WATER ABSORPTION AND METABOLISM DURING THE EMBRYONIC DEVELOPMENT OF THE HOUSE CRICKET ACHETA DOMESTICUS (L.) (GRYLLIDAE, ORTHOPTERA)

By

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A THESIS

Presented to the Faculty of Graduate Studies and Research of McGill University, in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

August, 1957

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I. INTRODUCTION

The eggs of certain species of insects, many of which are of great economic importance, absorb water from the external environment. Water uptake in these insects occurs in a definite stage in embryonic development. Previous studies on the egg of the house cricket, Acheta domesticus (L.), have shown that contact water is necessary for the development of the egg. The stage of embryonic development at which water absorption occurs, and the mechanism of absorption, however, have not been studied.

In the present investigation, water uptake by the egg is studied, the stage of embryonic development at which this uptake occurs is determined, and the mechanism of absorption is analyzed. A study is also made of the metabolic changes in the egg, because of the possibility that there may be some correlation between metabolic activity and water absorption.

II. REVIEW OF THE LITERATURE

A. THE WATER RELATIONS OF THE INSECT EGG

Insects, like all terrestrial animals, require water for survival. Water is generally obtained from the food, and some also drink water. The absorption of water by the egg, however, is of a comparatively rare occurrence, as the eggs of most insects are relatively impermeable to water and are provided with enough water to complete their development.

Perhaps the earliest reference to the increase in size of eggs as a result of absorption of water from the environment was noted by Rathke in 1844 (Roonwal, 1936). He observed an increase in the size of the eggs of Gryllotalpa (Orthoptera) and certain acquatic Trichoptera. Water uptake has since been observed in a number of species.

1. ORTHOPTERA - ACRIDIIDAE

Water absorption has been most extensively studied among the Acrididae, insects which are of great economic significance in most countries.

The first quantitative study of the changes in weight and water content of the egg was made by Bodine (1929). He found that during the early part of development before diapause, there was a marked increase in weight of the eggs

of Melanoplus differentialis. During diapause the water content remained practically constant, and there was a further increase in the post-diapause period.

Bodenheimer (1929), as a result of experiments on the eggs of Schistocerca gregaria taken from the field at different times, concluded that eggs which had completed one-third of their development under normal conditions (a moist soil and a saturated atmosphere) gave a reasonable hatch when placed in a desiccator at 100 per cent. relative humidity in the laboratory. In a later paper (Bodenheimer, 1932), he showed that newly deposited eggs were so sensitive to desiccation that it was impossible for hoppers to hatch unless the eggs had been placed under optimum conditions (100 per cent. relative humidity in the soil).

Bodine (1933) investigated the effect of immersing the eggs of Melanoplus differentialis in various solutions. He found that the eggs developed normally when placed in hypotonic or isotonic solutions. However, in hypertonic solutions the oxygen consumption fell more or less in proportion to the concentration used. Prolonged exposure resulted in marked morphological changes in the embryo.

Roonwal (1936) observed changes in weight in the eggs of Locusta migratoria migratorioides R.& F. during embryonic

development as a result of absorption of water from the soil. He showed that most of the water was taken up before katrepsis and that water was necessary for the completion of development.

Hamilton (1936) studied the effect of different humidities on the development of the eggs of Schistocerca gregaria and Nomadacris septemfasciata. He showed that when eggs were placed in moist sand at 100 per cent. relative humidity, a high percentage of the eggs hatched. Eggs placed in a desiccator at 100 per cent. relative humidity did not hatch. He concluded that it was essential for the eggs in the early stages of development to be in contact with free water and that they were unable to absorb water from a saturated atmosphere.

Thompson and Bodine (1936) studied the effect of desiccation on oxygen consumption and concluded that if desiccation had not been excessive, recovery both of water and respiration was possible. If, however, water loss had exceeded a certain limit, 2.5 to 3 mg.per egg, the eggs subsequently gained water far in excess of that lost, and this sometimes resulted in the rupture of the egg membranes.

Slifer (1938, 1950) has shown that in the eggs of Melanoplus differentialis water is taken up through a small, circular, specialized area (the 'hydropyle') in the yellow

cuticle. This structure consisting of two layers is situated at the posterior end of the egg and is a secretory product of a group of enlarged serosal cells ('hydropyle' cells).

Birch and Andrewartha (1942) studied the absorption of water by eggs of Austroicetes cruciata and concluded that the movement of water into diapause eggs could be described by Lillie's formula,

$$kt = \ln \frac{Veq. - Vo}{Veq. - Vt}$$

where Veq. is the volume at equilibrium, Vo is the volume at the first instant, Vt is the volume at time t, and k is the velocity constant. (Lillie,1916). They also studied the rate of water loss at different stages of development. The rate of water loss was greatest in the newly laid eggs, relatively slow for eggs in diapause, and progressively greater for eggs in post-diapause. The difference in rate of water loss between pre-diapause and diapause eggs, they claimed was due to the differences in the egg membranes, while the progressively greater rate of water loss in post-diapause eggs as compared with diapause eggs was due to increased metabolic activity.

Slifer (1946) has suggested that in the eggs of the grasshopper, Melanoplus differentialis, a wax or wax-like material is deposited at the hydropyle shortly after diapause

begins and this water-proofs the egg so that additional water required to complete development is prevented from entering. Development is resumed when the wax layer is broken down. In a later paper (Slifer, 1949), she carried out experiments which supported the idea that during diapause the hydropyle of the egg is water-proofed by a thin layer of wax.

Salt (1949) described a hydropyle in the posterior end of the eggs of the two-striped grasshopper, Melanoplus bivittatus, and determined the water uptake of the eggs. He found that the water uptake during the first four days at 25°C. was negligible, and after this it increased rapidly, the peak of absorption being reached on the eighth and ninth days. In several of the groups he also noted a second minor increase. The eggs absorbed about 60 per cent. of their original weight in water. Eighty-eight per cent. of this water was taken up during anatrepsis. Eggs deprived of free water from the time of oviposition and subjected to desiccation which was not severe, developed to the end of anatrepsis and remained viable for a long time. When water was provided such eggs absorbed it rapidly and continued their development. Salt (1953) later investigated the quantitative aspects of gain and loss of water under various conditions. He also confirmed his previous observations as regards water uptake.

Bodenheimer and Shulov (1951), studying the conditions necessary for the development of Dociostaurus maroccanus (Thunberg), came to the conclusion that the exact timing of water uptake is one of the principal factors of egg development. In Dociostaurus, loss of water in the initial stage of development appears to be obligatory, as is its intake when the eggs are morphologically and physiologically ready for katrepsis.

Matthée (1951) made a detailed study of the structure of the egg of Locustana pardalina Walk. and the mechanism protecting the egg against desiccation. He also studied the absorption of water and analyzed the factors involved in this process. Newly laid eggs kept at 35°C. absorbed very little water, if any at all, during the first two days but with the completion of the hydropyle on the third day, water was readily absorbed. Quiescent eggs which have a hydropyle absorbed water readily from the beginning. In both types of egg most of the water was taken up prior to katrepsis. Matthée observed that water was absorbed from glucose solutions having an osmotic pressure of 14.56 atmospheres (equivalent to a 2.1 per cent. solution of sodium chloride when the osmotic pressure of the yolk of the eggs was only 11.92 atmospheres (equivalent to a 1.7 per cent. solution of sodium chloride). When the eggs were deprived of oxygen

in an atmosphere of nitrogen, they did not absorb water though they remained alive. When oxygen was again supplied, normal water uptake was soon restored. On this basis, Matthée concluded that water uptake must be regarded as an active physiological process in the sense that it depends on active respiring cells. He also showed that water uptake was in part due to passive diffusion of water through the egg membranes, but this was only of significance during the early period before stretching of the egg membranes occurred.

Shulov (1953) studied the role of water in the embryonic development of the desert locust, Schistocerca gregaria. He noted that at 27°C., the water uptake was very slow during the first four days. A rapid increase in weight began on the fifth day when the embryos were in the state of late anatrepsis, the rate of increase being greatest during katatrepsis.

2. HEMIPTERA - CAPSIDAE

Water uptake has not been studied to any extent in the Hemiptera. The only notable studies were those made on the capsid bug, Notostira erratica. Johnson (1934), in a very detailed study of the development of the egg, observed that as the egg became swollen the yolk extruded as a plug under the operculum or micropylar cap. He then measured the water-content and found that it rose from 0.052 mg. per egg initially to 0.148 mg. per egg at hatching; correspondingly

the dry weight diminished from 0.059 mg. to 0.050 mg. per egg. Johnson (1937) later made a quantitative study of the increase in volume, weight and water content of the eggs at 28°C. He observed a sudden increase in water content about 55 to 60 hours after oviposition and a continuation of this increase until the time of hatching. He was unable to account for the sudden increase but thought it might be due to the production of osmotically active substances from the yolk by the developing embryo. He found that if eggs were deprived of contact water embryonic development was suspended, recommencing only when water was supplied.

3. COLEOPTERA - SCARABAEIDAE

The absorption of water by the eggs of coleopterous insects has been studied experimentally in three species of scarabaeids. Kerenski (1930) showed that the increase in weight of the eggs of Anisoplia austriaca Reitt. during development was due to absorption of water. The increase, he claimed, was not due simply to osmosis for the eggs increased in weight when placed in a 4 per cent. solution of sodium chloride, a 2 per cent. solution of potassium nitrate and a 2 per cent. solution of barium chloride. He concluded, therefore, that the eggs of A.austriaca were covered by a membrane which excluded the salt but absorbed

water from the various solutions.

Ludwig (1932) found that the water content of the eggs of the Japanese beetle, Popillia japonica, increased from 49 per cent. in the newly laid egg to 84 per cent. at the end of 14 days at 20°C.

Rothstein (1952) observed a change in the weight of the eggs of the Japanese beetle, Popillia japonica, during the first four days of embryonic development at 30°C. This was due to the imbibition of water. The water content of the eggs during this time increased from 50 per cent. to 81.3 per cent.

Laughlin (1957) observed that the egg of the garden chafer, Phyllopertа horticola L., absorbed nearly twice its own weight in water during the early part of embryonic development. A close correlation was shown to exist between the water content of the egg and the stages of embryonic development at different temperatures.

4. LEPIDOPTERA - PIERIDAE

Water absorption from the external environment is a rare phenomenon among lepidopterous insects. Beament and Lal (1957) showed that the increase in weight of the eggs of the cabbage butterly, Pieris brassicae (L.), was mainly due

to the absorption of moisture by the cement layer (outer layer of the chorion). They concluded that it was unlikely that this water could be used by the embryo, but might materially affect the success of eclosion.

5. ORTHOPTERA - GRYLLIDAE

The absorption of water by the eggs of crickets has not, until quite recently, received any attention. Heymons (1895) noted that during embryonic development the eggs of Acheta domesticus and A.campestris increased in size. The exact cause of this swelling, however, was not diagnosed.

The first important study on the effect of moisture on the development of the eggs of crickets was made by Browning (1953). He found that when eggs of Gryllulus commodus Walker were incubated on a moist surface at three constant temperatures, the rate of water uptake was much more rapid at the higher temperature. He also studied the rate of water loss, and concluded that water loss to the extent of more than 20 per cent. of the original weight generally resulted in the egg's death.

Busvine (1955) noted that contact water was necessary for the development of the eggs of Acheta domesticus, but no quantitative studies were made.

Ghouri (1956) observed that contact water and a saturated atmosphere were necessary for the development of the eggs of the Pakistani and Canadian strains of Acheta domesticus as well as for two related species of crickets, A.configuratus and Gryllodes sigillatus. He showed that eggs placed on a moist surface absorbed a quantity of water equal to their original weight, 24 to 50 hours after oviposition, depending on the species and temperature. The more rapid the rate of development the sooner was water uptake begun. He concluded that water uptake appeared to be correlated with the same stage of embryonic development in all species studied.

B. METABOLISM IN THE INSECT EGG

1. RESPIRATORY METABOLISM

Fink (1925) studied the rates of respiratory metabolism during the embryonic and metamorphic development of several species of holometaboleus insects. He found that in these eggs (Leptinotarsa decemlineata, Crioceris asparagi and Anasa tristis) which are laid on foliage or on the soil surface there was a short 'formative' period during which respiration was lowest, followed by a period of increasing respiration as development proceeded. In those species which deposit their eggs in the soil, such as Cotinis nitida

and Popillia japonica, the 'formative' period was much longer. The respiratory quotient in all these insects was low, varying from 0.42 to 0.71.

Melvin (1928) made a study of the oxygen consumption of the eggs of four species of insects, Anasa tristis DeG. (Hemiptera); Tropoea luna L. (Lepidoptera); Samia cecropia L. (Lepidoptera); and Pyrausta ainsliei Hein. (Lepidoptera). He observed a 'formative' period similar to that described by Fink (1925). However, the determination of the 'formative' period in these eggs appeared to be due to the length of incubation period rather than site of egg deposition.

Bodine (1929) studied the rates of respiratory metabolism during the entire developmental period of the eggs of several species of grasshoppers. He showed that grasshopper embryos could be grouped into three classes according to type of development. In Class I are those eggs that normally are subjected to low temperatures but will hatch if kept at high temperature, e.g., Melanoplus differentialis. Such embryos show a cyclic respiration with a peak preceding diapause, a decline during diapause followed by an abrupt rise as development resumes. The position of the respiratory curve can be influenced by changes in temperature, but the shape of the curve remains constant. Representing Class II are the eggs of Chortophaga and Romalea, eggs which are not

normally subjected to low temperature. The respiratory curve for such embryos is a continuous hyperbola with the initial, low level representing the 'formative' period. In Class III are such grasshoppers as Circotettix, whose eggs can only hatch if kept at low temperatures. In Melanoplus differentialis, Bodine observed that the respiratory quotient through embryonic development was between 0.8 - 0.7 indicating that fat was the chief metabolite.

Burkholder (1934) made a quantitative study of the oxygen consumption in the egg of Melanoplus differentialis and correlated it with the morphological development of the embryo. He observed that during the pre-revolution period, as the rudiments of the embryo were being formed, there was a gradual increase in oxygen consumption. At this stage, the embryo generally goes into a diapause which is characterized by a low respiratory rate. With the termination of diapause the oxygen consumption rose rapidly with a series of maxima at the times of (a) early revolution, (b) late revolution, at which stage dorsal closure takes place, and (c) post-revolution, from yolk engulfment to hatching.

Boell (1935) showed that in the developing eggs of Melanoplus differentialis, the respiratory quotient fell rapidly from an initial value of 0.95 (range 0.87 to 1.05) to approximately 0.6 in prediapause. It remained constant at

this value until diapause was completed. In early post-diapause, the R.Q. showed a slight decline followed by a rise and a levelling off at the time of hatching. This seemed to indicate that during embryogenesis fat is being metabolized.

Bodine and Boell (1936) compared the respiration of the naked grasshopper embryo with that of the intact egg. They showed that respiration of the naked embryo was lower than that of the whole egg during the pre-diapause and diapause stages, indicating the importance of extra-embryonic structures in metabolism. In the post-diapause stage, as the embryo engulfed the yolk, its respiration increased relatively until it equalled that of the intact egg.

Tuft (1949) observed a close correlation between developmental stages and respiration for the egg of Rhodnius prolixus at different temperatures. He showed that at both 25°C and 21°C., the oxygen consumption gradually increased in the pre-revolution period. As revolution commenced, the oxygen uptake rose rapidly with the maximum 24 hours after revolution. There was a slight decrease in oxygen consumption in the early post-revolution period as the embryo engulfed the whole yolk, and then it increased again to eclosion. Developmental studies showed that in this later period cell division predominates.

Ludwig and Wugmeister (1955) found that in Popillia

japonica the oxygen consumption at 33°C. increased rapidly during the first day of embryonic development, remained constant during the next two days, and then increased steadily until the time of hatching.

2. BIOCHEMICAL CHANGES

The biochemical changes in the egg during embryonic development have not been studied to any great extent.

Rudolfs (1926a,1926b and 1929) investigated the chemical changes during the embryonic and postembryonic development of the tent caterpillar, Malacosoma americana Fab. He found that the ether soluble materials (fats) of the egg masses were about 4.45 per cent. of the dry weight and that they decreased rapidly during the first few weeks, until at hatching only 0.56 per cent. remained. The average nitrogen content was about 13 per cent. In the initial stages of development, the nitrogen content of the egg masses was more or less constant but just prior to hatching, there was an increase. Glycogen remained practically constant at 0.28 per cent. during embryonic development.

Slifer (1930) observed that in Melanoplus differentialis higher fatty acids composed 17 - 22 per cent. of the dry weight of the eggs at the time of laying; these diminished rapidly during the period of growth before and

after hibernation and at the time of hatching less than half remained.

Hill (1945) studied the changes in reducing substances and in acid hydrolysate (glycogen) during the embryonic development of Melanoplus differentialis and concluded that the role of carbohydrate as a source of energy was more evident in the first five days than in later stages of development.

Needham (1950) presented the theory that in embryonic development carbohydrates, protein and fat are utilized in that order. The evidence for this theory was based primarily on the value of respiratory quotient as an index of metabolism.

Biochemical changes in the egg have been extensively investigated in the Japanese beetle, Popillia japonica Newman. Rothstein (1952) found that the nitrogen content was fairly constant during embryogenesis. Glycogen decreased rapidly from an initial value of 0.021 mg. per 100 eggs to 0.012 mg. after 3 days at 33°C. A second decrease in glycogen occurred just before hatching. There was no change in the free lipid (ether-soluble content) during early embryogenesis; however, beginning with the fourth day, a decrease began which continued until the time of hatching, the total loss amounting to approximately 58 per cent. of the available

supply. On the basis of these observations, Rothstein concluded that glycogen furnishes the main source of energy during the early part, and free fats the main source during the latter part of embryogenesis. Ludwig and Rothstein (1952) corroborated Rothstein's (1952) observations that nitrogen content remained constant during embryonic development.

III. MATERIALS AND METHODS

A. REARING THE CRICKETS

The insects used in these experiments were obtained from the cultures of Dr. A.S. Ghouri, a former graduate student. The specimens of Acheta domesticus (L.) were collected at Macdonald College, Quebec, while the adults of A.configuratus Walk. were reared from eggs sent out by the Department of Plant Protection, Government of Pakistan, Karachi.

The method adopted for rearing both adults and nymphs was the same as that described by Ghouri (1956) with very few modifications. Instead of the D_4 diet, used by Ghouri, the insects were fed on a diet of ground commercial animal food*. The adults were kept continuously in large wooden incubators at 28°C. and 50 per cent. R.H. and the nymphs at 33°C. and at a R.H. of 50 - 60 per cent. Humidity was controlled by means of saturated salt solutions as described by O'Brien (1948).

* Baby rabbit pellets, sold by Ogilvie Flour Mills, Montreal, P.Q.

B. COLLECTION OF EGGS

The method used for collecting eggs was similar to that described by Ghouri (1956). Small dishes containing moist sand were supplied to the adults when eggs were required. These were always removed after 12 hours so that the age of the eggs within the samples was never more than 12 hours.

The eggs from all oviposition dishes simultaneously removed were mixed and divided into small lots. Each lot was placed on absorbent paper and left for 3 hours on the laboratory desk to allow the excess water to evaporate; this facilitated subsequent separation of the eggs from the sand. The eggs were picked out of the sand with a fine camel hair brush, counted, and washed in several changes of distilled water. The eggs were examined under a binocular microscope and all unhealthy looking eggs were discarded.

C. INCUBATION OF EGGS

The eggs were incubated in batches of 50 or 100 in 4 oz. jars in water baths. The temperature of the water baths seldom varied more than $\pm$ 0.5°C. (Fig.1). A piece of moist cotton wool was placed at the bottom of each jar and this was over-lined with a piece of moist filter paper. The eggs were then uniformly distributed over the filter paper.

Fig. 1 - Water bath with 4 oz jars.

D. DETERMINATION OF WEIGHT OF EGGS

All weighing of eggs was done on a direct-reading balance* of sensitivity 0.01 mg.

The determination of the change in weight of the eggs of A.domesticus during embryonic development was made on batches of 100 eggs. The eggs were counted, washed, and air-dried on filter paper. The eggs were then weighed. One batch was selected and the initial oven-dry weight determined by drying the eggs in an electric oven at 100°C. for 24 hours and weighing. The remaining batches of eggs were incubated in water baths at 33°C. and 28°C. At daily intervals, one batch from each water bath was selected out, air-dried, and the final wet weight and oven-dry weight determined as above.

* Spoerhase Model 10M, Cave & Co., Vancouver, B.C.

IV. EMBRYOLOGY

The embryonic development of A.domesticus (L.) has been previously described by Heymons (1895).

In the present studies, the external morphological changes of the embryo, during its development, were observed. These observations were made under the same experimental conditions as those under which the physiological studies were carried out.

A. TECHNIQUE

The development of the embryo was studied at 28°C. and at 33°C. Batches of 50 eggs were incubated on moist filter paper and at daily intervals, one batch from each temperature was selected and fixed in Bouin's solution heated to 60 - 70°C. The chorion of each egg was punctured shortly after the solution had cooled, so that the fixative could enter the egg much more rapidly. The eggs were kept in the Bouin's solution for 24 hours, then washed in distilled water and stored in 70 per cent. alcohol. For a better study of the embryo prior to the formation of the eye spots, eggs were stained in toto with borax carmine and destained in acid alcohol until only the embryo retained the stain. The eggs were then transferred to a slide containing a few drops of glycerine and examined under

a binocular microscope. Portions of the chorion were removed and some of the yolk was dissected out.

B. FORM OF THE EGG

The newly laid egg of Acheta domesticus has an elongated rod-like appearance. It is usually concave ventrally and convex dorsally. The anterior pole is pointed while the posterior pole appears to be rounded. The micropyle is situated at the anterior end. As development proceeds, the external variations in form and shape of the egg are gradually levelled out by a swelling of the whole egg, and the shape is then cylindrical.

According to Heymons (1895) the chorion consists of two layers, an outer exochorion and an inner endochorion. The vitelline membrane lies just below the chorion in the newly laid egg.

C. MORPHOLOGICAL CHANGES IN THE EMBRYO

For descriptive purposes, the stages in the development of the embryo were divided into four periods: (1) Pre-revolution, (2) Revolution, (3) Early post-revolution, and (4) Late post-revolution.

1. PRE-REVOLUTION

In the newly laid egg, there was no sign of the germ

band. This first became apparent on the posterior part of the convex ventral side after 24 hours of incubation at 33°C. and 28°C. (Pl. I, Fig. 1). At this stage it was relatively small, and it extended scarcely one-third of the length of the whole egg. In the course of further development, the posterior end of the embryo pushed towards the dorsal side and after 48 hours at 28°C., it assumed a hook-shape (Pl. I, Fig.2). The embryo subsequently became completely sunken within the yolk with the head facing posteriorly. This stage of development was reached after 48 hours at 33°C. or 72 hours at 28°C. (Pl.II, Fig.3). Heymons (1895) has shown that the amniotic folds fused at this stage so that the embryo was covered on the ventral side by the amnion and the entire yolk was surrounded by the serosa.

Segmentation became apparent at the time of the dorsal flexure and was completed by the time the embryo became stretched out on the dorsal side. The rudiments of the gnathal and thoracic appendages were also apparent at this stage. There were no further external changes in the embryo during this period. It merely increased in size (Pl.II, Fig.4). The entire pre-revolution period took 72 hours at 33°C. and 120 hours at 28°C.

2. REVOLUTION

During the period of revolution, the embryo again reverted to the ventral surface of the egg by a counter-movement

that restored it to its original position with its head facing anteriorly (Pl.III, Fig.5). The revolution of the embryo was completed after 96 hours at 33°C. and 144 hours at 28°C.

The rotation of the embryo resulted in the rupture of the serosa. Heymons (1895) showed that the serosa at first covered the yolk at the anterior end, but later sank into the yolk to be absorbed. The amnion formed the provisional dorsal closure, but degenerated after final dorsal closure took place.

3. EARLY POST-REVOLUTION

The dorsal body wall was completed after 120 hours at 33°C. or 168 hours at 28°C. At this stage also, the eye spots appeared (Pl.III, Fig.6). The embryo subsequently grew anteriorly absorbing the yolk up to the anterior end and by 144 hours at 33°C. and 216 hours at 28°C., it occupied the whole egg (Pl.IV, Fig.7).

4. LATE POST-REVOLUTION

The period of late post-revolution was characterized by few external changes in the embryo, but there must have been marked internal changes in which the various organs were completed before hatching occurred. This period was completed between 168 hours and 216 hours at 33°C., and 240 hours and 336 hours at 28°C.

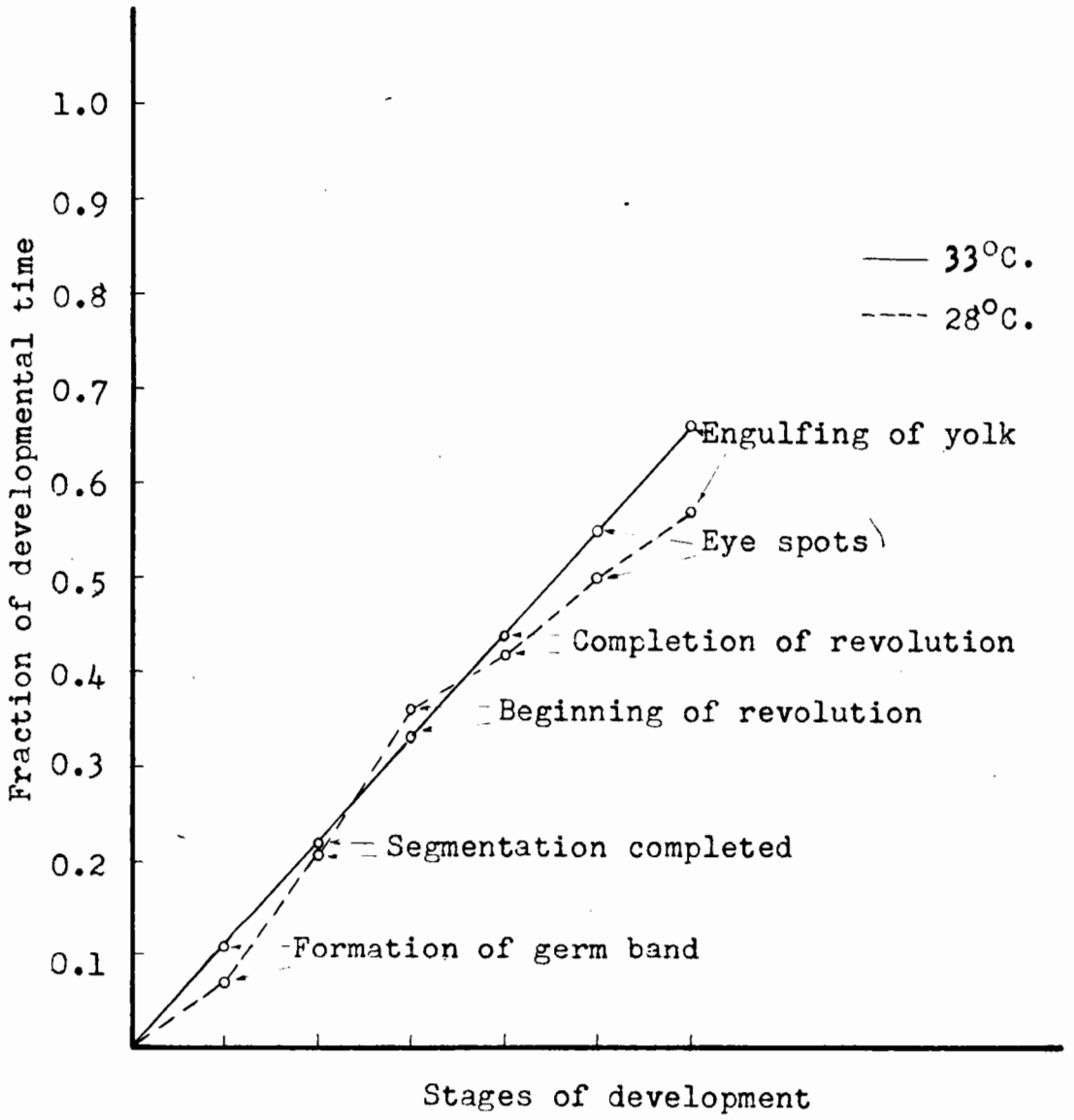

Fig.2 - The development of the eggs of A.domesticus at 28°C. and 33°C.

D. DISCUSSION

Fig. 2 shows that the various developmental stages did not respond in exactly the same way to changes in temperature. There was apparently a close correlation in the time at which segmentation was completed at 28°C. and 33°C. The engulfing of the yolk, on the other hand, was completed more rapidly at 28°C. than at 33°C. Within the batches of eggs examined, there was also some variation in the stage of development reached. Since development is not uniform, it is unlikely that a simple mathematical formula can be used to express precisely the rate of development of eggs at different temperatures.

V. WATER ABSORPTION

A. THE COURSE OF WATER ABSORPTION DURING EMBRYONIC DEVELOPMENT

Earlier studies by Busvine (1955) and Ghouri (1956) have indicated that contact water was necessary for the development of the eggs of *Acheta domesticus*. Ghouri (1956), in his studies on water absorption in three species of crickets - *A.domesticus*, *A.configuratus* and *Gryllodes sigillatus* - observed that uptake occurred at the same relative time in development in all three species and at all temperatures. He concluded that the commencement of water uptake appeared to be correlated with the same stage of embryonic development. In the following experiments, water absorption was studied and the stage of embryonic development reached was noted.

1. WATER UPTAKE BY EGGS OF *A.DOMESTICUS*

The changes in weight of the eggs of *A.domesticus* during embryonic development are presented in Fig. 3 in the text and in Appendix Table 1. The values for the change in weight at 33°C. are based on the means of four replicates, and that at 28°C. on three replicates of 100 eggs each.

During the first 24 hours of incubation at 33°C., there was a slight decrease in the weight of the eggs. The eggs

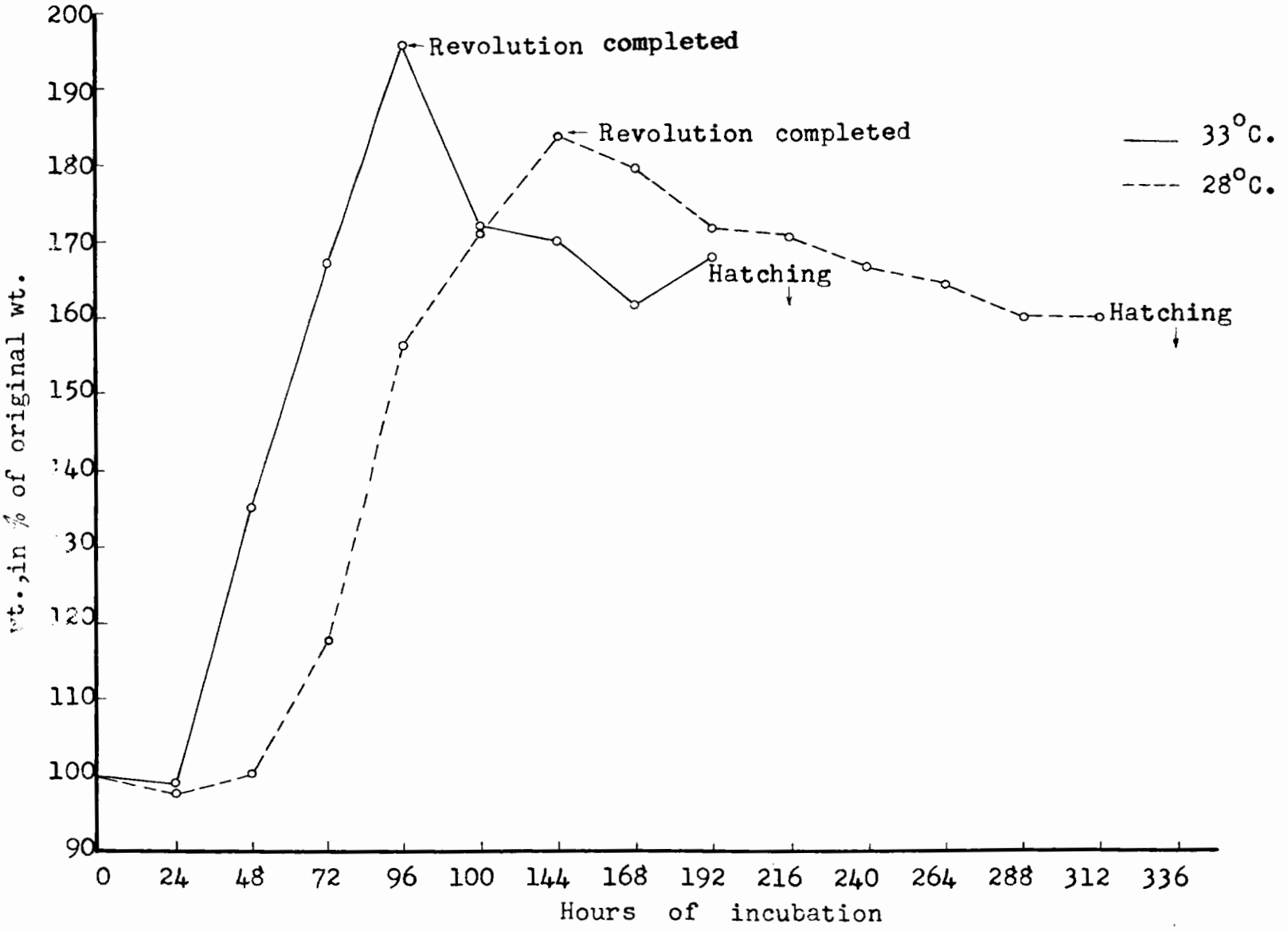

Fig.3 - Change in weight of eggs of A.domesticus incubated at 33°C. and 28°C. on moist filter paper.

began to absorb water rapidly after 48 hours of incubation, and after 96 hours the eggs had almost doubled their weight. A rapid decrease then followed, the weight of the eggs dropping from 195 per cent. to 173 per cent. in 24 hours. The weight of the eggs remained more or less constant during the next 48 hours, but there was a slight decrease with a minor increase 24 hours before hatching.

At 28°C., no water was absorbed during the first two days. A decrease in weight, similar to that observed at 33°C., was also noted at this temperature during the pre-absorption period. Water was absorbed after 72 hours, and the peak of water absorption was reached after 144 hours. The weight of the eggs at this stage was only 183 per cent. of the initial weight, as compared with 195 per cent. at 33°C. The greater extent of uptake of water at a higher temperature was also noted in A.configuratus, by Ghouri (1956). He attributed this to the differential effect of temperature on the speed of various physiological processes, since it seemed unlikely that the eggs required a larger quantity of water for completing development at the higher temperature.

A decrease in weight following the peak of uptake was noted at 28°C., but this decrease was more gradual than that observed at 33°C. This could be attributed to the effect of temperature on development. At 28°C. development is slower, consequently the developmental process would be more

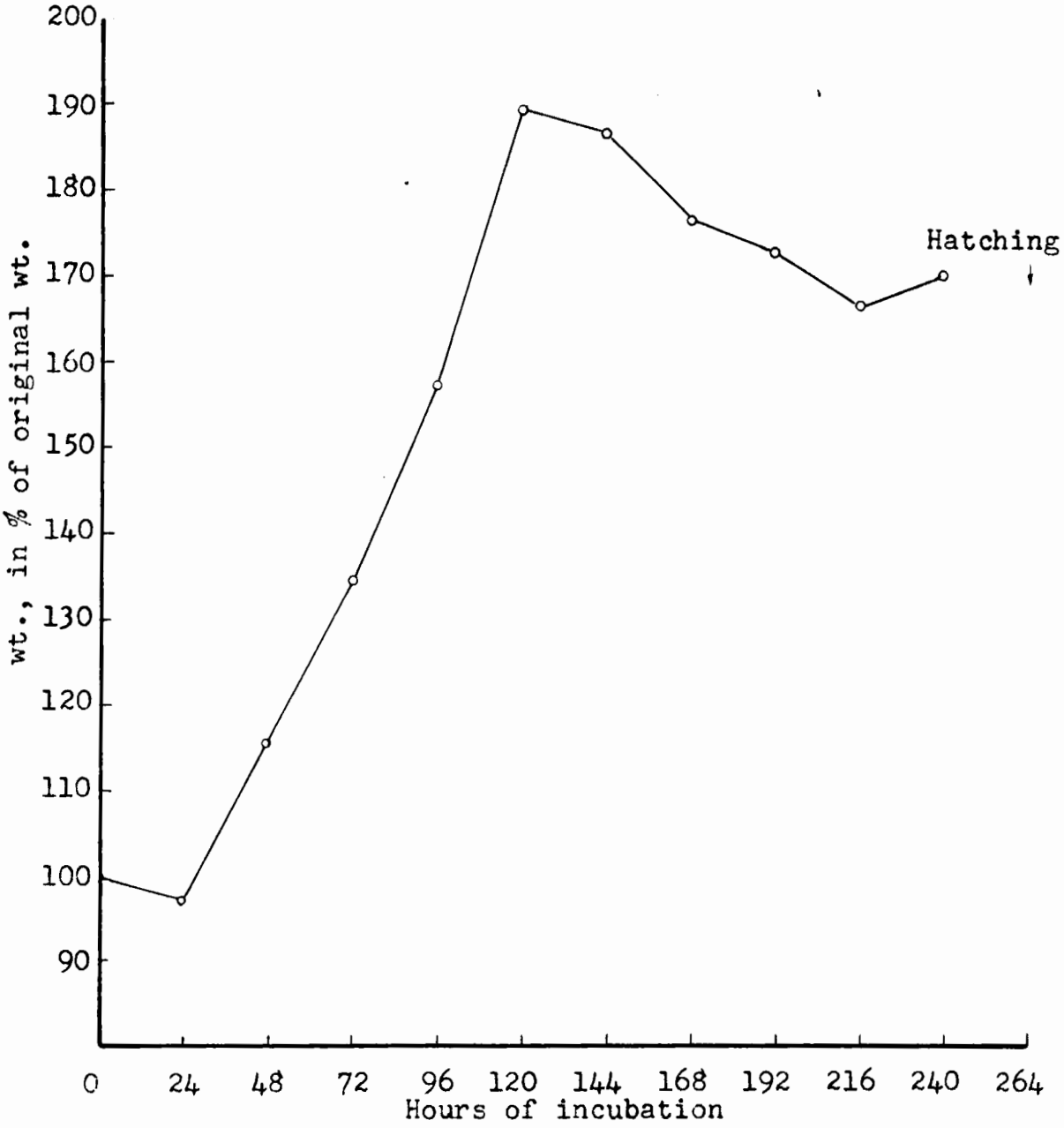

Fig.4 - Change in weight of eggs of A.configuratus incubated on moist filter paper at 33°C.

drawn out. At both 28°C. and 33°C., water uptake commenced when the serosa was completed around the embryo, as a result of the sinking of the embryo within the yolk, and ceased when the serosa was ruptured following revolution.

2. WATER UPTAKE BY THE EGGS OF A.CONFIGURATUS

For comparative purposes, the water uptake by the eggs of A.configuratus was studied at 33°C. Twelve batches of 100 eggs were used for these determinations. The initial wet weight of the eggs was determined after which they were incubated on moist filter paper. At daily intervals, one batch was selected and the final wet weight recorded.

Fig. 4 shows the changes in weight of the eggs of A.configuratus during embryonic development. The change in weight of the eggs of A.configuratus was somewhat similar to that of A.domesticus at 33°C. The initial decrease in weight at 24 hours was noted, as was the increase after 48 hours. However, the peak of uptake was reached after 120 hours as compared with 96 hours for A.domesticus. The longer period of absorption is probably due to the fact that the developmental period of A.configuratus is slightly longer. The mean incubation period of A.configuratus is 11.4 days, and that of A.domesticus is 9.1 days at 33°C. The decrease following the peak of absorption was much more gradual than that for A.domesticus. The eggs decreased in weight from

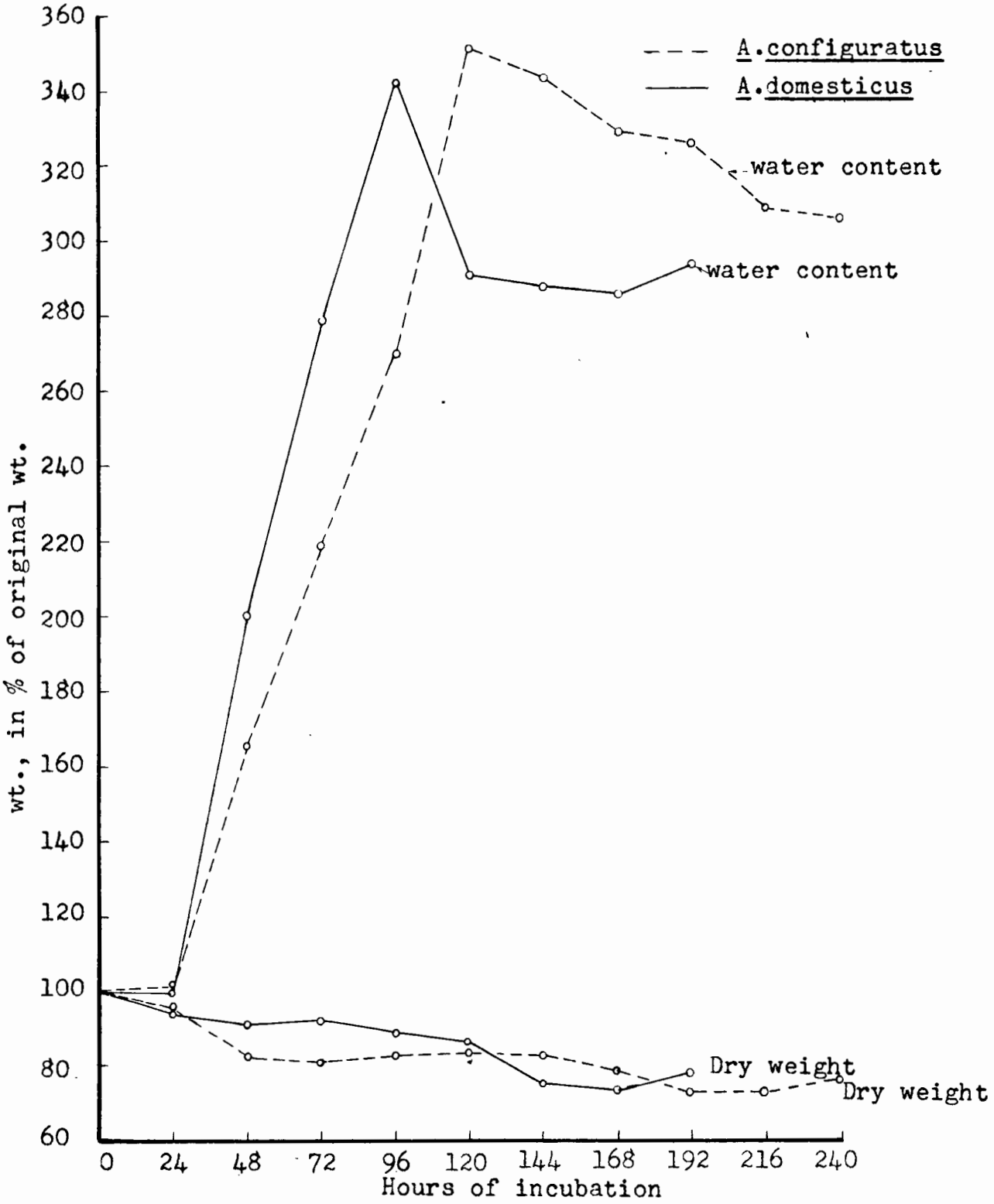

Fig.5 - Change in water content and dry weight of eggs of A.domesticus and A.configuratus incubated on moist filter paper at 33°C.

189 per cent. to 166 per cent. in 4 days, as compared with the 23 per cent. lost by A.domesticus in 3 days. A minor increase before hatching was also noted.

B. CHANGES IN DRY WEIGHT AND WATER CONTENT OF EGGS OF A. DOMESTICUS AND A. CONFIGURATUS

In the preceding experiments, it was observed that during the pre- and post-absorption periods the eggs of both A.domesticus and A.configuratus decreased in weight. This loss in weight may be attributed to either or both of two causes, namely, a loss of water or a decrease in oven-dry weight. To establish the nature of this decrease, the oven-dry weight of the eggs was determined over the periods of incubation. The results are presented in Figs. 5 and 6 in the text and in Appendix Tables 2, 3 and 4.

There was a drop in dry weight in the eggs of A.domesticus at both 28°C. and 33°C. during the pre-absorption period. During the absorption period at 33°C., the dry weight was more or less constant but decreased in the post-absorption from 89 per cent. to 78 per cent. The decrease in oven-dry weight at 28°C. was gradual over the entire incubation period. The oven-dry weight diminished from an initial value of 100 per cent. to 79 per cent. 24 hours before hatching.

The loss in dry weight in the eggs of A.configuratus

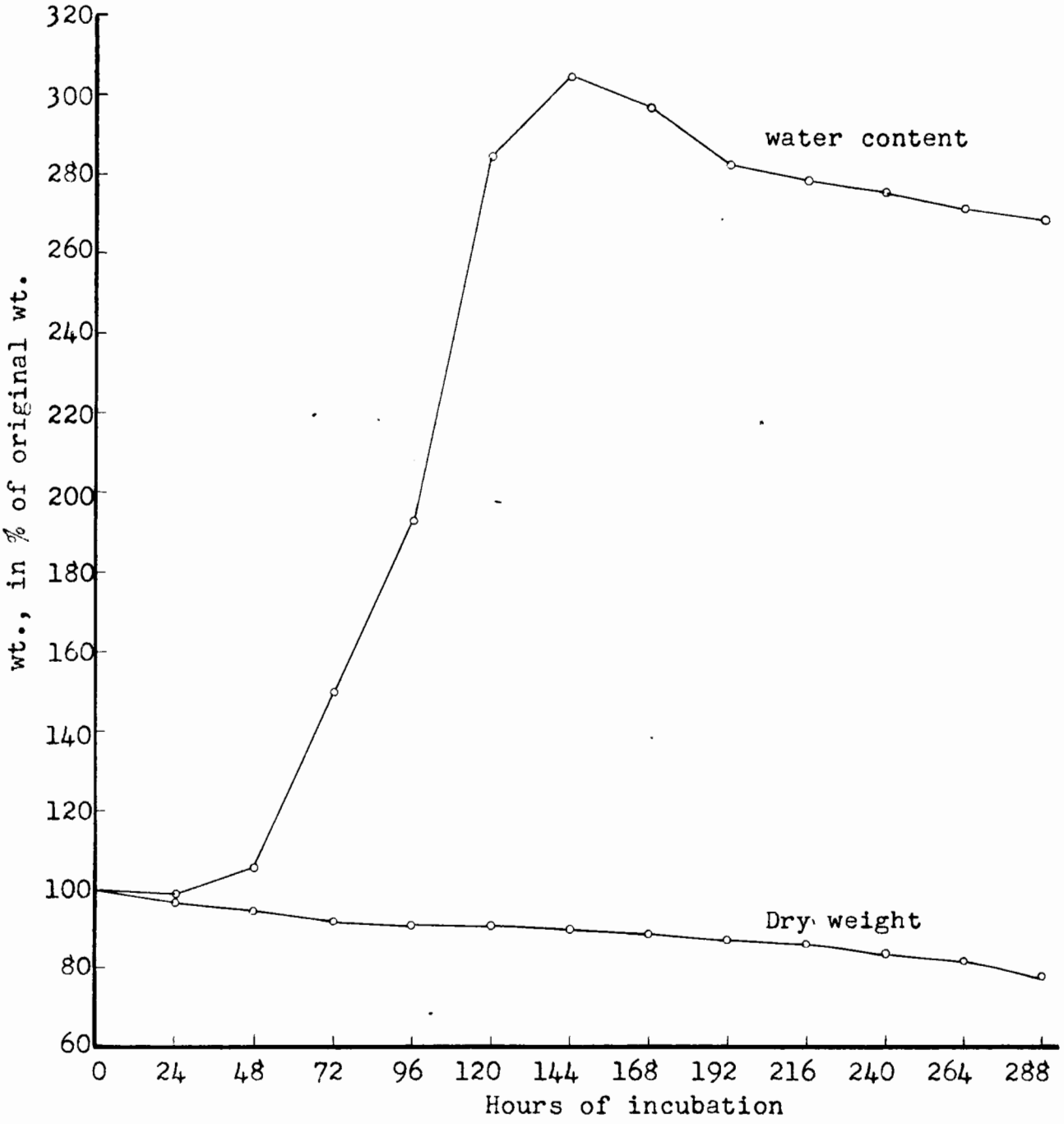

Fig.6 - Change in water content and dry weight of eggs of A.domesticus incubated on moist filter paper at 28°C.

was much more marked in the initial stages of development. The oven-dry weight dropped from 100 per cent. to 80 per cent. during the first 72 hours of incubation, thereafter it remained constant until a minor decline occurred after 168 hours, when the oven-dry weight decreased from 79 per cent. to 73 per cent. in 24 hours.

These observations showed that the initial loss in weight of the eggs in A.domesticus and A.configuratus was mainly due to a drop in dry weight. The decrease in weight of the eggs after the peak of uptake, however, was too great to be accounted for by the loss in dry weight. The reason for this decrease is not clear. A plausible explanation might be that the loss is due to chemical dehydration of the embryonic cuticle which is laid down at this time. Fraenkel and Rudall (1940), working with two species of blowflies, Calliphora erythrocelpha Meigen, and Sarcophaga falculata Pand., found that water was lost rapidly during the transformation of the larval cuticle into the puparium, and that water was lost from the puparium in contact with the body, even in a saturated atmosphere. They claimed that this loss of water might be due to the condensation of the chitin structure resulting in the squeezing out of water from the protein molecule, or to changes in the protein itself with the elimination of water and loss of ability to attract water.

C. SITE OF WATER ABSORPTION

In many locusts and grasshoppers, water is absorbed through a specialized part of the cuticle known as the hydropyle. The hydropyle is situated at the posterior end of the egg pole, and is a secretory product of a group of enlarged serosal cells (hydropyle cells). The hydropyle was first demonstrated in the differential grasshopper Melanoplus differentialis by Slifer (1938). Since then it has been found to occur in other grasshoppers and locusts; the Australian plague grasshopper Austroicetes cruciata (Birch and Andrewartha, 1942), the two striped grasshopper M.bivittatus (Salt, 1949), and the South African brown locust Locustana pardalina (Matthée, 1951). Slifer (1936) and Matthée (1951) showed that water uptake commenced only after the completion of the hydropyle and the underlying hydropyle cells.

Johnson (1934) showed that water was absorbed through the micropylar end (anterior end) in the capsid bug Notostira erratica. Kerenski (1930), on the other hand, found no special water absorbing area in the beetle Anisoplia austriaca and Browning (1952) found no evidence for a hydropyle in the cricket Gryllulus commodus.

In the eggs of A.domesticus, water uptake commences only after the completion of the serosa around the yolk.

The possibility, therefore, that water might have been absorbed through a specialized part of the serosa could not be overlooked.

The anterior third and posterior third of 10 eggs were each coated with "liquid solder". Ten eggs with both anterior and posterior thirds coated and 10 untreated eggs served as control. The eggs were incubated on moist filter paper at 33°C.

After three days on moist filter paper, all the eggs, save a few that died, were noticeably swollen and by the fourth day all of them appeared fully hydrated. The eggs which were coated with "liquid solder" were swollen to the extent that they gave the appearance as if they would burst. Owing to a heavy growth of mold, none of the eggs hatched. The experiment was repeated, but after the fourth day the liquid solder was softened with ethyl acetate and carefully removed. The eggs were then incubated on moist filter paper at 33°C. All the eggs, apart from a few which were killed during the removal of the solder, hatched.

These results seem to indicate that water is absorbed through the general surface of the egg membranes. To test this conclusion further, a number of eggs were incubated for 48 hours at 33°C., after which the anterior ends of some of these, and in other cases the posterior ends, were

attached by solder to a glass rod. The free ends of the eggs were then immersed in 0.1 per cent. basic fuchsin. After two hours the eggs were examined under a binocular microscope and a number of eggs dissected. The eggs were subsequently incubated on moist filter paper and the percentage of hatch recorded.

Only those parts of the chorion which were immersed in basic fuchsin were stained externally. However, the embryos were noticeably stained irrespective of the ends which were immersed. All of the eggs hatched and a number of nymphs on emerging had their abdomen slightly stained. The above observations showed that water is absorbed through the general surface of the egg.

D. MECHANISM OF WATER ABSORPTION

In the preceding experiments, it was shown that during a comparatively short period in embryonic development, i.e., between 48 to 96 hours at 33°C. and 72 to 144 hours at 28°C., the egg of A.domesticus took up a considerable amount of water through its entire surface. The following experiments were, therefore, designed in order to determine whether water uptake was an active process or a passive process. Although the eggs of many species of insects have been shown to absorb water, the actual mechanism of absorption, however, is not clear.

1. WATER ABSORPTION IN SODIUM CHLORIDE SOLUTIONS

Four batches of 50 eggs each were incubated for 48 hours, weighed, and then immersed in small phials containing distilled water and 0.6, 0.9 and 1.2 per cent. sodium chloride. The phials containing the eggs were placed in a constant temperature cabinet at 33°C. where they were kept for 18 hours. After this period, the eggs were taken out of the phials, washed in distilled water, air-dried on a filter paper and weighed. To determine whether the eggs were in any way affected by immersion in the various solutions for 18 hours, they were incubated on moist filter paper at 33°C.

None of the eggs, except those immersed in distilled water, hatched, neither was there any change in weight. The eggs placed in distilled water increased in weight by 23 per cent. of their initial weight. This increase was similar to that obtained when eggs were incubated on moist filter paper.

The failure of the eggs to hatch might have been due to either of two causes - a lack of oxygen or the penetration of salt resulting in the death of the embryo. The above experiment was repeated therefore, but during the period of immersion, air was bubbled through the various solutions. After immersion a number of eggs were selected at random, fixed in Bouin's solution and the embryos were dissected out.

No hatching was recorded in the eggs which were immersed in the various sodium chloride solutions. When compared with a normal developing embryo, it would appear that very little, if any, development took place when the eggs were immersed in sodium chloride solutions. In a subsequent experiment, it was observed that if eggs in a much later stage of development (eggs 96 hours old) were immersed in similar concentrations of sodium chloride for 24 hours, and then transferred to a moist filter paper and incubated at 33°C., normal hatching took place. It would seem, therefore, that the eggs were permeable to the salt during the period of uptake but after such period the salt was unable to enter into the egg.

2. WATER ABSORPTION IN GLUCOSE SOLUTIONS

It was shown in the previous experiment that no development occurred and consequently no water was taken up when eggs were immersed in various concentrations of sodium chloride solutions. The following experiment was carried out to determine whether they could take up water from glucose solutions at equivalent and lower concentrations to the sodium chloride solutions tested.

Five batches of 50 eggs taken from virgin females and 5 batches from normal fertilised females were used in this experiment. The eggs were incubated for 48 hours, weighed and then immersed in the various solutions in a similar way as

described for the sodium chloride solutions. They were immersed for 18 hours, following which they were weighed and incubated on moist filter paper at 33°C.

As was expected, none of the unfertilized eggs hatched; 60 - 70 per cent. hatching was observed in all other treatments.

The results are presented in Table 1. The concentrations of the various glucose solutions used are expressed in molarity and equivalent sodium chloride.

TABLE 1 - Changes in weight of fertilized and unfertilized eggs when immersed in various concentrations of glucose for 18 hours at 33°C.

Molarity of glucose solutions	Equivalent in % NaCl	Percentage increase or decrease in weight	
		Living eggs	Unfertilized eggs
Dist.water	0	+19.6	+ 2.1
0.0625	0.2	+ 3.9	- 1.5
0.1250	0.4	0.0	- 4.2
0.2500	0.6	- 1.0	- 9.1
0.3750	1.2	- 5.7	- 6.6

From the results in Table 1, it is seen that the unfertilized eggs were unable to take up water from various concentrations of glucose. Furthermore, the water uptake from

distilled water was negligible. The increase was only 2 per cent. while the living eggs increased their weight by 19 per cent. Equilibrium in the latter was reached at a concentration of 0.1250M glucose solution (equivalent to a 0.4 per cent. solution of sodium chloride). The increase in weight of the eggs in a 0.0625 M solution of glucose (equivalent to a 0.2 per cent. solution of sodium chloride) was only 4 per cent.

The failure of unfertilised eggs to take up water can be attributed to the fact that there is no development and consequently there are no changes in the egg membranes.

3. WATER UPTAKE FROM A SATURATED ATMOSPHERE

Lees (1946) has shown that water was taken up by the tick Ixodes ricinus from an unsaturated atmosphere. In the unfed adult tick the haemolymph has an osmotic pressure equivalent to that of a one per cent. sodium chloride solution and would therefore be in equilibrium with the water vapour in air in which the relative humidity was 99.4 per cent. But the tick can absorb water from an atmosphere in which the relative humidity is 92 per cent. This movement of water molecules against a vapor-pressure gradient involves the expenditure of energy in secretory activity. It was necessary to establish whether, in the case of the eggs of A.domesticus, water could be taken up from the vapour phase.

Two batches of 50 eggs after being weighed were incubated on a dry glass surface in a desiccator at 100 per cent. R.H. Two batches on moist filter paper were used as control. The desiccator containing the eggs was kept in an incubator at 33°C. The eggs were weighed at daily intervals until no significant change in weight was detected. A number of eggs were also dissected at regular intervals to determine the stage of development reached.

The results are shown in Table 2. Since there was no significant difference between the replicates, only the treatment means are given.

TABLE 2 - Changes in weight of eggs of *A.domesticus* when incubated at 100% R.H. (Temp. 33°C.)

Treatments	Weight (percentage of initial weight) recorded after:- 24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	168 hours
100% R.H.	95.7	93.4	89.5	108.2	139.1	142.1	141.5
Control	98.7	128.0	163.2	191.1	179.1	177.1	174.5

In the initial stages of development, between 48 to 72 hours, when water was rapidly taken up by the control, no increase in weight was recorded at 100 per cent.R.H. The development of the embryo in the latter was also much slower.

The increase in weight observed after 96 hours was probably due to the condensation of moisture. Water could be detected on the sides of the containers and on the eggs themselves. The eggs were, therefore, not only in a saturated atmosphere but in contact with free water. The eggs did not increase in weight as much as those which were incubated on moist filter paper. The increase was only 142 per cent.of the initial weight as compared with 191 per cent.by the control. After the sixth day no further increase in weight occurred and this was the stage at which revolution was completed and the serosa ruptured. The entire development period of the eggs was 20 days,while the mean incubation period of the control was 9.1. Only 20 per cent. of the eggs hatched at 100 per cent. R.H.

These observations indicate that the eggs are unable to absorb water from a saturated atmosphere.

4. DEVELOPMENT IN DISTILLED WATER

The aim of this experiment was to ascertain whether the eggs of A.domesticus could complete their development when completely submerged in distilled water.

Nine batches of 50 eggs were weighed and then placed in small phials containing distilled water. These were incubated in a constant temperature cabinet kept at 33°C. During the first eight days of development, one batch was

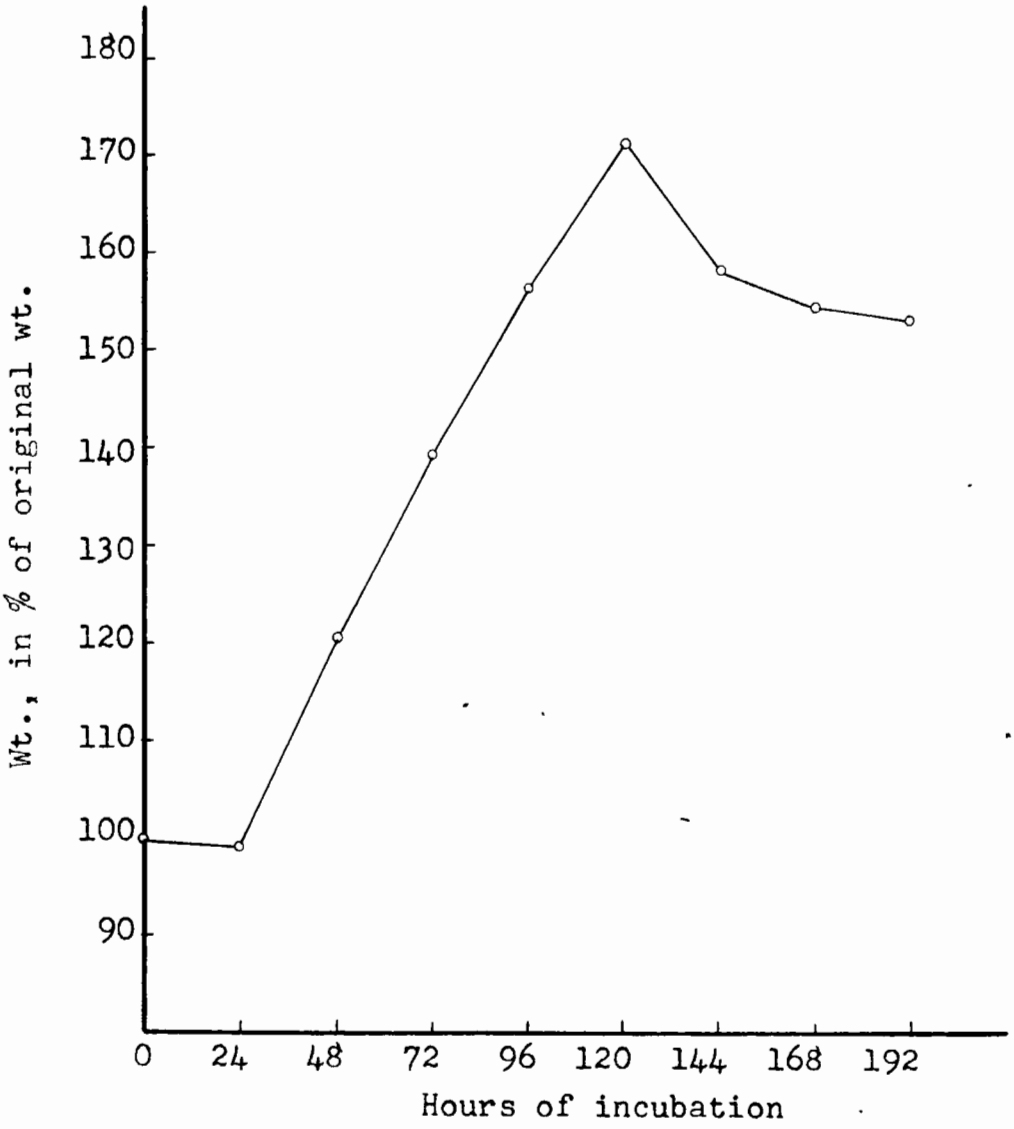

Fig.7 - Change in weight of the eggs of A.domesticus when completely immersed in distilled water and kept at 33°C.

selected at 24-hour intervals, air-dried and weighed. The ninth was allowed to continue its development completely submerged, until six hours before the expected time of hatching (215 hours at 33°C.), when it was transferred to moist filter paper and incubated at 33°C.

Seventy per cent. of the eggs which were immersed for 209 hours hatched. However, the mean incubation period was significantly longer than that of eggs incubated on moist filter paper, 12 days as compared with 9.1 days. The period of water uptake extended from 48 hours to 120 hours and the eggs only increased in weight by 171 per cent. (Fig.7). The longer period of water uptake was probably due to the fact that development was slower, as a result of an oxygen effect.

The fact, however, that the eggs were able to complete their development when completely submerged indicates that the eggs are in some way able to negate osmotic forces.

5. WATER ABSORPTION FROM FILTER PAPER MOISTENED WITH VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE SOLUTIONS

In a previous experiment (water uptake in sodium chloride solutions), it was noted that the eggs of A.domesticus failed to develop when immersed in sodium chloride solutions. However, in view of Korenski's observations (Korenski, 1930) that the eggs of Anisoplia cruciata were able to absorb water and develop if incubated on filter paper moistened with various

solutions, the following experiment was carried out. The aim was to ascertain whether the eggs of A.domesticus could develop on filter paper moistened with various concentrations of sodium chloride solutions, and whether there was any difference in the rate of uptake.

Four batches of 50 newly laid eggs were weighed and incubated on filter paper moistened with distilled water, 0.6, 0.9 and 1.2 per cent. sodium chloride solutions. The eggs were kept in a water bath at 33°C. The eggs were weighed after 48 hours and again at 96 hours. The percentage of hatch was also recorded.

TABLE 3 - Increase in weight of eggs incubated on filter paper moistened with various concentrations of sodium chloride solutions.

Treatment	Per cent. increase in weight after - 48 hours	96 hours	% Hatch
Dist. water	129.1	189.9	69
0.6% NaCl	131.5	190.2	63
0.9% NaCl	129.7	191.1	67
1.2% NaCl	128.2	188.4	66

The results in Table 3 show that the eggs were able to develop when incubated on filter paper moistened with various concentrations of sodium chloride solutions. There was also

no difference in the rate of water uptake.

6. RATE OF WATER LOSS DURING VARIOUS STAGES OF DEVELOPMENT

It is well known that the surface membranes of insects show asymmetry, in that water passes more rapidly in one direction than in the other (Beament, 1954). The rate of water loss, therefore, gives little information about the rate at which water can enter the egg. However, changes in the rate of water loss should reflect changes in the permeability of the eggs to entry of water.

To measure the change in the rate of desiccation, eggs of different ages were placed in a desiccator at 95 per cent. R.H. The eggs were first incubated in batches of 50 on moist filter paper at 33°C., and at varying intervals, one batch was air-dried on filter paper, weighed and placed on dry glass in the desiccator. The eggs in the desiccator were weighed at daily intervals. The desiccator was also kept at 33°C., and the relative humidity was controlled by means of a potassium hydroxide solution as described by Solomon (1951).

Fig. 8 shows the rate of water loss during the first 24 hours of desiccation. In the newly laid eggs, the loss was only 7 per cent. of the original weight. In eggs desiccated after 48 hours of incubation, the loss was

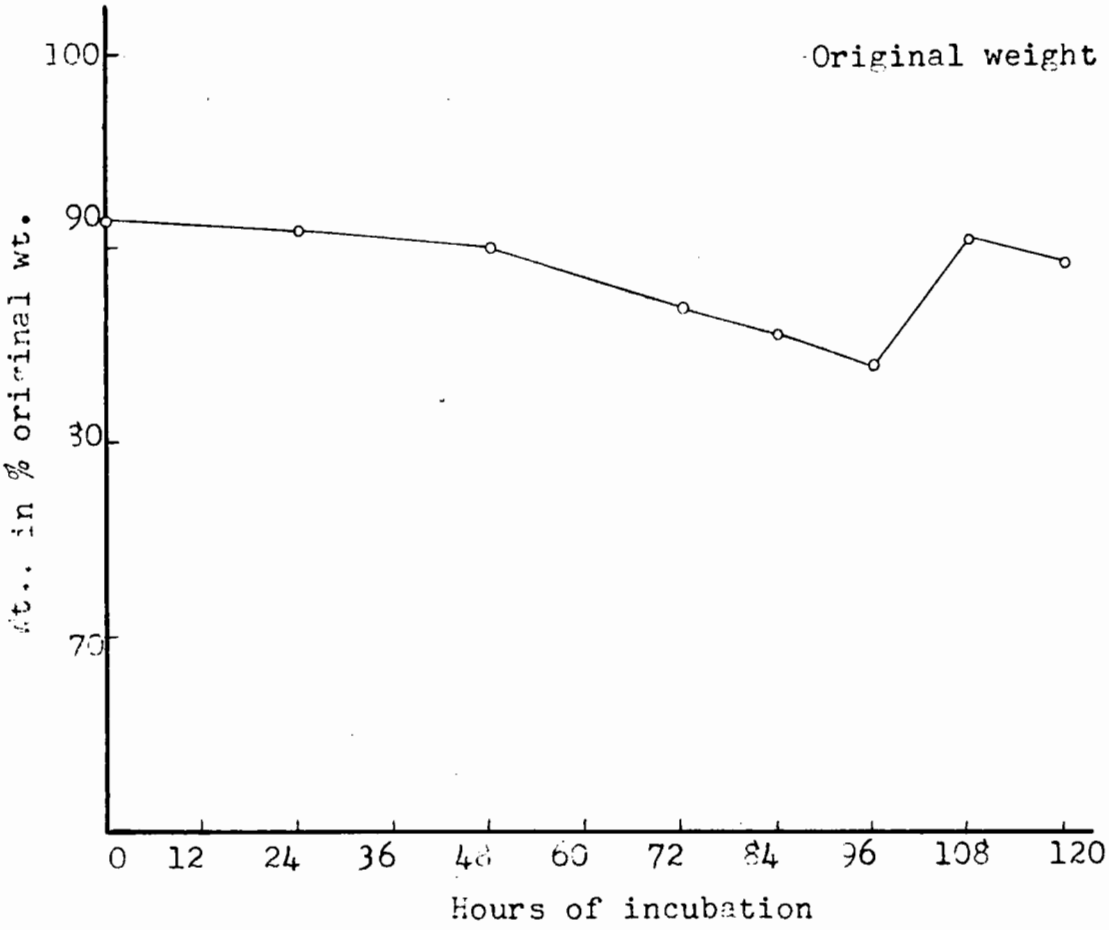

Fig.8 - Loss of weight of eggs at various periods of development after 24 hours exposure to 95 per cent. R.H.

10 per cent. A maximum weight loss of 16 per cent. was recorded in eggs 96 hours old. The loss then decreased to 9 per cent. in eggs which were desiccated after 108 hours of incubation. The increase in the rate of water loss during the period of water uptake (between 48 and 96 hours) indicates that the eggs are more permeable to water at this time. The relative impermeability of the eggs during the post-absorption period can be attributed to the presence of the embryonic cuticle.

The total amount of water lost over the period of desiccation had a marked effect on the development of the eggs. Eggs transferred to 95 per cent. R.H. after 84 hours of incubation lost about 47 per cent. of their wet weight and no hatching was recorded (Fig. 9, Table 4). The water loss of eggs which were incubated for 108 and 120 hours prior to desiccation was only 17 and 19 per cent. and 62 and 63 per cent. hatching,respectively,was observed. The incubation period of eggs desiccated after 72 hours was significantly longer than that of eggs which were desiccated after 96, 108 and 120 hours. Similarly the incubation period of eggs desiccated after 96 hours was longer than that of eggs which were placed at 95 per cent. R.H. after 108 and 120 hours of incubation. These results were similar to those obtained for A.domesticus by Ghouri (1956). It was noted in three

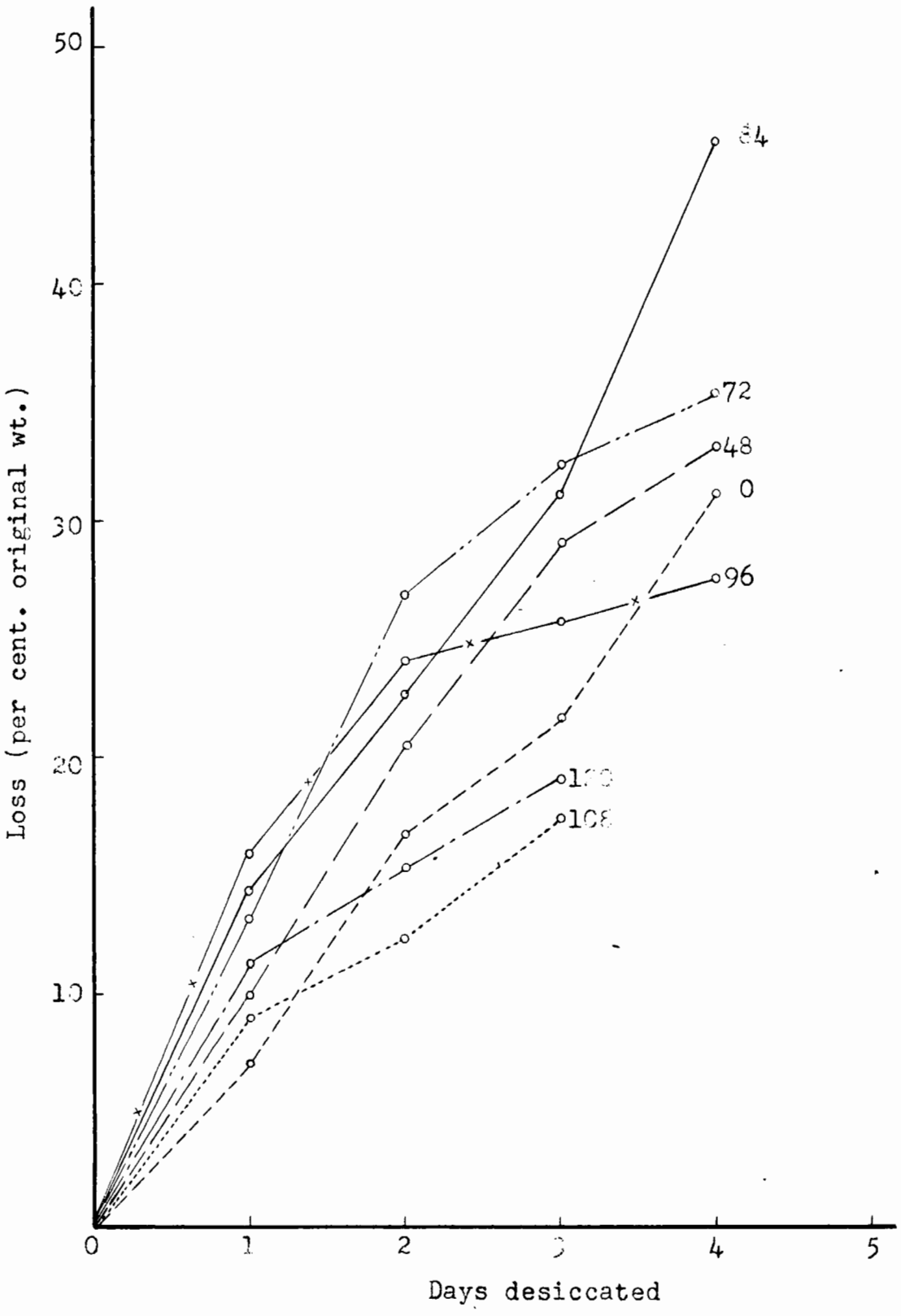

Fig.9 - Rate of water loss from the eggs of A.domesticus at 95% R.H. at the various stages of development indicated at the end of each curve.

TABLE 4 - Percentage hatch and mean incubation period of eggs desiccated at 95 per cent. R.H.

Age of eggs (in hours)	% Hatch	Mean incubation period (in hours)
0	0	-
48	0	-
72	16	263.2
84	0	-
96	34	243.5
108	62	233.7
120	63	228.3

successive trials that eggs which were incubated on moist filter paper for 84 hours prior to desiccation failed to hatch, while some hatching was recorded for eggs which were desiccated after 72 hours of incubation. The increased rate of water loss recorded for the eggs 84 hours old was due to the increased water content of the eggs.

7. PERMEABILITY OF THE EGG TO IONS

In the experiments designed to determine the site of water absorption, it was observed that when the eggs were immersed in a 0.1 per cent. solution of basic fuchsin, there

was some staining of the embryo. The possibility was indicated that the egg membranes might be permeable to ions and small molecules. It was necessary, therefore, to establish whether the egg was actually freely permeable to ions.

Eggs in batches of 50 were incubated on moist filter paper for 48 hours at 33°C., after which they were weighed. The eggs were then immersed in the various solutions for 18 hours and incubated at 33°C. Following the period of immersion, the eggs were air-dried and weighed to determine whether there was any change in weight. All eggs after immersion were placed on moist filter paper and incubated at 33°C., and percentage hatching recorded.

The substances tested and the concentrations used were as follows:-

1 per cent. basic fuchsin.
1 per cent. methylene blue.
1 per cent. cresyl violet.
1 per cent. tyrosine.
1 per cent. glycine.
1 per cent. tryptophane
0.1 per cent. potassium iodide.
0.1 per cent. potassium cyanide.
0.1 per cent. sodium arsenite.
0.1 per cent. potassium fluoride.

None of the eggs which were immersed in solutions of the various metabolic poisons (potassium iodide, potassium cyanide, sodium arsenite, and potassium fluoride) hatched. Sixty to seventy per cent. hatching was recorded in all other treatments. No increase in weight occurred in any of the batches of eggs during the period of immersion. The embryos and the yolk of the eggs which were immersed in the dyes were stained. The eggs immersed in tyrosine were uniformly darkened (Fig. 10). In a subsequent experiment, it was also noted that eggs immersed in 3,4-dihydroxyphenylalanine (Dopa) were similarly darkened. The dissected embryos in both of these cases were also uniformly darkened.

The above observations showed that the membranes surrounding the egg were permeable to ions. The failure of the eggs to develop when immersed in sodium chloride solutions, noted in a previous experiment, was apparently due to the penetration of salt. The increase in weight of eggs in the glucose solution could have been due to penetration of the glucose molecules. It is evident, therefore, that since the egg membranes are permeable to ions and small molecules, the determination of the osmotic pressure of the egg by immersion in glucose solutions of varying concentration as was done by Matthée (1951), may not be accurate.

Fig.10 - Egg of A.domesticus following immersion in tyrosine (upper) and normal egg (lower) x20.

It is well known that tyrosine is the precursor of melanin (Fruton and Simmonds, 1953). The fact that eggs when immersed in tyrosine or its derivative Dopa were darkened indicated that some of the substrate entered the egg and was oxidised by the enzyme tyrosinase (polyphenol oxidase) to melanin. The uniform darkening of the eggs showed also that the enzyme is generally distributed in the embryo, since melanin is always deposited where it is formed. Bodine and Boell (1935) have shown that in the eggs of Melanoplus differentialis, the tyrosinase activity rose steadily for the first 20 days of development and then remained fairly constant. The enzyme was almost entirely confined to the yolk and serosal cells until the time of yolk engulfment when activity appeared in the embryo.

E. DISCUSSION

Kerenski (1930) observed that the eggs of Anisoplia austriaca increased in weight and developed normally when placed on filter paper wetted with distilled water, soil filtrate, insect Ringer's solution, sodium chloride solutions up to 4 per cent., 2 per cent. solution of potassium nitrate or 2 per cent. barium chloride. He concluded that the eggs of Anisoplia are covered by a membrane which excludes the salt, but actively absorbs water from them. However, no figures were given by him to show the rate of absorption from the

various salt solutions. Neither was any experiment carried out to show whether the eggs could absorb water when completely immersed in the solutions. In A.domesticus, it was observed that the eggs could develop if placed on filter paper moistened with various concentrations of sodium chloride solutions. On the other hand, when the eggs were immersed in the solutions, not only did they fail to take up water but no development occurred. Kerenski is not fully justified, therefore, in concluding from his observations that the eggs of Anisoplia are covered by a membrane which excludes the salt and actively absorbs water from them, although this may actually be so in this species. When eggs are incubated on moist filter paper only a number of cells are in direct contact with the solutions. At 100 per cent. R.H. condensation of moisture is bound to occur. The increase in weight of the eggs may be due to the condensation of moisture on the surface of the eggs rather than to the absorption of water from the solutions.

Birch and Andrewartha (1942) studied the absorption of water by eggs of Austrobetes cruciata and concluded that the movement of water into diapause eggs could be described by Lillie's formula (Lillie, 1916). Accepting Lucké and McCutcheon's view (Lucké and McCutcheon, 1932) that the semi-permeability of biological membranes was not only dependent on morphological features but was also largely governed by

the metabolic state of the protoplasmic layer, they concluded that the change in permeability of the eggs to water from the diapause to the post-diapause stage probably was due to the increased metabolic activity of the latter. In other words, the egg became more permeable to water with increased metabolic activity.

Matthée (1951) from his studies on the absorption of water by the eggs of Locustana pardalina concluded that water uptake was in part an active physiological process dependent on respiration, and in part due to passive diffusion of water through the hydropyle membrane. The latter was of significance only before stretching of the egg membranes occurred; once the egg had become fully turgid, water had to be actively taken in. This conclusion was based as mentioned before (section on permeability of the eggs to ions) on the osmotic pressure of the egg by immersion in glucose solution of varying concentration. He also observed that the eggs were unable to take up water in the absence of oxygen and concluded that water was actively absorbed through the hydropyle by the hydropyle cells. However, Slifer (1938) has shown that the hydropyle cells often became detached from the hydropyle in Melanoplus differentialis at the beginning of blastokinesis (revolution), and yet water was still absorbed.

In A.domesticus, the mechanism of water absorption

appears to be a passive one. This is supported by the evidence that eggs in the water-absorbing state cannot take up water from various concentrated solutions, indicating that entry is simply by diffusion. The fact that the eggs are unable to absorb water from a saturated atmosphere and that the increase in weight of the eggs even in a very dilute glucose solution is small indicates that probably no expenditure of energy is required for water transport per se. The newly laid egg of the house cricket is enclosed by an outer chorion and an inner vitelline membrane. The chorion apparently does not play an important part in water absorption and the egg depends on the vitelline membrane to keep water out. Unfertilized eggs do not take up water when immersed in distilled water and remain unswollen for days until the egg decomposes. In the fertilized egg, the vitelline membrane disappears as development begins and as the serosa is formed. It is at this stage that water enters the egg. The entry of water through the serosa is probably due to the production of osmotically active substances by the embryo. With the rupture of the serosa after revolution and with the formation of the embryonic cuticle, the egg is rendered impermeable to water.

VI. METABOLISM

No study has so far been made on metabolic changes in the eggs of crickets. Most of the metabolic studies on Orthoptera have been confined to Melanoplus differentialis (Bodine, 1929; Burkholder, 1934; and others).

A. METHODS

1. CHEMICAL DETERMINATIONS

Batches of 100 eggs of A.domesticus and A.configuratus were incubated at 33°C. and at daily intervals, during the period of development, a number of these were selected out and the oven-dry weight determined by drying in an electric oven at 100°C. for 24 hours, cooling and finally weighing. Three replicates of 100 eggs each were used for the determinations of fat and glycogen in A.domesticus, while single determinations were made for A.configuratus.

a. Determination of Fat

The determinations of free lipids were accomplished by extracting the oven-dry samples in a Soxhlet apparatus with diethyl ether for 18 hours.

b. Determination of Glycogen

The method given by Good et al. (1933) was used for the

digestion of the samples. The samples were digested in 30 per cent. KOH for 30 minutes; 0.6 N HCl was used for hydrolysis.

Glucose was estimated by the colorimetric method of Somogyi (1952). Two cc. of the copper reagent were used and Nelson's reagent was used as the chromogenic reagent. The solutions were read in a Klett-Summerson photoelectric colorimeter at 520 mμ.

2. MEASUREMENT OF OXYGEN CONSUMPTION

The oxygen consumption of the eggs of A.domesticus at 33°C. was measured by the Warburg direct method described by Umbreit et al (1951). One hundred eggs were placed in each flask on a piece of moist cotton covered with a piece of moist filter paper. Readings were taken at eight-hour intervals during the entire period of development.

B. RESULTS AND DISCUSSION

Figs. 11 and 12 show that there was very little change in free lipids (ether soluble) in both A.domesticus and A.configuratus during early embryogenesis. However, a rapid decrease began on the fourth day in A.domesticus and on the fifth day in A.configuratus. By the seventh day in A.domesticus and ninth day in A.configuratus, the free lipid

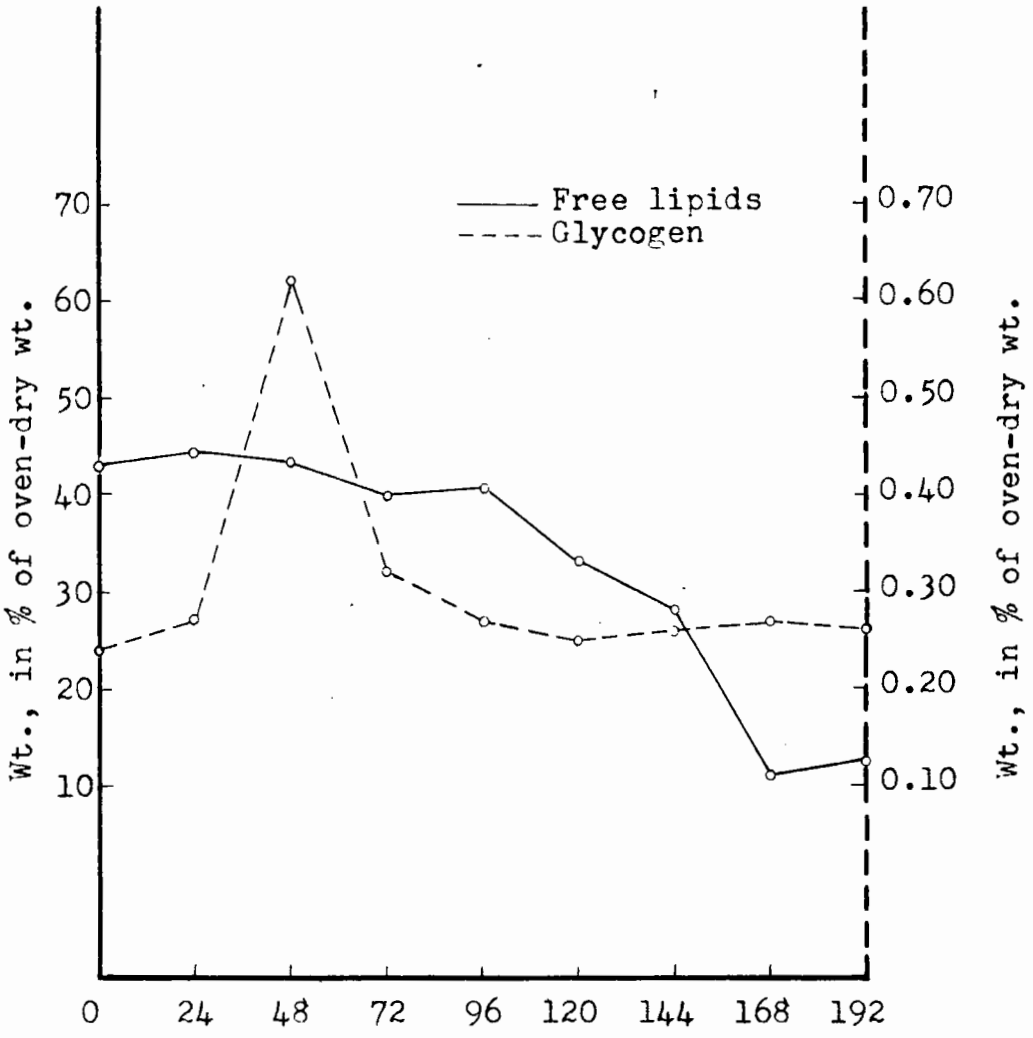

Fig.11 - Changes in glycogen and free lipids (ether extract) during the embryonic development of the eggs of A.domesticus at 33°C.

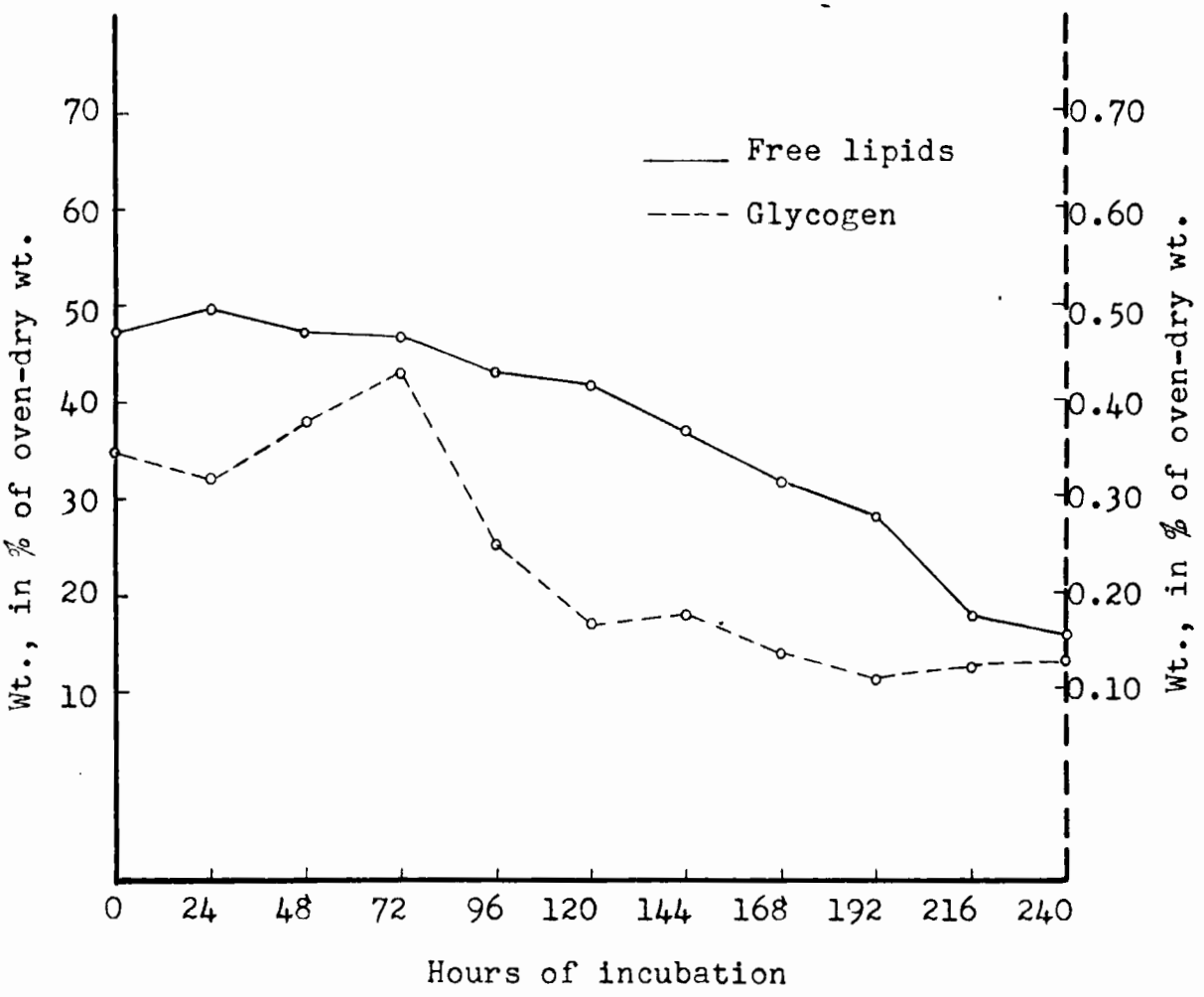

Fig.12 - Changes in glycogen and free lipids (ether extract) during the embryonic development of the eggs of A.configuratus at 33°C.

content of the eggs was only 11 per cent. and 17 per cent., respectively, of the dry-weight.

The glycogen content of the newly laid egg was very low, only 0.24 per cent. of the dry weight in A.domesticus and 0.35 per cent. in A.configuratus. An increase in glycogen was recorded after 48 hours in A.domesticus and after 72 hours in A.configuratus. In both species, there was a rapid decrease in glycogen 24 hours after this increase, and this was followed by a gradual decrease until hatching.

These observations showed that glycogen probably supplied the energy necessary for revolution which occurred between the third and fourth day in A.domesticus. In A.configuratus revolution probably occurred at a latter stage because of the fact that the developmental period at 33°C. was longer than that of A.domesticus; this would account for the decrease in glycogen 24 hours later. After revolution, free fat appeared to furnish the main source of energy.

The changes in oxygen consumption during the embryonic development of A.domesticus are shown in Fig.13. The oxygen uptake of the eggs rose gradually during the pre-revolution period and this increase continued until 8 hours after revolution when a decrease in the oxygen uptake of the eggs was noted. This decrease which continued until 136 hours of incubation coincided with the decline in wet weight

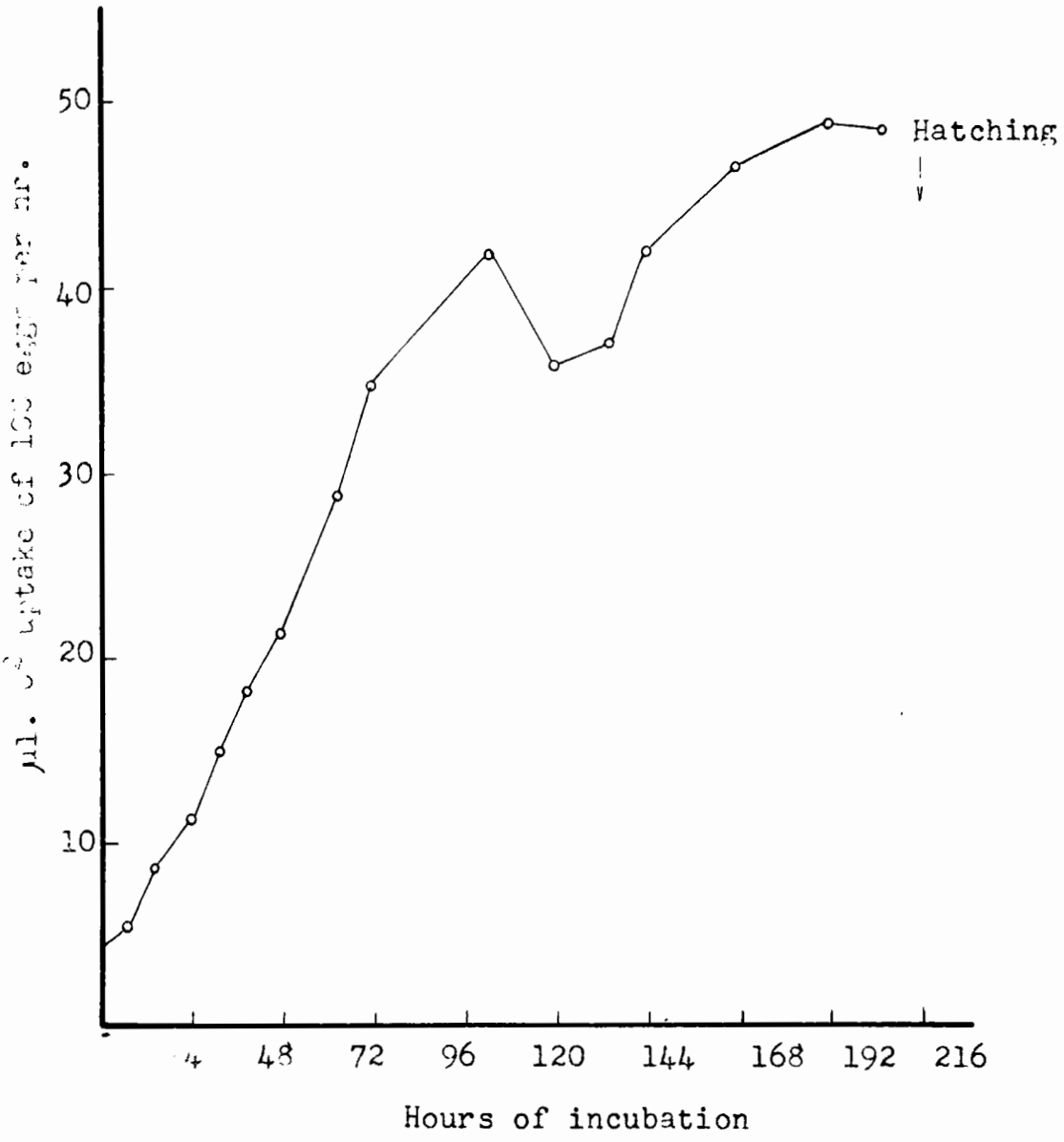

Fig.13 - Oxygen consumption of A.domesticus during embryonic development at 33°C.

during the post-absorption period (Fig.3) and with the engulfing of the yolk. A decline in the rate of oxygen uptake during the engulfment of the yolk was also noted in Melanoplus differentialis by Burkholder (1934) and in Rhodnius prolixus by Tuft (1949). This decline in the rate of oxygen consumption may be due to the fact that the embryo remains inactive while changes are taking place in the embryonic cuticle. Following this, the oxygen consumption again rose and this increase continued until the time of hatching.

VII. SUMMARY

1. The eggs of the crickets, Acheta domesticus (L.) and A.configuratus Walk., absorbed a quantity of water nearly equal their newly-laid weight during the early stages of development.

2. Water was taken up by the eggs of A.domesticus prior to revolution of the embrye and the rupture of the serosa.

3. In the eggs of both A.domesticus and A.configuratus, there was a decrease in dry weight during development.

4. Water was absorbed through the entire surface of the egg of A.domesticus, there being no special water absorbing area equivalent to the hydropyle and hydropyle cells of locusts and grasshoppers.

5. The eggs of A.domesticus could not absorb water and develop when immersed in sodium chloride solutions.

6. Very little, if any, water was absorbed by the eggs of A.domesticus from very dilute glucose solutions.

7. The eggs of A.domesticus were unable to absorb water from a saturated atmosphere.

8. The eggs of A.domesticus could complete their development when completely submerged in distilled water.

9. Water was lost more rapidly by the eggs of A.domesticus when desiccated at 95 per cent. R.H. during the period of water uptake than before or after.

10. The mechanism of water absorption in the egg of A.domesticus appears to be due to the passive diffusion of water through the serosa as a result of the production of osmotically active substances by the embryo.

11. The egg of A.domesticus was shown to be permeable to ions. The permeability of the insect egg to ions had, hitherto, not been demonstrated.

12. Tyrosinase (polyphenol oxidase) has been shown to be generally present in the egg of A.domesticus during early embryonic development.

13. Glycogen appeared to supply the energy necessary for revolution, while in later stages of development fat was the chief source of energy.

14. Oxygen consumption rose steadily during the embryonic development of the eggs of A.domesticus at 33°C., except for a decline just after revolution.

VIII. ACKNOWLEDGEMENTS

I wish to acknowledge with thanks the invaluable time and assistance given me by Dr. J.E.McFarlane, my Director of Research, both in the planning of the experimental work and the preparation of this manuscript.

The author is also grateful to the following:

Dr. F.O. Morrison, Associate Professor in Entomology, for the photograph on page 57.

The Government of British Guiana for the scholarship given to him.

Miss B. Robinson for typing the thesis.

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APPENDIX TABLES 1 to 4 (inclusive)

APPENDIX TABLE 1 - The change in weight of eggs of A.domesticus during incubation on moist paper at 33°C. and 28°C.

Hours of incubation	No. of egg	33°C. Initial wt. (mg.)	33°C. Final wt. (mg.)	33°C. Final, as % of Initial	28°C. Initial wt. (mg.)	28°C. Final wt. (mg.)	28°C. Final, as % of Initial
0	100	33.5			34.4		
24	100	32.4	32.1	99.0	35.3	34.5	97.7
48	100	33.33	45.2	135.7	36.4	36.4	100.0
72	100	33.3	55.8	167.5	35.3	41.5	117.5
96	100	34.9	68.1	195.4	36.0	56.3	156.3
120	100	33.7	58.1	172.4	34.4	59.1	171.8
144	100	32.8	55.9	170.4	35.5	65.3	183.9
168	100	32.2	52.1	161.8	33.3	59.8	179.5
192	100	33.5	56.5	168.6	35.7	61.3	171.7
216	100	Start emerging			32.8	55.9	170.4
240	100				35.9	59.9	166.8
264	100				33.3	54.8	164.5
288	100				35.9	57.8	161.0
312	100				34.8	56.3	161.7
336	100				Start emerging		

APPENDIX TABLE 2 - Change in water content and dry weight of eggs of *A.domesticus* incubated on moist filter paper at 33°C.

Hours of incubation	No. of eggs	Initial wt. (mg.) Wet	Initial wt. (mg.) Dry	Final wt. (mg.) Wet	Final wt. (mg.) Dry	Final water content (%)	Final dry wt. / Initial dry wt.
0	100	32.7	19.6			40.1	
24	100	32.4	19.4	31.5	18.6	41.0	0.958
48	100	33.3	19.9	45.2	18.2	59.8	0.914
72	100	33.3	19.9	55.8	18.4	67.1	0.924
96	100	34.7	20.7	66.6	18.6	72.1	0.898
120	100	33.5	20.0	56.5	17.2	69.6	0.860
144	100	34.4	20.6	57.4	15.6	72.1	0.757
168	100	32.2	19.2	52.1	14.2	72.8	0.739
192	100	33.5	20.0	55.3	15.6	71.8	0.780
216	Start emerging						

APPENDIX TABLE 3 - Change in water content and dry weight of eggs of *A.domesticus* incubated on moist filter paper at 28°C.

Hours of incubation	No.of eggs	Initial wt. (mg.) Wet	Initial wt. (mg.) Dry	Final wt. (mg.) Wet	Final wt. (mg.) Dry	Final water content (%)	Final dry wt. / Initial dry wt.
0	100	34.4	19.6			43.1	
24	100	35.3	20.0	34.5	19.4	43.8	0.970
48	100	36.4	20.7	36.4	19.7	45.9	0.951
72	100	35.3	20.0	41.5	18.4	55.7	0.920
96	100	36.0	20.4	58.8	18.6	68.4	0.911
120	100	34.4	19.6	59.1	17.9	69.8	0.913
144	100	35.5	20.1	65.3	18.2	72.2	0.905
168	100	33.3	18.9	59.8	17.0	71.6	0.899
192	100	35.7	20.3	61.3	17.7	71.2	0.871
216	100	32.8	18.6	55.9	16.2	71.1	0.870
240	100	35.9	20.4	59.9	17.1	71.5	0.838
264	100	33.3	18.9	54.8	15.5	71.8	0.820
288	100	35.9	20.4	57.8	16.1	72.2	0.789
312	100	34.8	19.8	56.3	15.8	72.0	0.797
336	Start emerging						

APPENDIX TABLE 4 - Change in water content and dry weight of eggs of A.configuratus incubated on moist filter paper at 33°C.

Hours of incubation	No. of eggs	Initial wt. (mg.) Wet	Initial wt. (mg.) Dry	Final wt. (mg.) Wet	Final wt. (mg.) Dry	Final water content (%)	Final dry wt. / Initial dry wt.
0	100	31.4	19.1			39.2	
24	100	30.6	19.0	29.9	18.2	39.2	0.957
48	100	30.8	18.7	35.7	15.6	56.4	0.834
72	100	30.8	18.7	41.4	15.3	63.1	0.818
96	100	27.3	16.5	42.9	13.7	68.1	0.830
120	100	28.3	17.2	53.6	14.5	73.0	0.843
144	100	27.3	16.5	51.0	13.8	73.0	0.836
168	100	28.1	17.0	49.7	13.5	72.9	0.794
192	100	27.9	16.9	48.3	12.4	74.4	0.733
216	100	31.8	19.3	52.9	14.2	73.2	0.735
240	100	31.8	19.3	54.1	14.8	72.7	0.766
264	Start emerging						

Plate 1

Plate 1.

Fig. 1. Lateral view of the egg of A.domesticus showing the germ band on the posterior part of the convex ventral side.

Fig. 2. Commencement of dorsal flexure.

Plate I

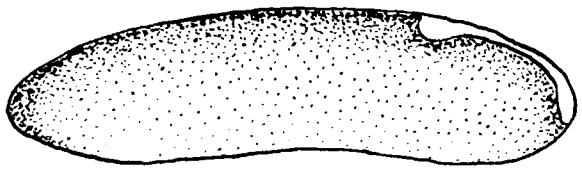

Fig. 1

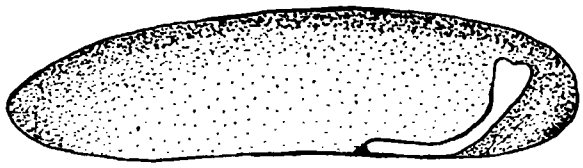

Fig. 2

Plate II

Plate II.

Fig. 3. Embryo completely sunken within the yolk and segmentation completed.

Fig. 4. Stage of embryonic development reached prior to revolution.

Plate II

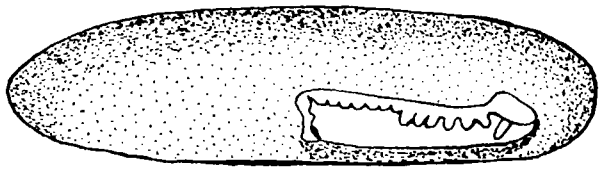

Fig. 3

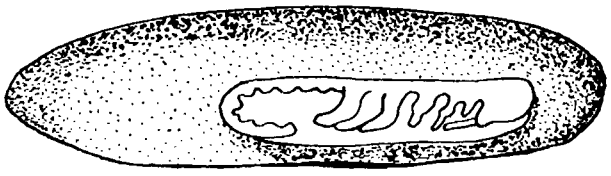

Fig. 4

Plate III

Plate III.

Fig. 5. Completion of revolution.

Fig. 6. Dorsal closure and formation of eye spots.

Plate III

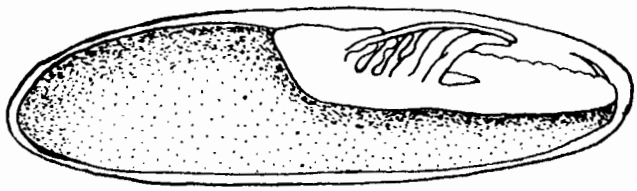

Fig. 5

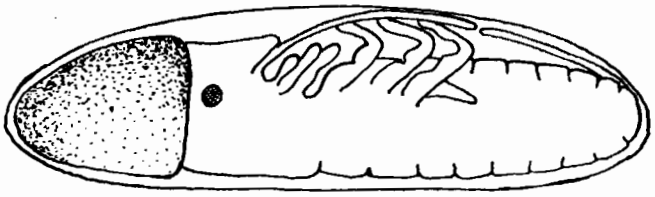

Fig. 6

Plate IV.

Plate IV.

Fig. 7. Engulfment of the yolk.

Plate IV

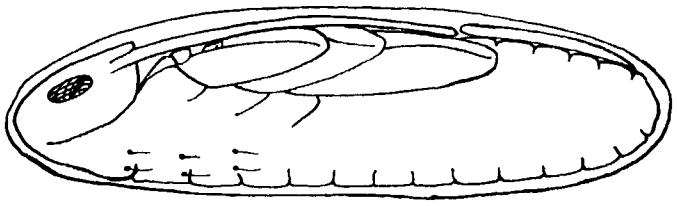

Fig. 7