EFFECTS OF CHANGES IN THE DENSITY OF WATER UPON THE BLOOD OF FISHES

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The fact of variation in the number of human blood corpuscles in health has been known for some time. Stierlin (1889) found variations in individual men of 1,650,000 per cubic millimeter and somewhat larger variations in women. Viault (1890) noted a marked increase in cases of residents at high altitudes. Some physiologists suggest that the increased number is probably not due to increased formation of red corpuscles but for one thing to evaporation of water from the surface and loss of water from the blood. Yet others have found in animals kept at low pressures that there occurs an increase in the number of corpuscles. Hill (1906), reviewing this work, notes an increase in hemoglobin and adds that it is a reaction on the part of the organism to compensate for low oxygen pressure at high altitudes. Although the other side of this question, the changes in the organism due to increased atmospheric pressure (caisson disease), has been investigated, the principal effect noted has been the presence of air bubbles in the blood and other tissues, the fatal effect of which is well known. The effect on the corpuscle count has not been noted.

Dr. F. B. Sumner, director of the biological laboratories of the Bureau of Fisheries at Woods Hole, Mass., who is interested in the cause of the death of salt-water fishes in fresh water and whose investigations will be referred to, suggested to the writer that he study the effect on the blood of fishes subjected to changes of density in the water. Part of my time has been devoted to this problem during the last three summers, with facilities and material afforded by the Bureau of Fisheries.

Sumner (1905) found that well-marked changes in weight resulted in certain cases where the density of the water in which fishes were kept was increased or decreased. He showed that although at first thought these changes might have been expected on the assumption that we were dealing with an osmotic action through semipermeable membranes, yet in other cases the changes did not follow these laws and the phenomena appeared to be complicated by physiological and chemical factors of an obscure nature. In one case (the carp) it was shown that the changes took place through the gills. In another paper (1907) the same author confirmed the last named result by experiments with the tautog. He also found seasonal differences in the osmotic phenomena and says that "these differences may perhaps be due to differences of temperature but are more probably due to seasonal variations in the physiological condition of the animals."

While not entering into the work in detail, such a course necessitating constant reference to figures, I will give the results of a few experiments and discuss the same.

I wish to corroborate the work of Sumner as to gain in weight of *Fundulus heteroclitus* when placed in fresh water. In this case the fishes were kept in separate dishes and the record of each individual fish was kept. They were taken out and weighed at intervals. The percentage of gain was obtained in each case and for each period. It was found that there was considerable variation. Thus in one experiment there is noted a gain of 13 per cent in one individual, while in the case of another fish of the same lot there was a gain of 2.1 per cent. The average gain was 5 per cent, or if we disregard the case of 13 per cent gain, the average was 4 per cent.

But it may be questioned what right we have to throw out the case of 13 per cent gain. In the first place, this is from two to six times as great as the gain in each of the other cases. What is more important, however, is that it is not a normal case—that is, the fish was not in normal condition, for on referring to the record it is found that at the end of six hours it had turned on its side and a few hours later was dead. This was true of other similar cases. Wherever there was an extraordinarily large initial gain it was noted that early death took place. Again, when in the course of the experiment a marked gain in weight occurred, it was found that this was soon followed by death. It thus appears that there is another factor here than merely osmotic phenomena—that there is a physiological (vital) reaction against osmotic pressures, and as death approaches, this physiological reaction ceases and the phenomena following for a short time at least are osmotic.

Now what is the effect on the blood when this species of fish is placed in fresh water? A familiar experiment in histology is to observe the effect on the corpuscles of distilled or tap water and of concentrated salt solutions. There are distinct effects which end in the destruction of the corpuscles. But do similar changes take place when the living organism (i. e., a salt-water fish) is placed in fresh water? An experiment was devised whereby one lot of *Fundulus heteroclitus* was kept in normal sea water and a second lot in fresh water. After certain periods of immersion (two hours, etc.) blood smears from fishes in each of the two lots were made on the same slide at the same time and stained simultaneously with Wright's blood stain, were then dried, and mounted in balsam at the same time. A careful examination with the twelfth oil immersion showed no measurable differences in the corpuscles themselves. This was true in other similar tests. In other words, whatever changes these different solutions have on blood it may be said that these changes do not reach the corpuscles themselves.

A series of experiments was made to ascertain whether there was any change in the corpuscle count due to changes in water density. In the case of mountain sickness it has been shown that at high altitudes there is a physiological reaction on the part of the organism to the effect that per cubic millimeter there are more corpuscles manufactured to take up more oxygen, and thus supply the organism with its normal amount. If fishes are placed in fresh water, is the blood diluted? This would be shown by a decrease in the number of corpuscles. To test this, hemocytometer tests were made upon the blood of normal *Fundulus heteroclitus*. The average blood count of eight was found to be 2,749,000. Four fish placed in distilled water for 1¼ to 2¼ hours were examined in the same way and the average blood count was found to be 2,171,000. In two experiments with distilled water there was at first a decrease in the number of corpuscles counted, then a gradual increase, which increase later passed up to and above normal. The last-named experiment indicates that there is at first a reduction, due to influx of water. After the reduction in number of corpuscles there is an increase, which indicates a reaction on the part of the organism to get back to the normal condition. Experiments in which *Fundulus heteroclitus* was subjected to fresh water show, in the main, that the gain in weight which is at first noticeable disappears after a few days and the weight of the organism falls below normal and the decrease continues. This is undoubtedly due to starvation. It may be objected that these fishes can not live very many days in fresh water. In distilled water, no, but in ordinary tap water, yes. On July 10, 1908, I placed ten *Fundulus heteroclitus* in a large rectangular dish containing about 20 liters of fresh tap water. In the bottom was placed some gravel from the shore near by, the gravel having been thoroughly washed. Most of the fishes died during the next three or four weeks, but one fish was alive and apparently in good condition on September 8, 1908, after sixty days. It should be said that the tank in which this specimen was kept was washed out thoroughly once a week and the fish fed. A rough test of the water with silver nitrate and nitric acid was made toward the end of the period, and a slightly greater amount of chlorides was indicated than was present in the fresh tap water. This agrees with Sumner's statement that a slight addition of salt water to fresh water was sufficient to keep the fishes alive, and therefore the death of fishes in dilute solutions was probably not due to lowering of osmotic pressures. To show the extreme diluteness of salt in the above case, I may add that it took the same reading with the specific gravity hydrometer as did tap water.

In another series of experiments the percentage of hemoglobin was determined with Dare's hemoglobinometer. Although this was less satisfactory, yet the average percentage of hemoglobin of 18 normal *Fundulus heteroclitus* was found to be considerably more than the average in the case of 29 specimens of the same species kept in fresh water for a certain length of time. Have we, however, a right to say that the corpuscles are thus deficient in hemoglobin? Since we have good reason for believing that there is an influx of water and that this takes place through the gills, the reduction in number of corpuscles and a lowering of percentage of hemoglobin shows that the blood has been diluted. We can also obtain the specific gravity of the blood. This was done, by the Hammerschlag method, in which a drop of blood is placed in a solution of benzole and chloroform and enough of each added so that the drop is suspended. The specific gravity of the mixture then would be the same as that of the blood and could be learned by the small hydrometer used for such work. The specific gravity of blood of 20 *Fundulus heteroclitus* was found to be 1.0510. In an experiment in which 22 *Fundulus heteroclitus* were kept in fresh water from two to eight hours the average specific gravity of the blood was 1.047+. In an experiment in which dogfish (*Mustelis canis*) were used, the average specific gravity of the blood of three was 1.0466. After about two hours immersion in fresh water the average specific gravity was 1.0417. Four determinations were made with each fish in the first part of this experiment and similarly in the second part. There was practically no loss of blood except that used in the determination, which fact is mentioned to anticipate the objection that the reduced specific gravity is due to loss of blood, as in the case of hemorrhage in man, when the specific gravity of the blood drops.

It is an established fact that the more dilute a solution is as compared with distilled water the nearer is its freezing point to that of distilled water. By the aid of a Beckman thermometer I obtained the freezing point of distilled water and by the same means the freezing point of the blood serum of a number of dogfish. The average lowering of the freezing point of blood serum of 7 dogfish taken from sea water was found to be 1.934°. Now if there is a slighter depression in the freezing point of blood serum of dogfish kept in fresh water for a certain period, then that means that the blood of such dogfish has been diluted, for such blood is nearer the condition of distilled water than the normal serum. The depression of the freezing point of the serum of 11 dogfish kept in fresh water for one hour was obtained. The average for five experiments was found to be 1.548°. We have already seen that the depression of the freezing point in case of normal dogfish serum was 1.934°. Hence we find that there was dilution of the blood.

In conclusion it may be said that when fishes are kept in fresh water there is a decrease in the number of corpuscles per cubic millimeter, a lowering of the hemoglobin percentage, a lowering of the specific gravity, and a lessening of the depression of the freezing point, all of which shows a dilution of the blood. But, on the other hand, is there shown a concentration of the blood when fishes are placed in a more dense medium? In the case of 19 *Fundulus heteroclitus* kept in sea water, the density of which was increased to about 1.040 by the addition of 25 grams of sea salt to each 1,000 of sea water, the average percentage of hemoglobin was found to be 77 per cent, as compared with 51 + per cent in the case of 18 normal fishes.

As to the number of corpuscles per cubic millimeter, the following experiment was devised: The average number of corpuscles in 8 individuals has been found to be 2,749,000 per cubic millimeter. A fish placed for an hour in a solution of sea water and sea salt so that the specific gravity was 1.050 showed a corpuscle count of 3,072,000 per cubic millimeter. After 2½ hours immersion in sea water and sea salt whose specific gravity was 1.068 the corpuscle count of another specimen was 3,912,000 per cubic millimeter. The average blood count of 14 *Fundulus heteroclitus* kept in a solution of sea water to every liter of which was added 25 grams of sea salt was found to be 3,539,000, while in another case the blood count of two fishes in a solution of sea water to every liter of which was added 45 grams of sea salt was found to be 4,217,000 per cubic millimeter.

At the same time in another experiment record was kept of the weight of the fishes and it was found that they lost steadily in weight while the corpuscle count increased. The same general results were obtained in all the experiments of this nature. From a great many experiments I can corroborate the statement of Sumner as to loss in weight when placed in more dense solutions. As to the change in specific gravity, fishes taken at the same time as those used in getting the normal specific gravity were experimented upon. On placing a lot of 10 in a solution of sea water and sea salt to raise the specific gravity to 1.045 for a period of nearly three hours, the specific gravity of the blood was found to be 1.054. During that time the fishes had lost 7.5 per cent in weight. On remaining in same solution nearly six hours, the average specific gravity was 1.060 and the fishes had lost 11 per cent in weight.

In another experiment with a solution of 1.040 specific gravity there was noted a gradual increase in specific gravity of the blood during the first day. This increase persisted in the case of those examined during the next day, while in 8 specimens examined two days later there was a decrease toward normal in the specific gravity noted. Although this one experiment is inconclusive, it indicates a reaction toward the normal. There is such a thing as normal specific gravity of the blood, and any marked departure is pathological. Hence the lowering of the specific gravity noted above might be interpreted as a reaction of the organism toward the normal condition. The water necessary to dilute the blood could be drawn in from the tissues.

An experiment was devised to test the relation between temperature and the lowering of the specific gravity of the blood. Three lots of fish were used.

Lot A.—Sea water at temperature of laboratory, 18° C.

Lot B.—Fresh water at 27° C.

Lot C.—Fresh water at 7° C.

The fishes were kept in these solutions from one to six hours. At the end of that period the average specific gravity of blood of—

Lot A was 1.0506, 18° sea water.

Lot B was 1.0463, 28° fresh water.

Lot C was 1.0501, 7° fresh water.

In other words, the cold water had prevented the decrease in specific gravity.

Now it is known that temperature is an important factor in osmotic exchanges and also in physiology of circulation. Therefore, whether the more sluggish movement of the blood through the gills is responsible for the difference between B and C or whether the lowering of temperature produced a condition in the gill membrane making osmotic changes more difficult can not be determined at present. We have seen from the previous experiments that if fishes are placed in a more dilute solution the blood is diluted; if placed in a more concentrated solution the blood is concentrated. It is not believed, however, that in the time during which the fishes were subjected to these various media there was any increased formation of red corpuscles or any destruction of the same.

Are the phenomena described above purely osmotic, and thus physical in their nature, or is there something more—that is, physiological or vital—concerned here? Certainly some of the phenomena observed above lead toward the latter interpretation. The great adaptability of the organism is well shown in the case of the specimen living in fresh water for two months, and again in the case of a few fish which lived for over a month in a solution the concentration of which was increased 50 per cent. It must be remembered that *Fundulus heteroclitus* is found not only in sea water but also in brackish water and inlets of fresh-water streams. Whether the above changes are true of the teleosts which are strictly marine, I can not say.