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Embryological and Ecological investigations on the development of the egg of Ammophila campestris Jur.*)

by

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*) After this paper was completed in 1944, WILCKE (Entomol. Ber., 264/266, XI, 1945) found that the *Ammophila* species we are dealing with is not the species *campestris* as described by LATREILLE but a new species he called *A. adriaansei*. However RICHARDS (Entmol. Monthy Mag., 82, 1946) is of opinion that the species was already described earlier by CURTIS under the name *Ammophila pubescens*. We think that it will give less confusion if we use here the same name as in the preceding paper on the life history (BAERENDS, 1941).

I. *Introduction*

Hitherto only a small number of ontogenetic researches on Aculeates have been carried out. *Apis mellifica* L. has been investigated by several researchers, a.o. NELSON (1915) and SCHNETTER (1934); *Chalicodoma muraria* Fabr. was thoroughly dealt with by CARRIÈRE & BÜRGER (1898) while a few ants and *Vespa vulgaris* L. were subjected to an embryological investigation by STRINDBERG (1913, 1914). No Sphegid, however, has been examined from this point of view.

As, during an investigation on the behaviour of *Ammophila campestris* Jur., we incidentally got the opportunity to collect well over a hundred eggs, we decided to give a description of the embryological development of this wasp. Although the quantity of eggs we were able to collect remained far below the number of eggs examined by for instance NELSON, SCHNETTER and CARRIÈRE & BÜRGER, this quantity seems to be sufficient to follow the general course of development, but often it proved to be inadequate for the solution of more detailed questions.

In broad outline the development of *Ammophila* agrees well with that of *Apis* and *Chalicodoma*. Nevertheless we are going to give in the following a full description of this development ; only in case of important differences we shall refer to literature. In doing this, we realise that we may repeat facts or processes that are already more or less known in detail. We feel, however, justified because a mere summing up of differences and additions to literature would be quite incomprehensible. Moreover, we think, that in some instances we are able to give a clearer picture of some processes and particularly of the sequence and the relative velocity of the pro-

cesses than can be found in current literature. Besides for the ecological considerations that will follow, this full description is necessary.

In collecting eggs of different ages in the field, it soon became clear to us that the age (in hours) is by no means an indication of development reached, for in eggs known to be of the same age this could be very different. ADLERZ (1903) already mentions this with regard to *Ammophila campestris*, he does not ascribe those differences to the influence of external circumstances but thinks that it is a peculiarity of wesp eggs. This seemed rather improbable to us for the following reason.

Ammophila campestris is one of the Sphegid species that goes on provisioning after the larva has hatched. The egg is laid on the first caterpillar and only after hatching the larva is provided with a second caterpillar. Subsequently 6 to 8 caterpillars are brought in during the next days. The activity of the imago is very much affected by weather conditions. Only on beautiful sunny days caterpillars are brought in. Between two periods of favourable weather, seldom lasting for more than a week, weather may remain bad for at least the same time. Under favourable weather conditions the development from egg to cocoon takes about 10 days. So, if the velocity of development of the eggs and of the larva is not affected by weather conditions the larva hatched after two days- would starve before it was brought another supply of food. As a matter of fact ADLERZ thinks that during spells of unfavourable weather numerous larvae die.

In the Netherlands, during the appropriate season, favourable weather is often very rarely (on an average in total about 30 not consecutive days) so, providing ADLERZ is right, it looks as if a colony of this wasp could hardly increase or even maintain itself over a number of years. If, however, weather conditions would effect the rate of development of the eggs and larvae in the same way as they effect the activity of the imago, the brood would not suffer from food shortage.

To solve this problem, micrometeorological observations in the nest-area of *Ammophila* have been tried and the effect of the microclimate on the velocity of development of the brood, in particular of the eggs, has been investigated. The influence of temperature, apparently the most important factor of the microclimate, was also tested under laboratory conditions.

The material has been collected at two places on the Veluwe (county Gelderland, Netherlands) one at Hulshorst (near Harderwijk) and another at Hoenderloo (near Arnhem). We are indebted to Ir. J. E. JURRIAANSE who kindly allowed us to work on his country-seat and to the Biological Laboratory „De Hoge Veluwe" (Dir. Dr A. D. VOÛTE) for its hospitality.

We owe much to Prof. Dr C. J. VAN DER KLAAUW for his criticism and advice and we are also thankful to Dr A. D. VOÛTE for some very useful remarks. Miss Dr K. SCHIJFSMA, who has been so kind to correct the english text, we particularly want to thank heartily at

this place. Finally we want to express our gratitude to Miss K. NEUMANN who made the microscopical slides.

II. Anatomical Part

A. Material and Technique

Ammophila campestris digs her nest about 3 cm deep in compact sandy soil. Having accomplished this work she brings a caterpillar (Noctuid or Geometrid) in the cell and lays an egg on it. Later, only after the larva has hatched, the female carries more caterpillars into the nest as food for the growing larva. From this it will be clear that the mere sight of the wasp paying a provisioning visit does not justify the supposition that an egg has been deposited. Fortunately the wasp when paying a mere provisioning visit to feed the larva spends only a few seconds in the nest, while the laying of an egg takes more time, usually the wasp remaining down for more than 30 seconds.

So to find a fresh egg we looked for an *Ammophila* with prey, followed her to the nest and noted the duration of her visit. If we wanted the egg to develop in the field for some time we only placed a mark near the nest (see for the technique of marking BAERENDS, 1941), in order to be able to find it back. If, however, we wanted to control the development of the egg in a thermostate, we immediately dug out the nest. With the boring apparatus shown in fig. 1 we took out a cylindrical lump of earth 5 cm in diameter in the middle of which was the nest. After opening the apparatus the lump could easily be taken out of it. Then we placed the lump in a brass tube of equal width and 5 cm high, closed at the bottom with a cork. These tubes we brought to the laboratory as quickly as possible and placed them in the thermostate.

When the eggs had been exposed to a certain temperature for the time required for the experiment, we took the lump out of the tube, broke it and fixed the caterpillar with the egg. We used Freyling's fixative at 50° C and kept the eggs in this fluid till it had cooled down, after which we transferred them to alcohol 96%. We always took care to use fixative not older than one month.

At first we mounted the eggs in paraffin after passing them from alcohol to xylol and then stained them with haematoxylin Ehrlich and eosin. Later we transferred them from alcohol via methylbenzoate to paraffin and dyed them with orange G instead of eosin. The last method yielded somewhat better results. It was not necessary to treat the eggs with diaphanol but to promote the infiltration by paraffin, it appeared recommendable to tear the chorion.

Our sections were 3 or 4 μ thick; we generally succeeded very well in making transverse sections, but longitudinal sections usually failed.

In this article we shall indicate the position of particular cells, cell groups, organs etc. by a relative measure. This measure we shall call a "unit". It is one hundredth of the length of the particular

egg under discussion, the number of units always indicating the distance of a certain point from the top of the egg.

B. Structure of the feminine sexual organs

As we wanted to investigate the influence of microclimatological factors upon the development of the eggs we had to know when development starts, i.e. when the egg is fertilized. For if fertilization could take place some hours or more before egg laying, development would proceed to a certain degree in the sexual organs of the wasp and it would be impossible to control external influences working upon the egg during the first period. To ascertain this we started to investigate the sex organs of the female.

On either side in the abdomen an ovarium is situated consisting of 3 ovarioles of the polytrophic type (fig. 2). These ovarioles open into two oviducts which enter the vagina from the dorsal side. The more the egg approaches the oviduct the more it reaches accomplishment. The 3 most caudal (eldest) eggs in each set of 3 ovarioles are not equally far developed but the 3 stages of these eggs at one side usually correspond with the stages of the 3 most caudal eggs in the ovarioles at the other side. This accounts for the female being able to lay 2 eggs in quick succession (BAERENDS, 1941).

Where the oviducts enter the vagina, the receptaculum seminis joins them. In the receptaculae of females we captured at the end of the season, we found less sperma than in receptaculae of females caught and fixed earlier in summer. This may explain that during September the females often lay eggs that do not develop because they have not been fertilized. For copulation takes place only at the beginning of the season. From the position of the aperture of the receptaculum in the vagina we may conclude that the egg is fertilized as soon as it enters the vagina. We never found an egg in the vagina although we examined females fixed when they were just about to lay an egg. We likewise never found cleavage stages in eggs fixed immediately after being laid. So we may conclude fertilization to take place just before the egg is deposited on the caterpilllar and from that moment we assume cleavage to start.

C. Structure of the egg.

The bilaterally symmetrical egg is about $2\frac{1}{2}$ mm long while its maximal cross diameter measures about $\frac{3}{4}$ mm. It is bean-shaped, concave on the ventral side and tapering towards the top. The surface is smooth, the colour milky white.

Stained with haematoxylin Ehrlich and eosin the ooplasma of the egg takes on a blue, the deutoplasm a red colour. Through the whole egg the ooplasm shows a spumoid structure, forming numerous cavities of different size which contain oil globules. These oil globules dissolved in our fixative. The size of the cavities is largest in the centre of the egg and diminishes towards the periphery. In the centre of the egg lies a cylindrical mass of denser ooplasm about half as broad as the egg and with the longitudinal axis corre-

sponding. SCHNETTER (1934) observing the same structure in eggs of *Apis mellifica* called it central column (Zentralsäule) (fig. 33). Nearer to the periphery the reticular ooplasm increases gradually in denseness, constituting near the surface the cortical layer of about 10 μ thick. SCHNETTER (1934) describes in the egg of the honeybee an ooplasmatic wedge penetrating from the cortical layer into the egg. He calls it „Richtungsplasma", which indicates the spot of fertilization. In the sections of younger cleavage stages we now and again found such concentrations of ooplasm near the apical pole, always in the neighbourhood of cleavage nuclei. Sometimes at this very place the periphery seemed to have been pierced. Occasionally we found in the neighbourhood of such a concentration of plasm some small nuclei, probably polar bodies. Moreover, ooplasm is concentrated round the original nucleus and round the cleavage cells („Hofplasma").

The deutoplasm is present as globules having a diameter from 2—12 μ, dispersed in the ooplasmic network.

The original nucleus can easily be found in the very young eggs in the ovarioles. Afterwards, when yolk has been deposited in the egg the nucleus is hardly discernible, only the concentration of protoplasm round it remains very distinct. In a few sections of a new laid egg in which the structure had been damaged, the nucleus presented itself as a light pinkish body of about 40 μ in diameter. In one section the centre of the nucleus lay at a distance of 17 units from the top in the other the distance amounted to 18 units. When cleavage has begun, the nuclei become better visible again. The same seems to be the case in other insects, for instance SPEICHER (1936) describes it in eggs of *Habrobracon*.

Close to the outside of the cortical layer clings a thin membrane, the vitelline membrane. Outside this membrane we find as a more solid envelope the chorion. In some sections we found places where chorion, vitelline membrane and cortical layer were in close contact and where these layers seemed to have been transfixed (fig. 5). We got the impression that those spots might be the micropyles; our material, however, does not permit us to assert this with certainty.

D. Cleavage

Eggs fixed only a few hours after having been laid proved to contain cleavage nuclei. In sections of the first stages the nuclei had retained little of the stain but in older stages the colouring was much better. Fig. 3 shows a number of consecutive cleavage stages. In the earliest stage in our possession, the 16-nuclei-stage, we found the cleavage cells from the 15th to the 25th unit in the longitudinal axis of the egg. In the 32 - nuclei-stage the cells are still arranged along the central axis, from the 7th to the the 25th unit. According to SCHNETTER (1934) in *Apis* the first nuclei constitute a spherical body in the foremost part of the egg. When the 16-nuclei-stage has been reached the constellation of the nuclei becomes more oblonged because of the cells migrating to the egg poles.

We could observe this situation in the eggs of *Ammophila* up to

the 256-nuclei-stage. In this stage (fig. 3) the nuclei are situated in the foremost $^2/_3$ part of the egg, on an almost cylindrical surface. The nuclei extend from the 5th to the 80th unit. The cleavage cells in the foremost part of the egg have moved somewhat towards the periphery.

In *Apis* this shifting of the nuclei towards the periphery takes place after the 128-nuclei-stage, which is in accordance with our data on *Ammophila*.

In moving towards the cortical layer the nuclei take some ooplasm with them (fig. 7), and consequently the network of protoplasm is torn up. So within the level occupied by the cleavage cells only a poor residue of the ooplasmatic reticulum persists. Outside this surface, however, it is still intact (fig. 8).

In the sections hitherto under discussion the nuclei were stained light pinkish, globular and about 10 μ in diameter. They were surrounded by a dark blue area of ooplasm. This appearance is shown by the nuclei when resting (fig. 6). During cleavage the nuclei are darker and hardly contrast with the surrounding plasm (fig. 9). From the fact that in the stages described hitherto, we always found resting nuclei, we may assume with probability that cleavage takes place quickly and simultaneously and that between two periods of cleavage activity there may be a rather long period of rest.

In the 256-nuclei-stage it appears that within the figure formed by the nuclei when moving towards the periphery, yet another group of nuclei may be distinguished. They look like ordinary resting cleavage nuclei, but they are somewhat larger. From these slides we could not conclude whether these nuclei had remained in their place when the other cells went to the periphery, or whether they had moved on their own account from the periphery back towards the centre of the egg. They are the yolk cells (fig. 10, dk). According to SCHNETTER (1934) the first yolk cells in *Apis* also appear after the 128-nuclei-stage.

In the next stages the nuclei move still nearer to the periphery and in the foremost part of the egg they wander quicker. Consequently the nuclei take on a pear-shaped arrangement.

Fig. 3 shows the position of the nuclei in a section of the 512-nuclei-stage. Here the nuclei range from the 5th to the 55th unit, so they have not penetrated backward as far as the nuclei in our slide of an egg of the 256-nuclei-stage. Such individual differences often seem to exist between equally old stages of different eggs, which is also corroborated by SCHNETTER's work on *Apis*. In this stage, the nuclei from the top to the 19th unit about reached the inner side of the cortical layer. In this and in the following stages, till the nuclei have passed the major part of the cortical layer, there are in all our eggs deeply stained nuclei. This indicates that cleavage processes are succeeding each other at a much quicker rate or that division is not simultaneous any longer. Naturally also both possibilities may obtain together. In *Apis* SCHNETTER (1934) too observed that as soon as the nuclei have reached the surface layer, cleavage becomes heterochronous. Both in *Apis* and in *Ammophila* the nuclei

reach the surface layer at about the 10th cleavage, that is at the 1024-nuclei-stage.

Cleavage occurring no longer in all nuclei at the same time the chance to find dividing nuclei becomes bigger ; so the fact that in the following stages we very often found dividing nuclei, does not necessarily mean that the rate of cell division has increased.

In the egg of the 512-nuclei-stage, within the surface formed by the cleavage cells, lie about 20 yolk cells from about the top to the 25th unit. They are looking like resting nuclei.

In the next egg, from the point of view of development, we counted about 1500 nuclei (fig. 3). This number is no longer a power of 2 which is in accordance with the idea suggested above that after the 1024-nuclei-stage cleavage becomes heterochronous. The fact that in this egg the foremost nuclei from the 3rd to the 36th unit are darkly coloured, while the nuclei from the 36th to the 94th unit seem to be resting, is in favour of the same view. Only the nuclei lying at the ventral side from the 6th to the 18th unit are situated within the cortical layer. We found about 100 yolk cells, looking like resting nuclei and occupying a space from the 4th to the 30th unit.

In further developed eggs counting of the cleavage cells in the whole egg was no longer faisible to judge the ratio of development, so we counted them in a number of successive sections at the same relative position through the eggs and took a mean value.

In the less developed eggs all nuclei of the foremost part are laying in the cortical layer ; at the ventral side they have approached the periphery somewhat more than at the dorsal side. From the 80th to the 100th unit the cleavage cells are still at some distance form the cortical layer. The maximum number of nuclei in a transverse section was found at about the 18th unit. In the older stages the nuclei keep moving through the cortical layer to the periphery, the plasm enveloping the nuclei accompanies them. SCHNETTER (1934) on the contrary states that in *Apis* the "Hofplasma" remains behind at the inner surface of the cortical layer. In *Ammophila* the ooplasm only stays behind when the nuclei assume their final position at the surface of the egg (fig. 10).

SCHNETTER (1934) found in *Apis* that the nuclei after having crossed the cortical layer, reenter it again, forming a single layer ("gleichmässiges Blastoderm"). Subsequently these cells divide and shift along each other till they are arranged in a single layer again. In the meantime a new cortical layer is formed inside this "gleichmässiges Blastoderm" by ooplasm migrating towards the periphery ("inneres Keimhautblastem"). When finally this plasm has also been taken up into the cells, SCHNETTER calls it "differenziertes Blastoderm".

Having only a limited number of eggs of these stages, we could not in every detail follow the formation of the blastoderm in *Ammophila*. As stated above the cleavage cells first pass the cortical layer and take position on the surface of the egg ; afterwards they move back into the cortical layer. As the number of cells finally

found in the cortical layer (mean number in some transverse sections at about the 18th unit) was twice the number of cells found on the surface of the egg in younger stages, we conclude that in *Ammophila* as in *Apis* the cells divide after having reentered the cortical layer. As we could not find any thickening of the cortical layer we think that an "inneres Keimhautblastem" is not formed. SCHNETTER (1934) observed that in *Apis* before the formation of the final blastoderm more nuclei concentrate on the ventral side, while the dorsal side of the cortical layer becomes thinner and poorer in nuclei. This certainly does not occur in *Ammophila*, the accomplished blastodermcells being all of the same height (20 μ x 5 μ). (fig. 34). Only later on, when the formation of the germ layers begins, a difference in dorsal and ventral blastoderm becomes visible.

During the formation of the blastodermcells lateral partitions between the cells appear first (fig. 12). Afterwards the basal membrane is formed (fig. 13). At the end of the delimitation of the blastoderm the yolk cells fuse to syncytia ; soon thereafter they have disappeared. In older stages, e.g. at the formation of the intestine, yolk cells appear again ; we could not ascertain where these originate from ; most probably they segregate from the surrounding cellayers, as proved to be the case in Apis.

When fitted into the picture of the blastoderm formation known in *Apis* and some other Hymenoptera, the few eggs we posess of these stages yield sufficient data to reconstruct the process in *Ammophila*. We could find only minor differences with *Apis mellifica* (DICKEL, 1904 ; SCHNETTER, 1934) with *Vespa* (STRINDBERG 1914) and with *Chalicodoma* (CARRIÈRE & BÜRGER, 1898).

From extensive statistical data SCHNETTER (1934) tried to deduce the position of the centre of differentiation in the egg of the honey bee. He found it at a distance of 24 units from the top. Our material is of course inadaequate to permit of such a statistical research, but in relation to SCHNETTER's work the following seems noteworthy.

The undivided nucleus we found at about the 17th unit. In the 512-nuclei-stage the nuclei lying at about the 13th unit have moved farthest to the periphery. The nuclei between the 10th and the 15th unit arrive first at the cortical layer. At the time that all nuclei have entered the surface layer we found the greatest number of nuclei in a section about the 17th unit. From these observation we might expect the centre of differentiation to lie between the 10th and 20th unit, that is in about the same area as in Apis.

E. Formation of the germ layers

1. General Survey

The final blastoderm of *Ammophila campestris* consists of cylindrical cells, 20 μ long and 5 μ broad. Their nuclei are spherical, 4 μ in diameter and coloured light pinkish (fig. 13).

Soon after the blastoderm has been formed, the cells lying in a broad strip at the ventral (concave) side of the egg become rounded off. This strip sinks below the surface and is subsequently

covered by a cell layer which is formed by the blastoderm cells formerly adjoining the rounded cells, multiplying in the direction of the ventral median. Later on this sunken strip is seen to produce fat tissue, muscles and reproductive organs, which proves it to be mesoderm. From the cells growing over the mesoderm at the poles and at the ventral sides of the egg, the integument, tracheal system, maxillar glands, stomodeum and proctodeum with Malpighian tubes are derived. So this layer is the ectoderm.

About simultaneously with the sinking in of the mesoderm plate, round cells from the poles of the egg migrate from the surface towards the yolk. From these cells originate the intestinal walls, so they represent the entoderm.

During the covering of the mesodermal plate by the ectoderm the form of the blastoderm cells at the dorsal side of the egg is changing. They become flatter and broader, therefore the cell layer becomes thinner. Soon a rupture between this cell layer and the ectoderm appears. The lateral margins of the dorsal layer grow towards the ventral side. Here they join each other, enveloping the egg and constituting the serosa. These processes, leading to the formation of the germ layers, are in broad outlines similar to those in *Chalicodoma muraria* Fabr. (CARRIÈRE & BÜRGER, 1898), *Apis mellifica* L. (NELSON, 1915; SCHNETTER, 1934) and *Vespa vulgaris* L. (STRINDBERG, 1914). Henceforward we will only cite the literature in case of differences, not when our statements agree with those of other authors or when our description is more detailed than theirs could be.

2. Mesoderm

The first mesoderm rudiment appears by the cells, in a ventral area covering about ¾ of the length of the egg, rounding themselves of and dividing. This area ranges from the 12th to the 88th unit. At the anterior end it occupies about $^1/_6$, at the posterior end about ¼ of the circumference of the egg (fig. 4a, 14).

By cell division the mesodermal plate becomes 4—5 cell layers thick, at the edges it is thinner. The cells have a diameter of 10—14 μ, when they are not in a cleavage stage they have spherical light coloured nuclei of 8 μ diameter. When cleavage takes place the nuclei are smaller and darkly stained. As far as we are able to deduce from NELSON's (1915) and SCHNETTER's descriptions the rounding off and the division of the cells giving rise to the mesodermal plate do not occur in the same way in *Apis*. Here cell division only starts after the mesodermal plate has sunken below the surface. According to STRINDBERG (1914) and CARRIÈRE & BÜRGER (1898) respectively in *Vespa* and *Chalicodoma* the mesodermal plate is more than one cell layer thick when it sinks in.

The sinking in of the mesodermal plate begins at the anterior end and gradually proceeds towards the posterior end (fig. 14, 15). The areas adjoining the mesodermal plate laterally, the so called side plates, first fold in (fig. 20) while remaining in connection with the mesoderm. Sinking in deeper the mesodermal plate is severed from

the side plates which grow towards the ventral median and join each other there (fig. 21, 22). Thus completed, the mesodermal plate lies against the yolk and is covered by the side plates.

In the meantime the mesodermal plate broadens, the edges penetrating farther and farther between the yolk and the ectoderm (fig. 4a–d). This proceeds very quickly; the mesodermal plate, at first consisting of 5—6 layers, is only 2 layers thick when it covers $^2/_3$ of the yolk. The quick rate of this broadening indicates that it is chiefly effected by shifting movements of cells that already existed. During this procedure the mesodermal cells have an elliptical shape, their long axis lying parallel to the surface of the yolk. The long axis measures 12 μ the short one 6 μ, the nuclei have a diameter of 4 μ and they are darkly stained, which indicates that cell division also occurs. This elliptical shape of the cells in the broadening mesoderm was also observed by STRINDBERG (1914) in *Vespa*.

As soon as the mesoderm covers about $^3/_4$ of the yolk surface, its dorsad extension stops and cell multiplication starts in a radial direction, consequently the mesoderm becomes thicker again. The cells resume their rounded shape, measuring 6 μ in diameter. The nuclei are faintly coloured. Now the mesoderm is 2—6 cell layers thick.

Before the mesoderm thickens and while its cells are still elliptical, the dorsal edges of that part of the mesoderm which lies behind the rudiments of the mouth parts, fold back (fig. 4d, 29). In this way 2 longitudinal tubes are formed. At regular intervals they are constricted, only the wider part (segments) being hollow. Soon however as the mesoderm grows thicker, the whole tube widens and its intersegmental parts also become hollow. The cells of those tubes are cylindrical, 8—10 μ high and 4 μ broad (fig. 48). Their nuclei are similar to those of the other mesoderm cells. In the eggs of *Chalicodoma muraria* similar tubes are formed in the same way; CARRIÈRE & BÜRGER (1898) call them "Mesodermröhren". NELSON (1915) found them in *Apis mellifica*, STRINDBERG (1914) in *Vespa vulgaris*. In many insects continuous tubes are not formed; in this case in each segment appears a separated mesodermal sac ("Mesodermsäckchen"). Such isolated pockets we find in *Ammophila* as in other Hymenoptera only in the antennal segments, where they give rise to the walls of the cephalic arteria (fig. 40). A rudiment of a mesodermal pocket in the segment of the second maxilla disappears soon.

The tubes as well as the pockets represent the primary coelom.

3. Entoderm

Shortly after the mesoderm plate begins to sink in, cell concentrations appear at the apical and caudal pole of the egg. These cells measure 10—14 μ, their faintly coloured nuclei having a diameter of 8 μ. The concentrations are in line with the mesodermal plate, they are broader than its ends. These cells proliferate from the surface of the egg (fig. 35). About the way in which this takes

place a number a different views can be found in literature. We too tried to find out more about this process ; our material however, was not sufficient to elucidate all details of the formation of the entoderm. DICKEL (1904) believes that the entoderm is formed both by invagination and by transformation of yolk cells into entoderm cells. The first idea, however, is based on evidence from one slide only ; the second idea this author got when he saw concentrations of yolk cells close against the inside of the polar cell masses. Such aggregations of yolk cells are often found near places where the cells are actively shifting or dividing. This activity having ended, the yolk cells degenerate. CARRIÈRE & BÜRGER (1898) think that in the mason bee the entoderm is formed by blastoderm proliferating into the yolk and that finally the outermost layer of these cell concentrations would become ectoderm.

NELSON (1915) in his work on *Apis mellifica* also believes the ectoderm to hail from immigrating blastodermcells. He, however, thinks that this entoderm finally is covered by the side plates extending themselves over these areas as they do over the mesodermal plate. STRINDBERG (1914), investigating the egg of *Vespa vulgaris*, shares NELSON's view.

We never found any sign of an invagination. In many sections we found entoderm cells lying in radial rows, which suggests that the blastoderm cells at the poles of the egg had simultaneously produced a number of daughter cells towards the inside of the egg (fig. 35). In accordance with NELSON's and STRINDBERG's statements, we observed, that the entoderm becomes covered by cells evidently originating from the side plates as these cells are lying entirely free from the coherent entoderm cells.

From the above it appears that there is no essential difference between the formation of the entoderm and mesoderm. Both start with the cells in the ventral median and at the poles rounding off themselves and dividing ; both, after differentiating, are covered by cells hailing from the side plates. Between both germ layers we could detect neither a difference in cell shape nor a clear line of demarcation.

In the Orthoptera, Lepidoptera, Coleoptera and some Diptera that have been investigated from this point of view (HIRSCHLER, 1928 ; KORSCHELT, 1936) there is no difference in the formation of mesoderm and entoderm from the blastoderm ; mesoderm and entoderm appear together. In these objects, within the sunken cell plate a differentiated median band and 2 polar cell concentrations can be distinguished. These 3 parts give rise to the mid-intestine, so they are representing the entoderm, the other parts of the cell plate prove to be mesoderm as from this part muscles, heart, fat tissue etc. are derived. So the distinction made between mesoderm and entoderm is not based on a difference of origin but on the character of the organs produced. In Hymenoptera the median band is missing and only the polar cell masses are present. This, however seems to us no reason to consider mesoderm and entoderm really different formations as CARRIÈRE & BÜRGER (1898) did. Never-

theless we will continue to use both terms as they facilitate descriptions.

By the time the mesoderm has been covered by the side plates, both egg poles are filled with entoderm cells, the hindmost cell mass being bigger and extending further dorsally than the foremost (fig. 14, 15). Until the enclosure of the mesoderm has been completed, the entoderm remains unchanged and is covered by the ectoderm.

Afterwards, simultaneously with the first formation of organs from the ectoderm, the caudal entoderm grows apicad, dorsal of the yolk (fig. 16, 17, 18, 30). At the same time, but at a much slower rate two separate tongues of the apical entoderm grow caudad and laterad of the yolk (fig. 17, 18, 32). The cells of these tongues are not different from those of the cell concentrations from which they are recruted. Many nuclei show cleavage stages, they are darkly stained contrary to the faintly coloured resting nuclei. The protruding tongues of the caudal and apical entoderm reach each other at a point 22 units from the top (fig. 18). The laterally tongues of the apical entoderm fuse with the sides of the dorsal tongue of the caudal entoderm. At their tips the tongues only are a few cells layers thick. At its base, the caudal tongue has many (maximally 8) cell layers. The apical entodermal tongues have a thickness of 3 cells at most. Only at the poles the entoderm lies close to the mesoderm, showing no line of demarcation. In the rest of the egg there is always some space between the derivates of these two germ layers.

4. Ectoderm

As the mesodermal plate starts to sink in, the areas adjoining it laterally, the so called side plates first fold inward (fig. 19). Later on, however, the side plates are severed from the mesoderm plate (fig. 20). The edges of the side plates, which became folded in, now continue their folding movement and apply themselves against the inside of he blastoderm. Out of this double layer a single layer is formed again. The cells of these layers first become wedge-shaped, move between each other till a single layer is formed and then reassume their cylindrical form (fig. 20, 21).

After this the cells of the side plates divide in a radial direction (fig. 21, 22), consequently the blastoderm ventrally becomes a double cell layer again. In the same way as described above, out of this double layer a single one is formed, the result of this being that the side plates grow towards the ventral median (fig. 28). Here their edges meet and join each other, in this way covering the mesoderm (fig. 4a-d).

This way of growing of a cell layer, viz. division followed by moving of the cells, we often encountered in our study of the development of the embryo of *Ammophila campestris*.

Laterally the ectoderm detaches itself from the dorsally situated extra-embryonic blastoderm (fig. 16, 29) and broadening grows towards the dorsal median. When, in a transverse section, it is occupying about 5/6 of the circumference of the egg no more cy-

lindrical cells are formed for the time being. The ectoderm now starts producing extremely flattened cells that shove over the dorsal part of the yolk, so establishing a thin connection between the lateral edges of the ventral ectoderm (fig. 4f, 18, 50). STRINDBERG (1914), observing this thin layer in *Vespa*, called it "provisorisches Rückenverschluss", NELSON (1915) described it in *Apis* as extra-embryonic ectoderm.

The embryonic ectoderm also gradually envelopes both egg poles. We do not know in detail how this is brought about, for, at the same time, at these places the formation of the organs begins, which confuses the picture.

During the enlargement of the ectoderm, the cylindrical cells are 12—20 μ long and 4—8 μ broad. The nuclei are 4—6 μ in diameter and, depending on their activity, darkly or faintly stained.

5. Serosa

When the cells on the ventral side of the blastoderm are beginning to form the mesodermal plate, blastoderm cells at the dorsal side also become active and divide (fig. 23). The daugther cells rearrange themselves to a single layer and so the blastoderm, that covers the yolk on its dorsal surface becomes wider. Consequently laterally on both sides of the egg a fold is formed, which becomes well developed at its caudal end only (fig. 25, 26). The cells of the dorsal blastoderm assume a cubic form and in each a number of spots can be found which are poor in plasm while vacuoles are discernible (fig. 24).

Soon the blastoderm is disengaged along the fold (fig. 29), beginning at the top of the egg and proceeding backwards. Only at the very caudal end the connection between the dorsal blastoderm and the side plates is maintained and here the fold prolongs itself. At the same time at this place the side plates are extending themselves dorsad over the yolk. As the edges of the side plates remain in contact with the dorsal blastoderm, the inner part of the fold is pulled over the yolk. At last the side plates join each other along the dorsal median and only then they are severed from the dorsal blastoderm, the edges of which also fuse together. By this time the hind part of the blastoderm, like a sac, covers the dorsal side of the caudal end of the egg with a double layer. The interior sheet of this sac may be called the amnion. Its cells are similar to the ectoderm cells. The exterior envelope is the serosa; like the extra-embryonic ectoderm of the rest of the egg, the serosa consists of flattened cells. Soon serosa and amnion are severed from each other.

In the meantime all round the egg the blastoderm cells are flattening and each cell stretches itself in all tangential directions. This combined with movements of the cells causes the extra-embryonic blastoderm to extend more and more to the ventral side (fig. 4, 15, 16, 31). According to STRINDBERG (1914) in ants the serosa is formed only by the apical part of the extra-embryonic blastoderm extending ventrad as well as dorsad. A similar process may take

place in *Ammophila*, as the mid part of the serosa is poorer in cell material than the egg poles.

In this way the whole egg is gradually enveloped by the extra-embryonic blastoderm. The hindmost part of this envelope we have already mentioned above as covering the amnion. The existence of this little amnion rudiment proves that in *Ammophila* the embryonic envelope is a serosa.

The cells of the amnion soon degenerate and disappear. The serosa also degenerates after some time, but during the whole course of development we can still find at least some remnants of it, the older the egg the less of it remains.

In other Hymenoptera that have been investigated from this point of view, only one envelope is present round the egg. This is, however, not in every species formed in the same way. CARRIÈRE & BÜRGER (1898) detected a fold between extra-embryonic blastoderm and embryonic blastoderm in the polar regions of the egg of the mason bee. KORSCHELT (1936) thinks this fold identical with the amnionfold, as appearing in other insects. As a matter of fact, we found in *Ammophila* that caudally a definite amnion rudiment developes out of that part of the blastoderm fold which lies closest to the embryo. In *Chalicodoma* the amnion folds do not develop further, the envelope which is finally formed is the serosa.

Although STRINDBERG (1914) in *Vespa* did not find an amnion fold, the development of the embryonic envelope, at least in the middle part of the egg occurs in the same manner as in *Ammophila*. In some ant species STRINDBERG (1913) did find an amnion fold, but here the development of the serosa passes off in a quite different way.

While in *Ammophila* the entire extra-embryonic blastoderm is involved in the formation of the embryonic envelopes, NELSON (1915) and SCHNETTER (1934) observed that in *Apis* only the lateral parts of the extra-embryonic blastoderm grow out to form the single envelope present. A strip of cells situated in the dorsal median (dorsal strip, Dorsalstreifen) does not take part in this development, and is finally absorbed by the yolk.

As SCHNETTER (1934) defines the amnion as an envelope covering the germ band, he calls the envelope formed in *Apis* amnion and the cells of the dorsal strip serosa. On the authority of BLÜTSCHLI, NELSON uses the name amnion in the same sense. However from SCHNETTER's photographs and NELSON's drawings of sections showing the envelope at the beginning of its extension the differences in the formation of the embryonic envelopes in *Apis, Vespa, Chalicodoma* and *Ammophila* seem to us much smaller than literature would suggest. The existence and subsequent resorption of the dorsal strip is the only essential difference we can see and such a strip does not occur in *Ammophila*.

F. Formation of the organs

1. General survey

Immediately after the closing of the ectoderm at the ventral side

of the egg, the formation of the ectodermal organs begins. In quick succession mouth parts and extremities, tentorium, maxillar glands and tracheal system, stomodeum and proctodeum with the Malpighian tubes appear. Subsequently at a slower rate, the rudiments of the nerve system and finally the integument arise (fig. 16, 17, 18, 59a).

The rudiments of the ectodermal organs are all clearly defined by the time that the caudal and apical tongues of the entoderm meet each other and the formation of the mid-intestine begins (fig. 59a—c).

Then, all ectodermal organs being clearly discernible the mesodermal organs arise. They however, are accomplished only after the wall of the intestine has been closed and when the integument begins to differentiate from the ectoderm (fig. 59a—d).

2. Ectodermal organs.

a. Antennae, mouth parts and extremities

As soon as the ectoderm is closed ventrally, in the apical part of the egg the rudiments of antennae and maxillae appear. In first appearance each of these rudiments consists of a slight elevation of the ectoderm, surrounded by a ring-shaped groove. The first and the sixth pair of rudiments lie at the lateral side of the embryo, respectively near the top and at about 20 units from it. The other rudiments are situated more dorsally between these two.

The foremost pair of rudiments which will become the antennae shows two slender elevations with their tips flexed dorsad (fig. 45). The second pair of rudiments represents the appendices of the intercalary segment. They soon degenerate.

The three other appendices, the mandibulae, the first and the second pair of maxillae first show themselves as oval elevations, with their longest dimension almost at right angles to the axis of the egg. With further development, the groove round each of the rudiments is smoothed out on their dorsal side, while ventrally it becomes deeper ; consequently the elevations are gradually heeling over towards the ventral side (fig. 46).

At the same time the germ band becomes longer ; the yolk maintaining its original length, the ectoderm extends over the apical pole of the egg towards the dorsal side. The distances between the subsequent appendices also increase and simultaneously the strip of ectoderm between the left and the right row of appendices narrows (fig. 15, 16, 17). Probably these changes are chiefly brought about by movements of cells in the ventral area.

When the ventral ectoderm strip narrows, the ectoderm which lies dorsally from the rudiments is pulled towards the ventral side. So dorsally, part of the yolk is uncovered again. Soon, however, by cell division in its lateral parts the ectoderm is again extended dorsally over the yolk.

The rudiments of the second maxillae, originally situated at the 22nd unit, now lie at a distance of 33 units from the top, which is the maximal relative distance ever reached.

Now just ventrally of the top, an invagination appears, which proves to be the rudiment of the stomodeum. This newly formed hole, is enlarged and deepens because a part of the caudally adjacent ventral ectoderm is taken up into it. By this movement of the ventral ectoderm in an apical direction the rudiments of the appendices are carried forward ; at the same time the distances between consecutive pairs of rudiments diminish (fig. 17, 18, 53, 54, 56, 57).

In the meantime, the above mentioned inclination of the rudiments towards the ventral median has continued, resulting finally in an overbridging of the deep ventral part of the groove round each rudiment. Soon this groove is closed for the greater part, at one point only an opening persists. In this way in each rudiment an ectodermal tube is formed.

The tubes that belong to the second maxillae give rise to the maxillary glands (see page 70). From the grooves near the first maxillae, the mandibulae and the antennae the tentorium is derived (see page 71).

The bases of the mouth parts, while increasing in size, move still further towards the ventral median (fig. 54, 55). The second maxillae are closely applied against the embryo over a distance of about 120 μ, only their foremost part protrudes freely from the surface of the egg. They approach each other ventrally and fuse together. The 2 maxillary tubes merge into a common outlet (fig. 54, 55).

Finally the mouth parts are situated as follows :

Apically of the mouth parts lie the antennae as very small elevations of the ectoderm. At both sides of the mouth we find the mandibulae and, immediately behind them, the first pair of maxillae. The coalesced second maxillae constitute a cross ridge behind the mouth opening. In the ,,axil' of the second maxillae the common opening of the maxillary glands is situated.

When the rudiments of the mouth parts are clearly visible another 3 pairs of ectodermal elevations arise caudally of the second maxillae ; these are the thoracal legs (fig 18, 53). Just as in the mouth parts we find invaginations at their bases ; those of the 2nd and 3rd pair of thoracal legs become tracheae, those of the 1st pair degenerate.

The formation of the tracheae out of these invaginations confirms the opinion of CARRIÈRE & BÜRGER (1898), that tracheal system, maxillary glands and tentorium are homodynamous. NELSON (1915) opposes this statement, as he considers the groove of the second maxillae to be a stigma and assumes that there is an essential difference between tentorium and maxillary glands on one side and stigma rudiments on the other. According to him, stigmata would always lie dorsally of the elevations while the other organs would appear at their ventral side. In our opinion this argument does not hold, as we observed that originally a ring shaped groove is present which gives rise to all the organs under discussion.

The thoracal legs hardly develop any more. They have already

degenerated to small ectodermal thickenings („Imaginalscheiben") when the mouth parts reach their ultimate positions round the mouth (fig. 54, 55, 56, 57).

b. Stomodeum.

About the time that the mouth parts begin to develop, a small invagination appears at the ventral side of the apical pole of the egg (fig. 45). This will become the stomodeum. The ectoderm, which in this stage is just covering the pole of the egg, still consists of rounded cells with dark nuclei; many cell divisions are still in progress. The rudiments of the stomodeum consist of similar cells.

While the ectoderm, shifting over the pole, is extending along the dorsal side of the yolk, the cells at the apical pole of the egg and those of the stomodeum assume a cylindrical shape. Then the cells of the stomodeum stop dividing, nevertheless, the stomodeum continues to sink in (fig. 16, 17, 18). In *Apis* this also occurs and to explain this, SCHNETTER (1934) assumes that ectoderm, lying caudally of the stomodeum, is gradually invaginated. The result of this is a ventrad movement of the stomodeum and a shortening of the germ band. Although we did not, like SCHNETTER, make our observations also on living eggs, our sections yield enough arguments to maintain that in *Ammophila* too the ectoderm layer in the foremost region of the egg furnishes the material for the stomodeum. During the growth of the stomodeum no more cell divisions occur in it, yet the distance between the mouth parts and the stomodeum keeps shortening (fig. 16, 17, 18, 53, 54, 55, 56, 57). As simultaneously the caudal edge of the stomodeum moves towards the ventral side (compare fig. 16 and 18), it actually looks as if the ectoderm material situated between the mouth parts is partially used for deepening the stomodeum. As at the same time ectodermal cells on the top of the egg are dividing, the apical edge of the stomodeum is "pushed" over the top in a ventro-caudad direction. In this way the stomodeum is not distended but its form remains unchanged during the movement (compare fig. 35 and 55).

The mouth parts having reached their ultimate positions, the cells of the stomodeum begin to divide. The thickness of its wall increases to several layers (fig. 47). These cells then are rearranged in a single layer, causing a lengthening of the stomodeum (fig. 53—57). At their free side the cells secrete a chitinous layer.

During its entire development, the caudal end of the stomodeum is applied against the yolk or, in older stages against the mid intestine. Shortly before the larva hatches, at this end, a ring shaped fold appears in the wall of the stomodeum. STRINDBERG (1914), who observed it in the embryos of *Vespa* and *Trachusa*, calls it "Proventrikelanlage", SCHNETTER (1934) describing it in *Apis*, speaks of "ringförmige Wucherung" or "pilzhutförmiges Organ". From our sections it appears that this thickening penetrates into and pierces the wall of the mid-intestine. In this way the communication between stomodeum and mid-intestine is established (fig. 60).

Meanwhile round the stomodeum a thin muscular layer has been

formed (fig. 44) and in the walls of the stomodeum longitudinal folds have appeared, imparting a star shape to it in cross section.

c. Maxillary glands.

On page 68 we said that originally the rudiments of the cephalic appendices are surrounded by a groove. The groove of the second maxillae develops into the maxillary gland (fig. 31).

While the part of the groove, which lies apically of the maxillar rudiment becomes shallower, the rest of it continues to invaginate. In this way an ectodermal sac is formed which penetrates into the mesoderm, just caudally of the maxillar rudiment (fig. 17, 18). Its cells are rounded ; they have a diameter of about 10 μ. Their nuclei are darkly stained, about 6 μ in diameter ; cell division is in progress.

The sac grows steadily backwards and extends between mesoderm and yolk. Its opening shifts somewhat to the ventral median while the sac flattens. Now the cells become cylindrical ; their nuclei are faintly coloured as cell division has stopped. By this time the sac is 100 μ long, 120 μ broad and 4—8 μ wide (fig. 17).

Then, quickly growing out to a length of about 400 μ the sac assumes a tubular shape. The tube finally measures 20 μ in diameter. The cylindrical cells of its walls are 12 μ high and 4 μ broad (fig. 49, 51, 52). By branching, it forms 2 or probably more diverticula which run winding between the fat cells and are very difficult to trace backwards.

When during the growing of the stomodeum the second maxillae are moving in an apical direction, both tubes are carried along. Moreover, at the same time both second maxillae and the openings of the tubes are approaching each other ventrally (fig. 54, 55). Finally the proximal ends of both tubes fuse over some distance in the ventral median. As the second maxillae then are situated alongside this superficial part of the joined tubes a short groove is formed. Subsequently the second maxillae bend over towards each other, fuse and cover the gutter, forming the labium. In this way the tubes get their common outlet in the "axil" of the labium.

The common part of the tubes lies ventrally of the nerve system ; behind the first furcation each tube curves laterad and dorsad till they are situated dorsally of the nerve system on either side of the ventral part of the intestine.

In literature these tubes sometimes are called silk glands, sometimes salivary glands. Surely in full grown *Ammophila* larvae the function of the glands justifies the first name. We will not, however, deny the possibility that as long as the larva takes food the tubes could act as salivary glands. So we prefer to refer to the tubes with the neutral name "maxillary glands".

d. Tentorium

On page 69 we described how, near each of the cephalic appendices, during its development an invagination in the ectoderm is enclosed. The grooves belonging to both antennae in this way become tubes which flatten out. They grow backwards in converging directions, so they meet in due course and fuse (fig. 53, 54).

The grooves belonging to the first maxillae develop in the

same way, they, however, grow out in converging forward directions. Finally they meet and fuse which each other and with the "antennal" tubes. So an X-shaped system of flattened tubes is established (fig. 53, 54) having outward apertures at a short distance ventrally of the antennae and somewhat dorsally of the initial basis of the first maxillae. The centre of the X constitutes a broad plate, which is situaed between stomodeum and suboesophagial ganglion (fig. 36). The entodermal cells, which originally were cylindrical, now have flattened and they secrete chitin.

The tubes appearing at the bases of the mandibulae, grow backwards over a short distance but do not flatten out. Chitin is also secreted in them ; they constitute the place of attachment for the strongly developed mandibular muscles (fig. 36). NELSON (1915) calls them "mandibular apodemes".

Together these tubes represent the inner chitinous cephalic skeleton, the tentorium (fig. 57, 60).

e. Tracheal system

When the formation of the mouth parts has begun 11 shallow invaginations appear in the ectoderm behind the 2nd maxillae at regular distances on both sides of the egg (fig. 17). As has been discussed on page 69, these invaginations are serially homologous with the invaginations at the bases of the cephalic appendices. The first pair soon vanishes.

Especially in older eggs it is clearly visible that the 10 remaining invaginations lie in the last 2 thoracal segments and in eight abdominal ones. At first these invaginations are oval-shaped in cross section, their longest axis lying about perpendicular to the longitudinal axis of the egg. In the centre it is shallower than at the circumference (fig. 48). Subsequently the ectoderm surrounding the invagination grows over it from all sides and finally covers it nearly entirely (fig. 49). Only in the centre outward communication persists.

The oval cavities soon become lozenge-shaped, their longest diameter lying parallel with the longitudinal axis of the egg (fig. 54). The 4 points of the lozenge grow out, giving rise to tubes that extend in the 4 directions of the diagonals of the lozenges. The apical and caudal tubes grow out first. In the more caudal abdominal segments the hindmost tube is longer than the foremost ; the reverse is the case in the more apical abdominal segments. In the intervening segments both tubes are equally long. The dorsal and ventral tubes grow out somewhat later, the latter are longer than the former.

The 10 discrete longitudinal tubes developed in this way on both sides of the embryo approach each other and fuse to one big tube, the tracheal trunk In each segment the dorsal and ventral tubes constitute branches of these trunks.

NELSON (1915) that states in *Apis mellifica* the branches of the left and of the right trunk communicate ventrally and dorsally. We could not ascertain whether this also occurs in *Ammophila*. In young larvae the branches certainly do not communicate, in older larvae

they are very difficult to distinguish between the other organs.

During further development the tracheal system sinks in, while its communication with the outside becomes longer, most probably by invagination of ectodermal cells (fig. 50). In this way on both sides of the embryo 10 short ectodermal tubes arise, connecting the tracheal trunk with the stigmata (fig. 60). Originally the cells of the invagination are cylindrical, later on they become round or elliptical, forming a kind of plaster epithelium (fig. 40). After the tracheal trunk has been formed, however, the cells become cubical. The nuclei do not change much, their size only decreases from 5 μ to 3 μ.

When the integument has secreted a cuticula, a chitinous layer also becomes visible in the tracheal trunk.

f. Proctodeum and Malpighian tubes

When the rudiments of the tracheal system have appeared a new invagination in the ectoderm becomes visible dorsally at the caudal pole of the egg. As this invagination develops, it branches into 2 tubes lying on either side of the yolk and making an impression upon it (fig. 17). Later on near the root of those tubes a second pair of similar tubes appear. All grow in an apical direction. At first the cells of these 4 tubes are cylindrical and similar to the ectoderm cells of the surface of the egg. Later on they become more cubiform and bear a great resemblance to the cells of trachea and maxillary glands.

The common outlet of those 4 Malpighian tubes is enlarged by ectodermal invagination and constitutes a sac (fig. 58a, b). At the same time its outward aperture moves form the dorsal to the ventral side of the egg. Probably this can be attributed to the same process that caused the shifting of the stomodeum described above, to wit to invagination of the ectoderm. Thus the proctodeum is formed, the final shape of which can be seen in fig. 58 and fig. 60. It lies on the ventral side of the embryo, close to the yolk and has two short coeca. The Malpighian tubes open into the apical part of the proctodeum. The cells of the proctodeum remain cylindrical. The communication between the proctodeum and the mesenteron only appears at a much more advanced stage as a narrow fissure. In older larvae already taking food this connection is still very narrow.

g. Nervous system

After the rudiments of the ectodermal organs described above have appeared, a small invagination in the ectoderm becomes visible in the ventral median (fig. 32). The first indication of it can be found near the second maxillae. It is prolonged in a caudal direction till a groove is formed, running from about the stomodeum to the proctodeum. This is the neural groove, laterally bordered by the two neural walls, protruding somewhat above the eggs surface. Perhaps there is some difference in the dimensions of the groove and the walls intra-and intersegmental. Our slides, being chiefly cross sections, did not give conclusive evidence on this point.

The ectoderm thickens in this neurogenic area. The neural walls increase in size and as there is little place for new cells in the narrow nervous groove, these protrude sideways into the embryo, constituting a fan shaped figure in each segment (fig. 52). From these cell concentrations the ventral cord is derived.

In addition to these thickenings in the ventral median, concentrations of ectoderm also appear in the apical part on the latero-dorsal side of the embryo (fig. 47). Examining successive sections here, we meet on either side of the embryo 3 thickenings of the ectoderm. These are the rudiments of the 3 pairs of cerebral ganglia.

In the ventral groove all cells are long drawn pear-shaped, 12 μ long with big globular nuclei of about 10 μ diameter. These neuroblasts we also found in the neural walls and in the ectodermal thickenings in the cephalic area. In the cephalic area the peripherical cells remain smaller and they soon constitute a new hypodermis covering the nervous system (fig. 38, 52). The neural groove is covered by hypodermis cells originating from the neural walls.

The neuroblasts give rise to a great number of smaller nerve cells with a diameter of about 6 μ and nuclei of 4 μ (fig. 52). Gradually these cells are severed from the hypodermis, the contact being maintained longest in the intrasegmental parts of the neural groove. In each segment a pair of ganglion rudiments is formed by cells from the neural walls. They are joined by nerve cells derived from the neuroblasts of the ventral groove, which in each segment move to the foremost part of it. Thus these cells from the neural groove constitute the commissures between the proper ganglia derived form the neural walls. Other cells of the neural walls build a double communication between consecutive ganglia, the connectives.

The nerve cells in the head of the embryo remained arranged in 3 groups. Some cells move a little and establish the mutual connections between the 3 cerebral ganglia and, passing on either side of the stomodeum, also the communication with the suboesophagial ganglia (fig. 43).

The nerve cells grow ramifications which arrange themselves in thin bundles of filaments which finally are situated in the centre of the ganglia and in the connectives.

The development of the nervous system begins at the head and preceeds in a caudal direction, so in one egg we can observe several consecutive stages.

In *Ammophila* we could distinguish 16 pairs of ganglia. The first 3 pairs have fused to one long drawn sub-oesophagial ganglion, the last 3 are nearly coalescent (fig. 60). In *Apis,* NELSON (1915) distinguished 17 pairs of ganglia and CARRIÈRE & BÜRGER (1898) counted the same number in *Chalicodoma.*

From the dorsal wall of the stomodeum a group of cells having the appearance of nerve cells is disengaged. In our opinion they represent the frontal ganglion, controlling the intestinal system. The rest of the "stomogastric system" as NELSON (1915) described it in *Apis,* we could not detect in *Ammophila.* Though we found small concentrations of ectoderm cells near the edge of all the

rudiments of the tentorium, they were not clearly defined groups, which we could identificate with the corpora allata. Nor have we been able to find in *Ammophila* the rudiments of the pharyngeal ganglia against the wall of the stomodeum.

The development of the nervous system in *Apis* has been minutely treated by NELSON (1915). Our scanty material does not allow us to deal with this subject as thoroughly as he did, so we had to confine ourselves to the rough description given above, which does not disclose important differences between the development of the nervous system in *Apis* and *Ammophila*.

h. Integument

As has already been described on page 65, when the rudiments of all mesodermal organs have arisen, the lateral edges of the ectoderm grow out in a thin layer over the dorsal side (fig 47). This epithelium remains till all organs have appeared, till the wall of the mesenteron has been formed and the intestine is closed at the ventral side. Then the cylindrical ectoderm cells covering the sides of the embryo, assume a cubical shape and move towards the dorsal side. This cubical epithelium takes the place of the thin layer mentioned above and soon its cells become cylindrical again.

In the foremost cephalic part of the embryo, by division of the ectoderm cells the integument acquires a thickness of several cell layers, while its cells are more or less oval (fig. 43). The rest of the embryo is entirely covered by a single layer of cylindrical cells (fig. 36, 39). On the whole surface of the embryo a thin chitinous cuticula is secreted.

3. Entodermal organs

a. Mesenteron

As has already been mentioned on page 65 the apical and the caudal entoderm approach each other, the former as 2 lateral tongues, the latter as one dorsal tongue (fig. 18). When these tongues have reached each other the apical entoderm begins to extend over the dorsal surface of the yolk. Its cells, first arranged in two or three layers, rearrange themselves in a single layer against the yolk. The moving cells are spindle shaped, their longest axis lying parallel to the surface of the yolk. Having covered the yolk dorsally the entoderm cells become cubiform. Only at the foremost part of the yolk, the covering is postponed till the formation of the mesenteron has been accomplished. Apically the caudal entoderm tongue is only a few cell layers thick, more backwards it consists of many cell layers. The cells in the median of the caudal entoderm tongue and in its apical part shift along each other till they constitute a single layer. Consequently the entoderm becomes thinner and it covers a greater part of the yolk. At both edges this sheet remains many cell layers thick.

By this time about the dorsal half of the surface of the yolk has been covered, exept apically. Then from the edges and the polar parts of the apical and of the caudal entoderm cells (surely also newly formed cells, as cell division is in progress) are moving

ventrad along the yolk (fig. 50). Finally the entire yolk is enclosed by a single layer of entodermal cells fig. 59a—d), which constitutes the intestinal wall. While arranging themselves into one layer the cells are cubiform, later on they become cylindrical.

During the development of the intestine the yolk shortens. Consequently the distance from the top of the egg to the apical end of the yolk increases. As the apical entoderm remains closely applied to the yolk it is also removed from the top of the egg.

From the moment that cell movement and cell division set in many yolk cells and ooplasm have been concentrating at places of greatest activity inside the yolk membrane (fig. 40, 50, 59). When the entoderm extends dorsad over the yolk, concentrations of yolk cells accompany it on the opposite surface of the yolk membrane. Later on, when the movement of the entoderm cells is ventrad, it seems as if the yolk cells are carried along with them to the ventral median. Near those cells of the migrating entoderm that have proceeded farthest we observe in the yolk filaments of plasm enclosing many yolk nuclei and rediating fan-like from spots on the yolk membrane corresponding to these leading entoderm cells. When finally the wall of the mesenteron is closed, ventrally over the whole length of the egg and dorsally in its foremost part, nearly all the plasm and yolk cells are accumulated in these regions. When the intestine has been enveloped, the yolk cells disappear.

The cells of the mid-intestine are cylindrical, 20 μ high and 6 μ broad. The nuclei lie close to the yolk, they are 8 μ in diameter; their chromatine mass is darkly stained, strongly contrasting with the rest of the contents of the nuclei (fig. 41). The other half of the cell is almost entirely occupied by a great vacuole. When cell division has stopped the nuclei are less visible in the cells. The vacuoles remain until resorption begins.

4. Mesodermal Organs

Soon after the first appearance of the mesoderm we can already distinguish some of its parts.

In all thoracal and abdominal segments and also in the antennal segment lies a coelomic sac (fig. 4e, f) on either side of the yolk. Ventrally of the yolk, over the whole length of the embryo, lies a 2—6 layers thick mass of mesodermal cells.

The wall of the coelomic sacs facing the yolk is the splanchnopleura, from which the muscular covering of the intestine is derived. The wall of the coelomic sacs lying close to the integument, the somatopleura, produces the muscles attached to the integument, the muscles of the mouth parts and those belonging to the stomodeum.

A number of cells situated at the dorsal side of the coelomic sacs in the transitional area between splanchnopleura and somatopleura develop to cardioblasts, the rudiments of the circulatory system (fig. 59). A few cells lying in the 3rd, 4th and 5th abdominal segment in or against the splanchnopleura give rise to the reproductive organs.

The fat cells originate from cells of the ventral mesoderm, facing

the yolk. Now the question has been put forward whether the fat cells are derived from the splanchnopleura or from the somatopleura. KORSCHELT (1936) identifying with splanchnopleura only the yolk facing wall of the coelomic sac, logically must consider the fat cells to be derivates of the somatopleura. CARRIÈRE & BÜRGER (1898) and NELSON (1915), however, call the inner surface of the ventral mesoderm mass splanchnopleura and the outer layer somatopleura although the lumen of the sac is missing in this part, as can be seen in fig. 37. However, a lumen does exist between the dorsal and the ventral groups of mesoderm cells in *Ammophila* and it actually seems as if there is a continuity between the true splanchnopleura of the coelomic sac and the dorsal layer of the ventral mesoderm. On this evidence we are inclined to believe that the fat cells hail from the splanchnopleura, our observations putting CARRIÈRE & BÜRGER and NELSON in the right.

a. Fat tissue

When through cell division in a radial direction the thickness of the mesoderm of the abdominal segment has increased to about 6 cell layers, a number of mesodermal cells lying closely applied against the yolk, concentrate in several groups (fig. 37, 38, 48). In transverse sections these groups appear as rings of cells, in longitudinal sections they look like short winding strains. As within these cell groups, usually a "lumen" is present, they give the impression of a system of very much ramified and anastomosing tubes, occupying from the 1st to the 8th abdominal segment but without any communication neither with any organ nor with the outside. Its cells are cubiform or polyedric, 4 μ in diameter. The nuclei are stained as dark as the plasm and they are not very sharply defined.

This tissue is present during the entire embryonic development (fig. 39, 50). Often it is difficult to distinguish between these mesodermal tubes and the ends of maxillar glands and Malpighian tubes. In the literature on Hymenoptera and other groups of insects, we did not come across any description of similar formations of fat cells.

When the intestine begins its resorbing activity the fat cells become much inflated, assuming the same appearance as the fat cells of other insects. The nuclei are situated against the cell walls, the cell increases to a diameter of 40—50 μ. After fixation a reticular structure remains, the alcohol of the fixative dissolving part of the cell contents.

b. Muscles

aa. Muscles round the mid-intestine

The small cells of the splanchnopleura multiply and give rise to thick strands of cells lying laterally alongside the yolk and making an impression on it (fig 38, 50). Originally the cells of the splanchnopleura were cylindrical, now they become polyedric, 4 μ in diameter. The nuclei occupy nearly the whole of the cell. A part of these cells move ventrad, alongside the yolk and when the ectoderm has entirely enveloped the yolk, a number of these cells also move dorsad (fig. 59a—d). In this way the whole intestine is covered by

a layer of spindle shaped cells, a muscle layer. Dorsally this layer is in connection with the parts of the coelomic walls surrounding the heart (fig. 41).

bb. Muscles of the stomodeum

Among the cephalic segments only the antennal segment has a coelomic cavity. Its walls, however, do not produce muscles. In the cephalic area the muscles are exclusively derived from the mesoderm that is situated ventrally in the embryo behind the invagination of the stomodeum (fig. 47m). A small part of these cells give rise to the muscles of the stomodeum, they apply themselves against its wall as it sinks in. The mesoderm cells originally round, now become spindle shaped and constitute a thin muscle layer (fig. 40). Its development begins at the forward end and proceeds backward. When the communication between the stomodeum and the mid-intestine has been established, the apical part of the latter is also surrounded by muscles established from the ventral mesoderm of the cephalic area. This muscle layer, which is somewhat thicker than that enveloping the rest of the mid-intestine, gradually merges into the latter.

cc. Muscles of the mouth parts

When the mouth parts have been differentiated most of the ventral mesoderm of the cephalic area concentrates on either side of the embryo inside the bases of the mouth parts. Together with the mouth parts these mesoderm cells move apicad. When all the organs in the head have been accomplished, these cells constitute a pair of big muscles bundles running from a latero-dorsal point of attachment via the tentorium to the strongly developed mandibulae (fig. 36, 43). Some less prominent muscles connecting the maxillae with the tentorium plate hail from the same origin.

dd. Muscles attached to the integument

Originally the mesoderm shows no distinct segmentation. When, however, the mid-intestine has been closed, the mesoderm cells concentrate in each segment. Between those concentrations only a small number of mesoderm cells remain. All cells arrange themselves in 4 groups of bands running from the dorsal as well as from the ventral side of the embryo to both flanks. Moreover, a few cells apply themselves against the inner side of the skin. The form of the cells changes from polyedrical to spindle shaped.

In the 4 groups of bands mentioned above the cells are not all directed in the same way. An outer layer of cells, which lie with their long axis perpendicular to the axis of the embryo, can be distinguished from an inner layer, the cells of which lie parallel to the longitudinal axis of the embryo.

From the inner layer are derived 4 groups of longitudinal muscles, the outer layer in the dorsal part constitutes thin muscle bands running from the heart to a region of the skin, somewhat dorsally of the stigmata. In the ventral part they connect an area of the skin just ventrally of the stigmata with the membrane surrounding the ventral cord (fig. 39). NELSON (1915) calls them dorsal and ventral diaphragma respectively.

In the foremost part of the head, on either side of the cephalic arteria, a small bundle of muscles connects the skin with the dorsal side of the stomodeum

c. Blood and circulatory system

aa. Blood cells

When the fat tissue has been differentiated from the mesoderm, a number of small round mesoderm cells, for the greater part originating from the most ventral part of the mesoderm greatly increase in size. They reach diameters of 8—10 μ and they grow small ramifications of plasm (fig. 32, 51, 52). We found them chiefly ventrally between the neurogenic area and the yolk. Later on they wander all through the embryo and when the mesoderm extends dorsad along the yolk these big cells move to the dorsal median where they are enclosed by the cardioblasts. In this way they get into the circulatory system so we may call them blood cells.

bb. Cephalic artery

Originally the walls of the coelomic sacs of the antennal segment consist of polyedric cells. When the mouth parts move apicad and the stomodeum lengthens, the coelom of the antennal segment also enlarges. While still growing it penetrates through the other cephalic segments and finally it reaches the first thoracal segment. Its cells flatten and become long drawn in a tangential direction (fig. 40).

When the embryo has been closed dorsally the coelomic cavities-originally situated laterally-move dorsad. The splanchnopleura of both sides become contiguous in one line closely above the stomodeum and there they constitute a groove which is open at the dorsal side. Subsequently the coelomic cavity is reduced as the somatopleura applies itself against the splanchnopleura. As finally these double walls approach and meet each other the groove is closed becoming the cephalic artery.

cc. Dorsal artery

Out of the dorsal wall of the coelomic cavities a row of cells arise, which increase in size beyond other coelomic cells. These cells are 6 μ in diameter and their plasm is faintly coloured; they are the cardioblasts. In a cross section 2—4 of these cells can be found close together on the dorsal surface of the coelomic wall (fig. 50). When the intestine has been closed these cells insert themselves between the intestine and the epithelium of the dorsal side which is simultaneously substituted by cylindrical epithelium. Arrived in the dorsal median the cardioblasts of both sides constitute a tube and then flatten out (fig. 42). The inner walls of the coelomic cavities, consisting only of thin, very much flattened cells have also moved towards the dorsal median. Here a part of the splanchnopleura gives rise to the dorsal part of the intestinal muscles. The rest of the dorsal parts of the coelomic cavities apply themselves against the dorsal artery and establish connections between the dorsal artery and the intestinal muscle layer and between this artery and the muscles of the skin (fig. 59a).

d. Reproductive organs

When the cells from which the intestinal muscles originate, are detached from the splanchnopleura, our attention is drawn by a number of cells remaining in the coelomic wall (fig. 50) and being somewhat larger than any other mesoderm cell (diameter 6 μ). In each transverse section through the 3rd, 4th an 5th abdominal segment about 2 or 3 of these cells can be found. They are the rudiments of the reproductive organs ; we did not notice any trace of them in earlier stages.

By the time the mid-intestine has been accomplished these cells are only present in the 5th abdominal segment. Now they have an elliptical shape, they are 10 μ high and 4 μ broad ; their number has increased. When the mesoderm extends dorsad they join in this movement (fig. 59). They multiply intensively and so they give rise to 2 nearly cylindrical cell masses (fig. 42, 60) being 150 μ long, 40 μ across and finally consisting of cells measuring 8 μ in diameter. These cell masses are situated longitudinally in the 5th abdominal segment, applied on either side against the dorsal artery and against the intestine. They are enclosed by an epithelium of flattened mesoderm cells.

In *Chalicodoma* CARRIÈRE & BÜRGER (1898) found the rudiments of reproductive organs in the 3rd, 4th and 5th abdominal segment. In *Ammophila* in the 3rd and 4th segment only a few big cells are formed, which subsequently disappear or perhaps wander caudad.

In *Chalicodoma* these big cells are already visible shortly after the formation of the coelomic sacs. Moreover, in *Chalicodoma* a rudiment of the efferent ducts was clearly visible ; we could not find a trace of it in *Ammophila*.

III. *Appendix*

Characterisation of the stages of development reached after cultivation during different numbers of hours at 30° C.

Stage A_1 *up to stage* A_{11}. Present are respectively 1, 2, 4 etc. up to 1024 nuclei. After 20 minutes no nuclei were distinctly visible, after 3 hours more than 1024 nuclei have been counted.

Stage B. 3 hours. 1500 nuclei could be counted. Nuclei in the foremost part of the egg are penetrating into the cortical layer.

Stage D. 6 hours. Blastoderm nearly differentiated, lateral partitions between the cells visible, basal membrane not yet formed. Yolk cells begin to fuse into syncytia.

Stage E. 8 hours. Edges of the mesodermal plate in the apical part of the egg partly covered by the side plates ; in caudal part mesodermal plate just sinking in. The rudiments of the apical and of the caudal entoderm become visible as rounded cells. At both poles of the egg a weak fold appears.

Stage F. 10 hours. In apical part side plates reach each other in the ventral median, in caudal part they sever themselves from the mesodermal plate and begin to cover it. Mesodermal plate 3—6 cell layers thick. Both ends of the egg show an entodermal cap, only the apical entoderm is covered yet by ectoderm. Serosa begins to cover the apical pole ; in the greater part of the egg the amnion fold is only faintly discernible, but caudally it is well developed.

Stage G. 12 hours. Ectoderm covers mesoderm and entoderm entirely. In transverse sections it covers about $^5/_6$ of the circumference of the egg. Mesoderm is shifting dorsad alongside the yolk, it is 2—3 layers thick. Its most dorsal edges fold down to ferm the coelomic sacs. The apical entoderm tongues have not

grown much, the caudal one now has a length of about 40 units. Serosa covers the apical pole. Caudally the amnion is fully developed. In the area between these parts the serosa is severed from the ectoderm. From about the top of the egg to the 22nd unit the rudiments of the cephalic appendices have appeared. The rudiment of the maxillary gland appears as a wide sac of 120 x 100 μ. The stomodeum is only a shallow invagination.

Stage H. 14 hours. Mesoderm 2—6 celllayers thick. Coelomic sacs have been formed from the thoracal segments to the 8th abdominal segment. The apical entoderm tongues reach the 25th unit, the caudal one the 50th unit. Serosa envelops the egg entirely ; amnion degenerates. The dorsal edges of the ectoderm are moving towards the dorsal median. The rudiments of the cephalic appendices have moved towards the ventral median, they are now situated in two rows behind the stomodeum, each row about 80 μ from the ventral median, towards which they are hanging over. The 5th pair lies at a distance of 33 units from the top. Behind this pair we find the 3 pairs of rudiments of the thoracal legs. Part of the cells of the stomodeum have become cylindrical, their depth is about 40 μ. The maxillary glands are about 250 μ deep and still sack-shaped. The rudiments of the stigmata are shallow oval invaginations. The rudiment of the proctodeum is 20 μ broad and 30 μ deep. In the lateral walls of the head the ectoderm thickens.

Stage I. 16 hours. Caudal and apical entoderm have reached each other. Except in the most caudal part, the ectoderm dorsally of the yolk forms a thin epithelium. In the serosa cells cannot be distinguished any more. The stomodeum is 100 μ deep and the cells of its walls are round now. The antennae lie just behind the stomodeum, the second pair of the cephalic appendices have disappeared, the others have approached the stomodeum and are situated in a V behind the antennae. The base of this V lies 22 units from the top. The thoracal legs have become longer and thinner. The maxillary glands are now tubes of 400 μ long and 20 μ wide. The rudiments of the trachea are lozenge-shaped 120 μ long and 70 μ broad. The proctodeum shows 2 short rudiments of the Malpighian tubes, which open into it with common outlet. Of the nervous system the cerebral ganglia appear as three thickenings of the cephalic ectoderm, while in the ventral median the neural groove and the neural walls are visible. Fat cells are being formed from the first to the fifth abdominal segments.

Stage J. 17 hours. The lozenges of the tracheal rudiments have become longer, their openings to the outside have narrowed. The Malpighian tubes are about 80 μ long, the proctodeum is 80 μ deep and 50 μ wide. The neural groove has developed over the whole length of the egg ; neuroblasts are being formed. The entoderm starts covering the yolk by expanding and thinning to a single layer. Mesodermal cells in the head arrange themselves round the stomodeum to build up a muscular layer. The formation of fat cells is now also proceeding in the 6th and 7th segment. Some of the ventral mesoderm cells enlarge to blood cells. The antennal coelom is about 60 μ long. In the 3rd, 4th and 5th abdominal segment the rudiments of the reproductive cells are visible.

Stage K. 20 hours. The antennae lie on either side of the stomodeum, the other mouthparts have moved further towards the stomodeum, the 2nd maxillae now lie 12 units from he top. The distance between the top of the egg and the 3rd pair of thoracal legs has been reduced to 25 units. The openings of the maxillary glands have shifted apicad and towards each other, but they have not yet fused. The caudal ends of the parts of the tentorium that are formed by the grooves of the antennae on either side of the embryo have fused. Those formed near the first maxillae have not yet fused. The mandibular apodemes are open grooves still. The stomodeum is 200 μ deep, its cells are cylindrical. The rudiments of the tracheae are now tube-shaped and the tubes of 2 adjacent segments have nearly reached each other. The Malpighian tubes have reached a length of 400 μ. The proctodeum has got its definite shape. In the cephalic part the neural tissue has been covered by a newly formed hypodermis. In each segment the nerve cells wander to its foremost part. Now 2/3 of the circumference of the yolk is covered by entodermal cells, giving rise to the mesenteron. More blood cells have been formed in the entire ventral part of the egg. The antennal coelem is 100 μ long. Reproductive cells are now only visible in the 5th abdominal segment.

Stage L. 22 hours. The antennae have been reduced to very small elevations, they have moved somewhat dorsad. Mouth parts have now reached their ultimate positions but second maxillae have not yet fused. Thoracal legs begin to degenerate. The stomodeum is 300 μ deep. Maxillary glands have fused but have not yet been completely covered by the second maxillae. Mandibular apodemes have closed to tubes with an opening to the outside. The X-shaped tentorium has been formed. The tracheal trunk has been constituted but still shows a widening in the centre of each segment at the mouthes of the branches. Rudiments of the suboesophagial and cerebral ganglia have been severed from the hypodermis. Ganglion frontale is visible in the wall of the stomodeum. More backwards the neuroblasts and nerve cells of the median cord are contiguous with the ectoderm at only one place in each segment. The rudiments of the ganglia are drawn out, they are connected by short thin connectives. In the foremost part of the eggs the intestinal wall has been closed ventrally, in the middle of the egg both parts have nearly reached each other, in the hindmost part the distance between both ventrad growing edges is greater yet. The cells are cylindrical, 20 μ high with big nuclei and vacuoles. Muscular layer around the stomodeum has now been formed. A number of mesodermal cells lie between the tentorium and the mouthparts. The splanchnopleura has formed a muscular layer round the ventral half of the intestine. The walls of the antennal coelom approach each other in the dorsal median. On the dorsal walls of the coelomic sacs cardioblasts can be found.
Stage M. 24 hours. The antennae have been reduced for the greater part. The mandibulae how sharp chitinuous furcated jaws. Second maxillae have fused now. Thoracal legs appear as slight thickenings only. Chitine has been secreted in the stomodeum. Ganglia more rounded and all free from the ectoderm ; connectives are longer than in the foregoing stages. The dorsal epithelium is thin still, cell division does not yet occur laterally in the ectoderm. The intestinal wall covers the yolk entirely. Mesodermal cells concentrate in the segments, giving rise to the muscles of the body. The splanchnopleurae are giving rise to muscles along the dorsal side of the intestine. Mandibular muscles have been differentiated. Cephalic arteria is still open at the dorsal side. Cardioblasts and blood cells are wandering dorsad.
Stage N. 26 hours. Stomodeum is 450 μ long, in transverse sections its lumen is star-shaped. In front of the mid-intestine the "Proventrikelanlage" has been formed. Maxillary glands have now been completely covered. The nerve cells have grown offshoots. A cylindrical epithelium now covers the dorsal side of the embryo. In the segments the mesodermal cells arrange themselves to the different muscle bundles. Heart has been closed over the whole length of the embryo, blood cells lie within it. The reproductive cells constitute 2 compact cylinders, lying in the 5th abdominal segment.
Stage O. 30 hours. Stomodeum as well as proctodeum in open connection with the mid-intestine. Chitine is secreted by all parts of the tracheal system. All muscle cells have become spindle shaped and constitute real muscles. The Malpighian tubes contain swollen cells, their lumina have become narrower. The hypodermis has secreted chitin.
Stage Z. (eating larva) 48 hours. The cells of the intestinal wall are full of a granulate substance. The fat cells have swollen, their nuclei lie against the wall of the cell.

IV. *Ecological part*

A. The microclimate to which eggs, larvae and imagines are exposed

As told above in the introduction, it soon became clear to us that the microclimate exerted a great influence on the activity of the imago of *Ammophila campestris* and on the development of its eggs and larvae. We now attempted to obtain a more detailed picture of this influence.

As we had to our disposal only rather simple instruments, the extension and exactitude of our meteorological observations have

been limited; for our purpose however, they were apparently sufficient.

1. Technique of the meteorological observations

During the day we registered every hour:

1°. The temperature of the air, 10 cm above the surface of the soil.
2°. The temperature of the air, just above the ground.
3°. The temperature in a nest of Ammophila (3 cm below the surface).
4°. The illumination on the ground.
5°. The relative humidity of the air 10 cm above the ground.

It is a pity that because of our rather tiring work during the day, it was hardly possible to do regular meteorological observations also during the night. The handicap which the lack of nightly series of observations meant to us, we partly overcame by occasionally making some registrations during the night and by using maximum and minimumthermometers. One of these we dug in at a depth of 3 cm, a second and a third we placed at 30 cm and 200 cm above the ground respectively. These two were protected from direct radiation by white painted metal screens which, however, permitted of a free circulation of the air round the thermometer.

We would have liked to measure the relative humidity in the cell of the nest. This, however, was technically impossible to us.

The temperature we read in tenth of degrees centigrade. To measure the temperature at a depth of 3 cm, we put the thermometer into the nest and covered it with a hull to protect it from direct radiation of the sun. To measure the temperature in the air at about 10 cm and just above the bottom, we placed the thermometers horizontally upon two iron stands of the required height. A white painted metal screen was placed in a distance of about 10 cm in such a way that its shadow fell upon the mercury of the thermometer.

In the beginning we registered these data at several spots in the colony. This, however, being a flat, sandy path nearly without any plant, showed a fairly homogeneous microclimate, so later on we did our registrations at one and the same place in the colony.

The illumination we measured with a Westron Phototronic cell, connected with a Ferranti galvanometer of 70 Ohm. When very intense illumination made the reading of the scale impossible, we used reducing filters (blackened photographic plates) which we placed upon the cell. Moreover we always placed an opal glass uppermost, in order to make the light falling on it diffuse and to prevent the setting of the cell from throwing a shadow upon the sensitive disk.

The relative humidity was measured with an ordinary framing of a wet and a dry thermometer.

2. Results of the meteorological observations

As for the mutual relations between the different meteorological

factors our observations of the different days generally agree. So we give here only some examples, namely our observations of August 16th, 17th, 18th an 19th 1941.

The curve representing the illumination often shows a sudden drop, usually followed by an increase to the original value. This sudden change is the effect of clouds, screening off the sun for a while (very often those clouds are the cumuli which may be formed at beautiful days during midday). Such a decrease of the illumination soon affects the temperature of the air at 10 cm above the bottom. On the contrary it hardly influences the temperature in the air just above the bottom and it never causes a change at 3 cm under the ground. Irregularities in the daily course of the temperature are intercepted by the "buffer action" of the surface layer of the soil. As soon as the illumination diminishes the ground gives out some heat which is absorbed by the layer of air just above the bottom and allows it to maintain its temperature for some time.

At a depth of 3 cm we only observed an increase at the beginning of the day and a decrease at its end. The heat penetrates more slowly into the bottom than through the air, so the curves of the temperature at — 3 cm run less steep than those of the temperature at 1 cm and at 10 cm above the bottom. The soil, however, gathers more heat and keeps it longer, so the curve of the temperature at — 3 cm often runs horizontally while the temperature of the air is already decreasing. This inertia causes that short alterations of the illumination do not act upon the bottom temperature. Another consequence is that during periods of bad weather and at night the temperature in the nest does not decrease as much as the temperature in the air.

The highest air temperature we usually found in the layer just above the bottom. Sometimes, however, as in our examples at August 16th and 17th the temperature just above the bottom did not exceed that at — 3 cm. Probably this occurs when the wind blows stronger. The curve of the temperature at + 10 cm usually lies lowest, as the air does not absorb as much heat as the soil.

Fig. 62 gives a survey of the maximum and minimum temperatures we registered during a period in August 1941. From these data it also appears that the oscillations are smallest at — 3 cm. Here the difference between the highest and the lowest maximum amounts to 9°, the difference between the highest and the lowest minimum to 5°. The oscillations at + 30 cm and at + 200 cm are much more considerable ; to wit those of the maxima respectively 12° and 15°, those of the minima respectively 11° and 8° centigrade.

In accordance with what we have learned from the daily curves of the temperature, the curves of the maxima show the highest peaks in the air layer close to the bottom, the maxima at — 3 cm have a lower value, those at + 200 cm are the lowest. The minima are highest at — 3 cm, they are lowest at + 30 cm, those of + 200 cm are intermediate. So the biggest changes in temperature occur in the layer just above the bottom. During the day it is heated

as well by direct radiation from the sun as by radiation of the soil. During the night, especially when the sky is cloudless the lowest layer of the air looses more warmth than the higher layers, because it is in contact with the strongly radiating surface of the soil.

The highest temperature observed in the nest was 37°. Probably this is an extremely high value, but during hot summer days we very often noted temperatures between 30° and 33°. The lowest temperature we found was 10° centigrade, during periods of bad weather temperatures between 15° and 20° were very common in the cell. So this is the wide temperature range to which the developing eggs and larvae of Ammophila campestris are submitted.

The highest temperature we found in the air at 30 cm above the bottom amounted to 40° centigrade, the lowest in this area was only 1½°. During periods of inconstant and rainy weather and cloudy skys the temperature seldom rose above 24°, at beautiful sunny days much higher values were reached.

The relative humidity at + 10 cm shows a close correspondance with the temperature at the same height (fig. 61). A decrease of this temperature immediately causes an increase of the relative humidity. Within somewhat wide limits the changes in temperature are inversely proportional to the changes they cause in the relative humidity. So the relative humidity behaves as in a closed system. At night and early in the morning the air is often saturated with water damp, at midday the relative humidity may have decreased to about 40 %. Therefore, we may conclude from the above that the chief variable factor determining the temperature is the illumination, while the temperature itself is regulating the relative humidity. The mutual relations between those three factors of the microclimate make it impossible to study their separate effect on the wasp and its brood in field observations. Therefore, in the following study we will consider only one representative of the microclimate, namely its most important factor in processes of life, the temperature.

B. The relation between the temperature and the activity of the imago

Fig. 64 shows the activity of the females of *Ammophila campestris* at different temperatures. These temperatures we measured at a distance of 10 cm above the bottom, being about the air layer in which the imagines live and work. The activity is expressed by the number of caterpillars that is brought in at a definite temperature, divided by the number of hours we registered that temperature.

From fig. 64 it will be clear that the wasps do not feed their larvae when the temperature is below 21° centigrade. Then their utmost activity is restricted to walking about in the colony and a little bit of digging. Temperature decreasing below 20° they do not even visit the nesting places. When temperature rises activity also increases, only at very high temperatures decrease in the activity of the wasps can again be noticed.

The daily course of the activity of the wasps is in accordance

with the above. In fig. 65 the full line represents the number of intensely digging wasps that could be counted every half hour of the day, the dotted line represents in the same way the number of caterpillars that have been brought into the nests. The data for those curves we only collected during bright summer days. Both curves show a slight drop at about midday, during the hottest part of the day. At that time the relative humidity is lowest ; we consider it improbable, however, that this would be the factor that inhibits the activity of the wasps, for, in the heather, the wasps are also less active during this time of the day, while here relative humidity certainly has a higher value.

So we have seen that the activity of the imagines of *Ammophila campestris* is entirely dependent on the temperature. The larvae get no food at all when the temperature above the bottom is not at least 21° during a period of at least 3 to 4 hours. So, if the metabolism of the brood should go on with the same intensity during bad as during good weather, the larvae would be doomed to starve. ADLERZ (1903) in fact asserted that a period of bad weather could become fatal to the brood of the wasps.

Studying the microclimate, we found that the temperature in the nest indeed follows the change in the air temperature but only to some extend. Now it seemed probable to us that the development and the metabolism of the brood would practically stop at a bottom temperature corresponding with an air temperature too low to permit of the wasps feeding their larvae.

Working from this hypothesis we wanted to ascertain to which degree the rate of development of the brood depends on the temperature of the nest.

C. The relation between the temperature and the development of the eggs

On page 83 we have already set forth that we could not measure the relative humidity in the nest. However, as the soil at the depth of the nest cell was always wet, we think we may assume that the relative humidity in the cell was rather constant and not far from 100 %. So, we only studied the influence of the temperature upon the eggs in the nest.

If the rate of development of the eggs in the field is actually regulated by the course of the temperature in the nests, it must be possible with the help of the knowledge of the relations between temperature and the development of the eggs and of continued observations of the temperature in the nests from the moment the egg has been laid, to calculate the stage of development of the egg at every moment.

First of all we wanted a more exact knowledge of the influence of the temperature on the development of the eggs. For this reason we started our experiments in the laboratory. By our first series of experiments we wanted to find out, how, at certain temperatures, the different stages of development described above, are related to time.

We collected freshly laid eggs in the field, brought them to the laboratory as quickly as possible and placed them, still in the lump of earth, in a thermostate of 30° (see page 56). Here we reared them for different numbers of hours. After the time wanted, we fixed the egg, examined it microscopically and determined the stage of development with the help of the criteria described in the Appendix. The result of this series is shown in fig. 67. In this nomogram the different stages of development have been arranged on the ordinate according to the time which it takes before they are reached. On the absis the time has been plotted out and so a straight line can be drawn in the figure, representing the relation between the stages of development and the time at 30°.

The second question we wanted to answer was, whether a change of temperature would cause the time intervals between the different stages to change all in the same proportion. This being the case, the points representing the stages found after rearing during different times at any given temperature would also lie on a straight line in fig. 67. So we attempted an other series of experiments at 25.7°. As can be seen in fig. 67, the points actually lie on a straight line. We also reared an egg at 33° during 18 hours ; the situation of the corresponding point too indicates that the curve representing 33° in fig. 67 will be rectilinear. As it now seemed very likely that the curves representing the different stages at other temperatures would be rectilinear too, we could locate the direction of this line by determining one point only. Those points we derived from fig. 68, which we obtained by the following series of experiments.

We cultivated a number of eggs all during the same time (24 hours) but at different temperatures, viz. about 10°, 16.5°, 20.5°, 24.5°, 30° and 36° centigrade. In fig. 68 these temperatures have been plotted out on the absis, while on the ordinate the stages of development have been arranged according to the number of hours elapsing before they are reached at 30°.

Much has been written about the form of the curve representing the relation between the rate of development and the temperature, especially because its shape is of great interest to practical entomologists when, for prognostic purposes, they want to calculate for instance the moment of hatching or something like that. (MAERCK, 1937). For if this relation could always be described by the same formula, a minimal number of observations or experiments would be sufficient to draw the curve as a base for further calculations. It is a pity, however, that till now nobody has ever been able to find such a formula and it seems very doubtfully that any general formula actually fits the facts (MARTINI, 1929, VOÛTE, 1935) Nevertheless it may facilitate the drawing of a curve when one knows a formula by which a greater part of the curve can be described.

According to BLUNCK's rule (BLUNCK, 1914; BODENHEIMER, 1924) the relation between the temperature and the rate of development would be linear. Many investigations, however, (see for instance SHELFORD, 1929 ; UVAROV, 1931) have shown that BLUNCK's rule

certainly does not hold at extreme temperatures, although it may be practically applicable within the temperature range in which a certain insect is living (ZWÖLFER, 1935 ; BODENHEIMER, 1938).

As far as low and intermediate temperatures are concerned our results can fairly well be represented by a logarithmic curve. At higher temperatures, however, this curve keeps rising steeper, while on the contrary the actual rate of development diminishes. JANISCH (1928) for this reason is of opinion that a catenery curve would give the best representation of the relation between temperature and development. Such a line is a combination of two exponential curves with opposite sign. One part expresses the increase in the rate of development when temperature rises, the other the injuring effect of higher temperatures (to get a real catenery curve it is necessary not to plot out on the ordinate the rate of development as has been done in fig. 68, but the time elapsing at different temperatures before an egg reaches a certain stage, i.e. the reciprocal value ; BLUNCK's straight line when plotted out in this way changes to a hyperbole). In general the catenery curve gives a better representation at higher temperatures, at the lower and intermediate temperatures there is hardly any difference between the catenery and the exponential curve.

In fig. 68 we have drawn through the points (each of which was determined twice) an exponential as well as a catenery curve. The appropriate exponential curve we found by plotting out the logarithms of the Y-value against their X-values, drawing a straight line through these points and deriving from this one the ideal exponential curve.

To choose the most suitable catenery curve we constructed with the help of the formula $y = (e^{\frac{x}{h}} + e^{-\frac{x}{h}}) = h \cosh \frac{x}{h}$ several catenery curves for different values of h. It appeared that the curve constructed with $h = 3^1/_2$ fitted in best with the points.
Below 31° the differences between the exponential curve and the catenery curve lies within the limits of the exactitude with which we could determine the points. At higher temperatures the course of the chain line shows a closer correspondance with the true curve than the exponential curve.

Above 32° not only the course of the curve gives difficulties but the exact location of the points also. It is difficult to identify the stages exactly as the slides show such a complicated picture at this age.

According to the catenery-curve, some development would occur even at a temperature as low as 0° centigrade. Whether this is actually true cannot be measured as at temperatures below 10° the rate of development is hardly measurable with our means.

We may say that from 10° to 32° the catenery curve gives a fair representation of our results, which means that we may use this curve for our calculations ; for the lowest temperature we observed in the nest was 13°, while, if temperature rose above 32°, it was always for a few hours only.

It will also be clear that in our case we certainly may not use BLUNCK's rule, as between 13° and 32° the curve is far from being a straight line.

With the help of fig. 68 we can now draw all the lines wanted in fig. 67.

Knowing the rate of development at each temperature, we now can calculate the stage of development of an egg when the course of the temperature in the nest during its development is also known. We can do this in two ways, first by reading it from the nomogram fig. 67, secondly by calculating the stage of development step by step in a manner similar to that indicated by SHELFORD (1929).

Before we may use these methods we have, however, to make a last series of experiments. It has been shown in some cases, especially when cultivating at very high temperatures (VOÛTE, 1935) that the rate of development increased when periods of high temperatures alternated with periods of low temperatures. Although we are inclined to believe, that this only holds for lethal high temperatures, we have done some experiments to test the effect of intermittent high and low temperatures.

Three eggs we cultivated during 36 hours for periods of 9 hours at 26° and at 17° alternately. Two eggs were first submitted to 26°, the third began its development at 17°. All three eggs finally reached stage J.

From the nomogram we can read off which stage should have been reached theoretically after 18 hours at 17° and 18 hours at 26°. We have to follow the line representing 17° for 18 hours and then to draw for another 18 hours a line parallel to the direction of the 26°-line. Then we also reach stage J, so we may conclude that changes of temperature as such do not affect the rate of development.

In stead of using the nomogram, it is easier to calculate the stages of development. We will describe the method with the help of the following example.

An egg has been laid Aug. 17th 1939 at 15.10 hour and it has been fixed the next day at 21.30 hour. From the determinations of the temperature in the field we know that on the first day from 15.10 till 18.10 the temperature was 30° ; on the same day the temperature was 29° from 18.10—18.40 and on the next day from 15.00 till 17.30. On Aug. 17th from 18.40—19.10 and on Aug. 18th from 14.00—15.00 and from 17.30—18.30 we observed a temperature of 28°, etc.

From fig. 68 it appears that after cultivating during 24 hours at 29° the same stage is reached as after 22.4 hours at 30°. So at 29° the rate of development is 22.4 times less as at 30°. In the same way we can express the development at each temperature in terms of the time of development at 30° ; so our final calculation looks like :

$$3 \times \frac{24}{24} + 3 \times \frac{22.4}{24} + 2.5 \times \frac{20.8}{24} + \ldots\ldots \text{ etc.} = 17.8 \text{ hours}$$

development at 30° centigrade. It appears that actually the egg has

just passed stage J, which is reached after cultivating 17 hours at 30°.

When the temperatures were higher than 25° we calculated to the nearest half hour and to 1°. Between 25° and 20° we calculated to the nearest hour and to 1°, below 20° to the nearest period of 2 hours and to 2°. At these low temperatures the rate of development is so small that a more detailed calculation would only mean a would-be exactitude, as the errors caused by the calculation lie within the limits of those made by the observations.

Table 1 shows the results of the calculations for the eggs reared in the thermostate, table 2 shows those obtained for eggs which developed in the field. These tables show that the calculated stages agree very well with the stages actually found. The agreement between the calculated and the actual stages is better still when one considers that it is very difficult to state differences smaller than 1, as entered in the last column of the tables.

So table 2 proves that in the field it is practically only the temperature which regulates the development of the eggs. Of the magnitude and the influence of the temperature one gets an idea by comparing in table 2 for instance the stage which is reached after cultivating 8 hours at 30°. In one case this stage is reached after 10 hours, in another only after 25 hours!

Table 1. Eggs cultivated during different times in a thermostate between 27° and 29° centigrade.

Age in hours	calculated stage*)	stage*) what was actually reached		difference
6	5.2	5	C	+ 0.2
9	7.8	8	E	— 0.2
$12^1/_4$	10.6	10	F	+ 0.6
15	13	12	G	— 1.0
$18^1/_2$	16	16	I	0.0
$20^3/_4$	18	17—20	J—K	— 0.5
21	18.2	17—20	J—K	— 0.3
$24^1/_4$	21.2	22	L	— 0.8
$25^1/_4$	21.9	22—24	L—M	— 1.1
27	23.4	24	M	— 0.6
$28^1/_2$	24.7	24	M	+ 0.7
30	26	26	N	0.0

*) The figures, indicating the stages as they have been calculated and as they have been reached, represent the number of hours it takes an egg to reach that particular stage when developing at 30°.

This chapter also proves the necessity of investigating the relation between temperature and development before making any calculation. It is certainly not allowed to assume a priori that this relation will be rectilinear within the limits of the temperature of the environment. In *Ammophila campestris* the relation is only approx-

imately linear between 22° and 30°, while as we have seen the temperature range of the environment is much wider.

ADLERZ (1903) found a large variation in the times of development for the eggs of *Ammophila campestris,* although, after his opinion, the external conditions remained equal. He thinks that this variation might be caused, either by differences in the completeness of the egg at the moment of depositing, or by a power of the female to keep the fertilized egg in the vagina until the propitious moment to depose it, has come. As by anatomical examinations of females just before they were going to lay an egg, we never found an egg in the vagina, ADLERZ' latter explanation had to be rejected. Now the former one is also shown to be incorrect as we could prove that all eggs when developed under the same conditions, reach the same stage of development. Therefore we must assume that in ADLERZ' observations circumstances have not always been equal.

Table 2. Eggs developed in the field under natural conditions during different numbers of hours.

Age in hours	Time at which the egg has been laid		calculated stage*)	reached stage*)		difference
6	13-8-'39	14.10	2.8	3	B	— 0.2
6	23-7-'38	13.40	3.2	3	B	+ 0.2
15 1/2	23-7-'38	16.25	5.8	5	C	+ 0.8
12 1/2	17-8-'39	17.46	4.5	5-6	C-D	— 1.0
8	14-8-'39	12.43	4.3	5-6	C-D	— 1.2
18 1/4	17-8-'39	16.20	6,4	6	D	+ 0.4
18 1/2	19-7-'38	16.20	8.2	6	D	+ 2.2
8 3/4	24-7-'38	13.30	7.4	6	D	+ 1.4
25	15-8-'39	14.45	8.6	8	E	+ 0.6
10	24-7-'38	12.20	7.7	8	E	— 0.3
10	24-7-'38	11.40	8,1	8	E	+ 0.1
21 1/4	16-8-'39	15.40	7.2	8	E	— 0.8
21 3/4	18-8-'39	14.58	9.7	10	F	— 0.3
21 1/2	19-7-'38	13.35	10.2	12	G	— 1.8
24 1/2	23-7-'38	15.25	12.7	12	G	+ 0.7
23	29-7-'39	17.25	14	14	H	0.0
27	18-8-'39	14.58	14.5	14	H	+ 0.5
30 1/3	17-8-'39	14.30	17.3	17	J	+ 0.3
39 3/4	17-8-'38	16.06	19.4	20	K	— 0.6
30 1/4	17-8-'39	14.11	17.8	17-20	J-K	— 0.7
31	24-7-'38	12.12	18.9	20	K	— 1.1
48	19-7-'38	12.50	20.6	20	K	+ 0.6
42	24-7-'38	13.40	22.3	22	L	+ 0.3
45	17-8-'39	15.30	22.5	22	L	+ 0.5
27	15-8-'39	10.55	23.4	22-24	L-M	+ 0.4
62	15-8-'39	16.30	25.7	24-26	M-N	+ 0.7

D. Relation between the temperature and the rate of development of the larvae

As measure for the rate of development of the larvae we shall use here the time wanted for the entire development, from the deposing of the egg till the formation of the cocoon. We do not possess complete information about the microclimatological factors during the whole development of a number of larvae, but we approximately know how many hours during the development of these larvae the conditions permitted the imagines to be active. As we have already shown above, this number of hours is a function of the temperature. In fig. 66 we have plotted out the time of development of a number of larvae against the main daily number of working hours of the imagines during the same time. It appears that the rate of development of the larvae increases at higher temperatures. The scattering of the points in the graph is undoubtedly due to the lesser exactitude of the data.

E. Conclusions

The activity of the imago as well as the development of the eggs and larvae are chiefly regulated by the temperature of the environment. The brood of *Ammophila campestris* depends on the temperature of the nest as well as on the temperature of the air outside the nest as the latter determines whether the imago will bring food or not. Now we found that the temperature at — 3 cm shows a close correspondence with the temperature at + 1 and at + 10 cm, the temperature in the soil generally being only lower. So when the temperature at the surface falls, the temperature in the nest will also decrease and with it the rate of development and of metabolism of the brood decreases. At about 20° the wasps stops provisioning ; this temperature being reached in the outside air the temperature in the nest does not exceed 18°. Now if the larva is not to starve the rate of development below 18° must be very slow and this in fact is the case as can be seen in fig. 68.

To depths over 15 cm the daily changes of the temperature hardly penetrate. At a depth of 7 cm for instance the daily amplitude of the temperature is only half that of the surface layer (MINNAERTS, 1939). From measurements we made at — 6 cm it appeared that at this depth the maximal difference of the temperature during a fortnight in August amounted to 5° only. Therefore it seems probable that brood of Sphegidae living at depths greater than 15 cm under the surface of the ground will develop with a constant rate even while the imagines are forced to idleness. If these larvae still had to be provisioned after they have hatched they would have a good chance to starve during a period of bad weather. Now those species, for instance *Philanthus triangulum* Fabr. and different species of the genus *Cerceris* first of all fill up the cell with enough food for the entire development of the larva and only then deposit their egg. On the contrary species which, like *Ammophila campestris* keeps provisioning after the larvae have hatched, must live in nests only a short distance below the surface of the soil. Such a species is for instance also *Bembex rostrata* L. which in fact digs shallow nests.

V. *Summary*

The mode and the rate of development of the eggs of *Ammophila campestris* have been investigated.

The egg is fertilized just before it is laid ; then cleavage sets in, leading to the formation of the blastoderm. The course of the cleavage, the formation of the germ layers and of the different organs has been described. These processes generally agree with the results of investigations upon *Apis mellifica* and *Chalicodoma muraria*. We will mention here only the most important discrepancies with literature. One of these is the formation of a real amnion element near the caudal pole of the egg, which is an argument in favour of the assertion that in *Ammophila* the final complete embryonic envelope is the serosa. A second is the formation by the mesoderm of a quaint system of cells in which fat is stored later on.

To get an idea of the micrometeorological influences to which the image and its brood are exposed, we attempted measurements of illumination, temperature and relative humidity. The temperature which depends very much on the illumination, chiefly regulates the activity of the imagines and the rate of development of the brood.

After having studied the relation between temperature and the rate of development in the laboratory we could calculate the stages of development of eggs developing in the field from the temperature records made in the nest. The relations between the temperature of the air, the temperature in the nest, the activity of the imago and the rate of development of the brood are such that the rate of development of the brood is about nil at an air temperature that forces the wasp to inertia.

VI. *References*

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VII. *Explanation of the symbols used in the figures*

a — antennae
aco — antennal coelom
am — amnion
amf — amnionfold
b — blastoderm
bl — bloodcells
bp^1, bp^2 — coeca of the proctodeum
c — heart
ca — cephalic arteria
cb — cardioblasts
cc — central column
cg — cerebral ganglion
ch — chorion
cl — cortical layer
coc — circumoesophageal commissure
cs — coelomic sac
dect — thin dorsal ectoderm
dp — deutoplasm
ect — ectoderm
ent — entoderm
exb — extra-embryonic blastoderm
f — rudiments of the fat cells
fg — frontal ganglion
g — ganglion
go — rudiment of the gonads
i — integument
ia — appendix of the intercalary segment
im — intestinal muscles
iw — intestinal wall
m — mesoderm
md — mandibula
mp — mesodermal plate
mt — Malpighian tubes
mu — muscles
mum — muscles of the mandibulae
mv — membrana vitellina
mx_1 — first maxilla
mx_2 — second maxilla
mxg — groove near second maxilla
mxgl — maxillar gland
n — nucleus
nc — nerve cells
nb — neuroblasts
ng — neural grooves
nw — neural walls
op — ooplasm
p — proctodeum
pa — „Proventrikolanlage"
s — serosa
sg — suboesophageal ganglion
si — stigma
sp — side plate
sr — rudiment of the serosa
st — stomodeum
$t_{1\text{-}3}$ — thoracal legs
$tn_{1\text{-}3}$ — parts of the tentorium
tr — trachea
vc — ventral cord
y — yolk
yc — yolk cells
ycs — syncytia of yolk cells

Fig. 1

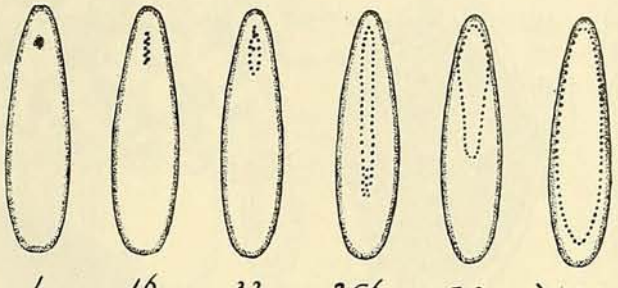


Fig. 3

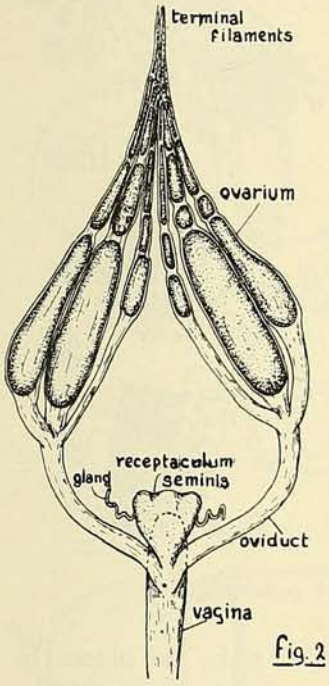
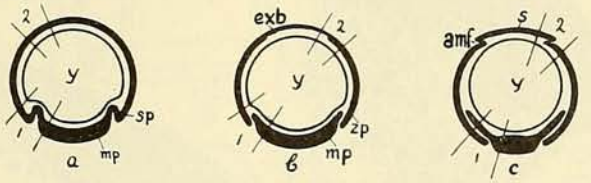


Fig. 2



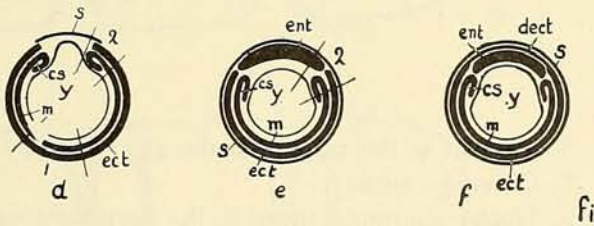


Fig. 4

Fig. 1. Boring apparatus for digging out the nests.
Fig. 2. Reproductive organs of the female of Ammophila campestris.
Fig. 3. Cleavage stages.
Fig. 4a—f. Diagrammatic transverse sections just behind the middle of the egg.

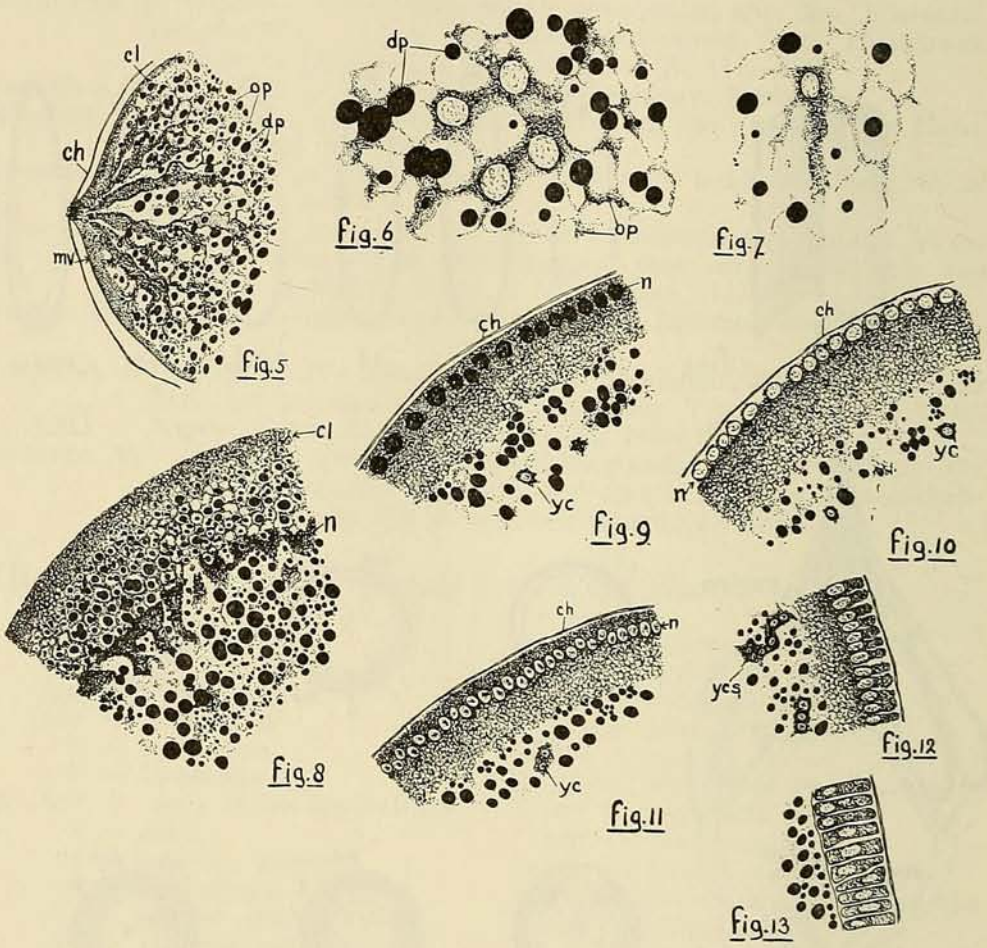


Fig. 5. A point at the surface of the egg that may be a micropyle.
Fig. 6. Cleavage nuclei.
Fig. 7. Nuclei migrating towards the periphery with a "wake" of plasm.
Fig. 8. Cordon of migrating nuclei; inside this cordon the plasmatic reticulum has been torn up.
Fig. 9. The nuclei have penetrated into the cortical layer.
Fig. 10. The nuclei lie on the outside of the cortical layer.
Fig. 11. The nuclei have multiplied and they have reentered the cortical layer.
Fig. 12. Cell delimitations appear in the cortical layer.
Fig. 13. The cells of the blastoderm have been formed.

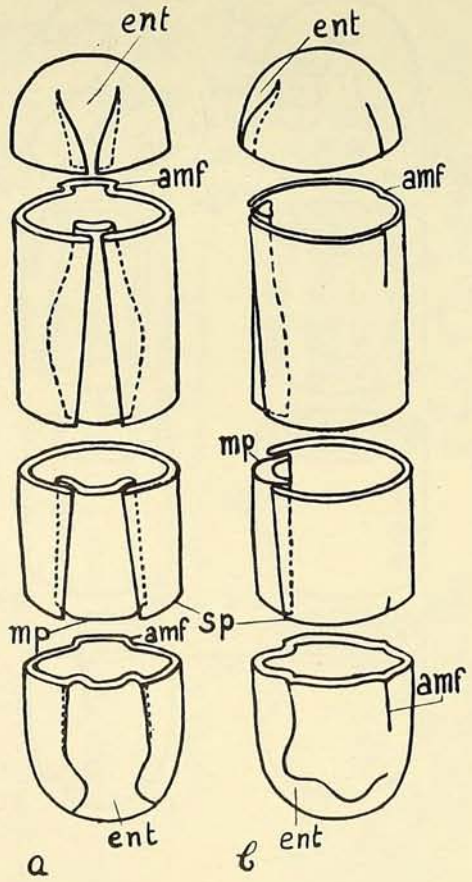


Fig. 14

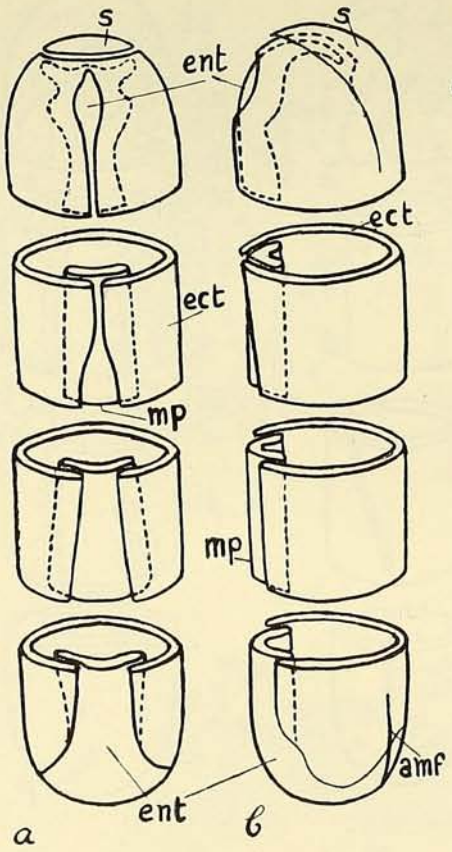


Fig. 15

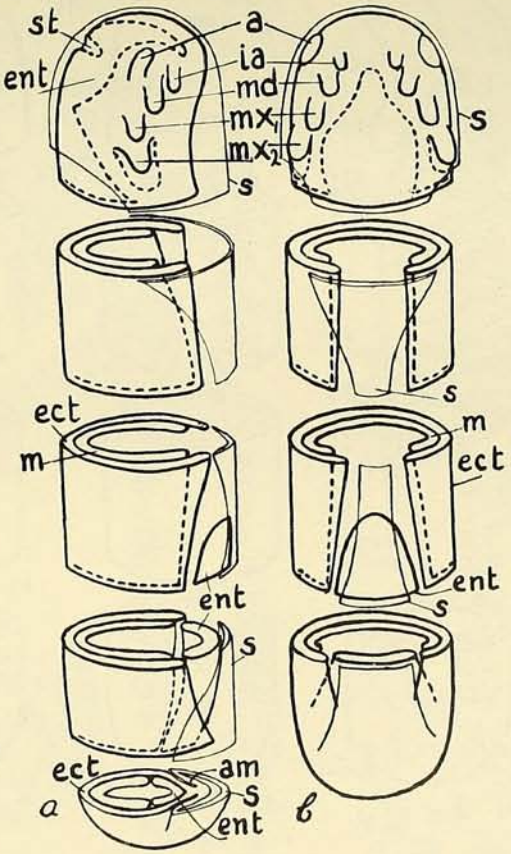


Fig. 16

Diagrams of the development of the egg; Fig. 14: stage E; a: ventrally, b: from the left. Fig. 15: stage F; a: ventrally, b: from the left. Fig. 16: stage G; a: from the left, b: dorsally.

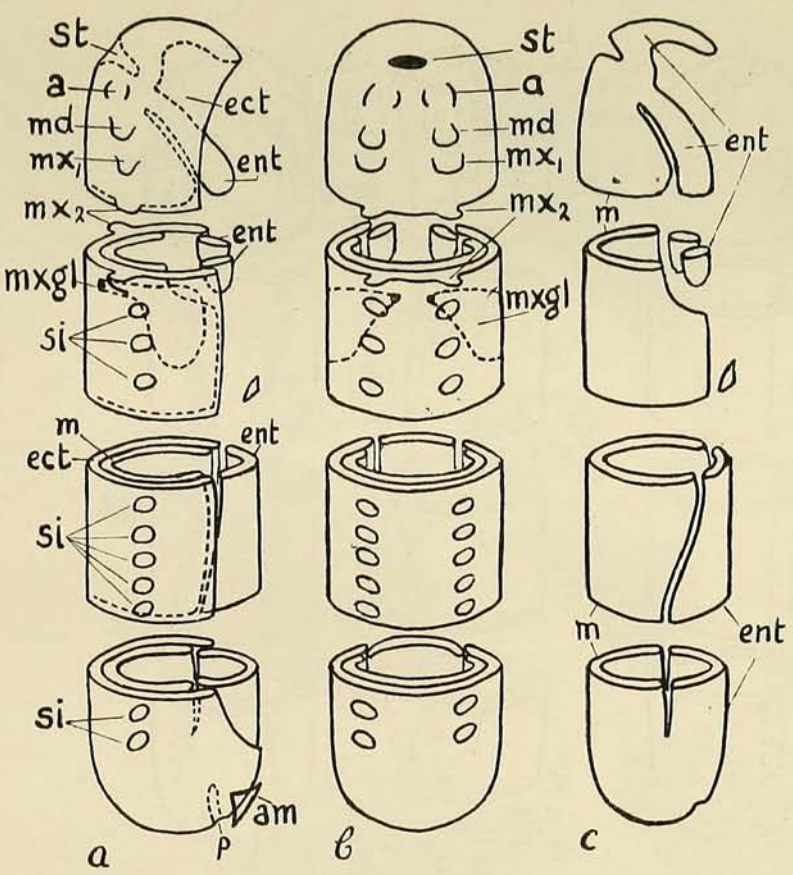


Fig. 17

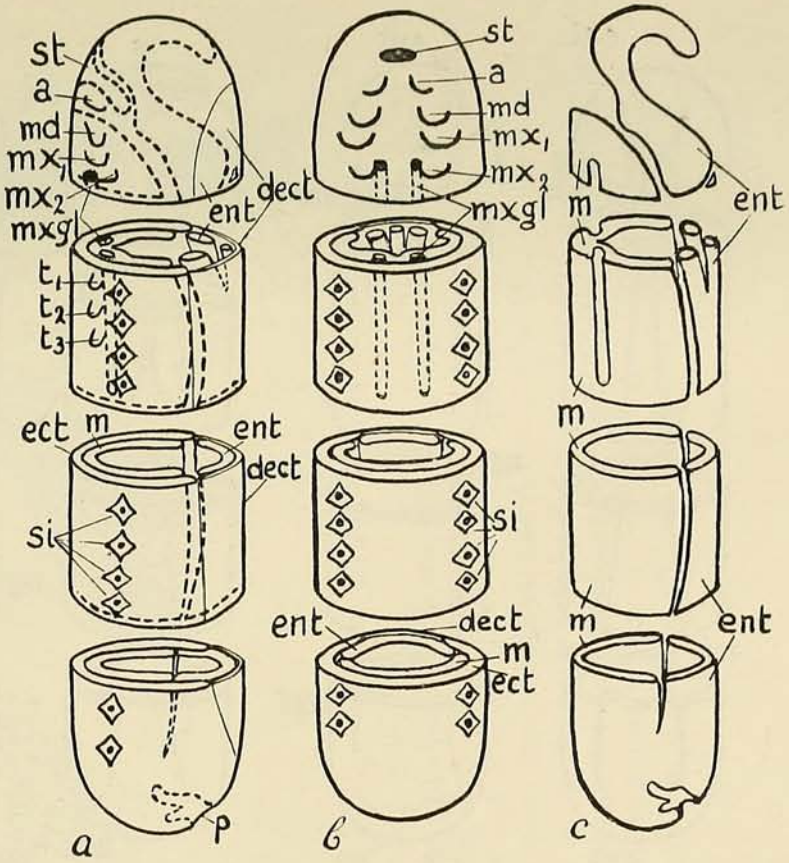
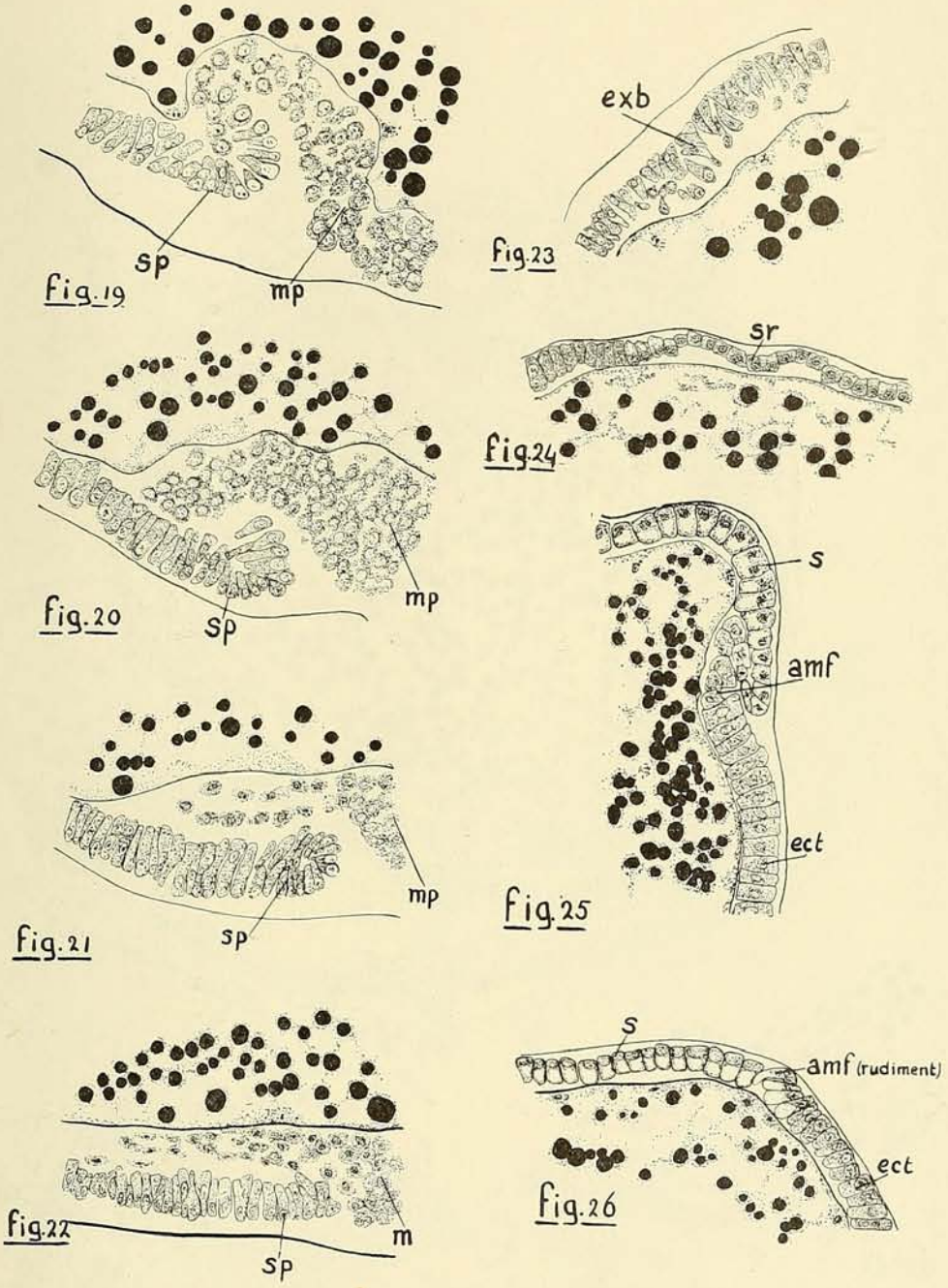


Fig. 18

Diagrams of the development of the egg; Fig. 17: Stage H; a. from the left (the serosa has been left out); b: ventrally; c: the same egg from the left, but the ectoderm has been left out. Fig. 18: Stage I; a. from the left (the serosa has been left out); b: ventrally; c: from the left, the ectoderm has been omitted.



Transverse sections through an egg in stage E.

Fig. 19. Through the caudal part, sector 1 of fig. 4a.

Fig. 20. Somewhat more apically, sector 1 of fig. 4b.

Fig. 21. Through the middle of the egg, about the same sector as fig. 20.

Fig. 22. Through the apical part, sector 1 of fig. 4c.

Fig. 23. Through the middle of the egg, the dorsal blastodermcells are dividing.

Fig. 24. Through the caudal part, sector 2 of fig. 4a.

Transverse sections through an egg in stage F.

Fig. 25. Through the caudal part, sector 2 of fig. 4c ; with the rudiment of the amnionfold.

Fig. 26. Through the middle of the egg, same sector ; with amnionfold.

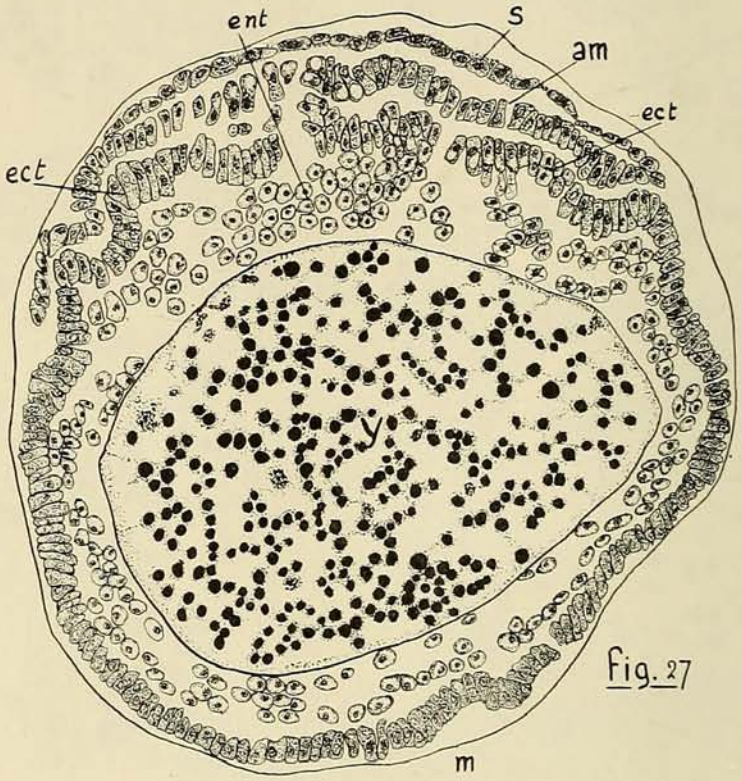


Fig. 27

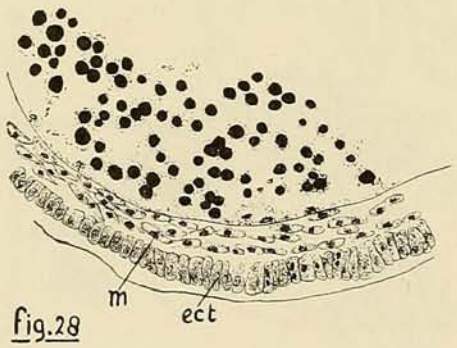


Fig. 28

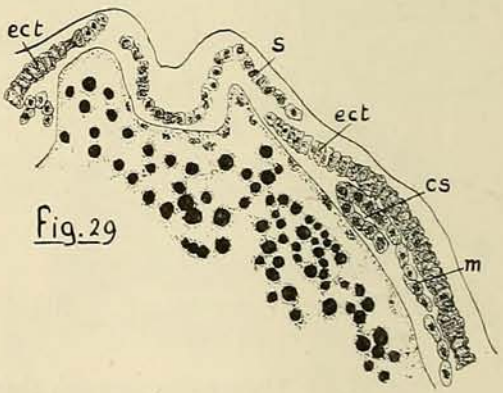


Fig. 29

Transverse sections through an egg in stage G, showing amnion and serosa.
Fig. 27. Through the caudal part.
Fig. 28. Through the middle of the egg ; sector 1 of fig. 4d.
Fig. 29. Through the middle of the egg ; sector 2 of fig. 4d.

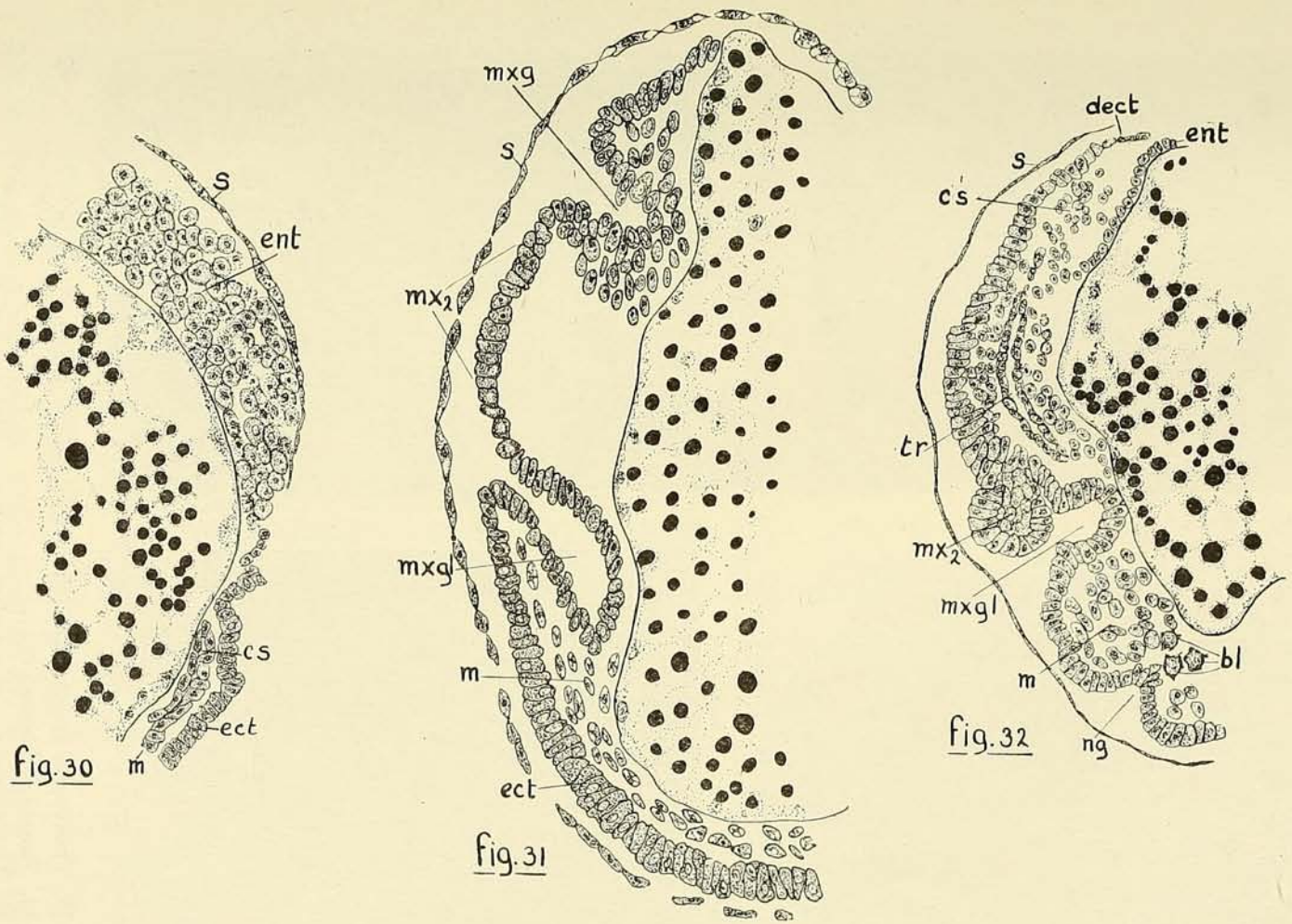
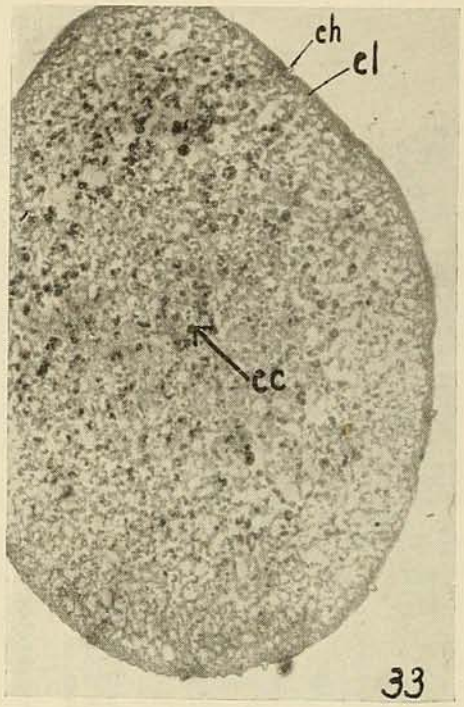


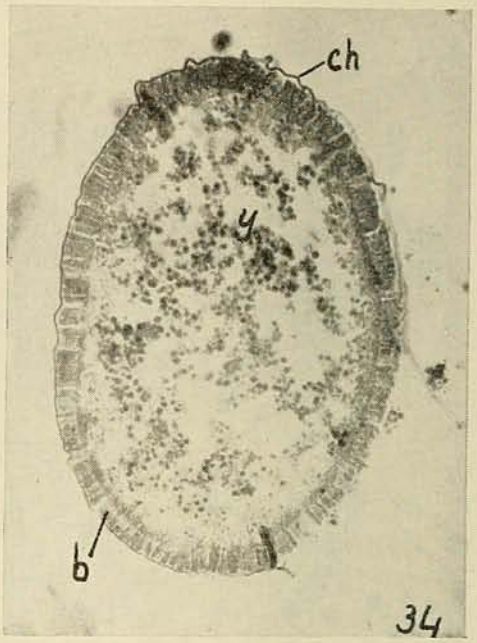
Fig. 30

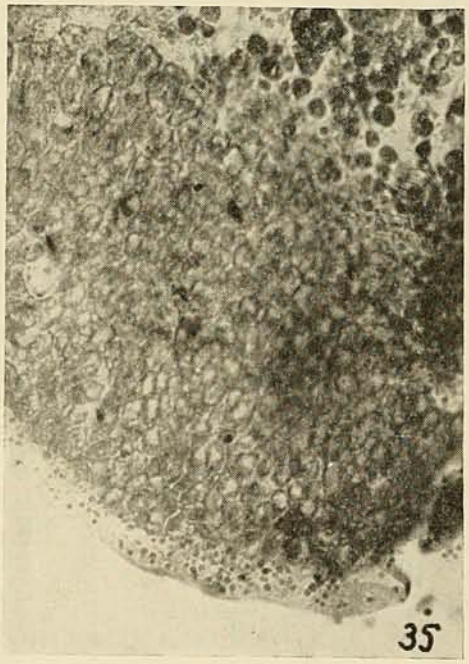
Fig. 31

Fig. 32

Fig 30. Transverse section through an egg in stage G, caudal part, sector 2 in fig. 4e. Fig. 31. Transverse section through an egg in stage H, with the grooves out of which the maxillar glands are being formed. Fig. 32. Transverse section through an egg in stage I, with the outlet of the maxillar glands.







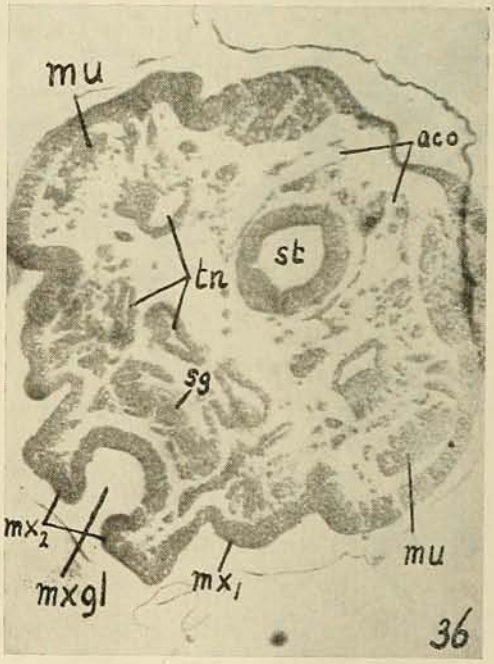


Fig. 33. Transverse section through an egg before cleavage sets in.
Fig. 34. The final blastoderm.
Fig. 35. The formation of entoderm at the caudal pole of the egg.
Fig. 36. Transverse section through an egg in stage L, through the 2nd maxillae which form a groove round the outlet of this gland.

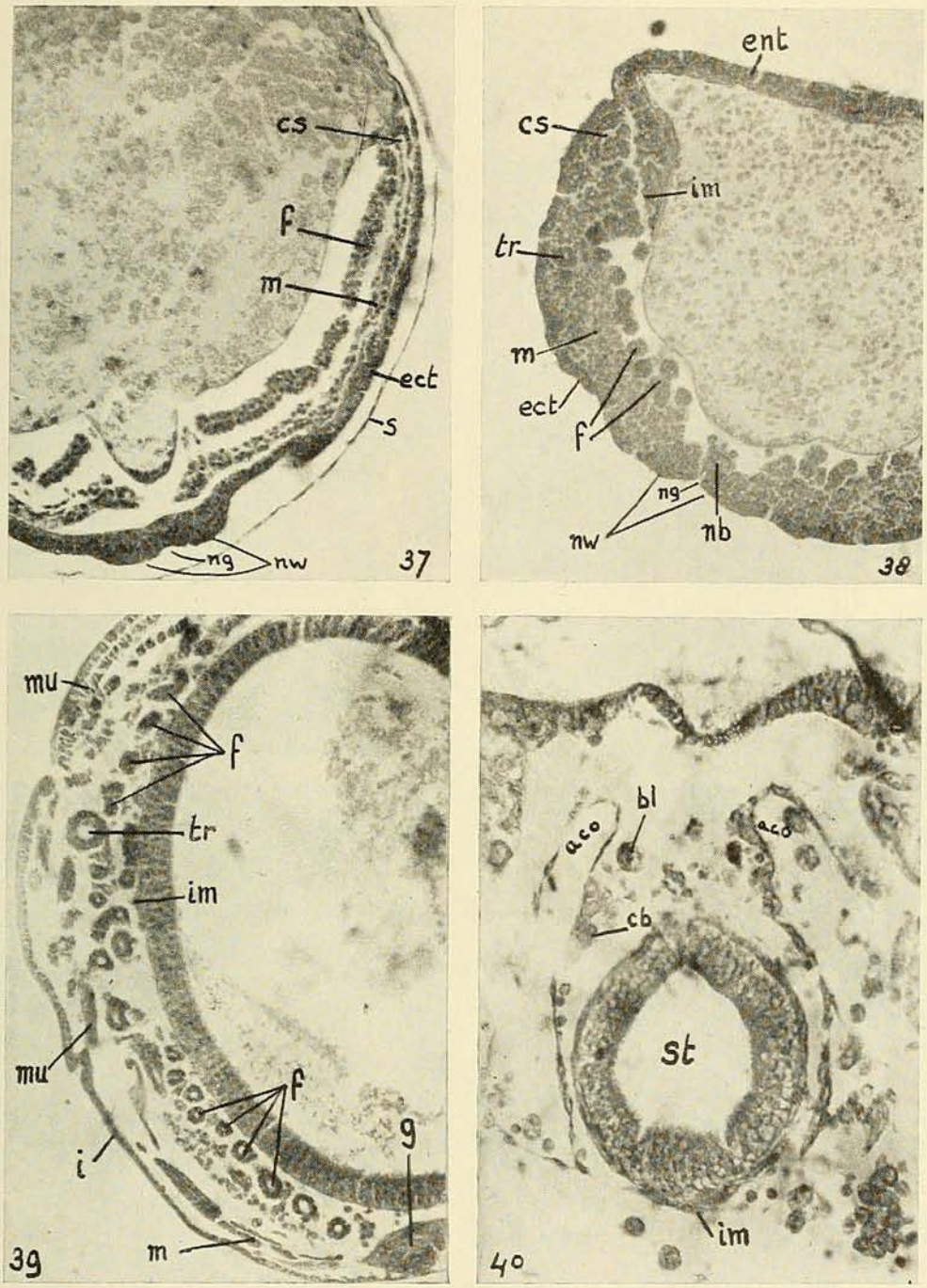


Fig. 37. Transverse section through the middle of an egg in stage H; the mesodermcells are configurating to form fat tissue.

Fig. 38. Transverse section through the middle of an egg in stage K. A part of the mesoderm cells have arranged themselves in „tubes".

Fig. 39. Transverse section through the middle of an egg just before hatching.

Fig. 40. Transverse section through the head of a larva in stage L, showing the muscle layer of the stomodeum and the antennal coelom.

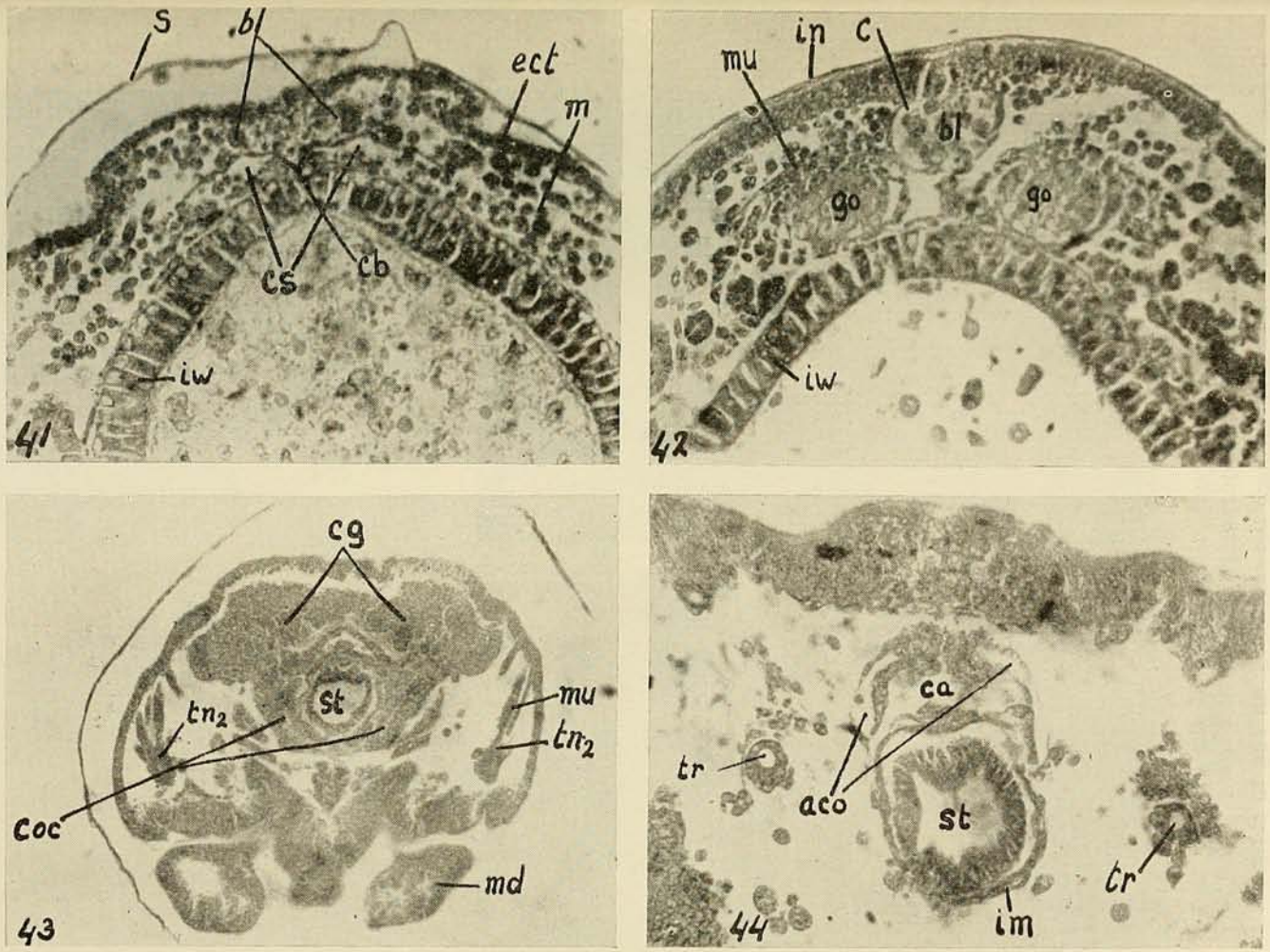


Fig. 41. Transverse section through an egg in stage M; the rest of the left and of the right coelomic sacs approach each other. Fig. 42. Transverse section through the fifth abdominal segment of an egg in state N, showing the heart, blood cells and the rudiments of the gonads. Fig 43. Transverse section through the head of an egg in state N, at the place where the circumoesophagial commissure is formed. Fig. 44. Transverse section through the head of an egg in stage N. The coelomic sacs of the antennae are forming the cephalic arteria.

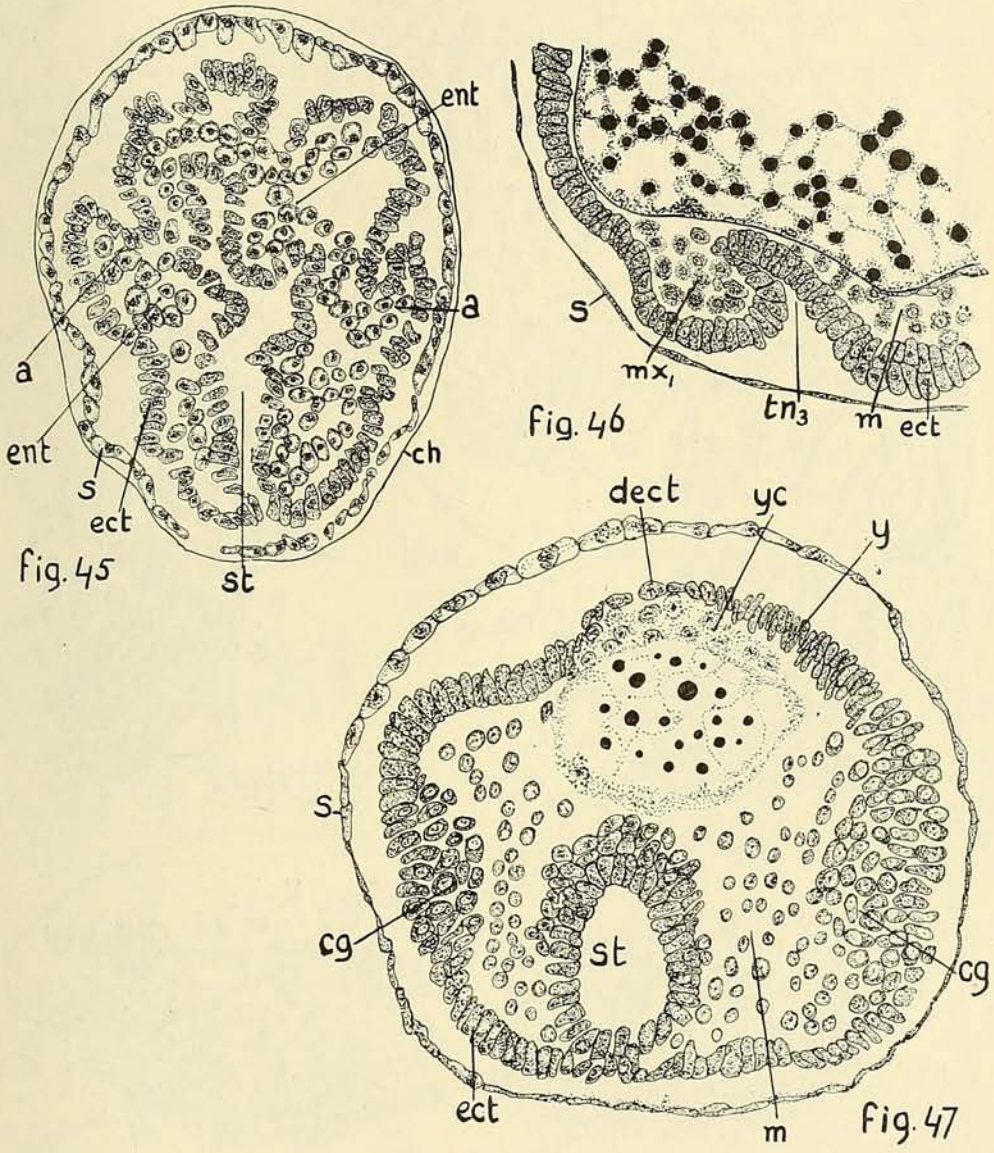


Fig. 45. Transverse section through the rudiments of antennae and stomodeum of an egg in stage G.

Fig. 46. Transverse section through a first maxilla of an egg in stage H.

Fig. 47. Transverse section through the most apical part of an egg in stage H, with stomodeum and ectodermal thickenings going to form the cerebral ganglia.

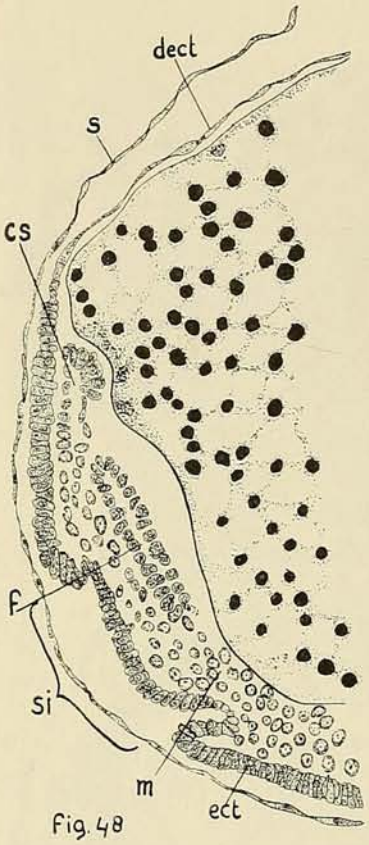


Fig. 48

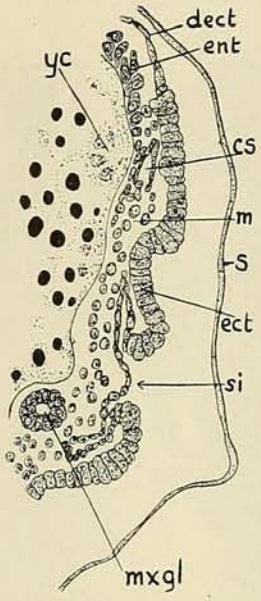


Fig. 49

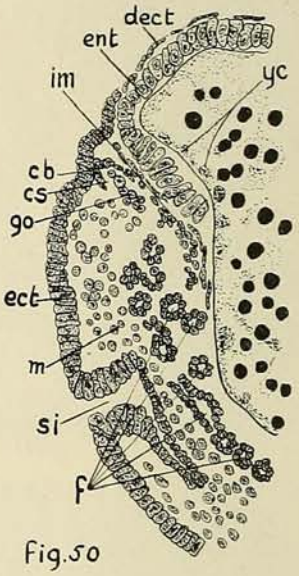


Fig. 50

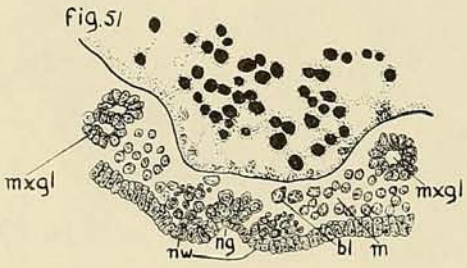


Fig. 51

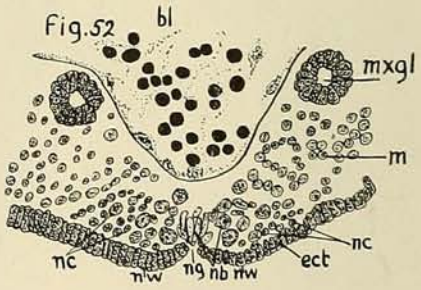
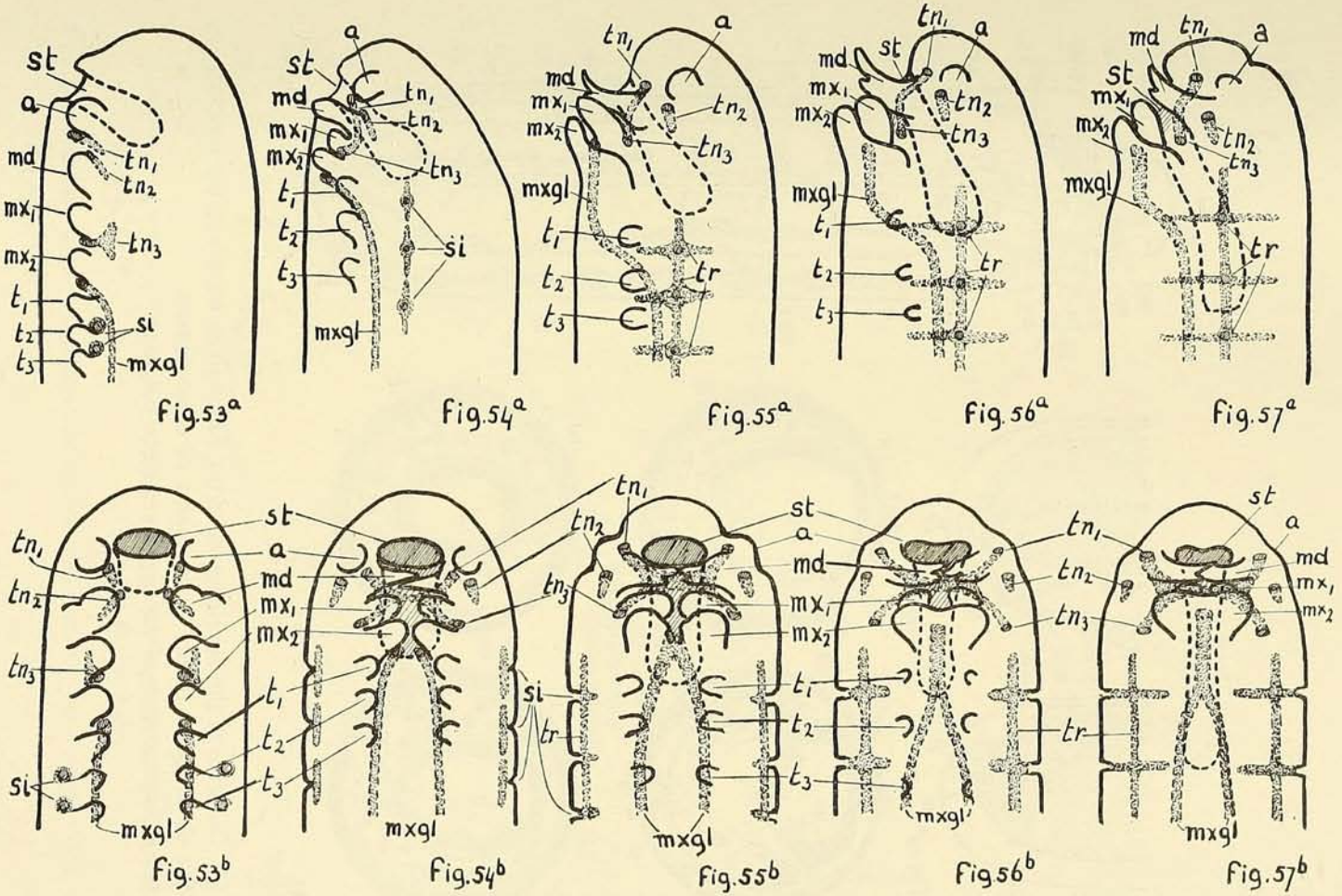


Fig. 52

Fig. 48. Transverse section through a stigma in stage H.
Fig. 49. Transverse section through a stigma in stage I.
Fig. 50. Transverse section through a stigma in stage K .
Fig. 51. Transverse section through the caudal part of an egg in stage K, sector of the ventral median.
Fig. 52. Transverse section through the some egg as in fig. 51, but somewhat more apically, sector of the ventral median.



Diagrams of the changes in position of the mouth parts and similar organs. a: from the left, b: ventrally. Fig. 53: Stage J; fig. 54: stage K; fig. 55: stage L; fig. 56: stage M; fig. 57: stage N.

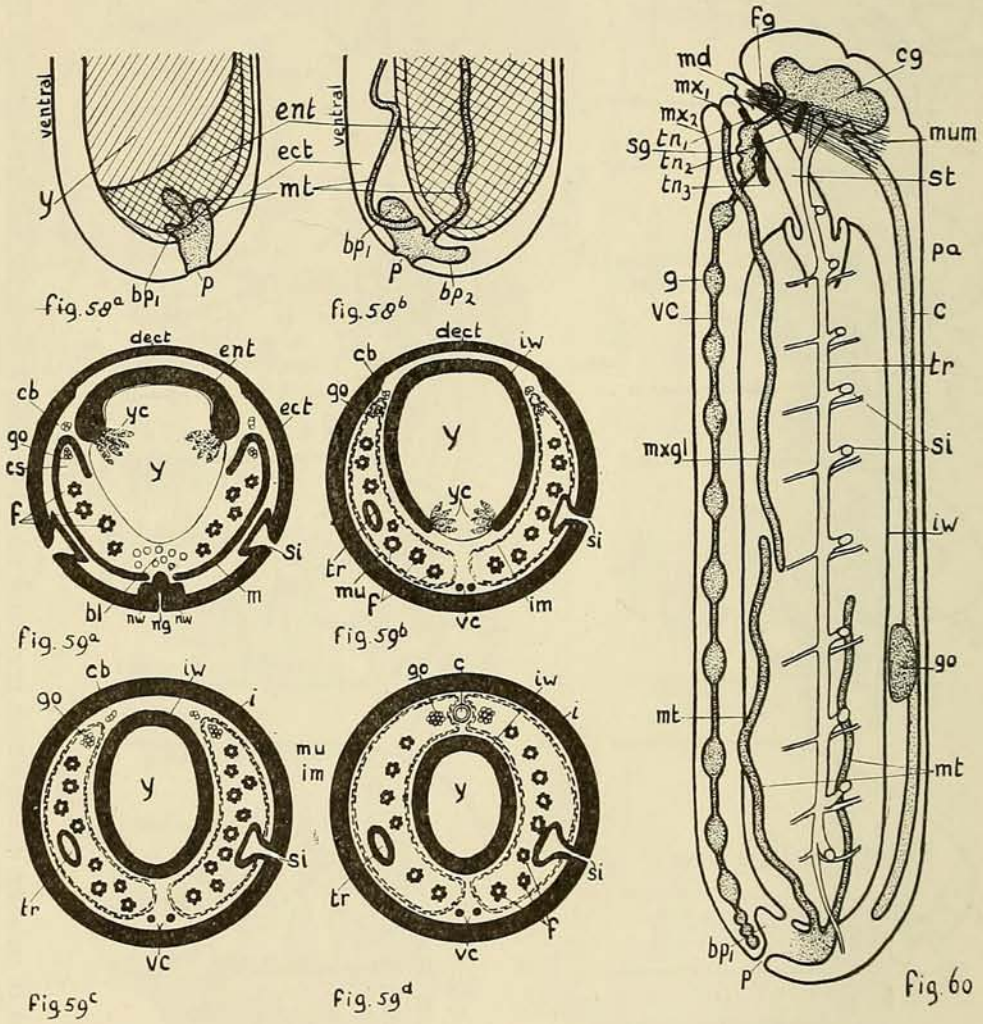


Fig. 58. Diagram of the development of the Malpighian tubes and the proctodeum.
Fig. 59a-d. Diagram of the development of some organs.
Fig. 60. Reconstruction of an egg just before hatching.

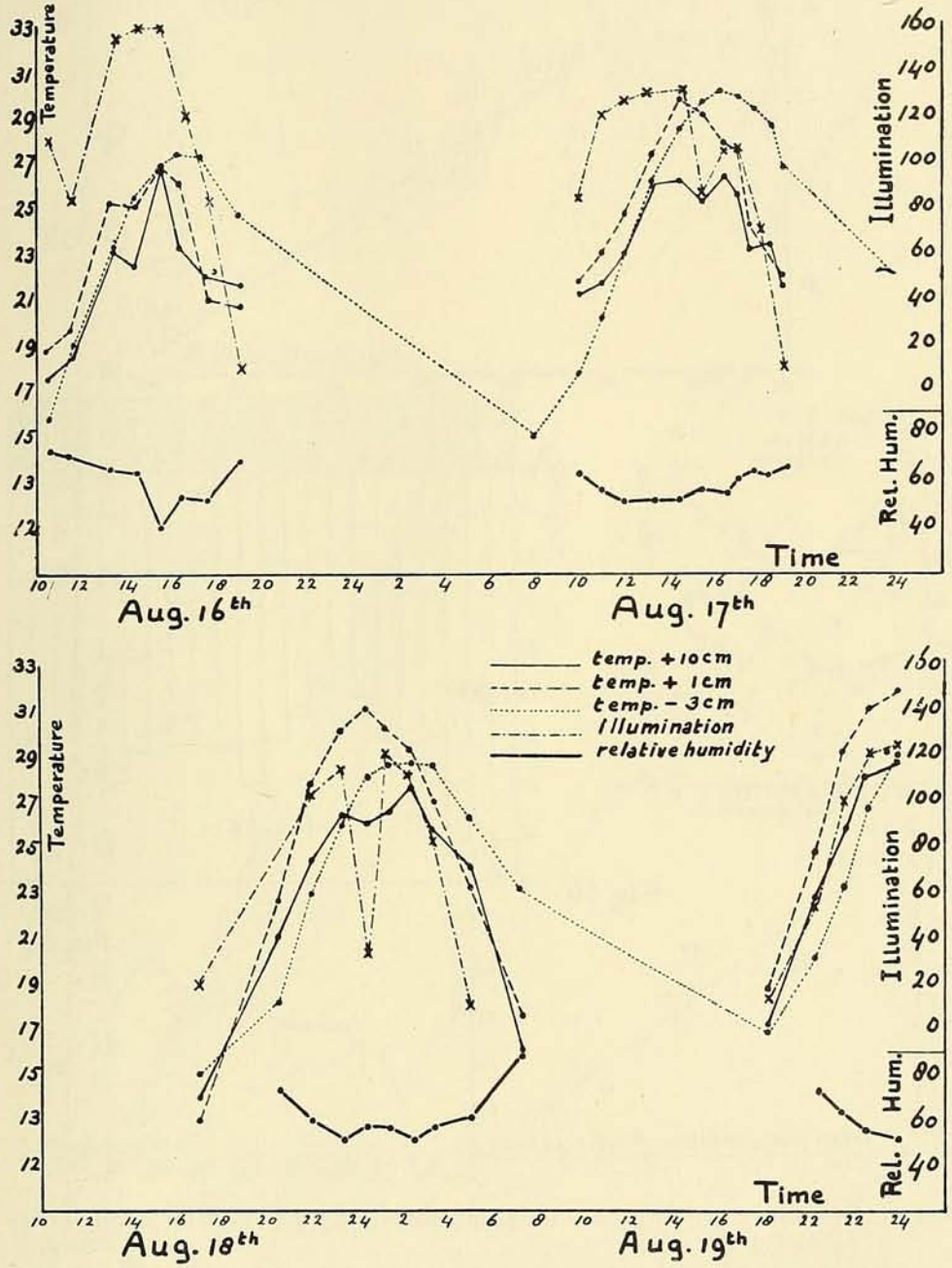


Fig. 61. Results of the micrometeorological observations on August 16th, 17th, 18th and 19th 1939.

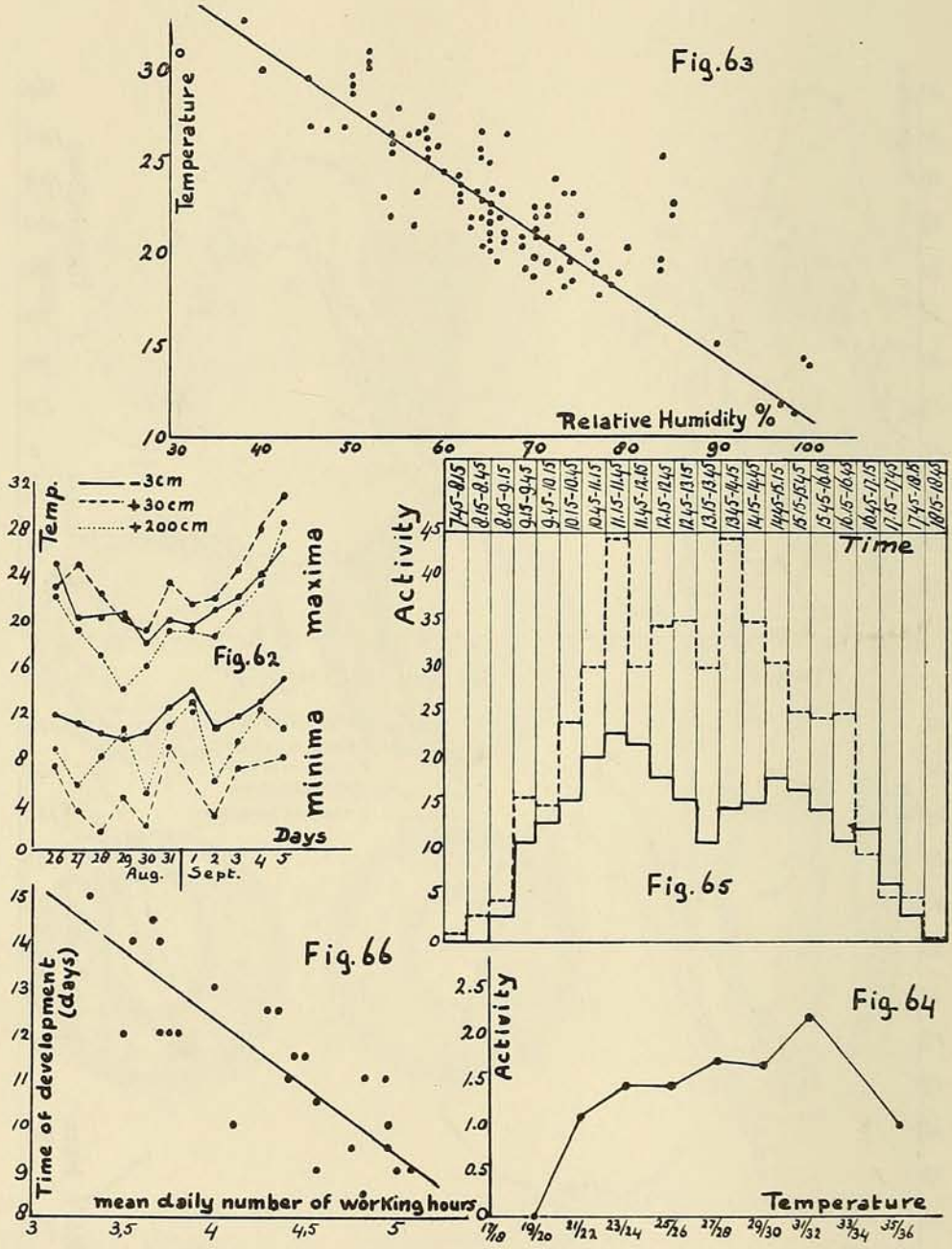


Fig. 62. Maximum and minimum temperatures during a period in August—September 1940.

Fig. 63. Relation between the temperature and the relative humidity 10 cm above the ground.

Fig. 64. Relation between the activity of the wasps and the temperature.

Fig. 65. Daily course of the activities of the females.

Fig. 66. Relation between the development of the larva and the temperature.

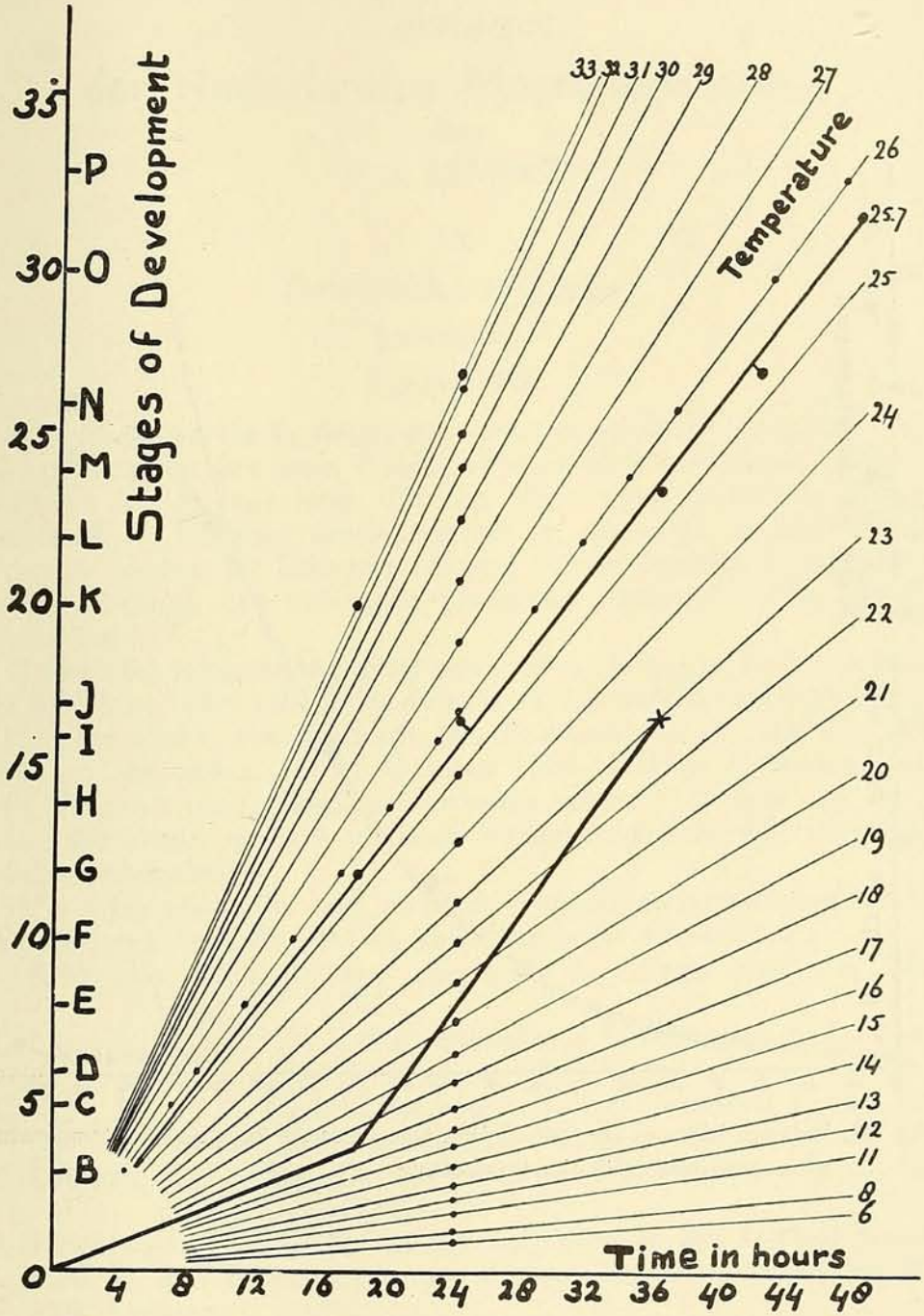


Fig. 67. Nomogram of the relation between the rate of development of the eggs and the time at different temperatures.

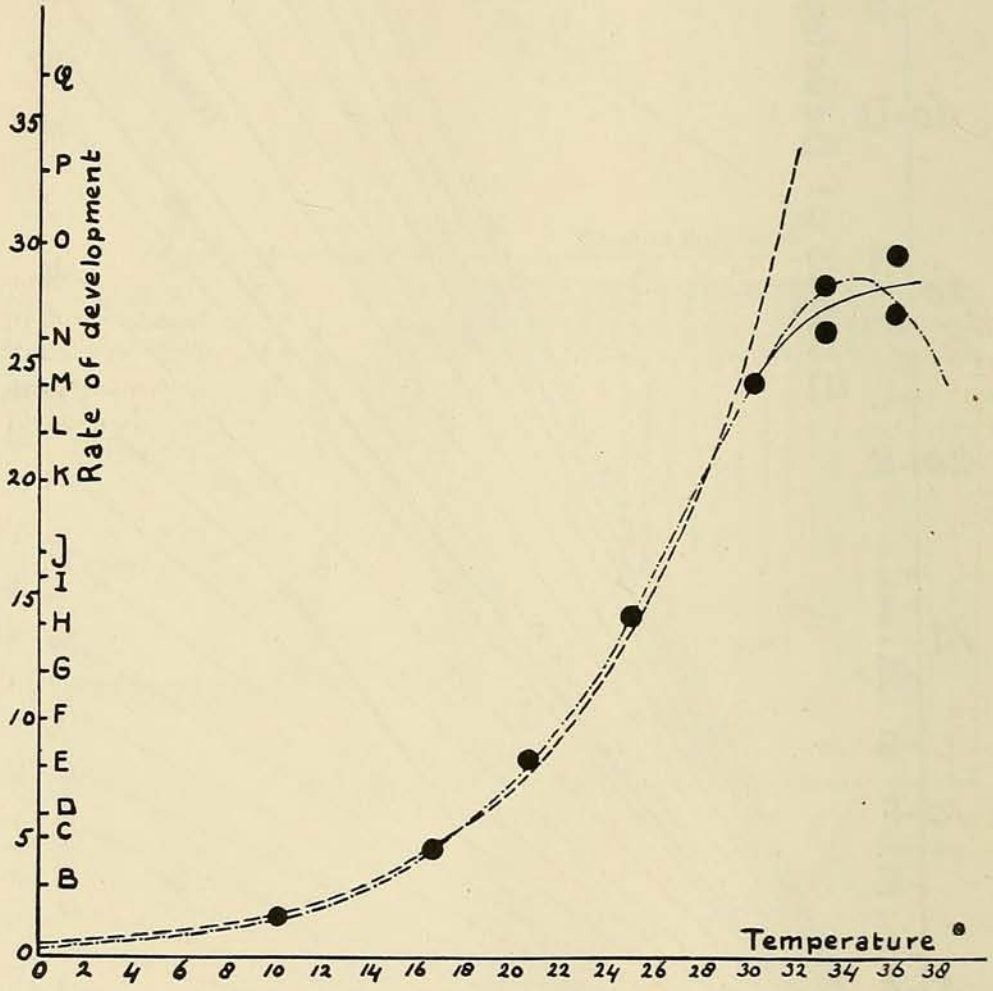


Fig. 68. Relation between the rate of development of the eggs and the temperature after experiments in the thermostate.