Observations on the Physiological Development of Trout Eggs.

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Introduction and Position of the Problem.

The physiology of the development of the Salmonids is followed by a series of observations which consider this problem from different points of view. Here we are mainly interested in the recently-published works of Runnström, Gray, Kronfeld, Scheminsky and others. These workers show that trout and salmon eggs are systems which are isolated to a large extent from external surroundings. According to Runnström (1920), salmon eggs have a high osmotic pressure throughout the whole of the embryonic development; after the eggs are placed in sugar solution of a comparable osmotic pressure, the osmotic pressure of the blood of the organism only decreases slightly (according to Runnström, the depression of the freezing point of eggs of Salmo salvelinus is as follows :- for eggs taken from the oviduct, before fertilisation, 0.645°C; after fertilisation, 0.599°C; in larvae, 0.580°C). Gray (1920) showed that the eggs of fresh-water trout also contain a considerable and constant amount of electrolytes, in consequence of which the depression of the freezing point (Δ) of the eggs has a constant value, 0.48°, during the whole period of development. Gray was unable to find any decrease in Δ in eggs after fertilisation. Moreover, he established, by measurements of the electrical conductivity, that some electrolyte passed out of the eggs during development. However, this begins to occur as soon as the eggs die. This was shown earlier for the eggs of other salmonids by Tangl and Farkas (1904).

Thus in salmonide eggs there is a regulation of osmosis; but the nature and mode of operation of this regulation, and above all the question of whether the isolation of the eggs is due to the presence of the egg membrane, or whether quite different factors play the main role here, has not hitherto been explained. The purpose of this work is to facilitate the solution of this question by the aid of experiments.

Strictly speaking, these questions are scarcely concerned in the work on the physiology of development of the salmonids. But several authors appear to incline to the opinion that the cause of the height and stability of the osmotic pressure of the eggs of salmonides lies mainly in the egg membrane; in other words, the regulation of the osmosis is considered as a purely passive process. At least Gray (1920 b), after studying the problem of the adsorption of hydrogen ions on trout eggs, writes that the equilibrium between the living cells and the hydrogen ions of the surrounding medium is due to the external membrane of the eggs. Runnström (l.c.) also maintains that the egg membrane of salmon eggs is considerably weakened by the poisoning effect of K ions. Probably such isolated opinions as those of Scheminsky and Gauster (1924) led to the statement that the "egg membrane (of trout) is impermeable to salts and albumen" (as we shall see later, only the second half of this statement is correct).

For the experimental illustration of the means by which salmonid eggs are isolated from the external medium, I posed the following questions, from the point of view of osmosis:-

1. As is known, there is a space between the egg membrane and the surface of the egg cells, which is filled with the so-called perivitelline fluid. The properties of this fluid are completely unknown, since the measurements of osmotic pressure, the chemical analyses, etc. of salmonid /eggs/ have hitherto only been made for the egg and perivitelline fluid combined (for the same reason the corresponding results for the egg cells are not entirely accurate). Kronfeld and Scheminsky (1926) believe that the perivitelline fluid "contains some dissolved substances", but this belief is not supported by any direct observations. But the question of the role played by the egg membrane in maintaining the constant amounts of electrolytes in the egg, can also be answered positively if the perivitelline space is filled with a liquid of high pressure. For the solution of this problem, it is first necessary to determine the osmotic pressure of the perivitelline fluid.

2. Extensive investigations of the question of the permeability of the egg membrane to various substances are naturally of the greatest importance here.

After I had stated these questions in a general form, some other problems occurred to me, which I shall outline later.

Material and Methods.

The material I used for this research was eggs of freshwater trout (Salmo trutta m. fario L.) In November 1927 I obtained some mature trout from the state fish-breeding station at Ropsha. The spawn was artificially fertilised in the laboratory and was then kept in running conductivity water in special frames at a temperature of 10 - 11°C.

The osmotic pressure of the perivitelline fluids was measured by Drucker and Schreiner's microcryoscopic method (1913). This method offers the possibility of measuring the depression due to very small quantities of substance (up to about 0.1 cmm) to be measured with adequate accuracy. For the examination, the liquid is sucked up into a glass capillary tube, which is then attached to a Beckmann thermometer (for the apparatus used in these estimations, see my work of 1928).

The perivitelline fluid was extracted for examination as follows :- the egg was first laid on filter paper and dried all over. A short vertical scratch was then made with a sharp needle. Since the egg falls to the bottom (owing to its weight) and the vegetative pole is thus in contact with the egg membrane, the main part of the perivitelline fluid collects at the top between the plasmatic disc at the animal pole and the egg membrane. When the egg membrane is penetrated in this area, a clear drop of the perivitelline fluid flows out through the hole. This drop is carefully collected in 4 - 5 capillaries (its volume being sufficient for this).

It was not difficult to be convinced that the liquid drawn up into the capillaries was not the inner content of the egg cell, but the true perivitelline fluid. A long-known property of the liquid yolk of salmonid eggs was used to confirm this belief, viz. that it coagulates on dilution with water. As Gray has shown in recent years, this phenomenon is due to the fact that a considerable amount of globulin is contained in salmonid eggs.

In order to test that the right substance has been withdrawn as a sample, I dipped the capillary tube containing the sucked-up liquid into water; in cases where the plasmatic surface of the egg itself had been damaged by penetration of the needle into the egg

membrane, and the drop contained a mixture of perivitelline fluid and egg-yolk, the liquid in the capillary congealed instantly on being dipped into water, and instead of a clear liquid drop, a small blob of white flocculant precipitate was visible. In cases where there was no penetration of the egg itself could be observed, I assumed that the capillary contained pure perivitelline fluid and I used the capillaries containing other parts of the same drop to estimate the depression of the freezing point (Δ) of the perivitelline fluid.

The Osmotic Pressure of the Constituents of the Egg.

In this way the value of Δ for the perivitelline fluid was determined at different stages of development. The samples were taken on the 2nd., 5th., 29th. and 50th. days of development (hatching began on the 45th. day in my culture. Hence the last sample corresponded to the end-stage of embryonic development.) Each time 5 to 6 samples were taken for measurement and the average value was calculated. The results are given in Table 1.

Table 1.

M.T.	Day of development	Δ of the perivitelline fluid
3. XI.	2	0.02
16. XI.	5	0.01
10. XII.	29	0.02
5. I.	50	0.02

It is seen from the figures obtained that the depression of the freezing point of the perivitelline fluid is very low and remains constant during the period of development. In its salt content, this liquid resembles the conductivity water in which the eggs were placed.

The Δ-value of the yolk at different stages of development was determined by the same method. In this case the perivitelline fluid was removed with filter paper after the membrane was penetrated, and a small glass tube was dipped deep into the interior of the egg, in order to take out a portion of the liquid yolk. It is seen from the results given in Table 2, that the Δ-values of the yolk are very different from those of the perivitelline fluid. The content of osmotic-active substances is fairly high in this case, approaching that of the blood of these creatures. It is constant (according to S. Schmidt-Nielsen, the Δ-value of trout blood is 0.567°C; Biological Tables, 1925?). It is noteworthy that our values are somewhat higher than those obtained by Gray for the summary depression of the freezing-point of trout eggs (0.48°).

Table 2.

M.T.	Day of development	Δ of the yolk
16. XI.	5	0.50
28. XI.	17	0.49
10. XII	29	0.49
5. I.	50	0.51

In order to compare our estimations with Gray's figures, the combined depression of the freezing-point of the system egg-cell + perivitelline fluid was measured by the usual cryoscopic method (Burian's method). Portions of about 20 eggs were used for these determinations. The eggs were dried on filter paper and then crushed in the test tube of the cryoscope. I was able to commence these observations (which are technically much easier) on the day of fertilisation. Thus I was able to estimate the value of the depression of the freezing-point of the eggs, on leaving the oviduct and 1 hour after fertilisation. The results obtained are given in Table 3.

Table 3.

M.T.	Day of development	Combined value for △ of eggs
11. XI.	before fertilisation	0.497
11. XI.	1 hour after fertilisation	0.468
28. XI.	17th. day	0.435
10. XXII.	29th. day	0.425

These values show that a measurable decrease in the combined △-values of the eggs takes place during development. The course of the change found in the △-values agrees with Runnström's results for salmon eggs (see p. 771); a difference in absolute values can be explained by the species characteristics of the eggs. Apparently Gray did not observe this decrease in the combined osmotic pressure during development although, as we shall see later, it has a certain meaning.

It is interesting that, in the usual cryoscopic estimations, I always obtained a somewhat greater depression in the freezing-point for eggs with penetrated membranes from which the perivitelline liquid had been partially removed with filter paper, in comparison with those for eggs in undamaged membrane coverings. This observation supports our microcryoscopic estimations reported above, of △ for the perivitelline fluid. The increase in △ in this case signifies that when the perivitelline fluid is drawn out, part of the system with a very low △-value is removed, and this results in a slight decrease in the total △-value found for the whole egg in the usual combined cryoscopy.

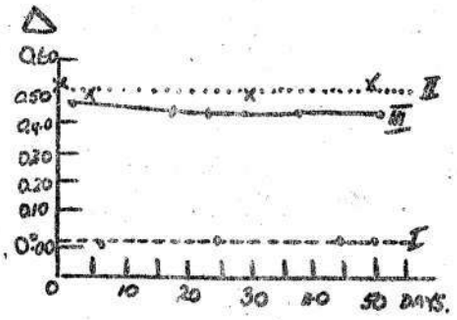
The Course of the Depression of the Freezing Point during Development.

Calculation of the Relative Quantity of Perivitelline Fluid.

Let us now compare three series of figures which we obtained. For clarity, these represented graphically in Fig. 1. This shows that the values of the depression due to the perivitelline liquid (I) and to the yolk (II) are represented by straight lines, running parallel to the abscissa axis. The first is a mirror image of the osmotic properties of the surrounding medium, the second is the physiological constant. As far as the total △ (I II) is concerned, this value falls somewhat during development; this decrease is initially very rapid (the steep gradient of the curve after deposition of the egg), and is later very gradual. It is evident that such a course of change of the total △-value, if the △-value of the constituents remains constant, is only possible in one case, if the quantity of the perivitelline fluid increases during development. The rapid fall in the combined value of △, coincides with the formation of the perivitelline space, which occursiimmediately after the egg is deposited in water. Apparently the liquid which is formed in this space does not differ essentially from water; it diffuses through

the raised membrane (this assumption does not, of course, in any way exclude the possibility of the passage of substances out of the egg-cell). Further; the quantity of the perivitelline fluid increases, as can be seen with the naked eye.

Fig. 1. Course of the depression of the freezing-point of the constituents of trout-egg during the development of the embryo. I. Δ -values for the perivitelline fluid. II. Δ -values for the yolk. III. Combined Δ-values for the egg.



Our figures, however, also show the possibility of a quantitative estimation of the relative amount of the perivitelline fluid. We know already that the total Δ -value of the egg (y) is a function of the relative amounts of the two liquids (x).

We can express this function by an equation. If we indicate the constant value of the depression of the freezing-point of the yolk and of the perivitelline fluid by Δ_d and Δ_o; if their volumes are V_d and V_p;

$$\text{then } x = \frac{V_d}{V_p}$$

if we assume that $V_p = 1$, then $V_d = x$. If we have x volumes of the first and 1 volume of the second liquid, then the volume of the whole system is $x + 1$.

From this, the equation for the combined depression of the freezing-point of the eggs is

$$y = \frac{\Delta_d x + \Delta_p}{x + 1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

from which x can be determined (1)

$$x = \frac{y - \Delta_p}{d - y} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

(1)

We admit a simplification here, in considering the egg to be divided into only 2 components (as far as osmotic pressure is concerned). There appear to be at least 3 "osmotic" components present in the egg:- perivitelline fluid, yolk and embryo (germ). A possible difference between the Δ-values of the yolk and the embryo has also been left out of account. In all probability the difference between the Δ-values of the yolk and of the germ tissue is not particularly large. Hence the errors arising from this source are not very important.

The values y, Δ_d and Δ_p are known and can be taken directly from Tables 1 – 3. If the x-values are calculated in percentages

$$(V_d \text{ in } \% \% = \frac{100\,x}{x+1} \text{ and } V_p \text{ in } \% \% = 100 - \frac{100\,x}{x+1})$$

then we obtain the values given in Table 4.

Table 4.

Day of development	y	x	Amount of yolk in %%	Amount of perivitelline fluid in %%
1	0.468	14.0	93.34	6.66
17	0.435	6.38	86.45	13.55
29	0.425	5.40	84.38	15.62

The accuracy of these figures is naturally not particularly great, since the y-values were estimated by the usual rough cryoscopy and not by precision cryoscopy; low accuracy of observations may thus be an important source of error in the calculations, but these figures may serve as a first approximation to the actual ratios. Gray (1926) assumes that the perivitelline fluid in trout eggs immediately before they are hatched amounts to about 10% of the fresh weight of the egg. Since the specific gravity of the yolk appears to be greater than that of the perivitelline fluid, the amount of perivitelline fluid must form more than 10% of the total amount. From this, it can be said that our figures do not differ so sharply from those of Gray. We are particularly interested here in the great increase in the perivitellin fluid during development; at the end of embryonic development, its amount is not less than twice as great as at the beginning. This increase in the perivitelline fluid probably precedes the swelling of the albumenous matter, the amount of which in the perivitelline fluid increases with the course of development. (I confirmed the presence of albumenous substances in the perivitelline fluid by Millon's reaction in the capillary tubes. This reaction in only doubtful at the beginning of the development and is very sharply defined at the end).

The rise of the swelling pressure in the perivitelline fluid probably plays an important part in the mechanism of the hatching of these fishes. Other opinions have also been expressed in this question (Winterbert, 1912, 1926); (Bourdin, 1926). I intend to discuss these opinions, as well as my own observations, on the hatching of trout eggs, in another place.

Thus the answer to the first of the questions posed at the beginning of this work has been found. The limiting capacity of the high osmotic pressure is not represented by the outer membrane, but by the surface of the egg cell. Thus it can be said already, that the outer membrane plays scarcely any part in the maintenance of a constant high osmotic pressure in the eggs. Let us now turn to the question of the extent to which the perivitelline fluid itself is isolated from the external medium, or in other words, to the question of the permeability of the egg membrane.

The Permeability of the Egg Membrane.

We have already some knowledge of the permeability of the egg membrane. In the first place, it is permeable to water. This can be seen from the work of Gray (1926) who showed that the embryo absorbs water from the surrounding medium. This absorption of water is particularly intensive after laying, but it is also recognised easily during the development of the embryo. The graph obtained by Gray for the percentage of the dry weight of the eggs of trout shows quite clearly that the absorption of water during the whole of the development of the embryo is an indivisible regular process, beginning at the commencement of the embryonic development and becoming further greatly accelerated (1) after hatching. Further, the egg membrane is permeable to some electrolytes. It is seen from Runnström's results (l.c.) that KCl at the isotonic concentration brings the embryonic heart almost to a standstill in 24 hours. We are naturally interested here in the permeability to dilute solutions, which presumably do not change the properties of the egg membrane. As far as strong salt or acid solutions, as well as fixing media, are concerned, it is known that they penetrate the membrane fairly easily.

I have carried out tests on the determination of the permeability of the egg membrane to NaCl, $HgCl_2$, KCN, saccharose and negative-stain suspensions.

The permeability of the egg membrane to common salt can be shown by quite an elementary test. Partially turbid eggs are very often found in the centre of the trout spawn. Observations show that these eggs are beginning to decay, although they remain alive for a long time and develop quite normally. (I observed on occasion that the embryo in a turbid egg developed normally for 2 weeks) That is, of course only possible in cases where the part of the yolk which begins to become turbid is in the immediate vicinity of the germ sheaths. If such eggs are placed in 0.7% NaCl solution (hypotonic solution), they become completely permeable after about 1 minute (2)

When the cleared eggs are dipped in water, they again become turbid. This type of effect is, of course, only possible if the egg membrane has a high degree of permeability to salt ions. Thus, it is not difficult to feel convinced that the turbidity of eggs is not due to an injury to the membrane, but is apparently caused by quite different factors. In the first place, penetration of the egg membranes by the needle does not damage the egg itself. I have pricked the membranes of a large number of eggs and observed their development. The surfaces of the embryo and egg-cell remained intack, the embryo then developed further and hatched afterwards in the same way as normal eggs. In the second place, the

(1) Thus the opinion of Kronfeld and Scheminsky (1926) that the trout egg is an absolutely enclosed system is not entirely accurate. The permeability of the egg membrane to water and the consequent embryonic absorption of water, is extremely important for the correct establishment of relations between the embryo and the external medium.

(2) As tests showed, flocculation of globulin in my material occurred with NaCl concentration of 0.59%, the depression being 0.36. The formation of turbidity in the eggs thus undicates that the osmotic concentration in some parts of the yolk is less than this limiting value.

turbidity may be caused by the action of a "poison" or an electric current on eggs with undamaged membranes. The formation of turbidity in eggs by the electric current was described by Scheminsky and Gauster (1924).

By immersing the eggs in dilute KCN and $HgCl_2$ solutions, I found that the egg membrane is very permeable to these poisons. In KCN solutions of about 0.1%, the embryonic heart came to a standstill after some minutes. In 0.2% solution of sublimate the embryos dies after about 15 minutes. The possibility of an osmotic effect is naturally excluded at these concentrations. The result obtained is obviously due to the diffusion of the poisons through the egg membrane.

Saccharose penetrates the membrane much more slowly than electrolytes. In solutions of 7% or more (weakly hypertonic solution), the egg membrane shrinks together slightly; after some minuted it regains its normal turgescence. Later the eggs begin to become turbid. This is obviously caused by the plasmatic skin on the egg surface bursting under the action of the hypertonic solution, the yolk flowing out into the perivitelline cavity and mixes with the liquid intthis space. This process can be observed easily under a magnifying glass. The flocculation of the globulin in the yolk takes place despite the presence of cane sugar in the perivitelline cavity. Observation shows that the egg yolk flocculates in aqueous solutions of cane sugar of any concentration.

I have used the following substances to determine the permeability of the egg membrane to collidal solutions and suspensions:- neutral red, Nile blue sulphate, trypan blue, suspensions of Indian ink. I used the intravitam dyes in 1% solutions and a commercial Indian ink preparation for bacteriological purposes (Grübler, Pelikan 541). The eggs were immersed in these liquids and after differing periods were opened in water to examine whether the embryo and yolk were coloured. These observations showed that only the two first of the above-named substances permeate the egg membrane. After remaining 48 hours in trypan blue and Indian ink, the perivitelline fluid, the embryo and the yolk all remain colourless; only the external surface of the egg membrane was coloured. Neutral red and Nile blue sulphate, on the contrary, give the yolk embry and perivitelline fluid an intensive colour; neutral red, a colloid with a high degree of dispersion, penetrates the egg membrane with greater speed. I have summarised the results of the tests in the following table, in which the (-) sign indicates failure to colour the membrane, (+) indicates slight colouration and (++) indicates intensive colouration.

	after 1 hr.	after 3 hrs.	after 12 hrs.	after 24 hrs.	after 48 hrs.
neutral red	+	++	++	++	++
Nile blue sulphate	-	+	++	++	++
trypan blue	-	-	-	-	-
Indian ink	-	-	-	-	-

Thus, from our results and from results given in the literature, the egg membrane is found to be permeable to the following materials:- KCl, NaCl, KCN, $HgCl_2$, $C_{12}H_{12}O_{11}$, neutral red, Nile blue sulphate. It is seen from this list that the egg sheath is completely passive to penetration by the substance, and only acts as a dead colloidal membrane. Different substances can diffuse through it, and the limit of its permeability is apparently only determined by the size of the particles of the solute. This limiting size is very significant, since it is

only highly colloidal substances and coarser suspensions which cannot permeate the membrane. We observe that the establishment of the minimum speed of diffusion of the substance through the membrane is important, since the development of the trout embryo under normal conditions takes many months; this time interval is sufficient for the production of the decisive ionic equilibrium by very slow diffusion. From this it can be deduced that it is only the albumen contained in the perivitelline fluid which cannot permeate the membrane; the remaining components of the perivitelline (if any are present) must be washed out by water during the development. It is, therefore, evident that the insulating role of the egg membrane is only operative as follows:-

1) it forms a definite filter to prevent the infusion of bacteria;
2) it protects the egg from injury by mechanical causes;
3) it prevents the albumen of the perivitelline fluid from passing out.

In addition, the egg membrane greatly reduced the velocity of diffusion. It thus plays a certain part in the physical and chemical relations of the embryo, chiefly by retarding the absorption of water considerably. But it cannot be responsible for the maintenance of a higher osmotic pressure in the interior of the egg. As we saw, the rate of diffusion of the electrolyte is so marked, that there cannot be any question of the amount of e electrolyte being kept constant during the whole period of development by the isolating action of the membrane.

It is evident that the level and stability of the osmotic pressure in trout eggs is not due to the presence of the dead colloid membrane, but to some other cause.

<u>The Osmotic Pressure of the Egg and the Surrounding Medium. Osmoregulation.</u>

As was seen earlier, the limit of the sharp decrease in osmotic pressure lies in the surface layer of protoplasm (or blastoderm and later skin surface). The cause of the isolation of the osmotic pressure of trout eggs from outside must be sought in the properties of this boundary layer. Such an interpretation makes the question more complicated; if the living constituent of the egg plays the main part in maintaining the high osmotic pressure, then the question is no longer a purely physicochemical one, but should rather be considered as physiological. Without attempting a complete solution of this question, we shall limit ourselves to the following problem: is there any functional connection between the osmotic pressure of the egg and the external medium or not? In other words, does the level of the osmotic pressure of trout eggs represent the result of the equilibrium between the external medium and the egg cell ions, or is it brought about by the activity of the organism ?

To elucidate this problem the following tests were carried out. Portions of trout spawn containing about 30 eggs were immersed in NaCl solutions of different concentrations, and the total depressions were determined by using the Burian cryoscope. The eggs remain there for different periods of time with continuous aeration by means of an air-water pump. The depression of the solutions in which the eggs were immersed were determined in each case. Table 5 gives the results of the estimations, which were carried out during 18 hours on the 17th. day of development after being placed in the solutions. The standard total depression of the eggs on this day was 0.435°C.

Table 5.

Molar concn. of NaCl in extl. medium	Δ of solns.	Δ of eggs (total)	Theoretical value of Δ of eggs with constants $\Delta_d = 0.50^\circ$ and $\Delta_p = \Delta_{soln.}$	
0.05	0.17	0.46	0.455	
0.1	0.34	0.47	0.478	living
0.12	0.39	0.50	0.485	
0.2	0.68	0.51	0.524	eggs
0.3	1.05	0.56	0.573	
0.5	1.68	1.52	0.660	eggs
1.0	3.34	3.02	0.885	decayed

In the analysis of the figures in Table 5 we shall first leave the third column of figures out of consideration. It is seen from this third column that the combined depression of the eggs is very little in solutions of up to 0.3 Molar NaCl. If the depression of the external solution is raised from 0.02° (conductivity water) to 1.05° (NaCl 0.3 Molar), the depression of the eggs only rises from 0.43 to 0.56° , i.e. it only rises 0.13°, although the osmotic pressure of the medium is increased 50 times. For further increases in the concentration of the external solution, this relationship changes very sharply: for concentrations of 0.5 molar upwards, the depression of the eggs rises almost to the value of the depression of the external solution. Thus there must be a critical point between the concentrations of 0.3 and 0.5 Molar, above which the physiological standard of the osmotic pressure in the egg cannot be maintained. It is not difficult to see the significance of this point; we observed that decay of the eggs under the injurious influence of the solution during the time intervals chosen, began to occur at this point. If the egg is still living, the plasma surface prevents the penetration of salt ions into the interior of the embryo and yolk, despite the strong excess pressure from the outside. If the egg is decaying, its interior becomes similar in respect of osmotic pressure to the external medium.

The behaviour described above shows that the osmotic pressure of trout eggs is largely independent of the external medium. In order to investigate this question more closely, let us turn to the explanation of the increase in the total depression which occurs in NaCl solutions of 0.05 - 0.3 Molar (with living embryos). Two cases are possible here: either part of the salt ions penetrate the plasma surface, and cause a slight rise in the osmotic pressure of the interior of the egg, or the solution only enters the perivitelline cavity where the osmotic pressure of the yolk and embryo remain constant. We can easily put this to the test by making use of the equation (1) given above (see page 776)*. On the basis of our results on the permeability of the egg membrane we can state that the osmotic concentration of the perivitelline fluid after the eggs have been immersed in the NaC solutions for 18 hours, certainly becomes equal to the osmotic concentration of these solutions. Thus we replace the constant Δ_p in our formula by a variable $\Delta_{soln.}$, i.e. the depressions of the NaCl solutions, and determine y from this -

* page 7 of translation.

equation, since the x value is already known (when $y = 0.435^{\circ}$, $x = 6.37$ - see Table 4). If we assume that $\Delta_d = 0.50^{\circ}$ remains constant, we can write the equation

$$y = \frac{\Delta_d x + \Delta_{soln}}{x + 1} = \frac{0.50 \cdot 6.37 + \Delta soln}{7.37}$$

and we can determine the summary depression of the eggs (y) for all the solutions which we used. These theoretical values are given in the third column of Table 5. If these figures are compared with the observed values of the depression for eggs in NaCl solutions, a fairly exact agreement between the corresponding values is seen (only up to the critical point 0.3 Molar mentioned above, of course). The maximum difference between them is 0.015, i.e. within the limits of experimental error. This can scarcely be a chance agreement. The only probable explanation of this agreement is that the depression of the yolk and embryo actually remain constant owing to an increase in depression of the external solution; if the value (for the yolk and embryo) actually increased; the observed depression of the egg would of necessity exceed the theoretical value of y. Actually this does not happen; as can be seen from Table 5, the observed values of Δ varied from the theoretical on both sides; more often they are somewhat less then the theoretical values.

The osmotic pressure of the yolk with the embryo thus forms a strictly constant value, which is independent of the osmotic pressure of the external medium. This value remains constant for any concentration of the external solution, as long as the embryo is alive. In contrast to this, the osmotic pressure of the perivitelline fluid varies very rapidly during increasing concentration of the external solution, and we can report that here again the perivitelline fluid appears to act as an external medium and not to play any other part with regard to osmotic pressure.

Conclusion.

We came to the conclusion that the high constant osmotic pressure of trout eggs is a function of the living constituent of the embryo. It is a result of the regulation of osmotic pressure of the organism which is isolated by the external medium. I should like to add here, that it is a result of active osmotic regulation. As far as the means of this regulation of osmosis is concerned, the cause lies mainly in the osmotic isolation of the egg at the plasma surface, which ensures the adequate supply of electrolyte to the egg, while preventing the entry of superfluous electrolyte from outside.

However, this is only one aspect of the process of osmoregulation. We know that water is absorbed during embryonic development. Consequently, dilution of the interior of the organism must compensate for this to some extent. In addition to the isolating plasmatic layer, we must assume the presence of other regulating factors. It may be that the active osmotic substance may be formed continuously at a regular speed. On the other hand, Kronfeld and Scheminsky found that the water content of the yolk had fallen greatly by the end of the embryonic development. These authors believed that the osmotic pressure of the yolk must rise sharply as a result, and this made the assimilation of the embryo a process which is very difficult to understand. But we have seen already that the osmotic pressure of the yolk actually remains very constant during the whole of the development. Such relations between the % water content and the osmotic pressure in the yolk are

only possible on the assumption that the osmotic pressure is self-adjusting during development; it may be thought that this adjustment is caused by the reduction of the active osmotic substance. We cannot, of course, say anything positive about the nature and method of this osmotic regulation at present, but it appears to me that this question can be approached by experimental work. It should be added that hitherto we have very little knowledge of the fine mechanism of the regulation of osmosis in the animal kingdom. Let us, therefore, be content with the statement that the regulation of osmosis in trout eggs appears to be a very complicated continuous and active process.

Summary.

The osmotic pressure of the perivitelline fluid and the yolk of trout eggs were measured separately by the Drucker-Schrein method. The permeability of the egg membrane and the variations in the osmotic pressure of the eggs when placed in salt solutions were also investigated. The following results were obtained.

1. The osmotic pressure of the perivitelline fluid is very low throughout the period of development; estimated by its salt content, this liquid is similar in osmotic pressure to the usual sugar solution. The osmotic pressure of the yolk, on the contrary, is fairly high and is constant; thus it resembles trout blood.

2. The total depression of the eggs increases slightly during development. The increase is due to increase in the quantity of the perivitelline fluid. The relative size of this increase is calculated from the elementary equation.

3. The egg membrane is permeable to water, many electrolytes, saccharose and some intra-vitam stains. It is only impermeable to highly colloidal solutions and suspensions. Thus the egg membrane may play an important part in maintaining the high osmotic pressure in the interior of the eggs (see also in 1. above).

4. The high and constant pressure of trout eggs is in no way due to an equilibrium between the concentration of the internal solution and the external medium. It has been proved by observation that the osmotic pressure of the yolk and of the embryo remain constant whatever the osmotic pressure of the surrounding medium, as long as the embryo is alive. If the embryo decays, the osmotic pressure of the yolk immediately becomes equal to that of the external medium.

5. The regulation of the osmosis of trout embryos is the result of the activity of the living constituent of the organisms.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.