THE OSMOTIC PRESSURE OF THE EGG OF THE COMMON FOWL AND ITS CHANGES DURING INCUBATION

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Having shown in a previous paper¹ that the blood and eggs of birds are not isotonic, it seemed of interest to study the changes, if any, taking place during incubation, thus tracing the pressure variations from germ cell to chick. This difference in osmotic pressure between the blood and the egg was quite unexpected; its magnitude may be seen from the following table, in which Δ denotes the depression of freezing point of the fluid below that of pure water and P stands for the osmotic pressure, which was calculated from the formula $P = 12.06 \Delta - 0.021 \Delta^2$.

Bird	Number of experiments	Δ	P in atmospheres
Common fowl (<i>Gallus bankiva</i>)	15	0.607° C.	7.31 Blood
	12	0.454° C.	5.47 Egg
Duck (<i>Anas</i>)	8	0.574° C.	6.92 Blood
	9	0.452° C.	5.45 Egg
Goose (<i>Anser</i>)	4	0.552° C.	6.65 Blood
	1	0.420° C.	5.06 Egg

The above difference in osmotic pressure is accounted for by the diminution in the inorganic salts of the egg as compared with the blood serum. This was ascertained by estimating the chlorides in egg-white and plasma by Volhard's method, after incineration, but as only 0.03 to 0.02 grm. of chlorine was found in the ash owing to the small quantity of material available, the following table is not of great accuracy:—

Plasma, per cent. chlorine	Duck	Egg white, per cent. chlorine
0.278	...	0.080
0.276	...	0.088
0.312	...	0.088
	...	0.104
Mean 0.287 %		Mean 0.090 %
As NaCl 0.473 %		As NaCl 0.148 %

To examine the changes during incubation, eggs were placed in a Hearson egg-incubator and maintained at 40° to 41° C. Samples were taken out at intervals, and their freezing point determined with a Beckmann thermometer, care being taken to avoid freezing out of the

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solvent, water, thus leaving a too concentrated solution. The zero of the thermometer was re-determined frequently, both in the apparatus used and in powdered ice and water. Vigorous and continued stirring is necessary in dealing with viscous liquids such as eggs. It is to be regretted that, possibly owing to the lateness of the season—August and September—most of the embryos had died before the shell was broken. This is not a very serious drawback, however, as owing to the variations in the freezing points of fresh eggs of Gallus, from 0.427°C. to 0.480°C. in a dozen determinations, quantitative results proportional to the time of incubation are not to be expected. The experiments are tabulated below:—

Period of incubation		Embryo present	No embryo	Musty eggs
Days	Hours	Δ	Δ	Δ
4	17	0.480°C.	—	—
4	17	0.458°C.	—	—
7	22	0.605°C.	0.532°C.	0.620°C.
11	18	0.565°C.	—	0.656°C. embryo present
13	17	0.605°C.	0.538°C.	—
14	17	0.565°C.	—	—
14	17	0.563°C.	—	—
14	22	0.590°C.	0.535°C.	—
14	22	—	0.550°C.	—
17	22	0.570°C.	0.538°C.	—
17	22	0.590°C.	—	—
18	17	0.611°C.	—	0.633°C. embryo present
18	19½	0.598°C.	0.533°C.	—
19	22	—	—	0.658°C. embryo present
19	22	—	—	0.680°C. embryo present

Control egg, in room at 15° to 20°C. for twenty days $\Delta = 0.480^{\circ}\text{C.}$

In these determinations the whole of the liquid contents of the egg were mixed, the embryo being rejected, and 12 to 15 c.c. poured into the tube containing the thermometer. It may be seen from the table that there is a rise in the numerical value of the depression of freezing point throughout the period of incubation, the final value, 0.590°C. to 0.611°C. , being about that given by the blood, 0.591° to 0.662°C. , mean 0.607°C. On the other hand, eggs containing no embryos show a similar rise, but to a much less extent; apparently, as the figures range from 0.532° to 0.550°C. , there is an initial rise due possibly to evaporation, although the egg chamber is nearly saturated with water vapour. Thus the rise in osmotic pressure during incubation may be divided into two parts:—

- (a) The rise, as shown by unfertile eggs, probably due to evaporation.
- (b) The rise due to metabolism of the embryo.

In connection with the latter it is to be noted that both yolk and white become much less viscous during incubation if there is a developing embryo, probably owing to the presence of an enzyme liquefying the reserve materials.

Eggs with or without embryos, but which were musty, gave high values in every case, 0.680° C. being the maximum obtained, though it is quite reasonable to expect that if bacterial action had been more extensive a much higher figure might have been reached. More attention was paid to this effect of bacteria in the second series of experiments than in the first, so this, combined with the impossibility of detecting putrefaction in its initial stages, may account for some of the high values of Δ obtained for eggs with embryos in the early stages of incubation.

The difference in osmotic pressure between the blood and eggs of birds, together with the gradual rise in pressure of the egg to approximate isotonicity with the blood, may, perhaps, be accounted for by the following speculation. There is much evidence that the ontogeny of an organism is a more or less abbreviated repetition of its phylogeny; by extending this view, based on morphological grounds, to physiology, there is reason to believe that birds are descended from ancestors with a lower osmotic pressure, about five or five and a half atmospheres. Fossil remains point to the reptilia as the class from which birds developed. It remains to examine the osmotic pressures of the blood of this group. The following determinations are available:—

	Δ	P
<i>Thalassochelys Caretta</i> , L.	= — 0.615	... Bottazzi and Ducceschi ¹
" "	= — 0.602	... Rodier ²
<i>Emys europaea</i> (freshwater species of above)	= — 0.463 to -- 0.485	... Bott. and Ducc.

Thus the osmotic pressure of the blood of the only freshwater reptile which I have been able to see recorded, is not very different from that of the eggs of birds, being, in fact, within the limits of variation. It may seem fanciful to regard the osmotic pressure as a hereditary character transmitted with great regularity, but such a possibility seems well worth serious attention in view of the high elaboration of the organs regulating this pressure in all the vertebrates, a regulation not quantitative only, but qualitative. In this connection Loeb's researches (see 'Dynamics of Living Matter') show how marked is the effect of a qualitative difference in the salts present upon developing

¹ Quoted from Rodier.

² Station Zoologique d'Arcachon, 1899.

embryos. The whole question of the constancy of osmotic pressure is discussed at length by E. H. Starling (see 'Fluids of the Body'), where Macallum's interesting views on the origin of the blood plasma salts from the waters of a pre-Cambrian ocean are also considered. So apart altogether from the, possibly misleading, agreement in osmotic pressure with certain reptilia, it seems not unreasonable to suppose that birds are descended from a stock which possessed a considerably lower osmotic pressure.

Turning now to the egg membranes which are easily observed, the existence of a fair degree of semi-permeability may be demonstrated by the experiment figured by Bergin and Davis ('Principles of Botany'), in which a chip having been taken out of the rounded end of an egg, over the air space, without piercing the outer membrane, a glass tube of narrow bore is inserted into the pointed end and cemented in position. The contents of the egg will slowly rise in the tube to a height of over a metre when the egg is placed upright in a few centimetres depth of distilled water. If the yolk be pierced it will colour the column, which will stay at its upper limit for several days and then slowly sink. That this membrane is somewhat permeable to sodium chloride may be seen by adding a few drops of silver nitrate solution to the water in which the egg is standing, when a turbidity or precipitate is produced. If, however, the outer membrane be pierced, the rise in the tube will only amount to a few centimetres, for the inner membrane is much more permeable than the outer, as seen by the silver nitrate test. It is to be noted that, while the two membranes are in contact throughout the greater part of the egg, at the blunt end they are separated by the air-space.

Acetic acid also penetrates the two membranes, for if an egg be placed in the dilute acid till the shell is dissolved, and then washed and placed in distilled water, it will be found that the egg swells greatly—to nearly twice its former volume, in fact. The water surrounding it becomes acid, even after numerous changes. If now the egg be placed first in a strong solution of sodium chloride, and then in water, it will swell again, and in this condition may be freely handled without risk of puncture. If the membrane be cut after the shell is dissolved, the egg will be found to be coagulated by the acid, having the appearance of having been boiled.

The yolk is also enclosed in a delicate membrane with some degree of semi-permeability, for on carefully breaking a fresh egg into water, and rinsing to remove the white, the yolk swells considerably and becomes a

paler yellow with a turbid appearance. The germinal disc soon disappears from sight, apparently sinking into the yolk, which by the dilution is now of a lower specific gravity, in which the disc can no longer float. This is evidence that the disc is surrounded by a membrane either impermeable, or more probably less permeable, to water than the yolk membrane. In this distended condition the yolk membrane is very delicate, being ruptured by the weight of its own contents if the water be drawn off from around it. In spite of this slighter permeability of the germinal disc as compared with the yolk membrane, there seems to be no doubt that the disc is normally approximately isotonic with the egg as a whole.

In the above experiment I have been unable to observe whether the disc really sinks into the yolk; it becomes lost to view, but it is just possible that in the imbibed condition it may not be practicable to distinguish it from its surroundings.

That the germinal disc is not necessarily absolutely isotonic with the fluid surrounding it, but is rather in a state of osmotic equilibrium with it, seems extremely likely from the researches of Moore and Roaf (Bio-Chemical Journ., p. 55, Jan. 1908) on the equilibrium between the cell and its environment. These authors showed that there was normally a difference of 0.02° to 0.03° C. between the freezing point of the serum and red blood corpuscles of the pig, and that diluting the blood affected serum and corpuscles unequally. The work of Dakin on the variations in the osmotic pressure of marine vertebrates by change in the external medium (Bio-Chemical Journ., p. 473, Dec. 1908), shows that in these cases also the systems are in equilibrium rather than isotonic.

SUMMARY

The osmotic pressure of the egg of *Gallus bankiva*, as calculated from freezing point depressions, rises during incubation from about 5.5 atmospheres to about 7.3 atmospheres, the latter value being approximately that of the osmotic pressure of the blood of the same bird.

Bacterial action during incubation may cause the pressure to rise to over eight atmospheres. The view is put forward that birds are descended from organisms with an osmotic pressure of five atmospheres or less.

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