the hemoglobin of the *Gastrophilus* larvae both have the more primitive hyperbolic dissociation curve and both autoxidize fairly rapidly.

Other important differences manifest themselves in the "span" (i.e., the distance between the peaks of oxyhemoglobin and carboxyhemoglobin absorption curves) and in the relative affinities for oxygen and carbon-monoxide (i.e., in the partition constant K). The blood hemoglobin of vertebrates has a "span" range of 43–56 A. and a K range of 125–550 (the highest value of K is found for the horse). Vertebrate myoglobin has corresponding values of 31–36 and 28–51 A., and for *Gastrophilus* larvae hemoglobin the values are 95 A. and 0.67. In other words, the *Gastrophilus* hemoglobin differs strikingly from vertebrate hemoglobin by its very large "span" and extremely low affinity for carbon monoxide.

Hemoglobin is not generally found in invertebrates, in which the respiration more commonly depends upon oxygen carriers which are not even heme compounds (hemocyanin, hemerythrin). The recent discovery of hemoglobin in *Paramecium* and in the root nodules of nitrogen-fixing leguminous plants is, therefore, of the greatest interest for the understanding of its biochemical origin. Surprisingly enough the hemoglobin which is synthesized by the *Gastrophilus* larva while it leads a parasitic existence attached to the stomach mucosa of the horse has a "span" very nearly like that of the hemoglobin (span 100) synthesized in the root nodules (incidentally, this synthesis cannot be accomplished either by the *Rhizobium* organisms or by the plant cells alone, but only by the coöperation of both). On the other hand, the root nodule hemoglobin has an affinity for carbon monoxide (K equals 37) which is of the same order as that of myoglobin, i.e., considerably less than of vertebrate blood hemoglobin but very much greater than that of *Gastrophilus* hemoglobin. The root nodule hemoglobin is thus somewhat intermediate between *Gastrophilus* hemoglobin and myoglobin, but all three are easily oxidized to methemoglobin. In other words, the ferrous iron in these compounds is much less stable than in blood hemoglobin.

Keilin points out that the very low value of the partition constant K of *Gastrophilus* larvae hemoglobin (0.67) is shared also by another heme compound, the cytochrome oxidase (0.1). Since all cells of aerobic organisms have the ability to synthesize hematin catalysts (catalase, peroxidase, cytrochrome system) they are, therefore, potential producers of hemoglobin. The only factor limiting the distribution of hemoglobin is the ability of the cells to synthesize the highly specific proteins which in combination with a heme prosthetic group manifest the truly remarkable property of reversible oxygenation. It is this property which no other compounds possess besides hemoglobin, chlorocruorin, hemocyanin, and hemerythrin.

Heme or hemochromogen are remarkable for the very great speed with which they are oxidized, much more rapidly even than *Gastrophilus* larva hemoglobin is oxidized to methemoglobin. But the native globin in hemoglobin protects the ferrous ion and thus stabilizes the oxygenated compound. As a result hemoglobin is an efficient oxygen carrier but an inefficient oxidizing catalyst unless it is denatured, when it ceases to function as an oxygen carrier and becomes a good oxidation catalyst. On the other hand, cytochromes (a,b,c) are neither autoxidizable nor can they be oxygenated, but their oxidation can be catalyzed by cytochrome oxidase which is a hematin protein. The biological importance of a substance like the *Gastrophilus* hemoglobin lies in the fact that it seems to narrow the gap between heme proteins which function entirely as oxygen carriers and those which function only as oxidation catalysts.

The oxygen carriers generally show what is known as the Bohr effect; namely, a displacement of the oxygen absorption curve to the right at the time when the partial pressure of carbon dioxide is increased (or the pH is decreased) (Fig. 6).

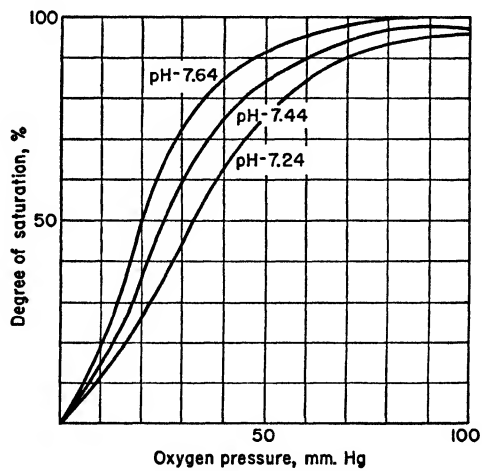


FIG. 6

Curve of dissociation of oxyhemoglobin of human blood at pH 7.24, 7.44, and 7.64. (Richards and Strauss, 221, according to the determinations of Henderson *et al.*)

The Bohr effect plays an important role in the transport of oxygen. In fact, at the tissue level, where the capillary blood becomes enriched with carbon dioxide, the dissociation curve is displaced towards the right and this is an important factor in the process of discharge of oxygen to the tissues. In the organ of respiration, where the carbon dioxide tension falls and the dissociation curve again shifts to the left, the Bohr effect favors

the uptake of oxygen by the carrier. An oxygen carrier which does not show the Bohr effect is a more primitive type of carrier. The hemoglobin of an echiuroid, *Urechis caupo* (Redfield and Florkin, 217) and of hemerythrin of *Sipunculus* (Florkin, 78) present this situation. Neither of these animals possesses a circulatory system and their oxygen carriers are contained in cells of the coelomic fluid. Hemoglobin (erythrocruorin) of *Urechis* appears, therefore, to be of a primitive type because it does not manifest the Bohr effect.

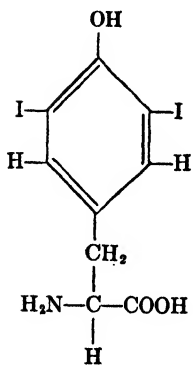
Evolutionary tendencies are recognizable not only in the variations of a large molecule like hemoglobin but also in the relation between the two categories of oxygen carriers represented by the hemoglobins and the chlorocruorins. Although the color of chlorocruorin solutions is definitely different from the color of hemoglobin solutions, the differences between their prosthetic groups are, from a chemical point of view, very slight. Hemoglobin contains the heme of porphine, 1,3,5,8-tetramethyl, *2,4-divinyl*, 6,7-diproprionic acid, whereas chlorocruorin contains the heme of porphine, 1,3,5,8-tetramethyl, *2-formyl*, *4-vinyl*, 6,7-dipropionic acid. In other words, the second compound differs from the first only by a slight chemical modification, namely, an oxidation of the 2-vinyl group. The peculiar carrier found among annelids comprising the families of Chloreminea, Sabellinea and Serpulinea appears, therefore, as an evolutionary form of hemoglobin, a truly *chemical mutation.*

We have seen that the phosphagens also differ; the invertebrates contain phosphoarginine and the vertebrates phosphocreatine. As can be seen from the structural formulas (p. 21), the replacement of one of the molecules by another furnishes another example of chemical mutation. Besides, in a general way, evidence of homology between biochemical constituents indicates the possibility of lineal evolution. A study of the chemical constituents of organisms makes it possible, for instance, to prove the homology between thyroxine, a component of the thyroid hormone in vertebrates, and 3,5-diiodotyrosine or iodogorgonic acid, so called because it was first isolated from the coral *Gorgonia* (Drechsel, 62), but found in many other invertebrates.

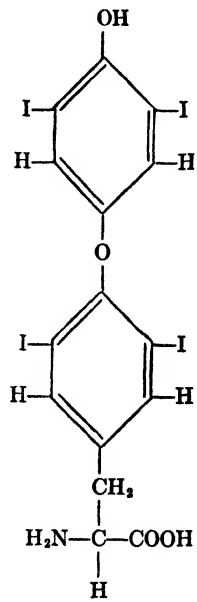
The sterol nucleus may give rise to a substance endowed with estrogenic activity, or to a substance playing a part in ossification, or to a substance partaking in mineral metabolism. Besides the active substances, other derivatives of the same sterol nucleus are found which have no biological activity at all. All these representatives of the same family of substances furnish new examples of the evolution of biochemical constituents, where the novel activity of a new derivative may depend upon a very simple chemical modification of the molecule.

Another way of evolution of biochemical constituents is represented by their acquisition of new functions. Urea is generally a product of protein

metabolism which is simply rejected with the excreta. But in the elasmobranch fishes, urea is retained in the organism and is utilized, as was said before, in maintaining osmotic pressure of the internal environment. In fishes it is possible to demonstrate a substance which acts on the uterus of mammals like oxytocin, the posterior pituitary hormone of these animals.



3,5-Diiodotyrosine, or Iodogorgoic Acid



Thyroxine

It is also possible to demonstrate in the nerve ganglion-neural gland complex of the ascidian *Ciona intestinalis* three active principles of the posterior hypophysis of vertebrates: the pressure, the melanophore dilating and oxytocic factors (Bacq and Florkin, 6). The ovary of Lepidoptera contains substances with estrogenic activity (Loewe *et al.*, 165) which act on the external genital organs of mammals. Substances with similar activity are found in different invertebrates. Without doubt we are dealing here with growth hormones upon which evolution has conferred a new role. The complex endocrine relations between the organs of higher vertebrates are not different from the particular instances where metabolic products of one organ may exert an influence upon the function of another organ. Evolution frequently utilizes an already existing substance for a new function, by creating a new system of biochemical receptors for its action.

CHAPTER IV

Orthogenetic Evolution of Biochemical Systems

Among certain biochemical systems it is possible to discern an evolutionary process which shows the same sequence in different phylogenetic branches. These systems, therefore, have evolved as if their variations were canalized along one definite direction in accordance with a biochemical design common to all animals.

A. "Milieu intérieur" (Internal Medium)

The "milieu intérieur," or internal environment, of organisms is a saline solution containing a number of organic substances. The latter represent either components which properly belong to this solution or substances simply in transit. The internal environment represented by the coelomic fluids is practically free from protein. But bloods, i.e., biological fluids which move within a genuine circulatory system, always contain protein. The protein content is incontestably correlated with the position occupied by the organism in the zoological scale, and the highest protein content is found in the most highly evolved groups. Among the mollusks, the proteinemia of a marine lamellibranch like *Mya arenaria* is of the order of 0.09%, that of a gastropod like *Helix pomatia* about 1.2 to 2.3%, and that of a cephalopod like *Eledone moschata* about 10%. Among the insects, likewise, the proteinemia of an orthopteron like *Dixippus morosus* is about 1%, whereas for a lepidopteron like *Bombyx mori* it is about 2%, and in an hymenopteron like *Bombus agrorum* it reaches 5% (Florkin, 87). One of the principal functions of plasma proteins is to maintain a colloidal osmotic pressure which is an important factor in the regulation of the water exchange between blood and tissues. When the colloidal osmotic pressure of blood plasma proteins is measured directly one observes, as is revealed in Table VI, that the highest pressures are found in animals with the highest type of organization, quite independently of the ecological conditions.

But proteinemia and colloidal osmotic pressure are not the only properties of the internal environment which vary with the degree of evolution of the animal groups. The same applies also to the content of protein sugar (combined sugar) in the internal environment. Contrary to what has been noted with regard to protein concentration, the protein or combined sugar content of plasma decreases as one goes up the animal scale. For instance, among mollusks, the protein sugar content of the plasma is 17% in the pond mussel, about 7% in the snail, and only about

TABLE VI

Colloid Osmotic Pressure (in cm. H_2O) of the Internal Medium of Different Animals

(Cardot and Meyer, 45)

Animal classification	Habitat		
	Marine	Fresh water	Terrestrial
Sipunculida	*Sipunculus nudus* 0.7–0.12		
Mollusca			
Lamellibranchia	*Pinna nobilis* 0.8–0.12		
	Opisthobranchia	Pulmonata	Pulmonata
	Aplisia fasciata 1.4	*Limnea auricularia* 1.3	*Helix pomatia* 1.8–2.2
	A. punctata 1.4	*L. stagnalis* 1.2–1.3	*H. aspersa* 1.5–1.7
Gastropoda	Prosobranchs		*H. aperta* 1.7
	Murex brandaris 1.2–1.4		*H. vermiculata* 1.6–1.8
	M. trunculus 1.5–1.9		*H. lucorum* 1.7
			H. pisana 1.5–1.9
	Cepia officinalis 2.9–3.5		
Cephalopoda	*Octopus vulgaris* 3.1–3.8		
	Eledone moschata 3.6–3.9		
	Carcinus moenas 3.2–4.0	*Astacus nobilis* 2.9–3.3	
Crustacea	*Dromia vulgaris* 3.1–3.6		
Decapoda	*Maia squinado* 3.4–3.8		
	Xantho rivulosus 4.3		
	Eriphia spinifrons 3.1–4.3		
	Portunus corrugatus 3.3–4.3		

Tracheata		Arachnida *Argiope Brunnichi* 4.1–4.3 *Araneus diadematus* 4.5 Orthoptera *Mantis religiosa* 5.0–5.4
Tunicata	*Phallusia mamillata* 1.9–2.2 *Microcosmus sulcatus* 1.3–1.5	
Fishes Selachii	*Acanthias vulgaris* 4.2–4.3 *Scyllium canicula* 3.1–3.6 *Mustelus hinnulus* 5.7–6.4 *Torpedo marmorata* 4.2–5.2	
Fishes Teleostei	Physostomi *Conger vulgaris* 14.6–17.3 Anacanthidae, Pleuronectidae *Rombus maximus* 17.4 Acanthopterygidae *Labrax lumpus* 17.4–25.0 *Trigla lucerna* 19.5–21.3 *Scomber scombrus* 19.6–19.8 *Scorpaena scrofa* 18.1–18.6	*Anguilla vulgaris* 22.5 Cyprinidae *Cyprinus carpio* 10–11.3 Esocidae *Esox lucius* 11.2–14.6

3% in the squid. In the vertebrates we find that the selachid *Scyllium canicula* contains about 13% combined plasma sugar, the carp about 5%, the frog 4%, and the horse only about 2% (Lustig and Ernst, 169). The protein sugar content of the internal environment does not behave at all like that of true glucemia (reducing and fermentable substances). The latter, in a general way, is low in invertebrates whose true glucemic level (except for bees) is at most 20 mg.% and frequently much less (Florkin, 88). Among vertebrates, while the selachid *Scyllium canicula* does have a true glucemia of only about 20 mg.% (Florkin, 83), man has 60–90 mg., and the chicken 160 mg.% (Erlenbach, 75). The orthogenetic behavior of the concentration of protein sugar in the internal environment is thus entirely different from that of true glucemia within the animal series.

B. Respiratory Function

The respiratory function of the internal environment is twofold: the transport of oxygen and the transport of carbon dioxide.

The transport of oxygen depends upon the presence of an oxygen carrier which is either dissolved directly in the plasma or enclosed in cells. An important property of the biological fluids containing the oxygen carrier is its oxyphoric power, i.e., the quantity of oxygen (in volume per cent) combined with the carrier when the latter is saturated. The oxyphoric power gives an idea of the content of oxygenable groups in blood or in coelomic fluid no matter what the nature of the carrier may be. Surveying the quantitative values of oxyphoric power, obtained from published data, the tendency of this biological property to enlarge gradually with a rise in the animal scale can be observed.

In each group there is a certain relationship between the degree of activity and the value of oxyphoric power. Since blood contains cells, the increased oxyphoric power could be due to two different causes. It could result either from greater concentration of the cells or from actual increase in the oxyphoric power itself. Examination of the literature shows that the increasing oxyphoric power of the blood, observed as one ascends the zoological scale, is accompanied by a tendency of the blood cells to acquire greater oxyphoric power.

The transport of carbon dioxide is due to the presence in the body fluids of substances the dissociation of which varies with the reaction, and likewise of molecules the dissociation of which differs with the oxygenation. The oxygen carriers belong to these latter substances. The penetration of carbon dioxide into the blood, which tends to cause acidification and concomitantly a reduction of the oxyhemoglobin, brings about a liberation of base which can combine with the carbonic acid, resulting from hydration of the carbon dioxide, to form bicarbonates. One can characterize the

efficiency of the system in taking on a charge of carbon dioxide according to its buffering capacity against this substance, i.e., by the quantity of carbon dioxide combined during the respiratory cycle that will cause a pH change by one unit. In the marine gastropod *Busycon canaliculatum* this value is 8.8, in the skate *Raja ocellata* it is 21, in the turtle *Chelydra serpentina*, 50, in the goose, 100, in the horse, 180, and in man, 210 (Florkin, 79). We see here most strikingly a progressive perfection of the mechanisms of resistance to changes in the internal environment.

C. Hydrolytic Processes of Digestion

We said that the process of preparation of cellular nutrients brings into play a number of hydrolytic enzymes. As we have already noted, this arsenal of enzymes presents a very great similarity in different groups. But the localization of these enzymes at the moment of their action is not always the same: they can be intracellular, or partly intracellular and partly extracellular, or entirely extracellular.

Intracellular digestion represents a most primitive mechanism of hydrolysis of foods. Among the Porifera (Sponges) it is the exclusive form of digestion. Even in the more evolved forms, like the brachiopods, in which alimentation is accomplished by a ciliary mechanism, it is maintained as the exclusive mechanism of digestion in correlation with the mode of their alimentation. There is no evidence that these animals secrete into the digestive tube. A unique intracellular digestion is found in Tardigrades. These animals absorb the soft portion of vegetable tissues and the chloroplasts may be detected in the cells of the middle intestine of the digestive tube.

Intracellular digestion also occurs to some extent in certain Metazoa, which Yonge (291) classifies into two groups: (1) certain primitive forms like coelenterates, most turbellarians, and *Limulus;* (2) and the more highly evolved forms in which intracellular digestion persists to a greater or less degree in correlation with their mode of alimentation, as in the brachiopods, rotifers, arachnids (except *Limulus*) and mollusks (except the cephalopods).

Extracellular digestion of a more or less marked degree is observed in the different groups of Metazoa, with the exception of sponges, brachiopods and tardigrades. It has completely replaced intracellular digestion in the nematodes, Polyzoa, annelids, myriapods, crustaceans, insects, cephalopods, tunicates, and vertebrates.

From all that is known relative to digestive processes in which hydrolases are involved, intracellular digestion must be considered to be the primitive process while extracellular digestion is an evolutionary acquisition. As we have said before, one can actually find intracellular digestion either in primitive groups or in some more highly evolved groups,

but in each case there is a peculiar mode of alimentation in connection with which variable degrees of intracellular digestion are maintained. This is found in animals which feed on fine particles gathered by ciliary movement or in animals which aspirate liquid nourishment, for instance by suction, as in the case of the arachnids.

A most striking example of the relation between the two digestive mechanisms in the course of evolution, as pointed out by Yonge, is found in mollusks. Here one discovers all stages from an almost exclusively intracellular to a completely extracellular digestion. With the exception of the Teredinea, which obtain part of their nourishment from wood in which they live, and the septibranchs which are carnivores (Yonge, 288, 290), the lamellibranchs feed by means of a ciliary mechanism which insures the collection of fine particles, principally phytoplankton. The only extracellular process in the digestion of the lamellibranchs is the amylase action, while all other hydrolytic activity is displayed intracellularly. The amylase is liberated into the gastric cavity by the dissolution of the crystalline style.* Besides the absorption by the digestive diverticula (hepatopancreas), small food particles are absorbed and digested by mobile amebocytes. The latter are absent in the septibranchs which are pure carnivores. In the Teredinea, which dig in the wood, the digestive tube has undergone characteristic modifications. In these animals a portion of the digestive diverticula has been modified for the intracellular digestion of wood (Sigerfoos, 247; Potts, 207; Yonge, 287). It is possible to extract from this portion a cellulase enzyme which transforms cellulose into glucose (Haring-ton, 120). Generally, the lamellibranchs are specialized herbivores (phytoplankton) which cannot digest intracellularly either starch or glycogen. Small particles of protein or lipide can be absorbed and digested in the digestive diverticula but much larger particles are taken care of by mobile amebocytes. The intestine contains no protease. As was pointed out by Yonge, this is really an indispensable condition for the existence of the crystalline style which is made up of globulins. A protease in the

* In a recent article Th. F. Lavine (*J. Cellular Comp. Physiol.*, **28**, 183, 1946) gives the following description of the *crystalline style*: "The crystalline style is an easily isolated organ present in various herbivorous marine mollusks (most lamellibranchs and a few gastropods) that is of interest because it contains the only extracellular enzymes found in these organisms. It is an elongated hyaline rod lying in a separate diverticulum, the style sac; the anterior end juts into the stomach where, with an intermittent rotary motion, it rubs against a horny part of the latter, known as the gastric shield. The composition of the styles of the various organisms is consistently reported to be 87% water, 12% organic matter and 1% inorganic matter. The organic matter is largely protein and the presence of a globulin, an albumin and a mucin have been reported. . . . The style's location early suggested a digestive function, and two enzymes have been found to be associated with it, an amylase and an oxidase."

The style of clams, according to Lavine, contains a cellulase, presumably a β-glucosidase active towards β-glucosidic linkages and an amylase which degrades the α-glucosidic linkages of starch and glycogen.

intestine would digest the style immediately. Yonge also noted that the presence of a crystalline style implies that the animal is a specialized herbivore.

Among the gastropods diverse forms of the hydrolytic mechanism are found. Yonge divides the herbivorous gastropods into two groups (excepting the Pulmonates): those which do possess, and those which do not possess a crystalline style. In the former, for instance in the Streptoneura like *Crepidula*, *Vermetus*, *Turitella*, etc., the conditions are very much like those in the lamellibranchs, in which the only extracellular enzyme found is amylase and the digestive diverticula are not at all organs of secretory activity but organs of absorption and intracellular digestion. The second group of herbivorous gastropods, those lacking the crystalline style, includes tectibranchs, such as the sea hare, and the nudibranchs *Caliphylla* and *Hermaea*. The stomach of the sea hare which feeds exclusively on *Ulva* contains a very acid juice. It can be shown that this contains the enzymes amylase, proteinase, and cellulase (Enriques, 73), which are secreted by salivary and gastric glands. Certain cells of the gastric glands have a secretory function but others are phagocytic and have reverted to intracellular digestion. *Caliphylla* and *Hermaea*, which feed on such algae as *Bryopsis* and *Codium*, suck up the fluid cellular protoplasm of the algae into the digestive tube. The fluid protoplasm penetrates into the digestive diverticula. These are provided with a muscular mechanism which makes it possible for the food to go in and out of the diverticula, thus promoting phagocytosis and intracellular digestion of the chloroplasts.

In the carnivorous Gastropods, such as *Murex*, one always finds an active proteinase in the lumen of the digestive tube. This is secreted by the digestive diverticula which, thus, function as a true gland. However, intracellular digestion also proceeds in the diverticula. The salivary glands, on the other hand, secrete an amylase.

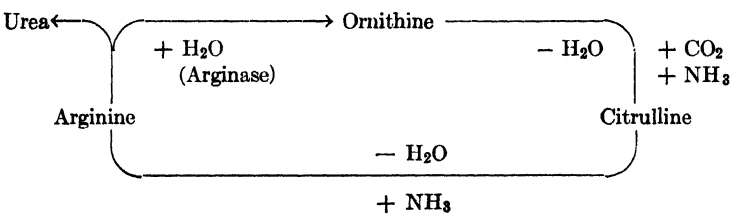
In the pulmonates, such as the snail, the hydrolytic processes are practically entirely extracellular and only the hydrolysis of protein is intracellular. In the cephalopods, on the other hand, digestion is exclusively extracellular, the intracellular digestion having completely disappeared (Romijn, 232).

D. Protein Metabolism

The metabolism of amino acids has been best studied in mammals, and they will be considered first. It is said that the food proteins, after being successively submitted to the action of pepsin in the gastric juice, of the trypsin in the pancreatic juice, and of peptidases in the intestinal juice, are finally absorbed as amino acids through the intestinal villi and enter the circulating blood. Part of these amino acids is utilized for constructive

purposes and the excess, as well as those amino acids which result from the endogenous protein metabolism, are degraded. The amino acids first undergo dehydrogenation, later hydration, then they form ammonia and ketonic acids. These operations, involved in the complex process of deamination of amino acids by means of different enzymes, occur in the liver and in the kidney.

The keto acid generally decomposes ultimately into carbon dioxide and water by a series of reactions which differs from one amino acid to another. The ammonia resulting from the deamidation of glutamine by the kidney is eliminated in the urine as ammonium salts of weak acids, as part of the system of physiological regulation of the acid-base balance. The ammonia formed by the deamination of amino acids in the liver of mammals is transformed there into urea by the mechanism known as the "ornithine cycle" (Krebs and Henseleit, 146). Ornithine, an amino acid found in the liver, plays a fundamental part in this cycle, and by combining with carbon dioxide and ammonia is changed to the amino acid citrulline. Citrulline, in its turn, takes up a molecule of ammonia and is transformed into arginine. By the action of the arginase enzyme, a molecule of urea is split off from arginine whereby ornithine is reclaimed and becomes again the starting point of the cycle. The cycle may be summed up in the following scheme:



Briefly, then, the protein metabolism of mammals has ammonia and urea as its ultimate products, the urea being in great preponderance. For this reason mammals are said to be *ureotelic.*

But in birds it has been known for a long time that the principal waste product of protein metabolism is not urea but uric acid. It has been actually demonstrated that urea is not being synthesized from ammonia in birds. Urea, therefore, can not be the precursor of the uric acid resulting from protein metabolism. Schuler and Reindel (241) and Benzinger and Krebs (18) independently of each other, have demonstrated that the liver and kidney of birds both can deaminate amino acids. They have also shown, that, at least in some of them, both organs perform the synthesis of uric acid. Ammonia is the substance from which the kidney and the liver can form uric acid, but urea is definitely not its precursor. This applies in the case of the chick or goose but not in the case of the pigeon.

Schuler and Reindel (243) brought forward evidence which shows that in the pigeon the ability to synthesize uric acid is not shared equally by both organs but that there is a certain division of labor. The liver, starting with ammonia, synthesizes a uric acid precursor which is then transformed to uric acid by the kidney. Schuler and Reindel identified this precursor as hypoxanthine, which is catalytically oxidized in the kidney by xanthine oxidase, an enzyme which is not present in the pigeon liver. A protein metabolism with a strong preponderance of uric acid formation is found among the Sauropsida, for example, the snakes and lizards, whose liver is very poor in arginase and for that reason cannot accomplish the ornithine cycle (Münzel, 182). But in the case of turtles one must distinguish between the aquatic and the terrestrial species. In an aquatic turtle like *Emys europea* urea strongly predominates among the nitrogenous excreta, and *in vitro* the liver synthesizes urea from ammonia while synthesis of uric acid is negligible. In a terrestrial turtle like *Testudo graeca* both urea and uric acid are abundant among the excreted nitrogenous substances. *In vitro* it is possible to show that its liver can synthesize uric acid from ammonia, but that urea is not formed as an intermediate in this synthesis (Münzel). Nevertheless, the liver of *Testudo graeca* can synthesize urea effectively by the ornithine cycle *in vitro* (Manderscheid, 177; Münzel, 182). One must, therefore, admit that in the liver of terrestrial turtles there exist side by side two different mechanisms of synthesis which, starting with ammonia, lead by different paths to the formation either of urea or of uric acid. Among the Amphibia, whose liver is rich in arginase and *in vitro* accomplishes the ornithine cycle (Manderscheid, 177; Münzel, 182), the protein metabolism is predominantly ureotelic. This is also true for the elasmobranch fishes whose tissues are all rich in arginase.

As for bony fishes, considering the over-all picture of excretion, we find that freshwater teleosts, such as the carp and goldfish, excrete ammonia and urea, with ammonia greatly in preponderance. The ammonia, however, is eliminated through the gills and its precursors are still unknown. In the Dipnoi, especially among the aquatic protopterans, both ammonia and urea are excreted, and the ammonia is again in great predominance (Smith, 250). But in the marine teleosts the total excretion is mainly made up of ammonia and trimethylamine oxide.

The data on the subject of protein metabolism in vertebrates are summed up in Table VII.

Among the invertebrates, as a general rule, we do not find the same well marked types of protein metabolism. In aquatic invertebrates ammonia definitely takes the lead over the other nitrogenous wastes, such as urea and uric acid. Among many invertebrates, for example, the earthworm, snail, etc., the influence of environmental or ecological conditions

upon the nature of their excretions can be discovered. But considering all the animals, it is apparent that excretion of ammonia is the primitive mechanism upon which evolution has superimposed the synthesis of such excreta as urea and uric acid, whereby the circulation and accumulation of ammonia in the internal environment has been avoided. A preponderant

TABLE VII

(Florkin, 93)

Animal classification	Principal product of protein metabolism	Hepatic arginase	Ornithine cycle
Mammals	Urea	+	+
Birds	Uric acid	Feeble	−
Reptiles			
Snakes and lizards	Uric acid	−	−
Turtles, aquatic	Urea	+	+
Turtles, terrestrial	Urea + uric acid	+	+
Amphibia (Anura)	Urea	+	+
Fish			
Elasmobranchs	Urea	+	
Teleosts, fresh water	NH_3 + Urea	+	−
Teleosts, marine	NH_3 + $(CH_3)_3NO$	+	
Dipneustes	NH_3 + Urea		

excretion of this substance is found only in the lower aquatic animals with an active circulation of water, which brings about a rapid purification of the internal environment. But as this purification becomes more difficult, the formation of urea by way of the ornithine cycle in vertebrates, and very probably by some other mechanism in invertebrates, becomes superimposed upon the more primitive protein metabolism. Among invertebrates which colonized dry land, as for instance among the terrestrial insects and the gastropods, when the unfavorable conditions for aqueous irrigation make the limitation of available water very critical, it is most probable that the accumulation of ammonia in the internal medium was the reason for the production of a much less soluble substance like uric acid, but we are ignorant as to the mechanism of this uricogenesis. In spiders uric acid is replaced by the purine base guanine. It is even less soluble than uric acid, and its origin is just as obscure.

In a very ancient group like the turtles a ureotelic and uricotelic mechanism coexist side by side (in the liver of terrestrial turtles) which may be traced to their amphibian ancestry and is perhaps evidence of phylogenetic succession. On the other hand, in contrast to the plasticity and ill defined nature of protein metabolism in some lower animals, in the more

highly developed animals we find that the ultimate protein metabolism is well defined and the process of degradation is nearly completely determined. In the insects or in the homeothermal vertebrates, which have achieved great independence from the external environment, the protein metabolism has become unilateral and is no longer affected by external circumstances. Insects, birds, snakes and lizards excrete the protein nitrogen almost entirely in the form of uric acid, while the mammals excrete it almost entirely in the form of urea. This illustrates the perfection of a biochemical mechanism for preventing the circulation of ammonia within the internal environment.

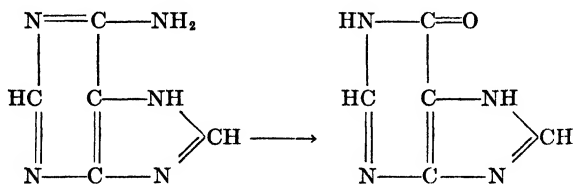
E. Ammonemia

A comparative study of the internal environment from the point of view of its ammonia content has long been frustrated by the formation of ammonia in shed blood, a phenomenon which only recently has been carefully studied. Almost as soon as blood of mammals leaves the vessels, a rapid production of ammonia (ammoniogenesis α) occurs, lasting about five minutes (Conway and Cooke, 47). This is followed by a small and prolonged rise in the blood ammonia. It had already been noted in many of the older studies, but was especially well studied by the school of Parnas. One can form an idea of the magnitude of the blood ammonia found *in vivo* by determining the curve of ammoniogenesis α and extrapolating to zero time, which indicates the initial level of preformed ammonemia. In mammals and birds the curve reveals that the initial blood ammonia level is extremely low, attaining at most the value of 0.01 mg. % (47). The same has been found (Florkin, 92) also in poikilotherm vertebrates (frog, *Emys orbicularis*, *Clemmys leprosa*, tench, and trout). It can be stated, therefore, that in vertebrates the ammonia content of the blood is practically nil. In insects, such as *Hydrophilus* and *Dytiscus*, it was also shown that *in vivo* the blood ammonia content is nil or at any rate very slight, as is also the case in vertebrates. On the contrary, in the snail, lobster, or crayfish the blood *in vivo* contains easily measurable quantities of ammonia: in snails 0.7–2.0 mg. %, in the lobster (Florkin and Renwart, 106) 1.6–1.8 mg. %, and in the crayfish (Florkin and Frappez, 104) 1.9 mg. %.

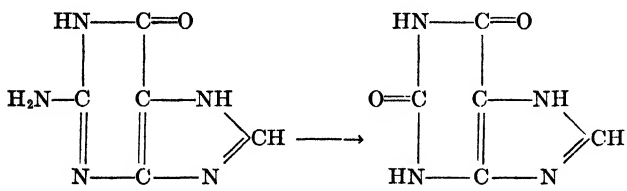
Thus, crustaceans and mollusks show a relatively high ammonia content in the blood while in vertebrates and in higher insects the ammonia content is practically nil. One perceives here the result of a more efficient synthesis of excretory waste products in the more highly evolved animals: the internal environment is thus disencumbered of circulating ammonia resulting from fluctuating rates of deamination. In other words, the synthesis assures the stability of the internal medium and at the same time the independence of the animal as far as its surrounding environment is concerned.

F. Purine Metabolism

All that is known of purine metabolism in living things indicates that this follows a very unique course and that the chain of reactions involved consists of the following steps: deamination of aminopurines, oxidation to uric acid, and finally uricolysis or degradation of the purine nucleus to a greater or less extent. Animal and vegetable nucleic acids are tetranucleotide compounds containing the two purine derivatives, adenine and guanine. These two aminopurines are, therefore, the substrate of purine metabolism in living things and the first stage of the metabolism is represented by deamination of adenine to hypoxanthine and of guanine to xanthine.



Adenine Hypoxanthine

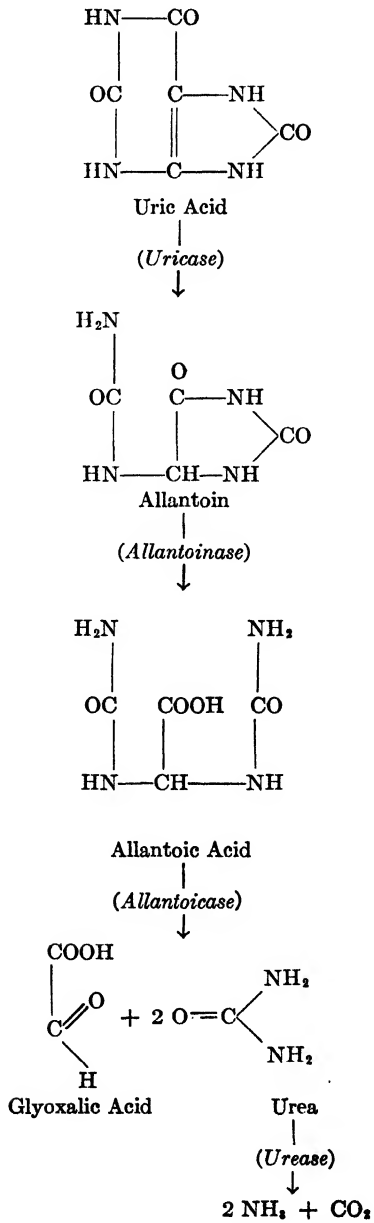


Guanine Xanthine

The existence of enzymes for the deamination of aminopurines has been demonstrated in representatives of the different animal groups (Schmidt, 238; Duchâteau *et al.*, 66). After deamination has been accomplished, xanthine oxidase is the agent which intervenes in the metabolism and, in the presence of molecular oxygen, brings about the oxidation of the hypoxanthine and xanthine to uric acid. The existence of the xanthine oxidase in the liver of mammals and birds has been established long ago. The enzyme is also widely distributed among invertebrates. It has been demonstrated beyond a doubt in the earthworm, pond mussel, *Planorbis*, *Dytiscus*, mole cricket (*Gryllotolpa*) and larvae of such different insects as *Aeschna*, *Tenebrio*, and *Limnophilus* (Florkin and Duchâteau, 96).

The first stages of purine metabolism terminate, therefore, with the formation of uric acid, which is the last metabolic stage where the purine nucleus is still intact. The degradation of this nucleus, or *uricolysis*, results

when a different series of enzymes come into play and, where the degradation has gone very far, can convert the nitrogen of the purine nucleus to ammonia:



The complete enzymatic system of uricolysis, which affects also the fate of the nitrogen of the purine nucleus, comprises the following constituents: *uricase*, *allantoinase*, *allantoicase* and *urcase*.

The enzymatic system of uricolysis is not the same in all groups of animals. Table VIII shows the results of studies on the components of this system in different zoological groups. A survey of the table enables us to gain an insight into the nature of the over-all purine metabolism.

TABLE VIII

(Florkin and Duchâteau)

Animal	Uricase	Allantoinase	Allantoicase	Urease	References
Actinia	+	..	..	..	Przylecki (210)
Starfish	+	+	..	..	Przylecki (210), Fosse and Brunel (109)
Sea urchin	+	+	..	..	Idem
Sipunculus	+	+	+	+	Florkin and Duchâteau (100)
Mytilus	+	+	+	+	Brunel (43)
Anodonta	+	+	+	+	Florkin and Duchâteau (100)
Crayfish	+	+	+	+	Idem
Lobster	+	+	+	+	Idem
Helix	+	..	..	..	Spitzer (258), Grah (114) and Plum (206)
Planorbis	+	−	−	..	Florkin and Duchâteau (100)
Earthworm	−	−	−	..	Idem
Hirudo	−	−	−	..	Idem
Distomum	..	−	−	..	Idem
Planaria	−	−	−	..	Idem
Hydrophilus	−	−	−	..	Idem
Tenebrio, adult	..	−	−	..	Idem
larva	−	−	−	..	Idem
Dytiscus	−	+	−	..	Idem
Aeschna, larva	−	−	−	..	Idem
Limnophilus, larva	−	−	−	..	Idem
Lucilia, eggs	+	−	..	−	Brown (38, 39)
larva	+	−	..	−	Idem
pupa	−	−	..	−	Idem
adult	+	−	..	−	Idem
Protopterus	+	+	+	..	Florkin and Duchâteau (100)
Calamoichthys	..	+	+	..	Idem
Raja clavata	+	+	+	..	Brunel (41)
Raja punctata	+	+	+	..	Idem

TABLE VIII (*Continued*)

Animal	Uricase	Allantoinase	Allantoicase	Urease	References
Cyprinidae, Esocidae, Scombridae	+	+	+	..	Brunel (41)
Salmonidae, Pleuronectidae, Anguillidae	+	+	−	..	Idem
Frog	+	+	+	−	Przylecki (209); Krebs and Weil (147); Brunel (42)
Various anurans and urodelans	+	+	+	..	Brunel (42)
Lamperta	−	−	−	..	Florkin and Duchâteau (100)
Lacerta	−	−	..	..	Przylecki (209)
Testudo graeca	..	−	−	..	Florkin and Duchâteau (100)
Clemmys leprosa	..	−	−	..	Idem
Pseudopus	..	−	−	..	Idem
Birds	−	−	..	..	Przylecki (208)
Mammals	+	−	−	−	Numerous authors
Man and other primates	−	−	−	−	Idem

Analysis of the excreta gives, indeed, little information on this score Since the excretory products of protein metabolism are quantitatively much more abundant than those of purine metabolism, the latter are literally swamped in the mass of the former. If uricase, allantoinase, allantoicase, and urease all coexisted, it could be assumed that purine metabolism, starting with the nitrogen of the purine nucleus, would be pushed ultimately to the production of ammonia. But if uricase, allantoinase, and allantoicase were present, it is likely that urea would be the ultimate product of purine metabolism. Furthermore, if it could be demonstrated that only uricase and allantoinase are present, the degradation would end with allantoic acid, whereas if the animal possessed uricase alone, only the stage of allantoin would be reached. If uricase is lacking, the purine nucleus will not split open and will be found in the excreta either as uric acid or as some compounds more closely resembling the aminopurines. By this reasoning we can gain information from Table VIII regarding the probable nature of purine metabolism in different groups of animals. A summary of the available information is presented in Table IX.

The one conclusion that can be drawn from the data presented in Table IX is that, contrary to the classical view, the most primitive types of purine metabolism found in the Metazoa, especially the types encountered in marine invertebrates, are not those which involve little transformation

TABLE IX

(Florkin and Duchâteau, 100)

↓	Man and other primates, birds, terrestrial reptiles
Uric Acid	Cyclostomes
|	Insects (except Diptera)
|	
(*Uricase*)	Mammals (except man and other primates)
↓	Diptera
Allantoin	Gastropods
|	
|	
(*Allantoinase*)	One group of Teleosts
↓	(Salmonidae, Pleuronectidae, Anguillidae)
Allantoic Acid	
|	
|	
(*Allantoicase*)	Selachii, Dipnoi, Crossopterygii
↓	One group of Teleosts (Cyprinidae, Esocidae and Scombridae)
Urea	Amphibia
|	Fresh-water lamellibranchs
|	
(*Urease*)	Sipunculids
↓	Marine lamellibranchs
Ammonia	Crustacea

of the aminopurines without destruction of their nucleus. On the contrary, the primitive types of metabolism bring about far reaching degradation, proceeding as far as the ammonia stage, as in the case of *Sipunculus*, mussel, lobster, and crayfish. The classical view, according to which purine metabolism in the lower forms of life should be very brief, is based on the assumption that the enzymes responsible for the deamination and oxidation of aminopurines are lacking in the great majority of invertebrates. But, as we have seen, this view is not acceptable. Comparing the different groups, one can see that in the most highly developed worms, for example, in the oligochetes and leeches, uricolysis progresses to a far lesser extent than in the sipunculids; it progresses to a far lesser extent in gastropods than in lamellibranchs; in fresh-water lamellibranchs than in marine lamellibranchs, in insects than in Crustacea, in Sauropsida than in Amphibia or fishes, in man and other primates than in all other mammals.

It thus appears that the evolution of purine metabolism operates by way of curtailing the enzymatic chain of uricolysis. The evolutionary process seems to manifest itself by a loss of enzymes, by a process of *enzymapheresis*, so to speak, whereby the primitive chain becomes progressively shortened by the loss of the terminal link. The complete chain

leading to ammonia formation is found in the marine invertebrates, such as *Sipunculus*, mussel, and crustaceans. By the loss of a terminal link the enzymatic chain leads next only to the formation of urea, as it is seen in the fresh-water mussel (*Anodonta*), fishes, and amphibians. A further shortening of the chain by one or two links, leading to the formation of allantoin or of uric acid, is found in animals which have become more highly evolved in the sense of attaining greater independence or by way of adaptation. This is the case in oligochetes, leeches, insects, reptiles, birds, and mammals.

A study of the zoological distribution of enzymes of the uricolytic system, thus, shows that the evolution of the purine metabolism is attained by a process of simplification through the dropping off of terminal links in the uricolytic enzyme chain. This evolution presents itself as a regressive biochemical orthogenesis through a process of enzymapheresis.

CHAPTER V

Biochemical Adaptations

Biochemical Characteristics in Relation to Anatomical, Physiological, and Ecological Characteristics

From the standpoint of general biochemistry, as the progress of research during the last decades has revealed, functional substances are able to control the organism's physiology. These special and exceptionally endowed substances belong to certain classes of chemical compounds which, however, are represented also by substances with no special biological function to perform. The sterol nucleus, for instance, may give rise to a substance endowed with estrogenic activity, or to a substance involved in mineral metabolism, or to a substance playing a part in ossification. But besides these functional substances one finds others, derived from the same sterol nucleus, which completely lack biological activity.*

It is an important fact that a change in the structure of the molecule may endow it with different activity. This depends sometimes upon slight modifications which, wherever or whenever they appear, impart special properties to derivatives from otherwise inactive compounds. The molecular changes may be responsible for some novel action occurring locally or they may create a new biochemical system which is affected by some already pre-existing substance. It is, therefore, not surprising to find that organisms may possess biochemical constituents which do not seem to have any significance for the life of the animal. For instance, what role can the melanophore-dilating hormone of the posterior hypophysis possibly play in mammals, or the oxytocic hormone in the ascidian *Ciona*? Nevertheless, there are biochemical peculiarities for which it is possible to establish a correlation with anatomical, physiological, or ecological conditions. In the living animal, as in a machine, the parts are adjusted to each other in the integration of the organism. The animal lives in a definite and separate medium but it is also in a continuous state of interchange with it and lives, therefore, in a certain characteristic manner. In the last analysis, the functioning of the animal as a whole is only the over-all effect of biochemical phenomena which unroll themselves on a submicroscopic scale. It would be very surprising if the biochemical systems, as the essential factors of functioning and of the mode of existence of the animal in its

* Marcel Florkin, Introduction à la Biochimie générale. Masson, Paris, and Desoer, Liége.

environment, were not likewise integrated. In fact, in a number of instances it can be demonstrated that a biochemical constituent or some peculiarity of a biochemical system determines the outward aspects of an animal's existence in nature. In other words, it is possible to show that life would be impossible in the absence of some biochemical constituent. Thus, if an organism lacks insulin, the biochemical constituent secreted by the Isles of Langerhans of the pancreas, diabetes develops and death may result from acidosis. But it is sufficient to inject the lacking biochemical substance to reestablish the normal existence of the organism. Similarly, the fatal Addison's disease results from a deficiency in the production of steroid hormones by the adrenal cortex. The biochemistry of internal secretions furnishes numerous examples where definite functional substances determine the morphology, the physiology, and even the very existence of animals. But these things are already so well known that it is not necessary to stress them further. The examples to be presented in the following pages are not limited to higher organisms but illustrate a pattern of biochemical adaptation which occurs in the entire animal kingdom.

A. Domain of Respiratory Function

1. *Protein Content and Anatomical Localization of the Internal Medium*

Biochemically it is of much importance whether the internal medium is a fluid contained in a coelomic cavity or a fluid within a circulatory system. Oxygen carriers within the coelomic fluid are invariably intracellular. Furthermore, the coelomic fluids are practically free from protein whereas the protein content in circulating bloods is an important variable. This correlation may also be considered as a special physiological function. In circulating bloods there is in effect an exchange of water with the tissues. The colloidal osmotic pressure of plasma, dependent upon the presence of proteins, plays an important role as regulator for maintaining the blood water content which the hydrostatic pressure tends to drive out of the vessels. The presence of proteins in the circulating bloods may, therefore, be considered a biochemical characteristic correlated with the special physiological problem of water exchange in the organism.

2. *Size and Localization of Oxygen Carriers*

Considering oxygen carriers according to their intracellular or extracellular localization, it appears that generally hemocyanin and chlorocruorin are dissolved in the blood, whereas hemoglobin may be either dissolved in the blood fluid or enclosed in the blood cells. But as far as the oxygen carriers found in coelomic fluids (hemoglobin or hemerythrin) are concerned, they are always enclosed in cells. Furthermore, there is a

definite correlation between the molecular weight of the oxygen carriers and their being enclosed in cells (Svedberg, 263).

Surveying Table X, it is clear that hemocyanins and chlorocruorins, which are invariably dissolved in the blood fluid, have high molecular weights (422,000 or more), but the molecular weights of hemoglobins vary all the way from 17,600 to 3,380,000. However, the hemoglobins which are dissolved in the plasma also have molecular weights of about 422,000 or more (the hemoglobin of the *Chironomus* larva is an exception to this rule).

TABLE X

(*Svedberg, 263*)

Oxygen carriers	Mol. weight	Localization I = intracellular E = extracellular
Hemoglobin of *Lamperta*	17,600	I
Hemoglobin of *Arca*	2 × 17,600 = 35,200	I
Hemoglobin of *Chironomus*		E
Hemoglobin of Mammals	4 × 17,600 = 70,400	I
Hemoglobin of *Sipunculus*		I
Hemoglobin of *Daphnia*	24 × 17,600 = 422,000	E
Hemocyanin of *Pandalus*		E
Hemocyanin of *Palinurus*		E
Hemocyanin of *Nephrops*	48 × 17,600 = 845,000	E
Hemocyanin of *Homarus*		E
Hemoglobin of *Planorbis*	96 × 17,600 = 1,690,000	E
Hemocyanin of *Calorcaris*		E
Hemocyanin of *Octopus*	168 × 17,600 = 2,960,000	E
Hemocyanin of *Eledone*		E
Hemoglobin of *Arenicola*	192 × 17,600 = 3,380,000	E
Chlorocruorine of *Spirographis*		E
Hemocyanin of *Rossia*		E
Hemoglobin of *Lumbricus*		E
Hemocyanin of *Helix*	384 × 17,600 = 6,760,000	E
Hemocyanin of *Busycon*		E

(The fact that hemerythrin of the *Sipunculus* has the same molecular weight as mammalian hemoglobin has been established by G. S. and M. E. Adair, and by A. and J. Roche, 223.)

In a general way, it can be said, therefore, that the oxygen carriers enclosed within blood cells are always small protein molecules.

Does the correlation between the particle size of the oxygen carrier and its localization within blood cells have a physiological counterpart? Where the blood contains practically no protein (as is the case in the octopus (Henze, 725) it is possible, knowing the molecular weight of the hemocyanin,

to calculate the colloidal osmotic pressure of the oxygen carrier. In the octopus this pressure can be shown to be of the order of 3 mm. of mercury (Florkin and Blum, 94) and the oxygen capacity is 4.5 volume % (Winterstein, 283). In man the protein osmotic pressure is abut 30 mm. mercury (Krogh, 148) and the oxygen capacity 20 volume %. Here, the colloidal osmotic pressure is due to the plasma proteins and the oxygen capacity to the hemoglobin in the red cells. What would the situation be if the hemoglobin necessary to attain an oxygen capacity of 20 volume % was dissolved in a plasma containing no other protein? In that case the colloidal osmotic pressure would be 175 mm. of mercury, whereas, as a matter of fact, it is only about one-sixth as great. The fact that human hemoglobin is a small molecule establishes, therefore, an incompatibility between the two functions which it would exercise if it were dissolved in the plasma; namely, the function of carrying oxygen and of maintaining the protein osmotic pressure. The mammalian organism could not exist if the hemoglobin was dissolved in the plasma instead of being enclosed in the cells. The two functions, that of creating a proper colloidal osmotic pressure and that of transporting oxygen, could not be served simultaneously by such large carrier molecules as those which are found dissolved in the blood plasma.

3. *Presence or Absence of the Bohr Effect and Localization of Oxygen Carriers*

If the partial pressure of the carbon dioxide gas is raised or if the solution is acidified, the oxygen absorption curve of an oxygen carrier, either in aqueous solution or in blood, is shifted to the right. The phenomenon is known as the Bohr effect. Among the many bloods and coelomic fluids which have been thus far investigated only two instances are known where this Bohr effect is lacking; namely, the coelomic fluid of an echiuroid worm, *Urechis caupo*, containing hemoglobin enclosed in cells (Redfield and Florkin, 217) and the coelomic fluid of *Sipunculus* the cells of which contain hemerythrin (Florkin, 78). Both of these animal forms are devoid of a circulation and their coelomic fluid contains *intracellular carriers of oxygen*. On the contrary, in all instances where the biological fluid containing an oxygen carrier is blood, enclosed within a circulatory system, one finds a more or less marked influence of the carbon dioxide tension on the position of the oxygen dissociation curve. In such animals, for instance in the lugworm (*Arenicola*), the blood shows the Bohr effect (Barcroft and Barcroft, 11), which aids in taking on or in setting free oxygen as the blood passes more or less rapidly from the organs of respiration to the body tissues. This does not occur in the coelomic fluid of *Urechis* or of *Sipunculus*. Here the absence of the Bohr effect appears to be a biochemical characteristic correlated with an anatomical peculiarity, namely, the absence of a circulatory system.

4. *Meaning and Magnitude of the Bohr Effect and Physiological and Ecological Characteristics of Animals*

In a general way, the Bohr effect is manifested in animals possessing a circulation. As can be seen from Fig. 7, if one considers the relation between

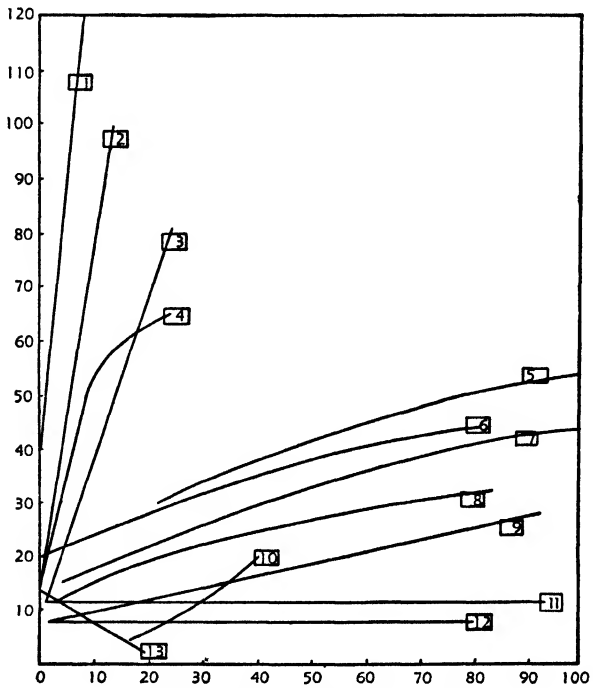


FIG. 7

(Florkin.) Relation between p_{50} and pCO_2. (p_{50}=partial pressure of oxygen in mm. Hg corresponding to a 50% saturation; pCO_2=partial pressure of CO_2 in mm. Hg.) 1. *Loligo*, at 23° (Redfield *et al.*, 216) 2. *Prionotus*, at 20° 3. *Opsamus* at 20° 4. Mackerel at 20° (Root, 235) 5. *Eumetopias* at 38° (Florkin and Redfield, 105) 6. Goose at 42° (Wastl and Leiner, 276) 7. Dog at 37.5° (Dill *et al.*, 60) 8. Man at 37.5° (Bock *et al.*, 26) 9. *Pseudemys* at 25° (Southworth and Redfield, 257) 10. Carp at 10° (Wastl, 275) 11. *Urechis* at 19° (Redfield and Florkin, 217) 12. Sipunculid at 19° (Florkin, 78) 13. *Busycon* at 25° (Redfield *et al.*, 216).

p_{50} (partial oxygen pressure corresponding to a 50% saturation) and pCO_2 (partial pressure of carbon dioxide), it is obvious that the amplitude of the Bohr effect differs greatly for different animal species. In animals like the squid or marine teleosts, which lead a very active existence in the waters of the ocean, this is very marked, but in air breathing animals or in fresh-

water animals the amplitude is much less pronounced. A great number of animals show a regular Bohr effect, but some others reveal unusual relations between pH and the position of the oxygen absorption curve. In certain animals, which contain hemocyanin in the blood, the Bohr effect is reversed; i.e., the affinity of the hemocyanin for oxygen is increased with a rise in the partial carbon dioxide tension. This has been observed in the blood of *Limulus* and *Busycon* (Redfield *et al.*, 216) and of the snail (Hogben and Pinhey, 130). The experiments of Redfield *et al.* (216), of Pantin and Hogben (197), of Hogben (129), of Hogben and Pinhey (130), and of Stedman and Stedman (260), all show that the affinity for oxygen of each hemocyanin presents a minimum at a given pH. The hemocyanins can be separated into two groups: in the first group, the minimum affinity for oxygen is on the acid side of the physiological range and the Bohr effect is normal (squid, crustacean); in the second group, the hemocyanins have a minimum affinity on the alkaline side of the physiological range and the Bohr effect is inverted (*Limulus, Busycon*). Where the Bohr effect is inverted, the affinity for oxygen is great and the Bohr effect is slight. A minimum affinity for oxygen at a pH strongly on the acid side of the physiological range has been shown in solutions of dog hemoglobin (Rona and Ylppö, 233), horse hemoglobin (Ferry and Green, 76) and in the blood of the fish *Tautoga onitis* (Green and Root, 115). The significance of the Bohr effect for each blood depends, therefore, upon the position of minimum affinity for oxygen of the oxygen carrier in relation to its physiological pH range.

The Bohr effect is especially prominent in such animals as the squid or in bony marine fishes such as the mackerel, which cannot exist except in a well oxygenated medium where carbon dioxide is practically absent. It is less pronounced in animals that live in fresh water or breathe through lungs. It is also very feeble in the skate. The inverted Bohr effect is found in animals like the snail, *Busycon* and *Limulus;* i.e., in animals whose mode of life brings them into contact with a medium poor in oxygen and rich in carbon dioxide.

5. *The Oxygen Tension at the Base of the Respiratory Cycle and the Activity of the Animal*

In the course of the respiratory cycle, as the blood turns venous, the content of oxygen and its partial pressure decrease. The partial pressure of the oxygen in venous blood depends upon the particular characteristics of the respiratory cycle of each animal. It reflects the oxygen tension prevailing in the ultimate ramifications of the circulatory system and, therefore, gives us an idea of the magnitude of the partial pressure under which oxygen is liberated in the tissues. As can be seen from Fig. 8, there is a

relation between this oxygen tension corresponding to the "base" of the respiratory cycle and the degree of activity of the corresponding organism.

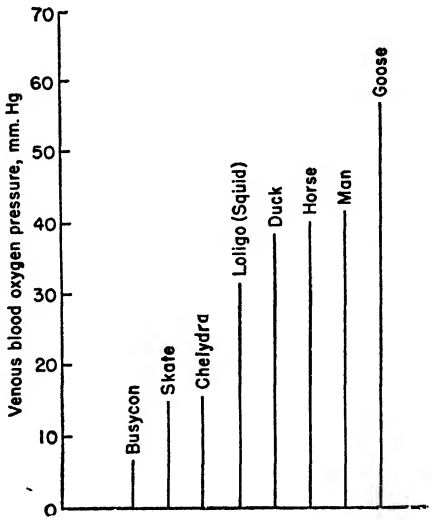


FIG. 8

(Florkin, 78.) The oxygen partial pressure at the "base" of the respiratory cycle.

6. *Loading Pressure of the Oxygen Carrier and the Partial Pressure of Arterial Oxygen*

When, in the course of the respiratory cycle, venous blood reaches the organ where the gas exchange with the environment ultimately takes place, it meets a much higher partial oxygen pressure than its own and, in consequence, becomes charged with oxygen. The physiology of the organs of respiration presents many variations which involve, under the conditions existing in the external environment (the atmospheric air), different levels of oxygen pressure under which the exchange is implemented. In the squid, oxygen is brought to the blood by the sea water circulating in the buccal cavity where the gills are found. The circulation is maintained with the aid of respiratory movements depending upon the animal's locomotion. In well-aerated sea water, at the partial pressure of 150 mm., the oxygen tension in the blood is 115 mm. mercury (Redfield and Goodkind, 218). On the contrary, in the marine gastropod *Busycon canaliculatum* we find a much less complete equilibration. In this animal oxygenated blood goes from the gills into the auricle, then into the ventricle, from where it is finally distributed by the vessels to the body tissues. The venous blood accumulates into a large sinus and from there passes into the gills. The

oxygen is brought to the gills by the circulation of sea water in the buccal cavity. The sea water is in equilibrium with air ($pO_2 = 150$ mm.) but the partial pressure of the arterial blood of Busycon is only 36 mm. of mercury (Redfield *et al.*, 216). In the skate *Raja ocellata* the blood, after being oxygenated in the gills, is distributed to the tissues and then returns to the heart, which contains only venous blood. From the heart it passes again into the capillaries of the gills, where the circulation is slow and sluggish. In the gills the equilibration of the blood with the external environment is not very perfect. Even when the sea water is well oxygenated, the partial pressure of oxygen in the arterial blood is only 70 mm. of mercury (Dill *et al.*, 59).

In the snapping turtle *Chelydra serpentina* the ventilation of the organ of respiration is not good and poorly regulated. Besides, although there is a double circulation, the cavities of the heart communicate and the arterial system, therefore, contains a mixture of arterial and venous blood. The imperfection of the circulatory system is associated with a low level of arterial partial oxygen pressure of only 57 mm. of mercury. In the homeothermic animals the partial oxygen pressure is also different from that of the external environment because it depends upon equilibrium with the alveolar air, which is different from atmospheric air. For instance, in the duck the partial oxygen pressure is 102 mm. (Wastl and Leiner, 276), in the goose 94 mm. (Wastl and Leiner, 276), in the horse 100 mm. (Henderson, 124), and in man 78 mm. (Henderson, 124).

TABLE XI

Organism	Arterial pO_2 in mm. Hg	Degree of arterial saturation	References
Sipunculus nudus	32	90	Florkin (78)
Urechis caupo	75	97	Redfield and Florkin (217)
Busycon canaliculatum	36	95	Redfield *et al.* (216)
Loligo pealei	115	97	Redfield and Goodkind (218)
Raja ocellata	70	93	Dill *et al.* (59)
Cyprinus carpio	85	93	Wastl (275)
Chelydra serpentina	57	95	Henderson (124)
Duck	102	98	Wastl and Leiner (276)
Pigeon	105	96	Idem
Goose	94	96	Idem
Horse	100	98	Henderson (124)
Man (A.V.B.)	78	96	Idem

It is obvious that, because of variations in the respiratory and circulatory systems, the levels of oxygen partial pressure, i.e., the pressures at the "peak" of the respiratory cycle, are very variable. And yet, if these values

of partial oxygen pressure are compared with the values of the degree of saturation of the oxygen carriers under arterial conditions, it is clear that a uniformity exists in the latter which contrasts with the diversity of the former. The degree of saturation of arterial blood (or, in the absence of a circulation, the degree of saturation of the coelomic fluid when the animal is in water equilibrated with atmospheric air) always falls within the range of 90 to 100%.

Thus, there is a correspondence between the position of the dissociation curve of the oxygen carrier and the conditions of respiratory physiology. The agreement is reflected in the fact that the loading pressure, i.e., the oxygen pressure where a 90–100% saturation is attained, corresponds on the graph of the absorption curve to the arterial partial pressure actually realized in the animal. Each drop in the partial pressure leads to a liberation of oxygen from the carrier, depending upon the shape of the oxygen absorption curve. In summary, the loading always operates at the highest point of the ascending limb of the dissociation curve independently of the position of the latter. This fact is illustrated in Fig. 9.

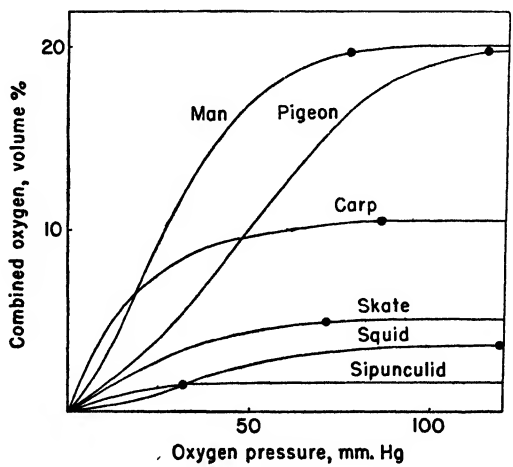


FIG. 9

Curves of oxygen absorption of different bloods under "arterial" conditions reproduced experimentally *in vitro*. • =arterial pO_2 *in vivo*. Man, at 38° and pH=7.47 (Henderson, 124), pigeon, at 42°, pCO_2=40 mm. (Wastl and Leiner, 276), carp, at 18°, pCO_2=30 mm. (Wastl, 275), skate, at 10.4°, pH=7.8 (Dill *et al.*, 59), squid, at 23°, pCO_2=0.5 mm. (Redfield *et al.*, 216), sipunculid, at 19°, pH=7.7 (Florkin, 78).

7. *The Oxygen Carrier and Metabolic Needs of the Animal*

Is the presence of an oxygen carrier of vital importance to animals? Very small animals can satisfy the metabolic needs of their tissues if the

fluid (blood) contains oxygen in physical solution. But there can be no doubt that for large and active animals the presence of oxygen carriers compensates for the big difference between the needs of the tissues and the oxygen brought in either by diffusion or by removal from the external environment as oxygen dissolved in the blood. In other words, the oxygen carrier compensates for the lack of harmony between the anatomical arrangement and the actual metabolic needs of the animal. Table XII reveals clearly the vital importance of the existence of a carrier for supplying oxygen to the tissues.

TABLE XII

The Cycle of the Respiratory Oxygen

(Florkin, 78)

Organism	Arterial pO_2 in mm. Hg	Venous pO_2 in mm. Hg	Oxygen transported in vol. %	Oxygen transported in dissolved state, vol. %	Oxygen transported in combined state, vol. %
Busycon canaliculatum	36	6	1.7	0.14	1.56
Loligo pealei	115	30	3.2	0.38	2.82
Raja ocellata	70	14	3.92	0.22	3.70
Chelydra serpentina	57	15	3.70	0.17	3.53
Duck	102	37	10.0	0.15	9.85
Goose	94	56	5.14	0.17	4.97
Man (A.V.B.)	78	40	5.3	0.10	5.20

The idea of the indispensability of an oxygen carrier is further corroborated by the results of the intoxication of mammals with carbon monoxide. Here death results from asphyxia because the respiratory function of the carrier is abolished through its combination with the carbon monoxide. The problem of the need for oxygen carriers can be approached also from another angle, as was done by Redfield and Goodkind in their study of the squid, *Loligo pealei*, by so altering the nature of the environment that the carrier can no longer perform its function. The results of their experiments show that the life of the animal becomes impossible under conditions where the oxygen content is lowered to 0.5 volume %, a concentration corresponding to the amount of oxygen that would be in physical solution in the internal medium in equilibrium with the outside environment, if it was devoid of an oxygen carrier.

The analyses of arterial and venous bloods in relation to the physicochemical properties of squid blood (previously determined by Redfield *et al.*, 216) enabled Redfield and Goodkind (218) to sum up the characteristics of the respiratory cycle. Fig. 10 furnishes a nomogram of the physicochemical system of the *Loligo* blood at 23°C. It can be seen from this nomogram that the blood leaves the gills almost completely saturated with

oxygen, as was already pointed out previously, and that the blood becomes venous by liberating its combined oxygen almost completely when the venous partial pressure of oxygen falls to 48 mm. The blood accumulates carbon dioxide as it becomes venous and shows only a 4 mm. increase in partial carbon dioxide pressure. In spite of the fact that the blood still

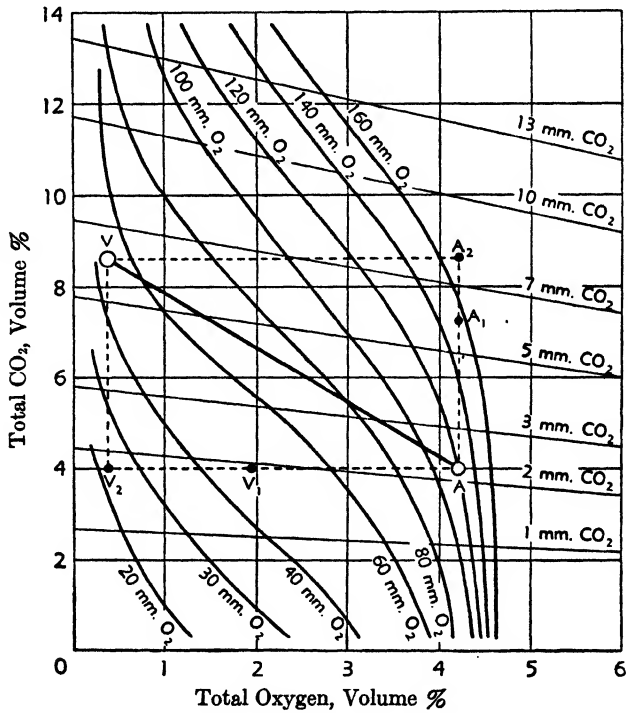


FIG. 10

(Redfield and Goodkind, 218.) Nomogram of the blood of the squid *Loligo pealei* showing the respiratory cycle at 23°C. The rectangular coordinates correspond to the O_2 and CO_2 concentrations while the inclined coordinates indicate the partial pressures, pO_2 and pCO_2.

contains an appreciable amount of carbon dioxide after it has become arterial on passing through the gills, the partial pressure of its carbon dioxide is only 2 mm. mercury; in other words, an equilibrium is practically established between the blood and the external environment.

The most striking characteristic of the respiratory cycle is the remarkable transport of oxygen, the mechanism of which is revealed in the nomogram. This shows that when the blood yields 3.9 volume % of oxygen to the tissues in the course of the cycle (from A to V in the nomogram), the

partial pressure of oxygen (pO_2) decreases by 70 mm. If there had been no accumulation of carbon dioxide during the change-over from arterial to venous blood, the curve expressing the respiratory cycle would follow along the line of a constant carbon dioxide content, i.e., from A to V_1 in the nomogram. For a drop of 70 mm. in the partial pressure of oxygen from its level at A, the point reached (provided the existing oxygen tension in the tissues, the constant of diffusion of oxygen and the rate of circulation remain unchanged) should be, therefore, V_1 corresponding to an oxygen content of 1.9 volume %. Since the arterial blood contained 4.2 volume % of total oxygen, the change from the arterial to the venous condition would set free 2.3 volume %. As a matter of fact, during the real respiratory cycle 3.9 volume % was set free because the Bohr effect was brought into play. To produce an oxygen discharge corresponding to the change in oxygen content from A to V, without the production of carbon dioxide, the oxygen partial pressure would have to drop to V_2, i.e., to 23 mm. But thanks to the shift in the oxygen dissociation curve, owing to the production of carbon dioxide, such a decrease in oxygen content occurs during the respiratory cycle while the pressure is maintained at 48 mm. as at V. To set free a given amount of oxygen, the Bohr effect aids in raising the partial pressure of the oxygen from 23 to 48 mm. mercury. By a similar reasoning it can be shown that the reduction of the hemocyanin to the tissue level, under the existing conditions of carbon dioxide pressure, raises the carbon dioxide content from 7.4 (A_1) to 8.3 volume % (V). If there had been no reduction and intervention of the Haldane effect, the partial pressure of the carbon dioxide (with the same amount of carbon dioxide) would have increased 8 mm., whereas the increase is only 6 mm. It must be admitted that the intervention of the Bohr effect and of its reciprocal Haldane effect is responsible for about one-third of the entire respiratory gas exchange. These facts illustrate the importance of the Bohr effect in the gaseous exchange of animals with a circulation, especially in very active animals like the squid which are dependent upon adequate aeration (see Fig. 7).

Redfield and Goodkind (218) produced asphyxia in a group of squids in ordinary sea water or in sea water to which carbon dioxide was added. The water was covered with a layer of paraffin. Under these conditions the animals gradually became less and less active and their respiratory movements finally ceased. One hour after respiration stopped the water was withdrawn and analyzed for oxygen and carbon dioxide. The composition of the water furnished information as to the conditions necessary for the existence of the animal. Since the blood in the squid is practically in equilibrium with its environment, it is possible to place the data obtained by analysis of the water in the nomogram, as was done in Fig. 11. This shows that the points corresponding to the lethal conditions, especially in

the sea water to which carbon dioxide had been added, correspond to different values of total carbon dioxide content and oxygen pressure, but they all fall in the same vertical column within the range of 0.5 to 1.5 volume % oxygen. These experiments demonstrate the indispensability

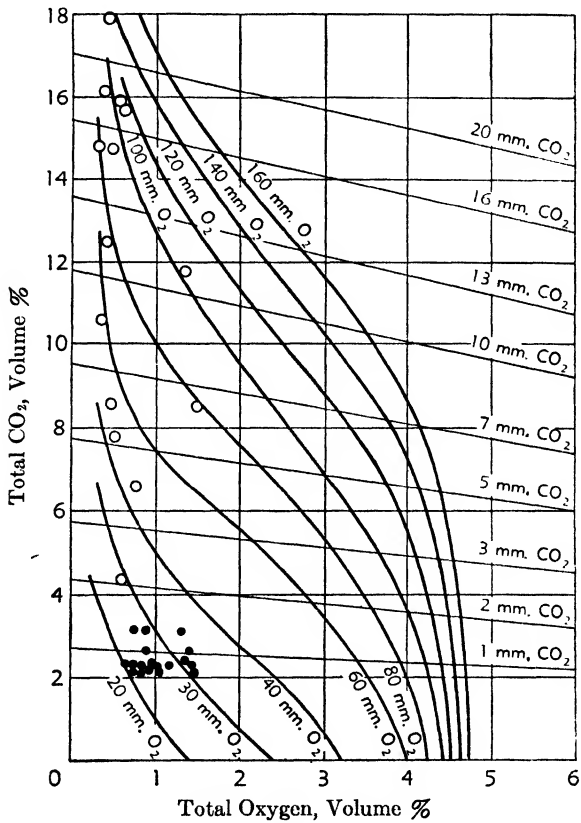


FIG. 11

(Redfield and Goodkind, 218.) Nomogram showing the oxygen and carbon dioxide content of the blood of the squid *Loligo* under different external conditions of pO_2 and of pCO_2, the values of which are indicated by circles. The black circles define the lethal conditions for animals in normal sea water. The open circles indicate the lethal conditions in the case of sea water previously enriched with CO_2.

of the oxygen carriers for the life of the squid. Life becomes impossible when the blood oxygen content falls to 0.5 volume %. Now, such an amount is contained in the internal environment lacking oxygen carriers and equilibrated with air. Such an internal environment could not sustain the life of the animal.

8. *Oxygen Carrier and the Special Ecological Condition of an Echiuroid, Urechis caupo*

Although it is true that in such cases as we discussed above it is possible to give the exact reason for the need of an oxygen carrier for the existence of the animal, it can be claimed that in other instances an oxygen carrier is not at all indispensable. If some marine animals are kept in an aquarium with well oxygenated sea water, the carrier retains its oxygen load and, therefore, seems to perform no function. This is the situation in the echiuroid worm *Urechis caupo*, the respiratory physiology of which had been studied in great detail by Redfield and Florkin (217). *Urechis*, as it became known only recently, is found along the Californian coast in the muddy sand exposed at low tide. It lives in a water filled U tube both ends of which open to the surface of the sand. At high tide the animal, assuming the position shown in Fig. 12a, drives the sea water by peristaltic movements of its muscular-cutaneous tube in the direction indicated in the figure by arrows and thus changes the sea water constantly during the periods of activity. This water brings food to the animal in the form of microorganisms which are caught in a very elegantly constructed mucous funnel. The water brings its supply of oxygen to the animal also. By means of a peristaltic movement opposite to that of the muscular-cutaneous tube, the animal "inspires" water through the anus into its highly developed lower intestine, the very thin wall of which is in contact with the fluid filling its large coelomic cavity. In the coelomic fluid are found cells containing hemoglobin. Several inspiratory movements are followed by one expiratory movement, during which the animal rejects all the water contained in its intestine. In well oxygenated water the hemoglobin in the coelomic fluid is nearly completely saturated (97%) although the partial oxygen tension may be only 75 mm. mercury, i.e., much lower than that of the surrounding water (about 150 mm.). If the animal stays in this condition, its hemoglobin remains almost completely saturated while the dissolved oxygen satisfies the needs of the animal. As was said before, the animal sucks up the sea water into its terminal intestine and rejects it after the coelomic fluid has been oxygenated at its expense. (The partial oxygen pressure in the expired water is about 100 mm.) Under these conditions the animal does not make use of its hemoglobin. The volume of coelomic fluid being about 20 cc. and its oxygen capacity about 4 volume %, the coelomic fluid of an animal contains a total of 0.8 cc. of oxygen. Since the average oxygen consumption of *Urechis* is about 0.01 cc./minute, it is obvious that the amount of oxygen in solution is quite sufficient to furnish the minute portion of oxygen which must be replaced during each minute (Hall, 118). During the periods of activity, which coincide with the periods of alimentation, *Urechis* lives at the expense of dissolved oxygen which is furnished by

its special respiratory apparatus. But after a period of activity the animal retires into the middle portion of the horizontal limb of its tubular dwelling and becomes immobile (Fig. 12b). It ceases to circulate the water or to suck it up into its digestive tube. The total oxygen content of its coelomic fluid and of the water in the intestine corresponds to the amount which would be consumed during 70 minutes. If the coelomic fluid contained no hemoglobin, the oxygen supply would be exhausted in 14 minutes. Thus, the presence of hemoglobin makes it possible for the animals to have a five times longer rest period between periods of activity.

This, as we said, demonstrates that the mode of life of *Urechis caupo*, and behavior under the natural circumstances of its existence, are directly dependent upon the presence of hemoglobin.

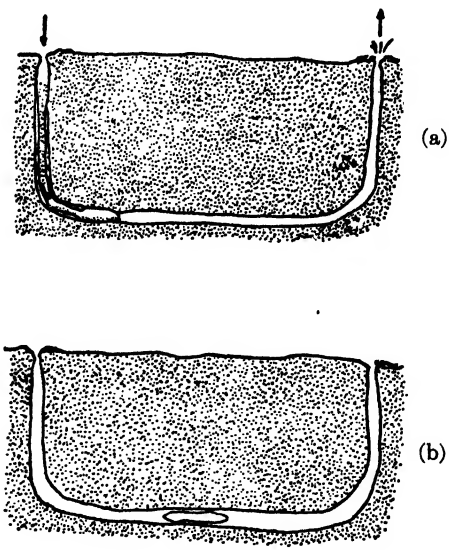


FIG. 12

(Fisher and MacGinitie, 77.) *Urechis caupo*

(a) During feeding and respiration
(b) During rest

B. DOMAIN OF GLUCEMIA

1. *Presence or Absence of a Circulatory System and Presence or Absence of Coelomic Glucemia*

As we survey the groups of annelids and sipunculids, we find that among these worms some of the annelids are provided with a closed circula-

tory system while some of the sipunculids are devoid of it and have only the coelomic fluid. For instance, the lugworm *Arenicola marina*, which belongs to the family of arenicolids, has a circulatory system, whereas *Dasybranchus caducus*, which belongs to the family of capitellids, and *Sipunculus* do not. In other words, *Arenicola* possesses two biological fluids, blood and coelomic fluid, whereas the *Dasybranchus* and the *Sipunculus* each have only a coelomic fluid. But these three animal species have one characteristic in common since they all live in and are nourished by the sand. In the natural habitat the digestive tube of these animals is permanently filled with sand. The outside environment, which is also a source of food, penetrates into the digestive tube. In the coelomic fluid of *Arenicola* it is impossible to detect any reducing substances, but the blood plasma does contain reducing substances. In the *Dasybranchus*, contrary to what was said about *Arenicola*, the coelomic fluid contains reducing and fermentable substances (8–9 mg. %) as is likewise the case in the *Sipunculus* (2.2–8.7 mg. %) (Florkin, 88).

It is especially noteworthy that among the animals under consideration, those containing reducing fermentable substances in the coelomic fluid do not have a circulatory system, and the coelomic fluid, in addition to its own functions, acquires also those functions which devolve upon the blood in animals with a circulation.

2. *Physiology of Glucemia and Ecological Condition of Sipunculus*

As was already pointed out, in the natural state these animals are filled with sand, the medium in which they live and which furnishes them with food. What happens if this ecological condition is altered by keeping sipunculids for several days in an aquarium, during which time the sand is discarded? Under these new conditions, after three days' sojourn in an aquarium, the intestine is completely emptied of sand; at the same time the true glucemia falls to zero and can no longer be measured (Florkin, 88). The sipunculid, therefore, has no power to regulate the glucemia, but under the special environmental conditions of its existence it is always protected from starvation and actually has no need for such regulation.

3. *Physiology of Glucemia and the Ecological Conditions of Two Decapod Crustacea*

The two decapod Crustacea, *Cancer pagurus* and *Carcinus moenas*, present an interesting relationship between the physiology of glucemia and ecological conditions. *Cancer pagurus*, which is characteristically of sluggish behavior, has an average true blood sugar level of about 22 mg. % (Roche and Dumazert, 228). Prolonged fasting for one month does not alter its blood sugar level. This crab, thus, has a mechanism for regulating

the blood sugar. In *Carcinus moenas* the true blood sugar behaves altogether differently. Ordinarily when this very active animal feeds, but not immediately after the ingestion of food, the glucemia is 12.5–16.5 mg. %. The glucemia decreases during inanition, and after 14 days of fasting one no longer finds fermentable reducing substances in the plasma (Florkin, 88). *Carcinus moenas* cannot maintain its glucemic level, whereas *Cancer pagurus* maintains a constant level in spite of prolonged inanition. It is interesting to compare the biochemical differences of these two crabs with their radically different modes of behavior.

4. *Physiology of Glucemia and Ecological Condition in the Bee*

Beutler's investigations show that the bee lacks glucemic regulation and glucide reserves. On leaving the hive on a collecting flight, the bee has an extremely high true glucemia, in the neighborhood of 3%. If she is enclosed in a latticed box, she can fly for 15 minutes, corresponding to a distance of approximately 3 miles. Then, when the blood sugar has dropped to a very low level (less than 1%), she flies or runs in spells and finally remains motionless, and the glucemia has fallen to about 0.5%. If at this time the bee's crop is still full of sugar solution, the blood sugar may increase again. The full crop, therefore, plays in the bee the same role as the liver does in vertebrates. The bee cannot actually regulate its blood sugar level, but the mass of bees are assured of a collective glucemic regulation by the accumulation of reserves in the hive. Just as the anatomical, physiological, or psychological peculiarities, so also the blood sugar physiology of the bee is correlated with its social life.

C. Domain of Digestion

1. *Nature of Hydrolytic Systems and Ecological Conditions*

Omnivorous animals, like echinoids, holothuroids, annelids in general and decapod crustaceans, digest proteins, glucides and lipides equally well. Carnivorous animals, like celenterates, turbellarians, asteroids, gastropods and cephalopods, have very powerful proleolytic enzymes but their glucidases are weak. The madrepores likewise can not digest starch (Yonge, 289) and in the digestive tube of gastropods neither amylase nor β-fructosidase can be demonstrated (Petrievic, 202). On the contrary, in herbivorous animals the proteases (proteinases) are poorly active (lamellibranchs, herbivorous gastropods, cirripedes, tunicates, etc.). The correlation between the arsenal of hydrolyases for digestion and the feeding habits is especially striking among the insects. The omnivorous cockroach possesses an arsenal of various enzymes (Swingle, 262). Insects, like the blood-sucking forms whose food is primarily protein, possess practically nothing

but proteolytic enzymes. For instance, the tsetse fly (*Glossina*) is provided with powerful proteases (peptidases, tryptases) but with a very weak amylase (Wigglesworth, 281). Among Lepidoptera the adults, which feed exclusively on nectar, have only an invertase, whereas the larvae which feed on vegetable matter have a varied enzyme collection (Stober, 261).

2. *Collagenase and the Ecology of Lucilia Larvae*

The larvae of the fly *Lucilia sericata* live in meat which they digest completely. This kind of digestion is not possible except through the secretion by the larva of a collagenase which dissolves the collagenous tissue surrounding the muscle fibers (Hobson, 128).

Maschmann (*Biochem. Z.*, **295**, 1, 1937) described an enzyme found in the young culture filtrates of *Clostridium welchii* (*B. perfrigens*) which digests gelatin and the trypsin-insoluble collagen. On injecting the filtrates into rabbits the collagen framework of the muscle was disintegrated leaving the muscle fibers intact. Recently Bidwell and van Heyningen (21a) have obtained highly purified preparations of κ-toxin from *Cl. welchii* type A which is essentially free from other toxins and is a proteolytic enzyme identical with Maschmann's collagenase. It apparently attacks only gelatin and collagen.

3. *Chitinase and the Special Ecology of Pseudagenia Larvae*

The larvae of this hymenopteron feed on spiders, on which they live as ectoparasites. The possession of a chitinase allows them to digest away the host's integument (Ramme, 213).

4. *Reductant and the Special Ecology of Keratinophages*

Several animals are capable of digesting keratin. The larvae of *Tineola biselliella*, the ordinary clothes moth, is endowed with special power to digest wool; the Mallophaga digest feathers and hair; and the larvae of the coleopteron *Anthrenus fasciatus* feed on horny substances. The mode of life among all these animals depends on their abnormal capacity to digest keratin. The digestion is not the result of some particular enzyme which these animals possess. As a matter of fact, their proteases are in every respect analogous to those of other animals, but their digestive juices contain some reducing agent (not yet isolated) which makes possible the digestion of keratin by the ordinary proteases (Duspiva, 69). The keratins are proteins insoluble in water, in organic solvents or in dilute alkalies, and are unaffected by proteinases. This special resistance of keratin to chemical agents results from the presence of disulfide linkages and from the peculiar structure of its molecule. In alkaline solution, the alkali sulfides or alkaline earth sulfides, potassium cyanide, and thioglycolic acid break

the disulfide linkages transforming them into sulfhydryl, SH groups: $-S-S- \rightarrow -SH\ HS-$, and under these conditions the keratins dissolve and are attacked by the proteinases. Under the influence of thioglycolic acid the reaction proceeds as follows:

$$R-S-S-R + 2\,HS-CH_2COOH \rightarrow 2\,R-SH + 2(-S-CH_2COOH)$$

and the keratin goes into solution. It is sufficient to add thioglycolic acid to the intestinal secretions of an animal which can not digest keratin and its digestive enzymes are able to carry out this digestion. Thus, the artificial reductant fulfills the same purpose as the physiological reducing substance which is found in the digestive juice of keratophages and which determines the behavior of these animals.

D. Domain of Photoreception

During the past half-century the idea has been gaining general acceptance that stimulation by light in both plant and animal organisms is mediated by a photosensitive pigment; the pigment is acted upon by light and is reconstituted by "dark" reactions. Although the photoreceptors, whether those found in plants and in the integument of lower invertebrates, or those represented by the various types of eyes, are similar only in their function, it is significant that they are bound together by a profound chemical homology based upon the common lineage and the preeminence of the group of carotenoid pigments in the various photochemical reactions. Apparently the carotenoids play an outstanding role in the photoreception of plants concerned with such phenomena as orientation to light, phototropic bending, the migrations of chloroplasts or directed movements of free-swimming forms. All studies made on the spectral sensitivity of these responses lead one to conclude that the process of the photoreception is mediated through the carotenoid pigments.

These highly unsaturated, fat-soluble pigments ranging in color from yellow to red are represented by carotenes, composed only of C and H, and by xanthophylls, containing in addition OH-groups. The carotenoids are easily and abundantly synthesized by plants. Animals, on the contrary, do not have the ability to synthesize them. Although they must obtain them through the ingestion of plant material, animals can modify and even degrade the molecule of the caretenoid pigments to serve their own special functional needs. This ability seems to have become the exclusive prerogative of the animal organism.

In some of the green flagellates the so-called eyespot is a photoreceptor organ which contains, besides the common plant carotenoids, also a new pigment astaxanthin, which is a dihydroxy-diketo derivative of β-carotene. The interesting thing about this pigment is that, as far as it is known, it is

found only in animal tissues. Although it occurs in the eyes and in the integument of crustaceans or as a screening pigment in the cones of certain eyes, it makes its first appearance as the main pigment of the eyespot in green flagellates.

Where and how the ability of animals to degrade carotenoid pigments had originated in the course of evolution, and what part, if any, it plays in the photic responses of eyeless invertebrates are questions to which, at least at the present time, there is practically no answer. What is definitely known, however, is that when image-forming eyes appear in the invertebrate kingdom, as in the case of mollusks and arthropods, — "animals have come to depend on plants for their carotenoids, and have developed the capacity to degrade plant carotenoids to yield vitamin A. The latter substances hereafter dominate the photoreceptor process" (Wald, 274).

It may be noted here that vitamin A is an alcohol formed by the splitting of a carotene molecule, and in the case of β-carotene (C_{40} H_{56}) the molecule divides into two symmetrical and identical fragments each representing a molecule of vitamin A ($C_{20}H_{29}OH$).

The origin of vitamin A from the carotenoid pigments is lost in the dim history of eye-bearing organisms. The important fact, however, remains that, regardless of the wide anatomical divergence between the existing optical systems, the latter all operate with a very limited group of chemical compounds. Furthermore, the photoreceptor process in all these different systems is limited within a narrow framework of reactions.

The apparently close association between the appearance of an image-forming, lens-bearing eye and of vitamin A suggests that the latter not only became the dominant factor in the photoreceptor process but may also have been the organizer for the development of the lens. While this suggestion is highly hypothetical, there is at least some embryological justification for it.

As is well known the embryological process is a sequence of events following each other with surprising precision. Only when a tissue has reached a particular stage of historical development does it become responsive to the morphogenetic influence of the so-called organizers which initiate a new departure or direction in the orderly process. Thus the formation of the eye in vertebrates not only presupposes the formation first of a neural plate, through the action of a primary organizer, but also subsequent evagination of an optic cup and induction of a lens proliferating from the overlying skin. From the point of view of this discussion it merits special consideration that in pigs, whose ration during gestation was lacking vitamin A, the succession of embryological events outlined above, whereby a lens is formed, is interrupted with the result that eyeless young are born. In other words, had an eye been normally developed the functional integrity of its photoreceptor mechanism would have been dominated by vitamin A.

But vitamin A is also the organizer or essential catalyst which starts the morphogenetic process leading to the development of the image-forming eye.

The vertebrate eye has two separate types of retinal photoreceptors: the system of rods which are specialized for vision in dim light, and the system of cones specialized for bright light and for color vision. The photosensitive substance of the rods is a rose-colored lipoprotein rhodopsin with a spectral absorption peak at 500 mμ. On exposing the retina to light the carotenoid is split off from the protein and goes through a series of changes from an unstable transient orange product to a more stable yellow pigment which finally bleaches to retinene$_1$ (387 mμ). If the bleaching is further prolonged the colorless vitamin A_1 (328 mμ) is produced. In an intact animal the retinal rhodopsin is resynthesized from protein and vitamin A_1 in the dark (24).

In the image-forming eye of invertebrates essentially the same photoreceptor mechanism is involved although there may be some differences in details. For instance, in the eye of the squid the retinene$_1$ seems to play the sole part in vision, while vitamin A_1 has an entirely subsidiary role. Likewise, astaxanthin is present in the eye of crustaceans but this pigment apparently acts only to screen the retinal cells and does not take any direct part in the photoreceptor process of vision.

It may, therefore, be stated with certainty that from the time animals have been in possession of image-forming eyes the photoreceptive process has been dominated by carotenoid degradation products, the vitamin A_1 and retinene$_1$. The multiple function which vitamin A fulfills in the vertebrates must be attributed to a secondary adaptation because there seems to be good reason to believe that among invertebrates the basic function of vitamin A_1 and of retinene$_1$ is associated exclusively with their photosensitive properties and is spatially limited to the photoreceptor organs. Perhaps the appearance of a liver among vertebrates and its ability to serve as a storehouse for vitamin A may have had something to do with the extension of its use by various other tissues. There can be little doubt that the extra photoreceptor functions of vitamin A represent a new departure and a new evolutionary path which must have been developed secondarily and thus increased still further the dependence of the vertebrate animal upon plants for supplying its needs for carotenoids. As for photoreception, the use of vitamin A_1 and of retinene$_1$ by image-forming eyes extends from the invertebrates clear through all land inhabiting vertebrates and marine fishes. A strange and curious break, however, interrupts this continuity. This poses a question regarding the chemical evolution of vision which is entirely unanswerable for the moment. The break appears among freshwater fishes whose retinas are purple instead of rose colored; instead of

rhodopsin, the retinas contain a porphyropsin system with a maximum spectral band at 522 mμ in place of 500 mμ.

This porphyropsin system seems to be similar to rhodopsin in every respect except that all its component parts have absorption spectra which are consistently displaced about 20–30 mμ towards the red, or the longer wavelengths (porphyropsin 522 mμ, retinene$_2$ 405 mμ and vitamin A$_2$ 355 mμ). The porphyropsin was first discovered in the retinas of freshwater fishes while permanently marine fishes (with one exception) have the vitamin A$_1$ or rhodopsin system. The biologically significant fact is that the euryhaline fishes, i.e., fishes which can tolerate wide variations in salinity and, therefore, can freely migrate between freshwater and the sea, possess predominantly one or the other of these photoreceptor systems. As Wald points out, the euryhaline fishes which realize in varying degrees their migratory potentialities are rigidly restricted to the environment where they spawn and develop embryologically. They fall into two groups: *anadromous*, which migrate from the sea to their spawning habitat in freshwater, and *catadromous*, which migrate from freshwater to their spawning habitat in the sea. The visual system, and the type of vitamin A, that these fishes possess is the one predominantly or even exclusively associated with their spawning environment, the ancestral home of their race.

If the beginning of the rhodopsin system (vitamin A$_1$, retinene$_1$) is lost in the evolutionary history of invertebrate eyes, the porphyropsin system (vitamin A$_2$, retinene$_2$) is so deeply imbedded in the origin of vertebrates that it almost appears like a chemical *fait accompli*, a basic mutation coeval with the very appearance of the vertebrate stock, and particularly with its relation to the spawning in a freshwater habitat. It remains one of the great mysteries of evolution as to how and why the porphyropsin photoreceptor system, which apparently made its appearance together with freshwater vertebrates, has changed back to the more ancient rhodopsin system with the migration of freshwater vertebrates in the direction of the sea or of the land. The change over from the porphyropsin back to the rhodopsin system is revealed strikingly during metamorphosis which transforms the bullfrog tadpole to the adult frog. This must be regarded as a recapitulation of the evolutionary process associated with the change from the aquatic to the terrestrial mode of existence. If we assume that the genetic pattern of porphyropsin of the ancient freshwater progenitor of vertebrates was determined by a new gene, the subsequent return to the rhodopsin must have been due to a loss of the gene. It would be of great biological importance to discover if the porphyropsin retinal system had any survival value in the freshwater environment, or was a purely fortuitous innovation in the adventures of the evolutionary process.

E. Domain of Osmoregulation

From the data obtained on the subject of osmotic pressure (measured by the lowering of the freezing point Δ of the solution) of the internal environment and, in the case of aquatic animals, also of the external environment, it is possible to draw the following conclusions:

1. The total concentration of the internal environment of marine invertebrates is close to that of the sea water ($\Delta i = \Delta e$).

2. The total concentration of the internal environment of fresh-water invertebrates is less than that of sea water but greater than that of fresh water ($\Delta i > \Delta e$).

3. The total concentration of the internal environment of marine teleosts is less than that of sea water ($\Delta i < \Delta e$).

4. The total concentration of the internal environment of fresh-water teleosts is nearly the same (or slightly less) as that of the internal environment of marine teleosts, and greater than that of fresh water ($\Delta i > \Delta e$).

5. The total concentration of the internal environment of marine elasmobranchs is slightly greater than that of the sea water, although the total concentration of inorganic substances is nearly the same as in marine or fresh-water teleosts ($\Delta i > \Delta e$).

6. The total concentration of the internal environment of fresh-water elasmobranchs is less than that of marine elasmobranchs but is much greater than that of teleosts; the concentration of inorganic substances is about the same as in marine or fresh-water teleosts or in marine elasmobranchs ($\Delta i > \Delta e$).

7. The total concentration of the internal environment of amphibians, reptiles, birds, and mammals is about the same as of the fresh-water teleosts.

This relationship between the internal and external environment is shown schematically in Fig. 13.

1. *Osmoregulation in Marine Euryhalines*

Although the invertebrates of the sea are generally in osmotic equilibrium with their external environment, still some marine invertebrates are euryhaline and can tolerate considerable variation in salinity of their medium. A dilution of the external environment does not occasion a similar dilution of the internal medium in these animals, a characteristic which makes it possible for them to enter the waters of estuaries. Such euryhaline organisms are more numerous than one would imagine. Among them is to be found not only *Carcinus moenas* but also other crustaceans, such as *Cancer pagurus*, *Eriocheir sinensis*, *Portunus puber*, *Heloecius cordiformis*, the annelid *Nereis diversicolor*, and the turbellarian *Gunda ulvae*. The degree of euryhalinity is itself highly variable. *Carcinus moenas*, which can live in the brackish waters of the estuaries, dies in fresh

water, whereas the Chinese crab *Eriocheir sinensis* passes with impunity from the ocean into the rivers. How do the euryhalines accomplish the regulation of the osmotic concentration of their internal medium in a much

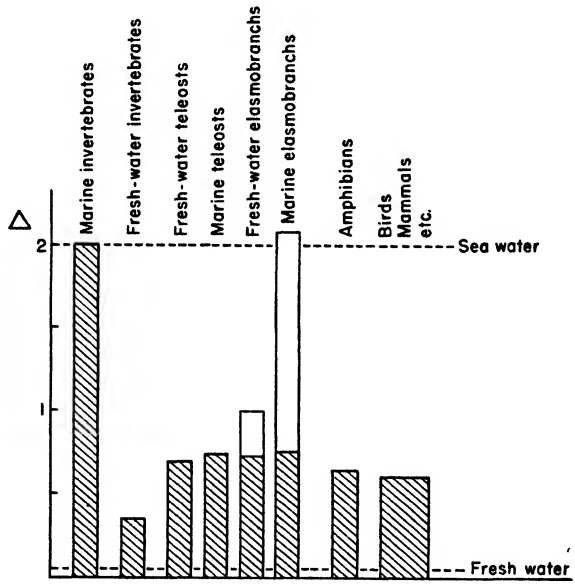


FIG. 13

(Baldwin, 9.) Lowering the freezing point (Δ) of the internal medium (osmotic pressures of the bloods) of different animals compared with those of sea water and fresh water. The shaded surface represents the part of the osmotic pressure due to the content of inorganic substances.

diluted external environment? This regulation operates through two mechanisms. On the one hand, they can actively absorb salts from the external environment into their system against the concentration gradient (Nagel, 184); and, secondly, their nephridia play a part in the osmoregulation, although this is apparently of small importance (Picken, 203).

2. *Osmoregulation of Fresh-Water Invertebrates*

The internal medium of fresh-water invertebrates is generally more concentrated than the external environment. In their natural habitat fresh-water invertebrates are, therefore, homeosmotic and maintain an inorganic composition which is, as is shown in Fig. 13, quite different from that of the fresh waters which they inhabit. For a long time it was wrongly assumed that the behavior of fresh-water animals can be interpreted on the basis of the impermeability of their enveloping membranes. We now know

that, in a general way, the maintenance of the concentration and composition of the internal medium depends upon a balance of different factors:

1. Penetration of water from the outside into the interior resulting from a difference in concentrations.
2. Absorption of inorganic substances from the hypotonic external environment.
3. The role of the nephridia, which eliminate more water than inorganic substances (a copious hypotonic urine).

An analysis of the part played by different factors in the osmoregulation of the pond mussel (*Anodonta*) has been made by Florkin and Duchâteau (103). If one suppresses the function of the nephridium of this fresh-water mussel by an injection of sodium veronal, it can be shown that there is a continuous penetration of water by osmosis through the surface of the body. In fresh-water mussels the pericardial fluid is formed by filtration from the blood through the wall of the ventricle, and it passes into the nephridium or Bojanus organ. The blood and the pericardial fluid have the same osmotic pressure (Δ) (Koch, 143), and if we determine their chloride concentration we find that these, too, are similar in the normal animal in its ordinary environment (Florkin and Duchâteau, 103). Thus, at the level of the heart, there is filtration of a fluid of the same chloride concentration as that of the blood. If the blood chloride concentration is altered by injecting either sodium chloride solution or distilled water into the mass of the foot, an equilibrium between the chlorides of blood and of pericardial fluid is rapidly established. The concentration of chlorides, as well as of calcium and phosphates, mirrors that of the blood of normal animals. By injecting sufficient barbiturate (for example, 200 mg. sodium veronal into a 150–200 g. fresh-water mussel) a condition of hypochloremia can be produced accompanying the narcosis. Under these conditions, while the chloride content of the blood decreases, that of the pericardial fluid remains high; the passage between blood and pericardial fluid has been suspended, the animal being in a veritable state of anuria resulting from a decrease of intracardiac pressure and from a general decrease in blood pressure, which is manifested in many instances in a great swelling of the foot. Observing the variations in weight of the pond mussel, whose nephridia have been put out of commission by an injection of barbiturate, it can be seen that the increase in weight is associated with an increase in water content. The progressive rise in weight of the narcotized animal shows the persistence of the entrance of water by osmosis while the loss of water is suspended. The degree of variation in weight of the animal is thus a measure of the penetration of water from the outside. The same phenomena are observed when a mussel is anesthetized by the addition of

ether to the water in which it lives. If the views expressed above are correct, there should be no increase in weight of a narcotized mussel if the concentration of salt in the internal medium were in equilibrium with that in the external environment. Such an equilibrium is realized when the external environment is more concentrated than the normal internal environment of the mussel. Under those conditions, the mussel ceases to behave like a homeosmotic organism and its internal medium shows the same total concentration and the same chloride content as the exterior environment, and it can be demonstrated that the weight no longer varies under the influence of narcosis. These experiments definitely demonstrate that in the fresh-water mussel there is a continuous penetration of water from the outside because the internal medium is more concentrated than the external. In aquatic larvae of insects the entrance of water from the outside operates either through the whole surface of the body, as is the case in the *Chironomus* larva (Harnish, 121) or through certain specialized portions, such as the anal papillae of the larvae of *Aedes* (Wigglesworth, 281). The water may also move along an osmotic concentration gradient through a specialized region like the gills (Maloeuf, 174), as is the case in the fresh-water crayfish.

The idea that fresh-water invertebrates absorb inorganic substances from an hypotonic external medium belongs to H. Koch (140), who in 1934 recognized that the anal papillae of the dipteron larvae can take up silver ions from a very dilute solution (0.001%). He developed a hypothesis of the part played by these papillae in the absorption of inorganic substances from the ordinary medium, and its correctness had been subsequently established beyond a doubt (Koch and Krogh, 142). This kind of absorption had been demonstrated in different fresh-water invertebrates: *Eriocheir sinensis*, *Limnea*, *Paliedina*, *Dreissena*, *Anodonta*, *Unio*, *Haemopis*, *Libellula* and *Aeschna* larvae, and the crayfish (Maloeuf, 174).

Although much is still to be explained in the mechanism of absorption, nevertheless Krogh and his collaborators have already collected much interesting material on this subject. Here belong the studies which Lundegardh and Burström (168) made since 1932 on the absorption of different anions and cations by plant rootlets from very dilute solutions (0.0050–0.0025 *N*). Where the phenomenon had been analyzed, it was demonstrated that the absorption of cations and of anions present, respectively, two different mechanisms. One mechanism, for example, would take Na^+ from $NaHCO_3$ or Na_2SO_4 without taking the anions, while the other mechanism would take Cl^- from NH_4Cl or $CaCl_2$ without taking the cations. Each of these mechanisms is more or less specialized in the different species. Evidently, this points to a different factor which intervenes in the regulation of the composition of the internal medium.

The osmoregulating function of the nephridium consists in excreting

much more water than inorganic substances. The principal function of the nephridium in most fresh-water invertebrates is the excretion of water.

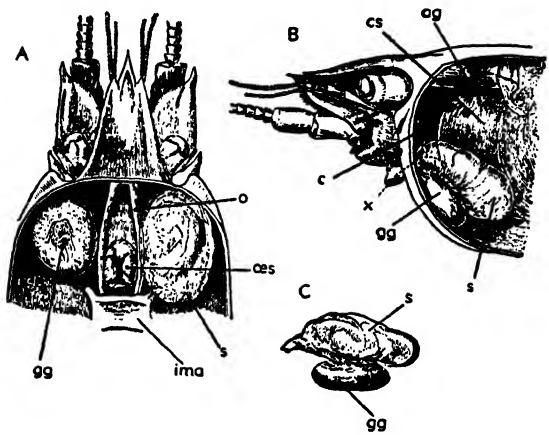


FIG. 14

(Huxley, 132.) A. The front part of the crayfish with the dorsal portion of the carapace removed, showing the position of the *green gland*. B. The same, with the left side of the carapace removed. C. The isolated green gland. Magnified twice. gg: *green gland* exposed in A by removal of the bladder; s: bladder; x: silk thread passed through the opening at the base of the antenna into the bladder.

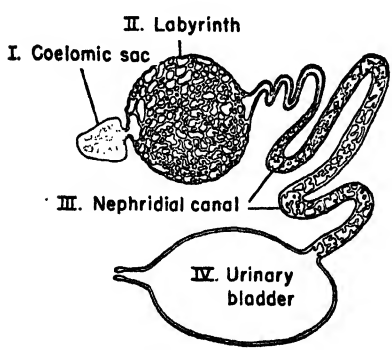


FIG. 15

(Schlieper, 236, modified from Marchal.) Diagram of the green gland of the crayfish.

In the crayfish the nephridia are represented by two round greenish masses (Fig. 14) which are situated close to the ventral side of the anterior extremity of the cephalothoracic cavity. They are called the *green glands*. The gland pours out its products into a vesicle which then expels its contents into a canal opening at the base of the corresponding antenna. Hence the name *antennary gland*, which is often given to this organ.

Anatomically, the nephridium of the crayfish is very different from that of a marine crustacean like the lobster. Here a long nephridial canal is found between the labyrinth and the vesicle which is not present in the lobster. The urine is formed by filtration across the coelomic sac, and the organic excreta are added to the urine as it passes down the labyrinth.

Reabsorption of inorganic substances occurs as the urine flows through the nephridial canal. This can be neatly demonstrated by examining the fluid in the different portions of the nephridium for Cl-ions (Peters, 199).

The osmoregulatory role of the nephridium in the crayfish *Astacus fluviatilis* can be shown very nicely by comparing the composition of the blood and urine (Scholles, 239):

	Δ °C.	Cl mg. %	Ca mg. %	Mg mg. %	K mg. %
Blood	0.81	691	41.8	6.4	20.2
Urine	0.09	35.7	11.0	2.9	2.5
$\frac{U}{B} \times 100$	11.04	5.16	26.3	45.3	12.3

These data reveal not only the hypotonicity of the urine in relation to the blood, but also the great difference in composition of the two fluids. This is an important factor in the regulation of the composition of the internal medium, which is so different from the composition of the external environment.

If a piece of the shell of a fresh-water mussel is lifted dorsally at the level of the hinge the heart can be seen through the roof of the pericardial cavity formed by the union of the two mantle lobes along the medio-dorsal line. If the roof of the pericardium is cut through, one enters into the pericardial cavity and reaches the heart with the intestine passing through it. Cutting the intestine where it leaves the liver and lifting it up with the heart, after sectioning the auricles, the intestine and heart can be detached and the floor of the pericardial cavity is uncovered, just as it appears in Fig. 16.

Two brown sacs of the nephridia, or organs of Bojanus, are thus brought into view. These two sacs are united in front along the median line and are separated behind. Close to the place where, on emerging from the liver, the intestine was cut, two small apertures appear which lead into the cavity of the organ of Bojanus and through which it communicates with the pericardial cavity. This orifice, or nephrostome, leads into the large lower cavity of the organ which extends back under the posterior adductor muscle and communicates with the upper chamber which extends forward, as is shown in Fig. 17, and terminates in a small orifice situated on the side of the body near the line of insertion of the internal gill.

It is possible to withdraw a brownish fluid by introducing a pipet through the nephrostome until the lower cavity, lined with connective tissue and covered with glandular elements, is reached. The fluid contains

dark colored particles which can be removed by centrifuging and the supernatant is designated the *Bojanus fluid.*

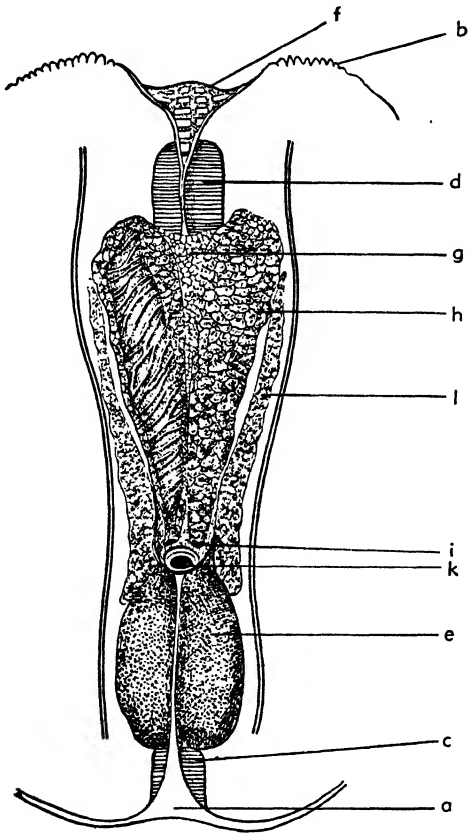


FIG. 16

(Vogt and Young, 269.) Dorsal view of the pond mussel through an incision in the mantle; the rectum and heart were removed to reveal the floor of the pericardial cavity. a, b, the anterior and posterior border of the mantle; c, anterior adductor muscle; d, posterior muscle; e, liver; f, gills; g, reservoir of venous blood; h, organ of Bojanus, which on the left side had been split open to reveal its cavity and the contents of its glandular portion; i, the orifices of the cavity of the organ of Bojanus opening into the pericardial cavity; k, intestine cut at the emergence from the liver; l, Keber's organ.

The function of the excretory organ of the mussel consists in producing the pericardial fluid, filtered through the wall of the ventricle into the coelomic cavity (pericardial cavity) under the pressure exerted in the circulatory system. Then, the fluid passes through the organ of Bojanus, where

it is modified into urine and excreted through the pore of each Bojanus sac. The blood plama and pericardial fluid lower the freezing point to the same degree (Koch, 143). The Bojanus fluid, too, has the same effect on the freezing point as the plasma (Florkin, 90). In other words, the three fluids are isotonic. On the contrary, the liquid eliminated through the excretory pore is definitely hypotonic to the blood or pericardial fluid (Picken, 204). It seems, therefore, that the principal osmoregulatory function of the nephridium is exercised beyond the ampullary portion in the superior chamber of the nephridium (Vorhöhle of Griesbach) (a′ in Fig. 17).

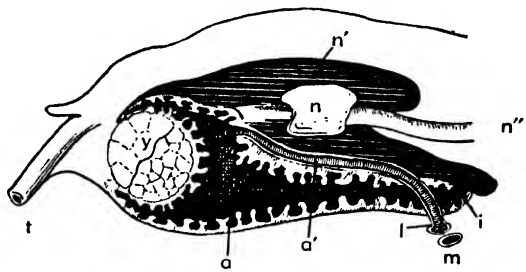


FIG. 17

(Bronn, cf. 37.) Diagram of the organ of Bojanus. a, glandular part; a′, non-glandular part; i, orifice of the glandular part opening into the pericardial cavity; l, external orifice; m, genital opening; n, ventricle; n′, pericardium; n″, intestine; t, rectum.

Still, the ampulla of the organ also plays a role in regulating the inorganic composition of the internal medium. Not only the excretion of organic wastes but the reabsorption of inorganic constituents as well takes place there, as the analyses of the Bojanus fluid reveal (Florkin and Duchâteau, 103).

A few examples are presented below showing the results of determination of chlorides in blood plasma, pericardial fluid, and in the Bojanus liquid of fresh-water mussels. All values are in mg./100 cc.

Anodonta (pond mussel)	Blood plasma	Pericardial fluid	Bojanus fluid
1	67.6	67.6	34.2
2	72.1	68.1	25.6
3	69.8	69.3	49.2
4	65.5	64.6	46.2
5	66.3	67.8	38.1
6	57.8	57.4	30.0
7	48.8	48.8	23.7
Average	64.0	63.4	35.3

Calcium and phosphorus determinations made on the same fluids show similar concentrations in plasma and pericardial fluid, while that of the Bojanus fluid is smaller.

	Mg. % Calcium		
8	30.2		24.4
9	32.7		22.9
10	29.1		20.0
11	29.0		21.1
Average	30.3		21.9
	Mg. % Phosphorus		
12	0.43	0.44	0.39
13	0.45	0.46	0.42
14	0.46	0.46	0.40
Average	0.447	0.453	0.403

It is, thus, certain that the organ of Bojanus exerts an osmoregulatory influence by means of the absorption of some inorganic substances. It also regulates the composition of the internal medium because of quantitative differences in the rate of reabsorption of different constituents.

The fresh-water mussel, just as the crayfish, possesses a mechanism for maintaining the water content of the animal and of its internal medium. This mechanism brings about a balance between the water which enters through the surface of the organism by osmosis and the water lost through the elimination of a hypotonic urine. Furthermore, the inorganic composition of the internal medium is regulated in both by a balance of two phenomena: an absorption of inorganic substances from a hypotonic medium and the excretion of a urine the composition of which is regulated through filtration and reabsorption by the excretory organ.

The role played by the excretory organs of larvae of aquatic insects in the regulation of the composition of the internal medium is also revealed by determining the chlorides in the urine as it comes out of the Malpighian tube and after its passage through the rectum (Boné and Koch, 29). The urine produced in the Malpighian tubes of larvae of *Chironomus* or of *Limnophilus* has a chloride content approximately the same as the blood, though, depending upon conditions, it may actually be somewhat greater or less. The Malpighian tubes, therefore, produce a secretion which partakes in the regulation of the inorganic composition of the internal medium. Besides, as the urine passes down the rectum, an active absorption of inorganic substances takes place against a concentration gradient, which is accomplished probably by the rectal glands. A regulation of the concentration and composition of the inorganic substances in the internal medium of insect larvae results because water is absorbed passively by osmosis and

inorganic elements are absorbed actively at the surface of the body, and a hypotonic urine is eliminated whose composition is regulated by two mechanisms, namely, the secretion by the Malpighian tubes and the reabsorption of salts in the rectum.

Although in most fresh-water invertebrates the organs of excretion play the principal role in osmoregulation, this situation is not found in all animals. In certain flatworms like *Gunda ulvae* (Pantin, 196; Weil and Pantin, 279; Beadle, 17) or in earthworms kept in water (Maloeuf, 176) the digestive tube excretes copiously a fluid which is hypotonic to the blood. The composition of the internal medium thus results from an equilibrium between this secretion and the active absorption through the surface of the body.

In general, it can be shown that in fresh-water invertebrates the maintenance of an internal medium, which differs from the external environment in its total concentration as well as in its composition, results from the balancing of two phenomena. At the surface of the organism, where it is in contact with the external environment, water enters passively by osmosis and salts are absorbed actively. In the region of the excretory organs, on the other hand, there is a copious elimination of a hypotonic fluid whose composition differs greatly from that of the internal medium.

The emphasis that was formerly placed on the supposed impermeability of the surface covering of fresh-water invertebrates must, therefore, be abandoned. In the normal animal, in its natural environment, water and salt enter the organism as a result of exchanges operating at its surface when the external environment is hypotonic. This absorption against the concentration gradient is a complex phenomenon integrated with the general functioning of the organism. There are circumstances under which an animal, living in its natural fresh water environment, does lose inorganic constituents from the internal medium through the covering of the body. This happens, for instance, in the fresh-water mussel, as was mentioned before, when the nephridium ceases to function after an injection of barbiturate. After such treatment the animal's internal medium suffers a decrease in concentration of chlorides, of calcium, and of inorganic phosphate (Florkin and Duchâteau, 103). We are dealing here with a diffusion from the inside to the outside since, following a similar barbiturate injection, the concentration of chloride, calcium, and phosphate remains unchanged if the animal is placed in a moist chamber instead of being kept in fresh water. The permeability of the body covering to salts can also be neatly demonstrated in larvae of *Chironomus* by the following experiment. If a ligature is tied around the last segment of the larva, both the elimination of urine and the absorption of salts localized at the anal papillae are simultaneously obstructed, yet the internal medium loses salts (Koch, 141).

As will be shown in the next section, there are marine animals which can migrate into fresh water and remain there with impunity. In such animals the mechanism of absorption through the body surface and the regulatory capacity of the nephridium are effective. Among the true fresh-water forms, however, such as the fresh-water mussel or crayfish, there are differentiated mechanisms for the reabsorption of inorganic substances. These mechanisms generally correspond to a special segment of the excretory organ responsible for the reabsorption. This segment makes possible a copious hypotonic urine which compensates for the continuous penetration of water through the body surface.

3. *Osmoregulation in Fresh-Water Teleosts*

It is now generally accepted that fishes have descended from fresh-water ancestors and have secondarily emigrated into the sea. For this reason fresh-water teleosts will be considered before the marine teleosts. The internal medium of the fresh-water teleosts, like that of the fresh-water invertebrates, is more highly concentrated than their external environment. The mechanism for maintaining the higher concentration of the internal medium is, in its main features, analogous to that of the fresh-water invertebrates: the surface of the body is more or less permeable to salts and to water, resulting in an osmotic penetration of water. But special mechanisms also exist for the absorption of inorganic elements from a hypotonic medium, which compensate the losses of salt through the urine and through the body surface. The mechanism of absorption is localized in the gills (Krogh, 149, 150). Fresh-water teleosts likewise produce a copious urine which is hypotonic to the blood. Their kidneys, which play an important role in osmotic regulation, consist of an enormous number (100,000 or more) of arterial glomeruli (Malpighian corpuscles) enclosed within a capsule. The cavity of the capsule extends into the urinary tubule consisting of several specialized portions. Under the influence of the hydrostatic pressure of the blood a filtration of considerable amounts of a blood ultrafiltrate takes place through the glomerulus. The composition of the ultrafiltrate, as it passes down the urinary tubule, is being modified through the processes of reabsorption and secretion. The existence of a multitude of filtering apparatuses, such as the glomeruli, represents an immense improvement over the coelomic sac of the crayfish or the pericardium of the fresh-water mussel. The glomerulus may be regarded as a remarkable adaptation to life in fresh water.

4. *Osmoregulation in Marine Teleosts*

The marine teleosts have an internal medium of about the same concentration as fresh-water teleosts, but their external environment is much

more concentrated than the internal medium. Instead of a tendency of the outside water to penetrate into the organism the osmotic conditions here are dominated by the water leaving the internal medium as well as by the permeation of salts. The circumstances are, therefore, the reverse of those which dominate the physiology of fresh-water teleosts. The difficult problem of conserving their water that faces marine teleosts cannot be solved except by the elimination of a hypertonic fluid. This cannot be the urine which, as a matter of fact, is of about the same concentration as the blood plasma or even slightly hypotonic to it. The marine teleosts, unlike their fresh-water relatives, swallow the salt water in which they sojourn. They absorb the water through the digestive tract together with a very large part of the salts dissolved in it. The absorbed salts are eliminated in large amounts from the gills, which thus assume the task of producing a hypertonic excretion to compensate the loss of water by osmosis (Smith, 251). The mechanism of osmoregulation of a marine teleost is compared to that of a fresh-water teleost in Fig. 18. This schematic representation shows that for the marine teleosts it is no longer a simple matter of eliminating water as in the fresh-water fishes, but, on the contrary, a serious problem of retaining this precious constituent of the internal medium.

What happens when a fresh-water organism, provided with a glomerular kidney, assumes either a marine or a terrestrial mode of existence? Contrary to the needs imposed by living in fresh water, where water penetrates the organism passively by osmosis, either of these two states of existence (in sea water or on land) necessitates the acquisition of mechanisms for the conservation of water. Obviously, under these conditions, the glomerulus, which is a mechanism for eliminating water, becomes a nuisance. The course of animal evolution runs along two different lines: either a segment is acquired by the urinary tubule, as is the case in mammals, the function of which is to reabsorb the water and to assure the elimination of dissolved substances; or the glomerulus disappears. In marine teleosts the latter occurs, and one finds all stages of reduction of the glomerular apparatus down to its total disappearance.

5. *Osmoregulation in Marine Elasmobranchs*

In Chapter III it was already suggested that the presence of urea in the internal medium of the elasmobranchs plays an important part in determining its molecular concentration. Furthermore, as can be seen in Fig. 13, the salt content in these animals is the same as in marine teleosts, but the urea and the trimethylamine oxide confer upon the internal medium a slightly greater osmotic pressure than that of the sea water. The conservation within the animal body of a substance so easily diffusible as urea is assured, in the elasmobranchs, by two mechanisms: (1) the almost com-

plete impermeability of the gills, oral membranes and skin to urea, (2) the presence in the urinary tubule of a segment specialized for the reabsorption of urea. The slight difference in osmotic pressure between the external and

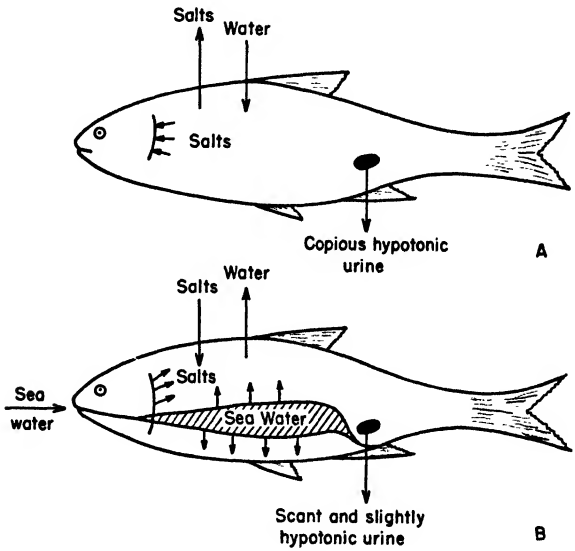


FIG. 18

(Modified from Baldwin, 9.) Diagrammatic presentation of the mechanism of osmoregulation in teleostean fishes. A. Fresh-water species. B. Marine species.

internal media insures a continuous inflow of water. The urine of marine elasmobranchs is slightly hypotonic to blood (Bottazzi, 33; Smith, 252). The mode of osmoregulation of marine elasmobranchs is, therefore, essentially of the same type as that of fresh-water teleosts since it involves absorption of water through the body surface and elimination of a hypotonic urine. Urea assures the proper concentration of the internal medium, a concentration which depends upon the existence of an osmoregulatory mechanism. At the same time, osmoregulation depends on the maintenance of urea concentration through the activity of the mechanism of reabsorption by the urinary tubule (Smith, 252). On the other hand, marine elasmobranchs, like marine teleosts, excrete salts extrarenally. Their kidneys are provided with glomeruli, which leads one to believe that they have descended from fresh-water organisms. The much larger excretion of water in marine elasmobranchs than in marine teleosts may be accounted for by the fact that their glomeruli have not degenerated.

6. *Osmoregulation of Fresh-Water Elasmobranchs*

There are some elasmobranchs that live permanently in fresh waters into which they migrated from the sea. The blood plasma of these fishes has a higher salt concentration than the fresh-water. The blood urea, though its concentration is less than in the marine forms, raises the osmotic pressure of the internal medium still more with the result that water from the external environment enters by osmosis. However, their much smaller

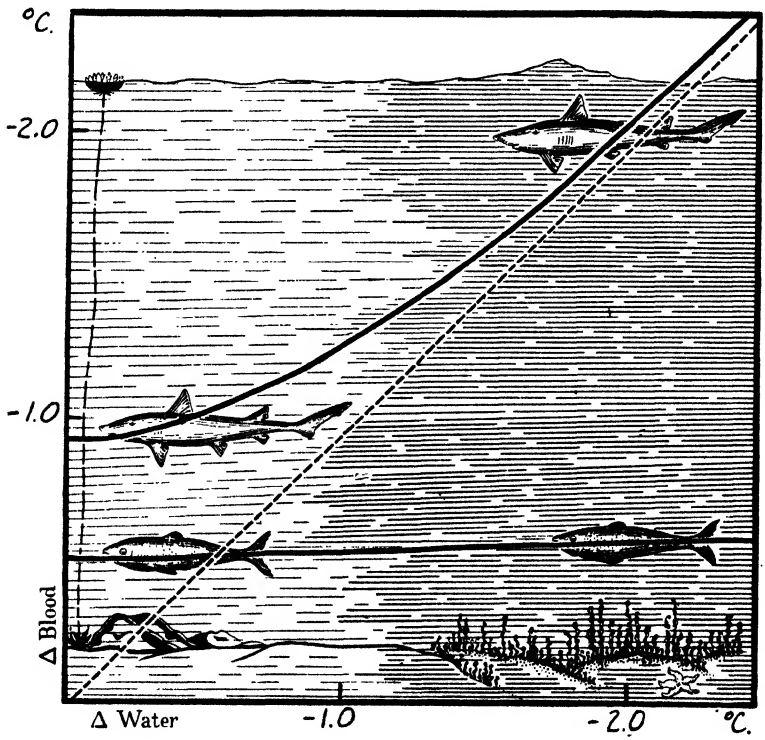


FIG. 19

(Smith, 254.) The greater or lesser shading of the animal and of its external environment in this picture corresponds to the degree of osmotic pressure. At the left, in fresh water, the teleosts and elasmobranchs have a Δi which is much greater than Δe; i.e., both are osmotically superior to their environment and, therefore, tend to absorb large quantities of water and have at all times adequate quantities available for the formation of hypotonic urine. At the right, in sea water, the Δi of the marine teleost is less than the Δe, i.e., the teleost is osmotically inferior to its environment and can obtain water for the formation of urine only at the expense of unremitting, extrarenal physiological labor (salt secretion). The elasmobranch, on the contrary, by virtue of its physiological uremia, is osmotically superior to its environment both in fresh and salt water.

urea content (0.6% in the fresh-water forms compared to 2% in marine elasmobranchs) (Smith and Smith, 256) truly represents an adaptation for limiting the penetration of water. The interrelationship between the internal and external environments of fishes is schematized in Fig. 19.

7. *Osmoregulation in Terrestrial Animals*

Among these animals the liquid external environment is represented only by the contents of the digestive tract. Hence the necessity for the development of mechanisms for conservation of water. As pointed out previously, this conservation can be realized by two means: either by the loss of the glomerular filtration apparatus, or by the acquisition of a mechanism for the reabsorption of water. The first method was adopted, for instance, by reptiles living in dry regions (snakes, lizards). But mammals and birds adopted the second method, by developing the loop of Henle, a specialized portion of the urinary tubule, where the greatest part of the water filtered by the glomerulus is conserved. In birds, as also in some other terrestrial animals, there is a supplementary mechanism for conservation of water: the cloaca formed by the fusion of rectum and urethra also absorbs water. The cloaca is analogous to the mechanism found in many insects whose urine, secreted by the Malpighian tubules, is concentrated in the rectum by the activity of rectal glands.

Speaking generally, it can be said that the ability to secrete a hypotonic urine is found widely among all vetebrates. In mammals this is revealed in the condition of polyuria. But the mechanism for regulation by secreting a hypertonic urine is found only in the higher animals like birds and mammals.

8. *Evolution of Osmoregulation in Vertebrates*

Considering the ensemble of excretory mechanisms upon which the composition of the internal medium depends, it is possible to select the nephrostome as the prototype of the secretory apparatus which belonged to the marine ancestors of the vertebrates and which gave rise to the filtration-secretion system of the most primitive fresh-water vertebrates. In the marine teleosts, by a regressive evolution through the loss of the glomerulus, this system became simply a system for secretion, whereas in mammals it evolved into a filtration-secretion-reabsorption system (Marshall and Smith, 179).

F. Marine, Fresh-Water and Terrestrial Animals

It is a generally accepted view that animals have originated in the sea from which they afterwards colonized the fresh waters and the earth.

Some of the animals have subsequently returned to the original marine environment.

An animal that lives in water establishes different relationships, depending on conditions, between the osmotic concentration of its internal medium (milieu intérieur) and that of its external environment. The conditions of the external aquatic environment compatible with life may vary within very wide limits. Fresh water may represent the most dilute environment in which life is still possible. But some animals may exist indefinitely even in distilled water. This is so in the case of the frog or the stickleback (*Gosterosteus*) (Gueylard, 116), etc. On the other hand, there are animals that can live in an extremely concentrated aquatic medium. Animals live even in the Salt Lake of Utah which contains 22% salt although its fauna is very specialized. Nevertheless animals that can subsist in aquatic media of very different salinity are not common. In many invertebrates the osmotic pressure is very close to that of the sea water in which they live, and the variations in the environmental concentration are reflected in the body fluids whose concentration is entirely dependent upon that of the environment. Therefore, animals whose bounding membranes are permeable to salts and water are called *poikilosmotic*. If one places the crab *Maia squinado* in diluted sea water, the salt from the internal medium leaves the animal and water enters until an equilibrium is established so that the concentration of the internal medium is automatically adjusted to that of the external environment. Other animals cannot be transferred from sea water into a more dilute medium without the danger of so severely modifying their "milieux intérieur" as to cause their death. They can live only in an environment of a definite concentration, as they do not tolerate concentration changes of the external environment except within narrow limits. Such animals are called *stenohaline*. Among these are many species of invertebrates living in the sea: ctenophores, echinoderms, brachiopods, pantopods, cephalopods, etc. which are all exclusively marine forms.

The fauna of the fresh waters consists of another group of usually stenohaline animals whose internal medium is generally more concentrated than the external environment. These animals maintain in their internal medium a concentration and composition of salts which differ from those of the outside environment, and they are called *homeosmotic*. The fresh-water fauna is very much less exuberant than the marine fauna. One finds there only a few coelenterates, bryozoans, turbellarians, annelids, some lamellibranchs and prosobranchs, and a somewhat larger number of crustaceans. The fresh-water fishes do not compare either in numbers or in variety of species with the marine fishes. Stenohalinity is not a general characteristic either of marine invertebrates or of fresh-water animals.

Let us consider, for instance, the crab *Carcinus moenas*. On being placed in diluted sea water its internal medium does not come into equilibrium with the external environment: it may become a little more dilute but retains a definite concentration. The crab behaves, under these conditions, like an *homeosmotic* animal. But it is *euryhaline* and this property makes it possible for the crab to invade the estuaries, where a *stenohaline* animal would soon perish through dilution of its internal medium. The mechanisms of osmotic regulation impose upon stenohalines of the high seas a "milieu" (internal medium) which reflects their external environment, and its concentration cannot be modified without bringing death to the animal.

The colonization of the biosphere by animals living in the sea required many adaptations. The animals had to be euryhalines to traverse the estuaries in order to reach the rivers from the high sea. Later, to adapt themselves to the special conditions of the fresh-water environment, they had to become homeosmotic, acquiring the necessary mechanisms for maintaining the osmotic concentration of their internal medium against a more dilute external environment. Since the lowering of the freezing point of the fluids of animals never falls below 0.1°, while that of fresh water is 0.02°–0.03° or even less, the fresh-water animals had to acquire homeosmotic mechanisms. This is only one of the many aspects of adjustment necessary to enable animals living in sea water to enter into and to maintain themselves alive in fresh waters. Another obstacle to this entrance is the existence of currents in the rivers. In order to stay in fresh water an animal, from its larval to the adult stage, would need to be fixed or it would have to be a very good swimmer to stay in its habitat in spite of the current which tends to carry it back towards the sea. This can be done only by those species which no longer reproduce by means of free swimming ciliated larvae but by means of eggs which hatch out organisms sufficiently strong and resistant to remain in the fresh waters. This is the reason why larval forms are so rare in fresh waters. The young generally remain inside the egg just long enough to pass through the larval stage, and emerge in a condition capable of resisting the current which tends to sweep them away (Sollas, cf. 189). This implies, as a biochemical corollary, that the egg must also furnish the young animal nourishment for a sufficiently long time. In a general way, it may be said that there is a definite tendency for the eggs of fresh-water invertebrates to become much larger in size and smaller in numbers than are the eggs of marine invertebrates. For instance, *Luccinum undatum*, a gastropod species living on the sea coast, lays 12,000 eggs, while the fresh-water gastropods do not lay more than 20 to 100 eggs (Carpenter, 46). The eggs of most fresh-water animals are surrounded by a mass of jelly and settle to the bottom. At the same time, fresh water

constitutes a much less constant medium than sea water (von Martens, cf. 189) and the extreme variations of conditions which prevail here make adaptation so much harder. Many eggs of marine animals depend upon their external environment to furnish inorganic elements for their development and are, therefore, incapable of developing in a medium poor in salts (Needham, 189). Maintenance of existence in fresh water implies that the eggs must be provided with enough inorganic substances to secure their development. This is another aspect of the biochemical problem of living in fresh water.

In the matter of terrestrial colonization, one of the first obstacles to its realization is owing to the fact that the supply of water becomes limited as compared with its easy availability in an aquatic environment. In terrestrial animals the outside liquid environment is restricted to the contents of the digestive tract and special mechanisms must be acquired for the purpose of conserving water. Terrestrial existence also entails many adaptations in ontogenetic development. Terrestrial embryos must be supplied not only with inorganic and organic nourishment, as is the case in fresh-water embryos, but they must also be supplied with water. In certain instances, as is the case in birds and in a great many reptiles, the egg is supplied with a reserve of water which is protected from loss by means of a more or less impermeable covering. Another method has been adopted by mammals which have become viviparous. Both methods of meeting the situation imply an intrauterine fecundation which may very well be considered also as an adaptation to terrestrial existence.

In discussing the characteristics of different biochemical systems in orthogenetic evolution, we called attention to the fact that in the higher animals ammonia has disappeared from circulation in the internal medium, but it is still present in the lower forms. We showed that this freeing of ammonia from the blood is due to the acquisition of excretory syntheses, the ammonia being transformed either into urea or into uric acid. We also showed that in the more highly developed forms one particular type of excretory synthesis is well defined and strongly predominant. Considering the various terrestrial animals, we note that birds and terrestrial reptiles, such as lizards or snakes, are *uricotelic* while the mammals are *ureotelic.*

According to Needham (187) there is a correlation between the nitrogen metabolism and the mode of reproduction. Among the terrestrial vertebrates ureotelic metabolism is associated with viviparity while the uricotelic process is associated with the existence of eggs provided with a more or less impervious capsule. Uric acid, which is a very poorly soluble substance, actually precipitates as soon as its concentration attains a certain level, while urea stays in solution even in a high concentration. Smith (255) raised serious objections to Needham's thesis that the form of nitrogenous

excretion of an animal depends principally on the conditions under which its embryo has to live. Needham (90) admits that the correlation between cleidoicity (i.e., production of eggs which are practically completely closed off from their environment) and uricotelism and urea retention may be far-fetched. However, he points out that this generalization of the relation between uricotelism and terrestrial oviparity is the only one which accounts for the lack of uricotelism among mammals.

CHAPTER VI

Systematic Characters

We have shown that there are biochemical characteristics which, when arranged in series as they are found in different branches of the animal kingdom, form a sequence manifesting general orthogenesis. This generalization falls within the scope of the rule known to morphologists under the name of *Deperet's law*, which states that the size of animals increases as they advance from the primitive to the most recent condition. The idea of a biochemical orthogenesis represents the unity of the biochemical plan of animals in its time dimension. In Chapter I we have discussed the unity of the plan from the point of view of its spatial dimension, manifested by the characteristics common to animal species which actually inhabit our planet. We have also shown the existence of adjustments between the biochemical and the anatomical, physiological, or ecological characters, and have emphasized the essential role which the integrated biochemical characters frequently play in the organism in its totality or in relation to its environment. We also presented evidence of what may be called biochemical adaptations. We continue to search in the total assemblage of biochemical characters for some which are significant for systematic classification and which are found in groups of more or less wide distribution. Supposing that such systematic characteristics do exist, is their distribution in accord with the classification of animals which morphologists have established by long and patient work? This question is of great general significance and its solution is a necessary preliminary to any discussion of the mechanism of evolution at the biochemical level.

We already pointed out that there are differences in the hemoglobins from different species of vertebrates (pp. 24–26). Specific biochemical differences have also been demonstrated in the proteins of the internal medium. For instance, the serum globulin of the horse differs from the serum globulin of man. The precipitin reactions furnish further proof of the specificity of the biochemical characteristics. It is known that, if human serum is repeatedly injected into a rabbit, the serum of the latter acquires the property of precipitating human serum. The antihuman serum obviously reflects the specific biochemical peculiarities of the human serum. However, the specificity of the antihuman serum so prepared is not absolute. Although it is true that the precipitating action is greatest for human serum, nevertheless it also causes a precipitation, though less marked, of

the serum of anthropoid apes, but the reaction is entirely negative with serum of lower apes or of other animals.

Comparing the secretion of the sebaceous glands of different mammals, it is found that this secretion contains a monovalent saturated arachidic alcohol (eicosanol) the composition of which in man corresponds to an empirical formula $C_{20}H_{42}OH$, whereas in sheep the empirical composition is $C_{26}H_{54}OH$ (Rothman, 234).

In mollusks and crustaceans the isoelectric points of their hemocyanins are about pH 5.0, but the pH values for the different species range from 4.34 to 5.50, indicating the existence of specific differences. Even within the same genus differences can be seen from one species to another. Still, if one considers, as Svedberg pointed out (263), a genus with many subgenera (for instance, the genus *Helix*), the isoelectric points are much more close to each other within the same subgenus. *Helix nemoralis* and *Helix hortensis* belonging to the subgenus *Tachea* have hemocyanins with isoelectric points of 4.57 to 4.63, whereas *Helix pomatia* of the subgenus *Helicogena* has a hemocyanin with an isoelectric point of pH 5.05 and *Helix arbustrorum* of the subgenus *Arionta* of pH 5.50.

But if from the biochemical point of view species differ widely while the genera do not, is it possible to discover in the biochemical order characters which are common to groups of more or less extensive distribution and which can serve as a basis for classification? The following examples will show that this is actually so.

A. Biochemical Characteristics of Vertebrates

One of the general characteristics of the vertebrates is the possession of a skeleton. It results from the impregnation of the connective tissue with a definite chemical substance. Generally, the skeleton of vertebrates consists of a collection of bony and cartilagenous pieces. In the former the basic substance of the connective tissue is impregnated with mineral matter constituting the salts of bone, a mixture of calcium and magnesium salts and a combination of tricalcium phosphate with some unknown organic radical (Dallemagne, 52). In the cartilagenous pieces the fundamental substance of the connective tissue is impregnated with chondromucoid. The chemical nature of the supporting tissue of vertebrates is characteristic for them and differs from the many chemical substances representing the organic portion of supporting tissues of invetebrates, such as spongin of the sponges, chitin of the arthropods, etc. Furthermore, the nature of the vertebrate skeleton also necessitates the existence of a special biochemical system responsible for the formation and maintenance of this structure. This system involves the participation of a number of func-

tional substances such as the phosphatase enzyme, the parathyroid hormone, the D vitamins, etc.

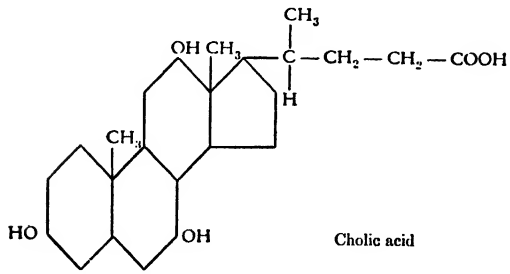
Another general characteristic of vertebrates is the presence in their skin, in a variety of forms, of keratin, a specialized protein whose properties we already had occasion to discuss (p. 67).

In the domain of digestive hydrolytic enzymes, vertebrates differ markedly from invertebrates. In man, for instance, digestion of polymerized glucides commences in the mouth under the action of salivary amylase. It continues in the duodenum under the influence of pancreatic amylase and terminates in the duodenum and ileum with the action of oligases present in pancreatic and intestinal juices. As for the digestion of proteins, it begins in the acid medium of the stomach by the action of pepsin, an enzyme found in the digestive juice of vertebrates but not in that of the invertebrates. In the intestine the digestion is continued by trypsin and by peptidases. This localization of the hydrolases in different portions of the digestive tract is one of the general characteristics of digestion in vertebrates as distinguished from invertebrates (Krüger, 151; Jordan and Hirsch, 133). In the invertebrates all the hydrolases are found in a single digestive juice. While the vertebrates are supplied with a *chain* of hydrolases, the invertebrates have only a *mixture* of the enzymes. The chain of hydrolyases characteristic of the vertebrates has been well studied in mammals, in reptiles (Wolvekamp, 284) and somewhat less thoroughly in birds. In fishes, as in mammals, there is a chain of hydrolases for the digestion of proteins but there is none for the digestion of glucids (Vonk, 270). In some invertebrates there is a crude scheme of an enzymatic (hydrolytic) chain, as for instance in cephalopods whose liver secretes a proteinase and a polypeptidase while their pancreas contains peptidases and an inactive proteinase which is activated by the enterokinase localized in the wall of the coecum (Romijin, 232). In the cockroach *Periplaneta orientalis* the distribution of proteases in the digestive tube also presents a rough approach to the hydrolytic chain, the proteinase being most abundant in the anterior while the dipeptidase is found in the posterior portion (Schlottke, 237).

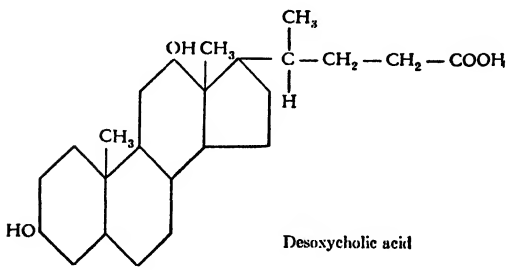
As for the secretion of glands annexed to the digestive tract, the vertebrates again display a characteristic peculiar for them alone, namely, the secretion of bile by the liver. The bile of vertebrates contains no enzymes, but it contains substances peculiarly characteristic to vertebrates, the bile pigments and substances which in the invertebrates are not secreted by a specialized gland, the bile salts.

The bile acids, which are steroid compounds, are found in the bile of vertebrates conjugated with either glycine or taurine and to a very small extent as salt of the free acid. The bile acids of the vertebrates are not

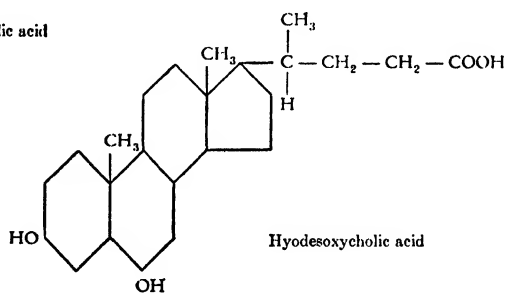
everywhere the same. Cholic acid seems to be common to all biles except those of certain rodents like the rabbit or guinea pig. In normal man, dog, and in most other vertebrates there is also found desoxycholic acid. Human



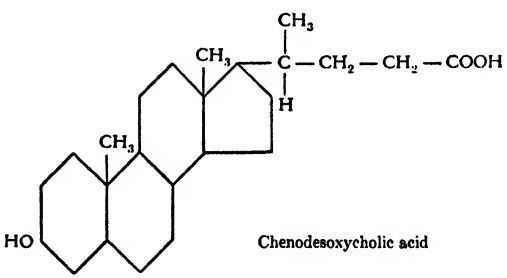
Cholic acid



Desoxycholic acid



Hyodesoxycholic acid



Chenodesoxycholic acid

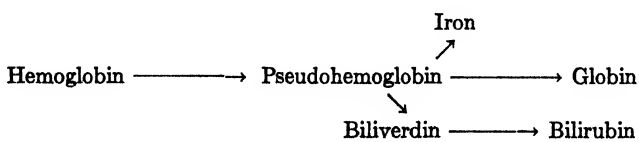
bile, as well as beef or pig bile, contain a small amount of chenodesoxycholic (anthropodesoxycholic) acid, which is the predominant bile acid of birds.

In the pig and in certain polar animals hyodesoxycholic acid is also

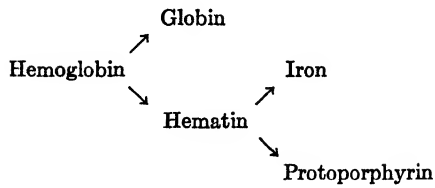
present. In fishes cholic acid is the chief component of the bile, as is generally the case also in mammals and snakes. The bile acids are conjugated principally with taurine, except in man, beef, kangaroo, hippopotamus and musk deer, whose bile acids are conjugated predominantly with glycine, or in the pig whose bile is almost entirely free of taurine and, furthermore, is distinguished by the presence of a special bile acid, the hyodesoxycholic acid.

Contrary to the generally prevailing idea, Vonk (271, 272) has demonstrated that bile salts of taurocholic and of taurodesoxycholic acids are found in the intestine of some decapod crustaceans where they are present together with digestive enzymes in the peculiar digestive juice and not, as in the case of vertebrates, in a specialized bile. It should also be noted that whereas taurine exists as the taurocholic salt only in the higher vertebrates, in the invertebrates, such as mollusks and crustaceans, it is found in the free state (Okuda, cf. 156).

The bile pigments, particularly bilirubin, are substances characteristic for the bile of vertebrate animals. All attempts to demonstrate these substances in the invertebrates have been in vain. The presence of biliary pigments in vertebrates is a corollary of the special mode of metabolism of their hemoglobin. Contrary to the classical theory, hematin cannot be considered an intermediate compound in the metabolism of hemoglobin in vertebrates (Brown, cf. 161; Duesberg, 68). The degradation proceeds instead through pseudohemoglobin with an open porphyrin nucleus as an intermediate stage. The pseudohemoglobin loses its iron and globin giving rise to biliverdin, which is then transformed to bilirubin (Lemberg, 161; Barkan, 12). Among invertebrates there is no formation of bile pigments, but certain facts suggest, and it has been repeatedly demonstrated, that in the metabolism of these animals hematin is formed as the normal end product. The blood cells of the echiuroid worm *Urechis caupo* contain, for instance, special granules (Redfield and Florkin, 217) consisting of hematin (Baumberger and Michaelis, 14). On the other hand, it was shown that the tissues of different worms contain hematin and protoporphyrin (Raphael, 214, 215). The hemoglobin metabolism in vertebrates operates along the scheme



It seems that the metabolic scheme in the invertebrates is as follows:



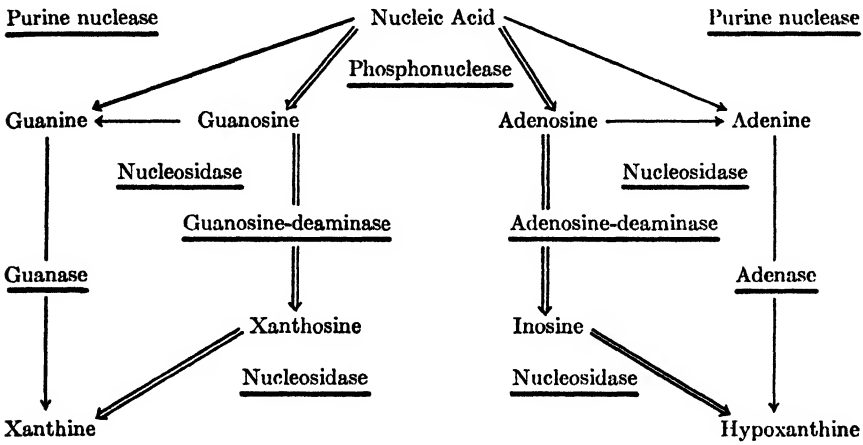
The blood of vertebrates contains a certain amount of easily split iron (Barkan, 12) which is thought to belong to the pseudohemoglobin. On the contrary, the hemoglobin-containing blood of an invertebrate, like that of the snail *Planorbis*, does not have any easily liberated iron (Liébecq, 162).

As was already pointed out, the hemoglobin of vertebrates presents special characteristics distinguishing it clearly from the hemoglobins of the invertebrates, which are frequently grouped into the class of erythrocruorins. The hemoglobin of vertebrates has a molecular weight of about 70,000; it contains 4 atoms of iron in its molecule; its isoelectric point is near neutrality. These characteristics differentiate it clearly from invertebrate hemoglobins which have quite different molecular weights, never corresponding to four units. Furthermore, the hemoglobins of invertebrates contain much greater amounts of arginine and of cystine and smaller amounts of histidine and lysine (Roche, 230). It must, therefore, be admitted that there is a characteristic vertebrate hemoglobin which is quite distinct from the hemoglobins of invertebrates.

We said previously (p. 44) that adenine and guanine represent two aminopurines which are the substrates of purine metabolism. The first stage of that metabolism is a process of deamination with the formation of hypoxanthine and xanthine, respectively. This deamination implies a liberation of purines from nucleic acids, which can be accomplished by means of purine nucleases. However, there is a second possible mechanism of deamination, with the aid of adenosine-deaminase and of guanosine-deaminase, which acts on the nucleosides (adenosine or guanosine) set free by the action of phosphonucleases on the nucleic acids. Aminopurine metabolism, thus, proceeds along two different paths of deamination leading either to aminopurines or to their corresponding nucleosides.

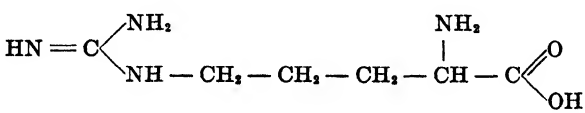
The liver of mammals contains guanosine deaminase, adenosine deaminase, and guanase (Schmidt, 238) but no adenase. Adenase is also lacking in the liver of birds (Florkin and Duchâteau, 97). In a general way, the same enzymatic ensemble is found in poikilothermic vertebrates, for instance, in the lamprey, tench, frog, and turtle *Clemmys leprosa* (Duchâteau *et al.*, 66).

All invertebrates, which have been investigated so far, were found to contain both adenase and guanase but not nucleoside deaminases which are present in vertebrates. These studies had been made on the

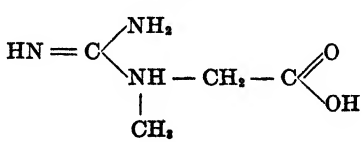


fresh-water mussel, crayfish, lobster, *Hydrophilus* and *Dytiscus* (Duchâteau *et al.*, 66). *The appearance of an enzymatic system for the deamination of nucleosides is a biochemical characteristic of vertebrates in contrast to invertebrates.*

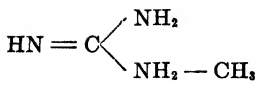
The distribution of two derivatives of guanidine, namely, creatine (or its anhydride creatinine) and arginine, is definitely different in vertebrates and invertebrates. Numerous observations confirmed Kutscher's (154) conclusion that arginine is a characteristic constituent of invertebrates and creatine of vertebrates. At the same time it was shown that in the invertebrates very small amounts of creatine may be found beside the strongly predominant arginine, while the reverse is the case in vertebrates.



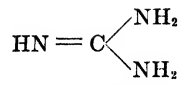
Arginine



Creatine



Methylguanidine



Guanidine

The particular interest of the studies on the distribution of arginine and creatine lies in the fact that these substances enter into the composition of muscle phosphagens (p. 20). As was shown in Table IV, the phosphagen of the vertebrates is phosphocreatine while that of the invertebrates is phosphoarginine. (Among echinoderms, the ophiuroidae form the single exception to this rule since they have phosphocreatine as their phosphagen, while the Echinoidea possess both phosphagens.) Methylguanidine, like the creatine of which it is probably the metabolic progenitor, is a characteristic component of vertebrates, whereas invertebrates do not possess this substance (Kutscher and Ackermann, 155).

As was already mentioned, the "milieu intérieur" (internal medium) shows wonderful uniformity among animals in regard to its inorganic constituents. If we determine, as far as published data on this subject are available, the ratio between the sums of the alkaline metals and alkaline earths $\frac{(Na + K)}{(Ca + Mg)}$ we note, as is shown in Table XIII, a clear difference between vertebrates and invertebrates, no matter what the habitat or mode of life of the species is. *The values of this ratio are definitely greater in the vertebrates.* This fact assumes special interest in the following connection. If the above mentioned ratio is increased in a solution bathing the heart of a snail, an electrocardiogram is produced resembling very closely the electrocardiogram of vertebrates. Conversely, if pieces of the auricles from frogs are placed in a solution approaching in composition a solution which is physiological for the snail, electrocardiograms of a simpler form are obtained (Cardot, 44). This demonstrates the possibility of a correspondence between the type of function of an animal organ and the inorganic composition of its internal environment.

On adding a mixture of mono- and dipotassium phosphate to the internal environment (serum) of vertebrates a curve of precipitation is obtained which is very characteristic for vertebrates. On a logarithmic plot one observes, first, a straight line corresponding to the precipitation of fibrinogen, followed by five segments which are observed in the precipitation curve of serum (Fig. 20). This characteristic curve is found in vertebrates as far removed from each other in the classification system as the turtle *Testudo graeca*, the cock, and man. On the contrary, in different invertebrates such as the *Hydrophilus*, the snails *Planorbis* and

Limnea or larva of *Bombyx mori* entirely different curves of precipitation are obtained (Florkin and Duchâteau, 101, 102).

Another general characteristic of vertebrates is the type of glucide metabolism. This type is characterized principally by the glycogenic

TABLE XIII

Ratios of the Sum of Equivalents of Sodium and Potassium and the Sum of Equivalents of Calcium and Magnesium in the Internal Medium of Animals $\left(\frac{Na + K}{Ca + Mg}\right)$

These Ratios have been Calculated from Data in the Literature.

Animal	Ratio	References
Marine invertebrates		
Echinus esculentus	4.2	Bethe and Berger (20)
Caudina chilensis	3.9	Koizumi (145)
Doris tuberculata	3.7	Bethe and Berger (20)
Aplysia punctata	4.4	Idem
Ostrea circumpicta	3.6	Kumano (153)
Limulus polyphemus	4.0	Macallum (172)
Hyas aranea	5.3	Bethe and Berger (20)
Fresh-water invertebrates		
Anodonta cygnea	1.3	Florkin (91)
Planorbis corneus	6.2	Idem
Astacus fluviatilis	5.5	Bogucki (27)
Terrestrial invertebrates		
Helix pomatia, summer	6.6	Lustig *et al.* (170)
Helix pomatia, winter	4.3	Item
Hydrophilus piceus	2.0	Florkin (91)
Vertebrates		
Marine elasmobranchs		
Raja stabuloforis	24.0	Smith (253)
Raja diaphenes	14.6	Idem
Carcharias littoralis	18.2	Idem
Mustelus canis	17.2	Idem
Fresh-water Ganoids		
Amiatus calva	10.5	Smith (253)
Lepidosteus osseus	11.1	Idem
Marine teleosts		
Lophius piscatorius	23.3	Smith (253)
Gadus callarias	10.7	Idem
Fresh-water teleosts		
Tinca vulgaris	15.1	Püschel (212)
Amphibia		
Rana virescens	10.6	Macallum (172)

TABLE XIII (*Continued*)

Animal	Ratio	References
Reptiles		
Crocodilus acutus	14.8	Dill and Edwards (58)
Chrysemys marginata	12.1	Smith (253)
Graptemys geographica	16.8	Idem
Emys blandingii	12.3	Idem
Pseudemys elegans	7.7	Idem
Chelydra serpentina	6.6	Idem
Caretta kempi	13.2	Idem
Caretta caretta	13.2	Idem
Birds		
Chicken	20.7	Morgan and Chichester (180)
Mammals		
Dog	21.1	Dill *et al.* (59)
Man	21.7	Dill *et al.* (61)

function of the liver and by the endocrine secretion of insulin, the hormone regulating glucid metabolism. *The existence of an organ specialized for forming glycogen reserves from the sugars absorbed from the digestive tract is a characteristic of vertebrates.* In the invertebrates glycogen is diffusely distributed in the organism, especially in the connective tissues, in the intestinal epithelium and in the organs of reproduction. The hepatopancreas of invertebrates is not an organ for the accumulation of glycogen and, in different mollusks, under normal conditions, it even contains no glycogen. *Furthermore, insulin is a hormone characteristic only for vertebrates.* Experiments on insects (Hemmingsen, 123), snails (Schwartz, 245) and crayfish (Florkin and Duchâteau, 95) definitely demonstrated that these invertebrates are insensitive to the action of insulin.

In the field of endocrinology one can find many other characteristics of vertebrates. *Thus the thyroid gland and its internal secretion, the thyroid hormone, are quite characteristic for these animals.* No substance is found in the invertebrates which is functionally active in the same manner as thyroxine. And, at the same time, invertebrate animals are completely insensitive to the action of thyroxine, which modifies neither their metabolism nor their development.

These examples will suffice to show that there are systematic biochemical characteristics which pertain to vertebrates. The latter are represented by bilaterally symmetrical Metazoa possessing a nervous system which consists of a cord terminating in a cerebral enlargement. This is contained in a cranial cavity formed by the expansion of skeletal vertebrae. But, in addition, these metazoans also have a keratin integu-

ment; they have a chain of digestive enzymes including pepsin, and a liver secreting bile containing both bile acids and bile pigments. These pigments owe their origin to the fact that the hemoglobin metabolism

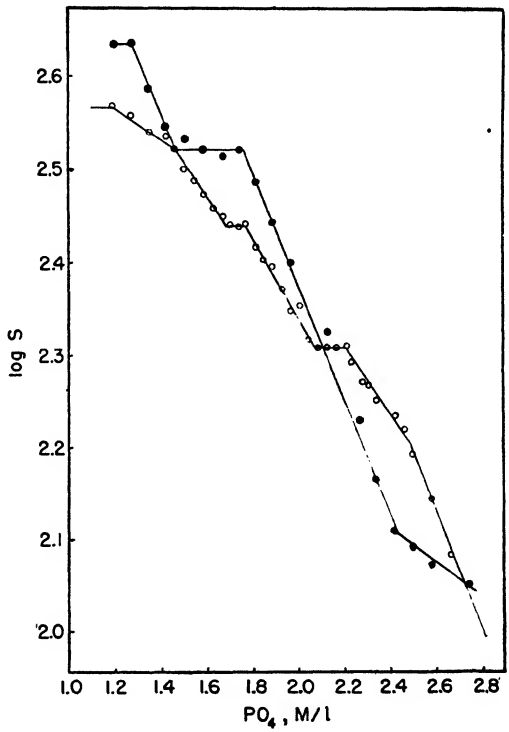


FIG. 20

(Florkin and Duchâteau, 101.) Curve of precipitation by phosphates of human serum (solid circles) and of plasma of *Bombyx mori* larvae (open circles) in the last larval stage before pupation.

passes through a pseudohemoglobin intermediate stage. The blood cells of these metazoans contain a hemoglobin consisting of four 17,600 units, with an isoelectric point near pH 7.0, and with definite proportions of arginine, cystine, histidine, and lysine. Their metabolism of purines is characterized by the presence of nucleoside deaminases in the system of enzymes; creatine is one of their characteristic constituents, and their phosphagen is phosphocreatine; the inorganic composition of their internal medium is characterized by a high ratio of $\frac{(Na + K)}{(Ca + Mg)}$;

the curve of precipitation of the proteins of their internal medium by phosphate is peculiar to them; and their liver possesses a specialized glycogenic function which is correlated chemically with certain hormonal activities (thyroid, insulin, etc.), and biochemical systems which render their tissues receptive to these actions.

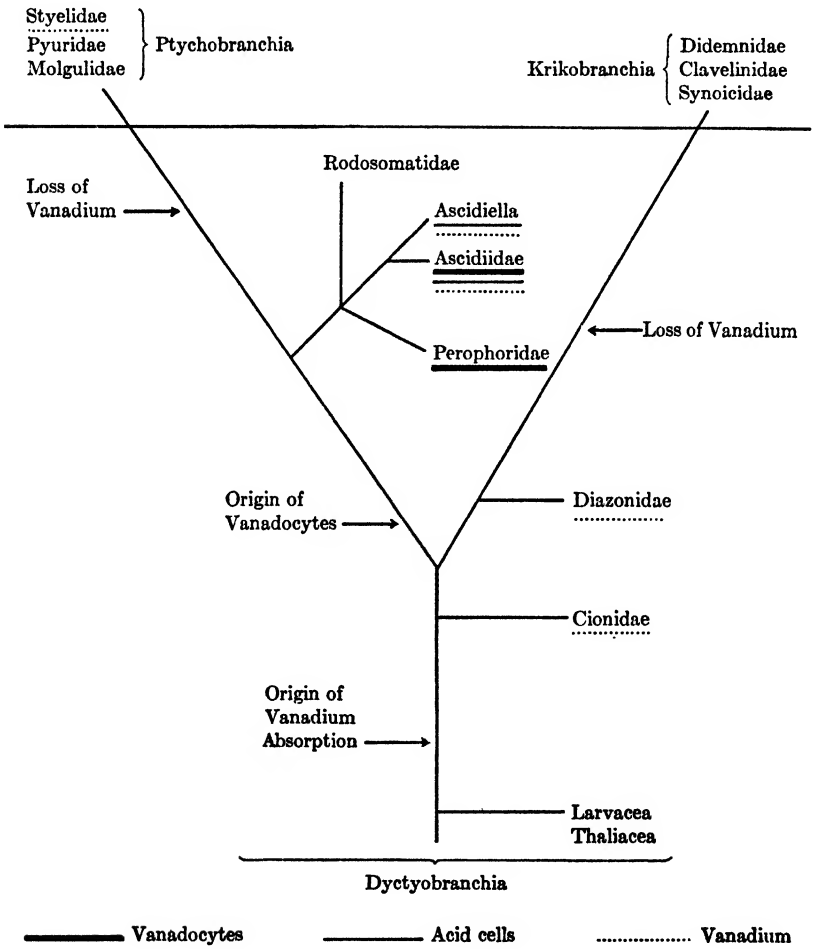
B. Biochemical Characteristics of Tunicates

One of the principal biochemical characteristics of the tunicates is their possession of a cellulose coat which covers the epidermis. The presence of cellulose in animals is an exclusive characteristic of the tunicates.

We have shown that so far as the zoological distribution of arginine and creatine is concerned, the latter is characteristically a vertebrate constituent whereas the former appears to be a characteristic constituent of the invertebrates. The tunicates do not share this characteristic with vertebrates since they contain arginine (Flössner, 107) and their phosphagen is represented by phosphoarginine (see Table IV). The blood of tunicates also displays a number of peculiar characteristics. First, one finds peculiar blood cells colored green, red, orange, or yellow, which have been the subject of important studies by Henze (126), George (112), d'Azema, and others. The accumulation of vanadium in the blood cells of some ascidians is a most astonishing biochemical idiosyncrasy of these animals, because the sea water which they inhabit fails to reveal traces of vanadium except by spectrophotographic methods. Another special characteristic of the tunicate green blood cells is that they are strongly acid (Henze, 126; George, 112) although they are suspended in a plasma which is practically neutral in reaction. Henze maintains that the cells of *Phallusia mammillata* contain as much as 3% of free sulfuric acid.

Webb (278) has shown that vanadium is practically a distinguishing possession of tunicates and outside this class of animals it has been found (though in much smaller amounts) only in the mollusk *Pleurobranchus plumula* (but not in a closely related mollusk *Oscanius membranaceus*) and in the holothurian *Sticopus möbii*. He also showed that the vanadium is present, either in organic combination as a chromogen in the greenish blood cells, the "vanadocytes," or without vanadocytes. The function of the chromogen is not known and there is no evidence that it plays a part in respiration. The chromogen has a molecular weight of about 900 (or perhaps a multiple of this) and consists essentially of a straight chain of pyrrols, very similar to bile pigments. It is not known where the vanadium is fitted into the pyrrol structure.

The vanadium chromogen is always associated with sulfuric acid in the vanadocytes present in the Ascidiidae and Perophoridae families. There is no evidence that other pigments found in the blood of tunicates contain vanadium. The interesting thing from a biochemical evolutionary



point of view is that the absorption and possession of vanadium is obviously a primitive character which has been lost in the more specialized families of the class. The diagram shows these relationships, giving the distribution of vanadium chromogen, free vanadium and sulfuric acid in the various families, which are best described in Webb's own words:

"The power to accumulate vanadium was acquired by an early form ancestral to all modern members of the group. After the cleavage into two lines of descent, one (Krikobranchia) lost this power after having given rise, soon after its differentiation, to the Diazonidae. The other line underwent a second cleavage; of the two branches thus formed one led, also with loss of vanadium, to the Ptychobranchia, whereas in the other vanadocytes and acid vesicular cells were evolved. Within the latter branch vanadocytes were in some forms (Rodosomatidae and 'Ascidiella') lost again."

Another special characteristic of the tunicate is the fact that its blood has a low carbon dioxide capacity. As was first shown by Winterstein (283), the carbon dioxide content of tunicate blood is even less than that of the water in which the animal lives. Starting from this observation, Henze (126) suggested that possibly ascidians, instead of excreting carbon dioxide into the surrounding water, absorb carbon dioxide from the latter to form the polymerized glucides of its mantle. This view has been accepted by different authors. Huus (131), in a recent monograph on the ascidians expressed this as follows: "The unusually low carbon dioxide content of their plasma indicates that ascidians actually utilize or bind carbon dioxide. Whereas 100 cc. of sea water contains 5.9 to 6.2 cc., Henze found in *Phallusia* only 0.73 to 1.56 cc. carbon dioxide in 100 cc. of plasma. Winterstein (283), who was the first to discover the low carbon dioxide content of ascidian blood, gives even lower values." And even more recently Bergmann (19) stated: "The (as yet not entirely certain) observation that the blood (especially of *Phallusia mammillata*) contains less carbon dioxide than the surrounding sea water, is an argument for the utilization by ascidians of carbon dioxide of sea water for building up their cellulose mantle."

The low carbon dioxide content is a real characteristic of the interior medium of ascidians, but the above argument unfortunately is based on a faulty interpretation of the facts. If one measures the total amount of carbon dioxide of the internal medium (fluid bathing the viscera collected under paraffin) of *Ciona intestinalis* and compares it with the carbon dioxide content of the sea water in which the animal lives, the following values are obtained (Florkin, 80):

CO_2 in sea water (equilibrated with air)	4.4 vol.%
CO_2 in internal medium	2.11 vol.%
CO_2 in internal medium (equilibrated with air)	2.11 vol.%

(The analyses were made with the Van Slyke constant volume apparatus.)

In the case of *Ciona* the total carbon dioxide content, thus, is considerably less than that of the sea water in which it lives. Still, as the results of equilibration with air show, both the internal medium and the sea water are equilibrated for the partial carbon dioxide pressure. In other words, the relatively small carbon dioxide content of the internal medium of *Ciona* does not indicate that the tissues remove carbon dioxide from the

internal medium but simply that the position of the curve of carbon dioxide absorption by the internal medium is low, or that the alkaline reserve of *Ciona* is small.

The carbon dioxide capacity at the partial carbon dioxide pressure of atmospheric air is always about 2 volume % in animals collected in their natural habitat, i.e., fastened to their support. But as soon as *Ciona* is kept in an aquarium detached from its natural support, this capacity decreases very rapidly, and after 1 or 2 days of sojourning in a perfectly irrigated aquarium or even in the sea, one no longer finds such high values (in one case it was 0.67 volume % after one day; in another case, after 4 days, it was 1.05, 0.65, 1.57 volume %) (Florkin, 80). The extremely low values of 0.24–0.29 volume % given by Winterstein for the carbon dioxide capacity (as defined above) of the internal medium of *Ascidia mamillata* is probably vitiated by this source of error.

The carbon dioxide content of sea water expelled by the cloacal syphon, in the course of very active circulation which irrigates the gill (branchial) cavity of *Ciona*, is always the same (within the limits of experimental error) as that of the sea water which enters the buccal syphon. The Van Slyke method is not sufficiently sensitive to detect any change in the water as it passes through the ascidian organism. If a *Ciona* is left in sea water covered with a layer of paraffin oil in a closed jar, after about 12 hours a measurable increase in the carbon dioxide content of the water is found (Florkin, 80).

The very low carbon dioxide content of the internal medium of tunicates does not, therefore, represent a utilization of carbon dioxide by the animal. The internal medium is in equilibrium with the external environment so far as the partial pressures of carbon dioxide are concerned. If, under these conditions, the total carbon dioxide content of the internal medium is much less than that of the sea water, this is because the carbon dioxide absorption curve of the latter is much higher than that of the internal medium. In other words, the alkaline reserve of the blood in tunicates is very small, and this must be regarded as a special characteristic.

Although the tunicates do not display biochemical characteristics of an endocrinological order, such as are found in vertebrates, nevertheless they manifest characteristics which do differentiate them from invertebrates. As we have already said, one never observes the secretion of an active hormone like the thyroid hormone or thyroxine in the invertebrate, nor is their metabolism or development modified by thyroxine. But in the tunicates there is an organ, the endostyle, which is homologous with the thyroid gland and, furthermore, the development of tunicate larvae is undoubtedly activated by the thyroid hormone (Weiss, 280). Besides, there is a little whitish gland in the tunicates, located in the adult between the two syphons

immediately under the nerve ganglion, which is the homolog of the hypophysis of vertebrates, as was demonstrated by Julin (134). It is said that neither the *neural gland* nor the nerve ganglion is of vital importance for the ascidian, and that the animal lives normally in an aquarium after their removal (Bacq, 5). Buttcher has demonstrated a substance in the extract of the hypophyses of *Molgula manhattensis* which exerts on the guinea pig uterus an oxytocic action strongly resembling pitocine, one of the active substances extracted from the posterior lobe of the hypophysis of mammals. Bacq and Florkin extracted from the nerve ganglion-neural gland of *Ciona intestinalis* the three active principles present in the posterior hypophysis of vertebrates: the hypertensive, melanophore, and oxytocic.

It can, therefore, be said that from the biochemical point of view the tunicates do not exhibit characteristics which belong to vertebrates. On the other hand, it can be said that they possess a cellulose covering, that they accumulate vanadium in their tissues and in some of their blood cells although the sea water contains only minute traces of this metal, that their blood has an extremely low alkaline reserve, that their blood has acid cells, that though they have no thyroid hormone they are sensitive to the action of this substance which stimulates the development of their larvae, and, finally, that even if they lack biochemical systems sensitive to the action of those hormones, their organism contains principles having the same effect as the hypertensive, melanophore, and oxytocic principles of the posterior lobe of the hypophysis of vertebrates.

C. Biochemical Characteristics of Cyclostomes

The true cyclostomes possess a skeleton which is largely membranous. Cartilage is found in the vertebral column, chiefly in the region of the tail, in the rays of the unpaired fins, and occasionally in the branchial arches, but there is no calcification of the cartilage nor formation of bone tissue. This is a particular biochemical characteristic of the cyclostomes which possess the general biochemical characteristics attributed to vertebrates; their epidermis is keratinized, their liver secretes a characteristic bile and is specialized for the glycogenic function, their blood cells contain hemoglobin, they possess nucleoside deaminases, they have a creatine containing phosphagen, etc.

Still, the cyclostomes, which constitute the lowest class of vertebrates, are close to the invertebrates in one particular respect, namely, the nature of their hemoglobin. The respiratory pigment of *Petromyzon fluviatilis* (*Lamperta vulgaris*) has been thoroughly investigated, so far as its molecular characteristics are concerned, in Svedberg's laboratory (Pedersen, Polson, Svedberg and Eriksson-Quensel, cf. 264). It has a sedimentation constant

of 1.87 and an isoelectric point of pH 6.0 (Svedberg and Eriksson-Quensel, cf. 264). These characteristics according to Svedberg indicate that this oxygen carrier is not, properly speaking, a hemoglobin, such as is found in other vertebrates, but an erythrocruorin. In fact, this is the smallest erythrocruorin known since the molecular weight of the oxygen transporter of the lamprey is only 17,600, or one weight unit. Although its oxygen absorption curve has not yet been established, it must be a hyperbola. A most interesting confirmation that the cyclostome respiratory pigments are erythrocruorins has been furnished by Roche and Fontaine (229). These authors studied the hemoglobin of lampreys from the point of view of the amino acid composition and found that it represents a rather peculiar type of erythrocruorin. The histidine and cystine contents are about the same as of invertebrate erythrocruorin, whereas the arginine and lysine content approaches that of a vertebrate hemoglobin.

The cyclostomes have also other characteristics which distinguish them from other vertebrates. The study of mammalian serum by Svedberg's sedimentation procedure reveals that the diluted serum contains an albumin component with a sedimentation constant of 4.5 and a molecular weight of 69,000; a globulin component with a sedimentation constant of 7.1 and a molecular weight of 160,000, and frequently also a globulinlike component of a molecular weight six times as great as that of other globulins. The serum of birds, reptiles, amphibians, and teleost fish all produce an analogous sedimentation diagram. On the contrary, the serum of the lamprey is altogether different (Svedberg and Anderson, 265). It contains one component with a sedimentation constant of 3.5 and another with a constant of 12. The first component probably has a molecular weight which corresponds approximately to the serum albumin fraction of mammals.

Still another peculiarity of the cyclostomes is the extremely low cholesterol content of their internal medium, as was shown by Fontaine and Drilhon (108).

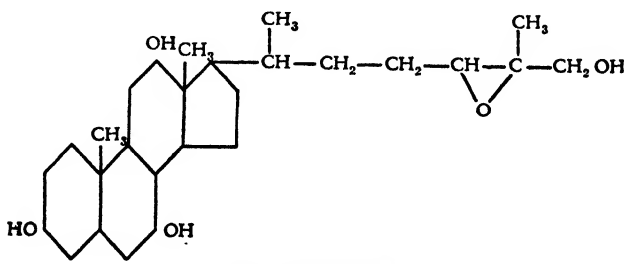
From the point of view of osmoregulation cyclostomes generally resemble the vertebrates which are homeosmotic. This, however, does not pertain to the marine cyclostomes known as hagfishes (Myxinoidea and Bdellostomoidea) which are poikilosmotic like the stenohaline invertebrates. The "milieu intérieur" of the hagfishes is more or less in equilibrium with their external environment so far as osmotic pressure and inorganic composition are concerned. The blood of *Myxine glutinosa* (marine cyclostome) contains about the same amount of urea as is found in the blood of regressed fresh-water elasmobranchs, but unlike the latter they contain sufficient inorganic salts to raise the total internal osmotic pressure to the same level as exists in the external sea water. Needham (192) suggests that *Myxine* may represent a tertiary return to a sea-water

habitat. At first there was urea retention for marine life, followed by virtual loss of it upon return to fresh-water existence, and when the animal became marine again readjustment to the new environment was now accomplished by a new mechanism of salt retention.

D. Biochemical Characteristics of Elasmobranchs

The elasmobranchs, a subclass of fishes, differ from the teleostomes by a peculiar biochemical characteristic: *their internal skeleton is essentially cartilagenous.* This skeleton is calcified in some regions but properly speaking it is never bone tissue. Bone tissue is found only in their scales and teeth.

But there are also other biochemical features especially characteristic for the clasmobranchs. They possess the general biochemical characteristics of vertebrates already enumerated before, and their liver secretes a bile containing biliary pigments and steroid compounds. But whereas in vertebrates generally these steroid compounds are bile acids conjugated with amino acids, the bile of elasmobranchs contains a special steroid *scymnol* which is found here conjugated with sulfuric acid (Hammarsten, 119). The scymnol has the following structure (Windaus *et al.*, 282; Tschesche, 267):



Scymnol $C_{27}H_{46}O_5$

We have already had occasion to point out that the organism and the blood of elasmobranchs contain considerable quantities of urea (p. 22). The uremia of marine elasmobranchs reaches a concentration of 26 g./l. and is never less than 18 g.; in other words, it reaches about the same level as the urea concentration in human urine. We are dealing here with a case of selective retention of urea since the other nitrogenous substances in the elasmobranch blood are not more concentrated than in the blood of other vertebrates. The discovery of this peculiarity, which has been found in all elasmobranchs studied (selachids, chimeroids, batioids) but not in any other animal group, is due to the work of Städeler and Frerichs (259) and to the

important comparative researches of Krukenberg (152). It is assumed that the urea exists in the tissues in some special combination. In 1890 von Schroeder (240) showed that the urea content of tissues of *Scyllium canicula* in relation to their water content is 2.95% (by weight) or very nearly the same as of the blood plasma. Léon Frédericq (111) studied the salt content of the blood plasma in elasmobranchs, and found this to be appreciably smaller than that of the sea water. Bottazzi (31), who was the first to apply the cryoscopic method to the study of biological fluids, was however greatly surprised to observe that the freezing point of elasmobranch plasma was very nearly the same as that of sea water, or even somewhat less. Rodier (231) explained the contradiction between the results of Frédericq and of Bottazzi when he demonstrated shortly afterwards that the urea accounts for this discrepancy, since it is an important component that maintains the osmotic pressure of the internal medium. Duval (70) admits that urea is responsible, on the average, for 44% of the osmotic pressure of blood plasma of marine elasmobranchs.

What is the mechanism of the physiological retention of urea in elasmobranchs? The answer is that the mechanism is primarily renal. Since 1906 Baglioni (7) has been insisting, first, that the urea concentration of the dogfish urine is very much smaller than that of its blood, and, secondly, that the urine excretion is not large (4–5 cc. per kg. and per hour). The determinations carried out by Kisch (137) on the blood and urine of the *Torpedo* illustrate these facts well:

	G. urea/l.		
Urine	1.4	4.4	2.1
Blood	16.6	20.0	18.7

The explanation of the renal mechanisms for the retention of urea, as was already pointed out in connection with our discussion of osmoregulation in elasmobranchs, is the reabsorption of urea in the uriniferous tubules (Smith, 255). The elasmobranch kidney, thus, presents a peculiar anatomical structure since it possesses a tubular segment interposed between the glomerulus and the proximal tube, which is not found in other vertebrates (Borcea, 30).

Another factor for the retention of urea by the elasmobranchs is the fact that the oral and branchial membranes as well as the integument are only very slightly permeable to urea (Duval and Portier, 71; Smith, 255).

We have already discussed the physiological role of the retention of urea in marine elasmobranchs in securing an osmotic pressure which permits the passage of water from the external to the internal environment (p. 84). The physiological uremia of elasmobranchs is, thus, to be regarded as a systematic characteristic. It is found also in the elasmobranchs which

have migrated into fresh waters (p. 83), though in the fresh-water species the uremia is diminished in consequence of adaptive variations. It is not to be confused with the urea accumulation in the mudfish, *Protopterus aethiopicus*, during its period of estivation (Smith, 250). In the course of its aquatic existence, this dipnoid excretes both ammonia and urea, the ammonia nitrogen constituting 41.2% and the urea nitrogen 18.5% of the excreted nitrogen. During the summer, the fish escapes into the mud of the dried out marshes and estivates there rolled up in a cocoon made of mud and mucus. During this estivation period it has a purely aerial respiration. In the course of this estivation it consumes its own proteins and the nitrogen is completely transformed into urea, which accumulates within the organism until it makes up 1–2% of the body weight. Returning to the water after the dry season, the animal revives, eliminates within the next 2 or 3 days a large quantity of urea, but little ammonia, and then again resumes its normal type of urea-ammonia excretion, with the preponderance of ammonia. In this case, however, we are dealing with a passive accumulation of urea and not an active retention, as is the case in elasmobranchs. In the case of the estivating mudfish the interesting fact is that the protein metabolism assumes a predominantly ureolytic type. This reflects a plasticity of the metabolic process which, as was mentioned previously (p. 41), is often observed in lower forms, and is an adaptation to a situation where the accumulation of ammonia presents a real danger.

E. Biochemical Characteristics of Sipunculids

Some years ago zoologists followed de Quatrefages in reuniting sipunculids with echiurids and priapulids into the class of gephyreans, which was considered a transitional group between annelids and holothurids. Nowadays the sipunculids are considered as a special group, a separate branch of the stem which gave origin also to annelids and mollusks. Be it as it may, the sipunculids "constitute a fairly homogenous group of exclusively marine animals, leading generally an endogenous existence the origin of which is probably very ancient" (Cuénot, 49). A peculiar biochemical characteristic of this group is the presence in their coelomic fluid of blood cells containing a special oxygen transporter *hemerythrin*. Hemerythrin has been found in the blood cells of all sipunculids examined, i.e., among diverse species of the following genera: *Phascolosoma* (Schwalbe, 244), *Sipunculus* (Lankester, 158), *Physcosoma* (Cuénot, 48; Kobert, 139), *Aspidosiphon*, *Phascolion* (Cuénot, 50). To Ray Lankester (1873) belongs the credit for having understood the nature of this respiratory pigment. In his discussion of the morphological relationship, which Brandt (35) established between cells containing hemoglobin

and the cells of the coelomic fluid of *Sipunculus nudus*, Ray Lankester showed that the pigment of the sipuncle is not a hemoglobin. As was shown in subsequent studies, this oxygen transporter is entirely different from the oxygen transporters of other animals (hemoglobins, hemocyanins, chlorocruorins). Its chemical nature represents a systematic characteristic of the group of sipunculids. Solutions of oxyhemerythrin are reddish in color, resembling the different red wines, according to the origin and concentration of the pigment (Cuénot, 50; Marrian, 178; Florkin, 78; Roche, 226). In the reduced state the solutions of hemerythrin are practically colorless. Hemerythrin is not a heme derivative like hemoglobin and chlorocruorin (Kobert, 139; Marrian, 178; Florkin, 78). It contains iron, but not copper (Andrews, 1) like hemocyanin. If an oxidizing agent is added to a hemerythrin solution, it assumes a brownish color which no longer disappears when the solution is subjected to a vacuum or to action of a reducing agent, because its iron has been transformed into the trivalent form, the methemerythrin (Marrian, 178; Florkin, 78; Roche, 226). The hemerythrin of *Phascolosoma elongatum* can be crystallized by adding alcohol and ether to its solutions (Florkin, 78), the crystals being pseudo-quadratic rhomboctahedrons (see Fig. 21). This method cannot be used to crystallize the hemerythrin of *Sipunculus nudus*, but this chromoprotein can be crystallized by prolonged dialysis at 0°, whereby crystals of variable shapes are obtained (Roche, 226). The hemerythrin crystals are insoluble in water but soluble in dilute salt solutions (Florkin, 78; Roche, 226), which indicates that the hemerythrins have gobulin properties. The elementary composition, the amino acid composition and the titration curve of hemerythrin of the sipuncle were studied by Roche who showed that the isoelectric point of this pigment is pH 5.8.

The visible spectrum of oxyhemerythrin of sipunculus is characterized by the existence of a unique, very feeble band (peak 4.950 A., Roche; or 5.020 A., Florkin). The absorption decreases towards the violet to a minimum (4650 A., Roche; 4690 A., Florkin), then increases again. When hemerythrin is reduced, the band is replaced by a scarcely perceptible hump and the very much weaker absorption rises gradually from about 5200 A. to the extreme violet (Roche). The ultraviolet spectrum of reduced hemerythrin is similar to that of the oxyhemerythrin, but in a general way the absorption is diminished (Roche). Hemerythrin combines with the same amount of oxygen whether the partial pressure of carbon monoxide is 4 or 150 mm. Hg, so that hemerythrin obviously does not bind carbon monoxide under conditions under which hemoglobin would be completely transformed to carboxyhemoglobin (Florkin). Similarly, bubbling carbon monoxide through a hemerythrin solution changes neither its spectrum nor its color (Roche).

The molecular weight of hemerythrin, determined by Adair's osmotic pressure method, is 66,000 (Roche and Roche, 223). Since hemerythrin contains 1.01% of iron (Roche) and binds oxygen in the proportion of one

FIG. 21
(Florkin, 78.) Hemerythrin crystals of *Phascolosoma elongatum*.

molecule oxygen to three atoms of iron (Florkin, 78), one molecule of hemerythrin ($Hr_{12}O_8$) transports the same quantity of oxygen as one molecule of hemoglobin (Hb_4O_8) (Roche and Roche, 223).

The presence of a special oxygen transporter in their blood cells is not the only biochemical characteristic of the sipunculids. Another characteristic peculiar to this group is the presence of considerable quantities of allantoic acid in their blood cells, those of *Sipunculus* containing about 1% (Florkin and Duchâteau, 98). When the fluid is deproteinized with phosphotungstic acid, the filtrate is slightly acid and the allantoic acid is spontaneously changed to urea and glyoxylic acid (p. 45). This gave Delaunay (54) the idea that the blood cells of the sipunculids are "surcharged with urea." Actually, however, what accumulates is allantoic acid, and this has been demonstrated in all sipunculids so far studied (*Sipunculus nudus*, *Phascolosoma Gouldii*, *Phascolion strombi*, *Phascolosoma elongatum*) but not in the coelomic cells of the echiuroid *Thalassema Neptuni*, of the polychete *Glycera gigantea*, of the capitellid *Dasybranchus caducus*, and of the holothurid *Thyone briareus*. Allantoic acid is missing also in the blood of the lamellibranch *Arca inflata*, of the gastropod *Busycon canaliculatum*, of the crustacean *Labinia emarginata* or of an arachnomorph *Limulus polyphemus* (Florkin, 81). What is the significance of this remarkable accumulation of

allantoic acid, which is peculiar to the coelomic cells of sipunculids? Are we dealing here with a synthesis from much simpler nitrogenous substances? Is the allantoic acid the material from which more complex nitrogenous

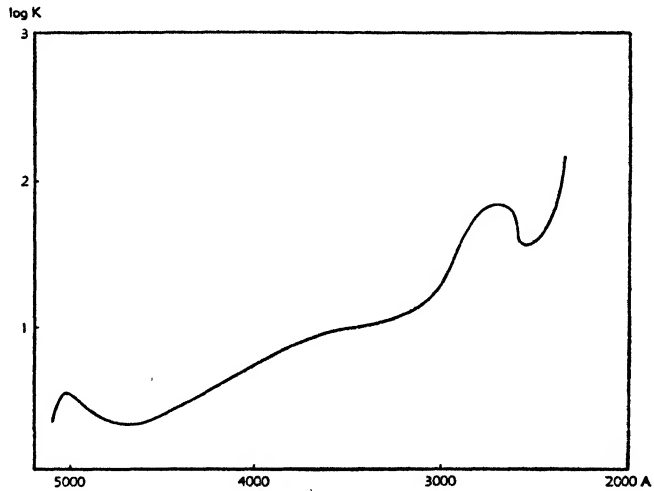


FIG. 22

(Florkin, 78.) Absorption spectrum of oxyhemerythrin of *Sipunculus*.

substances are being built up, as in the case of nucleic acids? It is claimed that in the sipunculids the eggs pass a period of most intense growth in the coelomic fluid during which an important biochemical synthesis of the thymonucleic acid occurs. It is not at all impossible that substances contained in the coelomic cells pass into the eggs. Baumberger and Michaelis (14) have shown that in the coelomic fluid of the echiuroid *Urechis caupo* the granules in the coelomic cells, which Redfield and Florkin (217) first described and which were later recognized as being hematin, pass into the eggs. Or is the allantoic acid perhaps a product of synthesis from some nitrogenous substance which accumulates in an excretory cell and is destined to be rejected to the outside? These questions need to be studied further.

F. Biochemical Characteristics of Insects

In Chapter III it was shown that the osmotic pressure of insect blood is to a very significant degree maintained by the presence of amino acids. In fact, the presence of a high concentration of amino acids in the blood plasma is a systematic biochemical characteristic of insects. The fact that the insects have a much higher amino acid concentration in their blood than any other animals has been known for a long time. This was already

demonstrated back in 1902 by Nazari (185) in the course of investigations carried out on larvae and pupae of Lepidoptera and on Coleoptera. Bishop *et al.* (23) found in the blood of larvae and pupae of the bee that the amino acid nitrogen concentration ranges from 250 to 300 mg. %. Duval *et al.* (72) determined the amino nitrogen in the trichloracetic acid filtrates from the blood of Coleoptera by the formol titration method and found 164 and 139 mg. % in the *Hydrophilus* and *Dytiscus* respectively. Using the colorimetric procedure on phosphotungstic filtrates of whole blood from the butterfly *Deilephila euphorbiae* on the day of hatching, Heller and Moklowska (122) found 164 mg. % of amino nitrogen. Babers (4) found 233 mg. % amino nitrogen in the whole blood of the *Prodenia* larvae. Obviously, the amino acid content of the blood plasma is what matters, and by means of an adequate micromethod it is possible to carry out a determination on the plasma of a single *Hydrophilus*. The values obtained from a series of analyses on a number of such individuals range from 40 to 80 mg. % of amino nitrogen (Florkin, 87). The changes in amino nitrogen content of blood plasma of *Bombyx mori* at different stages of its development were also studied by this procedure (Florkin, 87). The plasma of late larvae contains 230 mg. % amino nitrogen. At beginning of pupation (spinning of cocoon) the aminoacidemia decreases to about 145–150 mg. %, but later the plasma amino acid content rises again to about its original level (Fig. 23). In the imago on the day of hatching the amino acid nitrogen content of plasma was found to be 250–265 mg. %. Thus, it is

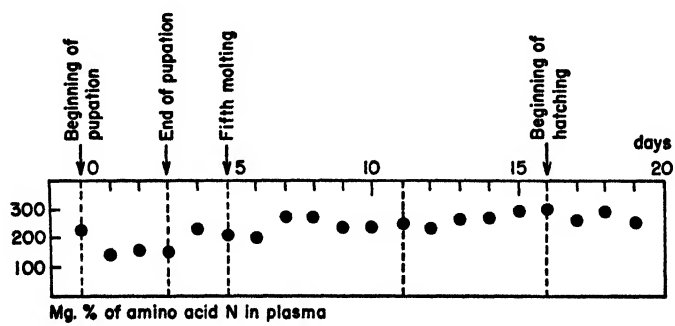


FIG. 23

(Florkin, 87.) Changes in the blood plasma amino acid content of silk worms during metamorphosis.

well established that the amino acid content of plasma in insects is very high. In animals of other groups the amino acid nitrogen in the phosphotungstic acid filtrate of plasma is quite generally somewhere about 10 mg. % or even less.

The nature of the amino acids found in the internal medium of insects is still insufficiently known. In *Dytiscus*, for example, the search for arginine, tryptophane, phenylalanine, or cystine gave negative results. The plasma concentration of histidine is about 30 mg. and of tyrosine 117 to 168 mg. % (106 mg. % of total amino nitrogen in plasma) (Florkin and Duchâteau, 99). The amounts of these two amino acids are very high as compared with other animals. The tyrosine content of the blood of the crab *Maia* is only 4 mg. % (Pinhey 205). Yet the tyrosine nitrogen represents only a small portion (8%) of the total plasma amino nitrogen of *Dytiscus*, and the proportion represented by histidine is still smaller. The nature of by far the largest portion of amino acids in the blood plasma of insects is, thus, still undetermined.

The very high uric acid content of the blood is another peculiar characteristic of insects, which distinguishes them from all other invertebrates. The uric acid content of the blood plasma of the *Hydrophilus* is 10.7 to 14.5 mg. %; that of the adult *Bombyx mori*, on the day of hatching, 12.6 to 14.5 mg. %; that of *Dytiscus* 18 mg. % and that of *Dixippus morosus* 10.4 mg. % (Florkin, 87). In the Crustacea, however, one finds only 0.3 to 0.9 mg. % in the plasma of *Maia squinado*, according to Delaunay (54, 55), or 0.2 mg. according to Boivin (28). Morgulis (181) found 2.0 to 2.4 mg. % uric acid in the blood of the lobster, 1.0 to 3.8 mg. % in the blood of the crab *Labinia* and 2.9 to 3.4 mg. % in the crab *Callinectes*. Only traces of uric acid, or even none at all, have been found in the blood of the amphineurid *Cryptochiton Stelleri* (Myers, 183); in different marine and fresh-water lamellibranchs (Myers, 183; DeWaele, 56), in the prosobranch *Haliotis rufescens* (Myers), in the gastropod *Limax agrestis* (Delaunay, 55) and in the cephalopod *Octopus vulgaris* (Delaunay, 55).

But we found a high blood uric acid content in the four adult insects studied (an orthopteron (*Dixippus*), lepidopteron (*Bombyx mori*), phytophage coleopteron (*Hydrophilus*) and carnivorous coleopteron (*Dytiscus*)). The larvae and nymphs of insects also present this characteristic. In the case of the silkworm, for instance, the plasma contains about 10 mg. % uric acid at the end of the late stage and just before pupation. From the beginning to the end of pupation the uric acid decreases somewhat progressively but returns to its original level during the prenymphal rest to again decrease slightly and progressively until just the eleventh day after the beginning of pupation. Then and until the end of the nymphal existence it rises again definitely and progressively (Fig. 24) (Florkin, 84). During these different phases of metamorphosis the lowest value for the plasma uric acid determined was 6.8 mg. %.

In fully developed larvae of the lepidopteron *Antheraea pernyi* the concentration of the plasma uric acid is 19 mg. % (Leifert, 160), in the larva

of the *Chironomus*, towards the end of the larval stage, it is 11.2 mg. %, and in *Hydrophilus* larvae 20 mg. % (Florkin, 84).

It is, therefore, obvious that the high plasma uric acid level of insects is a general characteristic, in spite of differences in the mode of their feeding and existence.

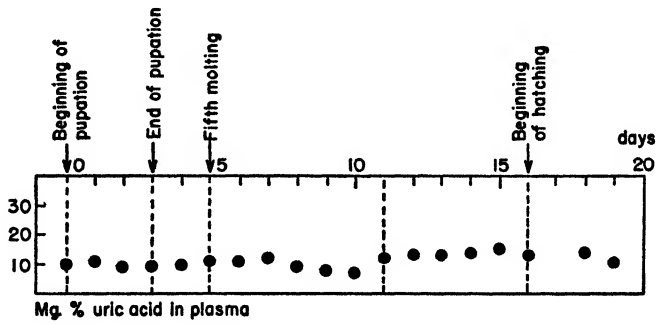


FIG. 24

(Florkin, 87.) Changes in the blood plasma uric acid content of silk worms during metamorphosis.

Quite characteristic for the class of insects is the high content of reducing nonfermenting substances in their blood plasma. Although these substances are lacking in the plasma of Crustacea (Florkin, 85) or of *Limulus* (Dailey *et al.*, 51), the plasma of insects does contain appreciable quantities. In *Hydrophilus*, for instance, the content of reducible nonfermentable substance may attain 75 mg. % (determined as glucose). In the *Bombyx mori*, in the imago stage, it corresponds to 139 mg. %, and during the metamorphosis of this lepidopteron it does not fall below 32 mg. %, the value found in advanced larvae, and increases during the different stages of metamorphosis (Florkin 85).

In describing the inorganic composition of the internal medium, it is customary to express the concentration of some constituent per unit of volume, or in terms of equivalents in relation to the concentration, also in equivalents, of Na or Cl ions which is assumed equal 100. This method yields results of little significance, because the over-all concentration of one internal medium may be very different from that of another, and because the proportion of Na or Cl ions in the sum of cation or anion equivalents is much more variable than it is generally believed. It is much more significant to compare what we call *indices* of the different cations and anions. One may define the alkaline component of the acid-base equilibrium by calculating first the sum of all the basic equivalents contained in the internal medium. The per cent of each cation is then calculated in relation to the

sum of basic equivalents (Σ cations), and this represents its index. The index of an anion can be similarly calculated in relation to the sum of basic equivalents which correspond to that of the acid equivalents. If one studies the indices for the different cations in the liquid phase of the internal medium of an insect like *Hydrophilus*, and compares the values with those of the indices found for other groups of animals, one is impressed by the high values for the magnesium and phosphate found in the insects. The magnesium index for sea water (Atlantic Ocean) is 17.8. Among marine invertebrates, except Crustacea, similar values are found (in *Limulus*, for instance, according to Macallum's data (173), the index is 17.0). Calculating the values for the magnesium index from data available in the literature (Bethe and Berger, 20) the following results are obtained: *Hyas aranea* 12; *Cancer pagurus* 8.5; *Carcinus moenas* 8.0; *Maia squinado* 11.5. In vertebrates and fresh-water invertebrates the values of this index range from 0.2 to 2.9. But the value of the magnesium index for blood plasma of an insect like *Hydrophilus* is 21.9 (Florkin, 91). The plasma of *Hydrophilus* contains 43–54 mg. % of Mg while that of the snail *Planorbis* contains only 2 mg., or that of the fresh-water mussel 0.5 mg. (Florkin), of the snail 2 mg. (Lustig *et al.*, 170), of the crayfish 6 mg. (Bogucki, 27) and of man 1.5–2.6 mg. %. The large content of magnesium in the blood of insects is a characteristic which is absolutely peculiar among animals and is found also in the whole blood of larvae and pupae of Lepidoptera (Brecher, 36; Heller and Moklowska, 122). The only exceptions to the above statement are the marine invertebrates whose internal medium, of course, reflects very closely the composition of the sea water.

The numerical value of the phosphate index, which for sea water is about 0.1, ranges from 0.5 to 3.2 for all the different fresh-water or terrestrial animals studied. But in *Hydrophilus* the value is 6.9 (Florkin, 92). The fact that insect blood is particularly rich in inorganic phosphate has been confirmed by a number of values for the whole blood established from published data: *Pieris brassicae* pupae 66 mg. % (Brecher, 36); *Deilephila euphorbiae* larvae 12 mg. % (Heller and Moklowska, 122); *Prodenia eridania* larvae 17.6 mg. % (Babers, 4); pupae of different Lepidoptera 22.5 mg. % (Drilhon, 63); bee larvae 31 mg. % (Bishop *et al.*, 23). The level of plasma phosphorus of *Hydrophilus* (14 mg. %) is much higher than that found in animals not belonging to the insect class. Thus, the inorganic phosphorus of the blood plasma of the snail is about 0.4% (Lustig *et al.*, 170), of the plasma of *Planorbis* 0.7, of the fresh-water mussel 0.5 (Florkin, 91) and of man 3.5 to 4.0 mg. %.

It can, therefore, be said that the internal medium of insects presents two systematic characteristics: high indices for magnesium and for phosphate.

On the other hand, insects are covered by chitin which is never impregnated with inorganic salts as is the case, for instance, in the crustaceans. Another biochemical characteristic of insects is the fact that they never contain glycine-betaine, a substance found in the crustaceans.

G. Taxonomy of Biochemical Characteristics

We shall take the insect *Hydrophilus piceus* (water beetle) as an example for the discussion of the classification of biochemical characteristics.

The surface of this water beetle is covered with chitin which is not impregnated with calcium salts. Its blood is of a pale yellow color which turns black on exposure to air; it has a freezing point $\Delta = -0.647°C$. (Barrat and Arnold, 13) and a pH of 6.6–6.8 (Kocian and Spacek, 144). The chemical composition of the plasma is as follows (Florkin, 91):

Hydrophilus				Indices of cations and anions (ϵ cations = 201.8)	
Proteins	3.50%	Na	275–278 mg. %	Na^+	59.8
Total Oxygen	0.11% (vol.)	K	50–54	K^+	6.9
Total CO_2	72–88% (vol.)	Ca	42–46	Ca^{++}	11.4
Amino-N	40–80 mg. %	Mg	43–54	Mg^{++}	21.9
Urea	2.6 mg. %	Cl	140–142	Cl^-	20.1
Uric Acid	10.7–14.5 mg. %	S	3–4	$SO_4^=$	0.9
True Sugar	5–31 mg. %	P	14	$HPO_4^= + H_2PO_4^-$	4.0
Reducing Substance (nonfermentable)	20–75 mg. %				

The existence of the chitin coat is a systematic characteristic of arthropods. The fact that the chitin is not impregnated with salts is a systematic characteristic of the class of insects. The proteinemia of *Hydrophilus* is 3.5%; that of *Dytiscus marginalis* 3.18%; of *Agelastica alni* 3.47%. In general the proteinemia of all the investigated coleopterons ranges from 2.7 to 4.1% (Florkin, 89). Since the proteinemia of the orthopteron *Dixippus morosus* is 1.04%, of the lepidopteron *Bombyx mori* 2%, and of the hymenopteron *Bombus agrorum* 5% (Florkin, 89), it is possible that the level of blood protein of the *Hydrophilus* is a biochemical characteristic of the order Coleoptera, belonging in the category of orthogenetic characteristics. The high amino acid content of the blood plasma of *Hydrophilus*, as was pointed out previously, is a systematic characteristic of the class of insects. This is also true for the uric acid content, for the

indices of magnesium and phosphate and for the concentration of reducible nonfermentable substances. A high level of potassium in insect blood has also been pointed out frequently. For example, the blood of the coleopteron *Leptinotarsa decemlineata* contains 111 mg. % K (Drilhon and Busnel, 64) while that of the crayfish contains only 19 mg. % (Drilhon-Courtois, 65). But the value of the K index in the internal medium of the *Hydrophilus* is the same as that of another terrestrial invertebrate, the snail, whose K index, calculated from the analyses of Lustig *et al.* (170), is 6.3. Until more extensive information is available, it is impossible to state definitely if the high K index might not express simply an adaptive characteristic developed in correlation with the special condition and mode of alimentation of terrestrial invertebrates. Certainly the high carbon dioxide content of the whole blood of the *Hydrophilus* represents an adaptive characteristic, and does not occur in a number of other insects (Florkin, 91). The *Hydrophilus*, although it lives in water, is actually an animal with an aerial respiration. Its mode of existence occasions an accumulation in the blood of carbon dioxide resulting from metabolism, as is generally the case in Amphibia. As was noticed elsewhere, animals of this category present a true physiological gaseous acidosis compensated by a rise in the carbon dioxide absorption curve. This is notably the case in aquatic turtles. The mode of existence of *Hydrophilus* is thus responsible for the high concentration of carbon dioxide in its blood, which seems to be an adaptation.

The fact that the blood of the *Hydrophilus* turns black on exposure to air is associated with the existence in its blood of a true phenolase. The existence of these enzymes in animals can not be demonstrated except in arthropods (Bhagvat and Richter, 21; Duchâteau-Bosson and Florkin, 67). The phenomenon of blackening is, therefore, a biochemical characteristic of the branch of arthropods.

CHAPTER VII

Perspectives

The study of biochemical characteristics depends upon techniques which frequently are complicated, and such a study is more difficult to accomplish than direct observation of morphological characters. Nevertheless, had naturalists started from these rather than from morphological observations they would have been bound to conceive the idea of evolution of animals. They could not have failed to recognize the phenomena of orthogenesis and adaptation, and they would have discovered biochemical characteristics on the basis of which to classify species into more or less extensive groups. Moreover, this classification, as far as our actual knowledge permits such a comparison, would be identical with the system elaborated by Cuvier and his collaborators at the beginning of the last century. This is the main thesis of this little book, the sole purpose of which is to lay before the reader biochemical evidence of animal evolution. To concede that the classification of morphological groups conforms to a biochemical classification is to favor the idea that morphological and biochemical characteristics are linked together and that both are governed by the same determinism. This offers an argument for the thesis that in the last analysis evolution is directed by biochemical phenomena. From this point of view it is possible to draw from the known facts a number of conclusions which are as yet hypothetical but which can serve as a basis of a program for future work.

A. Biochemistry and Morphology

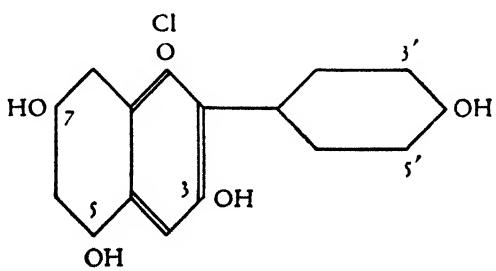
Soon after publication of the celebrated *Mikros opische Untersuchungen*, in which he developed his theory of cellular organization of animals, Schwann, who was barely thirty years old at thetime, came to Belgium to occupy a professorship first in the University of Louvain and later in the University of Liége, where he remained for a long time. It is amazing that, following a period of veritable fireworks of outstanding discoveries, the life of this man of genius became entirely engulfed in silence. But Schwann never really ceased to meditate and to experiment until the very eve of his death. The problem which was constantly before his mind was to correlate the molecular nature of living matter with the cellular organization of the animal. Schwann delved into this problem when the scientific world was

still too immature and totally unprepared for it. It was years before the submicroscopic structure of cells was revealed thanks to the employment of new techniques like the polarizing microscope, the diffraction of x-rays or the electronic microscope. The body of knowledge acquired by general biochemistry compels us now to accept the idea of structural continuity at the different levels of aggregation of living matter, and consequently the idea that microscopic structure depends upon submicroscopic structure which, in turn, depends upon the structure of large molecules or aggregates of molecules. These views have been developed by Needham (190) and by Baldwin (8) and they inspire further research. It is possible to conceive of the cytoplasm of the cell as a mass of interlacing protein fibrils constituting a *cytoskeleton* (Peters, 201). In the meshes of these fibrils other constituents are found, especially mitochondria and granules of different sizes. It is probable that the structure of the protein molecules of the cytoskeleton is one of the important elements of *chemical differentiation* of the specialized cells of different organs. But in the last analysis such chemical differentiation occurs during embryological development. The elucidation of its mechanism is one of the future tasks reserved for chemical embryology, which aims to define the biochemical basis of organogenesis by regarding such things as induction, individuation, morphogenetic field, morphogenetic potential, etc., from the viewpoint of biochemistry. The reader will find in the recent works of Jean Brachet (34) and of Joseph Needham (194) brilliant discussions of what lies between the known facts and the perspectives of future research. The role of the fibrils of the protein cytoskeleton in morphogenesis is certainly very considerable, as was suggested by Koltzov back in 1928. The idea according to which the activity of substances with specific functions may be associated with a definite architectural orientation of their cytoskeleton fibrils is one which demands experimental elucidation. Modern biochemistry has revealed the participation in metabolism of a number of substances with specific functions which truly assure the coordination of the organism. Genetics, chemical embryology, and physiology present many facts showing the preeminent role played by different functional substances in the realization of animal forms: the genes, with their cellular hormones, organizers and inductors, circulating hormones, etc. In the last analysis, one can conceive of the external form of an animal as being an expression of the specific rates of growth of its parts and, therefore, of their metabolism. In other words, form is the resultant of an interaction of biochemical systems with molecular and submicroscopic architectural patterns which are themselves the products of activity of specific functional substances arising in the course of ontogenesis.

B. Mechanism of Biochemical Evolution

Assuming that the continuity between molecular structures and morphological structures is real, the appearance of new molecules or of new combinations of molecules in an evolving process defines this evolution. The various evolutionary lines represent what is recorded in the archives of activity as well as the experience of the biochemical mechanism in the animal's adventures either in different environments or in an environment which changes considerably in the course of time.

The mechanism by which a biochemical constituent may be modified in the course of evolution presupposes knowledge of the mechanism which governs its appearance, with its particular structure, among the representatives of one or another group of animals. Chemical embryology unfortunately is not sufficiently advanced to furnish answers to this question. In some favorable instances, as for instance where pathological effects follow a change in a definite gene, genetics has given us some particularly interesting facts (113, 117, 285). In the human species, for example, the X chromosome carries a gene exerting an influence over the blood coagulation process. When this gene is altered, the coagulation is retarded and severe hemophilia results from complete inactivation of this gene. In the mouse an alteration of a definite gene leads to nanism because of the failure of formation of two anterior pituitary hormones, the growth hormone, and the thyreotropic hormone. But in most instances, the genes exert their effects locally, at the cellular level, where they control biochemical events. The altered gene which provokes albinism in the rat acts locally in each cell. The skin of an albino rat does not darken when it is grafted onto a normal rat. The biochemical component, which is lacking in the albino rat, is an enzyme whose production is governed by a definite gene.



The idea of the control of a hereditary biochemical character by the activity of genes is especially well illustrated by genetic studies relating to the production of anthocyanins by plants (Scott-Moncrieff, 246). The

anthocyanin pigments which are responsible for the blue, purple, and red colors of many flowers are derived from pelargonidine.

The different anthocyanins are derived from this molecule by substitution of methoxyl or hydroxyl groups in position 3′ or 5′, by methylation of the hydroxyl at position 7, by substitution of a sugar at position 3. The genetic analysis has shown that there are separate genes controlling the following operations within the family of anthocyanins: oxidation at 3′; oxidation at 5′ when oxidation at 3′ has already taken place; oxidation at 3′ and 5′; methylation at the OH in position 3′, or at both 3′ and 5′ positions; finally, the substitution of sugar at position 5.

In the instances above discussed the genetic control of the appearance of a biochemical constituent of some definite structure is indirect. The control operates through a more or less complicated mechanism of synthesis which results in the production of the particular constituent.

There are, however, cases where a biochemical constituent is produced directly by a gene. The determination of sex and the fusion of gametes in the alga *Chlamydomonas eugametos* (R. Kuhn, F. Moewus *et al.*) furnish examples of this type (cf. 34).

This alga exists in a synoica form, where the sexes are not separated, and a simplex form with the sexes separated. If a filtrate of male gametes is added to the undifferentiated sex cells, all cells become male. A termone was added (i.e., a specific chemical which determines sex in an organism of mixed sexuality) which in this case is an androtermone. On the other hand, if an extract from female gametes is added, a gynotermone, all the cells become female. This gynotermone is the aglucone portion of a glucoside and is probably the methyl ester of quercetine, whereas the androtermone is an oxyaldehyde related to safranal. Picrocronine, which is the precursor of androtermone, is found in the cells and is transformed to androgamone by an enzyme present only in the male gametes. The existence of the enzyme depends upon the presence in the male gametes of a gene M_D which produces the enzyme directly. Indeed, many of the facts, as was also emphasized by Richard Kuhn, favor the identification of the gene with the enzyme.

In the fruit fly *Drosophila* the vermillion gene (v^+) is responsible for the appearance of the vermillion color of the eye. The control operates through the formation, under the influence of the gene, of a biochemical substance related to tryptophane. This substance is kynurenine which diffuses into the interior medium and is transported to the eye where it enters the chain of reactions leading to the synthesis of the vermillion pigment (cf. 34, 194). Many biochemical hereditary substances are thus directly related to the nature of certain particular genes. A gene reproduces itself at each cell division; however, it can also reproduce itself independently

because it is not permanently fixed in the chromosome but diffuses into the cytoplasm. It is obvious that such direct products of gene activity present great interest.

The intervention of genes in the enzymatic direction of biochemical reactions is brought to light in a particularly striking manner in the researches of Beadle and collaborators (15) on the mold *Neurospora*. These admirable studies have shown how definite genes determine the production and the specificity of enzymes which enter the process of synthesis of different amino acids and of different vitamins at particular points. These researches lead experimentation into the region where gene control of cellular biochemistry becomes dominant.

The reproduction of a biochemical constituent like horse hemoglobin, for instance, is evidently an epigenetic process. What is inherited is the mechanism for producing this substance, but the substance itself is not formed anew in the embryo from a hemoglobin molecule obtained from the parents. In the case of the genes it is altogether different since they are the direct descendants from corresponding molecules of the progenitors. The production of a gene follows the model of another existing gene, of which the new gene is the exact copy.

The biochemical units which compose the genes may occasionally be reproduced inexactly and a mutation results. The mutated gene reproduces itself again in its new form.

The evolution of biochemical as well as morphological characters seems, in the last analysis, to be related to the variation in properties of the genes; in other words, to biochemical phenomena.

C. Biochemical Aspects of the Law of Serres

To develop successfully into the adult state the embryo passes through a series of stages of increasing heterogeneity and complexity just as its ancestors did in the course of their evolution. It is not surprising, therefore, that in the earliest stages of ontogenetic development one discovers biochemical characteristics, and consequently morphological characteristics, similar to those observed in the simplest animal forms. As Needham (188) pointed out, the existence of these characteristics undoubtedly plays an active part in the development of the animal form, in all probability through the production of specific chemical substances, the so-called organizers. One can enumerate many instances of recapitulation in the biochemical order of things. As was said before, vertebrates are characterized by the existence of specialized organs for the storage of glycogen whereas in the invertebrates the glycogen is widely diffused. But the same characteristic (the wide diffusion of glycogen) is found also in the vertebrate

embryos. Likewise, we considered the succession of systems of protein metabolism ending respectively in ammonia, urea, or uric acid as an expression of orthogenesis. Needham demonstrated that although the chick, like all birds, is an uricotelic organism, its embryo behaves like an ammonotelic organism, at first, and later becomes ureotelic and finally uricotelic (Fig. 25) Similarly, adult frogs are definitely ureotelic but the embryo excretes 50% of its nitrogen in the form of ammonia (Bialaczewicz and Mincovna, cf. 188).

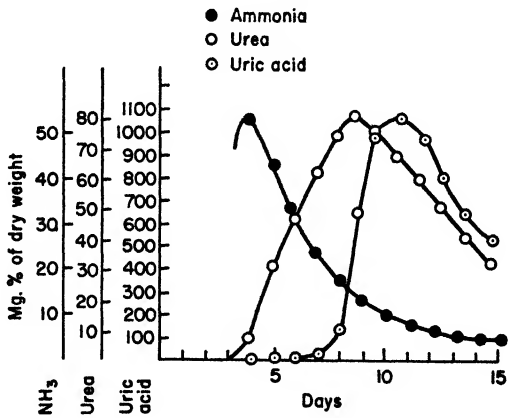


FIG. 25

(Needham, 188.) Excretion of nitrogen by the chick embryo during development.

The succession of enzymatic systems involved in uricolysis has been considered a manifestation of general orthogenesis which has been evolved by successive losses of the enzymes urease, allantoicase, allantoinase, and uricase. Thus, uricase is present at first in the chick embryo but disappears in later stages of its development (Przylecki and Rogalski, 211).

We also noted that arginine is a typical constituent of invertebrates whereas creatine is a systematic biochemical characteristic of vertebrates and, notably, of elasmobranchs. However, arginine is found in elasmobranch embryos but disappears in the adult organism (Kutscher and Ackermann, 155).

Wald (274) has shown that the porphyropsin system in the retina of bullfrog tadpoles changes over completely to the rhodopsin system precisely at the time of metamorphosis to the adult condition. This phenomenon represents a recapitulation just as truly as do the more obvious morphological changes, and both illustrate an abridged summary of the evolutionary changes involved in the passage of vertebrates in general from a fresh water to a terrestrial environment.

The hemoglobin of this animal likewise manifests changes suggestive of chemical recapitulation. The oxygen dissociation curve of the bullfrog tadpole hemoglobin is hyperbolic, indicating a high oxygen affinity, but during and after metamorphosis the curve assumes an inflected S-shape corresponding to a great fall in oxygen affinity. Similar changes have been described in the oxygen dissociation curve of the hemoglobins of the developing chick and of the mammalian fetus.

D. Reversibility of Biochemical Evolution

Biochemical evolution of animals is the expression of a balance between the loss of biochemical constituents and biochemical systems, on one hand, and the acquisition of new systems, on the other. The first, which may be designated the apheretic aspect of animal evolution, can be illustrated by many facts. In this sense it may be said that evolution is reversible since a character acquired can be lost again. Purine metabolism presents such an illustration of a system which evolved through the loss of enzymes, or enzymapheresis (p. 48).

In the field of general biochemistry the evolution of metabolism confirms the reversibility of biochemical evolution.

The idea prevailed for a long time that autotrophic bacteria (i.e., microorganisms which use only inorganic substances) preceded heterotrophic bacteria (i.e., microorganisms requiring complex sources of carbon and nitrogen) as denizens of the earth's surface. But Oparin (195) furnished good reasons for assuming that various organic substances existed on the surface of our globe before living things appeared. Recent discoveries with regard to viruses of molecular dimensions give a new orientation to these ideas. One also observes the great biochemical complexity of chemoautotrophic bacteria with an arsenal of vitamins of the B group. All this has led different authors to suggest that the first living things must have been monomolecular and strongly heterotrophic. According to some, these first living things must have been analogous to the virus of tobacco mosaic, but with an essential difference: they were capable of self propagation in the free state in a medium containing a variety of organic substances which are no longer found now, because they had been exhausted by the countless unicellular and multicellular living organisms which inhabited this medium.

It is not difficult to imagine the progressive complication of a simple organism which would follow the appearance of a gene governing the formation of enzymes or to forecast the probable effect of these hypothetical events from the point of view of natural selection. One can also realize how the complex biochemical system of autotrophic organisms could

develop. Then, through the loss of genes commanding one or another intervening reaction in the metabolic chain, autotrophs could again give rise to heterotrophs. The origin of heterotrophy among autotrophs has been achieved experimentally by Beadle (15, 16) and his collaborators in their studies on *Neurospora*. Many examples of lost functions are to be found along different evolutionary paths. Genetic analysis shows that loss of genes is statistically much more common than the acquisition of new genes. One need, therefore, not be surprised to note frequently the disappearance of genes which no longer confer any advantage within the sphere of natural selection. It is also possible to find more or less orthogenetic series characterized by losses of function. Thus, the heterotrophs which must obtain carbon in the form of organic compounds but can utilize nitrogen of the air, ammonia, or nitrates as sources of nitrogen, resemble most nearly the autotrophs. The prototrophs, or nitrogen-fixing bacteria, as well as many saprophytes belong in this category.

A new stage in the heterotrophic evolution is the loss of capacity to utilize ammonia as a source of nitrogen, which must now be provided in the form of organic compounds such as amino acids. Tryptophane is most frequently the indispensable amino acid. The necessity of supplying it in order to maintain the life of an organism marks the beginning of a diminishing ability to synthesize. The bacteria under consideration, having lost the mechanism for synthesizing tryptophane, can no longer build protein or perhaps certain fundamental substances unless this amino acid is furnished intact. Other bacteria, besides requiring carbon in the form of organic compounds and nitrogen in the form of some of the various amino acids, must also be supplied with various other substances for their existence. For instance, *Hemophilus influenzea* requires two substances found in blood (but not in serum) and for a long time designated as factor X and factor V. But factor X is really protoheme and factor V is cozymase. Without these preformed constituents the bacteria cannot manufacture cytochromes and some of their dehydrogenases.

Staphylococcus aureus cannot grow aerobically in a synthetic medium containing hydrolyzed gelatin, different amino acids such as tryptophane, tyrosine, and cystine, and glucose but must be supplied with nicotinic amide. Under anaerobic conditions *S. aureus* must be supplied with uracil. Bacteria present many instances of heterotrophy and, when cultivated in synthetic media, reveal the need for particular substances because with a defective mechanism of synthesis they fail to build some essential wheel of their chemical machinery. These substances are true bacterial vitamins.

In Procaryota biochemical evolution proceeds by the loss of synthesizing capacity; in other words, in the direction of heterotrophy. A similar

situation is found in the evolution of Eucaryota, as has been shown so well by Lwoff,* in the evolutionary series Chlorophyta-Leucophyta-Protozoa. The most primitive Chlorophyta can utilize nitrates as a source of nitrogen, whereas the Leucophyta need either ammonium salts or amino acids but cannot utilize nitrates. The Protozoa require complex polypeptides and can no longer use amino acids or mixtures of these substances. Animals present an extreme degree of heterotrophy which manifests itself strikingly in their need for vitamins. These substances are frequently the intact moiety which the organism uses for building up its functional substances. This is clearly seen in the relationship between coenzymes and different vitamins like thiamine, lactoflavine, niacin and pyridoxine. The first, in combination with phosphoric acid, yields cocarboxylase which forms carboxylase in combination with a specific protein; the second is the coenzyme for yellow enzyme; the third is a constituent of cozymase, and the last is the coenzyme of decarboxylase.

Fresh-water elasmobranchs also furnish an example of the reversibility of biochemical evolution. As was mentioned before (p. 85), the characteristic uremia decreases in this subclass of elasmobranchs which migrated from the sea back into fresh water.

Parasitism likewise presents many examples of such reversibility. If one compares parasitic flat worms with free-living representatives of the same class one finds that the latter possess a lipase which is as active as that found in other invertebrates but parasitic trematodes and cestodes have a very feeble lipase. Furthermore, the parasitic cestodes, unlike the free-living forms, no longer possess proteolytic enzymes (Pennoit-DeCooman and van Grembergen, 198).

E. Irreversibility of Lost Biochemical Characters

Although evolution is reversible in the sense that what had been acquired may be lost again, paleontologists have adduced many arguments to show that an organ lost no longer reappears in evolution. In this sense evolution is irreversible, and the phenomenon is known as the law of Dollo.

Does the law of Dollo apply also to biochemical phenomena? We have shown, in the discussion of the problem of osmoregulation, that in fresh-water teleosts a mechanism appeared for the elimination of water entering the organism by osmosis; namely, the acquisition of the renal glomerulus. This mechanism for elimination of water disappeared in the

* Chatton (cited by Lwoff, 171) classifies the Protista into two main groups: Procaryota, which lack a nucleus or mitrochondria; and Eucaryota, which have these structures. The Eucaryota in turn are subdivided into (1) Chlorophyta, possessing plastids and chlorophyll, (2) Leucophyta possessing only plastids, and (3) Protozoa possessing neither of these structures.

marine teleosts (p. 83), but when the marine teleosts return to fresh water the glomerulus does not reappear. The fresh-water pipefish *Microphis boaja*, related to the typically marine Syngnathidae, is aglomerular like its marine cousins, and the glomerulus once gone cannot be regained. As Baldwin (8) pointed out, if we should assume that the ensemble of complex biochemical structures which make up a living organism is determined by the nature and properties of submicroscopic and molecular elements it is necessary to consider the successive levels of the "spatial hierarchy." We may represent symbolically by letters A, B, C . . . the progressively complicated levels of a spatial hierarchy evolved for a particular species existing in a particular environment. If the conditions of the environment are modified in such a manner that the structures represented by M and N, for instance, become useless, it is very improbable that a new system or a new organ could be produced since only a modification at the lower level of the "spatial hierarchy" can determine morphological change. Under such conditions the system will atrophy or even disappear. If these systems happen to function during embryonic development as transitory organizers, they may be seen to appear and disappear again. Adaptation to a new environment will result in a modification of the arrangement of biochemical constituents to produce a new form of spatial hierarchy and to develop a new system or a new organ but not in the reappearance of an old one.

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Zoological Classification

Protozoa	Unicellular Organisms
Porifera	Sponges
Coelenterata	Jellyfishes, sea anemones
Platyhelminthes	Flatworms
Turbellaria	Free living
Trematoda	Parasitic flukes
Cestoidea	Parasitic flatworms
Annelida	Segmented worms
Polychaeta	Marine worms
Oligochaeta	Fresh-water or terrestrial worms
Hirudinea	Leeches
Echiuridea	Marine cylindrical worms
Sipunculoidea	Unsegmented marine worms
Mollusca	
Gastropoda	Snails, slugs
Lamellibranchiata	Clams, mussels
Cephalopoda	Squids, octopuses (free swimming, marine)
Arthropoda	Segmented, chitinous exoskeleton
Crustacea	Aquatic, gill-breathing
Copepoda	
Cirripedia	
Decapoda	Shrimps, crabs, lobsters
Insecta	Terrestrial, never marine
Orthoptera	Grasshoppers
Hemiptera	Bugs
Lepidoptera	Butterflies, moths
Diptera	Flies
Coleoptera	Beetles
Hymenoptera	Ants, bees
Arachnida	Spiders, scorpions
Echinodermata	
Asteroidea	Starfishes
Ophiuroidea	Brittle stars
Holothuroidea	Sea cucumbers
Echinoidea	Sea urchins

CHORDATA	Dorsal cartilagenous rod (notochord), dorsal tubular nervous system, pharyngeal slits
Protochordata	
Enteropneusta	Marine wormlike animals
Tunicata	Sea squirts
Cephalochordata	Lancelets (*Amphioxus*)
Vertebrata	
Cyclostomata	Lampreys, hagfishes
Pisces	
Elasmobranchii	Cartilagenous fishes, sharks, etc.
Teleostei	Bony fishes
Amphibia	Larvae aquatic, adults aquatic or terrestrial
Anura	Frogs, toads
Urodela	Newts, salamanders
Reptilia	Terrestrial, lung breathing
Chelonia	Turtles, tortoises
Crocodilini	Alligators, crocodiles
Squamata	Lizards, snakes
Aves	Terrestrial, with wings and feathers
Mammalia	Terrestrial, air-breathing, with hair covering and mammary secretion

Author Index

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INVERTEBRATA