VARIATIONS IN THE OSMOTIC CONCENTRATION OF
THE BLOOD AND COELOMIC FLUIDS OF AQUATIC
ANIMALS, CAUSED BY CHANGES IN THE EXTERNAL
MEDIUM

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From the 'Königliche Biologische Anstalt' on Helgoland

(Received October 27th, 1908)

In a previous paper in this journal, the results of a series of experiments were given, made to determine to what extent the constitution of the blood of fishes was dependent upon the concentration of the water, sea or fresh, bathing their bodies.

It was shown then, that although the teleostei maintained an almost constant osmotic pressure of the blood, which was only about one-third that of sea water from the open sea, yet at the same time they were slightly dependent upon the outer medium, and the blood was not absolutely unaffected by changes in the latter as affirmed by many writers. The concentration of elasmobranch blood was on the other hand practically identical with the sea water.

The following is a continuation of this work; and though the conditions prevailing in invertebrates has also been considered, they will only be touched upon here since a very good summary of researches on osmotic pressure has been published this year by Bottazzi.¹

The study of the physico-chemical conditions of the salts of the blood and internal media of the body, can be followed along three closely related paths dealing with:—(1) The relation of the constitution of the blood of aquatic animals to the medium bathing their bodies. (2) The processes by which changes in the external

1. Bottazzi, *Ergebnisse der Physiologie*, Ab. I and II, Jahr. 7, 1908.

media affect the blood. (3) The effects of such changes in the external medium upon the life of the animal. This report deals chiefly with the first of these, and the results tend to show that the blood and coelomic fluids of both aquatic invertebrates and vertebrates are in equilibrium with the external media though perhaps differing very much from it, and any change in the constitution of the latter brings about a gradual change in the blood, to a greater or less degree in the different groups, until a new equilibrium is set up.

VARIATIONS IN WEIGHT, AND CONSTITUTION OF THE BLOOD OF INVERTEBRATES, ACCOMPANYING CHANGES IN THE EXTERNAL MEDIUM

It is now well known that the blood and internal media of marine invertebrates is practically identical with the sea water in which they are living. Fredericq in 1885¹ showed that by altering artificially the concentration of the sea water, the blood of the animals changed similarly, and stated, moreover, that the time taken for the blood to become once more like the altered external medium, was not the same for different species. Garrey² found also that the exposure of *Limulus* to the drying influence of the atmosphere caused the freezing point to change, and similarly a sojourn in a damp cellar affected the concentration of the blood. He showed further that when other marine invertebrates, *Nereis*, *Homarus*, *Chaetopterus*, etc., were kept in diluted sea water, the concentration of the blood became less, and entrance of water took place, since the animals were frequently swollen after such immersion.

The following experiments indicate the changes in weight that I found to take place with some common marine invertebrates. The animals were all taken direct from sea water, the surplus water removed as far as possible, and then weighed. A control experiment showed that the amount of error due to adherent water was not great enough to influence the results.

1. Fredericq, *Archiv. de Zool. exper.*, 2 série, T. III, 1885.

2. Garrey, *Biol. Bull.*, Vol. VIII, 1905.

Species	Weight when taken from sea water	Time of immersion	Weight after immersion
<i>Cancer pagurus</i>	311 grms.	4 hrs.	320 grms.
(edible crab)	355 grms.	6 hrs.	362 grms.
„	170 grms.	6 hrs.	180 grms.
„	302 grms. (two specimens)	3 hrs.	312 grms.
	These two specimens after the three hours in fresh water were replaced in sea water, and after	3 hrs. 20 mins.	305 grms.
<i>Hyas araneus</i>	118 grms. (two specimens)	3 hrs.	123 grms.
(sand crab)	36.5 grms. (two specimens)	1 hr.	38.25 grms.
	These two specimens were now replaced in sea water, and after	2 hrs.	34 grms.
<i>Doris tuberculata</i>	52 grms. (three specimens)	2 hrs.	55 grms.
<i>Arenicola marina</i>	34 grms. (two specimens)	$\frac{1}{2}$ hr.	38.5 grms.
(lug worm)	These two specimens were now replaced in sea water, and after	$\frac{1}{2}$ hr.	35 grms.

These experiments indicate the rate of increase in weight, due to absorption of water, when marine invertebrates are placed in fresh water; and it will be seen from the above results with the edible crab, the sand crab, and the lug worm, that if they are replaced in sea water after a short interval, before they are seriously injured by the fresh water, they recover, lose water and diminish in weight accordingly.

Variations in Freezing Point.—The following experiments show the effects of a sojourn in hypertonic and hypotonic sea water, on the freezing point of blood and coelomic fluid of invertebrates. The hypertonic sea water was made by adding NaCl, KCl and CaCO₃ (in the proportions in which they occur in sea water), to that medium; the hypotonic by dilution of ordinary sea water with fresh.

Species	Depression of freezing point of blood or coelomic fluid in sea water: ($\Delta - 1.91^\circ$)		Hyper and hypotonic solutions used		Time of Immersion hours	Depression of freezing point of blood or coelomic fluid at end of experiment	Cl contents of blood at end of experiment per cent.
	Δ	Cl					
<i>Cancer pagurus</i> (blood)	-1.845°	-1.915°	-0.575	0.575	11	-1.402	1.05
<i>Cancer pagurus</i> (blood)	-1.845°	-1.915°	-3.070	3.075	11	-2.785	2.3
<i>Echinus esculentus</i> (sea urchin)	-1.860		-0.76	—	3½	-1.77	Note.—Cl per cent. in the normal blood is 1.7 per cent.
(coelomic fluid)	-1.860		-2.98	—	3½	-2.065	
<i>Asterias rubens</i> (starfish)	-1.860		-0.965	—	3½	-1.36	So much water was withdrawn by the hypertonic solution, that the amount of coelomic fluid was insufficient for determination
(coelomic fluid)			-3.00	—	3½		

In all cases, in the above experiments the normal equilibrium of the blood or other internal fluid with the sea water was disturbed, and an attempt was made to set up a new one. Eleven hours were insufficient for the blood of the crab to attain its normal relations to the external medium, and any longer immersion than this would have been fatal; just as longer immersion than three and half to four hours would have caused death of the *Echinus* or *Asterias*, and also without an equilibrium having been reached.

The osmotic pressure of the blood of invertebrates has been regarded as wholly dependent upon the external medium, that is, identical with it, the relations of the external and internal media being determined simply by osmosis through the body membranes. It is more likely that here also, there is a definite equilibrium between the blood and internal media on the one hand and the sea water on the other, even though normally the osmotic pressure of both is almost identical. Small differences very often occur, and I do not consider these due to errors in observation. Furthermore, it has been often forgotten that the blood of the freshwater crayfish has a Δ of -0.8° though living in water whose depression of the freezing point may be almost nil. This is a perfect case of an equilibrium between

the blood of an aquatic invertebrate and the external medium, yet both fluids differ considerably in concentration. The freshwater crustacea are, therefore, very similar to the freshwater fishes (Teleostei).

OSMOTIC PRESSURE OF BLOOD OF TELEOSTS AND ELASMOBRANCHS AND
CHANGES PRODUCED BY ALTERATIONS IN THE CONCENTRATION OF
THE EXTERNAL MEDIA

It has already been shown by me¹ that the concentration of the blood of teleosts is not altogether independent of the external medium.

Thus the teleosts in the fresher water of the Baltic have a slightly lower osmotic pressure for the blood, than the same species in the North Sea. Freshwater teleosts also have a slightly lower osmotic pressure and salt contents than marine species, and the eel (*Anguilla vulgaris*) when placed in sea water was shown to change so that after several hours the osmotic pressure of the blood had risen to about the average for marine teleosts.

The following are the results of experiments made to determine the effects of dilution of the sea water upon the blood.

Pleuronectes flesus (flounder).—This fish though a typical marine flat fish, often penetrates a considerable distance into river estuaries, so that it may even be found in fresh water. It returns, however, to the salt water of the sea at spawning times, and hence resembles the eel to a certain extent, since both move back into the sea—a reversal of the rule of the salmon, which ascends the rivers for spawning.

The freezing point and Cl contents of the blood have been determined for fish caught round Helgoland in water whose $\Delta = -1.91^\circ$, and also for some fish caught in the fresh water of the Elbe and forwarded by steamer.

HELGOLAND.—*P. flesus*—

Δ for blood from caudal artery -0.883° , -0.903° , -0.903° .

RIVER ELBE, HAMBURG. *P. flesus*—

Δ for blood from caudal artery -0.68° .

1. *Bio-Chem. Journ.*, Vol. III, p. 258, 1908.

There is, therefore, a very considerable difference in the osmotic concentration of the blood of the flounder, according as it is living in fresh water or sea water respectively.

The following experiments were all carried out in two large concrete tanks into which sea water or rain water from a reservoir, could be pumped as desired. The rain water was examined and found to be much purer than the fresh water from springs on Helgoland. When fresh water or a mixture of this and sea water was used, the water was well aerated by compressed air driven through pieces of cane. With sea water, the water was also kept running.

Cyclopterus lumpus (the Lump-sucker)—

Normal fish from aquarium tank. Blood $\Delta - 0.648^\circ$

Normal fish from aquarium tank. Blood $\Delta - 0.658^\circ$

Experiment I.—One specimen placed in a mixture two-thirds fresh and one-third sea water, six hours, then in three-quarters fresh and one quarter sea water for two hours. Killed after eight hours in diluted sea water. Blood $\Delta - 0.620^\circ$.

Experiment II.—One specimen placed in diluted sea water ($\Delta - 0.70^\circ$) for two hours and finally in fresh water for 24 hours. Blood $\Delta - 0.597^\circ$.

Experiment III.—One specimen in diluted sea water ($\Delta - 0.37^\circ$) for 48 hours. Blood $\Delta - 0.610^\circ$.

In each case a reduction of the osmotic pressure took place, or in other words, a new equilibrium was either set up or in process of being set up. Taking the mean for the normal blood as $\Delta - 0.653^\circ$, the amount of change in the three experiments was -0.033° , -0.056° , and -0.043° respectively.

The coelomic fluid and fluid from the stomach of the last specimen of *Cyclopterus* was also examined in order to determine their relations to one another and to the external medium. The results were :—

Fluid from coelom $\Delta - 0.617^\circ$

Fluid from stomach $\Delta - 0.36^\circ$

From a normal fish in sea water—

Fluid from coelom $\Delta - 0.690^\circ$

Fluid from stomach $\Delta - 1.593^\circ$

Thus, the coelomic fluid has its own equilibrium, the freezing point being a little lower than that of the blood but very similar. The contents of the stomach on the other hand have a Δ which is directly dependent upon the external medium swallowed, the

food and secretion. The very different Δ 's of the coelomic fluid and contents of the alimentary canal in the last experiment are striking, since both are only separated by the thin walls of the intestine.¹

The Eel. Anguilla vulgaris.—In my previous paper, the results of some experiments made on the eel, taken from fresh water, were given, and it was shown that the concentration of the blood increased when the fishes were placed in sea water.

The blood of three freshwater species then examined gave the following figures :—

Carp	...	...	...	$\Delta - 0.487^{\circ}$
<i>Abramis brama</i>	...	...	...	$\Delta - 0.510^{\circ}$
Eel	...	...	...	$\Delta - 0.570^{\circ}$

After twenty-four hours in concentrated sea water, sp. gr. 29.3, the Δ of the blood of the eel had increased to -0.745° .

The following supplementary experiments have been carried out with the same species of eel, but caught in the sea off Helgoland :—

Blood from normal specimens taken direct from sea water ...	$\Delta - 0.649^{\circ}$
" " " " ...	$\Delta - 0.620^{\circ}$

These figures are very interesting, for in the first place they confirm the aquarium experiment at Kiel in showing that the concentration and osmotic pressure of the blood of the eel is greater in the sea than in fresh water, and further show that the osmotic pressure reached by the blood ($\Delta - 0.745^{\circ}$) in the Kiel experiment was too high. Perhaps the rapid change from fresh water to sea water at Kiel caused the blood to pass beyond the normal equilibrium

1. It had been noticed that when marine fish were kept in fresh water, there was a gradual, but small, lowering of the osmotic pressure until death. Just before death, however, the diminution was considerable. It was in fact as if the teleost resisted the action of the external medium as far as possible. This was partially confirmed by a determination of the freezing point of a *Cyclopterus* in aquarium water, but in a pathological condition.

Cyclopterus appears in the shallow water round Helgoland, often adhering to the rocks and left dry by the tide, during the spawning season in spring only. In May and June it begins to disappear, and in summer seems to have left for deep water, since very few are caught. The fish kept in the aquarium, though living well in spring, almost all die as the time approaches when they should normally leave for deep water. Death comes gradually, and the jaws and bony scutes appear to decay, so that the fish presents for some time a very unpleasant appearance.

The freezing point of the blood of all the normal specimens of *Cyclopterus* was about -0.658° . One of these pathological specimens which was examined had the remarkable value $\Delta - 0.765$, or -0.107° more than the normal. It may be said, therefore, that the concentration of the blood of pathological specimens tends to approach that of the fluid in which they are living.

point for sea water, and after a longer immersion in that water it would probably have attained the more normal $\Delta - 0.634^\circ$, the mean for those specimens taken direct from the sea at Helgoland.

As converse experiments to those at Kiel, some specimens were placed in aerated fresh water, the change being gradual.

Eels placed direct in mixture half sea and half fresh water, and fresh water pumped in at intervals until the whole was perfectly fresh. The eels finally remaining in fresh water for three days.

Results :—(a) Blood $\Delta - 0.580^\circ$
(b) Blood $\Delta - 0.583^\circ$
(c) Blood $\Delta - 0.582^\circ$

Thus the osmotic pressure had decreased, and the freezing point ascended until it was practically the same as that recorded at Kiel for the fish from fresh water.¹

All the experiments, therefore, made on the teleosts show that the blood is in equilibrium with the sea water or the fresh water in which the fish are living, and a change in the external medium is followed by processes setting up a new equilibrium. This change may or may not prove fatal to the animal. The relation of the osmotic pressure of the blood of teleosts to the external medium is very like the relation of the temperature of mammals to their surroundings. In the mammals the temperature is normally nearly constant, yet not altogether independent of the temperature of their environment, for a great rise or fall in the temperature of the latter produces a corresponding but smaller change in that of the body. In short, the temperature of the body is in equilibrium with the temperature of the surroundings though both may or may not be identical.

1. It is interesting to note that the freezing point for the eels from the sea is the *biggest* of any marine teleostean examined by me, and further that the freezing point of the blood taken from freshwater specimens was *lower* than that from the other species experimented with. Hence, under normal conditions, the concentration of the blood fluctuates but little, compared with the change it would have to pass through if the blood of eels from sea water had an osmotic pressure equal to the average for marine teleostei. This feature can be correlated perhaps with the power the eel has of resisting sudden changes in the constitution of the external medium.

ELASMOBRANCHS

The blood of these fishes has usually the same osmotic pressure as the surrounding sea water. Experiments were made at Helgoland to determine how long elasmobranchs could live when transferred directly to fresh water, and the condition of the blood just before death. Mosso,¹ states that sharks die after some hours, and further, that after half an hour in fresh water, no more blood flows out from the caudal artery if the tail be cut, even though the heart pulsates. He states further that if a solution of NaCl is injected it will not pass through the gills, showing they are no longer permeable and that the blood of the fishes dead in fresh water is almost normal, death being due to stoppage of the gill capillaries. The first experiments were performed with very large *Acanthias vulgaris* (piked dog fish).

Average Δ for blood of normal fish from sea water — 1.90° and Cl 0.88 per cent.

I.—Specimen placed directly into fresh water and after 4 hours 10 minutes blood taken.

The fish was nearly dead, but even after this long period blood was easily obtained from the tail and gave Δ — 1.455° and Cl 0.48 per cent.

II.—*Acanthias vulgaris*. Placed directly in fresh water—

Blood abstracted after 3 hours 45 mins. Δ — 1.448° .

III.—*Acanthias vulgaris*. Placed directly in fresh water—

Blood abstracted after $4\frac{1}{2}$ hours Δ — 1.400° , Cl 0.455 per cent.

In all these cases, after more than three hours in fresh water, blood was easily obtained from the tail, though not in such large amounts as from the normal fish. Moreover, it can hardly be said from the above results, that at the time of death almost no change had taken place in the blood. There is no doubt, however, that the result varies greatly with the species, and perhaps that experimented with by Mosso was more like the Rays, the results of which follow.

With regard to the power of resisting fresh water, *Acanthias* almost if not quite equals the cod in this respect, and if the cod only was used as typical of the teleosts, and *Acanthias* as an elasmobranch, the great difference existing between these groups would not be so conspicuous.

1. Mosso, *Biol. Centralblatt*, 1890.

Another interesting point in the above results is that the reduction in the salt contents of the blood, as indicated by the chlorine contents, is much greater than the lowering of the osmotic pressure would lead one to expect.

Experiments with other Elasmobranchs.—

Raia clavata.—Average Δ for blood from normal specimens, = -1.90° .

One large specimen was placed in well aerated fresh water. This fish secreted large quantities of mucus, and at the end of two hours was almost dead. The heart was still beating, and blood was obtainable from the tail, but the quantity was small.

Δ of blood -1.645°

An elevation of the freezing point had thus taken place, though at the time of death it was much lower than that of the *Acanthias* blood.

The same speedy death was exhibited by other specimens of the same species. Two specimens (small) of *Raia batis* died within two hours after direct immersion in fresh water, but not enough blood was obtained from the caudal artery to allow of a determination of the freezing point.

At the suggestion of Dr. Franz, some of the fluid was taken from the eyes of *Acanthias*. These were removed carefully from the fish, the sea water removed, and the fluid abstracted. The freezing point was remarkably low, lower even than that of the blood, viz. $\Delta -2.028^{\circ}$, and the amount of chlorine, 0.946 per cent., was greater.

The flesh of elasmobranchs, like that of teleosts, contains much less salt than the blood, the figure for the muscles being only 0.15 per cent.

Some very interesting experiments made by Baglioni¹ show that to sustain the normal beat of the heart from an elasmobranch (*Scyllium catulus*), an artificial solution containing 2 grammes urea and 2 grammes NaCl for every 100 c.c. of tap water (in which calcium is present) is necessary. The urea aided contraction and the

1. Baglioni, *Zeit. für allgem. Physiol.*, 1907.

NaCl regulated the relaxation. Hence the urea is present not only to bring the osmotic pressure up to that of sea water, but as a necessary constituent of the blood, without which the regular beating of the heart is impossible. It follows, therefore, that the alterations in the constitution of the blood in the preceding experiments would interfere with physiological processes in addition to any blocking of the gill capillaries that occur as stated by Mosso.

The average results of the resistance of elasmobranchs and teleosts to fresh water, show that the latter can resist much greater changes than the former, and this agrees with the distribution of the elasmobranchs for they are typically marine fish and do not extend far into the Baltic. Bridge¹ states that some species are permanent inhabitants of fresh water; it would be most interesting to determine the freezing point and salt contents of the blood of these representatives of a group so characteristic.

VARIATIONS IN THE BLOOD OF *Pleuronectes platessa* (PLAICE) WHEN TRANSFERRED FROM THE SEA TO THE AQUARIUM

Some experiments were made to observe the change in the osmotic pressure of the blood of this marine teleost when immersed in diluted sea water the osmotic pressure of which was not less than that of the blood of the normal fish. These experiments led to a remarkable change being discovered, which took place under apparently normal conditions in the aquarium. The first experiments were as follows:—

A number of large plaice, caught off Helgoland, were brought back living, and eight set in a mixture of sea and fresh water, the proportions being about two-thirds sea and one-third fresh. After two hours this was changed by addition of more fresh water until the Δ was -1.125° , and the fish remained in this for fourteen hours.

Blood from three plaice, out of this mixture $\Delta - 0.640^\circ$

Blood from eight plaice, out of this mixture $\Delta - 0.630^\circ$

Blood from plaice out of same catch but kept in normal sea water in aquarium:—

Blood from three specimens $\Delta - 0.665^\circ$

Blood from four specimens $\Delta - 0.695^\circ$

Two things are noteworthy here: (1) The osmotic pressure

1. Bridge, *Cambridge Natural History*, Vol. VII, London, p. 432, 1904.

of the blood has decreased in this diluted sea water ; (2) the osmotic pressure of the blood of those fishes kept in the aquarium, but in sea water, is much lower than the figures obtained previously¹ for the same species direct from the sea.

A second experiment was consequently performed ; a considerable number of fishes were brought back, and divided into two lots.

First Lot.—Examined immediately. Blood gave—

For three specimens $\Delta - 0.715^{\circ}$

For three specimens $\Delta - 0.710^{\circ}$

For four specimens $\Delta - 0.705^{\circ}$

Second Lot.—Kept in *sea water* in aquarium for 34 hours.

Average result blood $\Delta - 0.630^{\circ}$

That is, for those specimens in sea water, under apparently normal conditions in the aquarium, the freezing point was higher by -0.08° than the specimens examined immediately after capture.

A third experiment was made to confirm the two previous ones by taking some of the blood from the fishes just after capture, the salinity of the bottom water being determined at the same time. The catch was divided into three lots. Two of these were brought back alive to land, the blood was taken from the others and brought back in tubes kept in ice. Of the two lots brought to land, one was examined immediately, and the other lot placed in an aquarium tank. The salinity of the bottom water and the aquarium tank water was about the same, viz. : Bottom water 36.55 per thousand, Aquarium water 36.00.

Results :— Blood taken from fishes immediately trawl was brought on deck—

First Series— $\Delta - 0.735^{\circ}$

Second Series— $\Delta - 0.753^{\circ}$

Third Series— $\Delta - 0.700^{\circ}$

Blood taken from fishes of same catch brought back to the Biological Station in running sea water. Time taken on way, $2\frac{1}{2}$ hours—

First Series— $\Delta - 0.741^{\circ}$

Second Series— $\Delta - 0.742^{\circ}$

Blood taken from fishes of same catch brought back to the Biological Station in running sea water, and kept for three days in large aquarium tanks—

First Series— $\Delta - 0.645^{\circ}$

Second Series— $\Delta - 0.655^{\circ}$

1. Dakin, *Bio-Chem. Journ.*, Vol. III, p. 264, 1908.

It will be seen from the above series of experiments that on every occasion there was a very considerable alteration in the freezing point after the fishes had been kept in the aquarium. Moreover, there was but little variation in these results; the fishes taken from the sea water direct had almost all the same Δ for the blood, those kept in the aquarium also agreed between themselves, but differed, as the results show, very much from those examined directly they were brought to land.

This remarkable change could not have been due to differences in salinity of the water, since analyses showed the salt contents was practically the same. If the fish had been pathological or weak through the change, the freezing point for the blood would be expected to have been lower, that is, more like the sea water. Instead of this, it was much higher in the aquarium fish, and they were quite healthy.

It is possible that this change was due to the difference in hydrostatic pressure, since in the sea the fish must have been living under about four atmospheres pressure. It is obvious that this would have no effect directly, but it may be responsible indirectly for changes resulting in the blood.

It was not possible at Helgoland to determine whether this change took place generally with fishes living on the sea bottom, when brought into the aquarium, but I hope to continue this series of experiments shortly.

I have, in order to compare the action of fresh water on marine teleosts, in this paper compared the results with the freezing points of blood from fish in the sea water tanks of the aquarium, though it does not seem from the figures for *Cyclopterus* that they differ from those caught in the sea. In any case, it will be seen that great care must be taken when examining the blood for purposes of comparison, to make certain the fishes are under the same conditions in each case.

INFLUENCE OF THE OSMOTIC CONCENTRATION OF THE SEA WATER
OR THE BLOOD UPON PELAGIC EGGS

The eggs of many marine teleosts are pelagic, and their specific gravity is so adjusted that but slight changes in the density of the water, if the salt contents is reduced, are sufficient to cause the eggs to sink.

Thus the eggs of the plaice or other pelagic eggs found floating twenty miles west of Helgoland, sink in the slightly less dense water round that island. In the Baltic Sea, where the difference between the *surface* and *deep* water in salt contents is very considerable, the eggs are very rarely found floating at the surface, but remain suspended at some point nearer the bottom. It has been found from measurements made chiefly by Ehrenbaum and Strodtmann,¹ that the diameter of these pelagic eggs is greater, the less dense be the sea water (with one exception—the plaice).

For example, the eggs of the flounder from the Baltic Sea had a diameter of 1.054 and 1.216 mm., and they occurred in water with salt contents 11.24 to 17.54 per thousand. The eggs of the same species from the North Sea had a diameter of 0.915 and 0.970 mm. It is still more interesting to find that the eggs while still in the ovary are larger in Baltic Sea specimens than in those taken from the salter North Sea water. For example, the ovarian eggs of *Clupea sprattus* have, according to Schneider, a diameter of 1.2 mm. in Baltic Sea specimens. The diameter of the eggs of the same species in the North Sea is, however, much less than this, and since these extruded eggs are larger than the ovarian, the ovarian eggs must be still smaller than those from fishes in the Baltic waters.

Ehrenbaum gives 0.94 mm. as the diameter of the ovarian eggs of *Pleuronectes limanda* in the Baltic Sea, whilst the floating eggs of this species in the North Sea only reached a diameter of 0.840.

The probability of this difference in size being correlated with the difference in salt contents of the blood was first suggested by Ehrenbaum and Strodtmann, who said: 'Vielleicht beeinflusst das

1. *Wiss. Meeresuntersuch*, Abt. Helgoland, Bd. VI, H. 1, 1904.

umgebende Wasser die Fische in der Weise, dass in der Blutflüssigkeit und vor allen Dingen in dem Liquor sich der Salzgehalt ändert. In der östlichen Ostsee befinden sich die Fische in der Regel in einem Wasser von geringem Salzgehalt, und hierdurch erhält auch die die Eier umgebende Flüssigkeit des Ovars eine geringe Konzentration. Ist nun die zur Bildung eines Eies erforderliche Salzmenge eine konstante, so wird in diesem Falle die entstehende osmotische Spannungsdifferenz eine Vergrößerung des sich bildenden Eies bewirken. Die Volumenzunahme hat dann den Vorteil, das spez. Gewicht zu verringern, so dass die Eier auch noch in weniger salzhaltigem Wasser zu schwimmen vermögen.'

This suggestion has been justified, since I found that the salt contents of the blood does differ, though slightly, in the fishes from sea water of varying density. The ovarian fluid, like the blood, has a salt contents much lower than that of the surrounding water. It is usually slightly lower even than the blood, and also dependent to a small extent, like that fluid, on the concentration of the sea water. These differences might account for differences in the size of the eggs in the ovary, but they would not account—they are so small—for differences in the specific gravity of the eggs from two places so near, as previously mentioned. Ehrenbaum and Strodtmann state, moreover, that by taking fertilized eggs of *Ctenolabrus* and *Sprat*, from North Sea water, gradually down to almost fresh water, no noticeable increase in size was found to take place. Hence, I think, the specific gravity of the eggs is determined to a large extent by the density of the water in which the fish are living at the moment of extrusion of the eggs, when an increase in size takes place; and the larger size of the eggs in the ovary in fishes living in much less dense water is due to the action of the outer medium on the blood and ovarian fluid.

CONCLUSION

The osmotic pressure of the blood of teleosts is, as we have seen, subject, though to a small extent only, to changes in the density

of the outer medium.¹ The river eel has a lower osmotic pressure for the blood in fresh water than in sea water, though the Δ for this fish from sea water is higher than that of any other marine teleost. Moreover, there is a greater percentage of salt in the blood of marine teleosts, and Sumner considers that both water and salts may pass osmotically through the bounding membranes.

He states² that control experiments excluded the possibility that the water or salts passed from the body through the alimentary canal, leaving as the only possible alternative an osmotic exchange through one or more of the limiting membranes. Further, he remarks (p. 97) 'The lining of the alimentary canal is, of course, readily permeable to fluids and to various substances in solution, but this, it is needless to say, is not freely exposed to the surrounding medium.' Now I do not consider the alimentary canal excluded, since water must pass in with the food; and my freezing point determinations on the fluid of the alimentary canal of *Cyclopterus* show that in sea water this fluid has a salt contents almost the same as sea water. Again, when *Cyclopterus* was immersed in fresh water, the fluid in the alimentary canal had a Δ much less than the blood and rather approaching the surrounding water in composition. This being the case, there is present the possibility of water or salts passing though the membranes of the alimentary canal by simple osmosis or by the more complicated chemical processes of physiological absorption.

On the other hand Sumner showed that keeping freshwater

1. The low osmotic pressure of the teleost blood, and its small range of variation compared with that of invertebrates, has led Dekhuijzen to suggest (*Ergebnisse von osmotischen Studien, Bergens Museums Aarbog*, 1904, No. 8, p. 7), the probability of the teleostean fishes having descended from Ganoids which lived in brackish water. They are assumed to have taken the osmotic pressure of the water there, and then having evolved the power of keeping this constant, wandered on the one hand to the sea and on the other to fresh water.

One would then have to assume also that the elasmobranchs had inherited the salt contents of their blood from ancestors living in brackish water, because though the osmotic pressure is the same as the surrounding medium, the salt contents is only about half that of the North Sea water.

It is much more likely that the teleostei have acquired the low osmotic pressure in the sea. Perhaps, as suggested by some authors, it represents the concentration of the primeval seas, which had a much lower density; or what is more probable, since all the vertebrates from teleosts to mammals have evolved the power of maintaining a definite salt contents for their blood, in defiance of solutions possessing higher or lower concentrations bathing their bodies, and passing through their alimentary canals, it is possible that at the same time the teleostean fishes acquired this low salinity, which is characteristic of vertebrates even though living in water of much higher concentration.

2. *Bull. of the Bureau of Fisheries*, p. 104.

fish with the body immersed in fresh water, but passing salt water over the gills, caused death, and there was a considerable loss of weight. No experiments, however, that have been made prove the permeability of the membranes, e.g. gills, for both water and salts by simple osmosis, though it seems certain that they are permeable for water, which would suffice to a certain extent for the toxic action of the salt water on the gills of freshwater fish. Botazzi and Enriques,¹ experiments indicate an impermeability of the alimentary canal walls of invertebrates to salts in solution except by nutritive processes, and Overton² after a number of experiments on the frog, as an example of the Amphibia, concludes that the skin is only with difficulty penetrated by salts, though very permeable for water. Schücking,³ on the contrary, finds that salts leave the body of *Aplysia*, when it is immersed in fresh water, though the mouth and anus were ligatured, and that the amount of water or salts lost or gained is affected by injection of small quantities of nicotine, strychnine, etc. He concludes that the membranes are permeable to both water and salts. It is impossible to say whether the increase in salt contents of the blood of teleosts, in water of greater concentration, is due to the passage of salts through the gills by diffusion or through the walls of the alimentary canal by diffusion or absorption. The sensitiveness of the teleosts to changes in the chemical constitution of the external medium, is indeed apparent, and it appears that the toxicity of fresh water for marine teleosts, and vice versa, is due more to the alteration in the chemical constitution than to differences of osmotic pressure, though in all probability both are concerned.

This certainly points to a permeability for salts, but does not indicate whether through the gills by diffusion, or the alimentary canal by absorption. Under normal conditions the Δ of the urine of the teleosts is *less* than that of the blood, yet at the same time the kidneys appear to regulate to a certain extent the concentration of the blood, for it was generally found that marine fishes immersed in fresh water excreted large quantities of urine.

1. *Archiv. f. Anat. u. Physiologie, Abt. Physiol.*, Supplement-Band., 1901.

2. Overton, *Verhandlung. der Physik-Medic. Ges. zu Würzburg.* N.F., Bd. XXXVI, No. 5, 1904.

3. Schücking, *Archiv. f. Anat. u. Physiol.*, 1902.

Whatever be the path taken by the water and salts, it is evident that a passage of both does take place both in vertebrates and invertebrates, and further that under normal conditions in fresh or sea water the blood and other fluids of the body are in equilibrium with the external medium. This condition is very like that suggested by Moore and Roaf¹ for the qualitative differences between the electrolytes of the cell and those of its environment, an equilibrium consisting not necessarily in an equality or isotonicism of the total osmotic pressure.

It has already been pointed out that the relation of the osmotic pressure of the blood of teleostean fishes to the water in which they live, is very like the relation of the temperature of 'warm blooded' vertebrates to that of their environment. This comparison might be carried still further and the aquatic invertebrates likened to the so-called 'cold blooded' animals whose temperature is practically identical with that of their environment.

Finally, it has been shown that any change in the chemical constitution of the surrounding water brings about for both invertebrates and vertebrates, sooner or later, an alteration in the electrolytes and non-electrolytes of the internal fluids. This is followed by an alteration of the chemical constitution of the cell, which draws inseparably after it an alteration or disturbance of its function.

I should like here to express my thanks to Professor Heincke, Director of the Biological Station on Helgoland, for his kindness in allotting me a work place and for providing me with the large quantity of material necessary, and also to the Staff of the Station, and in particular Professor Ehrenbaum for his interest and helpful suggestions. I have also to thank Professor Benjamin Moore for his many suggestions. ©

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1. Moore and Roaf, *Bio-Chem. Journ.*, Vol. III, p. 55, 1908.