The Formation and Structure of a special Water-absorbing Area in the Membranes covering the Grasshopper Egg.

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CONTENTS.

NEEDHAM (p. 1272, 1931) divides all eggs into three classes: (a) those which, when first laid, contain too little inorganic matter and water for their complete development and which, consequently, must take in these substances, as well as oxygen, from the medium surrounding them; (b) those which are dependent upon their environment for additional water and oxygen; and (c) those which need only oxygen. Many insect eggs, available data indicate, belong to the second group, and during their development require, besides oxygen, a supply of water greater than that with which they are furnished at the time when they are laid.

As indirect evidence for such a view we may take those cases reviewed by Needham (pp. 904-5, 1931), Uvarov (pp. 70-3, 1931), and Buxton (pp. 306-13, 1932) where, with few exceptions, high humidities have been found to favour the develop-

ment of the eggs of various species of insects. Indirect evidence
of another sort consists of observations on the increase in size
of the eggs as they develop. It is probable that Réaumur
(pp. 127–9, 1740) was the first to record this phenomenon. He
described the eggs of a saw-fly as doubling in volume during
their development, and wondered whether the shell of the egg
might act as a 'placenta' to draw nourishment from the willow
leaf to which it is fastened. He allowed leaves with eggs
attached to dry, and states that the eggs soon shrivelled. The
stems of other leaves with eggs on them were placed in water,
and the eggs on these remained turgid and hatched from
four to seven days later. Réaumur (1926)[1] noticed, also, that
the eggs of ants increase in size as they develop. In the two
centuries following Réaumur other workers have recorded
such a size increase in the eggs of insects belonging to many
different orders. Blunck (1914), Needham (1931), Johnson
(1934), and Roonwal (1936) list numerous observations of this
type.

In 1929[2]—and now the evidence becomes direct—Bodine
published curves demonstrating that the increase in weight of
the egg of the grasshopper, Melanoplus differentialis,
as it develops, is due to an actual increase in water content.
This was the first detailed, quantitative, and extensive study
of the subject. Somewhat earlier Hoffman, Dampf, and Varela
(1925) had reported a few experiments in which they showed
that the eggs of another grasshopper, Schistocerca para-
nensis, which had been shrunken by exposure to dry air were
able to absorb water and to regain their original turgidity when
returned to a moist atmosphere. In 1930 Kerenski proved that
the eggs of a beetle, Anisoplia austriaca, are able to
increase in weight when moistened with nothing but water.
Johnson (1934, 1937) has shown that the increase in size of

[1] Réamur's notes on ants, overlooked for nearly two centuries, were
finally found and prepared for publication by the late W. M. Wheeler.

[2] This article, as it appeared in *Physiological Zoology*, contained graphs
from which the numbers had been removed through an oversight on the
part of the publishers. The reprints, however, contain the numbers. This
difference has been the cause of more than a little confusion in the literature.

the eggs of Notostira erratica is due to an absorption
of water. Quite recently Roonwal (1936) has found, as had
Bodine, that the water content of the grasshopper egg rises as
it develops. Roonwal worked with the eggs of Locusta
migratoria migratorioides.

Jahn (1935 a) has shown that the chitinous cuticle of the egg
of Melanoplus differentialis is impermeable to such
materials as $K_4Fe(CN)_6$ and $FeCl_3$. This cuticle, a secretion
product of the serosa, is laid down during the fifth and sixth
days of development at 25° C. and remains intact until shortly
before the animal hatches (Slifer, 1937). The delicate, yellow,
outer portion of the cuticle is highly resistant to wetting, and
closely resembles the epicuticular portion of the body-wall of
an adult insect; while the tough, white, inner portion contains
chitin and has properties similar to those of the exocuticle and
endocuticle of the adult's exoskeleton. In fact there is reason to
believe that the chitinous cuticle represents the first embryonic
exoskeleton. During the latter half of the incubation period,
and after the embryo has undergone blastokinesis, a second
embryonic exoskeleton is secreted. This is shed immediately
after the insect leaves the egg. Since the outer yellow layer of
the first embryonic cuticle is so resistant to wetting, and since
it is present during all but the first few days of embryonic life,
how, then, is water able to enter the grasshopper egg? The pre-
sent paper deals with this problem.

PART I. THE FORMATION AND STRUCTURE OF A SPECIALIZED
AREA IN THE CHITINOUS CUTICLE.

A number of years ago while studying the fatty acid content
of the eggs of Melanoplus differentialis (Thomas) the
present author noticed that the portion of the chitinous cuticle
remaining after the eggs had been boiled in a strong solution
of KOH showed at the posterior end a small, circular area
which was so excessively thin as to be almost transparent. In
contrast to this, the cuticle covering all other parts of the egg
was, after such treatment, still tough, rather heavy, opaque,
and of a whitish colour. The idea immediately presented itself

that this circular area might be specialized for the exchange of materials between the egg and its environment. Attempts to prove this hypothesis were made by coating the posterior end of the egg with such materials as paraffin and asphalt varnish. These experiments were unsuccessful—for reasons not apparent at the time but now quite clear—and the work was eventually abandoned.

After Jahn (1935 a, 1935 b) had published his observations on the properties of the membranes which surround the grasshopper egg the presence of this thin area assumed a greater interest. It now seemed even more probable than before that this region might serve for the exchange of gases and liquids between the egg and its environment.

For microscopical studies a large number of Melanoplus differentialis eggs of various known ages were fixed in Bouin's or in Carnoy-Lebrun's solution. The eggs were sectioned longitudinally, at 7·5 microns, with the aid of the phenol water method described in an earlier paper (Slifer and King, 1933). Heidenhain's iron haematoxylin, Mallory's connective tissue method, and the Feulgen technique were used for staining.

A longitudinal section through the posterior tip of a 3-day old egg is shown in fig. 1, Pl. 30. It will be noticed that except at the extreme posterior end the chorion is thrown into depressions. These are the imprints of the ovariole epithelial cells which secreted the chorion. A fragment of one of the micropyles, which penetrate the chorion, is visible well back from the tip of the egg in the upper left corner of the drawing. The outermost layer of the chorion in the region posterior to the micropyles, is not clear and transparent, as it is elsewhere, but stains deeply and has a granular appearance. That this region differs from the rest of the surface of the egg can be demonstrated by placing whole eggs for a short time in Carnoy-Lebrun, then washing and staining in Mallory's connective tissue stain. The results are striking. The surface of the greater part of each egg, except in places where it has been injured, retains its original yellow colour, but the chorion covering the posterior tip is reddened with the fuchsin, indicating that the outer less permeable layer of the chorion is lacking here. In addition, the

contents of the micropyles stain brilliantly and, if the egg has been laid recently, each depression left by an ovariole epithelial cell will be filled with a blue-stained secretion, the so-called temporary coating of a previous paper (Slifer, 1937).

The cells of that part of the serosa which lies directly beneath the region of the chorion which contains no epithelial imprints are still thin and flattened in the 3-day-old egg (fig. 1, Pl. 30), but two days later they are found to be greatly enlarged, while the serosal cells elsewhere remain flattened. During the fifth and sixth days at 25° C. the serosa secretes the yellow cuticle over its outer surface (Slifer, 1937), and at the same time the enlarged serosal cells at the posterior tip of the egg secrete a membrane several times thicker than the rest of the yellow cuticle and distinctly different from it in structure (h, figs. 4, 5, and 6, Pl. 30). When examined with the aid of an oil-immersion lens this material, instead of appearing homogeneous, shows close-set and delicate striations running at right angles to the surface (fig. 4, Pl. 30). Following the formation of this striate layer the enlarged serosal cells begin the secretion of a second layer which is continuous with the white cuticle which covers the remainder of the egg and which is secreted by the ordinary serosal cells (Slifer, 1937). It never becomes as thick as the white cuticle found elsewhere but has the same structure, and stains in the same way.

Throughout the rest of this article this special area in the yellow layer of the chitinous cuticle will be referred to as the h y d r o p y l e (Gr. water-gate) and the enlarged serosal cells which produce it will be called the h y d r o p y l e c e l l s[1]. The introduction of these terms at this point is somewhat premature, but it will prevent much use of long descriptive phrases, and their suitability will become apparent towards the close of the paper.

By the time that the embryo is ready to undergo blasto-kinesis—which occurs on approximately the twenty-second day of active development at 25° C.—a considerable quantity of liquid has accumulated in the space between the white cuticle

[1] Roonwal (1935) noticed these enlarged serosal cells but was unable to assign them a function.

and the typical, flattened serosal cells found near the posterior
end of the egg (fig. 6, Pl. 30). The hydropyle cells are still
firmly attached to that portion of the cuticle which they secreted.
From this time on the history of the hydropyle cells can be
followed conveniently by making observations on the living egg.
This is best accomplished by placing an egg, from which the
chorion has been removed, in a drop of water on a cover-glass
to which several blobs of paraffin have previously been attached.
A smaller cover-glass is then laid over the egg and the whole
is inverted over the well of a depression slide. With such a
preparation it is possible to study the entire egg with a low-
power objective and the half (or more) near the observer with
a 4 mm. objective. Nuclei and cytoplasmic granules, vacuoles,
and filaments stand out with surprising clearness in the hydro-
pyle cells at the posterior end of an egg mounted in this way.
If it is desired to make a series of observations extending over
a long period the egg, when not being studied, may be removed
to an incubator and returned to the slide for examination at
selected intervals.

In order to learn the fate of the hydropyle cells eighty-one
eggs were chosen which were at the stage shown in Text-fig. 1 A.
These were studied with the help of the method just described.
During the initial stages of blastokinesis the strand of serosal
cells by which the embryo and yolk are attached to the hydro-
pyle cells becomes more and more slender (Text-fig. 1 B). Waves
of contraction, meanwhile, sweep along the lateral body-walls
of the embryo from the posterior towards the anterior end and
eventually the attaching strand breaks. In seventy of the
eighty-one eggs examined the break occurred in the thinner part
of the strand, the condition shown in Text-fig. 1 c resulting.
Here the hydropyle cells remain firmly attached to the inside
of the tip of the egg. The embryos in such eggs completed their
development and hatched, each leaving the hydropyle cells
behind inside the discarded yellow cuticle. In the remaining
eleven eggs the hydropyle cells were pulled loose entirely from
the end of the egg, and for some time could be seen as a
conspicuous lump on the serosa (Text-fig. 1 D). Later they
disappeared and were, presumably, taken into the mid-gut along
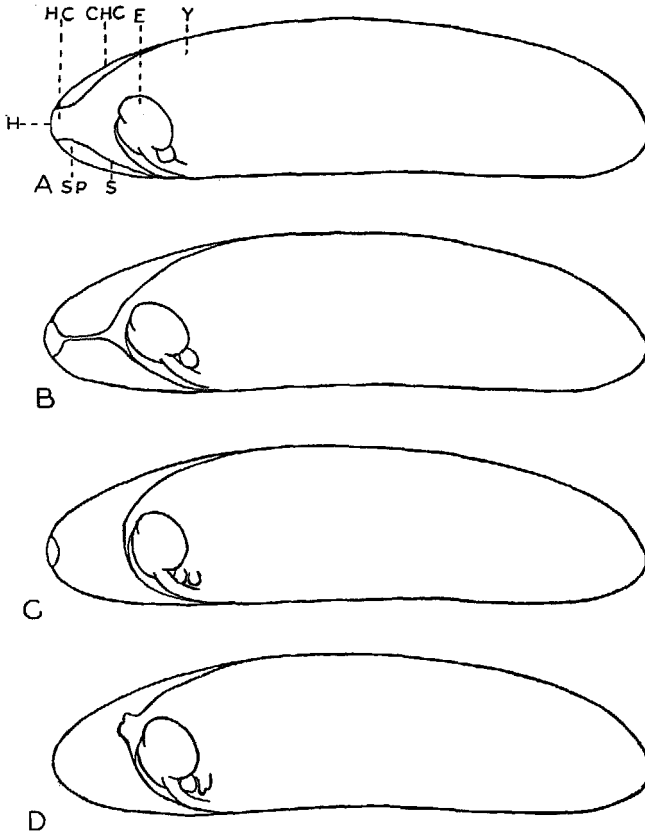
TEXT-FIG. 1.

Diagram representing Melanoplus differentialis eggs in stages preparatory to blastokinesis. The chorion has been removed from each egg. A, an egg in which the serosa (s.) has withdrawn from the chitinous cuticle (chc.) so as to leave a liquid-filled space (sp.). The head of the embryo (e.) is visible but the rest of it is covered by the yolk (y.). The hydropyle (h.) and the hydropyle cells (hc.) lie at the extreme posterior end of the egg. B, an egg at a somewhat later stage. c, an egg at a still later stage. The serosal strand has broken and the hydropyle cells remain attached to the inside of the posterior tip of the egg. D, an egg in which the hydropyle cells have been torn loose from the end of the egg and are visible, attached to the serosa, just above the embryo's head.

with the ordinary serosal cells and the yolk. Eggs in which this had occurred also hatched in an entirely normal manner.

From these results it may be concluded that the special function of the hydropyle cells is to secrete the hydropyle and that portion of the white cuticle which lies directly beneath it. After this has been accomplished they are no longer needed, and whether they remain attached to the posterior tip of the egg or are pulled away from it and digested makes little difference to the developing embryo. In eggs where the cells remain attached to the tip they may still be found, in sectioned material to retain a more or less normal appearance close to the time of hatching.

PART II. EXPERIMENTS ON THE REACTION OF THE HYDROPYLE AND OF OTHER PARTS OF THE EGG TO VARIOUS REAGENTS.

It has been stated in the preceding section that the thinness and unusual structure of the chitinous cuticle at the posterior tip of eggs which had been boiled in KOH, had aroused the suspicion that this particular area might be more permeable than other parts of the surface. With this in mind a number of reagents were selected which were apt to have some visible effect upon the surface or, should they succeed in entering, upon the contents of the egg. In all of the experiments which follow eggs were used from which the chorion had been removed. Observations were all made under a binocular dissecting microscope.

(A) KOH.

The effect of strong solutions of this reagent are especially conspicuous if eggs are used in which the embryos have just completed blastokinesis. The tip of the abdomen lies close to the posterior end of such an egg and a long, double row of cells containing white urate crystals is visible on either side of the abdomen. The alkali enters rapidly, and within a few minutes the extreme tip of the embryo's abdomen begins to show the first signs of disintegration. As the KOH diffuses inwards the urate crystals nearest the posterior tip of the egg suddenly disappear. When they have gone, those just anterior to them dissolve. This continues in a regular succession

from the posterior end forwards. The phenomenon is so striking and so orderly that only one explanation is possible. The alkali is entering through the posterior end and is diffusing anteriorly.

(B) Fixatives.

For experiments of this type eggs were used which were about to begin blastokinesis. If such eggs are placed in Carnoy-Lebrun the cells at the posterior end quickly coagulate and this coagulation—which is plainly visible—extends in a regular manner away from the posterior end. Eggs placed in Bouin's solution behave in the same way, but the fixative enters more slowly. Eggs exposed to 0·5 per cent. osmic acid are particularly interesting. The hydropyle turns brown almost instantly, then, very rapidly, becomes an intense black. Since the rest of the cuticle retains its original yellow colour the area blackened by the osmic acid stands out in sharp contrast.

(C) $KMnO_4$.

The yellow cuticle of an egg placed in a 0·05 per cent. solution of $KMnO_4$ soon turns brown while the hydropyle remains uncoloured. Eggs exposed to this solution for hours are unharmed and hatch at the usual time.

(D) $AgNO_3$.

Eggs about to undergo blastokinesis which are treated with 0·3 per cent. $AgNO_3$ and then placed in the sunlight retain their original colouring except at the hydropyle. Irregular brown patches appear slowly on this specialized area of the cuticle, and finally the whole circular area becomes black. The hydropyle cells also turned black in eggs which were exposed to $AgNO_3$ for an hour. After being washed these eggs were transferred to an incubator. No ill effects of the treatment could be observed. The eggs developed and hatched as usual. In some the blackened hydropyle cells were left attached to the posterior tip of the egg at blastokinesis and in others they were pulled away from it and, later, breaking loose from the serosa, were seen floating around in the fluid which bathes the embryo. The blackened cells from an egg of the former type were examined

in a yellow cuticle from which an embryo had just hatched. They were extremely hard and brittle.

(E) H Cl.

Eggs containing 17-day-old embryos were allowed to stand in concentrated HCl at room temperature for two weeks. At the close of this period the eggs had become coal-black but were still entire. The eggs were washed in distilled water and then transferred to 0·3 per cent. $AgNO_3$. At once a circular column of milky precipitate gushed from the posterior end of each. After a short time white spots appeared at other places on the surface of the eggs. These evidently marked the sites of small wounds. The forcibly ejected column appeared only at the posterior end. Eggs allowed to remain in the $AgNO_3$ solution eventually burst. This was clearly due to an inward passage of water more rapid than the outward diffusion of HCl. The hydropyle was examined microscopically in eggs which were removed to water from the acid and opened before bursting had occurred. No visible pores could be found in the hydropyle.

(F) HNO_3.

When placed in concentrated HNO_3 a white precipitate appears almost instantly at the posterior end of the egg and spreads rapidly away from it. A moment or two later the egg undergoes a miniature explosion, and a portion of the contents are thrown to a considerable distance through the ruptured hydropyle.

(G) Stains.

A number of eggs from which the chorion had been removed were exposed for a short time to Carnoy-Lebrun. Later these were washed and some treated with triosin and others with fast green. In both cases the hydropyle became brilliantly coloured while the rest of the cuticle was only faintly tinged by the stain.

PART III. THE EFFECT OF APPLYING AN IMPERMEABLE MATERIAL TO THE HYDROPYLE.

From the experiments just described it is apparent that the hydropyle differs markedly from other parts of the cuticle in

its reaction to various non-biological materials. It remained to be seen whether such physiological materials as O_2, CO_2, and H_2O enter and leave with more ease at this special area than at other locations on the egg surface. The simplest way to solve such a problem would be to cover the hydropyle with some impermeable material, and to compare the subsequent history of such eggs with the history of other eggs similarly treated but with the impermeable material applied to some other spot on the egg. A number of impermeable or relatively impermeable materials were tried, but only one gave satisfactory results. This was a commercial product sold under the name of O.K. Liquid Solder. This solder dries very quickly, sticks to the cuticle with great tenacity, has no toxic effects, and proved to be very impermeable to water. In all of the experiments described below the chorion was first removed from the eggs. If this were not done the sponge-like chorion (which often has minute cracks in it by this time) would very readily conduct water up under the solder and so defeat the purpose of the experiment. Moreover, in eggs from which the chorion has been removed it is possible to observe the condition of the embryo as often as desired through the transparent chitinous cuticle. Such eggs are much more delicate than ordinary eggs, and extreme care must be taken in handling them. If the slightest wound is made in the cuticle a portion of the liquid bathing the embryo oozes out and hardens into a small red spot. Eggs of this sort, unless the injury has been too great, develop normally; but the presence of these spots would be a possible source of error in experiments of the type to be described below. Consequently all eggs which developed red spots were discarded at once.

(A) Respiration.

One series of 20-day-old and one series of 22-day-old Mela-noplus differentialis eggs in which the diapause had been broken by exposure to low temperature were prepared as described above. The tips of the posterior ends of some, and the tips of the anterior ends of others, were then dipped in solder. The amount of oxygen consumed by each lot was then

determined in Warburg respirometers.[1] The results are shown in Table I. The figures are remarkably close and leave no doubt that the yellow cuticle as a whole is readily permeable to both O_2 and CO_2.[2]

TABLE I.

Two series of experiments in which the rates of oxygen consumption of post-diapause Melanoplus differentialis eggs with the anterior ends covered with solder were compared with the rates of oxygen consumption of similar eggs, the posterior ends of which were covered.

| Stage of development of embryo.	No. of eggs.	Anterior ends covered.	No. of eggs.	Posterior ends covered.
20-day	59	0·21 mm.$^3O_2$/egg/hr.	136	0·21 mm.^{3}/egg/hr.
22-day	43	0·41 ,, ,,	55	0·39 ,, ,,

(B) Weight (Water-content).

Bodine's (1929) curves demonstrating the increase in the water-content of the eggs of Melanoplus differentialis as they develop show a gradual but steady rise from the time when the eggs are laid to the onset of diapause at the close of the third week of development at 25° C. The curves then flatten out and remain so for a long period. At the end of diapause the water-content again begins to rise, and does so with increasing velocity until the eggs hatch. The curves secured by the present writer for eggs of the same species agree in their general trend with those of Bodine, but—owing perhaps to the closer intervals at which determinations were made—two rather interesting differences have been found. As may be seen in Text-fig. 2 (which is based on data secured when the eggs were

[1] The author is indebted to Dr. E. J. Boell who very kindly made these determinations.

[2] It might be objected that the solder had not been shown to be impermeable to gases. To test this point a piece of glass tubing tapering to a capillary at either end was filled with carbon dioxide gas. One capillary end was sealed in a flame and the other covered with the liquid solder. The piece of tubing was then suspended in a corked test tube over a solution of brom-thymol blue. At the end of seventeen hours the colour of the indicator still matched that of the standard. The tubing was then removed, a fine needle prick was made in the solder, and the whole quickly returned to the test tube. Within a few moments the indicator changed from bluish-green to a distinct yellow.

analysed for fatty acids, but which have not been published previously) the average wet weight of the eggs remains almost constant during the first week at 25° C. instead of increasing rapidly from the first day as Bodine's curves would indicate. This is a point of considerable interest, for it is at the close of the
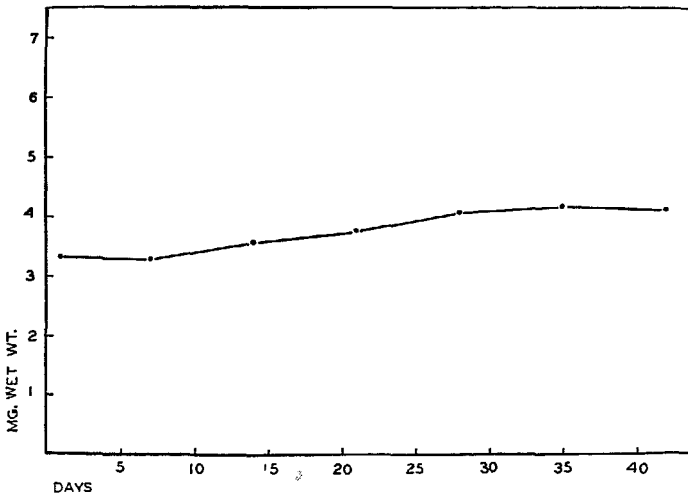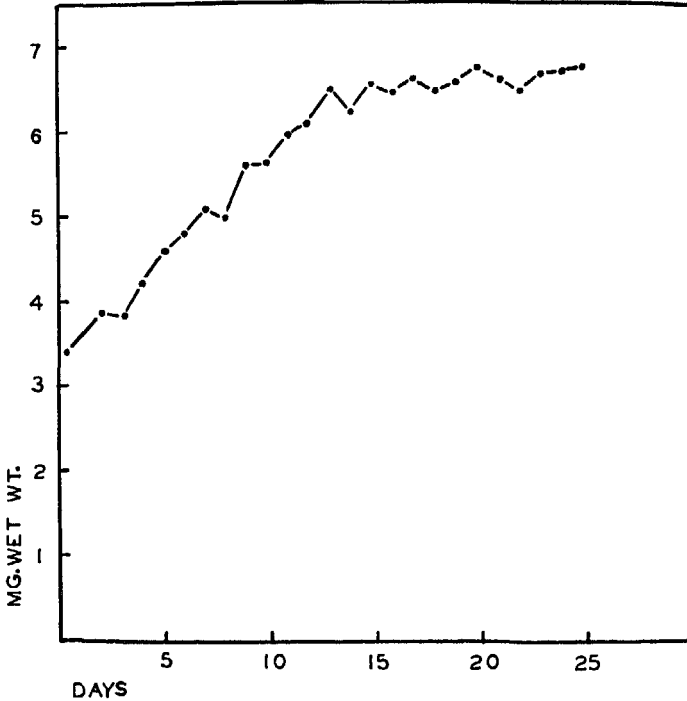

TEXT-FIG. 2.

Curve showing the increase in wet weight of the eggs of Melano- plus differentialis kept at 25° C. from the day when they were laid. The eggs enter diapause during the fourth week. Each point on the curve represents the weighted average for twenty experiments. A total of 20,441 eggs was used in securing the data for this curve.

first week that the serosa completes the secretion of the yellow cuticle. The acquisition of the power to absorb water in significant quantities is almost exactly coincident with the formation of this membrane.

In one other respect the curves secured by the present author differ from those given earlier. Instead of rising with increasing rapidity between the time when diapause is broken and hatching, as do Bodine's, those of the present writer show a rapid rise just before, during, and after blastokinesis. This is succeeded by a period of slower water uptake during the later stages of

incubation. The curve, some days before hatching, shows a tendency to flatten out parallel to the base line. It is characteristically concave rather than convex to the abscissa during

TEXT-FIG. 3.

Curve showing the increase in wet weight of Melanoplus dif-
ferentialis eggs during the last twenty-five days of incubation
at 25° C. These eggs had been exposed previously to low tempera-
tures in order to prevent the occurrence of a diapause. Hatching
began on the twenty-fifth day (thirty-eighth day of active
development at 25° C.). Each point represents a weighted average.
A total of 14,086 eggs was used in securing the data for this curve.

this period (Text-fig. 3). As the egg increases in size due to the uptake of water the pressure inside the egg naturally increases. The membranes are capable of considerable stretching, but after a time the resistance to further stretching tends to balance the forces leading to the further imbibition of water and the two

approach an equilibrium. The great turgidity possessed by the egg during its later development is well known to any one who has attempted to operate on eggs of this sort. A good portion of the egg contents spurt out at a slight prick of a needle.

For the experiments designed to test the effect of an impermeable coating applied to the hydropyle, eggs were chosen which had been kept at 15° C. for seventy-one days after they were laid. Such eggs contain embryos at a stage comparable to that reached after seventeen days at 25° C. (Slifer, 1932). These, when placed at 25° C., begin to develop more rapidly, and the majority hatch about twenty days later. The occurrence of a diapause is thus avoided. Seventeen-day-old eggs are particularly suitable for this purpose for the water-content is still low and the white cuticle is so well developed that the chorion can be removed without much difficulty. In the first few experiments of this type the eggs were weighed daily, but the eggs are delicate and the handling and drying preliminary to weighing caused the appearance of many small scars on the cuticle. It was necessary to discard so many eggs because of this that a new set of experiments was begun in which the weighings were made at longer intervals. The results of this experiment are shown in Text-fig. 4. Curve A shows the increase in weight of normal, untreated control eggs; curve B shows the weight of eggs from the same lot, the anterior ends of which had been dipped in solder, and curve C shows the weight of eggs the posterior ends of which had been similarly coated. Only one conclusion is possible. An impermeable covering applied to the posterior end almost completely prevents the uptake of water, while a similar coating on the opposite end has no such effect. Curve C does show a slight rise. This may be attributed either to some slight permeability of the yellow cuticle, or of the solder, to water, or to minute, unnoticed injuries of the yellow cuticle. Water, it may be concluded, enters (or is lost) in significant quantities through the hydropyle and not elsewhere.

(C) Development.

In addition to the weight measurements made on the eggs used in the experiments described in the preceding section,
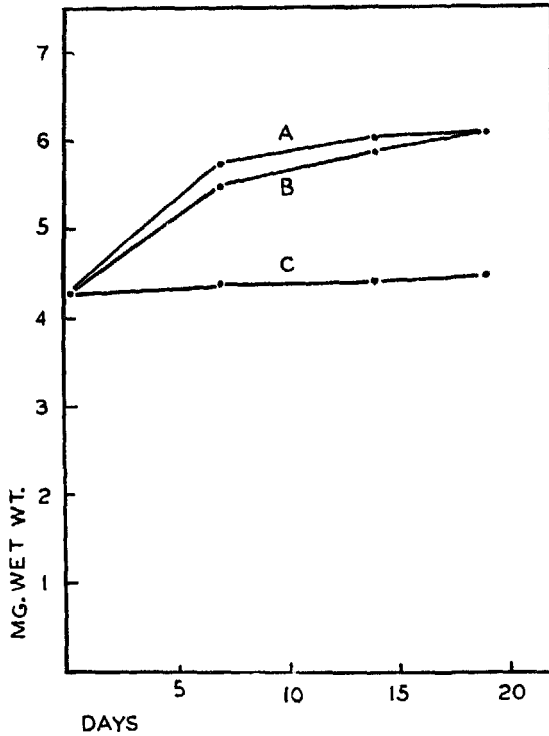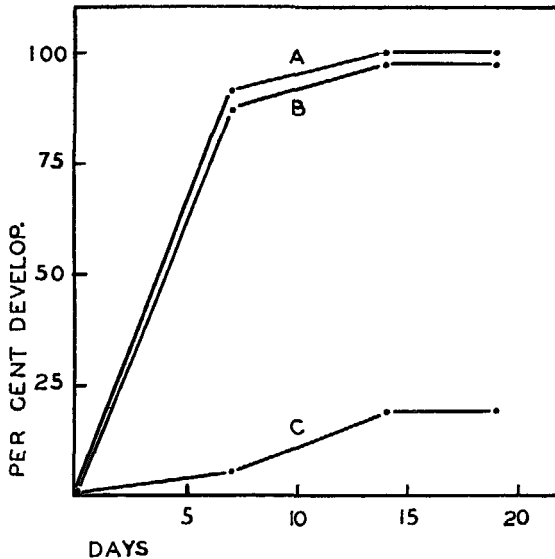
TEXT-FIG. 4.

Curves showing increase in wet weight of eggs with and without
the hydropyle covered with solder. A, untreated, control eggs;
B, eggs from which the chorion had been removed and the
anterior ends of which had been covered with solder; C, eggs from
which the chorion had been removed and the posterior ends of
which had been covered with solder. Curves B and C have been
corrected for the loss in weight due to the removal of the chorion.
The eggs at the beginning of the experiment were at the 17-day
stage of morphological development. Hatching, in the controls,
began on the nineteenth day of the experiment. 199 eggs were
used in securing the data for these curves.

observations were made on the course of development. Two
useful and conspicuous landmarks in the latter part of the
embryo's history are (1) the occurrence of blastokinesis and

(2) hatching. In Text-fig. 5 the percentages of the total number of eggs which had completed blastokinesis are given for the same eggs whose weights are shown in Text-fig. 4. The number of embryos which succeeded in completing blastokinesis is very much lower in the eggs which had the posterior ends covered

TEXT-FIG. 5.

Curves showing percentage of eggs in which blastokinesis had been completed. Eggs from same experiment as those in Text-fig. 4. A, normal, untreated controls; B, eggs with anterior ends covered with solder; C, eggs with posterior ends covered with solder.

than it is in either of the other two groups. By the twenty-first day, at 25° C., 73·5 per cent. of the control eggs, 60 per cent. of the eggs with the anterior end covered, but only 2·8 per cent. of the eggs with the posterior end covered had hatched. Development, then, stops at once or progresses at a greatly retarded rate in eggs when the entrance of water is prevented. It is conceivable, of course, that the solder placed in such close proximity to the head of the embryo might have some toxic effect. This objection was met by removing the solder from twenty-seven

of those eggs which had had the posterior end covered for four weeks, and which had showed no signs of developing during that period. This was easily accomplished by using acetone as a solvent for the solder. One week later 60 per cent. of these eggs had completed blastokinesis.

One point remained to be settled. Would the application of solder to the posterior ends of eggs after the greater part of the water had already been taken up have any retarding effect on development? This question was answered by placing solder on the posterior tips of thirty eggs which were scheduled to hatch about a week later. A like number had the anterior ends covered. The results were clear-cut. All hatched at the same time as the controls. The solder, then, does no harm. It is only the lack of water which stops or slows down development.

DISCUSSION.

A search through the literature on embryology in an attempt to learn whether or not the special area in the chitinous cuticle, here called the hydropyle, is present in other insect eggs has produced little in the way of results. It has, apparently, been the practice of nearly all those who have worked with insect eggs to remove the secreted membranes while preparing them for study. Structures such as the curious 'dorsal organ', of unknown function, described for certain apterygote insects, as well as for the eggs of a number of arthropods other than insects,[1] arouse the suspicion that this may correspond to the mass of cells which secretes the hydropyle in the eggs of Melanoplus differentialis. For example, the 'precephalic organ' described for the egg of Anurida maritima by Imms (1906) and the 'indusium' found in eggs of the Paratenodera sinensis by Hagan (1917) both bear a strong resemblance to the hydropyle cells of the grasshopper egg. But the illustrations accompanying the descriptions are not sufficiently detailed to tell whether or not such a special area exists. Moreover, little is known concerning the water metabolism of these forms.

More data, pertinent to the problem under discussion, are

[1] Hirschler (1928) and Korschelt and Heider (1936) may be consulted for references which deal with these structures.

available for the eggs of Notostira erratica than exist for those of most other forms. The eggs of this hemipteran absorb water, as Johnson (1934, 1937) has demonstrated, and a comparison of Johnson's figures of sectioned eggs (especially of fig. G, pl. II, 1934) with fig. 6, pl. 30 of the present paper is illuminating. The cells which Johnson describes as 'columnar epithelium of cells lining the yolk-plug wall' closely resemble the hydropyle cells of the grasshopper egg. The two-layered 'yolk-plug membrane' corresponds, apparently, to the chitinous cuticle of the present paper but it is not possible to tell, from Johnson's figure, whether the membrane is modified above the columnar epithelium to form a hydropyle. It would be interesting to test this point experimentally with the Notostira egg. Finally, Weber's (1931) description of the uptake of water by the eggs of a coccid, Trialeurodes vaporariorum, from the tobacco leaves to which they are attached by a small, thin-walled stalk should not be overlooked. A short time after the eggs are laid a brown membrane is formed below the chorion. This extends into the stalk and is thinner in that region than it is elsewhere. Weber states that when leaves with eggs attached to them are allowed to wither the eggs on them soon collapse and die—which recalls Réaumur's experiments, long ago, with the saw-fly eggs. After the inner brown membrane has formed the eggs are less sensitive to changes in the turgidity of the leaf than they are when newly laid. It seems probable that conditions in the coccid egg bear a rather close resemblance to those in the egg of the grasshopper.

CONCLUSIONS.

1. Water enters (or leaves) the egg of the grasshopper, Melanoplus differentialis, after the sixth day of development at 25° C., through a small, circular, specialized area in the yellow cuticle located at the posterior end of the egg. This area has been named the hydropyle.

2. The hydropyle is secreted by a group of enlarged and modified serosal cells. These are called the hydropyle cells.

3. The outer layer of the greater part of the chorion consists

of a clear material which is not readily permeable to dyes. At the posterior tip of the egg this impermeable layer is lacking.

4. The grasshopper egg first begins to absorb water in considerable quantities immediately after the yellow cuticle is formed.

5. Towards the close of embryonic development the rate of water uptake falls off markedly.

6. The chitinous cuticle as a whole is readily permeable to O_2 and CO_2.

7. If water is prevented from entering the egg by covering the hydropyle with a water-impermeable material development is stopped, retarded, or unaffected depending upon the water-content of the egg at the time when the impermeable coating is applied.

REFERENCES.

Blunck, H., 1914.—"Entwicklung des Dytiscus marginalis L. vom Ei bis zur Imago. 1. Teil. Das Embryonalleben", 'Zeit. wiss. Zool.', 111.

Bodine, J. H., 1929.—"Factors influencing the rate of respiratory metabolism of a developing egg (Orthoptera)", 'Physiol. Zool.', 2.

Buxton, P. A., 1932.—"Terrestrial insects and the humidity of the environment", 'Biol. Rev.', 7.

Hagan, H. R., 1917.—"Embryonic development of the mantid, Paratenodera sinensis", 'Journ. Morph.', 30.

Hirschler, J., 1928.—"Embryogenese der Insekten", 'Handbuch der Entomologie', von Chr. Schröder. Jena.

Hoffmann, C. C., Dampf, A., and Varela, G., 1924.—"Informe de la comision cientifica exploradora de la plaga de la langosta en el estado de Veracruz", 'Mono. Instit. Higiene, iii, secc. Parasitol., Mexico'.

Imms, A. D., 1906.—"Anurida", 'Proc. and Trans. Liverpool Biol. Soc.', 20.

Jahn, T. L., 1935 a.—"Nature and permeability of the grasshopper egg membranes. I. The EMF across membranes during early diapause", 'Journ. Cell. Comp. Physiol.', 7.

—— 1935 b.—"II. Chemical composition of membranes", 'Proc. Soc. Exp. Biol. Med.', 33.

Johnson, C. G., 1934.—"Eggs of Notostira erratica L. (Hemiptera, Capsidae)", 'Trans. Soc. British Ent.', 1.

—— 1937.—"Absorption of water and associated volume changes in eggs of Notostira erratica", 'Journ. Exp. Biol.', 14.

Kerenski, J., 1930.—"Beobacht. ü. d. Entwickl. der Eier von Anisoplia austriaca", 'Zeit. angew. Entomol.', 16.

Korschelt, E., und Heider, K., 1936.—'Vergl. Entwickl. der Tiere.' Jena.

Needham, J., 1931.—'Chemical Embryology.' Cambridge.

Réaumur, R. A. F. de, 1740.—'Memoires pour servir a l'histoire des insectes', 5.

—— 1926.—'Histoire des fourmis.' New York and London.

Roonwal, M. L., 1935.—"Fate of embryonic membranes in insects", 'Current Sci.', 4.

—— 1936.—"Growth-changes and structure of the egg of Locusta migratoria migratorioides", 'Bull. Ent. Res.', 27.

Slifer, E. H., 1932.—"Insect development. IV. External morph. of grasshopper embryos", 'Journ. Morph.', 53.

—— 1937.—"Origin and fate of the membranes surrounding the grasshopper egg", 'Quart. Journ. Micr. Sci.', 79.

Slifer, E. H., and King, R. L., 1933.—"Grasshopper eggs and the paraffin method", 'Science', 78.

Uvarov, B. P., 1931.—"Insects and climate", 'Trans. Entomol. Soc. London', 78.

Weber, H., 1931.—"Lebensweise und Umweltbeziehungen von Trialeurodes vaporariorum", 'Zeit. Morph. Ökol. Tiere', 23.

EXPLANATION OF PLATE 30.

Fig. 1.—Longitudinal section through the posterior tip of a Melanoplus differentialis egg which has been kept at 25° C. for the three days since it was laid. The serosa (s.) and germ-band or embryo (e.) lie directly beneath the chorion (ch.) and at the surface of the yolk (y.). A fragment of a micropyle is visible in the chorion in the upper left-hand corner of the drawing. ×160.

Fig. 2.—Diagrammatic, lateral view of an egg, from which the chorion has been removed, to show the size of the hydropyle at the posterior end of the egg. The concave side of the egg represents the ventral surface. ×10.

Fig. 3.—Diagrammatic view of an egg, from which the chorion has been removed, as seen from the posterior end. ×10.

Fig. 4.—A section through the hydropyle at right angles to the surface of an egg fixed twelve days before hatching was due. Note the striated appearance of the modified yellow cuticle as seen under high magnification. ×1,678.

Fig. 5.—A section through a part of the hydropyle and a few of the hydropyle cells cut at right angles to the surface of an egg fixed twelve days before hatching was due. ×680.

Fig. 6.—Longitudinal section through the tip of an egg fixed shortly before blastokinesis (Text-fig. 1 A represents an egg at this same stage). The serosa (s.) has withdrawn from the white cuticle (wc.) leaving a liquid-filled space between the two. A few particles of yolk are enclosed within the serosa. m., fragment of micropyle; ch., chorion; yc., yellow cuticle; h., hydropyle; hc., hydropyle cells. ×160.