normal conditions, the products of change pass into circulation and are gradually eliminated, either because they are assimilated elsewhere or because they are excreted. There are undoubtedly other effects to be taken into account but we reserve the discussion of these until we are able to deal with them on an experimental basis.

The Inorganic Composition of the Blood in Vertebrates and Invertebrates, and its Origin.

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I.—*Introduction.*

The first suggestion bearing on the origin of the inorganic composition of the blood of animals was that made by Bunge,* who, pointing out that we have inherited the notochord and the branchial clefts from marine ancestors, asked why the high percentage of sodium chloride in our tissues should not be an heirloom from life in the sea of that remote past. Eight years later R. Quinton† enunciated the view that in the great majority of animal organisms the internal medium, the circulatory fluid, or hæmolymph, is from its inorganic composition but sea water. In support of this he advanced a number of facts bearing on the composition of the internal medium of animals as compared with the composition of sea water, but the parallelism was only in a few instances extended beyond the amounts of sodium chloride in the two media. This view he unfortunately overlaid with a number of speculations, some of which prejudiced its acceptance amongst physiologists and biologists, and in consequence it did not attain the currency to which it was entitled.

In 1903, in discussing the inorganic composition of certain Medusæ the

physiologists without suffering material change ; if however, the solution be saturated with chloroform or ether, the tissue breaks down very rapidly, the proteins passing into solution. The only agent comparable in disintegrating effect with chloroform and ether is ammonia, according to Vernon.

* 'Lehrbuch der Physiologischen und Pathologischen Chemie,' Leipzig, 1889, pp. 120 and 121.

† 'Comptes Rendus Soc. de Biol.,' 1896, 1897, 1898, 1899. 'Comptes Rendus Acad. Sci.,' vol. 131, pp. 905 and 952. Also "L'Eau de Mer Milieu Organique," Paris, 1904.

author,* unaware of the speculations of Bunge and Quinton, advanced the view that the blood plasma of Vertebrates and Invertebrates with a closed circulatory system is, in its inorganic salts, but a reproduction of the sea water of the remote geological period in which the prototypic representatives of such animal forms first made their appearance. It was pointed out that in many Invertebrates with a vascular system still freely communicating with the exterior, the circulatory fluid is sea water, and this was probably the case also with ancient oceanic forms. The tissues in these latter had through a long period of time become so accommodated to the composition of the sea water of the period that when the circulatory system acquired the closed condition, the composition of the sea water of that period was, with slight modifications, reproduced in the vascular fluid, and thus transmitted to the descendant forms living in different habitats.

As corroborative of this view it was shown that even between the inorganic composition of the blood serum of mammals and that of the ocean of to-day there is a striking resemblance. This is not in concentration, for the salinity of the ocean is about three times that of mammalian blood serum, but in the relative proportions of the sodium, potassium, and calcium, as indicated thus:—

	Na.	K.	Ca.	Mg.
Blood serum	100	6·69	2·58	0·8
Ocean	100	3·66	3·84	11·99

The resemblances are very close except in the magnesium, but this exception and the minor differences were explained as due to the alteration in composition which the ocean has undergone since the Protovertebrate form arose, for not only has there been an increase in the saline concentration of ocean water, but there has obtained a change in the proportions of the basic constituents. This has been brought about by the continual elimination of the potassium and calcium, and the retention of the sodium and magnesium derived from the river discharge from the land areas of the globe.

In a subsequent communication† these observations were amplified, and evidence was advanced to show that the history of the composition of the

* "On the Inorganic Composition of the Medusæ, *Aurelia flavidula* and *Cyanea Arctica*," 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

† "The Palæochemistry of the Ocean in Relation to Animal and Vegetable Protoplasm," 'Canadian Institute, Transactions,' 1904, vol. 7, p. 535.

ocean fully accounted for the difference between the composition of the sea water of to-day and the inorganic composition of mammalian blood plasma.

A difficulty which lay in the way of definitely establishing the oceanic origin of the inorganic composition of the blood plasma was the fact that no analyses of the inorganic salts of the plasma of any Vertebrate below mammals had then been made. Even of the plasma of mammals only few analyses had been on record, and of these only the more recent were from all points of view wholly acceptable. Amongst these came the analyses by Bunge* of the sera of the ox, pig, horse, and dog, by Abderhalden† of the sera of the ox, sheep, goat, horse, pig, rabbit, dog, and cat. Of the analyses of the serum of man the more important recorded are those of C. Schmidt‡ and Bunge.§

Though there was in all these analyses a marked similarity of proportions in the potassium, calcium and magnesium in relation to the sodium, it was open to doubt whether these proportions would be found to obtain in the blood plasma of birds, reptiles, amphibia, and fishes.

There have been made a number of analyses of the blood in Invertebrates. Those of Griffiths‖ bear on the blood in a number of Crustacea, including the lobster, and in a number of Mollusca, including the Cephalopods, *Sepia officinalis* and *Octopus vulgaris*. In his results there is a remarkable similarity in the composition of the ash in all these forms, and the only noteworthy difference is the comparatively low proportion of lime (CaO) in Cephalopod blood. How far these analyses are representative one cannot say, for his results have not hitherto been checked, but the composition of the blood of the lobster, *Homarus vulgaris*, as given by Griffiths, does not correspond with what I have found in *Homarus americanus*, and it is, therefore, necessary to hold in suspense any opinion as to their general acceptability.

That one must adopt a critical attitude towards the results of analyses of this kind is emphasised by a consideration of those which have been furnished by a number of observations in this respect made on the blood of a single species. Genth,¶ Gotch and Laws,** and McGuigan†† have analysed the

* "Zur quantitativen Analyse des Blutes," 'Zeit. für Biologie,' 1886, vol. 12, p. 191.

† "Zur quantitativen Analyse des Blutes," 'Zeit. für Physiol. Chemie,' 1897, vol. 23, p. 521. Also "Zur quantitativen vergleichenden Analyse des Blutes," 'Zeit. für Physiol. Chemie,' 1898, vol. 25, p. 65.

‡ "Charakteristik der Epidemische Cholera," Leipzig und Mitau, 1850.

§ *Op. cit.*, pp. 221 and 222.

‖ 'Roy. Soc. Edinburgh Proc.,' 1890—91, vol. 18, p. 288.

¶ 'Annalen der Chemie,' 1852, vol. 81, p. 68.

** 'British Association,' 1884, p. 774.

†† 'Science,' 1907, vol. 25, p. 68.

inorganic composition of the blood of the horseshoe crab, *Limulus polyphemus*, and their results are in agreement only in the most general way. While the discrepancies are in some cases at least extraordinarily great, Genth's results even as regards the composition of the blood of forms from two different points on Chesapeake Bay are quite unlike, and the question is raised whether the variations are due to imperfect methods of analysis or to actual differences in composition. That his methods did not give exact results may be gathered from the fact that the percentages of potassium in the samples of blood from the two different sources were widely different, the element appearing to be twice as abundant relatively in one case as in the other. This is to a certain extent intelligible, for the methods of estimating potassium employed 60 years ago were much less exact than they are now, but even a defective method ought under like conditions to give uniform results.

The differences also between the results of McGuigan, on the one hand, and those of Gotch and Laws on the other, would, if accepted, make it impossible to regard the blood in *Limulus* as uniform either relatively or absolutely in its inorganic composition. That would entail the further conclusion that in *Limulus*, after a life in the sea almost co-extensive with geological history, there is a tendency to wide variation in the inorganic composition of the blood, irrespective of the changes in the concentration of the salinity of the sea water of its habitat. If that conclusion were correct it would indeed be difficult to understand how fixed relations in the inorganic composition of body fluids ever could arise, and the primal causation of such fixed relations in, for instance, mammals would be an enigma.

So far, therefore, the results of analyses hitherto made on the blood of Invertebrates leave the question of the origin of the fixed proportions of the inorganic elements in the blood of the higher Vertebrates still in doubt.

In order, therefore, to dispose of the question of the origin of these fixed proportions it was necessary to analyse the blood of representatives of the lower classes of the Vertebrates and of Invertebrate types with a closed circulation. This involved the collecting of quantities of blood plasma from a number of forms, many of which are not accessible ordinarily and, in consequence, the determination of the problem has been delayed far beyond the limit originally set in the writer's plans.

Within the last two years he succeeded in obtaining a quantity of material which has enabled him to undertake a partial investigation of the question. Through the kindness of the Director of the Woods Holl Biological Station he received a large quantity of the blood of the horseshoe crab, *Limulus polyphemus*. During his visit to the Canadian Marine Biological Station at

St. Andrews, New Brunswick, in August of last year, he obtained a quantity of the blood serum of the cod, *Gadus callarias*, also about 200 c.c. of blood serum from the pollock, *Pollachius virens*, and about 500 c.c. of blood plasma from three large specimens of lobster, *Homarus americanus*. Later, through the kindness of the Acting Director of the Station, Prof. Penhallow, considerable quantities of the blood and blood serum of the dogfish, *Acanthias vulgaris*, were collected and placed at the author's disposal.

These have been analysed to determine their inorganic composition, and the results, while limited, and therefore inadequate to determine finally the solution of the question, are contributory to that end and are of sufficient interest to justify their publication.

II.—*Methods of Analysis.*

The methods of analysis followed were on the whole those employed by Bunge in the analysis of the inorganic composition of milk, and subsequently adopted by Abderhalden in his investigations on the composition of the blood in a number of mammals. Modifications were introduced into these methods as the description of them indicates.

For the determination of the potassium and the sodium a weighed quantity, varying in volume from 30 to 80 c.c., of the blood or the serum was evaporated to dryness in a large platinum dish and then, after being heated for five hours at 115° C. to determine the residue, the latter was carefully carbonised at a low red heat till all the volatile organic matter was destroyed. The carbonised residue was then extracted several times with hot water, and the residue, after being dried and incinerated, was dissolved in dilute hydrochloric acid. This latter solution was added to the volume of the extraction fluids, and to the resulting mixture saturated baryta water was added to precipitate the sulphuric and phosphoric acids; the mixture was filtered, the filtrate reduced in volume by evaporation on a water bath, and then treated with crystals of ammonium carbonate to precipitate the calcium, magnesium, and the excess of the barium from the solution. The filtrate from this was evaporated to dryness in a large platinum dish, the residue fused with anhydrous oxalic acid, then dissolved in water and the solution filtered. The filtrate, containing the carbonates of sodium and potassium, was now evaporated to dryness in a small platinum capsule, the residue heated to dull redness and then dissolved in 5–8 c.c. of water. If the solution was not clear it was filtered through a filter paper of smallest possible superficial area, the solution again evaporated to dryness

in the capsule and the residue heated to dull redness. If, now, on dissolving this residue in 5–8 c.c. of water, the solution was not clear, it was filtered, the filtrate evaporated, and the residue again heated to dull redness, then dissolved in not more than 5 c.c. of water and the solution filtered. This solution, which contained only the carbonates of sodium and potassium, was treated with hydrochloric acid to convert them into the chlorides; the solution was now evaporated to dryness in a weighed platinum capsule, and the weight of the chlorides of sodium and potassium determined.

To determine the potassium the chlorides were dissolved in water; to the solution 3–5 c.c. of a 10-per-cent. solution of platinum chloride were added, and after the addition also of 3 c.c. of concentrated hydrochloric acid, the mixture reduced almost to dryness in a porcelain evaporating dish on a water bath. The residue was covered with 40 c.c. of absolute alcohol, the mixture stirred for a few moments, and then allowed to stand under a bell jar for an hour, at the end of which time 20 c.c. of ether were added, and a further extraction of an hour was allowed. After decanting the alcohol-ether mixture, a fresh mixture, consisting of 40 c.c. of alcohol and 20 c.c. of ether, was added and allowed to extract for an hour. On decantation of this a third supply was added, and the extraction continued for another hour. The supernatant fluid, which was quite colourless, was also removed by decantation; the residue was then dried, and the platinum salt in it was reduced to metallic platinum by heating it to 250° C. in a current of dry hydrogen. On cooling, the salt was dissolved in water, then evaporated to dryness, and once more subjected to reduction in dry hydrogen gas. This latter procedure was adopted in order to ensure complete reduction of all the platinum salt present.

The weight of the platinum thus found, multiplied by the factor 0·40195, gave the amount of potassium. This factor corresponds approximately to the theoretical value of K in the formula K_2PtCl_6.

The determination of the iron, copper (in Crustacean blood), calcium, magnesium, and phosphoric acid was, except in cases to be mentioned subsequently, made in the following manner:—To the weighed quantity of the blood or serum held in a platinum dish about 2 grammes of pure sodic carbonate were added; the mixture, after being carefully stirred, was evaporated to dryness, the residue carbonised, then extracted several times with hot water, acidulated with hydrochloric acid, the remainder of the residue completely incinerated, the ash extracted with hot dilute hydrochloric acid, the fluid filtered, and the filtrate added to the volume of the united filtrates previously obtained. What remained undissolved was ferric oxide (Fe_2O_3), which was weighed. Into the acid solution, when it contained

copper, as was the case when the preparation was derived from Crustacean blood serum, sulphuretted hydrogen gas was passed, the sulphide of copper formed was separated by filtration, and the copper determined as Cu_2S or CuO. The filtrate from this, after being boiled for some minutes to drive off the dissolved sulphuretted hydrogen, was nearly neutralised, acetic acid and crystals of ammonium oxalate were added, and, after standing for some hours, the calcium oxalate precipitate was removed and weighed either as CaO or $CaSO_4$. From the filtrate the magnesium was precipitated as magnesium phosphate by the addition of ammonium phosphate and ammonia.

In the case of the solutions made as described from the ash of Vertebrate blood or serum, ammonium acetate was added, and the mixture allowed to stand for 24 hours in order to precipitate some of the iron and all the phosphoric acid as ferric phosphate, which was removed by filtration. If there still remained iron in solution (as in the case of Vertebrate blood), the fluid was rendered alkaline with ammonia, the resulting precipitate of oxide of iron, lime, and magnesia was removed by filtration and, while still moist on the filter, extracted several times with a hot solution of ammonium chloride, which dissolved out the lime and magnesia and left the ferric oxide. The extract containing the dissolved lime and magnesia was added to the filtrate previously obtained, the whole reduced in volume by evaporation, the calcium precipitated as oxalate and from the filtrate the magnesium as phosphate in the usual way.

Recognising that the precipitate of calcium oxalate obtained in each case might, in spite of the method of separation used, contain also some magnesium oxalate, it was incinerated, the ash dissolved in acetic acid, and ammonium oxalate added to the solution to precipitate the calcium, which was weighed as lime or as sulphate. The filtrate from the precipitate was then appropriately treated, to separate and estimate the traces of magnesium present.

For the determination of the chlorine, 1–2 grammes of sodium carbonate were added to a weighed quantity of the serum or blood contained in a platinum dish, the mixture, after being carefully stirred, was evaporated to dryness, and the residue thoroughly carbonised at a dull red heat. The mass was extracted with hot water several times, and then completely incinerated at a low heat. The ash was dissolved in cold dilute nitric acid, the solution filtered, and the filtrate added to the volume of the extracts previously obtained. The chlorine in this was determined as chloride of silver in the usual way.

The sulphuric acid was determined only in the blood of the lobster and of the horseshoe crab (*Limulus*). A weighed quantity of the blood was

slightly acidified with acetic acid and heated, in order to coagulate the proteids, which were then removed by filtration. To the filtrate, which was clear, or had only a faint opalescence, some hydrochloric acid and a solution of barium chloride were added, and, after the fluid was heated almost to boiling, it was allowed to stand for 24 hours, when the precipitate of barium sulphate was removed by filtration and its weight determined.

The determination of that part of the depression of the freezing point (Δ) of a specimen of plasma or serum, due to the salts in it, was carried out in the following way: The weighed quantity of the blood (lobster and horseshoe crab) or serum (cod, pollock, and dog-fish) was evaporated to dryness, first on the water-bath, then in an oven at 115° C. for six hours. The residue was now carbonised at a low heat, and the carbonised residue then extracted several times with hot water containing hydrochloric acid, the remaining material completely incinerated at dull red heat, the ash dissolved in dilute hydrochloric acid, and the solution added to the volume of the extracts previously obtained. This fluid was now evaporated on a water-bath to complete dryness, and the residue then heated carefully, in order to convert any ferric chloride present into ferric hydrate. A few drops of dilute hydrochloric acid were added to dissolve all the magnesium salts present, then the preparation was carefully evaporated and heated to expel all traces of free acid. It was now dissolved in sufficient water to make the total weight that of the plasma or serum taken, and the Δ of this solution was then determined. In the majority of cases, two such solutions were made from two weighed portions of the same plasma or serum.

The values of the Δ so obtained cannot be regarded as free from objection. The solutes in such fluids are not under the same conditions as in the plasma or serum. In the latter, their dissociation is diminished by the colloids present. This, however, is compensated for in such solutions to a certain extent, since, owing to the absence of colloids, there is a slightly greater degree of dilution of solutes, and though there must, therefore, be a very slight increase in dissociation, the depression of the freezing point must be lessened. On the whole, consequently, while the values of the Δ ascertained may not be absolutely accurate, they still are data which can be compared with the values for the blood and serum.

Such solutions were further employed when the quantity of the plasma or serum was limited, as was the case with those fluids from the dog-fish and the cod. In this case the material had to be so used as to permit of the determination of as many constituents as possible. The iron in such solutions was precipitated by the addition of strong acetic acid and some ammonium phosphate, and from the filtrate ammonium oxalate precipitated

the lime as oxalate. The magnesium in the filtrate from this was precipitated on the addition of more ammonium phosphate and of strong ammonia, and the filtered fluid evaporated to dryness and heated to expel the acetic acid and ammonium acetate. To the residue, dissolved in a small quantity of dilute hydrochloric acid, saturated baryta water was added to remove the sulphuric and phosphoric acids, the excess of the baryta was precipitated with ammonium carbonate, and the filtrate from this was treated in the way described above, to determine the sodium and potassium.

III.—*The Results.*

A. *The Blood Serum of Limulus.*—The blood of *Limulus* was coagulated, and contained a large quantity of fibrin, which was removed by straining through fine muslin cloth. The serum was deep azure-blue in colour, and had a specific gravity of 1·03847. Its Δ was −2·04° C. The Δ of the salts in the blood was −1·875° C. The total salts amounted to 2·982 per cent. The percentages of solids in four different estimations were 7·960, 7·822, 7·942, and 8·056. The different results in the four estimations were due to differences in the time during which the residue was dried, first in a steam-heated oven, and then at 110° C. The average per cent. of these determinations was 7·945. The analyses of the ash gave the following:—

	I.	II.	III.	Mean.
Na	0·8944	0·8864	0·8848	0·8885
K	0·04951	0·0500	0·05017	0·04989
Ca	0·03614	0·03621	0·03604	0·03613
Mg	0·0995	0·0996	—	0·09955
Cl	1·6526	1·669	—	1·6608
SO_3	0·11718	0·11976	—	0·11847
Cu	0·00742	0·00773	0·00806	0·00773
				2·86107

B. *The Blood Serum of the Lobster.*—The blood of the lobster was rich in fibrin, and was of a light blue colour. The specific gravity of the serum was 1·0337. The solids in two estimations were 8·354 and 8·351 per cent., and the mean 8·3525 per cent. The total salts in two estimations were 2·855 and 2·849 per cent., the mean 2·852 per cent. The Δ due to the salts ascertained from these two determinations were −1·73° and −1·735°. The Δ of the serum in two different determinations was −1·78° C. The sea water from the bottom of the bay (at St. Andrews, New Brunswick), where the lobsters were caught, gave a Δ of −1·76°, that of the surface at the same point −1·635°. The analyses of the ash gave:

	I.	II.	III.	Mean.
Na	0·90294	0·90052	0·9065	0·90335
K	0·03491	0·032806	0·03361	0·03377
Ca	0·04394	0·04379	—	0·04387
Mg	0·01636	0·01468	0·01579	0·01561
Cl	1·5501	1·5439	—	1·547
SO_3	0·06107	0·0597	—	0·06038

C. *The Serum of the Dogfish.*—Two determinations of the Δ of the serum gave −2·035°, and two of that of the salts of the serum gave −1·075° and −1·0725°, the mean of the two being −1·0737°. The total salts of the serum in these two cases amounted to 1·775 per cent. and 1·7729 per cent., and the mean was 1·7739 per cent. The total solids, as ascertained in one determination, were 5·956 per cent. The analyses gave:

	I.	II.	Mean.
Na	0·59216	0·59158	0·59187
K	0·02742	0·02717	0·02729
Ca	0·016105	0·016011	0·01606
Mg	0·01402	0·01519	0·0146
Cl	0·9802	0·9837	0·9819

The blood (corpuscles and plasma) of the dogfish gave on analysis the following:—

	I.	II.	Mean.
Na	0·4959	0·4931	0·4945
K	0·0844	0·08255	0·08348
Cl	0·9391	—	—

D. *The Serum of the Cod.*—The solids of the serum of the cod gave in three different determinations 7·165, 7·1006, and 7·170 per cent., the mean of which is 7·1452 per cent. In one determination the total salts were 1·2823 per cent., and this gave a Δ of −0·71° C., while that of the blood of two very large cod, ascertained within two and a-half hours after their capture, was −0·765° C. The chlorides of sodium and potassium present amounted in three determinations to 1·1326, 1·1349, and 1·1293 per cent., the mean of which was 1·1322 per cent. The results of the analyses of the ash were:

	I.	II.	III.	Mean.
Na	0·4174	0·4147	—	0·41605
K	0·03912	0·03998	—	0·03955
Ca	0·0163490	0·01638	0·01617	0·016299
Mg	0·006375	0·00542	—	0·005897
Cl	0·6224	0·6252	0·6189	0·62217

E. *The Serum of the Pollock.*—The solids in the serum of the pollock amounted in one determination to 7·095 per cent. The total salts in another estimation were 1·2934 per cent., and the Δ for the salts of this determination was −0·737° C., while the Δ for the serum was −0·825° C. The analyses of the ash gave :

	I.	II.	III.	IV.
Na	0·4133	0·4156	—	0·41447
K	0·01873	0·01721	—	0·01797
Ca	0·01198	0·01355	0·01306	0·01286
Mg	0·005686	0·00618	0·0064	0·00608
Cl	0·5626	0·55997	—	0·56128

IV. *Ratios of Values on the Basis of Na* = 100.

A. In Marine Invertebrates and in Sea Water.

	Na.	K.	Ca.	Mg.	SO_3.	Cl.
Ocean water* (Dittmar)	100	3·613	3·911	12·106	20·9	180·9
Limulus polyphemus†	100	5·62	4·06	11·20	13·33	186·9
Aurelia flavidula‡ (Macallum)	100	5·18	4·13	11·43	13·18	185·5
Homarus americanus	100	3·73	4·85	1·72	6·67	171·2

* Calculated from Dittmar's analyses, '*Challenger* Report, Physics and Chemistry,' vol. 1.

† The earlier analyses of the blood of *Limulus* gave ratios (Na = 100) which are not in accord :—

	Na.	K.	Ca.	Mg.	SO_3.	Cl.
Gotch and Laws	100	5·30	3·83	11·67	7·65	157·8
Genth (*a*)	100	12·4	5·49	7·97	8·87	170·3
Genth (*b*)	100	6·11	4·86	11·06	8·56	161·5
McGuigan	100	4·72	5·51	10·29	4·83	168·5

Of these, as may be seen by comparison, Gotch and Law's results approach more closely those given by my analyses.

‡ 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

B. In Elasmobranchs, Teleosts, Mammals.

	Na.	K.	Ca.	Mg.	Cl.
Dogfish, *Acanthias vulgaris*	100	4·61	2·71	2·46	165·7
Cod, *Gadus callarias*	100	9·506	3·93	1·41	149·7
Pollock, *Pollachius virens*	100	4·33	3·10	1·46	137·8
Dog*	100	6·86	2·52	0·81	128·5
Mammal (average)*	100	6·69	2·58	0·80	118·3
Man†	100	9·22	3·37	1·76	101·4

* Calculated from Abderhalden's results, 'Zeit. für Physiol. Chem.,' 1898, vol. 25, p. 106.

† Calculated from the results of Bunge's analyses, 'Lehrbuch der Physiologischen und Pathologischen Chemie,' 1889, p. 221. The values for the K, Ca, and Mg appear high as compared with those obtained by me in analyses of the blood serum of man (unpublished).

V.—*General Observations.*

It will be noted on inspection of the results of the analyses that considerable differences exist between the blood of the Invertebrates, *Limulus* and *Homarus*, on the one hand, and the marine Elasmobranch and Teleost forms on the other.

In *Limulus* the amount of the total salts of the blood, 2·982 per cent., approaches that of the sea water, not indeed of the highest concentration, but of that which may be found at points along the Atlantic coast. At St. Andrews, New Brunswick, the total salts of the sea water collected in April were 2·417 per cent., but in sea water obtained in August 3·165 per cent.‡

In the blood of the lobster the total salts as ascertained were 2·852 per cent., which is between the two concentrations given above for the salinity of the sea water at St. Andrews, where the lobsters from which the blood was taken were obtained.

It is, however, when the ratios of the inorganic constituents based on the value Na = 100, as given above, are examined that one sees the close parallelism between the blood of *Limulus* and sea water. Only in the K and SO_3 are there important differences. The parallelism in ratios is all but complete between the blood of *Limulus* and the fluid in the disc of the Medusa, *Aurelia flavidula*. The Medusæ have probably always been marine forms, and *Limulus* and its ancestral prototypes have been oceanic as far back in geological history as the Cambrian. The parallelism in ratios not only between these forms but also between them and sea water, though striking, is not surprising. The blood of *Limulus* is but slightly modified sea water.

‡ Macallum, "On the Inorganic Composition of the Medusæ, *Aurelia flavidula* and *Cyanea Arctica*," 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

In the blood of the lobster the ratios, though they are on the whole parallel to those of sea water, differ from the latter, particularly in regard to the SO_3 and the magnesium. These are toxic constituents in sea water, and though in *Limulus*, because of its association since remote geological time with the ocean, a considerable degree of tolerance for them has been established, only a very limited adjustment to them has been developed in the lobster, whose marine history can be traced as far back as the Jurassic period only, that is to a far less remote period than in the case of *Limulus*. The ancestors of the *Homaridæ* are supposed to have been fresh-water forms of an astacoid character and a species of *Astacus*, *A. vectens*, is found in the so-called Lobster Beds in the Greensands of the Isle of Wight, belonging to the Cretaceous period. In the earlier period, the Jurassic, the macrouran decapods arose and their remains are found in deposits formed in more or less still waters, such as the Solenhofen slates, in which they are mingled with terrestrial and fresh-water forms. This suggests that the Mesozoic ancestor of the lobster of to-day must either have been of a fresh-water type or one that resorted to embayments, lagoons, or stretches of water more or less surrounded by land, and therefore of a fresh or slightly brackish character.

It is to be noted, further, that in the blood of the lobster the percentages of sodium and chlorine are 0·9033 and 1·547, while in *Limulus* they are 0·8885 and 1·6608. As the sea water at St. Andrews, New Brunswick, in April and August yielded on analysis 0·7423 and 0·9882 per cent. of sodium and 1·347 and 1·7473 per cent. of chlorine the sodium and chlorine concentrations in the blood of the lobster probably are approximately the mean values for the sodium and chlorine of its habitat. From this it would appear as if the sodium chloride of sea water passes freely into the blood till the sodium chloride concentration in both is approximately balanced.

In the serum of the dogfish, cod, and pollock the total salts are much less than in sea water. In the dogfish they are 1·7739 per cent., while in the cod and pollock they are 1·2823 and 1·2934 per cent. respectively. This marked difference is undoubtedly due to the difference in the length of time in which the Elasmobranchs and Teleosts have been associated with the ocean. The former have always been marine* since their origin in the Silurian period, while the Teleosts date only from the Jurassic and were probably derived from an *Amia*-like Ganoid. The Ganoids were abundant in Palæozoic and Mesozoic Seas, but these were probably also fresh-water forms, although through the scantiness of fresh-water deposits no evidence of such is known. The present day Ganoids, including *Amia*, are all fresh-

* A shark, *Carcharias nicaraguensis*, is native of the fresh-water Lake Nicaragua.

water forms, and it is not improbable that the Ganoid ancestor of the Teleosts was a fresh-water form or one which, like the sturgeon, occasionally occurred in brackish water.

The ratios of the sodium, potassium, calcium, and magnesium in the serum of these marine fishes are not on the whole very different and they approach those in the mammal. In the cod the potassium ratio (9·506) is high, and this may be due to a slight laking of the red corpuscles, which are rich in salts of that element. The ratio value for potassium in the pollock (4·33) is less than half of that in the cod, and it closely approximates that in the dogfish (4·61). The ratios in the dogfish and pollock are almost the same, and the difference is most marked only in the magnesium, which is more abundant in the former.

This excess in magnesium is apparently one of the results of the action of the sea water on the blood of the dogfish for all the time which has elapsed since the Vertebrate type arose, for the Elasmobranchs have, as already stated, been marine since their origin, which is, at the latest, Devonian and, if the Ostracoderms are Protoelasmobranch, probably early Silurian. The ratio for magnesium in the dogfish is, however, only a little more than one-fifth of that found in *Limulus,* which has been also since its origin in the Cambrian a marine form.

That the Teleosts have been oceanic for a much shorter time than the Elasmobranchs is shown in the osmotic pressure of their blood as measured by the Δ. In Elasmobranchs this varies somewhat with the species and the habitat, but from the determinations of Bottazzi,* Rodier† and others it has been found to lie between the values −2·03° C. and −2·44° C., while the sea water of the habitat of the animals, as a rule, gave a slightly lower Δ than the serum. In the dogfish the Δ was −2·035° C., of which the salts contributed −1·0737°. In the Teleosts it is always much less, rarely does it exceed −1°‡ and it ordinarily ranges between −0·466° in *Tinca vulgaris* and −0·838° in *Gadus virens.*§ In *Gadus morrhua,* according to Dekhuyzen, the Δ varied according to the locality from which the fish was taken and the minimum and maximum values were −0·644° and −0·811°. In the blood of *Gadus callarias,* as shown above, the salts gave a Δ of −0·71° C., while that

* 'Arch. ital. de Biol.,' 1897, vol. 28, p. 61.

† 'Travaux des Lab. de la Soc. Scientif. et Station Zool. d'Arcachon,' 1899, p. 103. Reference in Hamburger, 'Osmotischer Druck und Ionenlehre,' vol. 1, p. 465.

‡ Bottazzi found the Δ in two specimens of *Charax puntazzo* to be −1·04° and −1·035°, and in a specimen of *Cerna gigas* it was −1·035°. Dekhuyzen questions these results as they were obtained with a cooling bath of −12° C.

§ M. C. Dekhuyzen, 'Kon. Akad. van Wetensch.,' Amsterdam, vol. 8, p. 537, 1905.

of the sea water from which the examples of the cod were taken was −1·80°.* The blood in Elasmobranchs thus has an osmotic pressure approximately like that of sea water, while in Teleosts it is much less, in fact, only in excess of one-third. Dekhuyzen's observations would show that there is a tendency in the blood of Teleosts to increase the osmotic pressure with the increase in the saline concentration of the sea water, but it does not in *Gadus morrhua* pass beyond the limit of −0·811° C. The sea water thus influences only to a limited extent the osmotic pressure in Teleosts, while it has affected the blood in Elasmobranchs to the extent that the Δ is the same as or greater than it is in sea water. This balancing of the sea water and the blood plasma postulates an association with the sea for Elasmobranchs, which in duration exceeds enormously the time which has elapsed since the Teleosts arose. Strutt,† basing his observations on the amount of helium enclosed in a sample of hæmatite from the Eocene and on the amount of this gas liberated in a measured time from a certain quantity of uranium, suggests that about 30,000,000 years have passed since the Eocene. What then must have been the length of the interval between the Silurian and the present in order to account for the development of the high osmotic pressure in Elasmobranchs?

The difference between the Δ of the serum (−2·035°) and that due to the salts in it (−1·0737°) depends on urea and other organic solutes. Urea is present in large quantities in the blood of the Elasmobranchs. The first to note its presence in extraordinarily large amounts ("colossale Quantitäten"), not only in the blood but also in the muscles, liver, kidney, spleen, pancreas, ovaries, and testes of these animals were Staedeler and Frerichs,‡ who obtained as much as two ounces from the liver of a single shark (*Scyllium canicula*). Later, in 1890, von Schroeder§ found that in *Scyllium catulus* the blood as a whole had 2·6 per cent. urea. Assuming that the blood corpuscles were free from urea, the latter, he calculated, would constitute 3·1 per cent. of the plasma. In the liver and muscles of this animal it amounted to 1·36 and 1·95 per cent. respectively. Rodier‖ also noted that one-third of the osmotic pressure of the blood of sharks is due to urea. As the Δ which he found in the blood of all the forms he examined amounted to −2·05°, as much as

* The Δ of the sea water of the Atlantic along the coast of Nova Scotia and New Brunswick would seem not to exceed −1·90°. The sea water of Canso, the most eastern point of Nova Scotia, gave −1·825° and the maximum value for the sea water at St. Andrews, New Brunswick, was −1·85°.

† 'Roy. Soc. Proc.,' A, 1910, vol. 83, p. 96.

‡ 'Journ. für Pract. Chem.,' 1858, vol. 73, p. 48.

§ 'Zeit. für Physiol. Chem.,' 1890, vol. 14, p. 576.

‖ 'Travaux des Lab. Soc. Scientif. et Station Zool. d'Arcachon,' 1899, p. 103.

$-0{\cdot}68°$ was, therefore, due to urea, which must, consequently, be present to the amount of 2·18 per cent.*

The amount of urea in the blood serum of the dogfish was determined. The material which served for this purpose was that, portions of which had been used for the inorganic analyses detailed above. The serum had been preserved with thymol and was in good condition. Weighed quantities were mixed each with five times its volume of absolute alcohol, and the mixture held in a bottle placed in an agitator which was kept in motion for 24 hours. It was then filtered, the precipitate on the filter washed with absolute alcohol, and the combined filtrate and washings, after the volume was accurately ascertained, used for the determination of the urea. The method employed to this end was that of Folin and consisted in heating a measured quantity of the extract with magnesium chloride and hydrochloric acid for two hours, then adding strong alkali and distilling the liberated ammonia into standard acid solution, which was subsequently titrated with standard alkali. By this method the urea in four determinations as calculated from the ammonia found was: 1·965, 2·107, 2·017, and 2·017 per cent. The mean of these was 2·026 per cent.

This would give in the serum a lowering of the freezing point amounting to 0·63°. The latter with the amount of the depression due to the inorganic salts in the serum would total $-1{\cdot}7037°$. As the Δ of serum is $-2{\cdot}035°$ there still remains $-0{\cdot}332°$ to be accounted for.

This is due to ammonia salts; although only infinitesimal traces of these were present in the alcoholic extract of the serum yet ammonia compounds were found in considerable amount in the serum itself. The explanation for this is that concentrated alcohol does not dissolve readily certain of the salts of ammonia, notably the phosphate,† and consequently, absolute or concentrated alcohol may be used to separate the urea and the ammonia salts in the blood.

The amount of ammonia in the serum of the dogfish was determined with the Folin method and the results of three estimations gave each 0·1727 per cent. of NH_3, or a concentration slightly greater than N/10. This would fully account for the depression $-0{\cdot}332°$.

The high ammonia content, the extraordinary concentration of urea and the high percentage of salts, namely 1·7739, in the serum of the dogfish, all are the results of the action of the osmotic pressure of sea water on the blood of the dogfish, not for one or two geological ages but for all the time which has

* Assuming that a gramme-molecular solution gives a Δ of $-1{\cdot}87°$.

† Erwin Herter ('Mitth. Zool. Stat. zu Neapel,' vol. 10, p. 342) found the urine of *Scyllium catulus* rich in ammonia and P_2O_5 and it was markedly acid.

elapsed since the Cambrian period. The Teleosts, as pointed out above, arose in the Jurassic, and it is probable that the *Gadidæ* have been marine since the Cretaceous, yet in the blood of the cod the saline concentration is 1·2823 per cent. as against 1·7729 in the dog-fish. The difference, 0·49 per cent., would seem to be attributable to the longer life which the Elasmobranchs have undergone in the ocean, and it might be made the basis for determining the relative length of the time which elapsed between the Cambrian and the Cretaceous, could we with certainty know what was the original concentration of the salts in the blood plasma in the Protovertebrates of the Cambrian or Silurian. What it was we can only approximately conjecture.

In the blood of mammals it is in the neighbourhood of 0·9 per cent.* The difference between this and the 1·282 per cent. in the serum of the cod, namely, 0·38 per cent., might be explained as caused by the action of the sea water for all the time since the Cretaceous. On this basis the length of the marine life of the *Gadidæ* would be to the length of the marine life of the Elasmobranchs as 0·38 is to 0·877.

It is, however, not well to base any views on these data. For all the length of time during which the *Gadidæ* have been associated with the ocean, the organic solutes in their blood must be very minute in quantity. The Δ of the serum and of its salts being respectively $-0{\cdot}765°$ and $-0{\cdot}71°$, the difference ($-0{\cdot}055°$) may be due to urea, ammonia salts, but even if due to urea alone the amount must be very small. The ratio between $0{\cdot}055°$ and $0{\cdot}63°$, the Δ due to the urea in the dogfish blood, is very different from the ratio 0·38 : 0·877, and in consequence the latter cannot be regarded as indicating the relative durations of the oceanic history of the Teleosts and Elasmobranchs.

It may be that 0·9 per cent. is too low an estimate of the amount of salts in the blood plasma of the ancestral type of Vertebrate, and that just as in oceanic forms the growing saline concentration of the sea water tends and has tended ever to increase, though slowly, the salts of the blood, so in terrestrial forms the feeble salinity of their food and their environment may possibly have in the long ages decreased the salts of the plasma considerably below the ancestral standard. If the latter were 1·2 per cent., the increases in the salts of the serum in Teleosts and Elasmobranchs would be 0·08 and 0·57, and this would give the relative durations of oceanic life in these classes as 1 and 7.

It is impossible, however, to accept anything on this point as definite, and

* According to Bunge (*loc. cit.*) the concentration of the salts in human blood serum is 0·842 – 0·867 per cent. Calculating from Abderhalden's analyses, the salts of the serum of the dog amount to 0·9354, of the cat 0·9331, and of the sheep 0·9053 per cent.

one may perhaps be in a position to speculate safely on such matters only when careful analyses have been made of the blood plasma of representatives of all the classes of Vertebrates, and specially of the fresh-water fishes.

It is, nevertheless, certain that the inorganic salts have increased very slowly, much more slowly in the blood plasma of Elasmobranchs than they have in the sea water and, further, that the urea and ammonia salts have attained an extraordinary concentration. The explanation for this slow increase of the salts cannot be found in any inactivity of the epithelial cells of the mucosa of the intestinal tract, for Erwin Herter* found in the urine of *Scyllium catulus* 0·0415 per cent. Ca and 0·1416 per cent. Mg, as compared with 0·0464 per cent. Ca and 0·1421 per cent. Mg in the sea water of the locality (Naples) from which the animal was taken. The total amounts of the sodium and potassium were not determined, but the chlorine was estimated and found to be 1·3543 per cent., whereas in sea water at Naples it is 2·1142 per cent. This discrepancy may easily be explained, for the hydrochloric acid in the gastric juice in *Scyllium catulus*, according to Richet,† is from 0·69 to 1·29 per cent., and as the acid of the gastric juice is neutralised in the intestinal tract the chlorine thus contained may pass out with the intestinal excreta, and not by way of the kidneys, while its place in the renal excretion is taken by phosphoric acid, which is exceedingly abundant in the urine of this form. The correspondence between the sea water and the urine as regards the amounts of calcium and magnesium would seem to indicate very clearly that the intestinal tract, and, perhaps, also the gills, in absorbing fluids make little distinction, if any, between the water and the salts of the sea; in other words, sea water finds its way into the blood stream of the circulation in the Elasmobranchs.

It follows from this that it is the kidneys which determine the inorganic composition of the blood plasma in these forms. The kidneys not only regulate the total quantity of salts in the blood plasma, but they also maintain the ratios, almost as they obtain in higher Vertebrates, existing in the plasma between the sodium, potassium, calcium, and magnesium, even after long ages of exposure to the ever increasing saline concentration of the sea. That exposure, it is true, has had its result in increasing the total salts of the blood plasma, but the increase is but sufficient to bring the Δ due to them up to a little more than half of the Δ of the sea water of the habitat, or of the total Δ of the plasma.

It is in this respect that the dogfish differs completely from *Limulus*,

* 'Mitth. Zool. Stat. zu Neapel,' 1892, vol. 10, p. 342.

† 'Journ. de l'Anat. et de la Physiol.,' vol. 14, p. 170; also 'Comptes Rendus,' vol. 86, p. 676.

although both they and their ancestral forms have been marine and contemporary almost throughout all the periods of geological history. In the king crab the renal organs do not influence the concentration of the salts of the blood, which amount to 2·982 per cent., and they appear to influence only extremely slightly the amount of the magnesium, and more considerably the sulphuric acid (SO_3). Even in the lobster, in which the saline concentration of the blood is 2·852 per cent., the renal organs are very active only in the elimination of these two elements.

It is thus a far cry from the renal organ of *Limulus* and the lobster to the kidney of the Elasmobranch and still more so to the kidney of Teleosts and higher Vertebrates. The salts of the plasma of the cod are less than half those of the blood of the lobster, yet both *Gadidæ* and *Homaridæ* have been marine since the Cretaceous.

In mammals, according to Abderhalden's analyses, there is an extraordinary similarity in the inorganic composition of the serum of the number of the forms taken, and the ratios of the sodium, potassium, calcium, and magnesium are, as shown in Table (IV), B, almost parallel with those in the Teleosts and Elasmobranchs.

It may be of interest here to refer to the inorganic composition of the blood in mammalia which lead a marine life. Of these, the Cetacea have been marine since their origin in the early Eocene. So far no opportunity has occurred of analysing the serum of any of these forms, but through the kindness of Mr. G. W. Taylor, Director of the Canadian Marine Biological Station at Nanaimo, British Columbia, the author obtained a quantity of clotted and partially laked blood of the whale common in the Pacific off the coast of British Columbia. This was analysed and found to be very rich in potassium, as is the blood of the horse and pig, much the greater part of the element being held in the red corpuscles. The analyses gave the following values in per cent.:—

	Na.	K.	Ca.	Mg.	Cl.
1	0·1808	0·19962	0·00693	0·00444	0·26758
2	0·1802	0·20118	0·006005	0·00453	0·2695

If we take the mean of the two determinations of each element and range them with the values for the blood (corpuscles and plasma) of the horse and pig, as calculated from the determinations made by Abderhalden, the parallelism is remarkable:

	Na.	K.	Ca.	Mg.	Cl.
Whale	0·1805	0·2004	0·00646	0·00448	0·26854
Pig	0·17859	0·1917	0·00485	0·00533	0·2690
Horse	0·19974	0·2273	0·00364	0·00384	0·2785

The fact that the inorganic composition of the blood in the whale is so like that of the pig* and horse strongly suggests that the inorganic composition of the serum is the same, although, almost as long as in the case of *Gadidæ*, the whales have been oceanic forms.

It would seem accordingly that in the power which the kidneys exercise of regulating and rendering uniform not only the saline concentration, but also the ratios of the sodium, potassium, calcium, and magnesium in the blood plasma of Vertebrates so far examined, we have to deal with an unalterable function of primal importance inherited from a Protovertebrate or Eovertebrate type of the Cambrian or even of the Pre-Cambrian.

The retention of urea and ammonia salts in the blood of Elasmobranchs undoubtedly developed as a result of the tendency of the blood to balance in itself the osmotic pressure of the sea water. The very fact that the kidneys in these forms exhibit some inertness in the elimination of urea, while they are very active in the elimination of the salts, is significant. What they do most rigorously is the regulation of the concentration and composition of the salts of the blood. *The firmly fixed physiological habit or function must be the more ancient one, and consequently the earliest function was not the elimination of waste metabolic products, but the regulation of the inorganic composition of the blood. The function of excreting waste products came later, and in the Elasmobranchs never acquired the fixity that characterised the function concerned in excreting the salts.*

That in the *Gadidæ*, although of marine habitat since the Jurassic, the kidneys rigorously keep down the saline concentration and regulate the inorganic composition of the blood, while the urea is readily eliminated, is not a difficulty in the way of accepting this view. The Ganoid ancestors of the Teleosts were fresh-water forms, probably throughout the latter half of the Palæozoic and, at least, the first half of the Mesozoic, and during all that time the conditions which would tend, as in the Elasmobranchs, to increase the osmotic pressure did not occur. There was nothing, then, to work

* The parallelism in composition between the blood of the whale and that of the pig and horse is so close that it is of special interest in connection with the origin of the Cetacea. Some anatomists relate them to the Ungulata; others question this on various grounds. The inorganic composition of the blood would seem to bring the whales very close to the Ungulates.

against the elimination of waste nitrogenous matter, and this function, after the lapse of a long period of time, became fixed in the Ganoid kidney, with the result that, when the Teleosts arose, their kidneys had developed two very fixed functions, instead of one as in those of the Elasmobranchs, and these two have been transmitted with undiminished or slightly lessened force to their descendants, whether of fresh water or marine habitat, through the millions of years which have elapsed since the Jurassic. Thus, in the blood of the cod there is no accumulation of urea beyond the limit that is found in the blood of the higher Vertebrates.

It is easy to understand how a uniformity of composition of the internal medium of animals is a powerful factor in influencing the course of evolution. The capacity of the organism to make and keep its own internal media uniform gives an enormous advantage to it, for it can change its habitat and adapt itself to a new environment without affecting the stable conditions under which its own tissues and organs do their best work.

This independence of external media is much more characteristic of Vertebrates than of any Invertebrates, and, in fact, it may be regarded as a special feature of Vertebrate life. It is, indeed, difficult to conceive how Vertebrates could have arisen and undergone the extraordinary development and adaptation to either terrestrial or aquatic life which they have experienced in geological time if their internal medium had not been maintained constant.

The establishment of that constant internal medium would therefore appear to have been the first step in the evolution of Vertebrates from an Invertebrate form. That, on the other hand, postulates that the kidney, developed to regulate and keep constant the internal or circulatory fluid, was essentially the first typically Vertebrate organ, and therefore of origin more ancient than that of the Vertebrate brain and spinal cord.

That to-day the earliest appearance in the Vertebrate embryo of structures which are subsequently to develop into the kidney is after the neural groove arises, constitutes, apparently, an objection to this view which can, however, be met. The distinctive parts of the ovary and testis of the Invertebrate form out of which the Protovertebrate developed undoubtedly gave rise to the distinctive parts of the ovary and testis in the Vertebrate. The reproductive cells of the ovary and testis are, therefore, of origin perhaps as remote in time as the origin of the Metazoa. In the Vertebrate embryo, however, the distinctively reproductive elements make their appearance at a date later than that at which the neural tube arises, and this retardation is, without doubt, due to the effect exercised by the postponement of the time when the sexual function begins to operate in the individual of a species. It may thus

readily be that the renal organs, which do not function in the embryonic life of the individual, may arise relatively late, and yet in the Eovertebrate embryo have been amongst the very earliest structures to appear.

It is evident from the analyses of the blood of *Limulus* that the inorganic composition of its internal medium is determined by the composition of the ocean. If it were now to develop an excretory organ, with a function like that of the Vertebrate kidney, in its descendants in the far future, many millions of years from now, their internal medium, the blood, would in its inorganic composition reproduce in the main the ratios of the sodium, potassium, calcium, and magnesium, and also the saline content of the ocean of this age, although in that long interval the ocean would undergo very considerable change in composition and in saline concentration. The composition of the blood in that far remote future could be used to postulate the composition of the ocean of to-day.

So, from the composition of the blood plasma in Vertebrates, we may infer the relative composition of the ocean in the remote past, when the Vertebrate kidney acquired the function of controlling the salts and their concentration in the blood. This would give for the ocean of that age the ratios in round values as follows:—

Na.	K.	Ca.	Mg.
100	6·00	3·00	1·50—2·00

Compare this with the ratios of the ocean of to-day, which are:

Na.	K.	Ca.	Mg.
100	3·613	3·911	12·106

This would place the origin of the distinctively Vertebrate type, that is, of a form possessing a kidney with a function like that of the Vertebrate excretory organ, at a time when the ocean had one-eighth to one-sixth of its present concentration of magnesium, and less than one-third of its present content of sodium chloride.*

How far back in time that is to be placed cannot be estimated with certainty, for we do not know what was the sodium chloride content of the earliest ocean of the globe. Joly,† in calculating the age of the earth from the total sodium chloride now in the sea, and from the annual increment of the salt due to the river discharges from the land surfaces of the globe, assumes that it amounted to 14 per cent., or approximately one-seventh, that

* The concentration of sodium chloride in sea water is, in round numbers, 2·8 per cent., while in mammalian blood plasma it is about 0·7 per cent.

† "An Estimate of the Geological Age of the Earth," 'Trans. Roy. Dublin Society Series 2, 1899, vol. 7, p. 23.

is, that the sea contained 0·4 per cent. of sodium chloride. If we accept this, it follows that, when the Vertebrate kidney began to evolve, the sodium chloride in the sea had increased only 0·3 per cent., and since then as much as 2·1 per cent. This would place the origin of the Eovertebrate form at a date not earlier than the second eighth, and not later than the first fourth of the whole geological period.

We know, from the results of the analyses given above, that the magnesium in the blood of *Limulus* lags behind that of the ocean, which is ever growing in amount. The ratio between it and the sodium in mammal's blood may be put approximately 1 : 100. The ocean, then, in Eovertebrate time would have a higher magnesium content in relation to sodium, approximately 1·50–2·00 : 100, or one-eighth to one-sixth of the magnesium concentration of the ocean of to-day. These estimates also would place the origin of the Vertebrate kidney at a time somewhere between the beginning of the second eighth and that of the second sixth of the whole geological period.

These are speculations which are advanced with reserve. They may be accepted absolutely, or rejected wholly, only when we are in possession of the results of analyses of the blood plasma in all the representative Vertebrate classes, as well as of the blood of the higher types of Invertebrates.

Enough, however, has been advanced here to make it extremely probable that the inorganic composition of the blood plasma of Vertebrates is an heirloom of life in the primeval ocean.