THE OSMOTIC CONCENTRATION OF THE BLOOD OF
FISHES TAKEN FROM SEA-WATER OF NATURALLY
VARYING CONCENTRATION

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It is now thirty-seven years since Bert (1), who was one of the first to consider the osmotic relations existing between the internal fluids of the animal body and the external fluids bathing their bodies, published a paper on the causes of death when freshwater fishes are plunged into sea-water. During the interval a great advance has taken place in physical chemistry, particularly with regard to the application of this branch of science to physiology and medicine, and numerous observers have turned their attention to the constitution and physical properties of the 'internal' and 'external media' both for invertebrates and vertebrates. Fredericq (4) published in 1885 an account of some investigations concerning the relation of the salt contents of the blood of Crustacea to the salt contents of the sea or fresh water in which the animals were living, and from that date to the present time the osmotic conditions of the blood and other body fluids together with the nature of the bounding membranes have been studied either by chemically estimating the constitution of the internal and external media or by determining directly the osmotic pressure with the aid of the Beckmann's freezing point apparatus.

It is not necessary in this introductory communication to go into the history of the discoveries made in this line of research, but it may be mentioned here that various problems which are linked together and stand in very close relation to the constitution of the internal and external media and to the bounding membranes have been considered from several points of view. For example, the relation existing between the sea-water and the blood and coelomic fluids of marine invertebrates has been investigated from the purely

physico-chemical point of view, and again, the physiological action of various fluids, such as sea-water, on freshwater animals, and fresh water on marine animals with regard to duration of life in these media, has been considered. The results of the various experiments made by different observers, often differ considerably, as do naturally the theories deduced from these results. Fredericq (5), Rodier (12), Quinton (11), Garrey (6) and others have shewn that the blood and fluids of the body cavity, coelom, or haemocoel, of invertebrates have practically the same osmotic pressure as the water in which they live, and contain almost the same percentage of salts in solution. Moreover, as the sea-water in the case of marine invertebrates varies in salt contents and osmotic pressure, so do the fluids of the body change accordingly. Turning now on the other hand to the vertebrates, we find in the highest vertebrates, the mammals, a *constant* or *practically constant* osmotic pressure for the blood, amounting to about seven atmospheres, and this is held in defiance of alterations in the constitution of the food. For other higher vertebrates this also holds, and though the Amphibia have a somewhat lower osmotic pressure corresponding to a 0.7 per cent. salt solution, Overton (10) has demonstrated the action of the organism in keeping this pressure constant.

One finds, however, on investigating the same question in the fishes that there is a surprising difference. The blood of Elasmobranchs possesses about the same osmotic pressure as the external medium, the sea-water, and further, this pressure is not constant but, as is the case for the invertebrates, varies with changes in the external medium. Teleosts, on the other hand, are quite different, and appear to resemble more the higher vertebrates in keeping a constant osmotic pressure. The freezing point depression of the teleost blood appears from Garrey's work to average about 0.872° , though it is subject to slight variations. The surprising feature here is that in the teleostei, though the blood is brought into close connection with the sea-water by the gills, the fish contrives to maintain an osmotic pressure which is only about one-third of that of the surrounding sea-water. It should be mentioned also that the Elasmobranchs, though resembling the invertebrates in having a varying osmotic pressure for the blood which

is almost the same as that of the external medium, have a salt contents much less, resembling in fact the proportion of salts in the blood of the teleosts. The osmotic pressure, however, is brought up by the presence of considerable quantities of urea in the blood. We have therefore, roughly speaking, the following three groups of animals, members of which live in water but do not breathe air directly :—

1. *Invertebrates*.—Osmotic pressure and salts contents of blood and internal media practically identical with external medium.
2. *Elasmobranchs*.—Osmotic pressure of blood practically identical with external medium, but salts contents much lower.
3. *Teleosts*.—Osmotic pressure and salts contents of blood much lower than that of external medium.

Thus it appears as if the independence of the constitution of blood is first established in the teleosts. What determines this difference? Is the membrane, either gills or body wall, of the invertebrates so different in constitution from that of the teleosts that in the first case perfect osmotic conditions are set up, and the internal fluids are directly dependent on the external, whilst in the latter the membranes are absolutely impermeable to the external medium?

For invertebrates Fredericq and Quinton state that the bounding membranes are permeable to both water and salts, whilst Botazzi and Enriques (2) state that the membranes are semi-permeable, that is, they allow water to pass through and, therefore, bring about the osmotic equilibrium but are impermeable to salts.

For teleosts, Dekhuyzen (3) states from a series of observations made at Bergen, that they have a definite osmotic pressure of the blood, and that any differences occurring are probably due to differences in pathological conditions or variations in observation. Sumner (13) has, however, quite recently conducted an extensive series of experiments for the purpose of investigating the conditions of the external membranes in teleosts, the fish used being chiefly three species of *Fundulus*, small fishes which pass into brackish waters and one species of which occurs frequently in fresh water. By

weight determinations before and after placing a number of these fishes directly or gradually into fresh water or into sea-water diluted considerably with fresh water, he found that a considerable increase or decrease in the weight of the fishes resulted from changes in the salinity and hence osmotic pressure of the external medium, and came to the conclusion that though the osmotic condition of the blood and the external fluids in the teleostei are so different, yet the membrane of the gills is both permeable to water and probably to a smaller extent to salts. This is opposed to the views of Fredericq (4) who states that the membranes of teleosts are effective barriers to the external medium, and to Garrey who also supposes an impermeability. Fredericq (4) makes, moreover, the statement that the blood of salt-water fishes does not taste or tastes scarcely more salt than that of freshwater fishes, and that the muscles and glands of a salt-water fish contain no more salt than that of a freshwater one. Griffiths (8) also states that the blood of a sole or haddock does not contain more soluble salts than that from freshwater fishes. With regard, however, to these estimations of chlorine and salts, there seems to be some confusion. Atwater gives figures which are 15 per cent. lower for chlorine in the constitution of freshwater fishes than in marine, his average being 0.235 per cent. Cl for marine fishes, Quinton (11) gives the chlorine percentage of the blood of eight species of marine teleosts as 0.651 and freshwater teleosts as 0.411. This, as Sumner (13) points out, is much greater than the chlorine contents of the body as a whole, and also shews that the salinity figures for marine fishes are almost 50 per cent. greater than for freshwater fishes. Sumner has, unfortunately, not given the osmotic pressures as determined directly by the freezing point method for the blood of the fishes in which the changes in weight occurred in his experiments. If the gills are semi-permeable, that is only permeable to water, or to a small extent for salts in addition to this, then the osmotic pressure and chlorine contents of the blood of freshwater fishes should be lower than that of marine fishes, and this change should be found to take place in those teleosts which pass from the one medium to the other, like the eel and salmon. Garrey states that transferring common eels

from salt water to fresh water did not lower the osmotic pressure of the blood. Greene (7), on the Physiology of the Chinook Salmon, finds a lower osmotic pressure in those caught in fresh water, but thinks the small difference may be due to absence of food or to changes in the metabolism due to the changes in habits and the breeding period.

In view of these conflicting results I was encouraged to take up this investigation through a suggestion of Prof. Brandt at Kiel, and to examine the osmotic pressures and chlorine contents of the blood of fishes living under natural conditions and not alone under the experimental aquarium conditions. This is in many cases a matter of great difficulty, and most of the experiments previously made, including Sumner's, have been performed under the somewhat artificial conditions of the aquarium. It seemed very important, therefore, to supplement the aquarium results, and I was fortunately able to do so by obtaining permission to take my apparatus on the German investigation steamer 'Poseidon' on one of the expeditions from Kiel through the Kattegat and Skagerack to the North Sea. In this way I was able to examine fish from water of gradually varying density, and the number was only limited by the very bad weather this last February rendering it often both impossible to trawl for the fish and to perform any other experiments.

The first determinations were made at Kiel University, where fish were easily obtained living, since they are brought into the harbour at Kiel in submerged boxes and kept in the water until the actual moment of selling. The osmotic pressure has been determined by the freezing point method, and in order to make more certain of the correctness of the results, the freezing point of distilled water was determined before and after each series of observations. The Beckmann thermometer differed slightly from those in general use in having a shorter bulb so that only 10 to 15 c.c. of blood were necessary instead of the 18 to 20 c.c. usually required. This I found to be a most important acquisition, since it is often difficult to obtain larger quantities of blood from small specimens. The thermometer was made by Goetz, Leipzig. In almost all cases the blood was obtained by cutting the caudal artery and withdrawing the blood with a pipette.

It was always easy to obtain blood in this way, if the fish was actually living at the time and the heart beating. This occurred in every case, so that the blood was taken from the living fish and the osmotic pressure determined immediately, allowing no errors to creep into the results from decomposition. It has been stated by Hamburger (14) and Hedin (15) that it is the same whether one uses the blood, blood plasma, or serum, because the blood corpuscles in suspension have as little effect on the osmotic pressure as sand grains, but no decomposition should have taken place. Further investigations are being made with regard to this point but need not be considered here since for purposes of comparison the blood for the following experiments was always taken in the same way and the whole blood was used in every case.

The *depression* of the freezing point is expressed here in the usual way as Δ and the comparisons are made in terms of this depression. The actual pressure in atmospheres can be found by multiplying the depression in degrees by $\frac{22.4}{1.85}$. After the determination of the Δ , the blood was removed from the Beckmann's apparatus, and a quantity carefully weighed and transferred to a porcelain crucible. Powdered chlorine-free sodium carbonate was added and the whole evaporated to dryness and then slowly incinerated by moderate heat to prevent any considerable loss of chlorine. The residue was extracted with hot distilled water, filtered, and determined by Volhard's method. To the filtrate nitric acid was added in slight excess. To this solution a definite quantity of $\frac{N}{10}$ Ag NO₃ was added from a burette, taking care to use more than required to precipitate the chlorine so that in the solution there is an excess of silver nitrate. The precipitated silver chloride was filtered off, and the amount of silver nitrate in the filtrate determined by titrating with potassium sulphocyanide, using iron ammonium alum as indicator. This gives the quantity of excess, and by subtracting it from the quantity originally taken one has the amount of standard silver equivalent to the chlorine in the solution, from which the chlorine percentage can be easily reckoned out.

The experiments made have been grouped into series according to time and place, beginning at Kiel and ending at Helgoland. In every case where the Δ is given, this is the average of three determinations, the degree of 'under cooling' being kept small and, as far as possible, the same throughout.

Series I—Feb. 5th, 1908, Kiel Harbour :—

Sea water from Kiel Harbour Δ — 1.093°

Chlorine contents of harbour water 1.125 per cent.

Salt contents 2.033 per cent.

Blood from cod (*Gadus morrhua*) Δ — 0.720°

Chlorine contents of blood 0.50 per cent.

Series II, Feb. 6th, Kiel Harbour :—

Blood from *Gadus morrhua* Δ — 0.750°

Blood from *Gadus morrhua* Δ — 0.751

Chlorine contents of blood 0.50 per cent. and 0.503 per cent. respectively.

*Series III, Feb. 10th, Kiel Harbour :—*Blood taken from three specimens of *Pleuronectes platessa* (plaice), a very small quantity of blood is obtainable from a single fish.

Depression of the freezing point Δ = — 0.66°

Chlorine contents of the same blood 0.500 per cent.

The difference in the osmotic pressure between the blood of the cod and the plaice is here notable, and illustrates the differences which are found to occur amongst different species of teleosts from the same water.

*Series IV, Feb. 12th, Kiel Harbour :—*Blood from three large *Pleuronectes platessa* taken as in the previous experiment—

Depression of the freezing point Δ = — 0.650°

Chlorine contents of the same blood 0.531 per cent.

The blood for both determinations was a mixture of that from the three fishes used.

Depression of the freezing point for ovarian fluid from the same fishes Δ = — 0.630 .

*Series V, Feb. 16th, 1908—On S.S. 'Poseidon.'—*Baltic Sea, just outside the Kieler Bucht. Depth 27 metres. Temperature at bottom 1.88° C. Salts contents of bottom water 2.6 per cent., and of surface water 1.46 per cent.

Depression of the freezing point for bottom water Δ — 1.3°

The following determinations were made :—

Gadus morrhua Δ — 0.758°

Gadus morrhua Δ — 0.710

Gadus morrhua Δ — 0.730

For each of the following determinations, three fish were used :—

Pleuronectes platessa Δ = — 0.718°

Pleuronectes platessa Δ = — 0.720

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Series VI, Feb. 17th, 1908, S.S. 'Poseidon.' — Station, Kattegat III. Depth 21-24 metres. Between Jutland and Seeland. Temperature at bottom 2.99° C. Sp. gr. at bottom¹ 24.6. Salt contents: surface 2.93 per cent., and bottom 2.97 per cent.

A sample of bottom water gave	...	$\Delta = -1.665^{\circ}$
<i>Pleuronectes flesus</i> (2 specimens used)	...	$\Delta = -0.96$
<i>Gadus morrhua</i>		$\Delta = -0.715$
• <i>Pleuronectes platessa</i> (4 specimens used)	...	$\Delta = -0.73$
<i>Raia radiata</i>		$\Delta = -1.51$

This last named was the first elasmobranch examined, the great difference from the teleosts and the resemblance to the Δ for sea-water is noticeable.

CRUISE OF 'POSEIDON,' Feb.-March, 1908.—The stretches in black connected by the dotted line indicate the places where the trawl was used, and the numbers the series of determinations referred to in the text.

1. The figure given above (and in the succeeding determination for the Specific Gravity of the sea water) + 1000 equals the true Specific Gravity, that of distilled water being considered as 1000.

Series VII, Feb. 17th, S.S. 'Poseidon.'—Kattegat, near coast of Sweden and about thirty miles North of Seeland. 37-57 metres deep. Temperature at bottom 3.7° C. Sp. gr. at bottom 26.0. Salt contents : surface 1.93 per cent., bottom 3.15 per cent.

A sample of bottom water gave				...	$\Delta = -1.71^{\circ}$
1.	<i>Gadus morrhua</i>	...	...	...	$\Delta = -0.8$
2.	<i>Gadus morrhua</i>	...	..	...	$\Delta = -0.77$
	<i>Gadus aeglefinus</i>	...	...	...	$\Delta = -0.75$
	<i>Raia batis</i>	...	...	...	$\Delta = -1.820$
1.	<i>Acanthias vulgaris</i>	...	...	...	$\Delta = -1.820$
2.	<i>Acanthias vulgaris</i>	...	...	...	$\Delta = -1.795$

For the determination of the blood from *Gadus aeglefinus*, five or six fish were used. All the remaining fish were large, the cod being 1.3 metres in length. The increase in the depression of the freezing point for elasmobranch blood is to be noticed here, and the likeness between the two species.

Series VIII, Feb. 18th, S.S. 'Poseidon.'—Kattegat, between Denmark and Sweden, and direct East of Frederikshavn. 24-40 metres deep. Temperature at bottom 4.9° C. Salt contents : surface 3.447 per cent., bottom 3.445 per cent. Sp. gr. : surface, 28.2, bottom 28.2.

A sample of bottom water gave				...	$\Delta = -1.86^{\circ}$
1.	<i>Gadus morrhua</i>	...	...	...	$\Delta = -0.75$
2.	<i>Gadus morrhua</i>	...	...	...	$\Delta = -0.76$
	<i>Rhombus laevis</i>	...	...	...	$\Delta = -0.071$

Series IX, Feb. 19th, S.S. 'Poseidon.'—In Kattegat, direct East of and not far from Frederikshavn, Denmark. Conditions practically the same as above, both catches were trawled in the same stretch of water and no further observations taken.

<i>Rhombus maximus</i>				...	$\Delta = -0.79^{\circ}$
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Series X, Feb. 20th.—In Skagerack, West of and not far from Skager Point. Depth 92 metres. Temperature at bottom 5.02° C. Sp. gr., surface 28.4, bottom, 28.4. Salt contents, surface and bottom, 3.472 per cent.

A sample of bottom water gave				...	$\Delta = -1.893^{\circ}$
	<i>Gadus morrhua</i>	...	...	...	$\Delta = -0.74$
1.	<i>Anarrhichas lupus</i>	...	...	...	$\Delta = -0.84$
	<i>Anarrhichas lupus</i>	...	...	...	$\Delta = -0.73$
	<i>Cyclopterus lumpus</i>	...	...	...	$\Delta = -0.66$

The *Anarrhichas* were large specimens, length respectively 78 and 71 centimetres. The rather remarkable difference between these two fishes with regard to the osmotic pressure is another case of the variation sometimes noticed for the same species, in the same water. The other interesting feature here was the low osmotic pressure of the *Cyclopterus* blood.

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Series XI, S.S. 'Poseidon,' Feb. 21.—Skagerack, middle of channel, between Norway and Denmark. Depth 130-192 metres. Bottom temperature 6.76° C. Sp. gr.: surface 28.1, bottom 28.6. Salt contents: surface 3.434 per cent., bottom 3.519 per cent.

Freezing point of the bottom water	...	...	...	$\Delta = -1.92^{\circ}$
<i>Lophius piscatorius</i>	...	...	...	$\Delta = -0.63$
<i>Gadus aeglefinus</i>	...	...	...	$\Delta = -0.74$

The *Lophius* weighed 14 kilos and was, therefore, of considerable size; the blood was easily obtained, and the extremely low osmotic pressure is rather remarkable for a marine fish.

Series XII, S.S. 'Poseidon,' Feb. 26th.—Middle of channel on the boundary of Skagerack and North Sea. Depth 54 metres. Bottom temperature 5.31° C. Sp. gr.: surface 28.55, and bottom 28.65. Salt contents: surface 3.499 per cent., bottom 3.511 per cent.

A sample of bottom water gave	...	...	...	$\Delta = -1.96^{\circ}$
<i>Anarrhichas lupus</i>	...	...	...	$\Delta = -0.74$
<i>Lota molva</i>	...	...	...	$\Delta = -0.66$
<i>Hypoglossus vulgaris</i>	...	...	...	$\Delta = -0.78$
<i>Lophius piscatorius</i>	...	...	...	$\Delta = -0.68$
<i>Gadus pollachius</i>	...	...	...	$\Delta = -0.73$
<i>Raia valonia</i>	...	...	...	$\Delta = -2.0$

The fish used for the above determinations were all of very large size, the *Lota molva* being 131 centimetres long and weighed 17.5 kilos.

Series XIII, S.S. 'Poseidon,' March 5th.—North Sea about 100 miles West of Stavanger, Norway. Depth 105 metres. Bottom temperature 6.78°. Sp. gr.: surface 28.2, and bottom 28.6. Salt contents: surface 3.467 per cent., and bottom 3.512 per cent.

Bottom water	...	...	...	$\Delta = -1.95^{\circ}$
<i>Gadus morrhua</i>	...	...	...	$\Delta = -0.70$
<i>Gadus virens</i>	...	...	...	$\Delta = -0.71$
<i>Gadus aeglefinus</i>	...	...	...	$\Delta = -0.78$
<i>Lota molva</i>	...	...	...	$\Delta = -0.68$

The *Gadus morrhua* has a remarkably low osmotic pressure here, which is probably pathological; extremely few specimens were present in the trawl.

Series XIV, S.S. 'Poseidon,' March 7th.—North Sea, about 70 miles North-West of Helgoland. 40 metres deep. Bottom temperature 4.53° C. Sp. gr.: surface 28.5, bottom, 28.5. Salt contents: surface 3.479 per cent., and bottom 3.485 per cent.

Bottom water	...	...	...	$\Delta = -1.90^{\circ}$
1. <i>Gadus morrhua</i>	...	...	...	$\Delta = -0.73$
2. <i>Gadus morrhua</i>	...	...	...	$\Delta = -0.79$
3. <i>Gadus morrhua</i>	...	...	...	$\Delta = -0.75$
4. <i>Gadus morrhua</i>	...	...	...	$\Delta = -0.77$
<i>Raia clavata</i>	...	...	...	$\Delta = -1.99$

With the exception of the Ray and the specimens of *Gadus morrhua*, the contents of the trawl were small plaice. The four specimens of *Gadus morrhua* were used to determine to what extent variations might occur in the same species caught in the same water.

Series XV, Helgoland, April, 1908.—

Blood from three specimens <i>Pleuronectes platessa</i>	...	$\Delta = -0.78^{\circ}$
" " three " " "	...	$\Delta = -0.848$
" " five " " "	...	$\Delta = -0.750$
" " three " " "	...	$\Delta = -0.773$

Chlorine estimation for blood used in the second and third of the above determinations 0.60 per cent.

Chlorine estimation for blood used in the last determination 0.537 per cent.

Series XVI, Helgoland, May, 1908.—

<i>Gadus morrhua</i> —blood	...	$\Delta = -0.748^{\circ}$
Blood from two specimens <i>Gadus morrhua</i>	...	$\Delta = -0.778$
Blood from five specimens <i>Gadus morrhua</i>	...	$\Delta = -0.748$

Chlorine estimation for blood from the first specimen 0.507 per cent.

Chlorine estimation for blood from the cod used in the last two determinations 0.530 per cent.

Freezing point for bottom, water $\Delta = -1.90$.

Salt contents of bottom water 3.485 per cent.

The first point to be noticed in this series of estimations, is the great variation that occurs both amongst different specimens of the same species as well as between the different species themselves. It shews the great necessity of making a number of determinations before deducing any theories. For the teleosts examined the Δ varied from -0.63 to -0.96 and this was not due to any great difference in the external medium since the fishes with the average -0.96 came from water with a lower Δ than the fish with $\Delta 0.63$. It was therefore obvious that the different species could not be compared directly together unless specimens of the same species had been caught at all

or at many stations. Now the most important determinations are those made at Kiel and Helgoland where the convenience of a laboratory was at hand, and unfortunately only two species could be obtained living at Kiel, namely *Gadus morrhua* and *Pleuronectes platessa*. Fortunately, however, the codfish were caught at almost every station, and so form an interesting series; and plaice were also caught in Helgoland to allow of a comparison with the Baltic Sea specimens. The following table gives the determinations made on the plaice:—

Place where fish were caught	Δ for blood	Δ for sea-water	Salt contents of sea-water per cent.	Chlorine in blood per cent.
Kiel	- 0.66 } - 0.65 }	- 1.093	2.032	{ 0.500 0.531
Series V.—In Baltic Sea ...	- 0.718 } - 0.72 }	- 1.3	2.60	—
Series VI.—Kattegat ...	- 0.73	- 1.66	2.97	—
Helgoland	- 0.78 } - 0.848 } - 0.750 } - 0.773 }	- 1.90	3.48	{ 0.60 0.537

It will be seen that a direct increase in the osmotic pressure of the blood takes place as the density and osmotic pressure of the sea-water increases. The average for Kiel is - 0.655, and for Helgoland - 0.787; that is, whilst the sea-water bathing the fish has increased in osmotic pressure so that the Δ has changed from - 1.093 to - 1.90, an increase of 74 per cent. or almost 10 atmospheres, the osmotic pressure of the blood has increased by 20.1 per cent. or about 1.5 atmospheres.

This is a very interesting result and shows that the plaice at least have an osmotic pressure which is dependent to a certain extent on the sea-water, though nothing like the Elasmobranchs, since an increase of 74 per cent. in that of the sea-water produces an increase of but 20.1 per cent. in the fish. The chlorine contents also shew an increase corresponding to the increase in the osmotic pressure; the average for Kiel being 0.515 per cent., and for Helgoland 0.557 per cent. The relation of these changes will be discussed after the results for the cod are given in tabulated form.

Results of Chlorine and Osmotic Pressure Determinations for the Cod (Gadus morrhua)

Place where fish were caught	Δ for blood	Δ for sea-water	Salt contents of sea-water per cent.	Chlorine in blood per cent.
Kiel	-0.720	-1.093	2.033	0.50
	-0.750			0.503
	-0.751			0.50
Series V.—In Baltic Sea ...	-0.758	-1.30	2.6	—
	-0.710			
	-0.730			
Series VI.—In Kattegat ...	-0.715	-1.66	2.97	—
Series VII.—In Kattegat ...	-0.80	-1.71	3.156	—
	-0.77			
Series VIII.—In Kattegat	-0.75	-1.86	3.444	—
	-0.76			
Series IX.—In Skagerack ...	-0.74	-1.893	3.472	—
Series XIII.—North Sea ...	-0.70	-1.95	3.512	—
Series XIV.—North Sea ...	-0.73	-1.90	3.485	—
	-0.79			
	-0.75			
	-0.77			
Helgoland	-0.748	-1.90	3.485	0.507
	-0.778			0.530
	-0.748			

The figures in the above table form a rather startling contrast to those for the plaice. More specimens have been used for the estimations of codfish blood than that of any other species, perhaps forty or more in all, and never are two alike. There seems to be a very considerable variation in specimens of the cod taken from the same place, considerable when compared with other teleosts that I have examined since, and this variation is greater than the actual change of the blood between the two places Kiel and Helgoland.

If the average be taken for the first two series Kiel and Baltic Sea specimens the Δ is -0.73 ; the fish are from water of $\Delta - 1.093$ and $\Delta - 1.30$. In the same way the average for the fishes from Series VI, VII and VIII is -0.759 , the sea-water being $\Delta - 1.66$ — $\Delta - 1.86$, and lastly Series IX—Helgoland gives an average $\Delta - 0.757$ (leaving out the fish with $\Delta 0.70$ caught in Series XIII and which

is probably in a pathological condition), with sea-water $\Delta - 1.89$ — $\Delta 1.90$.

Thus if the averages of these three sets are taken, there is a slight increase between the fishes of the Kiel and Baltic Sea examined, and those of the Kattegat, Skagerack and North Sea, but instead of an increase of 20 per cent. as occurs with the plaice, there is only an increase of about 3.9 per cent. in the blood for an increase of 74 per cent. in the sea-water. I mention these averages in order to shew that if any steady variation can be said to take place it is in the direction of an increase in the denser water of the North Sea. The difference in the averages of the Kiel and Helgoland fishes taken separately from the others is as follows:—Kiel average $\Delta = - 0.740$, Helgoland $\Delta - 0.758$ —an increase of $\Delta - 0.018$, whilst the variation amongst specimens caught at the same place is in one case $\Delta - 0.73$ — $\Delta - 0.79$.

The conclusion arrived at therefore here, is that a slight increase of osmotic pressure occurs in the blood of cod caught in water of higher salt contents, as the North Sea, but that this increase is only small compared with that taking place in *Pleuronectes platessa* under the same conditions, and is furthermore overshadowed by individual variation.

Another point to be noticed in the series of experiments made on the 'Poseidon,' and which has been verified by further observations made at Helgoland, is the differences occurring between the species. The numbers given by previous observers, often from single observations, have not sufficiently emphasised these differences, since they may be put down in the same category as those occurring between individuals of the same species. If, however, averages are taken for different teleosts, it will be found that in spite of variation there is a 'mean' for each species which is not the same. Thus, for example, *Lota molva* and *Lophius piscatorius* have a low osmotic pressure average $\Delta = - 0.65$, whilst that for *Pleuronectes flesus* is much higher averaging about $\Delta = - 0.85$, for fish caught in sea-water of $\Delta - 1.90$.

The figures for the Elasmobranchs agree very satisfactorily with what was expected from the results of Botazzi; there is little variation between different species caught in the same water, the

osmotic pressure is almost the same as that of the surrounding sea-water, and as this increases in density so does the osmotic pressure of the blood change. Thus the first Elasmobranch caught on the voyage, a specimen of *Raia radiata*, gave $\Delta - 1.51$, the Δ for the sea-water being $- 1.66$, and later when the osmotic pressure of the sea-water had increased and the Δ was $- 1.95$ — $- 1.98$, two specimens, *R. valonia* and *clavata*, gave for the blood $\Delta - 2.0$ and $- 1.99$ respectively.

In view of the small amount of change taking place in the blood of the cod and on account of the above experiments generally, it was thought advisable to determine the osmotic pressure and chlorine contents of some freshwater teleosts that were obtainable in Kiel, and particularly for the eel, in order to see what difference existed in the blood of typical freshwater fishes. The specimens had all been kept in fresh water in large well-aerated tanks for a few days from the time that they had been removed from the breeding ponds and freshwater lakes in Schleswig Holstein in the neighbourhood of Kiel.

Tank water in which fishes were living				...	$\Delta - 0.020^{\circ}$
Carp	...	...	...	blood	$\Delta - 0.487$
<i>Abramis brama</i>	...	...	...	„	$\Delta - 0.510$
Eel (<i>Anguilla vulgaris</i>)	...	...	...	„	$\Delta - 0.570$

The chlorine estimations for the blood of the above fishes gave the following results:—

Carp	blood contained...	...	0.2 per cent. Cl
<i>Abramis brama</i>	do.	...	0.253 „ „
Eel	do.	...	0.277 „ „

Thus the osmotic pressure and chlorine contents of the blood of freshwater fishes is much below that of marine teleosts, and the statements of Griffiths and Fredericq with regard to the salt contents of freshwater fishes cannot be regarded as correct, for the marine teleosts have about 50 per cent. greater chlorine and, therefore, salts contents. These figures agree with those of Quinton's for other freshwater teleosts, and it must be observed that the chlorine contents of the blood is much higher than the chlorine contents of the body, or the whole fish.

These determinations were completed by investigating the

changes taking place in the blood of the freshwater eel when placed in sea-water, and I am at present engaged on the complementary experiments with the same species of eel caught in the sea. A large eel taken from the freshwater tank was placed abruptly into slightly diluted North Sea water, the Δ for which was -1.59 . This abrupt change was followed by a copious secretion of mucous, but after a few hours the specimen appeared quite normal. After an interval of six hours in this water the blood was taken and examined.

Blood	...	...	$\Delta - 0.66^\circ$
Chlorine	...	...	0.367 per cent.

There is evidence here of considerable change, for, compared with the Δ for the eel in fresh water, the freezing point is 16 per cent. lower and the chlorine contents have also increased considerably.

Another still larger specimen was therefore taken, as before, directly from fresh water and placed this time in sea-water rather more diluted with fresh (sp. gr. 20.3). This specimen endured the change more calmly and without such a mucous secretion. The following was the procedure :—

10.30 a.m.—Eel placed abruptly from fresh water into diluted sea-water, sp. gr. 20.3 .

3.45 p.m.—Sp. gr. of water increased to 25.5 by addition of North Sea water.

6.45 p.m.—Sp. gr. of water, further increased to 29.3 by addition of some concentrated sea-water.

The eel remained overnight in this water and at 10.30 a.m. the next morning, after a sojourn of twenty-four hours in sea-water, the eel being quite healthy in appearance, the osmotic pressure of the blood was determined.

Blood from eel after 24 hours in sea-water ... $\Delta = -0.745^\circ$

Hence the osmotic pressure had increased until it attained a pressure which is about an average for marine teleosts.

The osmotic pressure of the Teleostei in fresh water averages about $\Delta - 0.527$, and for those in North Sea water the Δ is about -0.750 , hence for those fishes like the eel and salmon which pass from the sea to the rivers there must be a place where the external water is isotonic with the blood. These fishes are, in short, capable

of passing from a hypertonic fluid to a hypotonic fluid without injurious effects following. Of the marine teleosts which do not pass far into the rivers there are many which are found in the Baltic Sea, far East of Kiel, for example, *Cottus scorpius*, *Pleuronectes flesus*, *Gadus morrhua*, *Lota vulgaris*, *Nerophis ophidion*, occurring frequently together with others only occurring as visitors. Whether these species that occur frequently have passed their whole lives in this area is a question. In all probability they do pass their whole lives and lay their eggs in this region. Now it will be seen from hydrographical tables that in the North-East Baltic where the fishes named above occur, the water has a salt contents below that of the fishes' blood and is hypotonic. Thus marine teleosts are often found in waters isotonic and hypotonic to the blood. The Elasmobranchs on the other hand are only rare and as visitors in the West Baltic, and altogether absent from the South-East and North-East Baltic. This appears to be correlated with the fact that the constitution and osmotic pressure of the blood is much more dependent on the osmotic pressure of the external media than is the case in the Teleostei, but further experiments on the resistance of teleosts and elasmobranchs to changes in the constitution of the sea-water are being made and the results will be discussed later.

CONCLUSION AND SUMMARY

It has been shewn by previous observers that the marine teleosts have an osmotic pressure and salt contents differing to a great extent from that of the sea-water bathing their bodies, and in this respect shew a remarkable contrast to both the marine elasmobranchs and invertebrates. Certain workers have assumed from this that the body membranes separating the blood and internal fluids from the sea-water were impermeable barriers to water and salts. Sumner, by weight and chlorine analyses came to the conclusion that this was not the case, and that to a certain extent both water and salts could pass through these bounding membranes.

Now we may assume here four possibilities for the bounding membranes :—

1. The membranes are impermeable to water and to salts.
2. The membranes are semi-permeable, allowing water to pass through but impermeable to the dissolved salts.
3. The membranes are permeable to water and in a slight degree to salts.
4. The membranes are permeable to both water and salts.

If the first of these possibilities held good, it would be easy to understand how the teleosts retained their low osmotic pressure and salt contents, against the influence of the sea-water, but no change either in osmotic pressure or weight should then take place if the animals are placed in different media. This change does take place, for the experiments shew that in natural conditions the freshwater teleosts have a lower osmotic pressure than the marine forms, that this changes with the alterations in the water (as for example in experiments with the eel), and that even alterations in the salt contents prevailing in the sea, influences the osmotic pressure of the blood. Thus we may consider the first possibility, of the teleosts having impermeable membranes, as quite disproved.

If the fourth possibility held good and the membranes were **directly** permeable to both water and salts, then the conditions for **an osmotic** change would not prevail and no change in weight should **occur** if the fishes were taken from salt-water and placed in fresh water. Sumner has shewn, however, that a change does occur and **that water** passes osmotically into the fish, increasing the weight. **Moreover**, if this was the case it would be difficult to conceive of **the alterations** in the osmotic pressure of the external media—for **example** the difference between that of the Baltic Sea water and the North Sea water, or the fresh and salt water in the case of the eel experiments—producing such a little change in the constitution of the blood. Furthermore, it would mean that some organ or organs were continually at work to such an extent that the osmotic pressure and salt contents of the marine teleosts were kept regularly at about one-third of that of the water bathing the outside of the membranes under consideration. This fourth possibility, is therefore, also impracticable, and we are reduced to the second and third.

If the limiting membranes of the body are semi-permeable, that is, permeable to water but not to salts, then perfect osmotic conditions are set up, and any such increase of the osmotic pressure on the outside of the membrane as takes place in passing from the Baltic Sea to the North Sea, would cause a corresponding increase in the osmotic pressure and chlorine contents of the blood, since more water would pass out from the blood and body fluids through the membranes into the sea and leave the blood and fluids more concentrated. Similarly, when placing an eel from fresh water into salt water, the great increase in osmotic pressure in the external medium should cause water to leave the blood and body fluids, making the concentration and osmotic pressure of these latter higher than before. This is exactly what takes place in the experiments. Sumner assumes also a slight permeability to salts, but only slight, so that the osmotic conditions are preserved and exactly the same state of things would occur. Hence we must conclude that the bounding membranes of the teleostei are either semi-permeable, permeable for water and not for salts, or to a small extent for salts. I am of opinion at present that the permeability is only for water, and that Sumner's experiments do not indicate conclusively a permeability for salts; this, however, will be discussed in a later paper. The increase in chlorine contents in these experiments does not prove that chlorine has passed into the blood, since the same result would be obtained by water passing out, and therefore increasing the concentration. If now we conclude that either of these two possibilities holds good, and the experiments prove this to be the case, then how do the marine teleosts contrive to maintain an osmotic pressure and salt contents much lower than that of the external medium, whilst freshwater teleosts have a higher osmotic pressure and salt contents than the external water?

Allowing that the membranes are permeable to water, then there must be a continual stream of this fluid from the bodies of marine teleosts outwards into the sea-water and *vice versa* in the freshwater teleosts, but in spite of this the constitution of the blood remains practically constant. I believe this to be accounted for by the three following assumptions:—

1. The permeability for water is not very great.
2. The permeable membranes are of small extent.
3. The actual loss or gain in water by the blood is counteracted by resorption and secretion.

The permeable membranes of the teleosts appear to be confined to the gills, the body walls of the fish being impermeable, because fresh water has practically no harmful effect on a marine teleost if the body part only is immersed in it, and sea-water flows over the gills. The permeability of the gills for water must not be great or one would have the secreting organs working continually at 'high pressure' to keep the blood under constant conditions against the action of the external media. Since, however, an increase of 74 per cent. in the osmotic pressure of the external fluid produces a much less increase in that of the blood, only 3.9 per cent. in the case of the cod, the amount of water passing from or into the fish must not be large. It is, however, large enough to show that the blood of the teleosts is not altogether independent of the external medium and a considerable alteration in the constitution of this latter is accompanied by a corresponding alteration in the constitution of the blood in defiance of any organs working to maintain this constitution constant.

SUMMARY

1. The blood of marine teleosts has a considerably higher osmotic pressure than that of freshwater teleosts.
2. The change of density in the sea-water from Baltic to the North Sea is accompanied by a change, though small, in the osmotic pressure of the blood.
3. This same change occurs when a common eel is taken from fresh water and placed in sea-water, the osmotic pressure changes from that typical of freshwater teleosts to a much higher pressure, about the average for typical marine teleosts.
4. These changes indicate a permeability of the bounding membranes, probably only the gills, to water.
5. The teleosts contrive to maintain, partly by physiological

means, an osmotic pressure for the blood which is almost independent of the external water, and only great changes in the constitution of the surrounding medium affect this constancy.

6. Though considerable variations occur in any one species of teleost, in water of the same density, yet there is a 'mean' for the osmotic pressure of the blood which is distinct and peculiar to the respective species.

7. Different species react differently to the same changes in the outer medium.

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