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American Naturalist, Volume 50, Issue 599 (Nov., 1916), 641-663.

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American Naturalist

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THE AMERICAN NATURALIST

VOL. L.

November, 1916

No. 599

THE EVOLUTIONARY SIGNIFICANCE OF THE OSMOTIC PRESSURE OF THE BLOOD¹

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THE facts of comparative anatomy, embryology and paleontology form the tripod of evidence on which rests to a great degree the validity of the doctrine of evolution. Accepting the doctrine of evolution as a working hypothesis has resulted in clearing up puzzling problems in the above named departments of biological inquiry. At the present time, more attention is being paid to physiological than to morphological problems. In physiology, the great emphasis is placed on mammalian problems with especial reference to man himself. Now if the mammals are the product of a long process of evolution from simple ancestors, it follows that not only has there been a morphological evolution, but also the present complicated functions of the higher animals have evolved from the simpler processes of primitive ancestral forms. In order to understand the significance of particular physiological facts, we must therefore view the matter in the light of evolution. It is not essential that all needful evidence be at hand to make perfectly clear the significance of the higher physiological activity. Indeed, it is well worth while at times to state clearly any of our problems in the evolutionary form and arrange the evidence accordingly.

¹ Read by title before The American Society of Naturalists, Philadelphia, Dec., 1914.

In this way we become aware of the need for information to clear up the question which inevitably arises.

It is commonly known that the blood and body fluids of animals possess a certain osmotic pressure. Life processes are constantly dependent on the passage of materials in and out of cells and differences in the osmotic pressure of substances within and without the cell are held to be one cause of this mutual movement. Variations in the osmotic pressure of the blood and body fluids of animals are not so generally known. In the case of severe hemorrhage it is a common practice to replace the lost blood by a physiological salt solution which has the same osmotic pressure as that of the blood. Formerly a 0.7 per cent. saline solution was used. This is isotonic with amphibian blood. The reason for this was that the fact was first discovered in a study of frog's blood. The saline solution (based on amphibian studies) of the physiological laboratories was considered proper for use in hospitals as well. Later it was found that a 0.9 per cent. saline solution represents more nearly the composition of human blood and this solution is in use at present.

But why does human blood have an osmotic pressure equivalent to that of a 0.9 per cent. saline solution? In order to answer this question we must examine all available data as to the osmotic pressure of the blood and body fluids of animals in general. When this is done it appears in many cases at least as though the osmotic pressure of the blood and body fluids were merely a direct adaptation to the environment. But in other cases this is not so clearly apparent, in fact the osmotic pressure possessed by certain forms shows no evident adaptation to the environment at all. The terrestrial vertebrates illustrate this last condition. It is only when we view the entire question from the standpoint of evolution that the main features of the puzzle become apparent.

It might be well to explain at this point the meaning of osmotic pressure. One gram molecule of hydrogen gas at atmospheric pressure occupies 22.4 liters space, and to confine this gas in a space of one liter would require a

pressure of 22.4 atmospheres. A gram molecule of any other gas under the same conditions has the same pressure. Van't Hoff in his theory of solutions established the fact that a substance in solution behaves as a gas occupying the same volume as the solution and the laws which solutions obey are analogous to those which are followed by gases. Therefore a gram-mol of a substance dissolved in a liter of pure water would have the same pressure as a gram-mol of gas, *i. e.*, 22.4 atmospheres. This pressure property of dissolved substances is called osmotic pressure. Since the blood and body fluids contain salts and other substances in solution, these fluids therefore have a certain osmotic pressure. It is well known that a salt solution has a lower freezing point than that of pure (distilled) water. The difference is proportional to the difference in concentration. Since the osmotic pressure depends on the concentration, it follows that the amount of the depression of the freezing point of the solution below that of distilled water is a measure of the osmotic pressure. The osmotic pressure stated in atmospheres can be readily obtained from the " Δ " or depression of the freezing point by the use of the following formula. Osmotic pressure in atmospheres = $(\Delta \times 22.4)/1.85$.

The blood of a vertebrate serves two double purposes. It carries oxygen to tissues and carbon dioxide away. This is its respiratory function. It also carries nutrients to tissues and wastes of metabolism from tissues. We might call this the nutrient function. But the blood of the earthworm is mainly a respiratory fluid. The body cavity is filled with foods absorbed directly from the intestine and distributed by the peristaltic movements of the body to the various tissues. In insects the air is carried directly to the tissues through tracheæ while a so-called heart lying on the dorsal side of the intestine and open at its anterior and posterior ends aids in churning and distribution of food absorbed into the body cavity from the intestine. The indefiniteness of the term "blood" is at once apparent. Most persons in using this

TABLE I
SHOWING THE FREEZING POINTS (Δ) OF THE BLOOD OF ANIMALS

Species	Δ Blood	Δ Water	Locality	Observer
I. Coelenterate:				
1. <i>Alcyonium</i>	2.195	2.29	Naples	Bottazzi
II. Echinodermata:				
2. <i>Asteropecten</i>	2.312	2.29	Naples	Bottazzi
3. <i>Asterias</i>	2.295	2.29	"	"
4. <i>Holothuria</i>	2.315	2.29	"	"
III. Annelida:				
5. <i>Sipunculus</i>	2.31	2.29	Naples	Bottazzi
IV. Arthropoda:				
6. <i>Homarus vulgaris</i>	2.292	2.29	Naples	Bottazzi
7. <i>Maja squinado</i>	2.36	2.29	"	"
8. <i>Maja verrucosa</i>	2.13	2.29	"	Fredericq
9. <i>Homarus americ.</i>	1.82	1.80	Woods Hole	Garrey
10. " "	1.78	1.76	St. Andrews	Macallum
11. <i>Limulus</i>	1.90	1.82	Woods Hole	Garrey
12. " "	2.04	?	?	Macallum
13. <i>Astacus</i>	0.80	0.03	Fredericq
14. <i>Barbus</i>	0.475	0.03	"
V. Mollusca:				
15. <i>Aplysia</i>	2.31	2.29	Naples	Bottazzi
16. <i>Octopus</i>	2.24	2.29	"	"
VI. Cyclostomata:				
17. <i>Polistrotrema</i>	1.966	1.924	Monterey	Greene
VII. Elasmobranchii:				
18. <i>Mustelus vulg.</i>	2.36	2.29	Naples	Mosso
19. <i>Trygon viol.</i>	2.44	2.29	"	"
20. " "	2.378	2.29	"	Bottazzi
21. <i>Mustelus laev.</i>	2.36	2.29	"	"
22. <i>Scyllium stell.</i>	2.31	2.29	"	"
23. <i>Torpedo ocell.</i>	2.351	2.29	"	"
24. <i>Torp. marmorata</i>	2.292	2.29	"	"
25. <i>Squatina angelus</i>	2.28	2.29	"	"
26. <i>Acanthias vulg.</i>	1.90	1.91	North Sea	Dakin
27. <i>Raxia clavata</i>	1.90	1.91	" "	"
28. <i>Carcharias lit.</i>	2.03	1.82	Woods Hole	Garrey
29. <i>Mustelus canis</i>	1.88	1.82	" "	"
30. <i>Mustelus canis</i>	1.869 ²	1.81	" "	Scott
31. <i>Squalus acanthias</i>	1.84	1.81	" "	"
32. " "	1.70	1.42	New York	"
VIII. Pisces:				
33. <i>Acipenser sturio</i>	0.76	2.00	Arcachon	Rodier
34. <i>Charax</i>	1.040	2.29	Naples	Mosso
35. <i>Serranus</i>	1.035	2.29	"	"
36. <i>Conger vulg.</i>	1.120	2.29	"	Bottazzi
37. <i>Deutex vulgaris</i>	1.022	2.29	"	"
38. <i>Oncorhynchus</i>	0.762	1.924	Monterey	Greene
39. <i>Pleuronectes fles.</i>	0.883	1.91	North Sea	Dakin
40. <i>Pleuronectes plat.</i>	0.71	1.91	" "	"
41. <i>Lophius</i>	0.80	2.00	Arcachon	Rodier
42. <i>Lump sucker</i>	0.648	1.90	North Sea	Dakin
43. <i>Gadus mor.</i>	0.72 (0.64)	1.80	Baltic	Dekhuysen
44. <i>Pleuronectes</i>	0.681	1.80	"	"
45. <i>Conger vulg.</i>	0.74	1.80	"	"
46. <i>Cottus scorpa.</i>	0.941	Amsterdam	"
47. " "	1.178	Helder	"

² Mean Δ of eighty specimens.

Species	Δ Blood	Δ Water	Locality	Observer
VIII. Pisces:				
48. <i>Gadus angel</i>	0.767	1.80	Baltic	"
49. <i>Gadus virens</i>	0.76	1.80	"	"
50. <i>Gadus merl</i>	0.86	1.80	"	"
51. <i>Molva vulg</i>	0.716	1.80	"	"
52. <i>Molva byrkel</i>	0.86	1.80	"	"
53. <i>Motella tric</i>	0.605	1.80	"	"
54. <i>Hippoglossus</i>	0.671	1.80	"	"
55. <i>Pleuronectes pl</i>	0.672	1.80	"	"
56. <i>Pleuron. micro</i>	0.681	1.80	"	"
57. <i>Labrus bergylta</i>	0.694	1.80	"	"
58. <i>Labrus mixtus</i>	0.681	1.80	"	"
59. <i>Conger vulg</i>	0.696	1.80	"	"
60. <i>Salmo trutta</i>	0.785	1.80	"	"
61. <i>Labrax lupus</i>	0.72	1.80	"	"
62. <i>Trigla hirundo</i>	0.669	1.80	"	"
63. <i>Anarrichas</i>	0.665	1.80	"	"
64. <i>Agonus cataphr</i>	1.095	Helder	"
65. <i>Zoarces</i>	1.30	"	"
66. <i>Tautoga onitis</i>	0.86	1.82	Woods Hole	Garrey
67. " ".....	0.70	1.42	New York	Scott
68. <i>Cyonoscion</i>	0.792	1.82	Woods Hole	Garrey
69. <i>Conger eel</i>	0.82	1.82	" "	"
70. <i>Anguilla</i>	0.90	1.82	" "	"
71. ".....	0.635	1.91	North Sea	Dakin
72. <i>Scup</i>	0.75	1.82	Woods Hole	Scott
73. <i>Morone am</i>	0.735	1.82	" "	"
74. <i>Oncorhyncus</i>	0.628	0.03	Fresh water	Greene
75. <i>Morone am</i>	0.571	0.03	" "	(Scott)
76. <i>Anguilla</i>	0.57	0.03	" "	Dakin
77. <i>Pleuronectes</i>	0.68	0.03	" "	"
78. <i>Perca</i>	0.507	0.03	" "	Dekhuyzen
79. <i>Esox lucius</i>	0.519	0.03	" "	"
80. <i>Salmo fario</i>	0.567	0.03	" "	"
81. <i>Abramis blicca</i>	0.497	0.03	" "	"
82. <i>Cyprinus carpio</i>	0.527	0.03	" "	"
83. <i>Tinca vulgaris</i>	0.519	0.03	" "	"
84. <i>Leuiscus eryth</i>	0.495	0.03	" "	"
85. <i>Erythrinus</i>	0.577	0.03	" "	"
86. <i>Abramis brama</i>	0.51	0.03	" "	Dakin
87. <i>Cyprinus carpio</i>	0.487	0.03	" "	"
IX. Amphibia:				
88. <i>Rana escul</i>	0.563	Bottazzi
89. <i>Bufo viridis</i>	0.761	Bottazzi & Ducceschi
90. <i>Bufo vulgaris</i>	0.445	Bottazzi
X. Reptilia:				
91. <i>Thalassochelys</i>	0.61	2.29	Naples	Mosso
92. <i>Emys europa</i>	0.463	2.29	"	Bottazzi & Ducceschi
93. " ".....	0.440	2.29	"	Bottazzi
XI. Aves:				
94. Capon.....	0.66	D'Errico
95. Turkey.....	0.75	"
96. <i>Gallus bank</i>	0.623	Bottazzi
XII. Mammalia:				
97. <i>Delphinus phocaena</i> ...	0.74	1.90
98. Horse.....	0.58	Findlay
99. ".....	0.565	Winter

Species	Δ Blood	Δ Water	Locality	Observer
XII. Mammalia:				
100. Ox.....	0.601	Findlay
101. ".....	0.55	Winter
102. Pig.....	0.625	Findlay
103. ".....	0.55	Winter
104. Dog.....	0.599	Findlay
105. ".....	0.565	Winter
106. Rabbit.....	0.578	Findlay
107. ".....	0.57	Winter
108. ".....	0.564	Bottazzi & Ducceschi
109. Sheep.....	0.55	Winter
110. Cat.....	0.615	Findlay
111. MAN.....	0.560	"

term think of the fluid circulating in the blood vessels of a vertebrate. The term body fluid is also ambiguous. In an invertebrate it has reference to that part which we call the blood of a vertebrate. In the vertebrate we usually think of the secretion of serous membranes as "body fluid." After all, the subject of discussion in this paper is the fluid by which food is carried to tissues and wastes carried away. Having thus defined the use of the terms, let us examine the osmotic pressures of the blood of various animals.

Table I, which follows, shows one hundred and eleven determinations of the osmotic pressure of the blood of representatives of nearly every animal phylum. Many of these determinations are averages. Some of the forms are wholly terrestrial, some live in fresh water, some in either fresh or seawater, some live wholly in the sea. Considerable variation in the osmotic pressure of the blood is shown.

Of the marine forms given some are found in the Mediterranean, while others for the most part occur in the ocean or in protected waters connected with it. There is great variation in the osmotic pressure of the blood of forms living exclusively in the Mediterranean. Great variation is shown in the case of those living in the ocean. In some cases in each environment, complete harmony with or rather isotonicity with the environment is apparent. In other cases this is not at all evident. For

example, the average Δ of twelve species of invertebrates from the Mediterranean is 2.281° , while the average Δ of the water in which they live is 2.29° . A simple case of adaptation is thus evident. But the bony fishes, teleosts, tell a different story.

It is worth while to contrast the osmotic pressure of the blood with that of the external medium. To do this we will break up all the forms into groups not according to the environment alone, but also according to relationship. If we should be guided by environment alone, the result would be a confused tangle. Table II shows the average Δ of these groups selected not only on the basis of relationship but also taking into partial consideration the environment.

" Δ ," Blood, 12 Invertebrates, Mediterranean	= 2.28°	—Water = 2.29°
" Δ ," Blood, 4 Invertebrates, Ocean, bays	= 1.82	—Water = 1.79
" Δ ," Blood, 3 Invertebrates, Fresh water	= 0.592	—Water = 0.03
" Δ ," Blood, 1 Cyclostome, Ocean, bay	= 1.966	—Water = 1.924
" Δ ," Blood, 8 Elasmobranchs, Mediterranean	= 2.346	—Water = 2.29
" Δ ," Blood, 6 Elasmobranchs, Ocean, bays	= 1.902	—Water = 1.85
" Δ ," Blood, 4 Teleosts, Mediterranean	= 1.054	—Water = 2.29
" Δ ," Blood, 32 Teleosts, Ocean, etc.	= 0.744	—Water = 1.82
" Δ ," Blood, 13 Teleosts, Fresh water	= 0.545	—Water = 0.03
" Δ ," Blood, 4 Amphibia, Fresh water	= 0.551	—
" Δ ," Blood, 6 Reptilia,	= 0.56	—
" Δ ," Blood, 3 Aves,	= 0.67	—
" Δ ," Blood, 8 Mammals,	= 0.577	—

From this table it is evident that the blood of the marine invertebrate is isotonic with the water in which it lives, whether this be the Mediterranean or the ocean. As stated above, it appears to be a simple case of adaptation. But in the other cases the relation is not so simple. If we compare the osmotic pressure of the marine teleosts, fresh-water teleosts and the amphibia, etc., with the osmotic pressure of the external medium great differences are evident. And yet it can not be said but what all these forms are adapted to their environment. But it is not enough to make this statement, but to try to explain why such a relationship becomes possible. The isotonicity existing between the blood of marine invertebrates and

their environment has been discussed by Fredericq ('85-'04), Rodier ('99), Dakin ('08), Garrey ('05) and Bottazzi ('97-'06). Now it is held that evolution of life began in the sea. The single celled forms were completely surrounded by the sea and it is easily understood why the osmotic relations would remain primitive in case of these forms. In gastrula type animals, such as *cœlenterata*, practically all cells of the body are bathed directly by the sea and as far as we know these forms also are in osmotic equilibrium with sea water. Now with the appearance of mesoderm and a body cavity much of the body is removed from direct contact with the sea. But the complete equilibrium remains. As Quinton ('00) says, the marine invertebrate, though anatomically independent of the sea in many of its organs, yet it is still physiologically open to the sea which in an osmotic sense still ebbs and flows throughout its body.

Protoplasm originating in the sea was built up with certain relationships with sea water, which relationships are still maintained throughout all marine invertebrates. May not the sparsity of fresh-water porifera and *cœlenterates* and the comparative failure of fresh-water algæ be due to the difficulty of maintaining the integrity of protoplasm when all cells of these forms are so freely bathed by fresh water, the osmotic pressure of which is nearly zero?

Next above the marine invertebrates is a single case of a cyclostome which is in osmotic equilibrium with the surrounding sea water. What the osmotic pressure of the blood of a cyclostome in fresh water is, we have no record. It should be noted here that cyclostomes are now regarded as degenerate fishes and on that account any evidence from these forms as to the higher course of evolution must be treated with care. In the next place we find that eight species of elasmobranchs from the Mediterranean and six from the ocean possess blood which is practically isotonic with the sea water outside. Apparently they do not differ from the marine invertebrates. But it is evident that the osmotic pressure of the blood is slightly

greater than that of the external medium. Furthermore, analysis shows that the osmotic pressure of elasmobranch blood is due to different substances from those which account for the osmotic pressure of the blood of marine invertebrates. Therefore the elasmobranchs belong to a second category. In the third group we will place the marine teleosts. The osmotic pressure of their blood is somewhat less than half that of the medium in which they live. We have the case of four species from the Mediterranean and thirty-two species from the ocean which show this. The osmotic measurements show a decided difference between the blood and the surrounding medium. A decided independence also. In the same group or possibly a fourth group we will place the fresh-water fishes and with these the amphibians, reptiles, birds and mammals. Thirteen species of fresh-water fishes possess blood with an osmotic pressure less than that possessed by the marine teleosts. Let us assume here that the fresh-water fishes were derived from marine ancestors. In becoming acclimated to fresh water, the blood suffered a decrease in its osmotic pressure. Whether this was in direct response to the great decrease in the osmotic pressure of the surrounding medium as compared with seawater is problematical, but appears probable. The amphibians were derived from the fresh-water teleosts. Some of the amphibians still retain their aquatic habits and structures. They in all probability possess the osmotic pressure of fresh-water fishes. Other amphibia metamorphosed into terrestrial forms, taking with them the osmotic pressures of the blood possessed by their fish-like ancestors. Blood with the same osmotic pressure as that of the fresh-water fishes flows on through the amphibia to the reptilia and on to the birds and mammals. An examination of Table II shows the close similarity between the osmotic pressures of fresh-water fishes, amphibians, reptiles, birds and mammals. According to the above hypothesis, the order of evolution was I. Marine invertebrates, II. Elasmobranchs, III. Marine teleosts,

IV. Fresh-water teleosts, amphibians, reptiles, birds and mammals.

Let us examine each of these groups with regard to their osmotic independence of the external medium. That is, what is the effect of changes in the concentration of the external medium on the osmotic pressure of the blood of these groups.

First, the invertebrates. Let us recall Quinton's statement that marine invertebrates are still physiologically open to the sea. For when the concentration of the external medium is changed, it is found that a change in the osmotic pressure of the blood takes place. Fredericq ('85 and '04) stated that the change in one was followed by an equal change in the other. In a few hours the new equilibrium is established. If the time of sojourn in the modified sea water was small the equilibrium with it was not completely attained. Moreover, all invertebrates did not adapt themselves with the same rapidity to changes in the external medium. On the whole, provided the external change was not too great, it was followed in time by complete equilibrium between the osmotic pressure of the blood and that of the modified sea water. This was true in the case of sea water made dilute by addition of fresh water and sea water made more concentrated by the addition of salt. In other words, the organism possesses no structures which render it independent of the changes in the external medium. There are three structures concerned in these changes. First the integument, second, the intestinal wall and third the gill membranes. With the appearance of gills, the body integument apparently is the first structure to become impermeable. The intestinal wall is the first to show a selective action.

Second, the elasmobranchs. These had been placed by investigators with the marine invertebrates not only because their blood possessed the same osmotic pressure as the external medium, but it was thought that when the external medium was changed, the same changes occurred in the blood of the elasmobranch. I made extensive ex-

periments to test this ('13) and found that when a change was made in the external medium, though considerable change took place in the blood of the dogfish, yet it was considerably less than the external change. In fact it appeared as though the change in the blood was roughly proportional to the change in the external medium (p. 20, Scott, '13). The condition was so marked as to show clearly that the elasmobranch belonged in a category differing from that of the marine invertebrate.

Third, the marine teleost. Much emphasis has been placed upon the claim that these forms are absolutely independent of changes in the external medium. With this claim, I must differ. The following evidence is the basis of this difference of opinion. In the first place Tables I and II show that the blood of teleosts from the Mediterranean has a higher osmotic pressure than that of blood of teleosts from the ocean. There is a corresponding though greater difference in the osmotic pressure of the water. Dakin '08 in a trip from Kiel to Helgoland found that the osmotic pressure of the sea water increased 74 per cent. and that the osmotic pressure of the blood of the plaice showed an increase of 20 per cent. The cod did not show so great a difference, being but 4 per cent.³ Garrey '05 reported Δ of the blood of the tautog at Woods Hole to be 0.86° while at the New York Aquarium, where the harbor water is much more dilute than at Woods Hole, I found the Δ of tautog blood to be about 0.70° . Therefore it would appear that even blood of the marine teleost is somewhat modified by changes in the external medium. And yet practical independence has been achieved. This is evident from the fact that the marine teleost lives in a medium which has an osmotic pressure over twice as great as that of the blood of the fish.

Macallum ('10) has explained the peculiar osmotic pressure of the blood of marine teleosts as due to their origin from fresh-water teleosts. This is based on morpholog-

³ On the other hand Dekhuyzen, '05, found a difference of 20 per cent. in the osmotic pressure of cod blood according to the locality from which the fish was taken.

ical evidence of the evolution of the true teleosts from ganoid ancestors from the elasmobranchs through forms similar to the sturgeons and the bow-fins. I doubt very much, however, whether ichthyologists would wish to conclude on this basis that all marine teleosts had their origin from fresh-water forms. In fact certain paleontologists trace the evolution of certain fresh-water teleosts from ancestral marine teleosts. The sea is the home of the preponderating fish population. Here the class of Pisces has found its greatest opportunities for range of movements to escape enemies, in search of food or place of breeding.

Facts concerning the osmotic pressure of the blood of anadromous fishes throw light as to the possible if not probable origin of fresh-water forms. Greene ('04) determined the osmotic pressure of the chinook salmon in Monterey Bay to be 0.76° . On the spawning grounds in fresh water its blood had a Δ of 0.628° , a decrease of 17.6 per cent. Flatfish are known to be somewhat anadromous. Dakin ('08) found the Δ of the flounder, *Pleuronectes flesus*, in the North Sea to be 0.83° , while in the River Elbe in fresh water its blood had a Δ of 0.68° , a decrease of 18 per cent. The same author found that the blood of the eel, *Anguilla*, in fresh water had a Δ of 0.57° , quite similar to that of fresh water fishes. After a day in sea water another specimen had blood with a Δ of 0.745° . Eels taken from seawater had blood with a Δ of 0.634° . Eels from seawater placed in fresh water for three days possessed blood with a Δ of 0.582° , practically the same as for fresh-water forms. At Woods Hole, ignorant of this work of Dakin's, I made observations on the Δ of the blood of the white perch, *Morone americana*. This form can live equally well in salt or fresh water. Taken from the slightly brackish waters of Tashmoo Pond, Marthas Vineyard, Massachusetts, the blood showed a Δ of 0.635° . The upper end of this pond is the source of drinking water for Oak Bluffs. A number of perch were placed in running tap water for a day, when the blood showed a

Δ of 0.571° , similar to the fresh-water fishes. Others of this lot were placed in sea water for two days, when the Δ of their blood was 0.766° . Others taken directly from the Eel Pond (sea water) showed a Δ of 0.735° . The result is similar to Dakin's. On the whole the conclusion seems justified that anadromous fishes are able to adapt themselves to a degree to the great changes in the osmotic pressures of the external medium, which they meet in passing from salt to fresh water or vice versa by a slight corresponding change in the osmotic pressure of the blood.

It is commonly known that sturgeons are anadromous. For some reason the elasmobranch has been shut out of fresh water. There is but one elasmobranch known to inhabit fresh water, *Carcharias nicaraguensis* of certain lakes in Nicaragua. Although the integument of the shark is impermeable, yet I have found the gills to be still permeable to salts (Scott & Denis, '13). The ganoids derived from elasmobranchs ventured up fresh-water streams. They returned to the sea. Rodier ('99) states the Δ of the blood of *Acipenser sturio* to be 0.76° , which places it in the same group as the marine teleosts. What the Δ is in fresh water is not known. The modern sturgeon is a long way from the modern shark. Nevertheless it is conceivable that the ancestral ganoids tried fresh-water conditions. Is it not possible that these conditions, fresh water and food found in fresh water had some influence on the change in structure. During all subsequent periods when evolutionary changes were taking place some forms went back and forth from sea to fresh water. Some forms remained in fresh water. During this period of experimentation, impermeable membranes were built up. In the meantime the blood had become modified, due to the temporary sojourn in fresh water. The osmotic pressure was reduced; the membranes once made practically impermeable remained so, and when those forms returned to the sea and remained there they retained *almost* unmodified the osmotic pres-

tures they had acquired during their fresh-water experience. We can thus speculate that in some such way the present osmotic pressures of the blood of marine and fresh-water teleosts were acquired. Whatever may be the case with the marine and fresh-water teleosts, it is more clearly indicated that the osmotic pressure of the blood of terrestrial forms is derived from fishes which lived in fresh water. The present day anadromous fishes constitute all that remains of a movement which at one time was far more general.

The chemical composition of the blood throws further light on the question. The osmotic pressure is due to substances dissolved in the blood. These are chiefly salts. Quinton ('00) states that sodium chloride represents from 85 per cent. to 90 per cent. of all the dissolved salts of the blood. The sodium chloride content can be ascertained from a study of the chlorides which are easily determined. Let us ascertain the changes in the sodium chloride content of the blood of the forms under discussion. In the first place what is the total salt content of sea water. According to Bottazzi ('97) the total salt content of water from the Mediterranean is 3.78 per cent. The water of the ocean contains about 3.22 per cent. salts. Of course there is some variation. The percentage of salts in fresh water is very small, 0.05 per cent. (Sumner, '05). What is the percentage of salt of the blood of forms living in the sea? Quinton ('00) made forty-nine determinations of the sodium chloride content of the hemolymph of ten species of marine invertebrates belonging to five different groups and found that these averaged 3.24 per cent. He made 26 determinations of the sodium chloride content of the sea water in which these forms lived, and found that it was about 3.31 per cent. According to these researches of Quinton, the blood of the marine invertebrate contains about the same percentage of salts as the water in which they live. Moreover, it follows that the osmotic pressure of the blood is determined almost wholly by the salts of the blood and not by any organic solutes. It was because

of this relationship that Quinton felt justified in making the statement that the marine invertebrate while anatomically closed to the external medium, is yet physiologically open to it. That functionally speaking the marine invertebrate is still freely exposed to the sea without, which still practically ebbs and flows through its body.

Macallum ('10) says:

In *Limulus*, the amount of total salts in the blood, 2.982 per cent., approaches that of the sea water,—which may be found along the Atlantic coast. At St. Andrews, New Brunswick, the total salts of the seawater collected in April were 2.417 per cent., but in sea water collected 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. The blood of *Limulus* is but slightly modified sea water. It would appear as if the sodium chloride of sea water passes freely into the blood of the lobster till the sodium chloride concentration in both is approximately balanced.

This agrees entirely with the work of Quinton. For some reason, the marine invertebrate has not been able to keep the sea out. One asks why the question of the permeability of membranes of fishes to salts is of such interest to the comparative physiologist? One answer is that impermeability represents independence of the sea the osmotic pressure of which differs so much from that of fish blood. And this independence is not to be found among the marine invertebrates.

As shown above, elasmobranch blood possesses the same osmotic pressure as that of the marine invertebrate and that of the sea without. But analysis shows that the osmotic pressure of elasmobranch blood is due to entirely different causes. For example, what is the salt content of elasmobranch blood? It should contain about 3.22 per cent. salts in order that its total osmotic pressure be due to salts. But Rodier ('99) found that the blood of elasmobranchs did not contain over 1.7 per cent. sodium chloride. Dakin ('08) found the blood of the dogfish to contain but 1.45 per cent. sodium chloride. My analysis of the blood of another species, *Mustelus*, at Woods Hole

showed 1.424 per cent. sodium chloride. Fredericq ('04) found the blood of *Scyllium* to contain but 1.71 per cent. salts, while Macallum ('10) found the blood of the dogfish, *Acanthias vulgaris*, contained 1.7739 per cent. sodium chloride. In other words the sodium chloride content of the blood of elasmobranchs will account for only about half of its total osmotic pressure. Evidently a great change has come about. "The difference between the Δ of the serum and that due to salts of the serum depends," as Macallum ('10) says,

"on urea and other organic solutes." Urea is present in large quantities in the blood of elasmobranchs.

Staedeler and Frerichs ('58) obtained as much as two ounces from the liver of a single shark. In '90 von Schroeder found that *Scyllium*, another dogfish, contained blood with 2.6 per cent. urea. Rodier ('99) computed that one third the osmotic pressure of the blood of elasmobranchs was due to urea.

In '13, I found that *Mustelus* blood contained 1.55 per cent. urea. Macallum ('10) in *Acanthias vulgaris* found an average of 2.026 per cent. urea. Due to dissociation, the salts have twice the osmotic pressure, approximately, as the urea, although the urea and salts are present in about equal quantities. But the urea and salts are not sufficient to account for the osmotic pressure of the blood. The difference is due to the presence of ammonia salts, as Macallum found. For example, he found 0.1727 per cent. ammonia in the serum of the dogfish. This would fully account for the remaining percentage of the depression of the freezing point unaccounted for by the presence of the salts and urea. So that we see, that while superficially the elasmobranch resembles the marine invertebrate in the osmotic pressure of the blood, yet below the surface a marked change has taken place. Several observers had noted that the osmotic pressure was slightly greater than that of the sea water. This at least is another indication that the equilibrium is not like that existing between marine invertebrates and the sea. For some reason the elasmobranch has lost in salts. Their place has been taken by *nitrogenous* solutes. The con-

dition is lacking in the marine invertebrate. Some one has characterized the jellyfish as organized sea water. According to Macallum the blood of *Limulus* is but slightly modified sea water. The blood of the marine invertebrate has remained at the same low level so far as the presence of nitrogenous compounds is concerned. To what may we ascribe this new condition? Is it due to great proportion of nitrogenous food? To the particular kind of liver? To the great development of the muscular system? To a peculiar function of the kidney? Questions can at present be asked only. We lack information as to certain aspects of elasmobranch physiology.

However much the elasmobranch may have experimented in the matter of unique nitrogenous content of the blood, it is certain that this condition is lacking in the teleosts. And the lack there is carried over to the forms which developed further. For the osmotic pressure of the blood of teleosts is again determined almost wholly by the salts present. The salt content of the blood of marine teleosts is considerably less than that of elasmobranchs. Quinton ('00) found the blood of eight species of marine teleosts to contain 1.076 per cent. salts. Rodier ('99) found that the blood of the ganoid, *Acipenser sturio*, had a salt content varying from 0.643 per cent. to 0.979 per cent. The blood of *Lophius*, a strictly marine form, contained 1.164 per cent. salts. Hamburger states that teleost blood contains 0.936 per cent. salts, but whether these are fresh-water or marine species is not stated. Macallum ('10) found that the blood of the cod, *Gadus callarias*, contained 1.2823 per cent., while that of the pollock, *Pollachius virens*, contained 1.2934 per cent. salts. It is evident, therefore, that the percentage of salts in the blood of the marine teleost has been decreased as compared with the total saline content of elasmobranch blood. Moreover, the osmotic pressure of the blood of the teleost is due almost wholly to the salts present. Macallum ('10) proved this. He found that the Δ of the salts of cod blood was 0.71° , while that of the entire blood was 0.765° .

There is a difference of but 0.055° . The Δ of the salts of the blood of the pollock was 0.737° while the Δ of the entire blood was 0.825° , showing a difference of but 0.088° . In other words, almost the entire osmotic pressure of the blood of the teleost is due to the salts. The urea, ammonia or other organic solutes present must be very small and are represented by the difference above mentioned, namely 0.055° in the case of the cod and 0.098° in the case of the pollock. How different is this condition from that found in the elasmobranch where in one case noted by Macallum, and which is typical, the difference between the Δ of the saline contents of the blood and the entire blood was 0.961° , a difference as great as the average Δ of the marine teleost and as stated due to the relatively enormous amount of urea and other organic solutes in the blood of the dogfish. Again the question arises: What brought about this change between the composition of elasmobranch blood and that of the teleost? Was it due to the migrations to and from fresh water before certain species of teleosts took up their home permanently in the sea? And yet the marked difference between the two is not alone a difference in salt content. It is far more the absence from the blood of urea, ammonia and other organic solutes. Let us use Macallum's data as a basis for comparison. The blood of marine teleosts contains about 30 per cent. less salts than the blood of elasmobranchs but it contains 90 per cent. less organic solutes. The distinct loss therefor is in organic solutes. This therefore must have been a significant factor in the evolution of the higher form. Now what is the most apparent structural difference between the elasmobranchs and teleosts? It is of course that the skeleton of one consists of cartilage and the skeleton of the other is bone? It does not necessarily follow, however, that the power to build a bony skeleton depends on the absence of organic solutes from the blood, nor is there apparently any close connection between them.

The fresh-water fishes in all probability agree with the

marine teleosts in low percentage of organic solutes and this characteristic is maintained by all the higher forms. Dakin found that the blood of the plaice at Helgoland contained 0.92 per cent. salts, while at Kiel in brackish water it had a salt content of 0.85 per cent. Mosso ('90) stated that marine teleost blood had a higher salt content than that of fresh-water forms. Dakin ('08) found the blood of the eel in sea water to contain 0.605 per cent. salts, while in fresh water its saline content was 0.466 per cent. Quinton ('00) found that the blood of fresh-water teleosts contained 0.7 per cent. salts. Atwater ('91) found that the flesh of fresh-water teleosts contains less salt (15 per cent. less chlorine) than that of marine teleosts. Sumner ('05) obtained a similar result.

The anadromous fishes possess blood that is less saline in fresh water than in sea water. It is also true that strictly marine teleosts of the present day vary a little in the saline content of their blood when the salinity of the external medium changes. These facts indicate that the decreased salinity of the blood of fresh-water teleosts was brought about in response to the low saline content of the external medium. During the migrations that took place in the past when there were probably more anadromous fishes, this diminution in salts took place. Those forms that remained in fresh water retained the percentage of salts they acquired by their sojourn in fresh water. At the same time they built up membranes which maintain an equilibrium in spite of the differences in the osmotic pressure of the blood within and the fresh water without. Similar membranes were formed in case of the marine teleosts, which maintain an equilibrium with the sea water in spite of the fact that the osmotic pressure of sea water is over twice that of the teleost blood. The evidence at hand indicates that the last membranes to become practically impermeable to salts were the gill membranes. And yet though impermeable to salts they still are required to be permeable to gases.

Now the blood of amphibia contains about 0.7 per cent.

salts. This closely resembles that of fresh-water fishes. The blood of mammals contains a slight increase in its saline content. Bunge ('89) states that human blood serum contains about 0.84 per cent. to 0.86 per cent. salts. Macallum ('10) calculating from Abderhalden's analyses, concluded the total saline content of the blood of the dog amounted to 0.935 per cent., that of the cat to 0.933 per cent. and that of the sheep to 0.905 per cent. To quote from Macallum:

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 almost parallel with those in the Teleosts and Elasmobranchs.

Macallum had an opportunity to analyze the blood of "the whale common in the Pacific off the coast of British Columbia," and the parallelism between the inorganic constituents of its blood and that of the horse and pig was remarkable, thus bringing the whales very close to the Ungulates to which some anatomists relate them.

The above studies of the osmotic pressures of the blood, the change in the permeability of the protecting membranes and the inorganic and organic composition of the blood are understood only by viewing them from the standpoint of evolution. The increase in saline content of mammalian blood as compared with amphibian and fresh-water teleosts can be ascribed to the regulative action of the kidney. Most investigators give the impression that the osmotic pressure of the blood of animals is definite and fixed. This is not true. Findlay calls attention to the variation in the osmotic pressure of human blood at different times of day. For example, a distinct though slight rise (0.03°) is noted after meals. This question needs further study. My investigations showed that *Mustelus canis* can pass with entire safety through a range of 0.15° (+ and -) in its osmotic pressure. The range through which invertebrates can pass is much greater. The observations of Dekhuyzen ('05) and Dakin ('08) show that the range becomes limited in the case of

marine teleosts. The range is very much more restricted in fresh-water teleosts and higher forms. Protoplasm is an ever-changing substance. There is a constant ebb and flow. Protoplasm of the higher forms has evolved through long ages to a condition wherein it is associated with the same salts it was entirely surrounded by when it first began to be. The amounts have changed, but the proportions have remained quite constant and the kinds have remained the same as those in the sea. And that is why the surgeon must inject a 0.9 per cent. saline solution into the veins of his patient suffering from hemorrhage. And that is why human blood has a certain osmotic pressure. Macallum ascribes the first great reduction in salts which took place in the elasmobranch to be due to the kidney, whose primary function was not the elimination of the wastes of metabolism, but the regulation of the concentration and composition of the salts of the blood. The elasmobranch kidney is very inert and sluggish in the matter of the elimination of the organic wastes. The teleosts acquired the habit of still further keeping down the saline content while at the same time they eliminated the urea readily. However, I do not see that the process is necessarily limited to the kidneys alone. A thorough study of the elasmobranchs and teleosts is needed to throw light on this puzzle. I can see why the migratory habits of teleosts or teleost ancestors (ganoids) would account for reduction in salt content of the blood, but this throws no light on the reduction of salts in elasmobranch blood as compared with invertebrate blood. Nor does Macallum indicate any use the large amount of urea might serve. Balgioni ('06) found that salt solution alone led to stoppage of the elasmobranch ventricle in diastole. It increased diastolic tonus, while urea increased systolic tonus. The presence of the two in about equal amounts mutually neutralized each other and made the continuous rhythm of the heart possible. All we can say is that for the kind of protoplasm of which the elasmobranch heart is composed, the urea is a necessary constituent of the blood. Furthermore it does not appear to be necessary

for the teleost heart. At any rate we are aware that once we begin to question further, the necessity of further knowledge becomes evident. This paper can be brought to a close in no better way than by quoting a statement made by Claude Bernard ('65). We may accept it as one of the laws of evolution and conclude that inquiries concerning the osmotic pressures of the blood of animals amply prove its truth.

Chez tous les êtres vivants, le milieu intérieur, qui est un produit de l'organisme, conserve des rapports nécessaires d'échanges et d'équilibre avec le milieu cosmique extérieur; mais, à mesure que l'organisme devient plus parfait, le milieu organique se spécifie et s'isole en quelque sorte de plus en plus du milieu ambiant.

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