THE ABSORPTION OF WATER AND THE ASSOCIATED VOLUME CHANGES OCCURRING IN THE EGGS OF *NOTOSTIRA ERRATICA* L. (HEMIPTERA, CAPSIDAE) DURING EMBRYONIC DEVELOPMENT UNDER EXPERIMENTAL CONDITIONS

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(With Three Text-figures)

INTRODUCTION

IT is well known that the eggs of some species of insects increase in size during embryonic development and that there is a simultaneous absorption of water by the egg. Kerenski (1930) has weighed swelling eggs of *Anisoplia austriaca* Reitt. (Coleoptera), Ludwig (1932) those of *Popillia japonica* Newman (Coleoptera) and Evans (1933) has estimated the volume changes which occur in the eggs of some hymenopterous parasites of certain Diptera. The relation between the volume change and the uptake of fluid by the eggs of any one insect species apparently has not been investigated.

The present paper deals with the eggs of the capsid *Notostira erratica* L. and their increase in volume, weight and water content during embryonic development at a constant temperature. These eggs possess a special structure, the subopercular yolk-plug, accommodating them to changes in volume; this structure, and its function, has been discussed elsewhere (Johnson, 1934) and the present paper only briefly considers its behaviour during water absorption by the egg.

METHODS OF REARING, WEIGHING AND VOLUME DETERMINATION OF THE EGGS

Rearing. The eggs used in the following experiments were from the summer form of *Notostira erratica* (Female 1 of Butler) (Butler, 1924) and were obtained by the method previously described (Johnson, 1934). During the experiments these eggs were reared at 28° C. (±0·05°) in direct contact with distilled water of approximately *p*H 7·0, in the following manner.

A silica combustion spoon was held by a rubber bung so that the bowl projected into a conical Pyrex flask. A little distilled water within the flask minimized evaporation from the few drops of distilled water which were held in the spoon. Upon the distilled water in the spoon, eggs in batches, from five to thirty, were floated. The flask containing the eggs was immersed in a water-bath, at constant temperature of 28° C., with the open end of a capillary tube through the bung projecting above the surface of the water. The flask possessed a thermometer with the bulb near the spoon.

Frequently after about the third day from oviposition the eggs sank. This was due to a lowering of the surface tension at the chorion, as the latter became saturated with water, and not to an increase in density of the eggs. Submersion seemed to make no difference either to the embryonic development or to the rate of water absorption by the egg. Except where otherwise stated, eggs were extracted daily from the flasks and weighed. At the same time, the pH of the distilled water in the spoon was tested with a B.D.H. capillator (bromo-thymol-blue) and the water renewed. The pH varied between 6·8 and 7·1 from day to day throughout the investigation.

Weighing. The eggs were weighed on a Kuhlmann micro-balance which read to 1/1000 mg. A small glass trough was weighed, first alone, then with the eggs. In order to remove surplus water from the surface of the eggs the latter were placed upon a fine filter paper before being weighed; in spite of the apparent roughness of this method great accuracy was possible since any significant amount of water present on the surface of the eggs was immediately detected by the inconsistent behaviour of the balance as the moisture evaporated. The total daily time during which eggs were out of the thermostat was from 15 to 20 min. The results set out below are given in milligrams: the third decimal place should be regarded with due caution although the analysis of the data shows that it may be legitimately used.

Volume determination. There appears to be no standard method for measuring the volumes of small living objects without involving their death. Several micro-methods were tried during the present investigations but the following procedure was finally adopted.

A small pyknometer was made from a thick glass capillary tube by drawing it out to a small diameter and bending it to the shape shown in Fig. 1 A. The internal diameter of the wider limb was 0·73 mm., i.e. just wide enough for an egg to slip down. A fine mark was made with indian ink (etching with hydrofluoric acid weakens such a narrow tube) near the top of this limb. The bent limb was drawn to a fine point with an exceedingly small internal diameter 0·07 mm. at the tip.

After a thorough cleansing with chromic acid and a subsequent washing with water the pyknometer was completely filled with boiled and cooled distilled water by immersing the fine capillary tip in water and attaching the wider limb to a filter pump. After wiping with filter paper, the pyknometer was attached to the bulb of a thermometer with an elastic band. Attached to the thermometer, it was then suspended in an air thermostat consisting of a test-tube held rigid by a cork block in a rectangular glass vessel through which flowed a constant stream of tap water. The whole apparatus was clamped above a sink and the tap water allowed to overflow at the top of the rectangular vessel (Fig. 1 B).

When the temperature of the air in the test-tube became constant, water was gently withdrawn, with a piece of damp filter paper, from the fine capillary tip of the pyknometer, until the meniscus in the wider limb (viewed through a horizontal microscope) stood at the ink mark. The pyknometer was then detached from the thermometer, quickly weighed, and replaced on the thermometer. The meniscus was then raised to the top of the wider limb by placing a brush full of distilled water on the capillary tip. (This makes it easier to insert eggs than if the meniscus was some way down the limb.) Then the eggs were inserted into the wider limb, one by one from the tip of a fine brush; it was sometimes necessary to push them below the meniscus with a fine wire. The thermometer with pyknometer were then replaced in the mouth of the test-tube and the temperature allowed to come to its original value. The meniscus was again withdrawn to the ink mark and the pyknometer with water containing eggs was weighed. It was found to be inadvisable to use more than ten or less than five eggs for one volume determination, and the lowest number used in the results published here was five.

For accuracy with this technique it is necessary that (1) the pyknometer should be quite clean, internally and externally; (2) that the pyknometer should be manipulated

with forceps so as to avoid greasing the outside; (3) that the tip of the bent limb of the pyknometer should have as fine an internal diameter as possible in order to minimize evaporation of water while the weighings are being made.

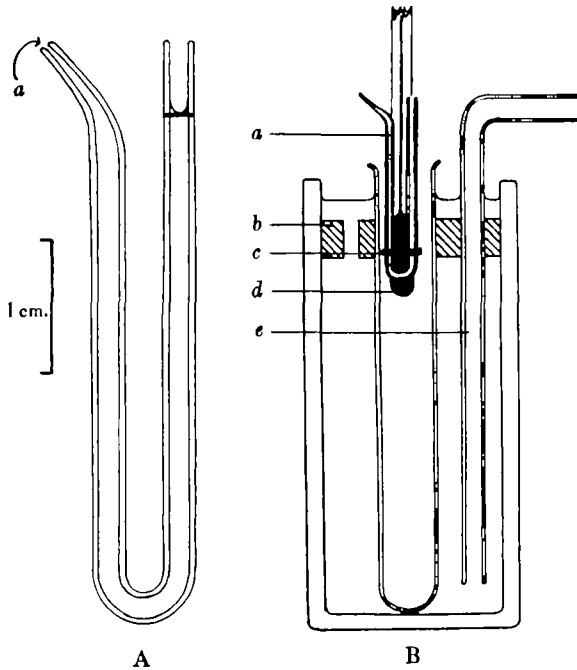Successive volume determinations with a small piece of platinum wire gave consistent results (0·4 cu. mm.).

Fig. 1. (A) The pyknometer: *a*, the capillary tip.
(B) The pyknometer, *a*, attached to thermometer bulb, *d*, and suspended in air in a test-tube; *b*, cork block; *c*, rubber band; *e*, inlet tube for tap water.

INCREASE IN WET WEIGHT OF EGGS DURING EMBRYONIC DEVELOPMENT

Three batches of eggs were weighed separately at intervals during embryonic development. The results showing the increase in wet weight are set out in Table I.

For the first 2 days after oviposition the increase in wet weight of the eggs is very slight. On the third day a large increase in weight occurs with a characteristic abruptness. From the third day until the eggs hatch there is a continuous increase in weight which follows a smooth and typical course (Fig. 2).

In the first batch the initial fall in numbers of eggs from forty-six to thirty-three was due to discarding apparently infertile eggs which failed to swell. This state can be detected after about 60 hours from oviposition when the eggs are incubated at 28° C. Correction was made for such discarded eggs in the weighings taken before their detection (except in the first weighing of the first batch) by subtracting the product of the mean weight of a single egg of the batch at oviposition times the number of eggs discarded, from the weighings preceding the detection of the in-

fertile eggs. Thus in the second and third weighings of the first batch, thirteen eggs were corrected for in this manner and the weight of the eggs given in the second and third lines of the third column is a value calculated from the weighing of thirty-three fertile and thirteen infertile eggs. Similarly the first five weights in column three of the second batch are calculated values from an original number of twelve eggs and the second and third values in the third column in the third batch are values calculated from an original number of eleven. The final drop in numbers of eggs in all three batches was due to eggs having hatched between successive weighings.

Table I. *Increase in wet weight of three batches of eggs of* Notostira erratica *L.*

Hours after oviposition	No. of eggs	Wet wt. in mg.	Mean wet wt. per egg in mg.	% increase in wet wt. per egg from wet wt. at oviposition
2	46	5·459	0·119	0
26	33	3·952	0·119	0
48	33	4·005	0·121	2·3
68	33	5·012	0·152	28·2
77·5	33	5·552	0·168	41·9
100	32	6·136	0·192	61·7
117·5	32	6·349	0·198	67·3
139	31	6·368	0·205	72·9
170	30	6·150	0·205	72·9
188·5	15	3·036	0·202	70·4
0	11	1·208	0·109	0
24	11	1·217	0·110	0·9
46	11	1·223	0·111	1·8
69·5	11	1·505	0·137	25·7
75	11	1·595	0·145	33·0
98·5	11	1·905	0·173	58·9
119	11	2·021	0·184	68·5
143	11	2·066	0·188	72·5
166·5	10	1·891	0·189	73·4
190·5	10	1·933	0·193	77·1
0	11	—	—	—
14	11	1·376	0·125	Taken as 0
39	8	1·030	0·129	2·2
63	8	1·239	0·155	24·0
87	8	1·516	0·189	51·2
97	8	1·571	0·196	57·0
135	8	1·712	0·214	71·2
159	8	1·738	0·217	73·7
183	8	1·760	0·220	76·0
188	7	1·427	0·204	63·2

All values in the fifth column showing percentage change from weight at oviposition are calculated from the mean weights for single eggs given in the fourth column. Reducing the data to a percentage enables the increase in wet weight of all three batches to be represented in a single graph with periods the same as those in the original data. It is seen from Fig. 2 that the three batches show similar amounts of percentage increase in wet weight and all points lie very close to a common curve. If a percentage is not used and the actual increase in wet weight or the actual wet weights themselves are plotted against time the points do not lie so close to a common curve as if percentage values are taken: this is due, in the case

of the actual weights, to a difference in the weights of the eggs at oviposition. In Fig. 2 the wide placing of the points from the general curve after 180 hours is not due to eggs losing weight but to an alteration in the mean weight of a single egg consequent upon the drop in numbers of the eggs in the batch as hatching occurs.
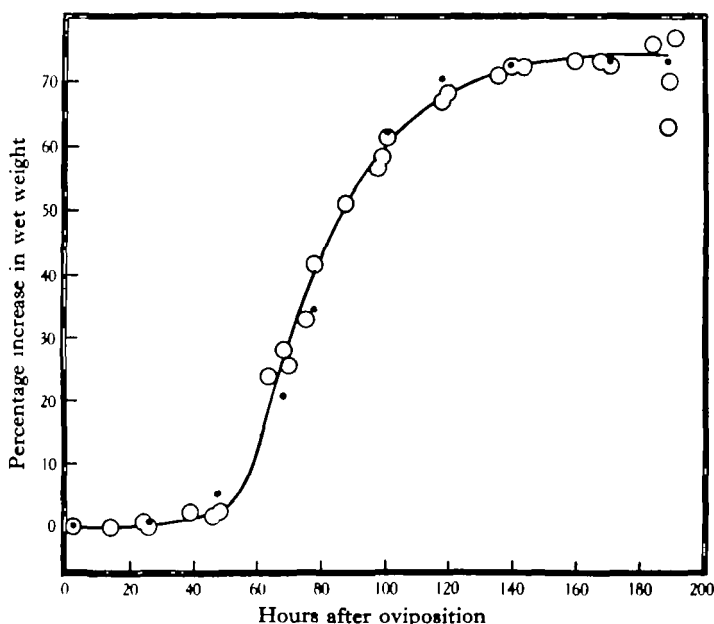

Fig. 2. Percentage increase in wet weight from wet weight at oviposition (Y_t) of eggs of *Notostira erratica* incubated at 28° C. in contact with neutral water.
O Experimental (observed) results (Table I).
● Results calculated from the equation $Y_t = 73\cdot3718/1 + 140e^{-\cdot0839(t-20)}$.

INCREASE OF WATER CONTENT OF EGGS DURING EMBRYONIC DEVELOPMENT

If when eggs are dried only water is lost, and if during embryonic development the dry weight of the eggs remains constant or decreases, then the increase in wet weight which takes place during the life of the egg must be due solely to the increase of water content. This increase must be due to the absorption of water from the external environment and probably also to oxidation of fat stored in the yolk of the egg. The water content of eggs was, therefore, determined.

Batches of eggs were weighed at oviposition, placed in the thermostat at 28° C. and taken out after a definite period of time, weighed and then dried at 100–110° C. until constant in weight. The weights of all batches of eggs which had been weighed and dried within 4 hours of each other were grouped, the corresponding time being the mean time for the group. The results are shown in Table II. It will be seen that there is a slight decrease in the weight of dry matter in the egg as development proceeds. The gross increase in wet weight of the egg does not, therefore, express as accurately as could be wished, the amount of water accumulating within the egg.

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An estimate of the amount of water in the egg at various stages during development is shown in Table II.

Table II. *Wet and dry weights of separate batches of eggs*

Time in hours	No. of eggs	Mean wet wt. per egg in mg.	Mean dry wt. per egg in mg.	$^0/_0$ water per egg
0	25	0·111	0·059	46·85
27	22	0·114	0·059	48·25
66	35	0·141	0·059	58·16
72	7	0·161	0·057	64·60
89	16	0·177	0·054	69·49
95	27	0·164	0·051	68·90
120	30	0·202	0·052	74·26
187	21	0·207	0·044	78·74
194	16	0·198	0·050	74·75

VOLUME CHANGES ASSOCIATED WITH THE INCREASE OF WATER CONTENT

Eggs were incubated under the conditions already described above. They were extracted after definite periods of time and their volumes determined with the pyknometer. A different batch of eggs was used for each determination. It will be seen from Table III and from Fig. 3 that, within limits which may reasonably be due to experimental error, the increase in volume of the egg is in direct proportion to the increase in wet weight. Neglecting the loss of dry matter during development for every fraction of a gram added to the wet weight of an egg there is the same fraction of a cubic centimetre added to its volume. In Fig. 3 the initial and final

Table III. *Weights and volumes of different batches of eggs*

Hours after oviposition	No. of eggs	Mean wet wt. per egg in mg.	Mean vol. per egg in 1000ths c.c.
0	6	0·111	0·097
0	5	0·128	0·105
20	31	0·126	0·120
24	8	0·115	0·100
27	8	0·116	0·110
28	6	0·111	0·099
47	25	0·126	0·113
67	9	0·142	0·131
69	8	0·148	0·136
71	25	0·131	0·119
91	8	0·167	0·158
119	12	0·186	0·175
119	6	0·205	0·195
119	10	0·214	0·206
120	6	0·188	0·180
186	15	0·202	0·193
187	11	0·204	0·189
189	8	0·205	0·195
191	5	0·177	0·162
192	5	0·211	0·202

drop in weight and volume is not real for each weighing is of a different batch of eggs and these differ in their weights at oviposition.

STAGES IN EMBRYONIC DEVELOPMENT AND IN THE BEHAVIOUR OF THE SUBOPERCULAR YOLK-PLUG DURING WATER ABSORPTION

The eggs when laid are yellowish white in colour and only begin to change to a paler tint as water is absorbed. This change in colour can be detected with the unaided eye about 60–65 hours after oviposition when eggs are incubated at 28° C. in contact with water. At this time the apparently structureless germ-band of the
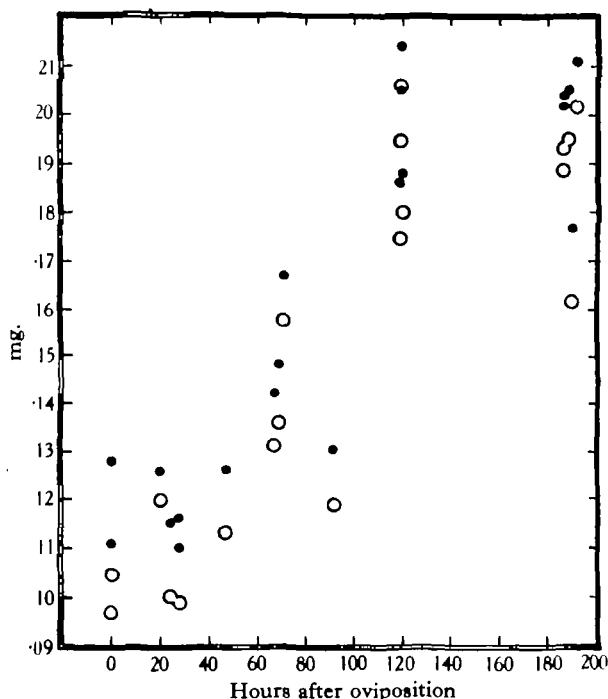

Fig. 3. Showing wet weights of different batches of *Notostira erratica* eggs, and the volumes of the same batches; 28° C., neutral water (Table III).
● Wet weight per egg in mg.   ○ Volume per egg in 1/1000 c.c.

developing embryo can be seen and the dark subopercular yolk-plug also commences to form beneath the rim of the cap. The rapid accumulation of water within the egg, which starts at about 55 hours after oviposition, coincides, therefore, with the laying down of the germ-band. When the yolk-plug is completely formed water absorption is proceeding rapidly and during this time, from 60 to 75 hours after oviposition the yolk, particularly at the posterior pole of the egg, becomes frothy. A vacuole filled with colourless fluid is eventually formed and this increases in size until it occupies at the posterior pole about one-sixth the length of the egg. This vacuole remains visible until about 120–140 hours after oviposition, by which time it has become filled with the developing posterior abdominal segments and

the tips of the legs and antennae of the embryo; thus, during the period of most active tissue formation the water content of the egg rapidly increases. The red elements of the eyes are by this time visible and the subopercular yolk-plug has extended from the chorion rim to its maximum distance. Hatching of the embryo takes place between 180 and 200 hours after oviposition, by which time increase in the wet weight of the egg has almost ceased. Immediately on hatching there is a slight drop in weight which is probably due to the loss of a small amount of unswallowed amniotic fluid. Apart from this slight loss, there is at no time during embryonic development a loss of wet weight by the egg provided that it is kept constantly in contact with water.

DISCUSSION

The eggs of *Notostira erratica*, like those of many insects (see Roonwal, 1936), increase in weight and absorb water. This phenomenon is also known to occur in animals other than insects. Thus the eggs of *Notostira*, like those of the trout (Gray, 1926), do not contain sufficient water when laid for the complete development of the embryo. And if *Notostira* eggs are deprived of water embryonic development ceases, only recommencing when water is again supplied.

Increase in water content of the egg can occur by the absorption of water from the external environment and by the production of metabolic water as yolk is oxidized within the egg. Increase in metabolic water would result in an increase of weight only if the yolk fats were fairly completely oxidized; and since the oxidation of fats equal in amount to the weight of the complete egg at oviposition would give an increase in weight only of about 16·5 per cent, we must conclude that the increase in wet weight of the egg is largely due to water absorbed from the external environment. The decrease in amount of dry matter during embryonic development will tend to balance any increase in weight due to oxidation of fats, so that the amount of water absorbed will probably be well represented by the increase in wet weight of an egg and also by the increase in volume.

Other authors who have published quantitative data on water absorption by insect eggs are Bodine, Kerenski, Ludwig, Evans and Roonwal. Unpublished figures of Evans for the eggs of some hymenopterous parasites of Diptera show that the increase in volume of the eggs is represented by equations similar to those which represent the increase in wet weight for eggs of *N. erratica*. Kerenski's figures also agree fairly well with the results expected with the equation for *Notostira* but with different constants, and the curve for *Popillia japonica* is also very similar to that for *Notostira*. In the experiments of Evans the possibility of the absorption of substances other than water from the host must be borne in mind.

Since Bodine (1929) has given only a diagram of water absorption by eggs of *Melanoplus differentialis* I have not made an analysis of his results. But the shape of the curve is different from those obtained by either Kerenski, Evans or myself for the other insects, in that the curve possesses a flat intermediate region representing a long period of almost complete suspension of water absorption.

No reasons for the sudden increase in water content in *Notostira* eggs after about

55–60 hours are put forward, but this increase coincides with the onset of visible tissue formation and is probably due to the production of osmotically active substances from the yolk by the developing embryo. This increase in water content begins long before the yolk-plug has commenced to extrude and absorption of water undoubtedly occurs over the whole area of the chorion: the sudden absorption of water does not take place at the rupturing of the chorion.

SUMMARY

The eggs of *Notostira erratica* L. (Hemiptera) swell during embryonic develop-ment. Increase in wet weight accompanies swelling.

Under the conditions of the experiments described in this paper the gross increase in wet weight of the eggs from oviposition till hatching is due entirely to the increase in amount of water. The weight of water per egg is, however, slightly greater than the increase in wet weight of the same egg, since the amount of dry matter per egg decreases slightly during development.

The increased amount of water must be largely due to absorption from the external environment: a certain amount, however, is probably metabolic water, which if due to oxidation of fat in the yolk would result in an increase in wet weight of the egg. This increase due to metabolic water will tend to be balanced by the decrease in dry matter during embryonic development.

The increase in volume of an egg during embryonic development is directly proportional to its increase in wet weight during the same period. Theoretically it would be more accurate to correlate volume change with the amount of water in the egg; this cannot be done accurately enough, however, with the present methods.

The embryonic development and the extrusion of the subopercular yolk-plug in relation to water absorption are briefly described. The work of other authors concerned with water-absorbing insect eggs is discussed.

The method used for finding the volume of the eggs (which does not involve their death) is described in detail.

ACKNOWLEDGEMENTS

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