INVESTIGATION ON EMBRYONIC CUTICLES IN INSECTS

by

RISHIKESH KANT SHARAN
M.Sc. (Benares)
Lecturer in Zoology,
(Science College, Patna, India)

Department of Zoology,
University of Edinburgh.

THESIS
Presented for the Degree of Ph.D.
in the University of Edinburgh.

November 1953
Resubmitted in August 1956.

CONTENTS

INTRODUCTION 1

MATERIAL AND METHODS 4

MAIN STAGES IN THE EMBRYONIC DEVELOPMENT OF A LOCUST . 10

STAGES IN THE METAMORPHOSIS OF DROSOPHILA 13

STRUCTURE OF THE PROVISIONAL CUTICLE 17

Histochemical methods 18

Results 20

CHANGES IN THE EPIDERMIS DURING MOULTING IN EMBRYOS OF L.PARADALINA AND T.MOLITOR 24

PROGRESS IN DEVELOPMENT OF THE INTERNAL SYSTEMS IN RELATION TO THE DEPOSITION OF CUTICLE 33

Study of a Hemimetabolous Insect 33

Study of a Holometabolous Insect 40

THE BEARING OF THE DEVELOPMENT OF THE MUSCLES UPON THE DEPOSITION OF EMBRYONIC CUTICLE 47

COMPARISION OF PROVISIONAL CUTICLES PRODUCED DURING EMBRYOGENESIS AND METAMORPHOSIS 56

The Development of the Different Systems of Organs and Tissues during the Metamorphosis in Drosophila ... 57

Changes in the Epidermis in relation to the different Moults which occur at Metamorphosis 72

MOULTING OF PROVISIONAL CUTICLE IN RELATION TO SOME OBSERVATIONS OF GLANDULAR ORGANS 79

CONCLUSION 88

SUMMARY 93

REFERENCES 96

INTRODUCTION.

The presence of a provisional embryonic cuticle had already been observed in several insects (Aphidae, Orthoptera, Hemiptera, Neuroptera, and some types of Coleoptera), and it was reasonable to assume that its occurrence was universal in the group (Wiggleworth 1950).

A protective role has been ascribed to it, on the basis of it assuming a protective function in some insects. For instance, in this case, of Grasshoppers they emerge from the egg invested with a cuticular envelope. This envelope affords protection as the young nymph climbs upward through the soil from the buried egg pod to the surface. But whereas this example is a legitimate one, it is difficult to maintain that its presence in all insects has a protective function. Often, the provisional cuticle is discarded before emergence.

A cuticular sheath of this kind also occurs in some spiders and crustaceans, implying that its production is an arthropodan feature.

Information on these provisional embryonic cutticle was hitherto very fragmentary. In fact, they had been regarded as structures that happened to be present. Whereas their protective role had been emphasised no explanation /

explanation has been put forward to account for the production of an extra cuticle when it was shed before the insect emerged from the egg.

Poyarkoff (1914) in his paper entitled "Essai d'une theorie de la nymphe des insectes holometabolous." suggested that the shedding of the pupal cuticle was associated with the attachment of the muscles to the cuticle. He maintained that the rest of the internal systems of tissues and organs did not themselves require this shedding of the pupal cuticle. Hinton (1947) has also referred to this problem.

The main purpose of this work has therefore been to attempt to investigate the formation and shedding of the provisional cuticles in insect development particularly in relation to the development of associated muscles. Observations have also been made on the origin of endocrine glands in the embryos of Locustana paradalina in view of their detection in Locusta migratoria.

I am grateful to the Government of Bihar, India, for the grant of scholarship, which enabled me to do my work here. I wish to thank Professor James Ritchie and Professor Michael Swann for giving all the laboratory facilities and suggestions. I also wish to thank Dr. B. M. Jones.

I want also to express my heartfelt thanks to all the members of staff of Zoology Department for their constant help; and especially to Dr. D.R.J.Wallace and Mr. Fox for the photomicrographs.

MATERIAL AND METHODS.

In this study large numbers of embryos of *Locustana paradalina* and of *Tanebrio molitor* were used. The different stages in the metamorphosis of *Drosophila* were also examined.

Embryos of *Locustana paradalina* were reared from pods of dried quiscent eggs. The egg pods had been collected from East African sites in 1950. They were kindly sent by Dr. J.J.Matthee. The period of diapause was potentially over, but the embryos within the dry eggs did not advance in their development if they remained dry. However, when the eggs were moistened and kept in closed dishes development recommenced. Thus developing embryos in any required stage were obtainable by simply transfering the pods of quiscent eggs from stock to the conditions described.

The large number of locust egg pods were therefore kept in stock until required. Development was restarted by wetting the eggs and keeping them at 30^{0}C, 100% R.H.

It was important to keep the eggs moist, right up to the time of the emergence of the nymph. Each pod was kept in a separate petri dish, lined with moist cotton wool and filter paper. At a uniform temperature of 30^{0}C it /

it took about ten to eleven days for the locust to hatch out. At about 25^{0}C the incubation period, after wetting the dried eggs, lasted twenty-two days.

The state of development and the position of the embryo was more easily observed by first removing the opaque chorion by immersing the egg in a saturated bleach solution. This treatment resulted in the exposure of the transparent serosal cuticle through which the embryo could be seen. The embryo was dissected out of the egg.

Fixation of the internal soft tissue of arthropods with their hardened exoskeleton, always presents a difficulty. This difficulty was overcome, however by using a very hot fixative and leaving the material in fixative for about twenty-four hours at 48^{0}C . This use of hot fixative was important because only in this state was it able to penetrate the cuticle. On the other hand cold fixative failed to do this.

Embedding was carried out under a binocular microscope so that the material could be properly orientated in the block to provide sections cut in the desired plane. Embedding of the material was carried out in a glass ring, about ½ inch in diameter, which rested on a glass slide. Moulten wax was first placed in the ring. The material was then /

then transferred into the wax. To do this a warm pipette was used. The material was then manoeuvred into place with the help of a warm needle. Four sets of scars at right angles were put at the edge of the hardened wax to indicate the two axes of the material. The slide and ring of wax containing the orientated material was first transferred to lukewarm water and afterwards to cold water. In this way it was then possible to press the wax out of the ring. Serial sections of the embryos at different stages in development were consequently prepared. Mayer's Haemalum and alcoholic Eosin were the principal stains used since they gave the best results. Alcohol of 96% strength was used during the final stages of dehydration. Absolute alcohol was avoided since final dehydration and clearing was done in amylacetate. The material was embedded in paraffin of 56°C melting point. Provided the edge of the razor remained particularly sharp the cuticle when cut did not impair the inner part of the serial sections.

The eggs of _Tenebrio molitor_ were obtained as follows. First, stocks of larvae were reared at about 30°C. When the adults were produced they were removed from the stock culture and transferred to petri dishes lined with black filter paper. The eggs when they were laid showed up against the black background of the filter paper. Some bran was also placed in the dish. The dish was covered with /

with a wire gauze and kept upon a stand inside a large jar containing an appropriate mixture of glycerine and water to provide a Relative Humidity of about 90 per cent. The adults were kept at a temperature of $30^{0}C$. As often as possible every day the dish was searched for newly laid eggs. If no eggs were laid, the old filter paper was removed. Afterwards fresh food was introduced, and a new filter paper was inserted. In this way any eggs that may have been present but not detected were not collected along with the freshly laid eggs the next day. The freshly laid eggs were transferred to solid glass cells at $30^{0}C$, 90% R.H.

Eggs were fixed at 24 hours intervals after being laid in hot alcoholic Bouin. Batches of eggs were fixed in this way at progressive steps in the development up to the time of hatching. The incubation period, under the contions described, lasted seven days. Whole eggs containing embryos at different stages in development were sectioned and stained with Mayer's Haemalum and Eosin.

Little difficulty was experianced in rearing <u>Drosophila</u> because there were several well tried methods to choose from, all of them being suitable for obtaining the insect at any required stage in their development. <u>Drosophila</u> was needed to study the differentiation of the tissues to the adult state and the formation of provisional cuticles.

Migratory /

Migratory larvae, togather with some food, were transferred from stock bottles to petridishes lined with black filter paper. The food consisted of a mixture of maize meal, treacle and agar which were thoroughly mixed with yeast a day before the food was actually used. The larvae, while burrowing in the food and feeding were kept at a temperature of 25°C. When the larvae had migrated from the food placed in the centre of the dish they were examined every fifteen minutes. In this way, the prepupae were separated as soon as they formed. It was very easy to detect the prepupal stage because immediately before it is formed the migrating larva becomes immobile and everts its spiracles. When this takes place the event is regarded as the beginning of the prepupal stage. The prepupae were transferred to moist rearing chambers and kept at a temperature of 25°C . From the time the spiracles were everted, <u>Drosophila</u> material was fixed at hourly intervals over the first sixteen hours period, so that the progress of development at the end of each successive hour could be followed in serial sections. Thereafter, <u>Drosophila</u> was fixed at longer intervals during the pupal phase and up to the time that the adult emerged. In the case of the <u>Drosophila</u> as with the other insect material previously mentioned the fixative used was hot alcoholic Bouin, and the principal stains used were Mayer's Haemalum, D. Haematoxylin and alcoholic Eosin. Peterfi's celloidin method /

method was also used for embedding. But the material was mostly prepared by using 96% alcohol and amyl acetate instead of absolute alcohol and Xylol which tends to harden the material. The puparium was removed before the pupa was prepared for embedding. However the puparium during the very early stages of metamorphosis cannot be removed. This is due to it still being attached to the epidermis for four hours after the eversion of the spiracles. The puparium even up to thirteen to twenty-five hours after eversion of the spiracles is still closely applied to the epidermis. Indeed, to avoid damaging the epidermis no attempt was made to remove it this time.

MAIN STAGES IN THE EMBRYONIC DEVELOPMENT OF A LOCUST.

It has already been mentioned that wetting of the quiscent eggs of Locustana paradalina induced a recommencement of development. (Matthee 1951).

After wetting the egg swells and the embryo enlarges. The present study of the development of Locustana paradalina was carried out at 30^{0}C and 100% R.H. Matthee (1951) has calculated that the dormant state is entered 24 hours after the eggs are laid. So the embryo was in an early state of development when the development recommenced after the wetting of the eggs. The eggs were full of yolk and the embryonic envelops were present. The epidermis had formed a mould, the cells were already differentiated and had taken their position to form different organs and tissues. From now onwards development proceeds briskly. The number of days referred in the text denotes the days after the wetting of the dorment eggs. Five days later the embryo bursts through the fused amnioserosal membrane and revolves round the posterior pole. The provisional embryonic cuticle is completed on the fourth day and it is loosened from the epidermis on the sixth day. A second embryonic cuticle is secreted in the place of the sloughed cuticle. The muscle fibres which were not fully formed when the provisional embryonic cuticle was secreted by the epidermis gain attachment to the /

the second embryonic cuticle. On eighth day the muscle fibres were fully attached to the second embryonic cuticle by means of tonofibrills.

The nymph emerged on 10th or 11th day. Thus at the time of its emergence the nymph is covered by two cuticles, the provisional embryonic cuticle which forms a cover all round the embryo and the second embryonic cuticle which is the functional cuticle of the nymph.

The newly emerged nymph was in a helpless condition, without any power of locomotion for sometime. Soon it freed itself from the envelope of the provisional embryonic cuticle. It then gained the power of locomotion and started hopping about.

It was observed that the development of L.paradalina is a continueus process. The development progressed uniformly without in any way effected by the formation of cuticles.

A brief chronological lists of events in the embryology of L.paradalina at 30^0C and 100% R.H. was therefore as follows.

Days	Events.
1	Epidermis has formed a mould.

Days	Events.
	Cells have taken their position to form different organs and tissues. Stomodaeum and proctodaeum were already differentiated. Brain had taken its shape.
2	Myoblast tissues were formed.
4	Epidermis showed activity and the provisional embryonic cuticle was secreted. Muscle fibres were not yet striated.
5	Blastokinesis completed.
6	Provisional embryonic cuticle moulted and in its place other cuticle was deposited.
8	Muscle fibres have gained attachment to the second embryonic cuticle. Almost all the organs and tissues like alimentary canal, Salivary glands, Brain and ganglia were more or less completely formed.
10	The young nymph emerged out of egg.

STAGES IN THE METAMORPHOSIS OF DROSOPHILA.

The interest in the developmental mechanism and genetics of Drosophila has augmented our knowledge of its development. Nevertheless since the present work was concerned with the times when the different cuticle loosened away from the epidermis and the times when the epidermis began to lay down a new cuticle it was desirable to investigate carefully the stages in metamorphosis at 25°C.

On being immobilised the larval shape gradually changed to that of pupa. This entailed the shortening of the larva brought about by muscular contraction. The larval cuticle also shortened and formed the puparium. Segmentation was obliterated. When the shape of the pupa had been assumed the prospective puparium was at first white. However the shaped puparium remained white for a short time only, about three to five minutes. Thereafter, it hardened and darkened very quickly. Two hours later the seams of the so called 'operculum' on the anterio-dorsal region of the puparium were formed. These seams are lines of weak resistance so that when the eversible sac or 'ptilinum' located just above the base of the antennae is pressed against this operculum it opens and enables the young adult to emerge from its hard protective case.

About /

About four hours following the time when the puparium was white, a useful mark from which to designate the different stages, the epidermis retracted away from the puparium. This step in development marked the beginning of the so called prepupal stage. The insect in this stage can be dissected out of the puparium. It was covered with a provisional cuticle the prepupal cuticle. This stage lasted for about five hours.

The end of the prepupal stage was signalled by a contraction of the insect, particularly at the anterior end. So once again the epidermis retracts from the cuticle, namely the prepupal cuticle and the insect entered the pupal stage.

The imaginal organs were all everted and the insect more or less assumed the form of the adult. It was now surrounded by both the puparium and the shed prepupal cuticle. When this has been accomplished the epidermis deposits the pupal cuticle.

It is therefore legitimate to regard the beginning of the prepupal stage with the accompanying unfoldings of the imaginal organs as marking the initiation of adult development. For at this time, it was also significant that the larval epidermis was replaced by a new layer of epidermal cells proliferated from the regenerative regions of /

of the imaginal buds. However this replacement process as will be pointed out later in this work extend over a long period. The pupal stage lasts about 25 hours. Its end is marked by the epidermis retracting from the pupal cuticle. This moult occurred when the epidermis was still composite, being made up of both imaginal and larval cells. Thus the insect was now enclosed by three loose envelopes the puparium, the prepupal cuticle and the pupal cuticle.

A chronological list of events in the metamorphosis of *Drosophila* at 25^0C was therefore as follows.

Hours.	Events.
0	Puparium shaped but still white.
1	Imaginal buds start unfolding. Puparium fully coloured.
4-5	Epidermis retracted from the puparium to mark the beginning of the prepupal stage.
11	Epidermis retracted from the prepupal cuticle to mark the beginning of the pupal stage. Imaginal organs everted.

Hours.	Events.
37	Moult of pupal cuticle and end of pupal stage.
96	Emergence of the adult.

STRUCTURE OF THE PROVISIONAL CUTICLES.

The cuticle of an insect is composed of two basic layers, the outer thin epicuticle and the inner endocuticle.

The epicutcle may be further divided into three and sometimes four layers. There may or may not be a thin outer 'cement layer' overlying an outer wax layer. Beneath this wax layer is the polyphenol layer. The inner layer is called the cuticulin layer.

The endocuticle particularly in a hardened exoskeleton possesses an outer region of tanned protein, namely sclerotin, and chitin and an inner region composed of chitin and protein which has not been tanned.

In the formation of cuticle the cuticulin layer is the first to be deposited. This is followed by the deposition of procuticle. The next step is the synthesis and release of polyphenol substances and the deposition of a wax layer. The final stages entail the hardening of a polyphenol substance secreted from the dermal glands onto the surface to form the protective cement layer and the deposition of the remainder of the endocuticle.

In the laying down of a provisional cuticle particularly during embryonic development it was to be expected /

expected since it was so thin that only some of the components had been deposited.

The aim of carrying out the histochemical tests, described in the next section on embryos of Locustana paradalina and Tenebrio molitor was to determine at what time during embryonic development deposition of the provisional embryonic cuticle commenced and what components were actually laid down.

Histochemical methods

In order to detect the first signs of the formation of embryonic cuticle, the silver test of Lison (1936) was adopted and used in the way devised by Wigglesworth (1948).

The provisional embryonic cuticle of L.paradalina were subjected to two type of tests the chloroform treatment and Acid and Caustic treatment.

This chloroform treatment test is based on the property of the cement layer of cuticle to resist dissolution in cold chloroform and so it protects the underlying wax layer which is soluble in cold chloroform. Both the cement and wax layers can be removed by immersing the cuticle in boiling chloroform for an hour. The cement and wax layers can be removed also by rubbing the surface with cotten wool soaked in chloroform.

Hence, /

Hence, when cuticle is pre-treated in cold chloroform, and after immersion in silver solution the surface becomes chestnut brown it implies that a wax layer is present as the outer layer of epicuticle . This is referred to as argentaffin reaction. If on the other hand there is no reaction after pre-treatment, the presence of an outer cement layer is indicated. For the determination of the presence of polyphenol layer the cuticle is boiled for about one hour in chloroform. It is then immersed into silver solution. The polyphenol layer then takes up stain.

A control test was run, that is to say, untreated cuticle was put into silver solution.

For the determination of cuticulin layer the provisional embryonic cuticle was subjected to Acid and Caustic treatment. In the Acid treatment test cuticle is cautiously heated in concentrated nitric acid. The endocuticle swells and becomes bright yellow; there is a vigorous evolution of gas and the endocuticle dissolves but the epicuticle remains.

In the caustic treatment test a piece of embryonic cuticle is boiled in chloroform for about onehour . This is done to dissolve the cement and wax layer. Then the cuticle is heated in 10% caustic until the endocuticle dissolves/

dissolves and a thin layer, the cuticulin layer is left. Further heating will cause this layer to break up into oily droplets, which suggest it is composed of a fatty substance. This cuticulin layer is thought to be a lipoprotein.

The above histochemical tests were performed on the embryos of locust at different stages to detect the beginning of secretion of cuticle. They were also performed upon the embryonic cuticle of newly emerged nymph. As mentioned before the newly immersed nymph is covered by the loose provisional embryonic cuticle.

In case of T.molitor these tests were performed only on the provisional embryonic cutice after the emergence of the larva.

Results.

The presence of an embryonic cuticle has been observed in several insects and as already mentioned, it has been suggested that its formation is universal in both holometabolous and hemimetabolous insects. In L.migratoria the formation of the embryonic cuticle has been described by Roonwal (1937), and he believed that its formation took place one day after the completion of the blastokinesis. In the present investigations serial sections of embryos in different stages of development were examined, and /

and as described above histochemical tests were made to detect the components of the cuticle. Histological evidence and, more important, the results of histochemical tests, showed that the provisional cuticle first began forming between third and fourth day after the egg had terminated its dormant state, on wetting (Fig.1) that is to say one day before the completion of blastokinesis.

The histochemical tests showed that the provisional cuticles of L.paradalina and Tenebrio molitor were composed of two primary layers. The epicuticula and the endocuticula. In both the insects the epicuticula was composed of four distinct layers. An outer thin colourless 'cement layer', a wax layer, a polyphenol layer and a cuticulin layer. A detailed chemical determination of the different layers of the provisional embryonic cuticle did not come within the scope of this work. Wigglesworth (1947) and Pryor (1940) have, among others, hitherto described the chemical nature of these different layers. Wigglesworth (1947) has provisionally regarded the cement layer as a protein with just sufficient preformed quinones to lead to a rapid spontaneous hardening. It may either be a modified loe or belong to some other undefined group of chemical substances containing protein as an admixture. The polyphenol layer is secreted through the pore canals, but /

but not a great deal is known about its chemical nature. Wigglesworth (1947) described it as being most probably made up of protein material, very rich in dihydroxy phenols. The tanning of the protein being due to the phenol becoming oxidised in part to quinones which in turn tan the protein. Cuticulin is provisionally regarded as being polymerized lipoprotein, subsequently tanned by quinones, (Pryor, 1940). The outer part of the provisional cuticle of Locustana took up the haematoxylin stain indicating an exocuticle.

Observations on the serial sections of Tenebrio molitor showed that the provisional embryonic was first formed on the fourth day after laying of eggs, as an extremely thin continuous layer upon the epidermal cells. Thereafter the deposition of cuticle followed the normal course and the provisional embryonic cuticle obtained the features of normal exoskeletal cuticle. Detailed results of histochemical examinations of the provisional cuticle has already been mentioned before in this section.

Histological evidences showed that this provisional embryonic cutice of Tenebrio molitor was also composed of an epicuticula and an endocuticula. But the outer region unlike the provisional embryonic cuticle of Locustana paradalina /

paradalina did not take up any stain, thus indicating a lack of tanning in the outer region. The endocuticle was therefore a uniform layer.

The important conclusion to be deduced from these observations on the structure of the provisional cuticle is that it is a normally formed cuticle. The use of the terms like pellicle, envelope and so on, previously used to describe the provisional embryonic cuticle, was apt to give the impression that it was composed of substances unlike those of normal cuticles.

The epidermis therefore deposits a provisional cuticle in the normal way, and all the complicated series of events connected with the formation of an epicuticula and an exocuticular region are carried out.

PLATE I

Illustrates the condition of the epidermis in L.paradalina during different stages of its embryogenesis.

Fig. 1. Condition of the epidermis, four days after the breaking of the dormant state of the embryo by wetting the egg.

Fig. 2. Condition of the epidermis, five days after the breaking of the dormant stage by exposure to moisture.

Fig. 3. Condition of the epidermis, six days after breaking of the dormant condition of the embryo by exposure to moisture.

a, provisional embryonic cuticle; b, nucleus;
c, chromatin material; d, moulting gland;
e, second embryonic cuticle.

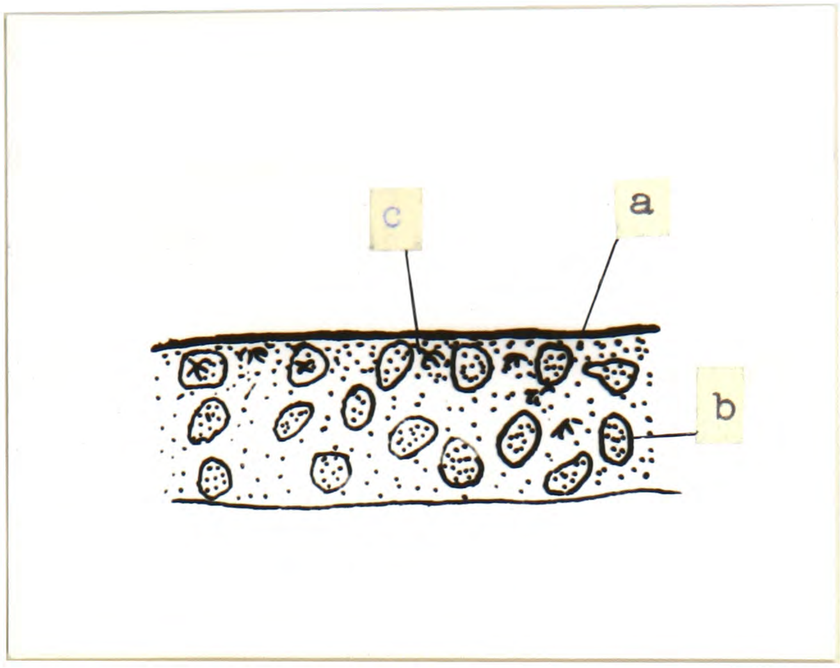


FIG 1

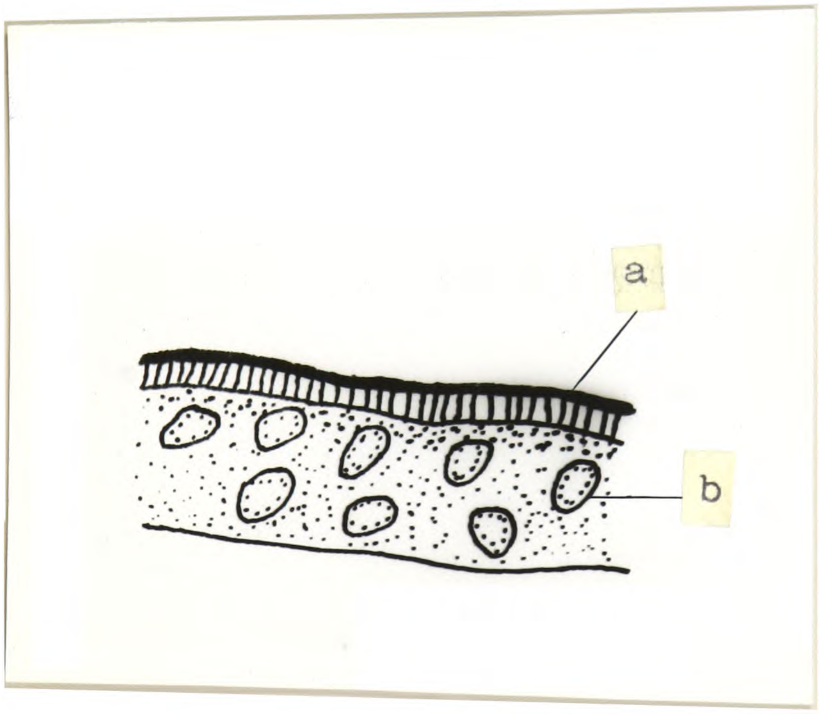
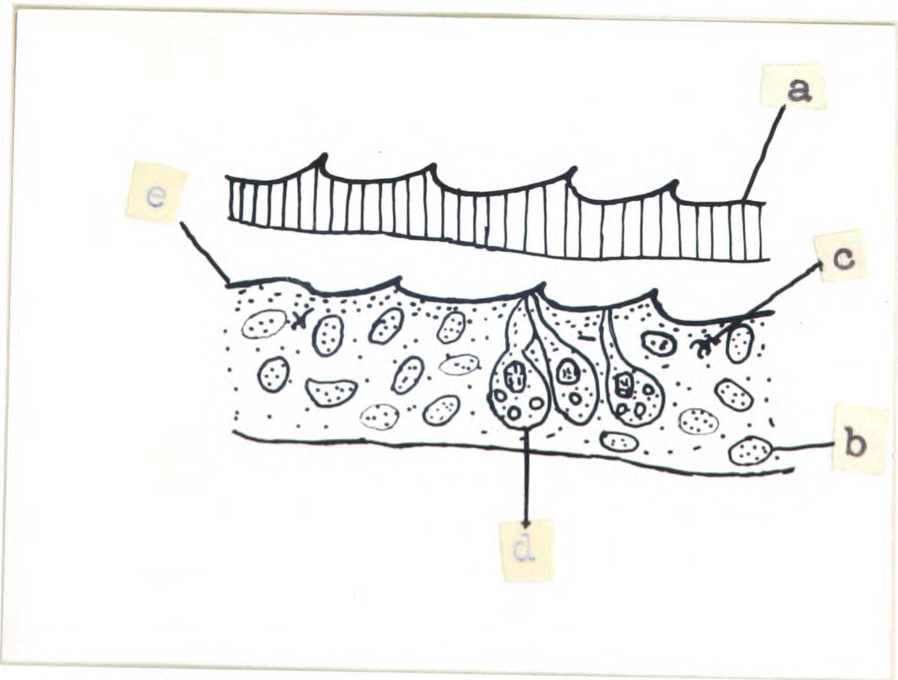


FIG 2.

20µ



CHANGES IN THE EPIDERMIS DURING MOULTING IN EMBRYOS OF L.PARADALINA AND T. MOLITOR

It has already been mentioned that the provisional embryonic cuticle of Locustana paradalina is first formed on the third to the fourth day after the development had been initiated by wetting the dry eggs. The epidermis was examined particularly with a view to detect any cyclical changes effected in it, coinciding with the embryonic moult and the subsequent deposition of the new cuticle after the sixth day, i.e., the day following the completion of blastokinesis. The epidermis in locust embryo is composed of tiers of cells, which are most certainly active during the first four days after the termination of the dormant period. At this time the myoblasts formed the muscle bands, which were in the process of differentiation (Pl.V, fig.1,2). The epidermis on the third day showed an intense activity, as denoted by the relatively large number of mitotic figures, and by the presence of chromatin globules in the cells. The activity of the epidermis corresponded with the laying down of the provisional cuticle. The epidermis was still very active on the fourth day, by which time the cuticle was being secreted (Pl.I, fig.1). The epidermis was less active on the fifth day (Pl.I, fig.2). As will be obvious from the figure, on this day the /

the nuclei of the epidermis showed lesser number of chromatin granules, which were mostly arranged towards the periphery of the nucleus. The number of mitotic figures were decidedly less at this time than they were at the four and six days stages (Pl.I, fig.1,3). But on the sixth day, i.e., one day after the completion of the blastokinesis, the epidermal cells became more elongated and once again became active (Pl.I, fig.3). Soon after this the epidermis retracted from the embryonic cuticle. Relatively large number of mitotic divisions and chromatin globules were found scattered in the cells, and these were regarded to constitute a visible sign of a process of "rejuvenation" which permits the cells to renew their growth (Wigglesworth 1950). Some of these may come from the dissolution of the entire nuclei.

Besides these, a large number of unicellular glands were also observed in the epidermis. These glands were two to three times the size of other epidermal cells and they mostly occurred in groups. These glands did not have many chromatin granules and their nucleus was characterised by the presence of clumps of chromatin material. The cytoplasm of these glands presented a vacuolated appearance at this stage, thus showing a stage of intense activity. /

PLATE II

Shows the condition of the epidermis at different times during the embryogenesis in *T.molitor*.

Fig. 1. In four days old embryo.

Fig. 2. In five days old embryo.

Fig. 3. In six days old embryo.

a, nucleus; b, provisional embryonic cuticle; c, definitive cuticle; d, chromatin material.

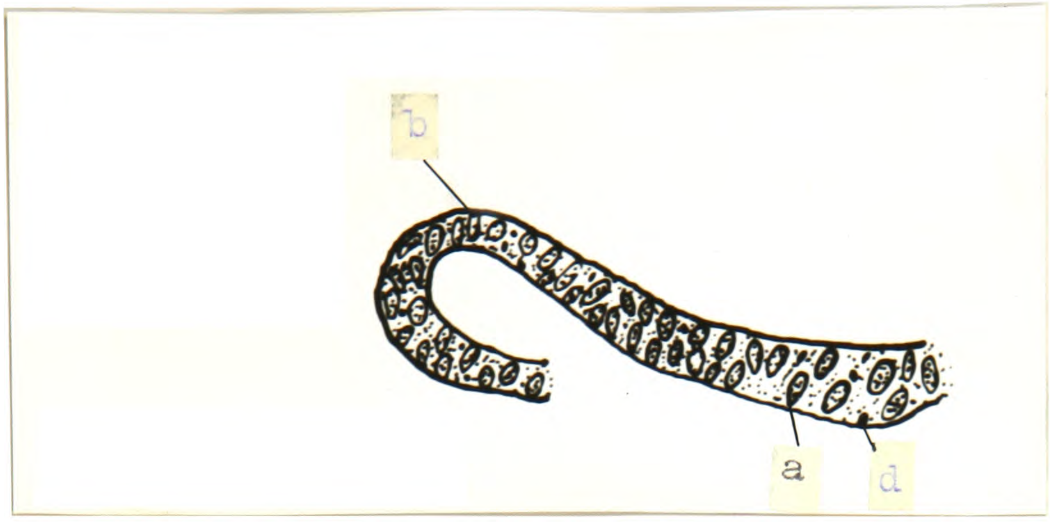


FIG 1

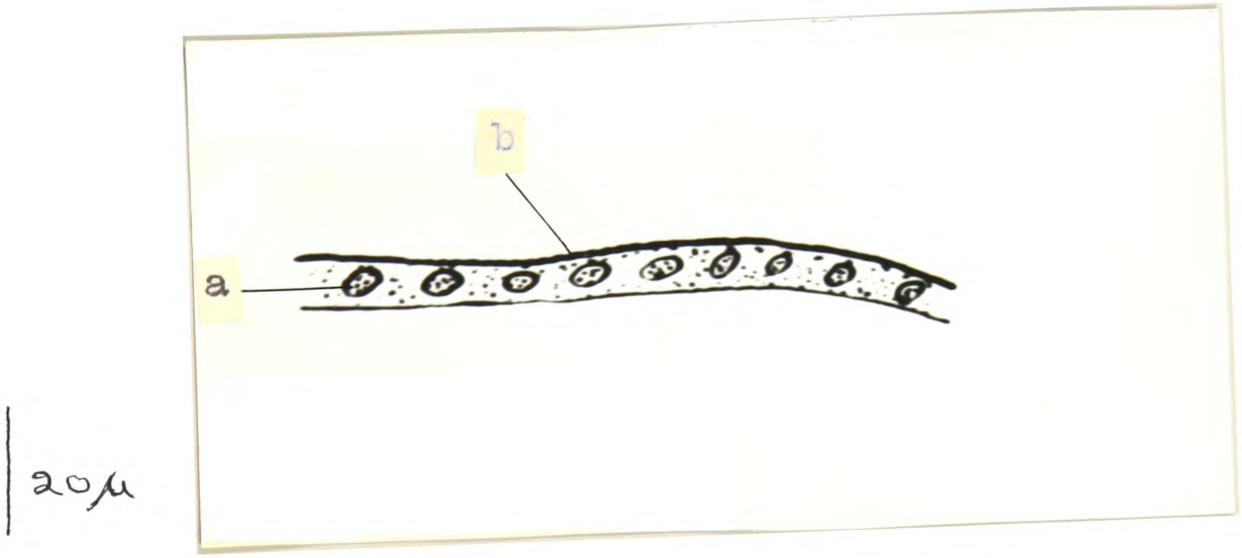


FIG 2.

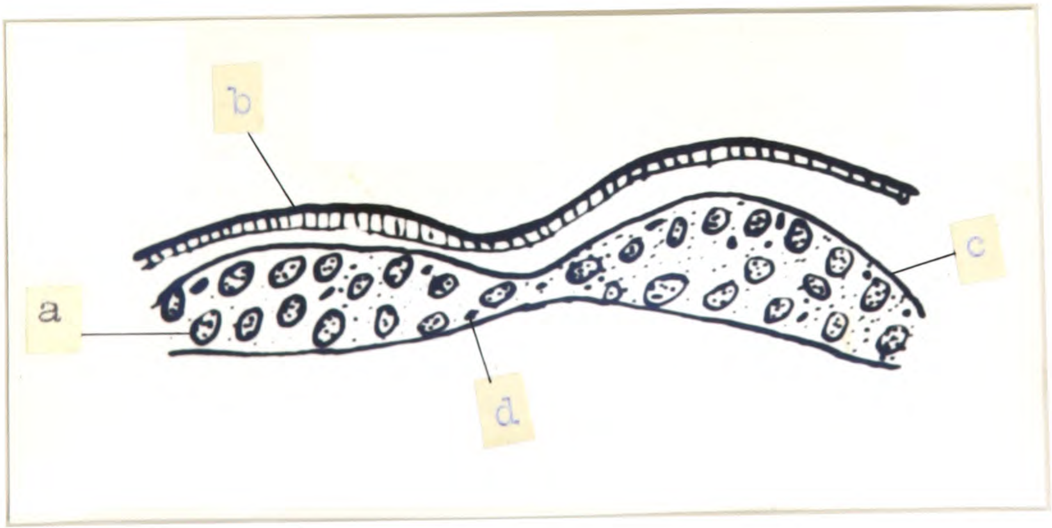


FIG 3.

activity. The glands opened to the surface by means of small ducts (Pl. 1, fig. 3).

The function of these glands is supposed to be to secrete the moulting fluid, which separates the provisional cuticle from the epidermis, so that a new cuticle might be secreted at their place. In the support of this idea, it may be pointed out, though a thorough search was made for these glands on other stages, specially on the three-four days stage, when the provisional cuticle was being secreted, they could not be located. Their presence only at the time when the moulting is taking place lends strong support to their being regarded as moulting glands.

The presence of these unicelluler moulting glands has been described by Tower (1906) to be present in embryonic as well as in later stages. Wigglesworth (1933) describes these unicelluler glands in Rhodnius prolixus.

Thus from the above description of the condition of the epidermis it will be noted that in L.paradalina an intense phase of activity in the epidermis during embryogenesis appeared to coincide with both the commencement of the deposition of the cuticle and the subsequent embryonic moult.

In /

In Tenebrio molitor the provisional embryonic cuticle started forming itself on the fourth day after the deposition of the eggs. The epidermis was intensely active on the fourth day. Mitotic divisions had increased and a relatively large number of chromatin globules were present (Pl.II,fig.1). These signs of activity were in marked contrast to the quiet state of epidermis on the second and the fifth day (Pl.II,fig.2). However, on the sixth day, when the embryonic moult occurred the epidermis again became extra active (Pl.II,fig.3), with a large number of mitotic divisions completed and the presence of chromatin globules scattered in the epidermis.

On the fifth and the sixth day a large number of dermal glands made their appearance in the region of epidermis (Pl.VIII, fig.2). The dermal glands of Tenebrio molitor were found to be multicelluler, and tabular in structure . They were composed of epidermal cells, which had become modified and sunk into the dermis (Pl.VIII, fig.2). These glands were composed of a large number of secretory cells and opened to outside by means of a narrow duct. It is difficult to say, whether parts or whole of these glands are secretory.

These glands were absent on the fourth day of embryogenesis, when the provisional embryonic cuticle was /

was being secreted. Their absence during that period suggests that they are not concerned with the secretion of the cuticle. On the other hand their presence, 6 during the time of moulting, suggests that the secretion of these glands seems to help in the moulting of the provisional embryonic cuticle.

Keeping the above description in view,it is reasonable to conclude that in *Tenebrio molitor* also the period of maximal activity of the epidermis coincides with that of cuticle secretion and its moulting.

At the time of their emergence from the egg, both *Locustana* and *Tenebrio* species were enclosed by a provisional embryonic cuticle. In each case this provisional embryonic cuticular envelope was cast off soon after the emergence. During this period the insect appeared to be in an quiescent stage. The cuticular envelope formed a loose covering, more or less as in a pupa. Its ~~this~~ condition is, therefore, not unlike the pupal state of a holo-metabolous insect, where the pupal cuticle corresponds to the provisional cuticle of the embryo. Since the embryonic provisional cuticle of locust was shed by retracting of the epidermis on the sixth day, i.e., about five days before emergence, this envelope, as one would expect, possesed slightly larger dimension than the enclosed /

enclosed body and it resembled a sack with out-pockets for housing the appendages. The provisional embryonic cuticle of Tenebrio was shed by the epidermis only two days before emergence. In a newly emerged Tenebrio larvae the provisional cuticle is much more closely applied to the newly deposited cuticle. This is shown very clearly in sections of newly emerged larvae of Tenebrio (Pl.VIII, fig.3). By contrast, in the locust, the exuvial space between the provisional cuticle and the newly deposited cuticle was relatively substantial (Pl.XVII, fig.3).

In view of the preceding results it is of interest at this stage to consider the suggestion that embryonic provisional cuticles have a protective function. As already mentioned the formation of an embryonic cuticle is common among arthropods and cursory observation of the cuticular envelope enclosing the larva, newly emerged from the egg would lead one to assumption that their role was a protective one. This would most certainly appear to be its function in the case of the Grasshoppers. The egg pods are laid in the ground by the grasshopper and the newly emerged nymphs have to crawl upward through the soil to the surface. During this ascent to the surface the nymph is still enclosed by the provisional cuticle and there is every reason to suppose that it does serve to give /

give protection against abrasion at a time, when the newly deposited cuticle is by no means complete.

But one need only consider the subject chosen for study in this work to question the validity of ascribing this protective function to insects in general.

In the Locustana paradalina the newly hatched nymph was immobile immediately after its emergence, when it was still enclosed within its provisional cuticle. This insect appeared at that time to be incapable of active locomotory movement, approaching anything like that, which would be required to force its way up through the soil to the surface of the ground. Even the definitive cuticle was by no means hardened sufficiently to take the pull of the muscles and this could account for the absence of active locomotory movement. After adequate hardening of the definitive cuticle had occurred it could take the pull of the muscles, and when this had occurred the insect shed the provisional cuticle and began its active life. In its natural environment it appeared more unlikely that the provisional embryonic cuticle gave a protective role like that of the Grasshopper mentioned above; it simply stayed as a loose coating around the nymph.

Again in the case of the provisional cuticle of

the /

the embryo of Tenebrio molitor , it would be difficult to attribute a pretective function to it. These beetles haunt sheltered sites in the flour mills and in store houses and live on spilled food, which accumulate in cracks and crevices. They are, therefore, usually found in small or large numbers in the immediate neighbourhood of the sites of food. The eggs are laid on food substances and so the larvae as they emerge from the eggs are provided with their food supply. Hence no active locomotory movements are required in their cases. Therefore, it is difficult to conclude that the provisional embryonic cuticle has any protective role to play in this case.

While the examples of embryonic cuticle taking up the function of protection are legitimate ones, it must, however, be borne in mind that there are probably a larger number of cases in which the provisional cuticle is formed and shed by the insect before it emerges from the egg shell. The provisional cuticle is seldom detected in such cases because it is left behind in the egg shell. In such cases the provisional embryonic cuticle cannot take up any of the above two functions.

Poyarkoff (1914) has suggested that provisional cuticle either embryonic or pupal, owe their existence to /

to the developmental factors, directly connected with an interesting inter-relationship between the cuticle deposition and the muscle development, which allows a proper attachment of the muscle fibrillae to the cuticle. The same author, has also pointed out that the provisional cuticle obtains a protective role as a secondary aquisition. A case of preadaptation. This is principally due to the insect acquiring a provisional cuticle of developmental origin and turning it into account after gravitating into a habitat and a mode of existence, in which the provisional cuticle take on a protective function.

PLATE III

Photomicrograph, showing the condition of the fore-gut and the hind-gut of *L.paradalina* at different stages of its embryogenesis.

Fig. 1. Fore-gut two days after the breaking of the dormant stage by exposing the egg to moisture.

Fig. 2. Fore-gut four days after the termination of the dormant condition by wetting the egg.

Fig. 3. Hind-gut four days after the termination of the dormant condition by wetting the egg.

a, fore-gut; b, phyrynx; c, oesophagus; d, crop; e, gastric ceaca; f, brain; g, anterior intestine; h, rectum; i, malpighian tubule; j, yolk. k, boundary of mid-gut and hind-gut.

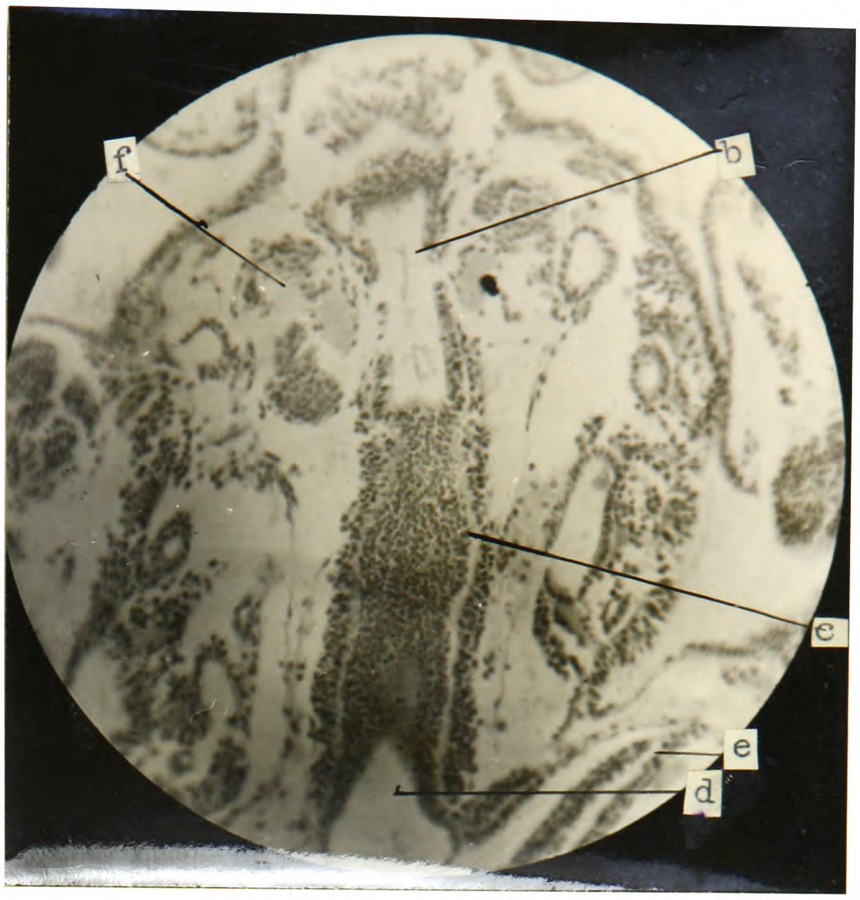


Fig 1 FIG 2.

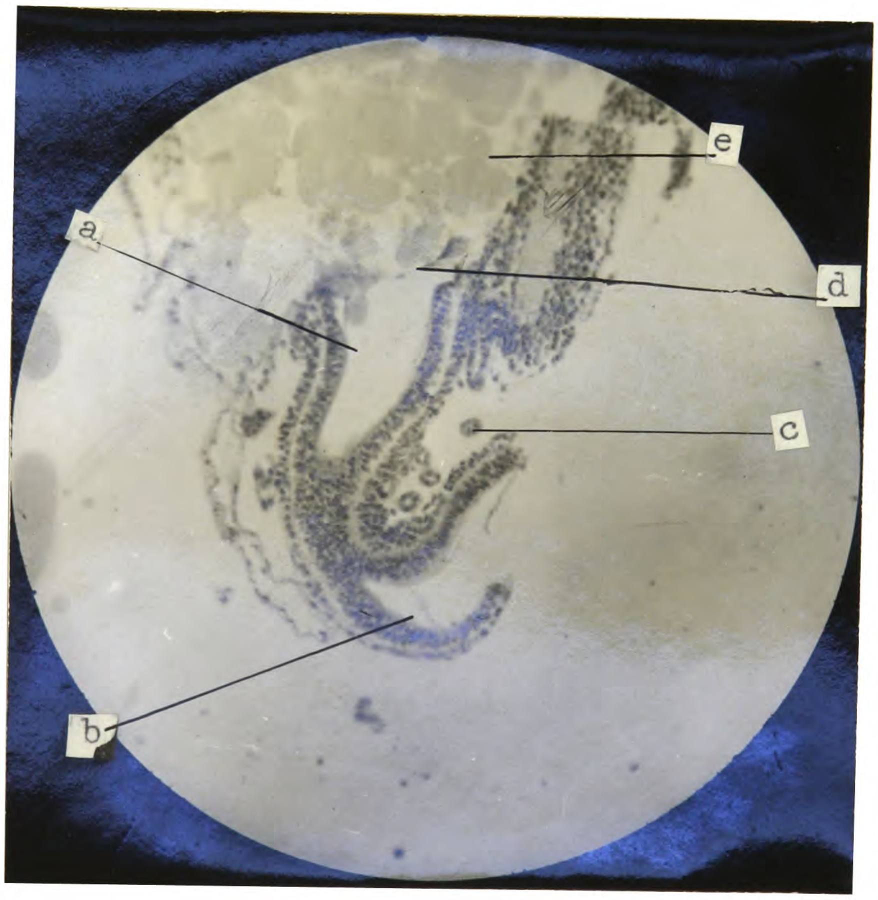


FIG 3

PROGRESS IN DEVELOPMENT OF THE INTERNAL SYSTEMS IN RELATION TO THE DEPOSITION OF CUTICLE.

Study of a Hemimetabolous Insect.

Locustana paradalina lays two kinds of eggs, one which will enter a diapause state, the other capable of continuous development until the emergence of the instar from the egg. When both the diapause and non-diapause eggs have sufficient moisture (all other conditions being favourable), they will develop at more or less the same rate. Non-diapause eggs take between five and six days to reach the step in development which precedes katatrepsis. Katatrepsis occurs on the fifth day and hatching of the hoppers begins on the tenth to eleventh day at 30°C and 100% R.H. In diapause eggs the embryo reaches the step in development just before katatrepsis on the seventh day, and it is at this time that the embryo enters into the diapause state, when all development is arrested. The embryo will enter a state of dormancy after the period of active diapause has terminated and, provided the external conditions remain harsh, the embryo will not develop. Matthee (1951) observed that it may take as much as thirty-six to ninety-five days or even longer to break the actual diapause condition if the eggs are kept continuously under favourable conditions. But after exposure to /

to dry conditions the diapause condition is "broken" in a much shorter time when the eggs are subject to a quick change from a dry to a wet atmosphere.

When conditions are harsh and dry at the time the eggs are laid, development in both diapause and non-diapause type is arrested prior to katatrepsis, and they will remain dormant until suitable conditions return. At the time of such an arrest in their development, both types of eggs more or less reach a similar step in their development. At this time too, almost all the organs have taken shape, for example, the rudiments of the eyes, antennae, mandibles, two maxillae, and the three pairs of jointed legs. The animal has become segmented into the usual divisions. The rates and manners of development of the different systems were compared from the time when development recommenced, on stimulating the dormant embryos by exposure to wet conditions. Matthee (1951) has calculated that the dormant state is entered twenty-four hours after the eggs are laid, but in the following text the age in days implies the time after the termination of the dormant state. Roonwal (1937) has studied the development from the four-cell stage, which occurs about five hours after the egg is laid. But since one of the aims of the present work was to investigate the developmental conditions of /

of the different systems of organs and tissues, at the time of the retraction of the epidermis from the provisional embryonic cuticle, it was essential to observe the progress of development from the termination of dormancy to emergence.

Moreover, since already it has been discovered in the present work that the deposition of cuticle begins a day before and not after katatrepsis (Roonwal 1937), it has been thought worthwhile to record here, although briefly, the sequence of events in the development of the different systems.

Particular attention was paid to the progressive development of the skeletal muscles in relation to the moult, and to the times when various incretory centres, including the newly discovered prothoracic glands, were located with confidence.

Alimentary canal. It was observed that the stomodaeum (Pl.III, fig.1) and the proctodaeum had taken shape before the beginning of the dormant state, but the mid-gut was not formed at this stage. The gastric caecae, which are six in number in this case, arose from the wall of the fore-gut on the fourth day (Pl.III,fig.2). By this time the crop, oesophagus and the pharynx were also /

also differentiated (Pl.III,fig.2). By the fifth day the proctodaeum became differentiated into its two basic parts, the rectum and the anterior intestine (Pl.III, fig.3). The malpighian tubules were also budded off from the anterior part of the proctodaeum on the fifth day (Pl.III,fig.3). The yolk mass was present until one day before the emergence, and it was at this time that the mid-gut became discernable (Pl.IV, fig.2).

The possible origin of the mid-gut has given rise to several opinions. According to Nelsen (1934), who worked on the differential locust (Melanopus differentialis), the mid-gut rudiment is derived from the end of the inner layer associated with the blind end of the stomodaeum and proctodaeum; although he does not deny the possibility of the ectodermal tissue migrating inward from the endoderm. Thus he maintains that it originates from anterior and posterior rudiments.

Stuart (1935), working on the same insect, arrived at a different conclusion regarding the origin of the mid-gut epithelium. He states that shortly before hatching, the yolk cells peripherally form a temporary lining upon the inner surface of the mesodermal components of the mid-gut. About the time the insect hatches, each yolk cell /

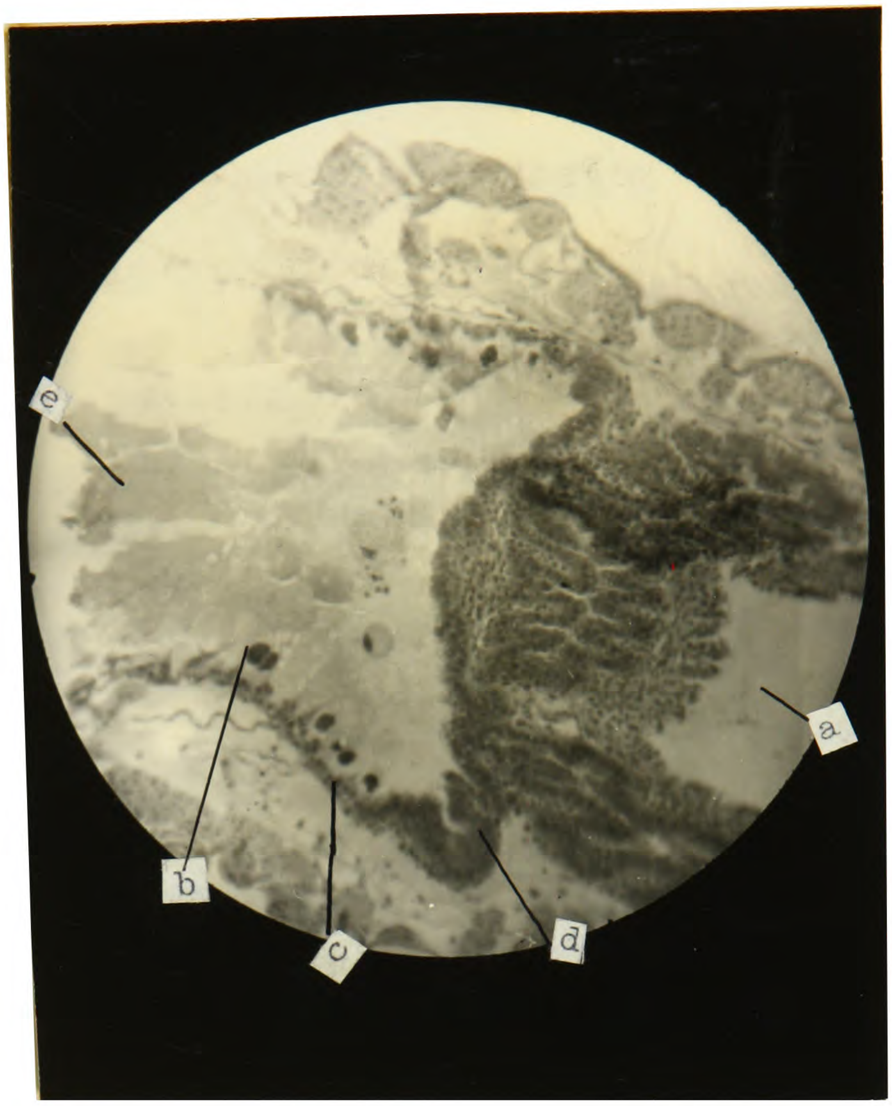
PLATE IV

Photomicrograph, showing the stage of development of the mid-gut of *L.paradalina*, subsequent to the breaking of the dormant state of the embryo by exposure to moisture.

Fig. 1. Condition of the mid-gut nine days after the termination of the dormant state.

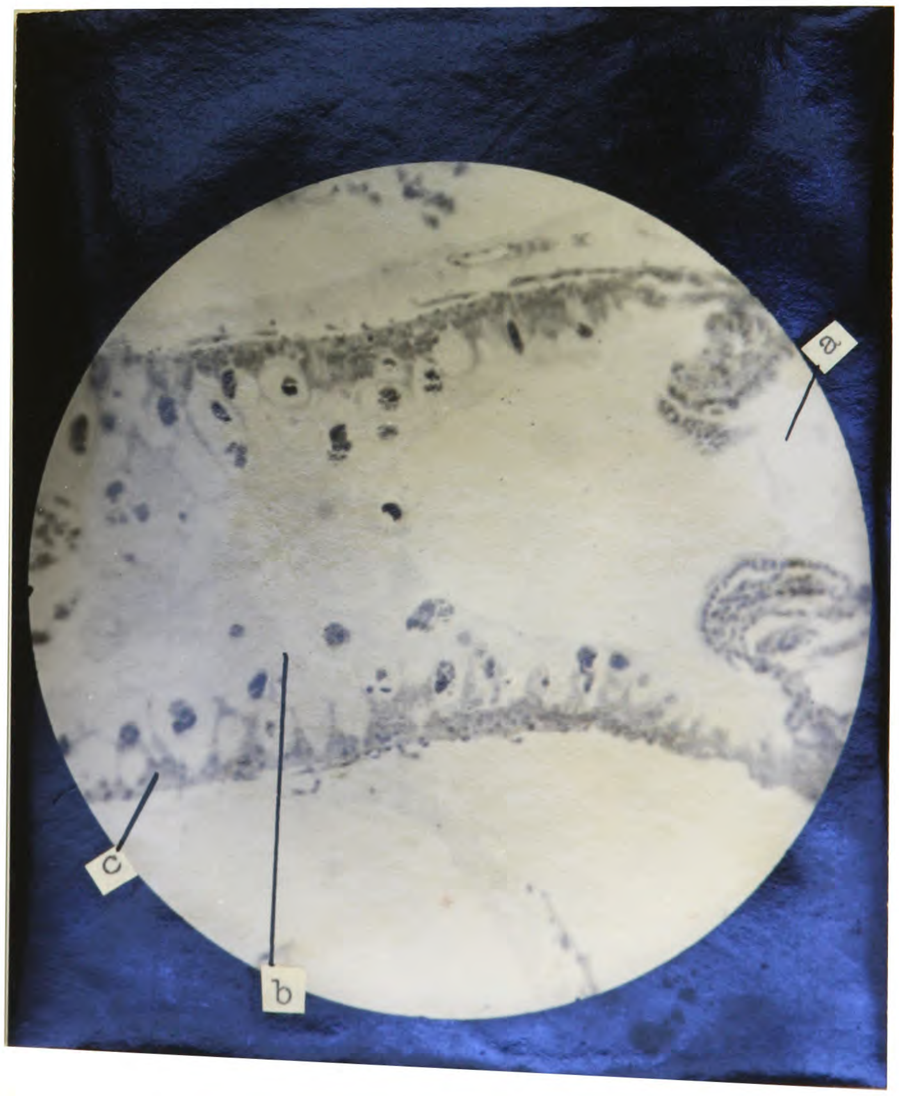
Fig. 2. Near completion of the wall of the mid-gut after ten days.

a, fore-gut; by, mid-gut; c, cells proliferating from the regenerative centres; d, regenerative centres; e, yolk.



|145μ

FIG 1



|145μ

FIG 2.

cell nucleus divides itself into a dozen, or more, smaller nuclei, which he designated as the "Presumptive Epithelial Nuclei." Each of these nuclei then takes a portion of the yolk cell cytoplasm and thus are formed the definitive mid-gut epithelial cells.

The present investigation indicated that the mid-gut originated from proliferating masses of cells, which were derived from the fore-gut and the hind-gut (Pl.IV, figs.1,2). Hence they are ecotodermal in origin. As mentioned above, soon after the completion of blastokinesis was found that the position of the mid-gut was occupied by a yolk mass. This yolk mass was first enclosed, from the dorsal side, by the thin dorsal closure. This provisional dorsal closure soon degenerated to leave the thin splanchnic mesoderm to enclose the yolk (Pl.IV,fig.1). Two days before hatching, that is to say, nine days after the termination of the dormant state, it was observed that the regenerative centres from the two blind ends of the stomodaum and proctodaeum began to spread over the splanchnic membrane bounding the incomplete mid-gut, as well as over the yolk mass; which became divided into compartments by accumulation of the yolk around the yolk cells. It was observed that the regenerative cells spread specially along the delicate membrane dividing the yolk mass /

PLATE V

Photomicrograph, illustrating the stages in the development of the muscles in *L.paradalina*, subsequent to the breaking of the dormant state of the embryo by exposure to moisture.

Fig. 1. Formation of the unstripped muscle fibres after two days.

Fig. 2. Development of the unstripped muscle fibres after four days.

Fig. 3. Condition of the unstripped muscle fibres six days after the breaking of the dormant state.

Fig. 4. Appearance of the stripped muscle fibres after eight days.

a, myoblast tissue; b, muscle fibres unstripped; c, muscle fibres stripped; d, epidermis.

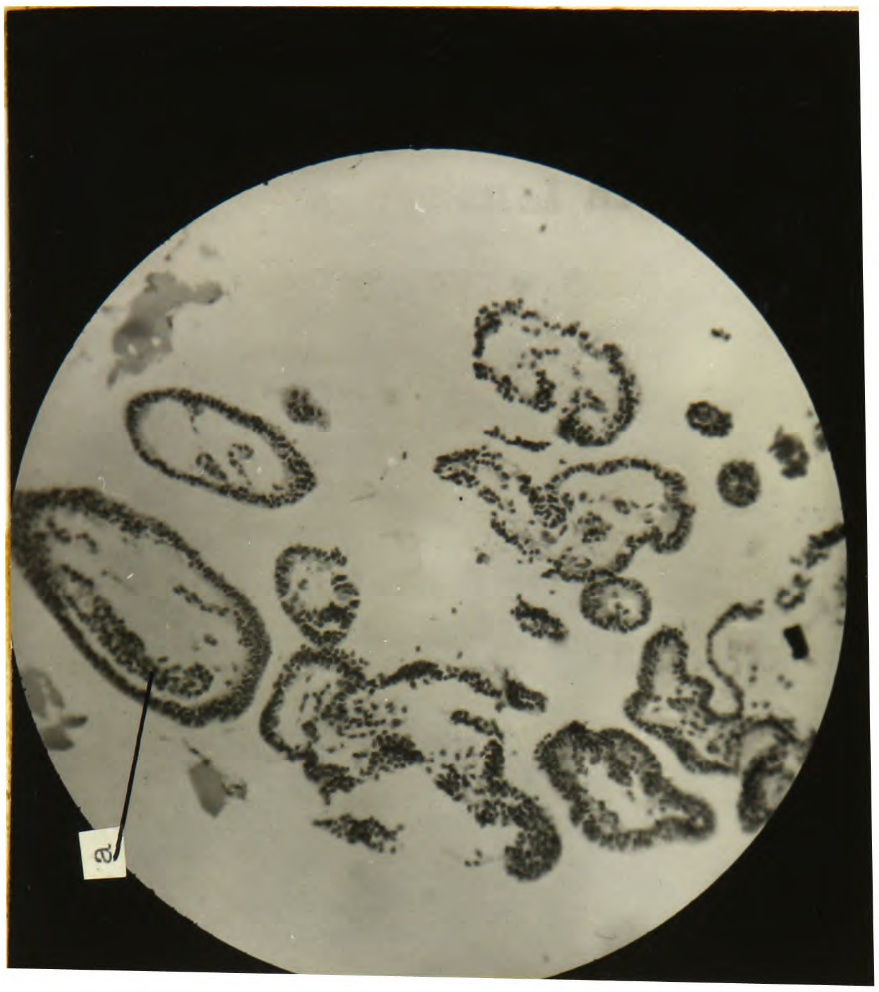


FIG. 1.

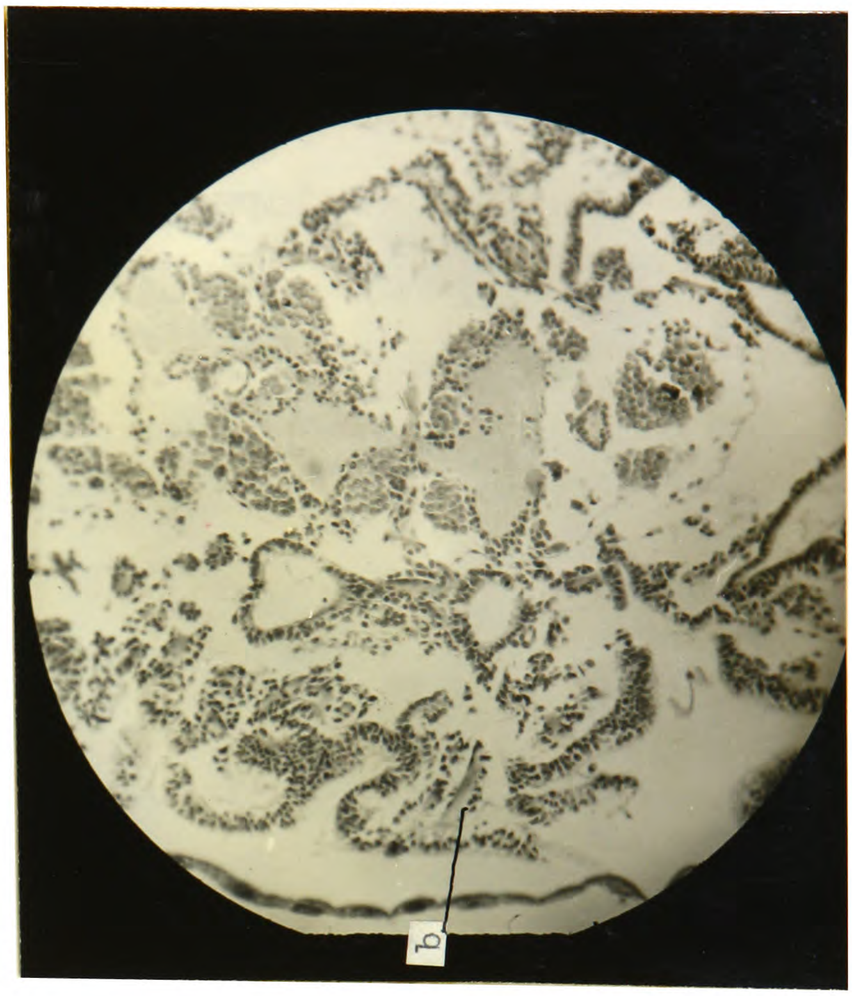


FIG. 2.

145μ

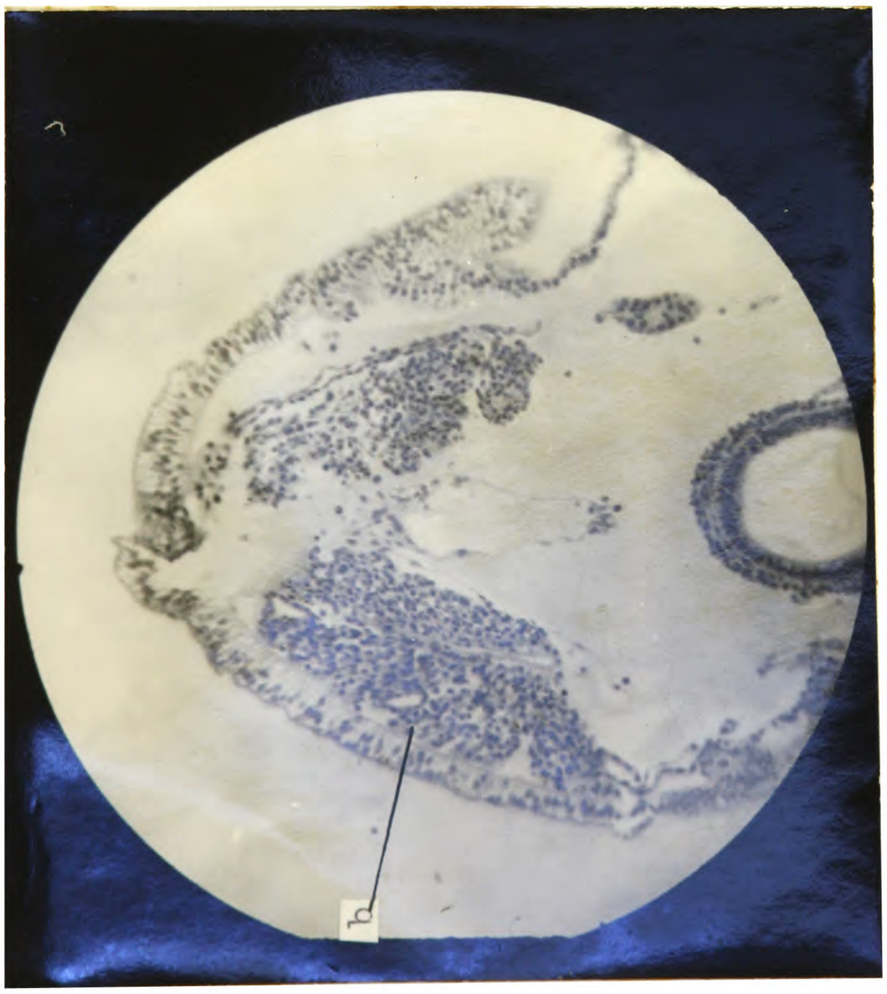


FIG. 3.

145μ

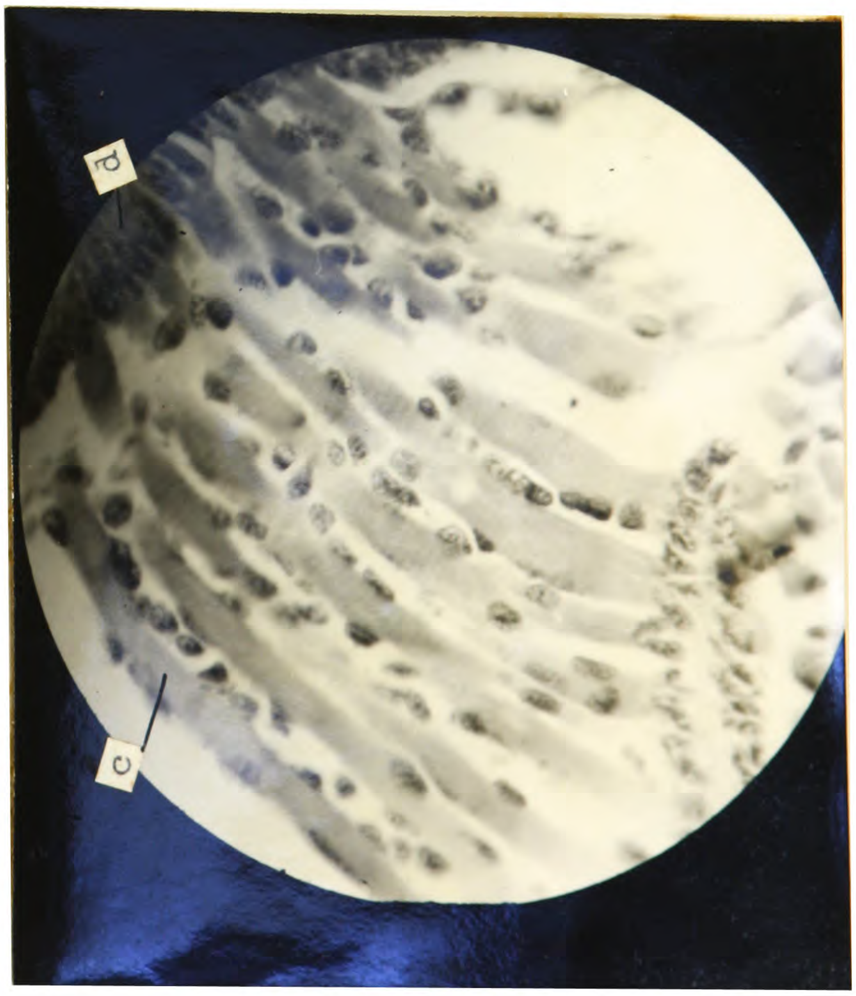


FIG. 4.

30μ

mass into compartments. It was on the tenth day (one day prior to hatching), that the mid-gut became more or less completed and the stomodael and proctodael blind ends ruptured to form a continuous canal, though some yolk, was still present at this stage (Pl.IV, fig.2). But on emergence, however, the alimentary canal became potentially functional.

Muscles. Two days after the wetting of the eggs, the first anlage of the muscles were located (Pl.V, fig.1) in thoracic pouches, in limbs and in abdominal pouches. The muscles were in the form of large number of myoblasts, surrounding definitive muscle tissues. Most of the muscles were found to arise in close connection with the epidermis. One could also expect this, of course, in the case of the skeletal muscles, with which the present investigation is principally connected. The muscle development progressed from the second day and on the fourth day additional muscles were formed. The muscles made their first appearance in the region of the head at that time (four days), (Pl.V, fig.2) . Muscles, at this stage, were elongated, but still very much in the formative stage . All the muscles took proper shape and were quite well formed by the sixth day (Pl.V, fig.3) but they were, as yet /

yet, unstriated and myoblast cells, persisted in between the muscle fibres. By the sixth and the seventh day muscles were in close contact with the epidermis, but, as yet, they had not secured proper integration with the definitive cuticle (Pl.V, fig.3). Muscles obtained their characteristic striated appearance on the eighth day (Pl.V, fig.4). Muscle fibres were seen to be joined to the cuticle by means of fine tonofibrils. A detailed description about the mode of attachment of muscles will be given later in this thesis.

Salivary Glands. On the sixth day salivary glands were well formed in the thoracic region, on the either side of the alimentary canal.

The name "salivary glands" is not very appropriate; the name "labial glands" seems to be more suitable, since it designates the place of their origin.

The function of these glands has not been established in all the types of insects, in which they are found.

Nervous system. The nerve cells were well differentiated before the katatrepsis, but the brain was observed to have taken shape on the day following the closing of the dormant period (Pl.III, fig.1).

The /

The observations on the neurosecretory cells of the pars intercerebalis will be described later on in this paper.

Prothoracic glands. These glands were discovered and identified for the first time, i.e., in the prothoracic region of the embryo (Pl.XX, fig.1,2,3). Each gland was observed to be spindle shaped and arose from the epidermis (Pl.XX, fig.1). They were first located on the fourth day after the wetting of the eggs. Thereafter they showed definite changes and reached their maximum size on the sixth day, which corresponded with the embryonic moult.

As in the case of the neurosecretory cells, detailed attention will be given later on to these glands.

Corpora-allata. These glands had taken up their customary form and position, on either side of the oesophagus by the sixth day (Pl.XIX, fig.1).

Further attention will be paid to these glands in a later section of this paper.

Study of a Holometabolous Insect

In Tenebrio molitor, the development was followed from the time the insect deposited its eggs. The development in /

in Tenebrio molitor has been followed by Ewest (1937), at 25^{0}C. The developmental period at this temperature was found to be much longer than at 30^{0}C. According to Ewest (1937), it took eleven days at 25^{0}C for the development to complete, whereas at 30^{0}C, it was observed by the author to take seven to eight days only.

The egg was found to be yolky and oval in shape. It was of creamy colour and was covered with sticky substances, to which food particles adhered.

At one day stage, a blastoderm was formed all round the yolk; on the second day, the coelomic pouches were formed. The epidermal cells at this stage were elongated. Blastokinesis was observed on the fourth day and it was found to have been completed by the fifth day. The provisional embryonic cuticle was first observed with certainty on the fourth day (Pl.II, fig.1) and it sloughed off on the sixth day (Pl.II, fig.3), when a new cuticle was secreted in its place to give attachment to the larval muscles.

As mentioned earlier, while dealing with the development of various systems of organs and tissues in <u>Locustana paradalina</u>, the present investigation was made chiefly to find out the condition of the different organs and /

and tissues at the time, when the epidermis retracts from the provisional embryonic cuticle; though a general observation has been made of the development of the different systems of organs from the very beginning.

Alimentary canal. It consists in its complete form of different parts derived from the three basic parts, viz. the fore-gut, the mid-gut and the hind-gut.

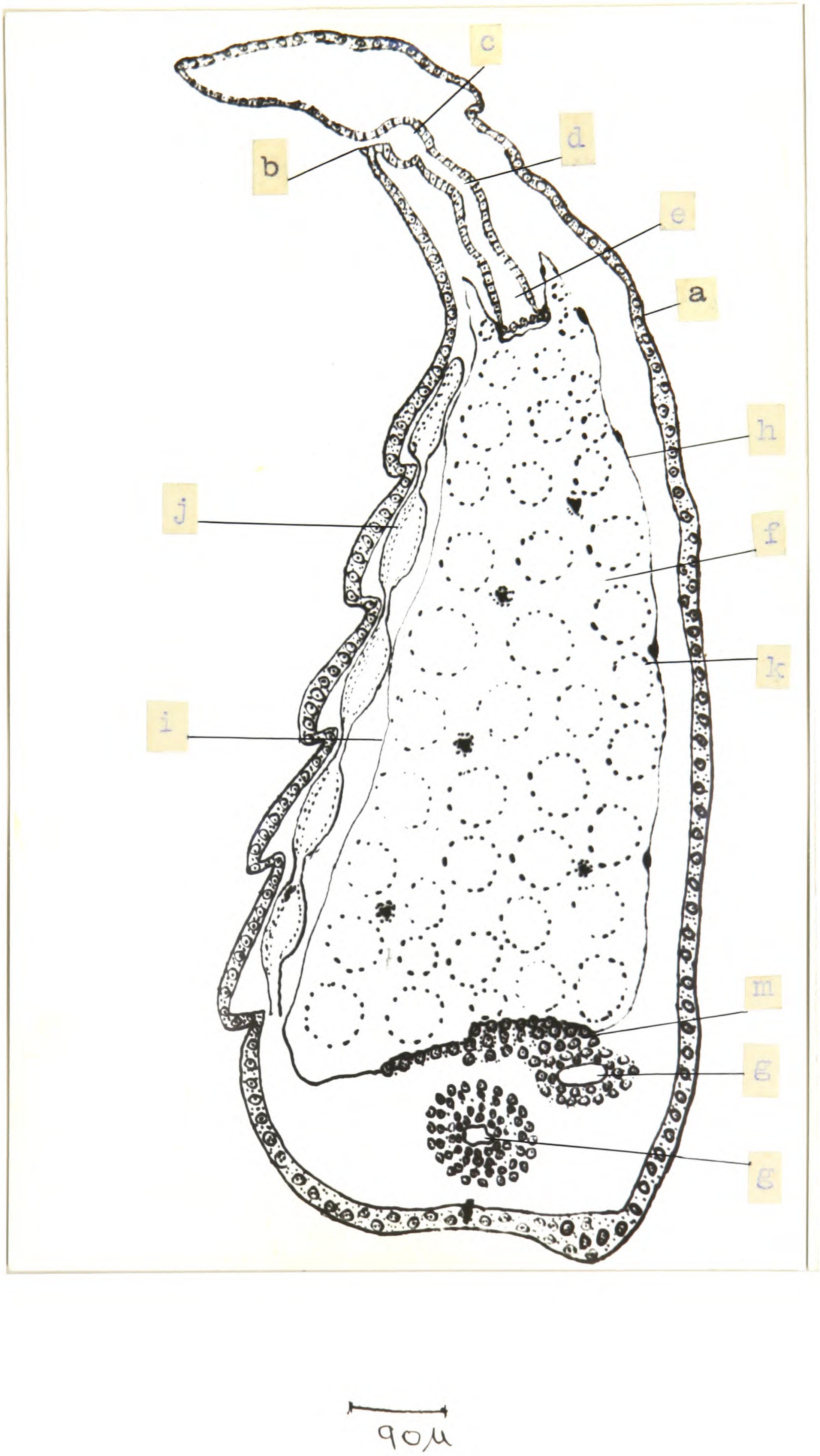
The stomodaeum was first observed in a three days old embryo. By the fifth day the differentiation of the stomodaeum into buccal cavity, pharynx, oesophagus, crop and the ventriculus was completed (Pl.VI,).

The outline of the mid-gut was first observed on the fifth day just after the completion of the blastokinesis. The yolk which was first a homogeneous mass, containing a large number of nuclei and structureless mass of cytoplasm, became divided into compartments by a reticulum. The reticulum, which is mesodermal in origin, seemed to form a provisional ventral mid-gut wall, which separated the yolk from the epineural sinus on the ventral side, and showed the provisional mid-gut wall. On the dorsal side the provisional mid-gut wall was formed by a portion of amnion (Pl.VI). This observation agrees with that of Mansour (1934), who observed the same fact in Brachyrhinus ligustici.

PLATE VI

Illustrates the condition of the developing alimentary canal and nervous system of a five days old embryo of Tenebrio molitor.

a, epidermis; b, buccal cavity; c, pharynx;
d, oesophagus; e, crop; f, provisional mid-gut;
g, hind-gut; h, amnion; i, provisional ventral mid-gut wall;
j, ganglia; k, yolk; l, boundary of fore-gut and mid-gut;
m, boundary of mid-gut and hind-gut.


c
b
d
e
a
h
j
f
k
i
m
g
g
90μ

But, on the seventh day, i.e. one day prior to the emergence, this part of amnion was replaced by the cells derived from ends of the stmodaeum and the proctodaeum (Pl.VI). By this time the cells from the stomodaeum and the proctodaeum were found to have spread all round the mid-gut, and thus formed its real wall (Pl.VII). The yolk was persistent in the mid-gut throughout the embryogenesis and was not absorbed as in the case of Locustana paradalina. , just prior to the emergence (Pl.VII).

The proctodaeum was found to have formed at the same time as the stomodaeum. Its two parts, the anterior intestine and the rectum were differentiated in a five days old embryo (Pl.VI).

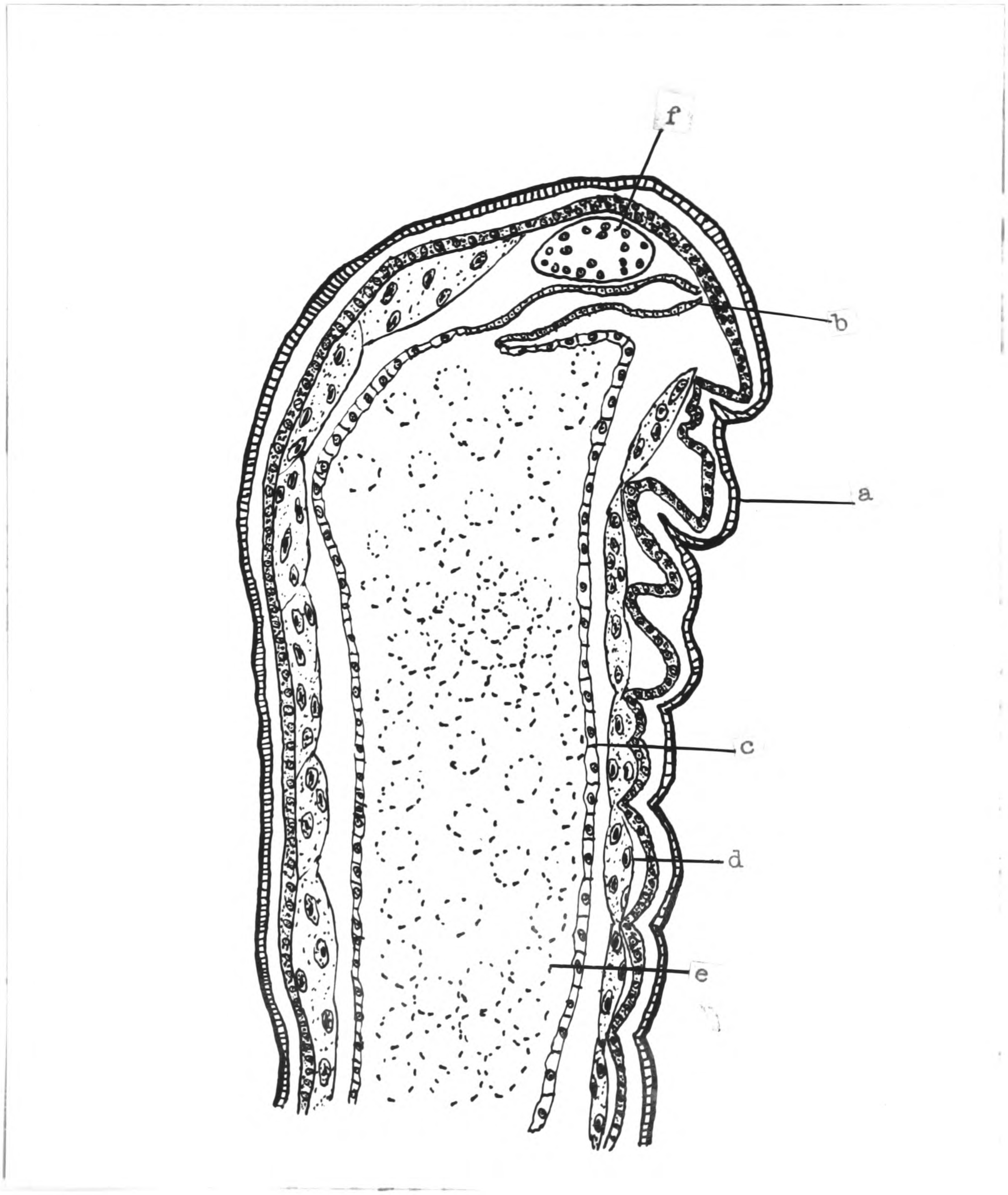
Salivary glands. No structure resembling the salivary glands were observed in Tenebrio molitor.

Muscles. The first appearance of the muscles was in a three days old embryo. The muscles first appeared in the coelomic sacs. They developed from the myoblasts in close contact with the epidermis. There were also to be seen a large number of myoblast cells, surrounding the developing muscle fibres (Pl.VIII, fig.1). The muscles in this /

PLATE VII

Illustrates the condition of the fore-gut and the mid-gut and the muscles in a seven days old embryo of *T.molitor*.

a, provisional embryonic cuticle; b, fore-gut; c, mid-gut; d, muscles; e, yolk; f, brain.



80 μ

this case also were found to be developing, without getting attached to the provisional embryonic cuticle, which, therefore, like the embryonic provisional cuticle of Locustana paradalina, was precociously formed. The muscles were quite well formed in a five day stage, but they were not yet striated (Pl.VIII, fig.2). By the seventh day the muscles were more or less completely formed and started getting attached at places to the newly laid larval cuticle. But the attachment and the striation of all the muscles were by no means completed. Attachment of all the muscles to the larval cuticle and also their striation were completed soon after the emergence (Pl.VIII, fig.3). The attachment of the muscles to the cuticle was effected by means of fine tonofibrills.The epidermal cells close to the region of attachment were absent (Pl.VIII, fig. 3) .

Nervous system. Brain was first observed with confidence in a three day old embryo. In the four and five days old embryo differentiation proceeded further and ganglia were found to have differentiated out in the most of the segments (Pl.VII). In the sixth day stage the differentiation of the nervous system was more or less completed, with the brain and a ganglion in each segment.

Prothoracic /

PLATE VIII

Shows the different stages in the development of the muscles in the embryos of T.molitor.

Fig. 1.	In a three days old embryo.
Fig. 2.	In a five days old embryo.
Fig. 3.	In a newly emerged larva.

a, myoblast; b, unstripped muscle fibres;
c, striped muscle fibres; d, tonofibrills;
e, provisional embryonic cuticle;
f, definitive cuticle.

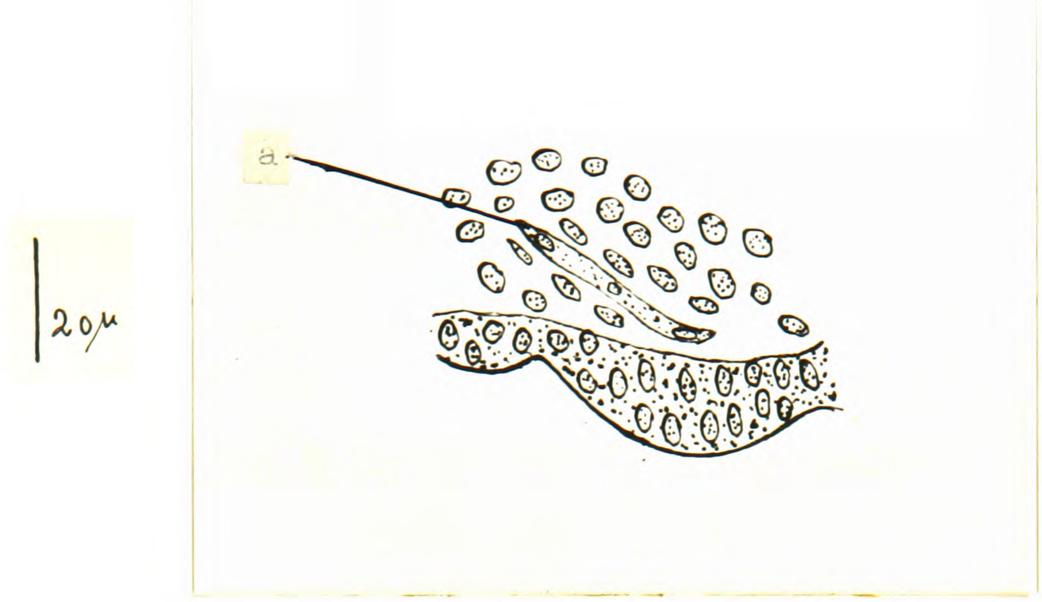


FIG. 1.

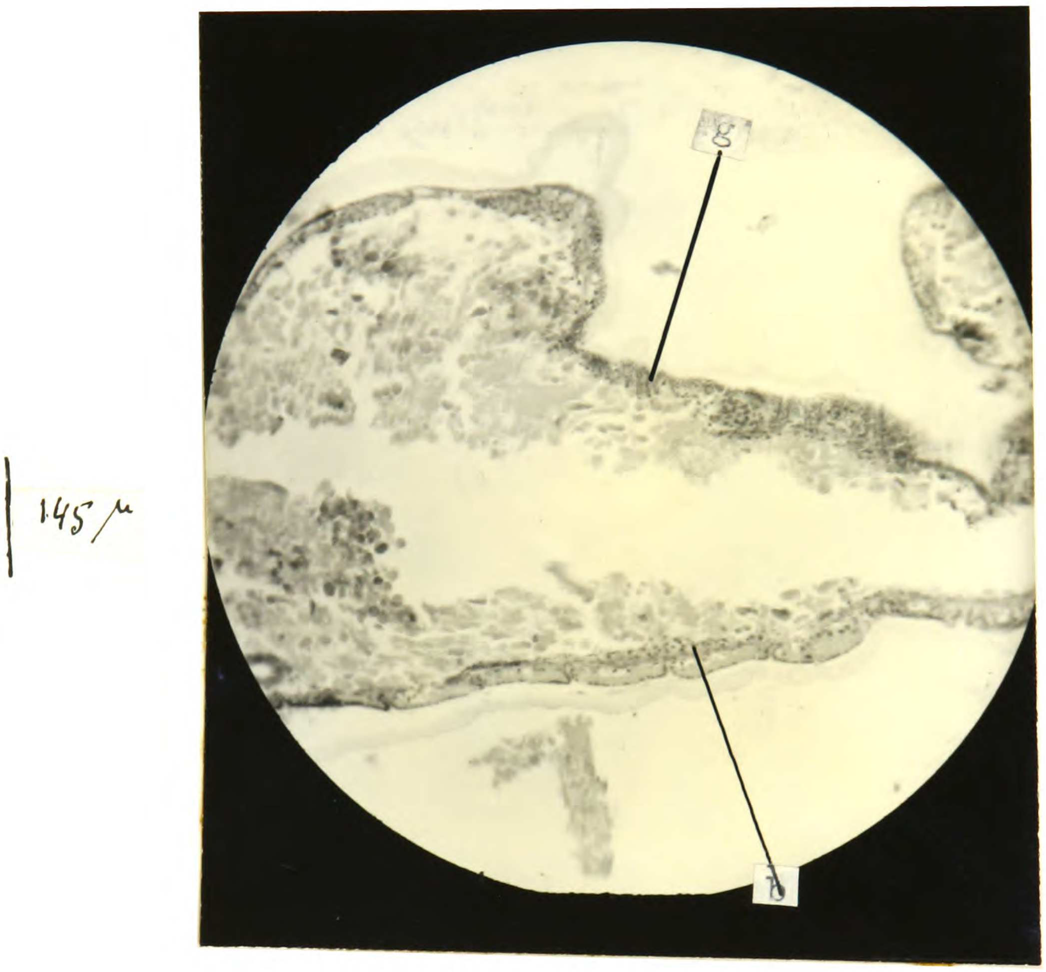


FIG. 2.

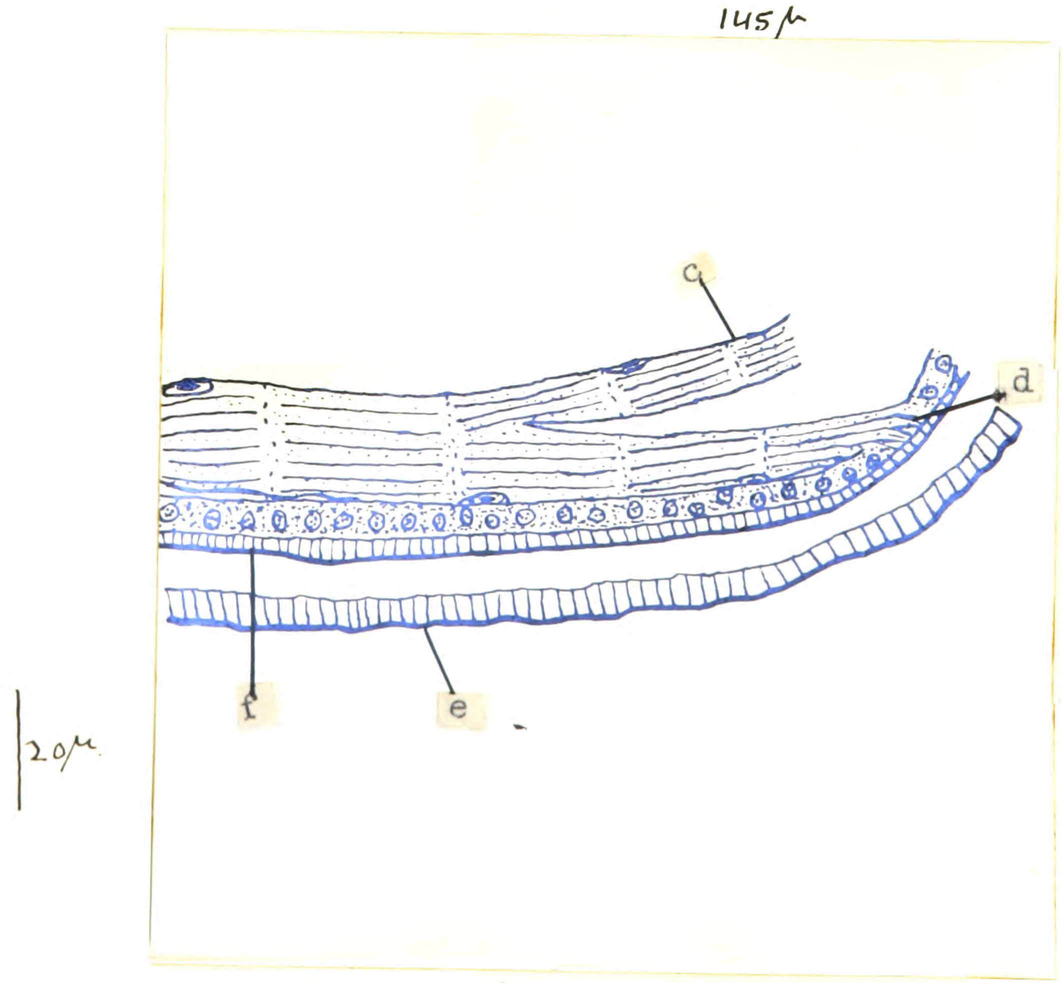


FIG. 3

Prothoracic glands. No glands resembling the prothoracic glands of *Locustana paradalina* were observed in this case.

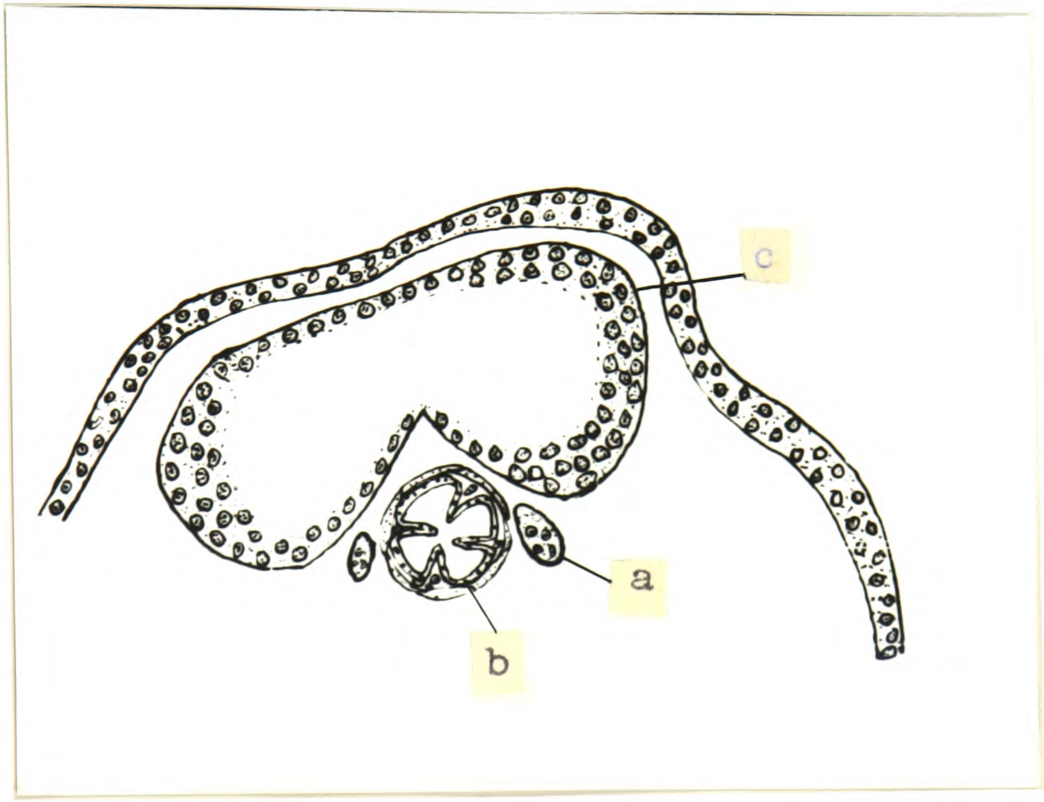
Corpora allata. A pair of structures, resembling the corpora allata, were observed on the sides of the oesophagus in a newly emerged larva. The presence of the corpora allata in the case of beetles has not been mentioned, to my knowledge, by any one else. They were first observed in their usual position, i.e. on the sides of the oesophagus in specimen (Pl.IX) just prior to the emergence of the larvae. They could not be located in the earlier stages.

It will be apparent from the foregoing description that almost all the systems of organs and tissues are more or less completely formed before the moulting of the provisional cuticle take place. The only system which is not properly formed, in the sense that it has not gained proper attachment, is the muscle system. Most of the muscles were seen to gain attachment with the second cuticle, soon after the emergence, that is, at a time when the second cuticle was still at an early stage of development. The epidermis had already constituted the mould of the larva by the time the provisional embryonic cuticle was secreted (four days). Hence, there seems to be no need for the epidermis to be without its inelastic cuticle /

PLATE IX

Illustrates the Corpora-allata in a newly emerged larva of *Tenebrio molitor*.

a, Corpora-allata; b, oesophagus; c, brain.



90µ

cuticle so far as its general moult was concerned. So like Locustana paradalina the only reason for the moulting of the provisional embryonic cuticle seemed to be to facilitate the attachment of the muscle ends to the second cuticle.

THE BEARING OF THE DEVELOPMENT OF THE MUSCLES UPON THE DEPOSITION OF EMBRYONIC CUTICLE.

In view of the fact, that different systems of organs and tissues progress without any significant punctuation in their development during embryogenesis, there was no reason to believe that this moult of the provisional embryonic cuticle indicated any kind of general upheavel. The epidermis at the time had moulded itself into the shape of the first instar. As far as the internal organisation was concerned, this mould was perfectly adequate to act as a guide for the developing systems.

But since the muscles must perforce gain attachment to the cuticular skeleton, it seemed probable that the purpose of the moult could be located in this association between the muscles and the cuticle.

Perhaps the most spectacular view of the epidermis of the insect is obtained, when one looks down upon a portion of it. Then it seems that the epidermal layer is essentially a net-work. Although primarily a single layer, yet the epidermis has the power of altering its appearance tremendously. It thickens before a moult occurs and becomes syncytical. During this period the epidermis is intensely active. There appear a large number of mitotic figures in it and rejected chromatin material /

material (Pl.I,fig.3 and Pl.II,fig.3). Throughout the developmental phase the epidermis is concerned not only with the deposition of the cuticle and the formation of the various kinds of sensory end organs, and other integumental structures, but also with providing a mould to act as a guide for the internal systems of organs and tissues developing within it. During the early part of embryogenesis, when the epidermis had already arranged itself as a guiding mould, the other organs **were** still accumulations of specialised histoblasts in the process of differentiation into the different tissues and organs. It is, therefore, reasonable to hold, on the basis of the above undisputed facts, that it is the epidermis that gives a lead to other organs and tissues.

In _Locustana paradalina_, the epidermis was found to be well formed following the termination of dormancy. The anlage of most of the organs like muscles, fore-gut, hind-gut and nervous system etc., with the exception of certain organs like mid-gut and the salivary glands, were well formed (Pl.III, fig.1).

But the epidermal mould of _Locustana paradalina_ was observed to be continuously changing and progressing towards the final shape. It appeared from the above facts, that /

PLATE X

Shows the condition of the brain, the fore-gut and the muscles in a two and six days old embryo of *L.paradalina* after the breaking of the dormant condition of the embryo by wetting.

Fig. 1. Two days old embryo.

Fig. 2. Six days old embryo.

a, epidermis; b, brain; c, fore-gut; d, myoblast; e, unstripped muscle fibre; f, trachea.

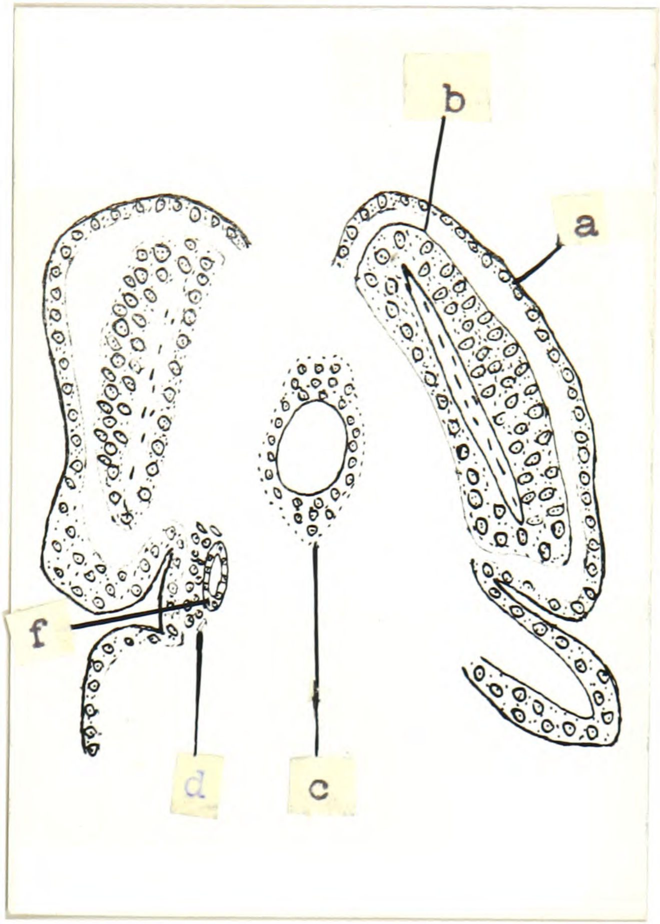
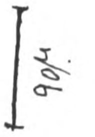


FIG. 1.



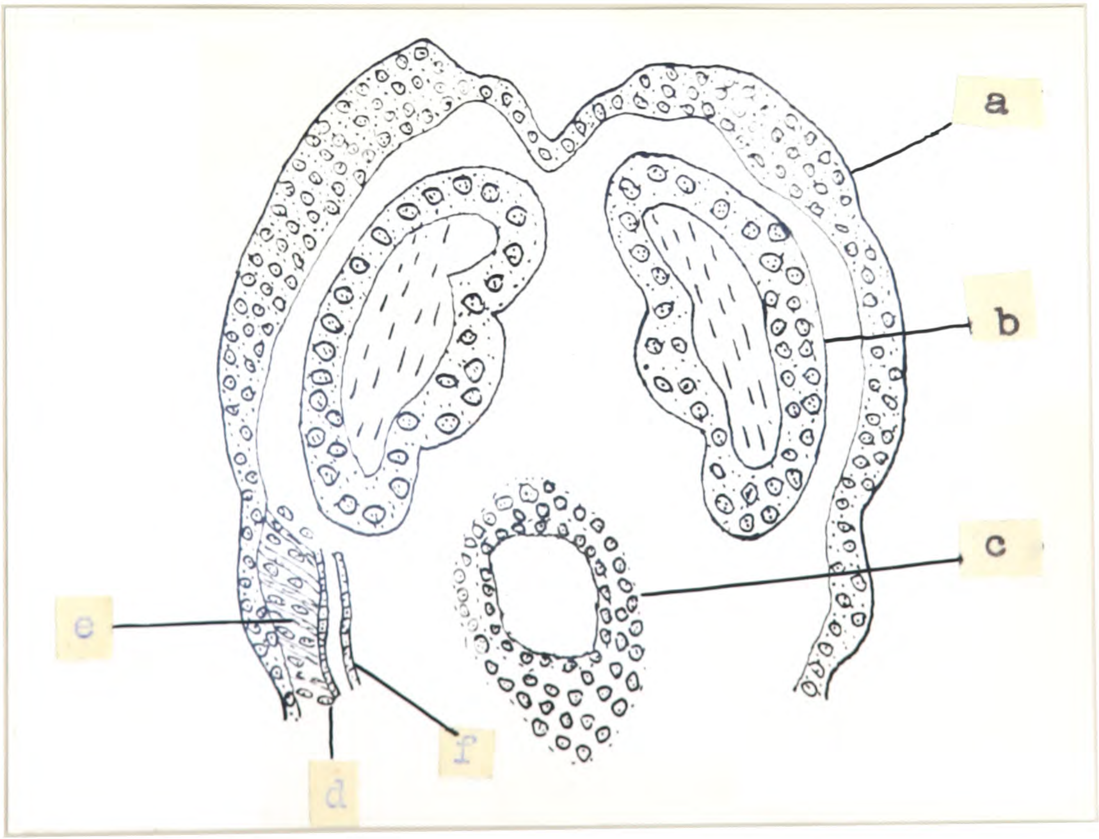


FIG. 2.

that, as the form of the mould was changing towards the final shape, so do other organs change, as if following in the wake of the epidermis. Thus with the elongation and broadening up of the epidermal mould of the locust at a later step in the development, it was found, as one would expect, that the alimentary canal had also elongated, broadened and differentiated into different component parts (Pl.III,fig.2). In the same way muscles had developed and became more definite (Pl.III,fig.2 , Pl.X,fig.1,2). The nervous system also showed the same trend of subsequent adaptation (Pl.X,fig.1,2 and Pl.XI,fig.1,2,3).

The epidermis is found to be plastic and to have the capacity to alter its shape. It must, therefore, influence the form of organs, which gain attachment to it at more than one point.

An important feature of the epidermis is, of course, its capacity to secrete cuticle. In Locustana paradalina, the provisional embryonic cuticle was first observed to be formed four days after the termination of the dormant period. By this time, the epidermis had acquired a mould, which, though more or less resembled the emerged nymph, still yet needed some minor changes to be exactly like the first instar. This change in mould was brought about /

PLATE XI

Illustrates the changes in the shape of the brain corresponding to the change in the mould of epidermis during different stages of embryogenesis of L.paradalina, after the termination of the dormant stage of the embryo by wetting.

Fig. 1. Two days stage.

Fig. 2. Five days stage.

Fig. 3. Six days stage.

a, epidermis; b, brain; c, provisional embryonic cuticle; d, definitive cuticle.

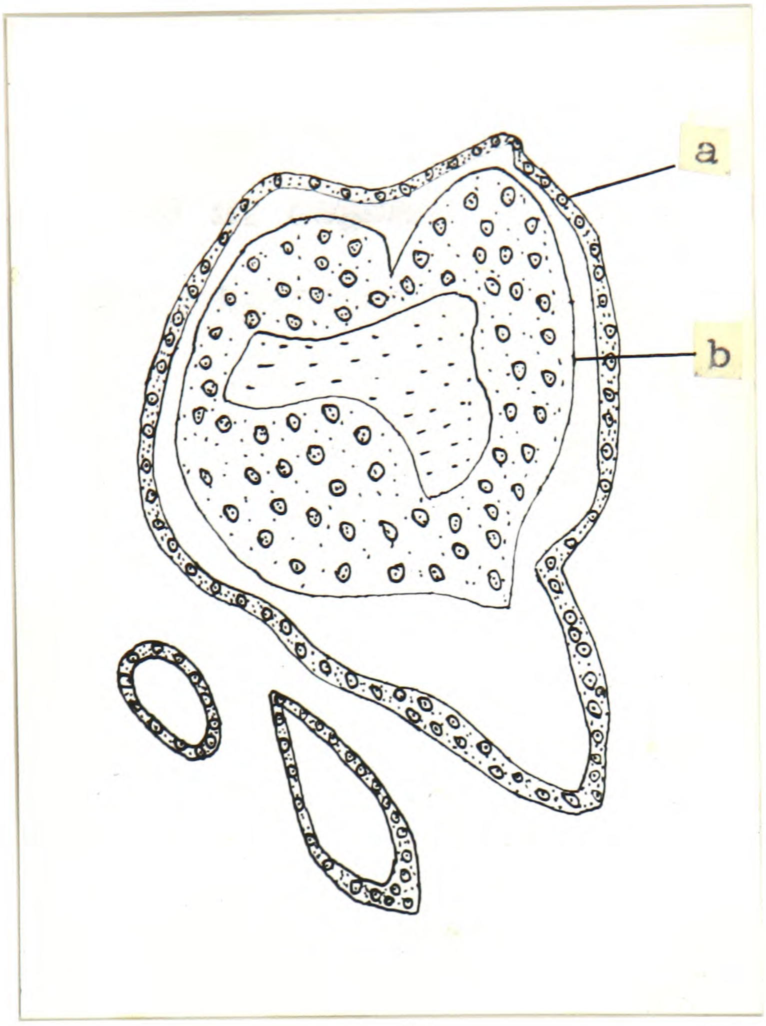


FIG. 1.

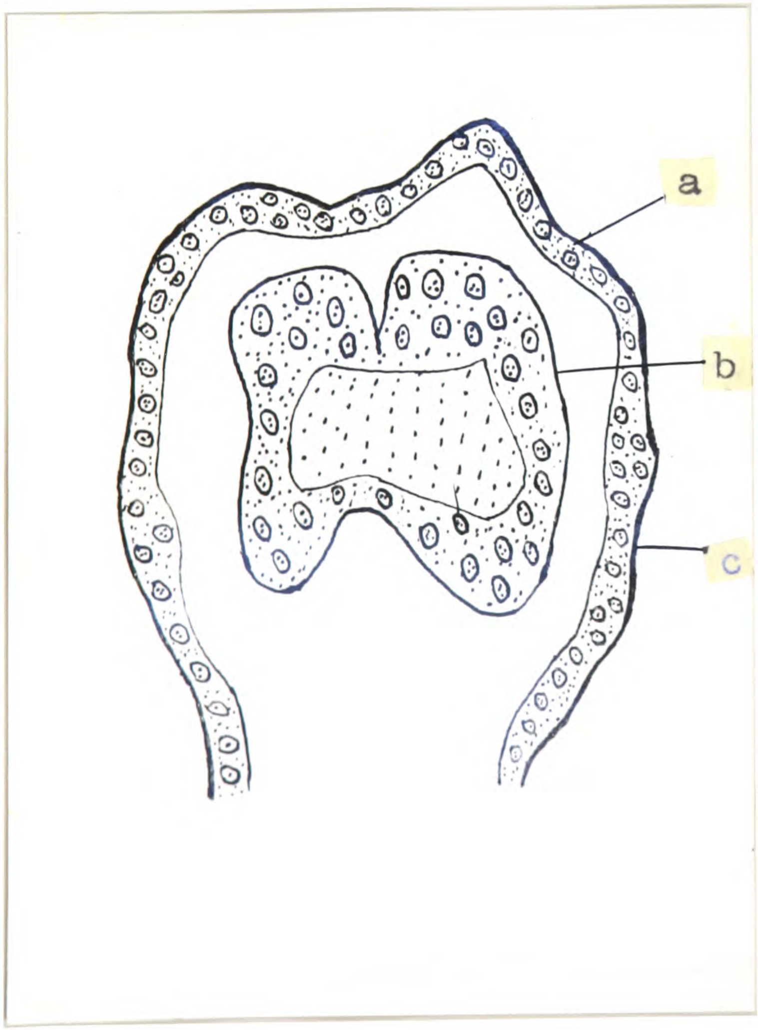


FIG. 2.

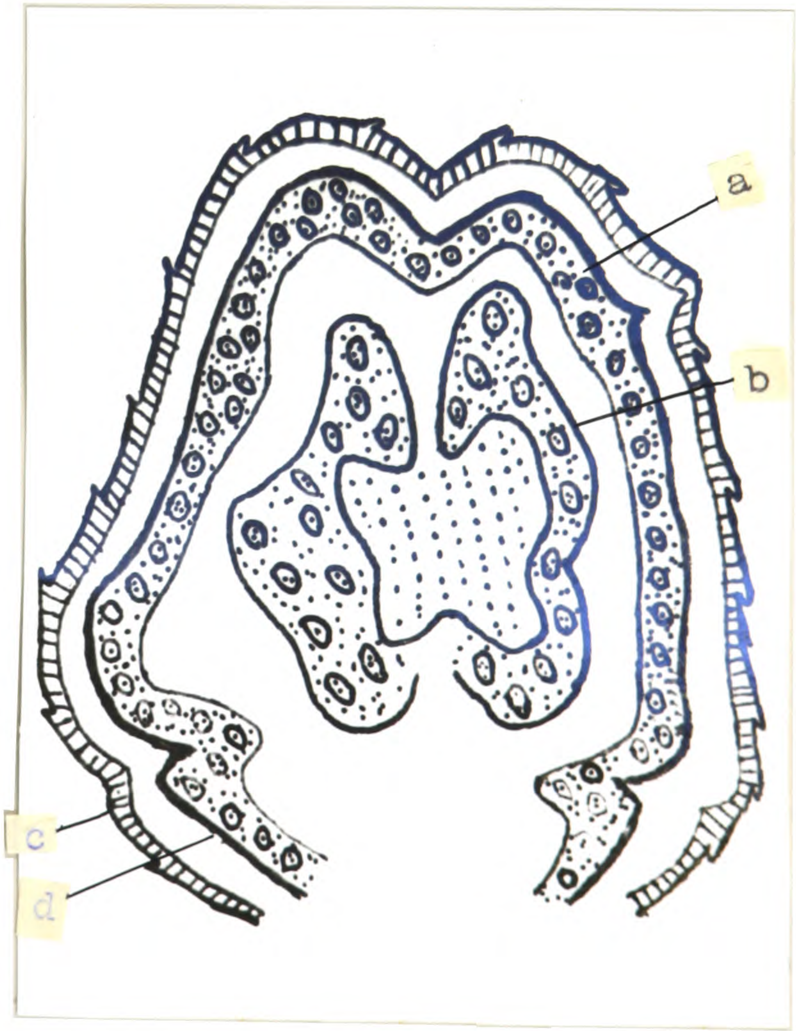


FIG. 3.

90μ

about at the time, the epidermis retracted from the provisional embryonic cuticle. For a short time, during this period, the epidermis was free from its inelastic cuticle and consequently it was in a position to make necessary changes in the mould. This was what appeared to have happened in L.paradalina. There was a definite change of mould during the short period on the sixth day after the breaking of the dormant stage by wetting, when the epidermis was free though for a very brief period from its inelastic cuticle. (Pl. XI,,fig.1,2,3 and Pl. X ,fig.1,2) .

In Tenebrio molitor also, the epidermis was found to have formed a mould, which resembled the larval form by the fourth day of embryogenesis (Pl.II,fig.1). It has already been pointed out before, that at 30^0C and 90% relative humidity, the embryonic development was completed in this insect in about eight days time. So by the time, the provisional embryonic cuticle was secreted, almost all other organs were found in the mid-stream of their development. Thus, by that time, as has been described in the preceding chapter, muscles, nervous system and alimentary canal were forming. The brain at that time was found to have clearly differentiated and other ganglia had started differentiating.

All /

All the organs from this stage onwards progress gradually in their development, without any interruption towards their completion, fitting themselves gradually within the mould of the epidermis.

Now one of the chief functions of the cuticle is to act as an exoskeleton to resist the pull of the attached skeletal muscles. This attachment is effected in many ways and will be described later on. In *Locustana paradalina* the embryonic cuticle is first secreted on the fourth day after the termination of the dormant period. The first rudimentary muscles are formed two days previously (Pl.III, fig.1). The embryonic cuticle of *Tenebrio molitor* was first observed in a four-day old embryo and the muscle rudiment was first observed one day earlier. The muscles at this time are being differentiated from a group of myoblast cells.

It is reasonable to suppose that the rate of deposition of cuticle and the progress of development of muscles must at some period during late embryogenesis, reach steps which coincide appropriately to allow the developing muscles to attach themselves properly to the cuticle.

One is therefore left to conclude that the reason for the moult of cuticle, while the progress of development of the systems of organs and tissues, including the muscles, /

muscles,.is in mid stream, is connected with the connection of the muscles to the cuticle. Since this integration is accomplished just before emergence, it follows that the rate of deposition of cuticle from the time this moult occurred is attuned to effect this essential integration. Hence the first formed cuticle may be said to have been deposited precociously in relation to the development of the muscles.

It is therefore reasonable to conclude that the moult of provisional cuticle is a gearing process, which allows cuticle formation to become properly attuned to the development of the muscles, so that the muscle ends, or fibrillae, may become properly integrated with the cuticle.

The attachment of muscles to the cuticle is a process of integration. For the proper attachment of the muscles to the cuticle there must of necessity be a proper relationship between the deposition of the cuticle and the development of the muscle ends or tonofibrillae. This attachment as one would expect, takes place subsequent to the moult of provisional cuticle. The appropriate hardening of the cuticle allows it to take the pull of attached muscles, and it is then that the insect is capable of locomotory activity. In <u>Locustana paradalina</u> and <u>Tenebrio molitor</u> /

PLATE XII

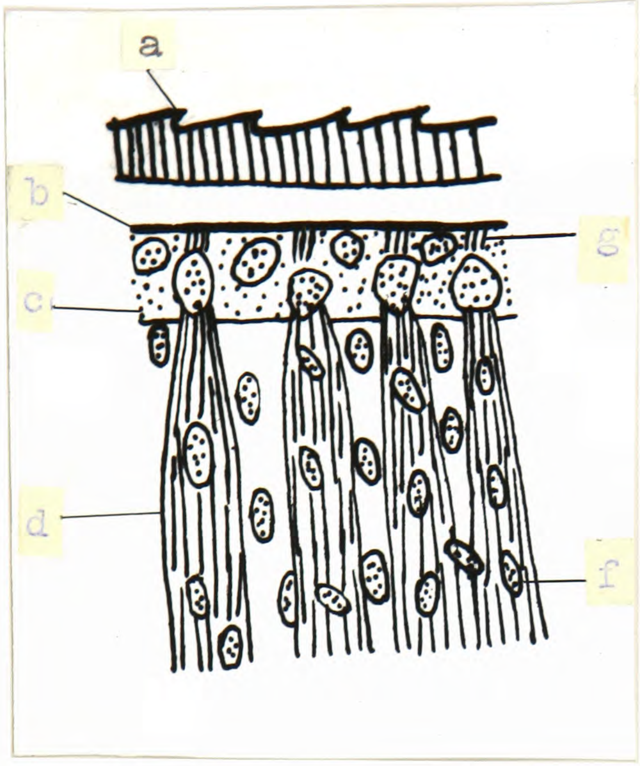
Shows the mode of attachment of the muscles to the cuticle, in the embryos of L.paradalina after the dormant state of the embryo has been broken by exposure to moisture.

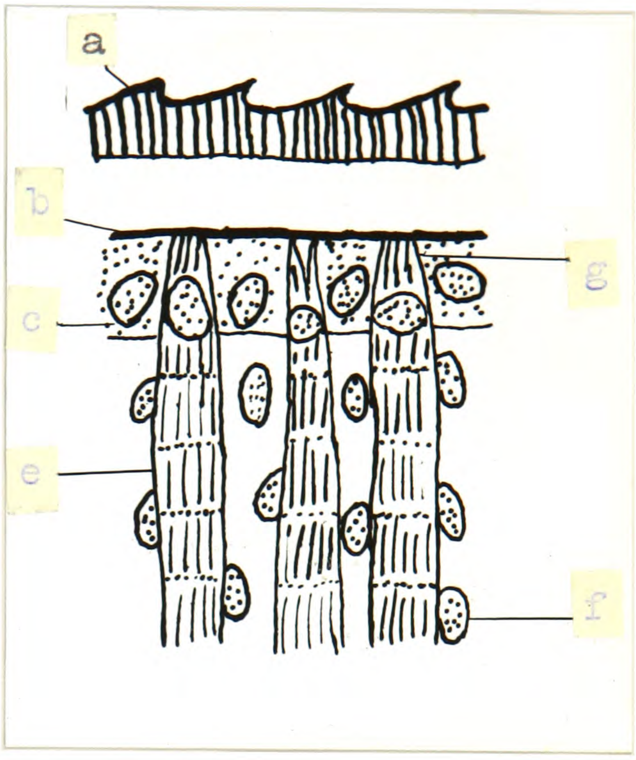
Fig. 1. Condition on the seventh day.

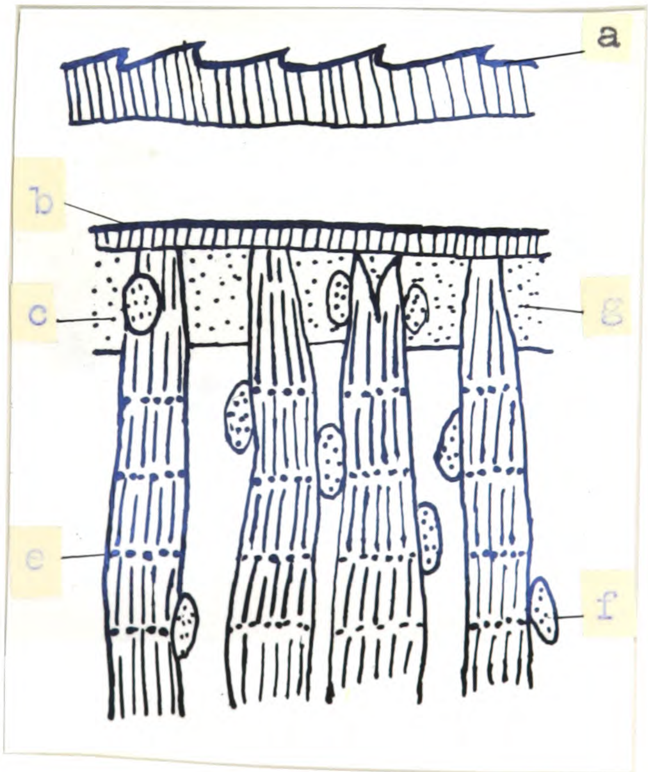
Fig. 2. Condition of the eighth day.

Fig. 3. Condition of the nineth day.

a, provisional embryonic cuticle; b, definitive cuticle; c, epidermis; d, unstripped muscle fibres; e, stripped muscle fibres; f, myoblast cells; g, tonofibrills.







20µ

molitor the muscle during embryogenesis, were contiguous with the epidermis. They were not fully developed at this time, and they were present in the form of aggregation of myoblast tissue.

The attachement of the muscle fibres to cuticle is usually effected by intermediary fine connective fibrils called tonofibrillae, that traverse the epidermal layer. Boelitz (1933) pointed out that the dilator muscles of the ectodermal part of the alimentary canal were attached by tonofibrillae to the cuticular intima. The tonofibrillae were produced by a transformation of the epidermal cells at the end of the muscles into cuticular fibrils that were continuous with the cuticula. So these tonofibrillae were not derived from the myoblasts but from the epidermis itself (Pl.XII, fig.1) when the tonofibrillae are forming in the plamatic part of cells they may entirely disappear, but in the case of Locustana paradalina the nucleated remnant of such cells persisted between the group of tonofibrillae (Pl.XII,figs.1,2,3). However, this was not the case in Tenebrio molitor. It was seen in many insects that the tonofibrillae not only traversed the epidermal layer but they also penetrated the cuticle for some distance, but this was not observed in L.paradalina or in Tenebrio molitor. This penetration of tonofibrillae was /

was explained by Henneguy (1906). He pointed out that the tonofibrillae were formed in the early stages from the epidermis when the epicuticula was being secreted. So they were first connected with the outer layers of the exocuticle, and their proximal extensions became embedded in the endocuticle, subsequently laid down beneath the former.

It has been mentioned that the deposition of embryonic cuticle is both Locustana paradalina and Tenebrio molitor is not in relation to the development of muscle fibres. In Locustana muscles were still in a rudimentary condition at the time the provisional cuticle was being secreted. In Tenebrio embryonic cuticle was not yet formed when the muscle fibres were beginning to take shape. This first laid cuticle was shed on the sixth day, after termination of the dormant period in Locustana paradalina. Immediately afterwards the epidermis began to deposit fresh cuticle, and it was to this cuticle that the muscles formed during embryogenesis, attached themselves (Pl.XII,fig.1,2,3). In Locustana proper attachment was obtained on the eight and ninth day, that is to say, two or three days after the commencement of secretion of the new cuticle. It was at this time that the striation appeared, thus indicating a proper attachment (Pl.XII, fig.2,3). However, in Tenebrio molitor, proper attachment was /

was not brought about until after emergence (Pl.VIII,fig.3).

It is therefore reasonable to state that the deposition of cuticle by the epidermis was laid down precociously during embryogenesis. In Locustana paradalina it was shown that proper integration by the muscles was effected two to three days after the commencement of the deposition of new cuticle.

It is clear that, if this shedding of the first laid cuticle did not take place, a continuous building up of cuticle would result, and the whole structure being completed before the muscle ends were ready to become attached through tonofibrillae.

One may conclude with the suggestion that the first laid cuticle is precociously laid, its deposition being therefore out of relationship with the rate of development of the muscles. To equilibrate cuticle deposition with the development of the muscle ends the first laid cuticle is shed, so that the rate of deposition of cuticle of the freshly laid cuticle becomes attuned to the formation of tonofibrillae. This simple adjusting process brings about a proper integration of muscle ends with the newly deposited cuticle.

PLATE XIII

Shows the condition of the epidermis at different times during the metamorphosis of *Drosophila*.

Fig. 1. Condition of the epidermis during the moulting of the 'prepupal' cuticle which occurs 11 hours after the eversion of the spiracles.

Fig. 2. Condition of the epidermis 18 hours after the eversion of the spiracles.

Fig. 3. Condition of the epidermis during the moulting of the 'pupal' cuticle, which occurs 37 hours after the eversion of the spiracles.

a, 'prepupal'cuticle; b, 'pupal' cuticle;
c, nucleus; d, chromatin granules.

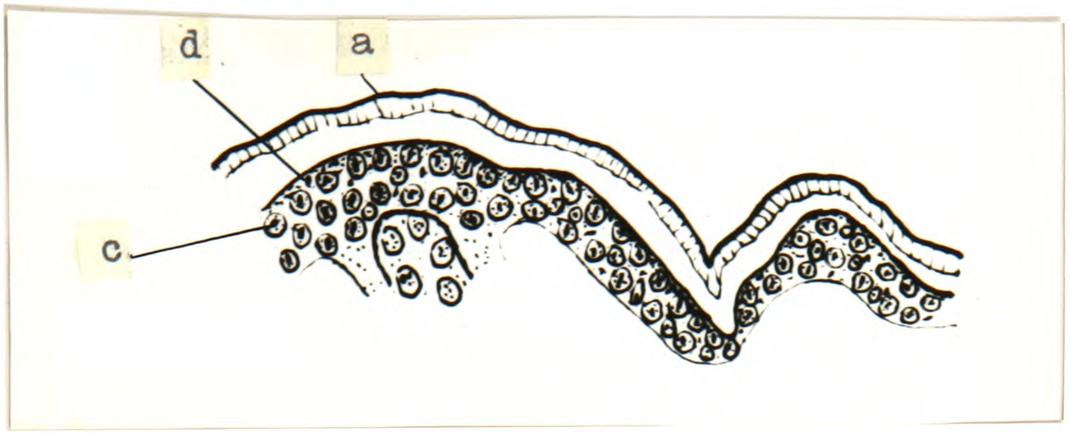
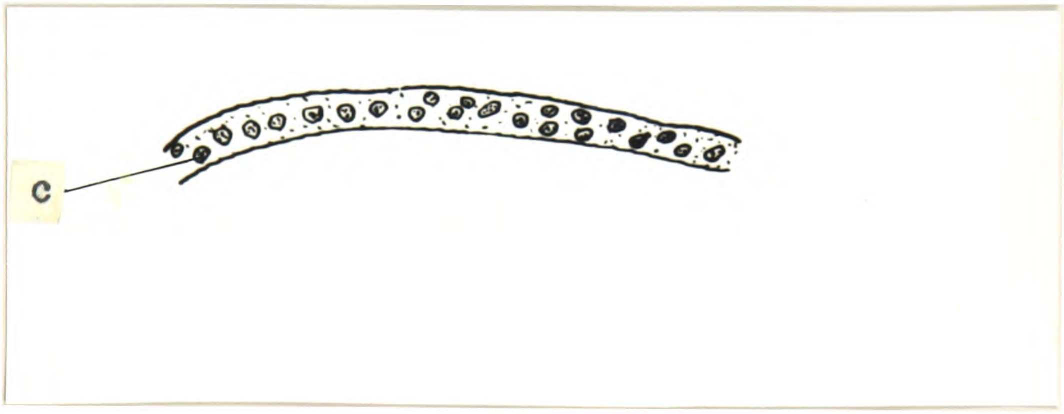


FIG 1



20µ

FIG 2.

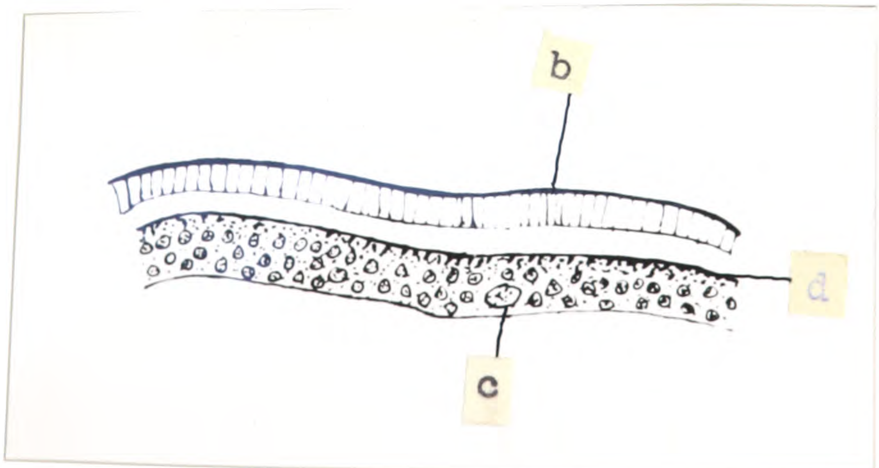


FIG. 3.

COMPARISION OF PROVISIONAL CUTICLES PRODUCED DURING EMBRYOGENESIS AND METAMORPHOSIS.

This section deals with the course of events that takes place in the development of Drosophila during metamorphosis. The chief aim of this investigation is to relate the events in the development of the internal systems and tissues in relation to the deposition of the cuticle and subsequent moulting. In effect, an attempt has been made to find out and verify whether or not the causes for the production of the provisional embryonic cuticle resembled those responsible for the formation of the provisional cuticles during the metamorphosis of a holometabolous insect.

A current interpretation of the events during metamorphosis in Drosophila, coveys to one the impression that the metamorphic development consists of a series of upheavals, punctuated by moults. There is, therefore, a tendency to believe that the metamorphic phase covers a series of suppressed instars, for example , the prepupal and pupal stages.

A am inclined to believe that the adult or imaginal differentiation begins, when the old larval cuticle is shed by the epidermis, and that the differentiation of the adult systems and the tissues is a continuous process /

process during the metamorphosis and that at certain steps in the metamorphosis the epidermis and the epithelia of the fore and the hind guts are induced to retract from the old cuticle. And finally, it appears that the development of the rest of the adult systems of organs and tissues progresses without any significant interruption from the time, the old larval cuticle is shed up to the time, when the adult organisation is fully completed.

The investigation on *Drosophila* was, therefore, conducted to test the validity of the above conceptions, and further to try and discover the true significance of the extra moult that occurs during the metamorphosis of a holometabolous insect, and also to find out if there is any thing like a "pupal stage."

The Development of the Different Systems of Organs and Tissues during the Metamorphosis in Drosophila.

It was found that the puparium (the larval cuticle) hardened soon after the beginning of the prepupal stage. The prepupal stage was marked by the eversion of the spiracles and the larva becoming quiescent. The puparium, which was creamy white in colour, became chestnut brown in about three hours. In four hours the puparium separated cleanly from the epidermis and the so called "prepupal" cuticle /

cuticle was secreted. This cuticle was very thin and could only be detected with great difficulty (Pl.XIII,fig.1). In about eleven hours, this cuticle was shed (Pl.XVIII, fig.3), and the epidermis deposited another cuticle, which is generally referred to as the pupal cuticle. From this point, the pupal stage is supposed to begin. This pupal cuticle was sloughed off at about thirty-seven hours, after the eversion of the spiracles (Pl.XIII,fig.3). A fresh cuticle, the imaginal one, was soon deposited by the epidermis after its retraction from the pupal cuticle.

Muscles. In the early stages of the prepupa the muscles were firmly attached to the puparium. There were two types of skeletal muscles in the larva (i) the longitudinal muscles running along the integument and bridging the body segments. These were attached directly to the larval cuticle (puparium); they showed striation at this time (Pl.XV, fig.1). (ii) The circular muscles, three in each segments, and running across the width of the larva (Pl.XV, fig.1), were properly connected to the puparium. When the epidermis retracted from the puparium, the muscles broke off their cuticular connections.

These types of muscles behave differently in the anterior and posterior segments. The anterior muscles underwent histolysis much more quickly than the posterior ones. /

PLATE XIV

Illustrates the development of the different imaginal muscles during the metamorphosis of *Drosophila*.

Fig. 1. Formation of the imaginal muscles of the anterior region in forty hours after the onset of the "Prepupal" stage.

Fig. 2. Unstripped longitudinal muscles formed forty-eight hours after the beginning of the "prepupal" stage.

Fig. 3. Anlage of the transverse abdominal muscle forty-eight hours after the beginning of the "prepupal" state.

Fig. 4. Shows the attachment of the abdominal muscle fibres to the imaginal cuticle in about seventy-nine hours after the beginning of the "prepupal" stage.

a, epidermis; b, myoblast; c, unstripped muscle fibres; d, stripped muscle fibres; e, tonofibrills; f, imaginal cuticle.

145µ

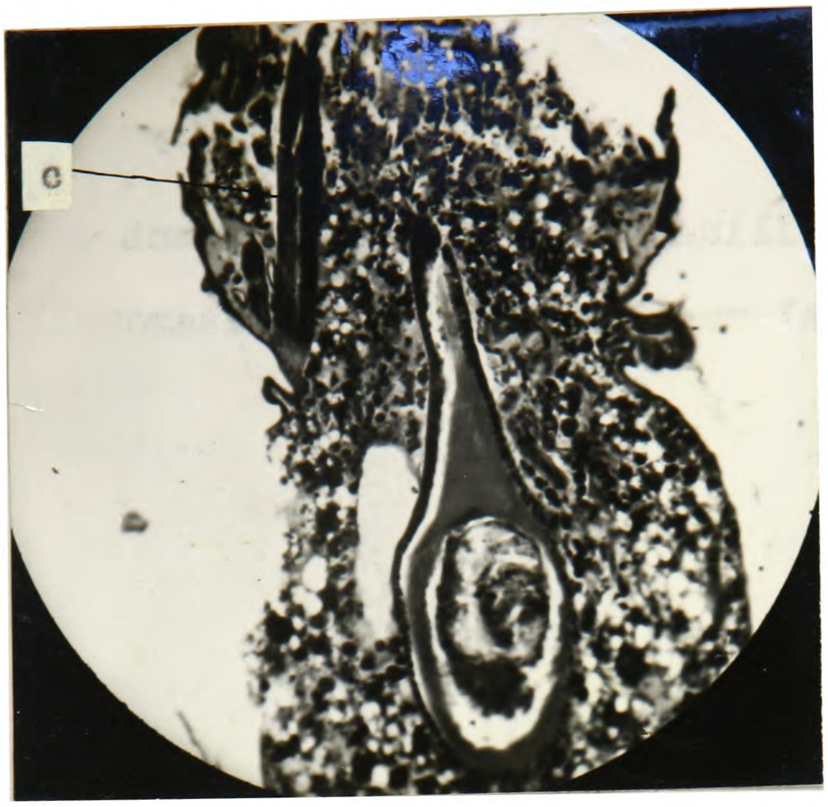


FIG 1

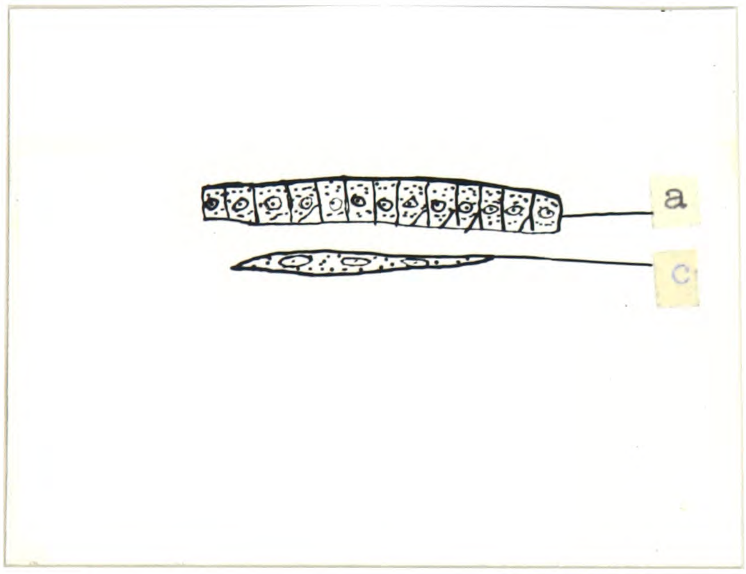


FIG 2

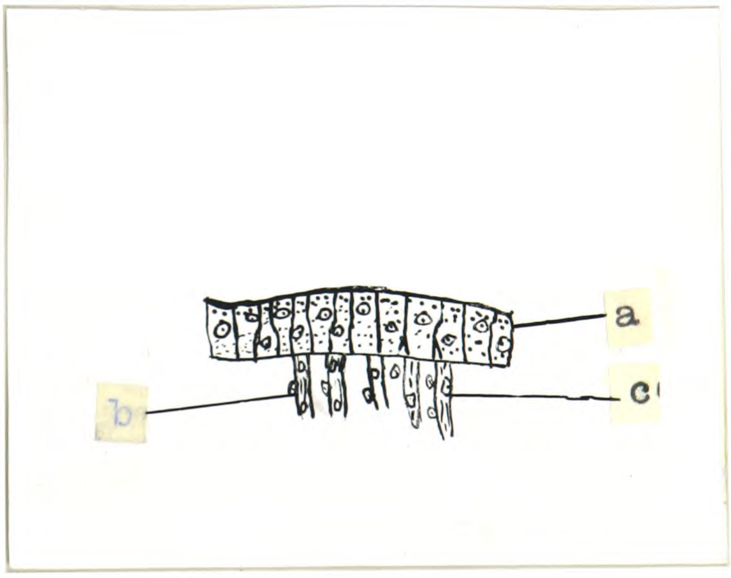


FIG 3

30µ

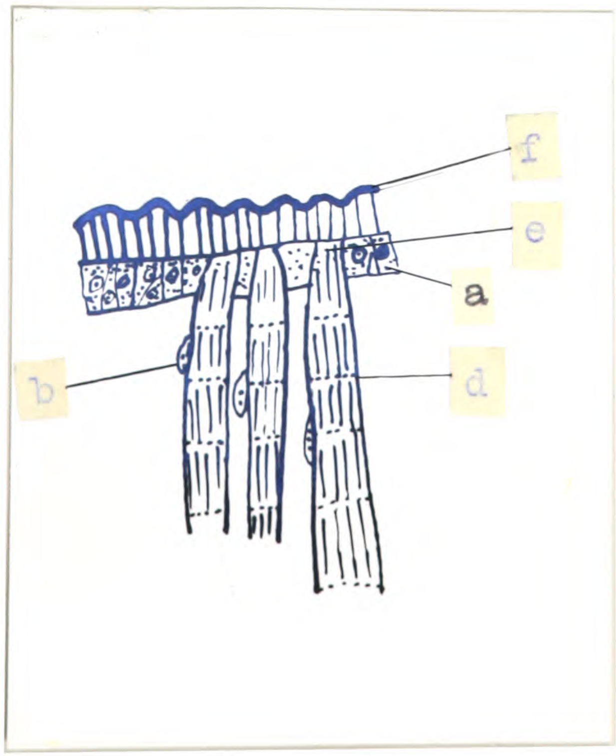


FIG 4.

ones. In seven to eight hours all the anterior muscles, with the exception of the three prothoracic and the pharyngeal dilators, had degenerated completely. This observation agrees with that of Robertson (1936). These two types of muscle systems disappeared after twenty hours.

The first traces of the development of the adult thoracic muscles were observed after forty hours(Pl.XIV, fig.1). They began to unfold earlier than the adult abdominal muscles.

The abdominal muscles showed also early signs of degeneration, but, nevertheless, they persisted for a relatively longer period.

The anlage of imaginal abdominal muscles were first observed in a forty-six hours stage.(Pl.XIV,fig.2). At this time, a large number of myoblast cells were seen to arrange themselves, close to the epidermis. Some of these myoblast cells arranged themselves along the length of the metamorphosing insect, while others arranged themselves along the breadth of the insect. At forty-eighth hour stage these myoblasts formed small bands muscles along both the planes (Pl.XIV, fig.3).

The significant point in the development of the muscles, during the metamorphosis, was that the formation of /

of the definitive adult cuticle began paripassu with the retraction of the epidermis from the puparium. Thus the adult systems of muscles took the place of the muscles of the final larval instar. No intergrading systems could be seen between the two. Henceforth, after forty hours, the development of the adult systems of muscles went on progressively till the muscles gained proper attachment at the seventy-ninth hour stage (Pl.XIV, fig.4).

Epidermis. The epidermis of the prepupa was composed of large larval cells. The larval epidermis gradually began replacement. The anterior portion of the epidermis first got replaced. This replacement was accomplished by new cells which were proliferated by the anterior thoracic buds.(Pl.XV, fig.2). In about four hours after the onset of the prepupal stage, the imaginal cells from the thoracic buds began to spread on the outer surface of the epidermal layer and in the meantime the invested larval cells degenerated (Pl.XV,fig.2). The old cells, as they sloughed off drifted into the haemocoel, and there they became engulfed by phagocytes. Three hours later, most of the anterior portion of the old epidermis was replaced by the imaginal epidermis   The abdominal region retained the larval epidermis for a relatively much longer period. Eventually, however, the imaginal cells encroached upon the /

PLATE XV

Shows the position of the larval muscles, the fore-gut; along with the gradual unfolding of the imaginal buds during the prepupal stage of Drosophila.

Fig. 1. Soon after the eversion of the spiracles.

Fig. 2. Four hours after the beginning of the prepuapl stage.
a, larval epidermis; b, imaginal epidermis;
c, imaginal thoracic buds; d, pharynx; e, oesophagus;
f, longitudinal muscle fibres; g, circular muscle fibres; h, puparium; i, brain.

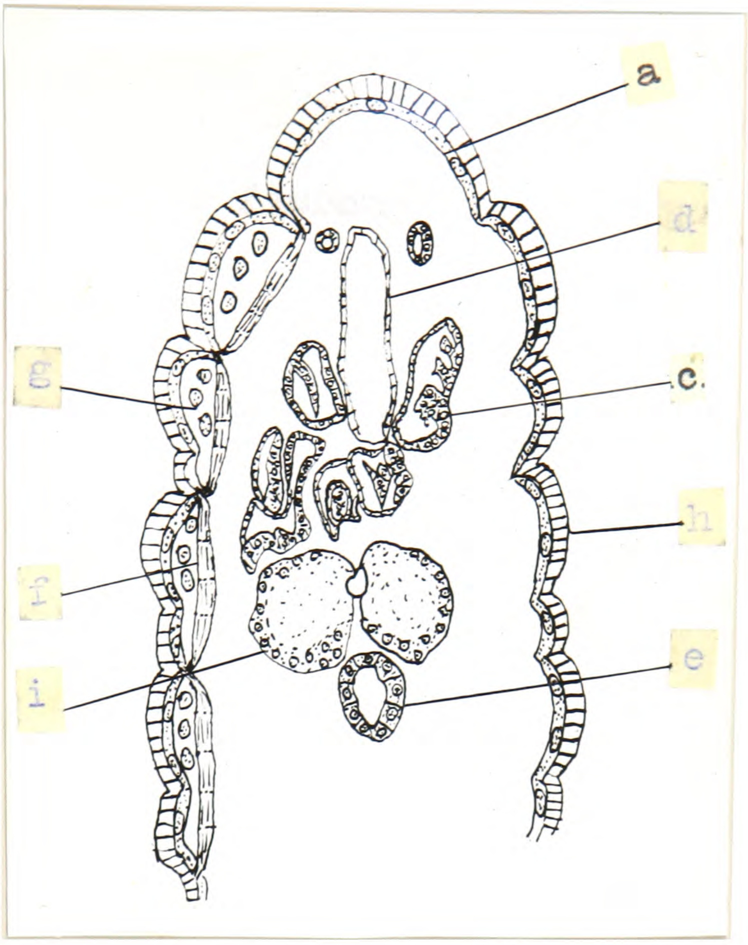


FIG 1

90μ

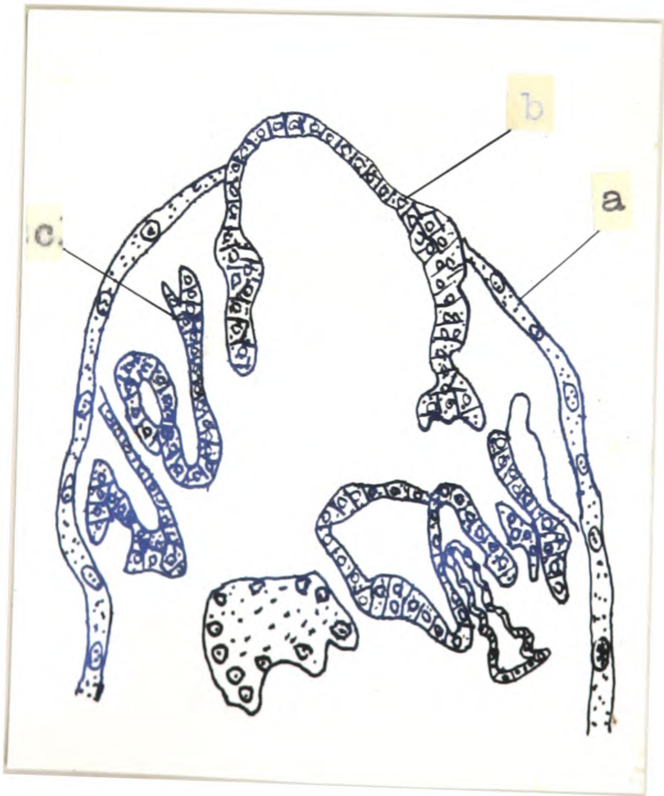


FIG 2.

the larval cells in the region of the abdomen and replaced them. The full replacement in the abdomen took about forty hours.

During the shedding of the prepupal and the pupal cuticle, as the replacement was going on a composite structure of the integument resulted in the sense that it was made up of both the larval and the imaginal cells were present.

Thoracic Buds. The imaginal buds of the thoracic region were composed of characteristic histoblasts (Pl.XV,fig.1). In fact they represented the potential systems of the adult. Their role in the Diptera was first appreciated by Weismann (1864-66), and, later on by Kowalevsky (1887), Van Rees (1888) , Pratt (1900) and Vaney (1902). The well defined thoracic buds in this case were the cephalic complex (antenna and mouthparts), the dorsal meso-thoracic buds (wings), the dorsal meta-thoracic buds (haltere), and the ventral leg buds (Pl.XV,fig.1).

These buds were well formed in the third instar of the larva.

The development of these buds, in the larval stage, has been described by Auerbach (1936), and in the metamorphic stage by Robertson (1936). But the description given in /

in these valuable works was not made from the angle of approach the present author has in mind.

Now, when the pupal stage set in the imaginal differentiation commenced. The imaginal buds, which were seen in the late larval stage, migrated to take up their definite places soon after the onset of the prepupal stage. They reached their set position in about four to five hours (Pl.XV,fig.2). There they developed gradually and completed their development at a late phase of metamorphosis, giving rise to the structure of the imago.

Salivary glands. The development of the salivary glands during the prepupal and the pupal stages has been already followed by Robertson (1936). In the present investigation the development of the salivary glands was studied with a view to seeing whether their development like the unfolding of the thoracic buds was a continuous process or it was interrupted during the moulting of the prepupal and pupal cuticles. The present investigation showed that the replacement of the larval salivary gland is a continuous process, which begins when the old larval cuticle is shed.

In the larva a pair of salivary glands resided on either sides of the alimentary canal. Anteriorly they extended up to the pharynx, where they eventually opened by /

PLATE XVI

Shows the state of the salivary glands during the different stages of metamorphosis of Drosophila.

Fig. 1. Position and condition of larval salivary gland in rellation to other structures, just after the eversion of the s;iracles.

Fig. 2. Condition of degenerating larval cells of the salivary glands five hours after the eversion of the spiracles.

Fig. 3. Shows the beginning of the replacement of the larval salivary glands by the imaginal cells proliferated from the anterior regenerative centre.

a, puparium; b, cells of the salivary glands; c, nucleus of the salivary gland; d, chromatin material; e, vacuole; f, brain; g, imaginal regenerative centre of the salivary glands; h, imaginal buds of the thoracic region; i, pharynx; j, larval epidermis.

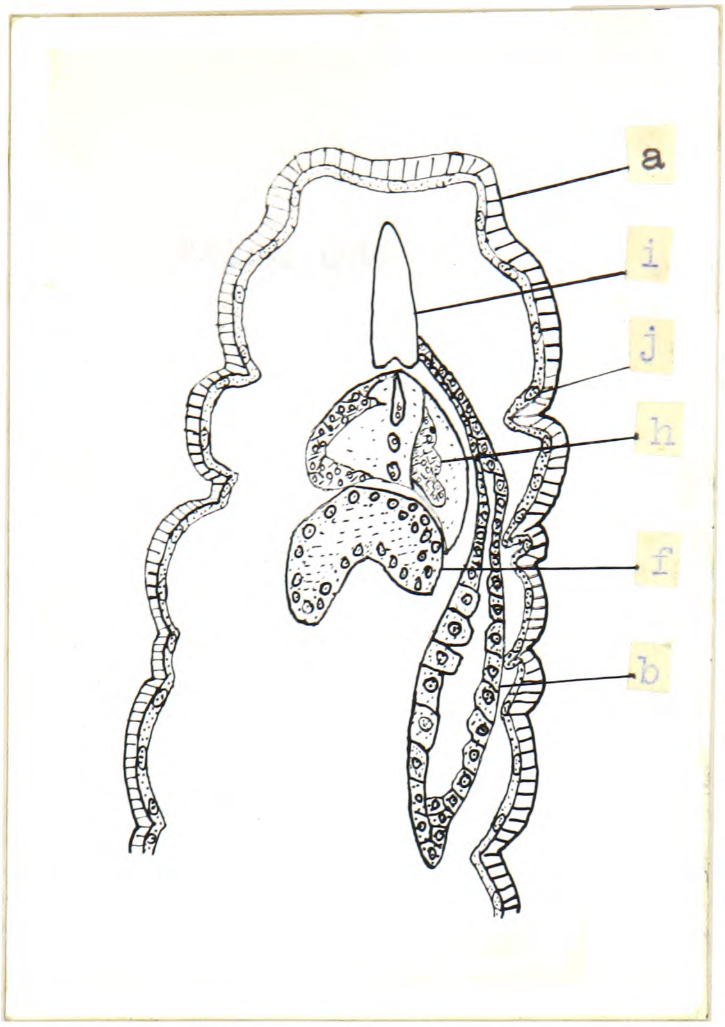


FIG 1

90µ

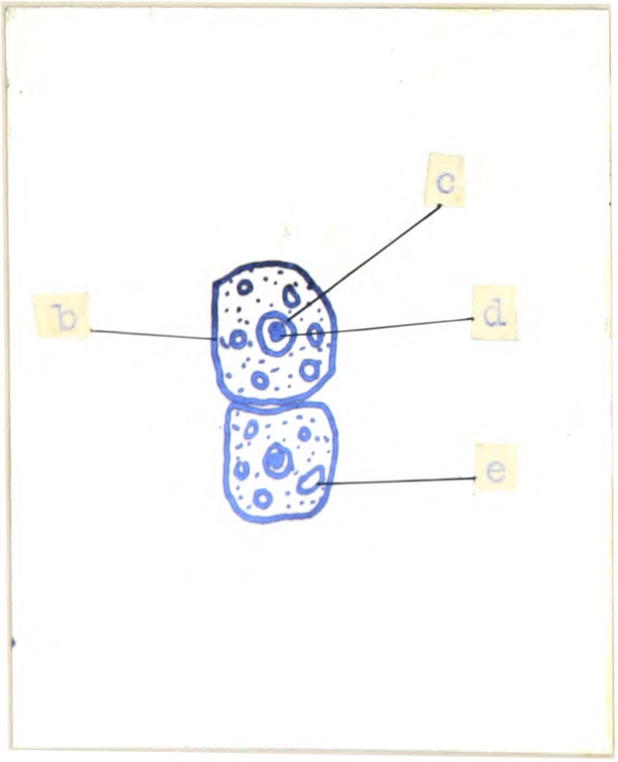


FIG 2.

20µ

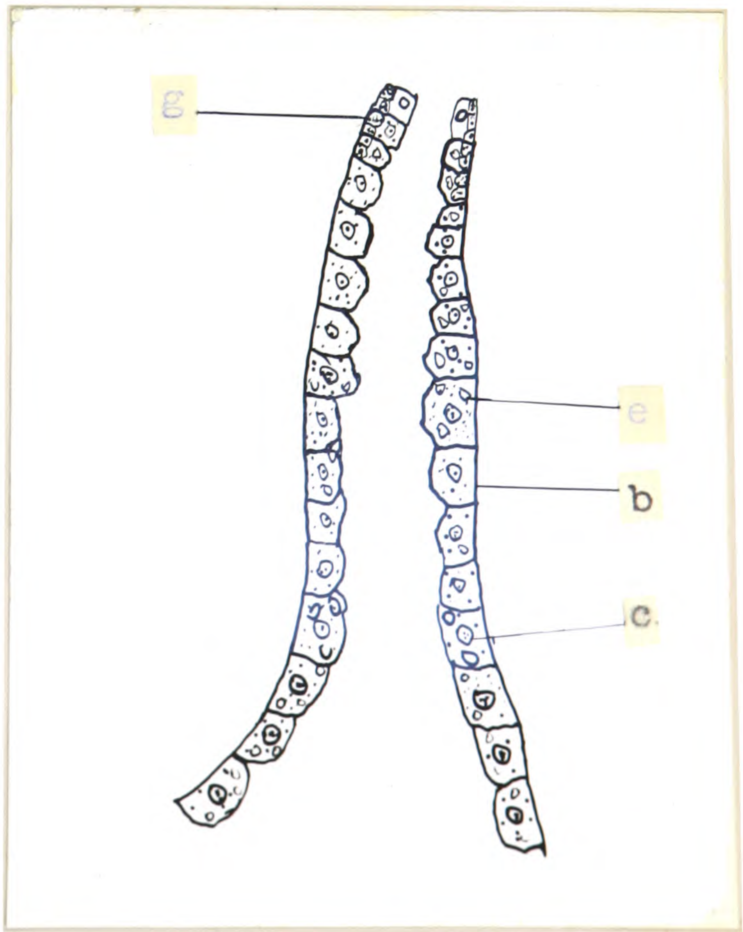


FIG 3.

60µ

by a common duct, whereas posteriorly they extended to half the length of the body (Pl.XVI,fig.1). In the preceding plate the salivary gland of only one side is shown observed in one of the sections. The common duct and the gland of the other side is not shown.

The cells of the glands were composed of exceptionally large cells, some of them being many sided with prominent nuclei (Pl.XVI,fig.1). During early prepupation period, the salivary glands apparently remained unchanged. After the moult of the adult cuticle, changes occurred in these glands. The glands degenerated and the cells became vacuolated and clumps of chromatin material appeared in the nuclei. About five hours, after the puparium formation, the histolysis of these glands began, the cell boundaries disappeared and the chromatin material aggregated in the centre of the nuclei (Pl.XVI,fig.2). About ten hours, after the puparium formation, their generative centres, situated on the anterior side of the salivary glands, began proliferating the cells, which replace the degenerated cells of the larval salivary glands.(Pl.XVI,fig.3). The old cells of the larval salivary glands were sloughed off and engulfed by the phagocytes. This process of replacement of the larval salivary glands by the adult salivary glands was a continuous process. In twenty-four hours, practically whole /

whole of the larval salivary gland was replaced, although some cells of the larval salivary glands had not yet been removed. In twenty-eight hours, however, all the larval cells had disappeared. There is no question of salivary glands being redifferentiated, when the moulting of the provisional (pupal) cuticle occurred after thirty-seven hours. The glands extended in the abdomen after thirty-six hours. They coiled into the abdomen at the end of fifty-four hours.

The Alimentary Canal. Since the provisional cuticle, lining the gut is shed by fore and hind parts of the gut, it was felt reasonable to expect that the activation of the epidermis coincided with the activation of atleast the fore and the hind region of the gut. In other words, one would assume that certain changes that occurred in the epidermis took place also in the epithelia of the fore and the hind parts of the alimentary canal. On observations being made about the state of activity of the fore and the hind parts of the gut during the moulting of the provisional cuticles from those parts of gut, it was found that the epidermis of those portion of the gut showed marked degree of activation.

A great deal is known about the regeneration of the /

the gut from proliferating centres (Robertson,1936). But there has been no serious attempt to differentiate between the degree of reconstruction, accompanying, and, subsequent to the moult of the old larval cuticle and that accompanying the moult of the provisional cuticles (prepupal and pupal). Previous observations led one to believe that a pupal stage would lead to the formation of some sort of organisation, which, by a process of change, develop into an adult organisation.

So far it has already been indicated that the muscles, the salivary glands, and the thoracic buds of the adult began to develop at various times, after the moulting of the old larval cuticle. Thereafter, the development progressed continuously until they obtained their final form.

This investigation regarding the gut was, therefore, aimed towards placing in their proper perspective the adult differentiations, when the moulting of the provisional cuticle occurred.

It was found, as was known before, that the emptying of the gut by the migrating larva is a prelude to the moulting of the larval cuticle, or, the puparium. Before adult differentiation was initiated, the regenerative centres had already made their appearance in the region /

PLATE XVII

Illustrates the replacement of the larval alimentary canal by the imaginal alimentary canal during the metamorphosis of *Drosophila*.

Fig. 1. Brain, larval oesophagus and ventriculus with imaginal regenerative cells, just after the beginning of the prepupal stage.

Fig. 2. Larval oesophagus replaced by the imaginal oesophagus in about three hours after the beginning of the "prepupal"state .

Fig. 3. Regenerative centres of the mid-gut soon after the beginning of the "prepupal state.

Fig. 4. Larval mid-gut completely replaced by the imaginal mid-gut; in three hours after the commencement of the "Prepupal state.

Fig. 5. Shows the formation if the crop, twelve hours after the eversion of the spiracles.

a, oesophagus; b, larval cells; c, imaginal cells; d, crop; e, ventriculus; f, mid-gut; g, meconium; h, brain.

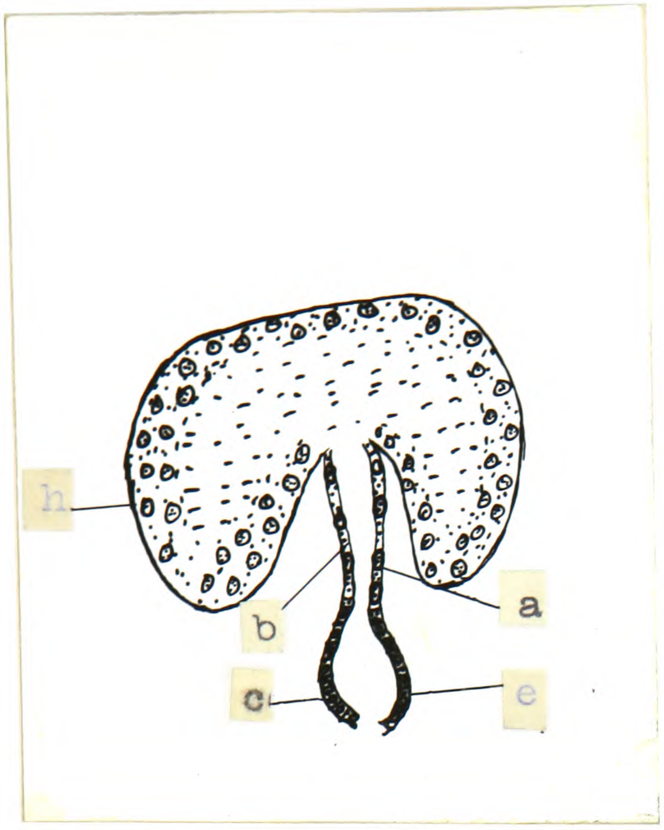


FIG 1

90 μ

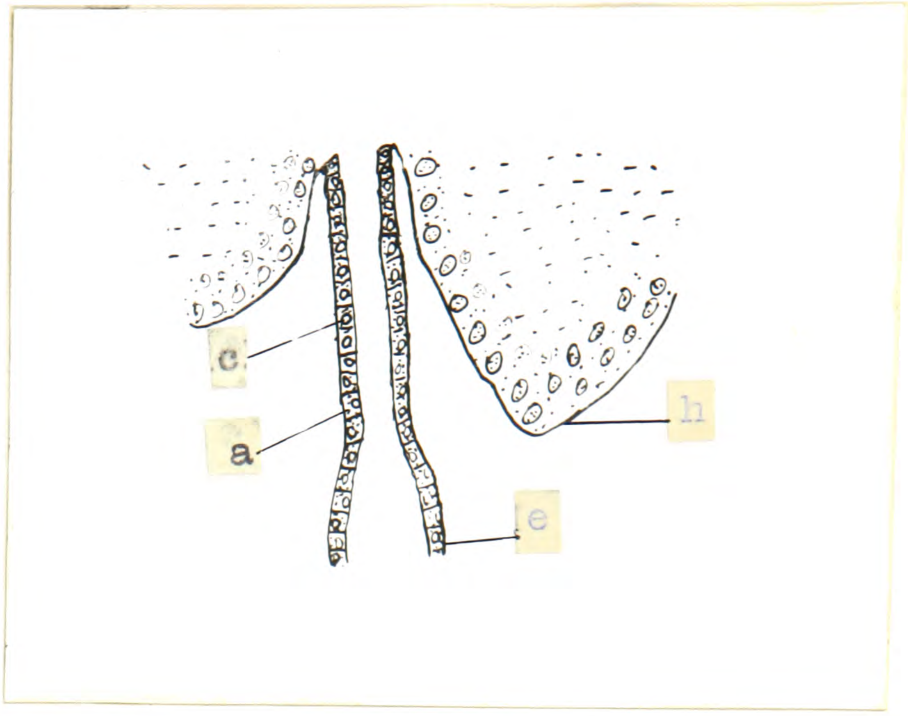


FIG 2.

60 μ.

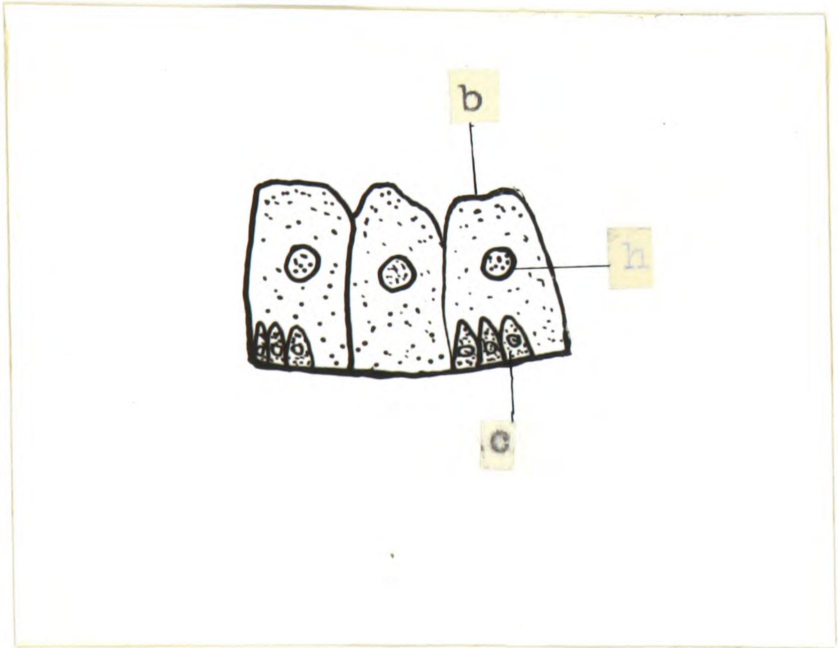
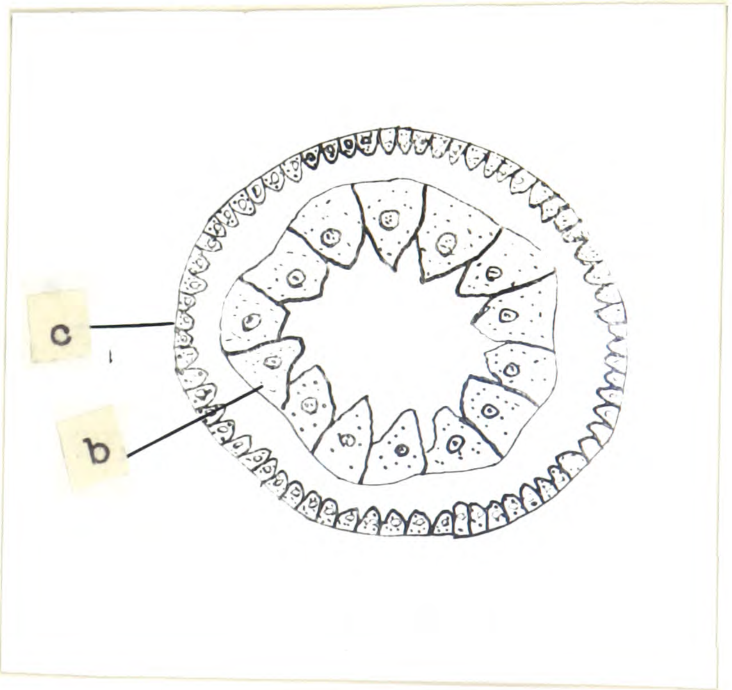
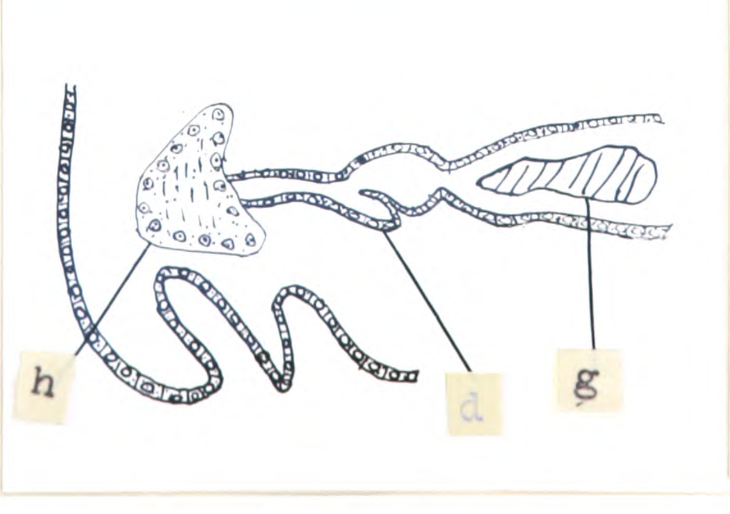


FIG 3

10 μ





region of the gut (Pl.XVII, fig.1 and 3).

Fore-gut. The fore-gut of Drosophila consisted of a muscular pharynx, which continued posteriorly as the short oesophagus. The oesophagus passed between the lobes of the brain and extended into proventriculus just behind the brain (Pl.XVII, fig.1 and 2). The proventriculus of the larva of Drosophila has been described by Strassburger (1935). A ring of small imaginal cells, the imaginal ring of the oesophagus marked the end of the fore-gut (Pl.XVII, fig.2). This ring of the imaginal cells gave rise to thee posterior epithelium of the fore-gut (Pl.XVII, fig.2). The anterior epithelium was renewed by the labial buds, situated on either side of the anterior part of the pharynx (Pl.XV, fig.1). The epithelial cells of the pharynx were replaced by the imaginal epithelial cells very early in the prepupal stage. By the time the puparium was sloughed off, practically the whole of the larval epithelial cells of the pharynx had been replaced by the imaginal epithelial cells very early in the prepupal stage. The new imaginal epithelial cells secreted their own cuticle, as they replaced the

larval/

larval epithelial cells the cuticle of which was sloughed off, along with the cells. At the end of four hours the pharyngeal region secreted a new cuticle, along with the other parts of the epidermis. The new imaginal epithelial cells spread and the larval epithelial cells, which broke up, were shed into the body cavity, from where they were removed by the phagocytes.

The crop, which was absent in the larva, made its first appearance soon after the beginning of the pupal stage. It first appeared as a slight ventral outpocketing of the oesophagus, just anterior to the position of the future proventriculus (Pl.XVII,fig.5). The enlargement continued to progress ventrally and posteriorly. It obtained its adult form at the end of sixty hours.

The condition of the fore-gut was examined most closely during the time that the moulting of the prepupal and pupal cuticle took place. It was found that when the prepupal cuticle was shed, the fore-gut was lined by imaginal epithelial cells, which showed an accompanying marked degree of activation.

The shedding of the pupal cuticle was also marked by a similar phase of the activation of the gut epithelia cells. /

cells.

Mid-gut. At the beginning of the prepupal stage the mid-intestine consisted of an inner layer of large epithelial cells, resting on the thin basement membrane. Among larval epithelial cells, there were found small groups of other cells contiguous to the basement membrane itself (Pl.XVII, fig.3). These other cells were imaginal epithelial cells, which gave rise to the mid-intestinal epithelium of the adult. These cells were also described in the pupal stage in Drosophila by Robertson (1936) and in larvae of several Dipteras by Ganin (1876).

During the first three hours of prepupal life the imaginal cells spread out, separating the intestinal epithelium from its basement membrane. This separations was completed in three hours, and by this time a tube around the old larval epithelial cells was also formed by the imaginal cells (Pl.XVII,fig.4). The intestine shortened after some time, and the diameter of the lumen increased, so that the tube of the larval epithelial cells became compressed.

There were four gastric caecae just posterior to ventriculus. They were present in the larval and early prepupal stages. At the end of a period of six hours they were /

were shed in the lumen of the mid-intestine. They were not replaced in the adult. The shed mass of the old mid-gut, gastric caeca, and the ventriculus remains are usually referred to as the 'yellow body', This shed mass persisted through out the prepupal and pupal periods. It was discharged from the alimentary canal of the newly emerged fly and has been called a meconium (Pl.XVII,fig.5).

Hind-gut. The replacement of the larval hind-gut by the imaginal cells took place in the same way as in other parts of the alimentary canal. The hind-gut was separated from the mid-gut by a ring of imaginal cells, similar to that which divides oesophagus from the mid-intestine. The process of the replacement of the larval hind-gut has been elaborately described by Strassburger (1935) and Robertson (1936). Briefly stating, the epithelium of the hind-gut was renewed by the anterior imaginal rings, proliferating backward and forward. In thirty-six hours, the old epithelia was more or less completely replaced.

It was rather difficult to detect any special activation of the epithelium of the hind-gut at the time the moulting occurred on account of the meconium contained within the lumen.

It took about sixty hours for the adult alimentary tract /

tract to become fully developed. It was also significant that when the shedding of the pupal cuticle occurred, the adult gut was very well advanced in its development.

In the face of the above mentioned evidences supplied by the observations made on the development of the thoracic buds, the muscles, the salivary glands and the alimentary canal, the idea held by many workers in this field of investigation that there is a pupal stage, equipped with pupal system, does not, in my opinion, seem justified. I think this conception arose because it was found that the epidermis and the hind parts of the gut were shedding the provisional cuticles. There is no need, therefore, to read into this activity a process of the replacement of the larval epidermis by a pupal epidermis. As a matter of fact, when the pupal cuticle was shed, the epidermis was composed partly of larval and partly of imaginal cells.

Moreover the thoracic buds give rise, by a process of development over the metamorphic period, to the specialised imaginal structures (cephalic complex, legs, wings and halteres), a fact which makes it unnecessary to post an intermediate stage of instar having its own adult like structure.

The /

The same fact is true, so far as the muscle system is concerned. It has already been seen that soon after the beginning of the metamorphosis the larval muscles started to degenerate and in its place imaginal muscles started forming. Muscles (imaginal) were completed in about seventy nine hours (Pl.XIV,fig.4), long after the pupal cuticle was shed (thirty-seven hours).

The larval salivary glands as has been pointed out before, showed signs of degeneration soon after the moulting of the larval cuticle (Pl.XVI,fig.2). They were completely replaced by the imaginal salivary glands on reaching the twenty-four hours stage.

The epithelium lining the larval alimentary canal was replaced by the imaginal epithelial cells. A detailed description of the mode of this replacement has already been given before.

It is, therefore, legitimate to conclude that a pupal instar is not a stage, in the proper sense of the term. A pupal organisation, as something distinct from the larval or adult organisation, does not exist. It is, for example, erroneous to suppose that the adult system replaces the pupal system on the basis of the evidence furnished by the observations made, regarding the various systems /

systems of organs and tissues described above.

Changes in the Epidermis in relation to the different Moults which occur at Metamorphosis.

As is well known, an imaginal epidermis, more or less, replaces the larval epidermis during metamorphosis. This fact has also been confirmed by Robertson (1936), in his work on the replacement of the larval epidermis during the metamorphosis in Drosophila.

As far as I could perceive, the larval epidermis, prior to its retraction from the larval cuticle (puparium), exhibited the expected changes. Subsequent to the complete retraction of the larval epidermis from the puparium, the imaginal epidermal cells, arising from the thoracic buds, began first to replace the larval epidermal cells of the anterior region of the insect. This observation agrees with that of Robertson (1936) made on the same insect. The imaginal epidermal cells, as they were proliferated from the regenerative centres, migrated over the old larval epidermal cells, which on disintegration were engulfed by the phagocytes. As this process advanced, fresh cuticle was a laid down as a continuous sheet, despite replacement of the larval epidermal cells by imaginal epidermal cells. Hence, both the imaginal and larval epidermal cells produced a composite cuticle. As pointed /

pointed out earlier, this is also true in regard to the imaginal cuticle, which is produced in thirty-seven hours after the commencement of the prepupal stage.

In seven hours from the beginning of the prepupal period, it was estimated, the anterior region had acquired the imaginal epidermis. At this stage that part of the larval epidermis, which was not yet replaced, began to show changes. In ten hours the cells enlarged, and there were signs of nuclear enlargement and of the appearance of chromatin granules. These signs of activity in the larval portion of the epidermis coincides with its retraction from the prepupal cuticle, which took place in eleven hours after the beginning of the prepupal stage. The imaginal region of the epidermis likewise retracted, but it was not possible to distinguish marked, cellular changes in the imaginal epidermis coinciding with the moult. This was due to their continuous active state.

A new cuticle was, therefore, deposited and once again the larval epidermal cells became reduced in size. By the time, this second provisional cuticle separated from the epidermis, the epithelial cells again showed a marked degree of activation. They became elongated, there were mitotic figures and patches of chromatin granules exuded out from the nuclei (Pl.XIII,fig.3). It also appeared /

appeared at this time that the replacement of the larval epidermis was not complete. This moulting of the provisional cuticle (pupal) happened at the twenty-eight hours stage. The next deposited cuticle was the definitive cuticle of the adult; and the muscles of the thoracic region for the first time became integrated with it.

It is, therefore, perhaps legitimate to conclude that the larval epidermis was first replaced by the imaginal epidermis at the anterior region of the insect during the metamorphosis.

The larval epidermis, during the deposition and moulting of the prepupal cuticle, showed the expected changes (Pl.XIII,fig.1), which were in sharp contrast to the larval epidermis observed at other period (Pl.XIII, fig.2). The second provisional cuticle (pupal) moulted at the end of thirty-seven hours (Pl.XIII,fig.3). At this time also, the larval epidermal cells were not completely replaced by the imaginal cells. Hence, the imaginal cuticle was composite, in the sense that some portions of it were formed by the larval epidermal cells; although later, the larval epidermal cells were replaced by the imaginal epidermal cells, which, in turn, deposited additional imaginal cuticle. Thereafter, all the muscles, when they had /

had completed their development, became attached to this cuticle (Pl.XIV,fig.4).

It has already been pointed out that in the embryos of *Locustana paradalina* and *Tenebrio molitor*, a second cuticle, was deposited, in order that the muscles might become properly attached to the cuticle. A brief description has been given before, specially in regard to *Locustana paradalina* and *Tenebrio molitor*, of what may be regarded as a gearing process, which allows the cuticle to become properly attuned to the development of the muscles for the purpose that a proper integration of the muscles-ends with the cuticle may take place.

Keeping this in mind, the significance of the two moulting of the provisional cuticles during the metamorphosis of *Drosophila* was looked into for the purpose of attempting to discover if these metamorphic moulting were likewise linked up with the muscle development.

Subsequent to the retraction of the epidermis from the larval cuticle, the muscles became detached in about three hours. Muscles then underwent a process of dedifferentiation and the degree of degeneration varied according to the particular systems of muscles concerned. During the time the muscles were differentiating there were /

were two accompanying moults, involving retraction of the epidermis on two occasions from the already laid cuticle. At no time did the new developing imaginal muscles gain attachment to these provisional cuticles. During the first moult practically nothing could be seen of the imaginal muscles, but the larval muscles were seen beginning to degenerate. By the time, the second moult of the provisional cuticle (pupal) had occurred, the imaginal muscles were not yet developed. These muscles did not, in fact, obtain the forms of fibres until about forty hours after the eversion of the spiracles.

It has already been pointed out that the imaginal muscles completed their development in about seventy-nine hours after the eversion of the spiracles (Pl.XIV, fig.3). These muscles were, therefore, not able to gain attachment with the second provisional cuticle (pupal cuticle). It was, therefore, reasonable to suppose that in the metamorphosis of Drosophila the purpose of moulting was essentially linked up with the muscle development. It has already been pointed out that the other internal systems progressed continuously in their development and in no ways appeared to need these moultings.

In Locustana paradalina, during embryogenesis the /

the cuticle was deposited for nearly forty-eight hours after the moulting of the provisional cuticle and before some of the muscles gained attachment.

In *Tenebrio molitor*, the larval cuticle was deposited for forty-eight hours, after the moulting of the provisional embryonic cuticle and thereafter the muscles were able to gain proper attachment.

In *Drosophila*, the second provisional cuticle (pupal), was shed at about thirty-seven hours after the commencement of the adult differentiation. At the end of forty-one hours after the adult cuticle started forming, the adult muscles became finally attached to it. It is noteworthy that the period taken for the muscles to gain proper attachment with the imaginal cuticle does not differ very much from that taken in the cases of *L.paradalina* and *T.molitor*.

It is, therefore, clear that a particular threshold was reached during the deposition of the cuticle, when the muscles could gain proper integration. An overdeposition of cuticle, by the time the muscle fibres were formed, would prevent integration. To counteract this overdeposition in *Drosophila*, the pupal cuticle was shed, and /

and the rate of deposition of fresh cuticle became properly related to the last phase of the development of the muscles.

All the evidence obtained from the observations on the embryogenesis of Locustana paradalina and Tenebrio molitor and the metamorphosis of Drosophila point to the fact the shedding of the precocious cuticle was a process exclusively connected with the muscle development.

The same fact has been observed by Poyarkoff (1914), in regard to the Endopterygota. He mentioned that the only internal organ that need two moults, during the metamorphosis, for its proper development and function, was that of the skeletal muscles.

For the exact development of the adult muscles a mould of the shape of the adult with its cuticular parts, having the same spatial relation as that of the adult, was required. This mould was the pupa and the pupal cuticle was sloughed off to give proper attachment to the muscle ends.

MOULTING OF PROVISIONAL CUTICLE IN RELATION TO SOME OBSERVATIONS ON GLANDULAR ORGANS.

It is now generally accepted that growth and moulting in insects are initiated by hormones released into circulating blood by glands, and the subject of insect endocrinology presents at the present time a particularly vigorous field. The operative process governing moulting and metamorphosis has now been described in many insects. Kopec (1922), working on the larvae of *Lymantria* (Lepidoptera) was the first to demonstrate the existence of growth stimulating factors. He showed that, if the brain was removed from the larva before a critical period has been reached, pupation did not take place. But, if it was removed after the critical period during growth, normal pupation was achieved.

However it was Hanstrom (1938), who detected in *Rhodnius prolixus* the large cells in the brain, which he thought gave rise to the pupation factor. The hormonal function of these cells in *Rhodnius* was later confirmed by Wigglesworth (1940), who called the secretion the "moulting hormone." But Fukuda (1940, 1941, 1944) provided evidence to show that in the larvae and pupae of *Bombyx mori* the prothoracic glands were the immediate source of the moulting hormone. So far as the Lepidoptera are concerned, the function of the factor liberated by the /

the prothoracic glands would seem to have been elaborately worked out by Williams (1947, 1948a, 1952). His beautiful experiment on *Cecropia* silk-worm has clearly shown that the factor liberated by the neurosecretory cells is necessary to activate the prothoracic glands, which in turn release their contributive hormone. Williams (1952) believed that this prothoracic gland hormone promoted growth and development. In cyclorrhaphous Diptera it has been recently proved by Possompes (1950) that the lateral glands, which are located in the ring gland of Weismann and which are supposed to liberate pupation hormone (Vogt 1943), are themselves first liberated by the factor released from the brain. These lateral glands are thought to be homologous with the pericardial or prothoracic glands of other groups of insects (Thomsen 1941).

The composite control mechanism responsible for moulting appears to be present among all the insects studied. Hence it is reasonable to suppose that neurosecretory cells and glands corresponding to prothoracic glands are universal among insects. The prothoracic glands have now been described in *Dixippus* by Pflugfelder (1947), in *Siales* (C.Geigy and Ochse), in *Gryllus* by Sellier (1951), and in *Periplaneta* by Bodenstein (1953).

Emphasis /

Emphasis has so far been directed upon the hormonal control of insect metamorphosis. Bodenstein (1936, 1953), Hare (1936), Koller (1937), Scharrer (1948), Piepho (1942), Williams (1941, 1948, 1949, 1952) and Wigglesworth (1934 to 1952) have been the main contributors to knowledge on this phase of development.

The bulk of evidence, therefore, provides abundant proof that a brain factor secreted by the neurosecretory cells of para intercerebalis induces the prothoracic gland (ventral gland, head gland, pericardial or peritracheal gland of Weismann), to secrete a growth and moulting hormone. The corpora allata, on the other hand, most certainly promote growth in the larval direction. Wigglesworth (1952) believed that the corpora allata prevents differentiation in the adult direction. Growth of the insect, therefore, depends on a delicate balance of the hormones present in the blood. When the hormone of the corpora allata is absent the moult occurs as usual and is followed by adult differentiation. Wigglesworth (1952) from his work on <u>Rhodnius</u> also held the view that the factor of the prothoracic gland induces moulting and growth only, differentiation of the adult organisation being the result of an absence of the secretion of the corpora allata.

But according to Williams (1952) adult differentiation in the silk-worm moth <u>Platycemia</u> depends upon the /

PLATE XVIII

Shows changes in the neurosecretory cells of the protocerebrum, after the breaking of the dormant state of the embryo of L. pardalina, by exposure to moisture.

Fig. 1. After two days.

Fig. 2. After three days.

Fig. 3. After four days.

Fig. 4. After five days.

a, neurosecretory cells; b, nucleus; c, ordinary nerve cells.

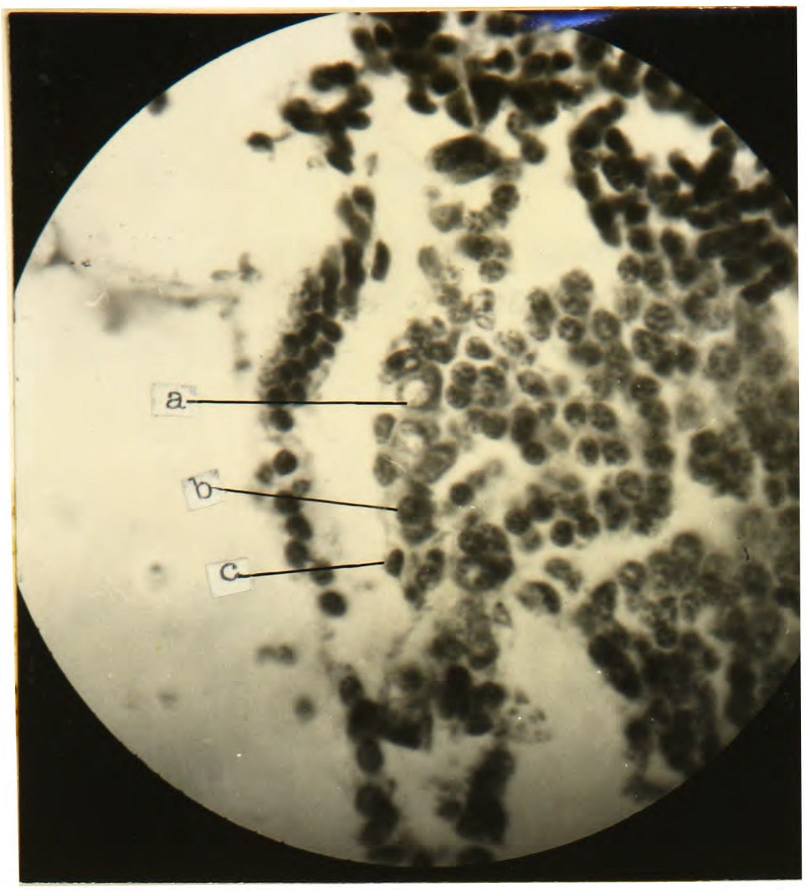


FIG. 1.

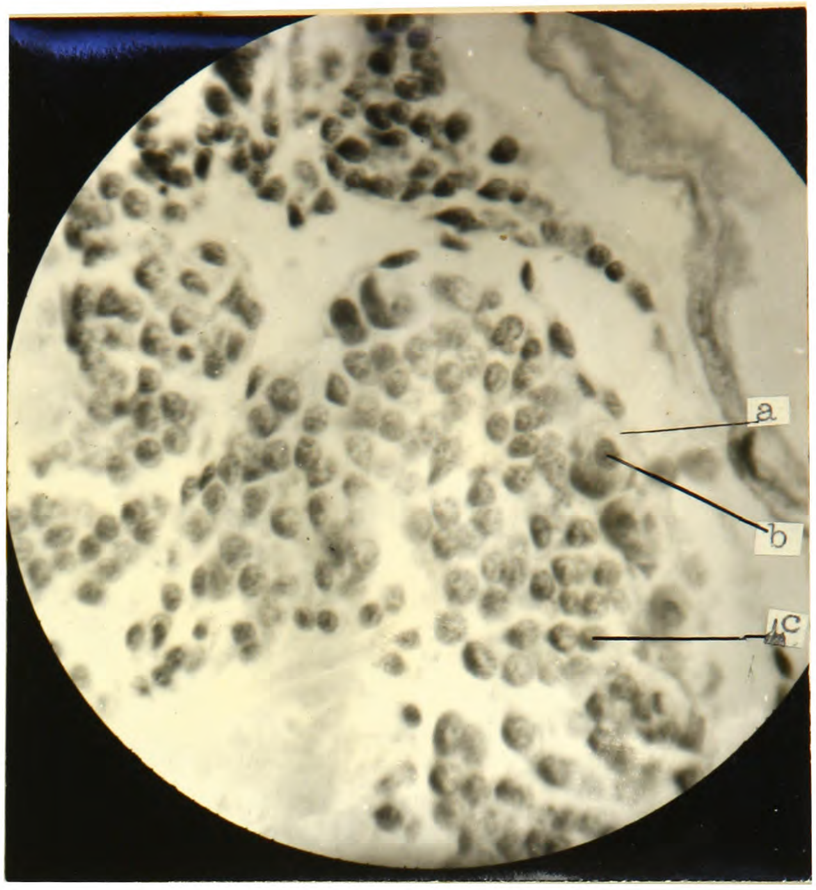


FIG. 2.

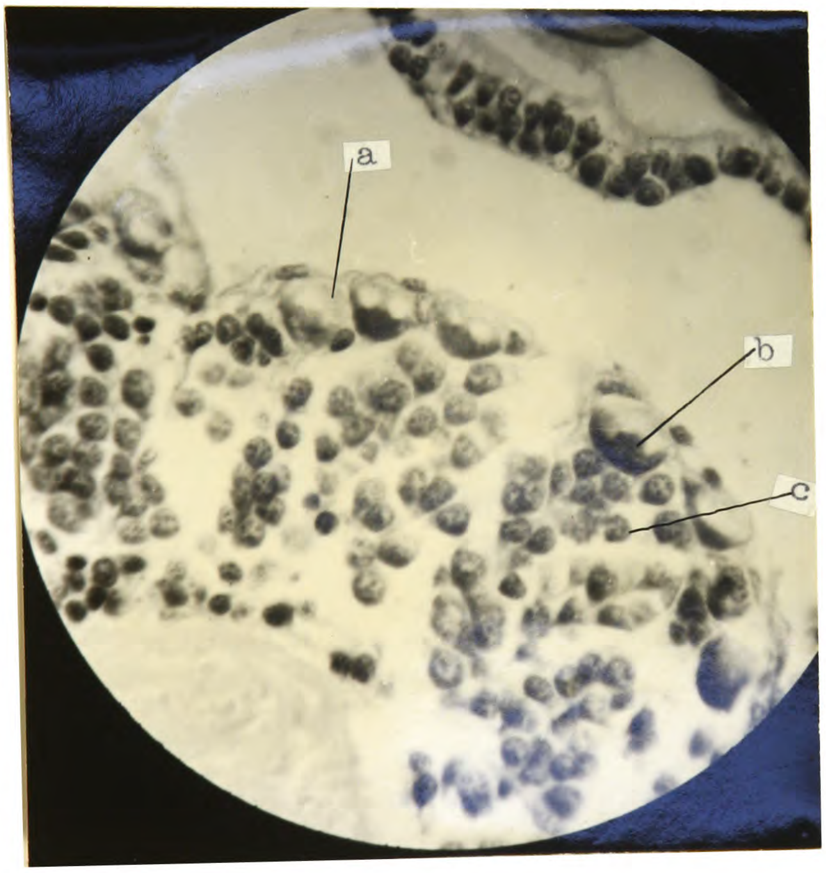


FIG. 3.

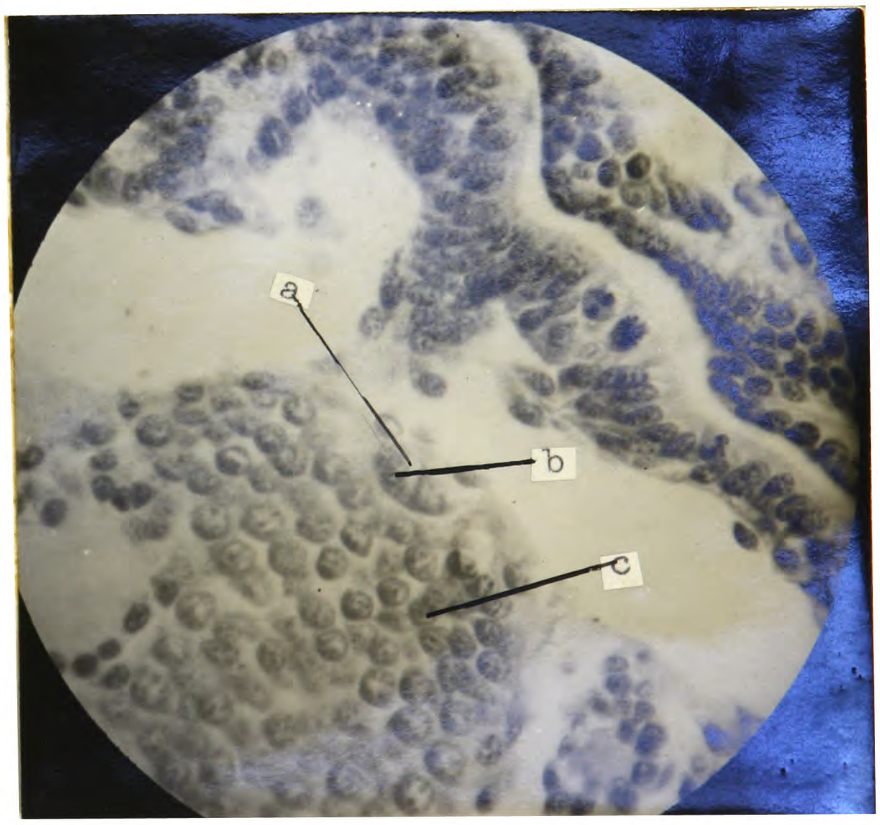


FIG. 4.

30μ

the presence of the hormone released by the prothoracic glands up to a critical step in the development marked by the inception of eye pigmentation. This view is shared by Sharrer and Piepho.

In the light of previous work in this field it was of interest to confirm the discovery of a pair of glands (prothoracic glands) in embryos of *Locustana paradalina*, whose activity was significantly related to the production of the embryonic cuticle. These glands had also been observed in *Locusta migratoria*.

The neurosecretory cells of the brain of *Locusta* were observed at different stages.

It was practically impossible to distinguish when these secretory cells were at rest, from the ordinary nerve cells. On reaching their maximal activity they became enlarged and exceeded the size of the ordinary nerve cells by three to four times. The nuclei became more intensely stained, and large vacuoles appeared in the cytoplasm (Pl.XVIII,fig.1,2,3).

In sections through the protocerebrum, the neurosecretory cells were distributed around the periphery, and therefore immediately beneath the outer membrane. They were either oval or square pear-shaped.

The /

PLATE XIX.

Shows the corpora allata in the embryos of *L. paradalina* at different times after the breaking of the dormant state by exposure to moisture.

Fig. 1. After six days.

Fig. 2. After nine days.

Fig. 3. Immediately after emergence.

a, corpora allata; b, nucleus; c, oesophagus.

Fig. 1.

145 μ

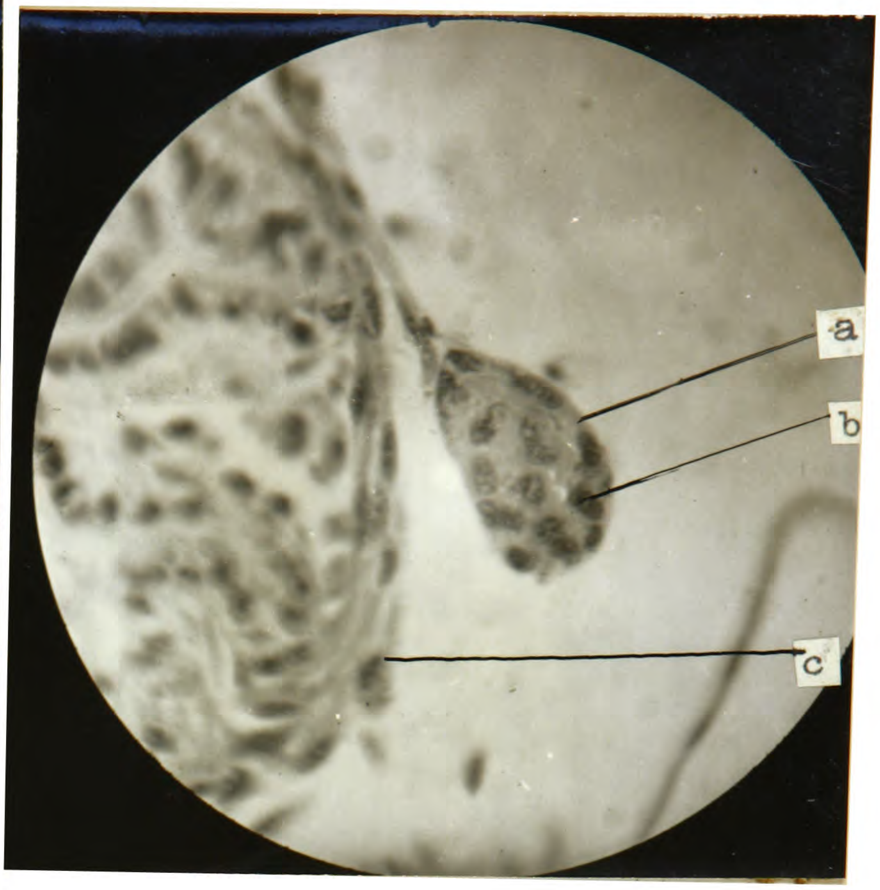


Fig. 2.

30 μ

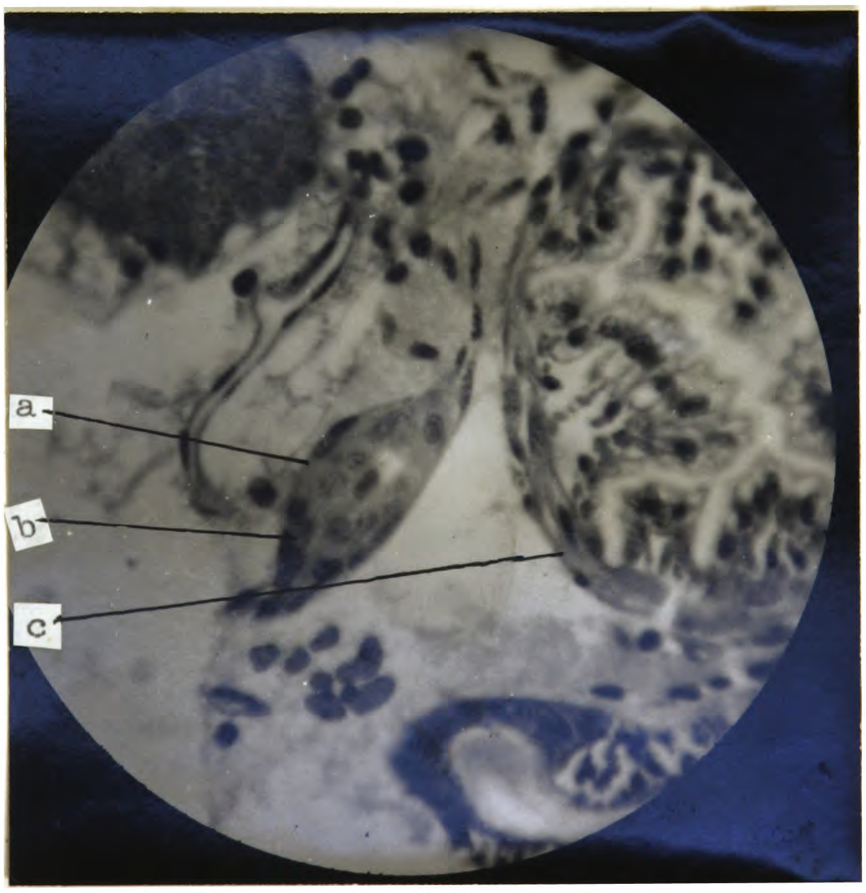


Fig. 3.

30 μ.

The corpora allata, when properly formed and in position on each side of oesophagus within the blook sinus, possessed the usual histological structure. They were quite distinct from the anterior median hypocerebral gland and the pair of smaller oesophageal glands (Pl.XIX,fig.1,2,3

They were oval in shape and in sections were seen to be composed of bean-shaped nuclei possessing deeply stained spheres of chromatin material (Pl.XIX,fig.1). The sizes of the nuclei varied. It is perhaps valid to describe the appearance as being syncytial because no cell boundaries were visible at any stage during the growth of the embryo.

It is known that Prothoracic glands vary considerably in different insects. In the Lepidoptera they are represented as syncytial strands containing relatively large nuclei. These strands of tissue are embedded in the fat body surrounding the large anterior tracheal trunks adjacent to the spiracle. They apparently have no nerve supply, but they have an abundant tracheal supply. It has been found to be practically impossible to isolate these glands, for experimental purposes.

In the Cockroach Lycophaea (Scharrer 1948) the prothoracic glands are composed of a pair of elongate conspicuous glands. They extend from the neck region to each /

PLATE XX.

Shows the size and position of the prothoracic glands glands after the breaking of the dormant state of the embryo of *L.paradalina* by exposure to moisture.

Fig. 1. After six days.

Fig. 2. After nine days.

Fig.3. On emergence.

a, prothoracic gland; b, oesophagus; c, trachea; d, muscles.

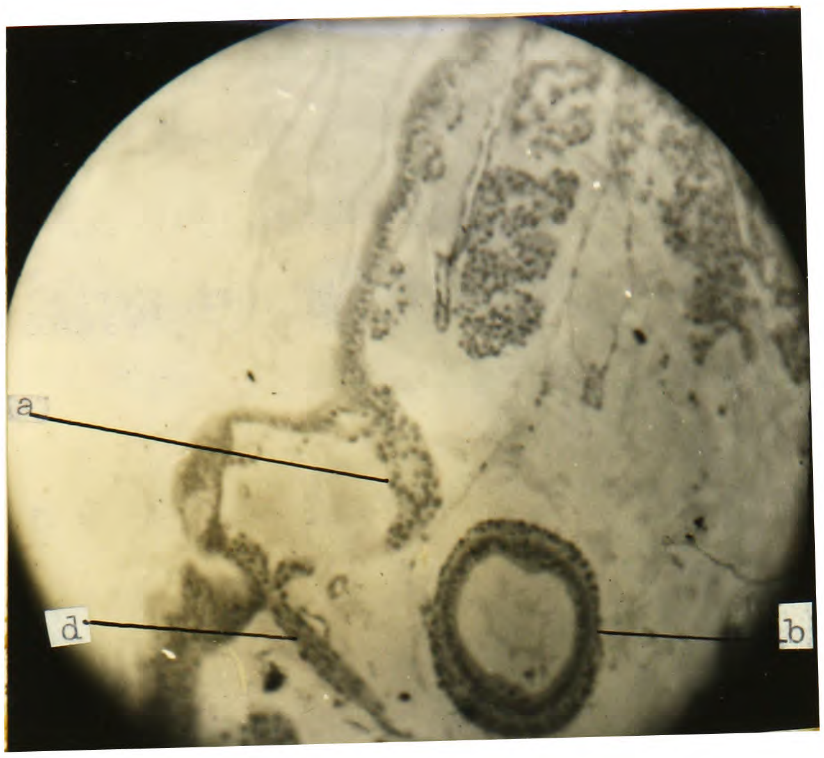


FIG. 1.

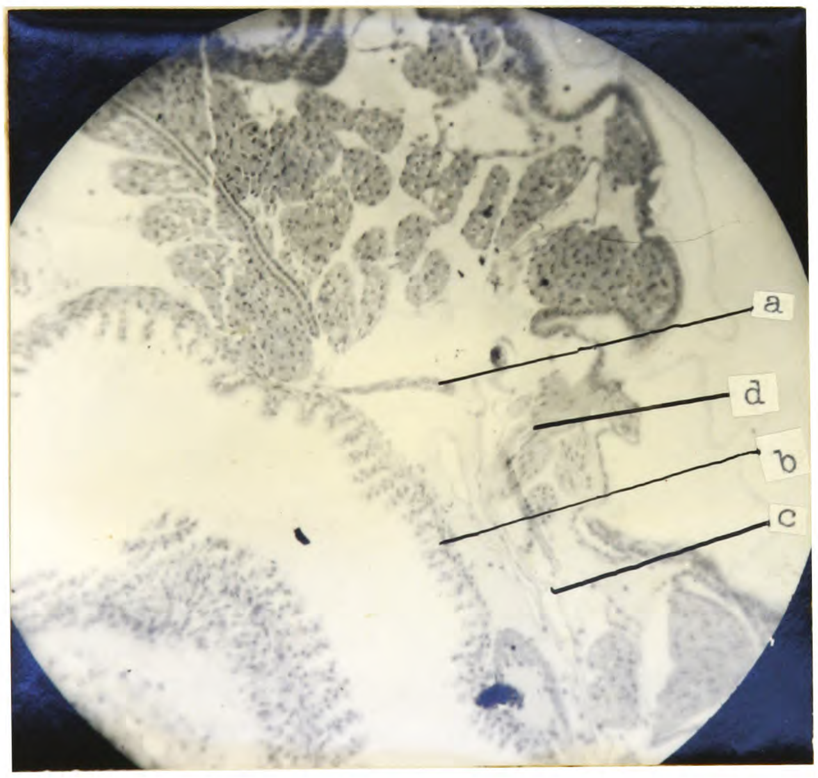


FIG. 2.

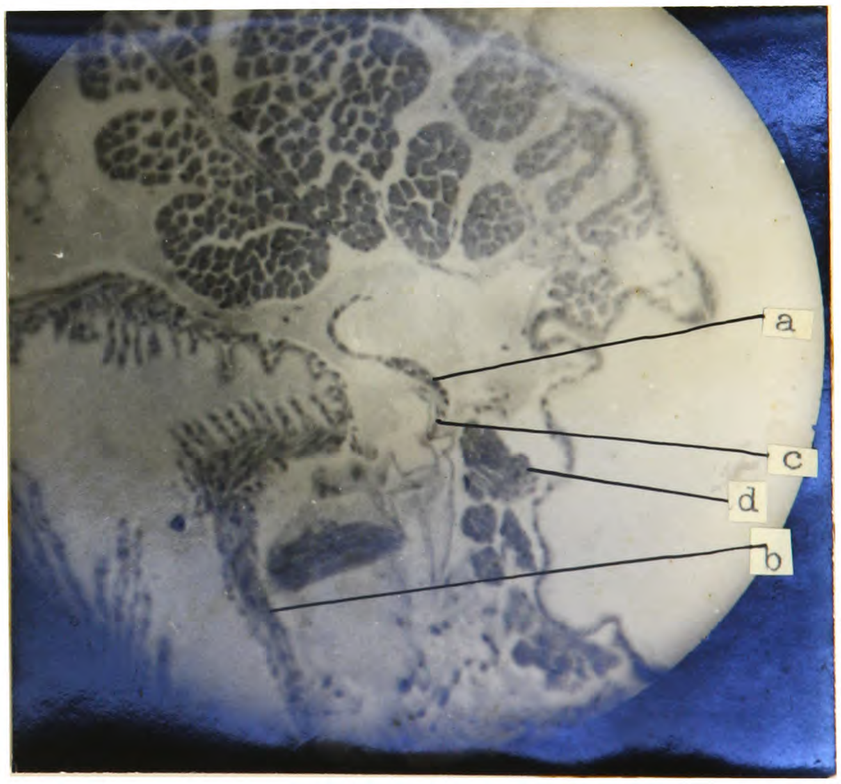


FIG. 3.

each side of the prothoracic ganglion. There is no nerve supply, but they are equipped with central muscular strands and supplied with trachae. There is a relatively large number of nuclei of different stages.

It has already been mentioned that the ventral glands, head glands, etc., are homologous with the prothoracic glands described in some insects.

In Locustana paradalina the prothoracic glands are paired structures. They are elongated and joined to the epidermis at their two ends (Pl.XX,fig.1). The larger middle part of the gland resides near the side of the oesophagus, and they extend posteriorly, just anterior to the first ventral ganglia. Their size varies considerably. The glands are syncytial and the nuclei vary considerably in size. They are usually bean-shaped and lobulated and they contain deeply staining spheres of chromatin material (Pl.XX,fig.1,2). The cytoplasm stained lightly.

It has been previously mentioned that wetting the quiscent eggs of Locustana paradalina was sufficient to stimulate development (Matthee 1951). The position of the embryo in the egg is such that it is situated at the ventral side with its head facing the hydropyle. The ventral part /

part of its body is adjacent to the egg shell. In my serial sections I was able to observe that the neurosecretory cells became conspicuously large, especially at the end of forty-eight hours (Pl.XVIII,fig.1). They attained their maximal size and histological activity at ninety-six hours. The nuclei became intensely stained, and large vacuoles appeared in the cytoplasm (Pl.XVIII,fig.3).

After ninety-six hours the embryo began to revolve , the amnio-serosal membrane having ruptured to allow the embryo to migrate around the end and take up a new position on the dorsal surface of the egg. This process of blastokinesis had caused the embryo to make a revolution, so that the head now pointed towards the opposite pole, with this time the tail pointed towards the micropyle. In this position the embryo develops and grows until emergence.

When the revolution was completed, the neurosecretory cells became smaller and thereafter it became difficult to distinguish them from other cells of the brain (Pl.XVIII,fig.4).

The prothoracic glands are ectodermal in origin (Pl.XX,fig.1), and are ventrally situated in the neck region. They first make their appearance in about seventy-two hours stage as inpushings of the epidermis. In ninety-six hours stage they were quite distinct, but it was at one hundred and twenty hours stage, they attained their maximum size.(Pl.XX,fig.1). The glands were relatively large at this time. The nuclei increased in number, and they assumed in same case lobulated appearance and more marked affinity for stains (Pl.XX,fig.1).

The corpora allata arose as involution of the epidermis. They were first observed in position seventy-two hours after the recommencement of development. At this time they flanked the oesophagus, after having migrated from the proliferating epidermal wall inward, towards, and adjacent to the oesophagus. The glands did not show any noticable cyclical changes during embryogenesis, and their size remained more or less constant. The nuclei did not change their appearance. The sizes of the nuclei varied, and all of them showed marked affinity for stains (Pl.XIX,fig.1,2,3).

The production of an embryonic provisional cuticle is probably universal in insects. It may be produced at any time during the embryogenesis. In _Locustana paradalina_ the provisional cuticle is normally shed about twenty-four hours after the completion of revolution of the embryo. As mentioned previously, the beginning of the formation /

formation of cuticle takes place about forty-eight hours before the revolution.

The main point of interest, as far as this work is concerned, was that the maximal activity of the prothoracic glands coincided with the retraction of the epidermis from the cuticle. When moult was accomplished, the prothoracic glands became reduced in size and showed histological signs of an abatement of activity.

CONCLUSION.

It is clear that the embryonic provisional cuticle is a relatively incomplete structure. It has been referred to as a pellicle or envelope, to distinguish it from the properly formed cuticular exoskeleton. It is, in fact, composed or parts or layers identical with those of a properly formed endocuticle.

The true significance of the provisional cuticle, although of common existence, had not previously been detected. Attention had been focused upon its functional value. It was assumed that its chief purpose was one of protection for the newly emerged insect, which like Grasshopper, had perforce to push its way up to the surface of the soil from a buried egg-pod. The cuticular envelope thus gave protection from abrasion by hard particles.

The functional aspect was certainly valid, but there was every reason to suppose that it was a secondarily acquired adaptation or preadaptation, as these kinds of adaptive structures are. They are of a purely developmental origin.

The present work has shown that in insects exhibiting either hemimetabolous or holometabolous development, the production of a provisional cuticle is also due to developmental /

developmental causes. The production of a provisional cuticle during either embryogenesis or the metamorphic phase of a homometabolous insect, is the result of a moult, initiated to bring the rate of deposition of cuticle into relation to the development of muscle ends. It is, in fact, a process which synchronises cuticle deposition with the development of the muscle ends.

The "pupal" cuticle is a provisional cuticle, and the "pupa" of an insect consists of the developing adult enclosed by a provisional cuticle. Jones (1953) has also pointed out that if the holometabolous insect during metamorphosis was stripped of this provisional cuticle, the "pupal instar" would cease to exist. In any case, it is very difficult to define the period which covers the "pupal stage". It has been pointed out in this work that the developing systems of internal organs and tissues are those of the adult, because the insect for a time is enveloped by a provisional cuticle which is shed. There seems little need to confuse this metamorphic period by attempting to inculcate a separate instar.

The question which arises, of course, is whether "pupation" also occurs within the egg. Provided the interpretation for the "pupal" state put forward in this thesis is accepted, there is no need to suppose that pupation /

pupation within the egg is fundamentally different from metamorphic pupation, because a provisional cuticle is produced in both cases.

The process is carried further in Drosophila because, during the metamorphic phase, that is to say, when the adult systems are actually unfolding, there occur two moults. In other words, the epidermis sheds provisionally laid cuticle twice. The end result is that the muscles become properly attached to the cuticle which is laid down subsequent to the shedding of the provisional cuticles.

It has already been clearly shown in this work that the internal systems of organs and tissues would not appear to be directly affected by these moults of the provisional cuticle. During embryogenesis the progress of this development is unimpeded. Neither do they show apparent signs of change coinciding with the actual moult. Only the epidermis, as one would expect, shows accompanying changes.

During metamorphosis the same procedure is evident. Immediately after the shedding of the cuticle of the final larval instar, the unfolding of the adult systems begins. From this point onwards, the differentiation of the internal systems is aimed towards the achievement /

achievement of the adult organisation.

The retraction of the epidermis from the provisional cuticle during the adult development would, therefore, appear to be a morphogenetic movement, more or less, independent of the rest of the organism. However, the fore-gut and the hind-gut are also involved in the moulting of the provisional cuticle, but no other organs and tissues are affected.

If the development process is a continuous one, it is not unreasonable to criticise the retention of the term "pupal instar" as a fresh load to the already over burdened terminology of Entomology. It must, however, be remembered that the "pupal instar" corresponds to a somewhat indefinable period of the metamorphic development of the adult and in that sense the retention of the term may not get us into much confusion.

Whereas, it was possible to detect and observe changes in the epidermis, which corresponded to the shedding of the cuticle, it was felt that more light could be thrown on the production of the embryonic provisional cuticle and its shedding by attempting to detect changes in the endocrine glands.

I, therefore, examined my serial sections of the embryos /

embryos of Locustana paradalina, in order to try and throw further light on the production of the embryonic provisional cuticle from the internal secretory activity.

It was clear that the moult of the provisional cuticle during the embryogenesis of Locustana paradalina was accompanied by significant changes in different glands.

As already described, the maximal activity of the neurosecretory cells was followed by the maximal activity period of the prothoracic glands. The activity of the prothoracic glands coincided with the actual moult of the provisional cuticle. This inference is drawn from the fact that the prothorocic glands attained their maximum size during the time the moulting of provisional cuticle was taking place.

Jones (1953), has pointed out the significance of the changes of the gland in Locust embryos in relation to diapause and his ligaturing experiments indicate the function of the prothoracic gland. My observation of the histological pictures obtained from my prepared sections of embryos of Locustana paradalina are therefore of interest in confirming their presence.

SUMMARY.

1. Provisional cuticles in insects usually consist of the epicuticle and incompleted endocuticle.

2. The embryonic provisional cuticle of *Locustana paradalina* has an epicuticle composed of the "cement layer", wax layer, "polyphenol layer" and cuticulin layer, and the endocuticular region is tanned. That of *Tenebrio* is similar except that the endocuticle is not tanned.

3. During a moult of provisional cuticle the epidermis shows, not unexpectedly, definite signs of increased activity.

4. The shedding of provisional cuticle is induced in order to synchronise the rate of cuticle deposition with the development of muscle ends, so that a proper integration between the muscles and the cuticular exoskeleton can take place.

5. The protective role of the embryonic cuticle, as illustrated by that of Grasshopper, is secondarily acquired. It may be described as a preadaptation.

6. The epidermis and the fore and hindgut only would appear to be directly connected with a moult of provisional cuticle. The development of the internal systems of organs and /

and tissues does not appear to be effected by, or in need of, such a moult, either during embryogenesis or during metamorphic development of a holometabolous insect.

7. Pupation in insects may be defined as a state of development which exists when the developing adult possesses a provisional cuticle. Despite this definition it has been pointed out that it is difficult to know what period the stage "pupal instar" covers.

8. The embryonic provisional cuticle of *L.paradalina* began to form four days after damping the dormant eggs, i.e., before the revolution of the embryo. It moulted after six days. In the case of *Tenebrio* it began to form on the fourth day after the laying of the eggs. It moulted on the seventh day.

9. In the embryo of *Locustana paradalina*, the muscles gained attachment to the second cuticle on the eighth day: in *Tenebrio* on the seventh day.

10. During the metamorphic development of the adult stage of *Drosophila*, two provisional cuticles are produced and shed subsequent to the moult of the "puparium".

11. The adult systems begin to unfold when the "puparium" is shed. Adult metamorphic differentiation begins at this point, and it progresses without hindrance from the moults /

moults of the provisional cuticle until the proper adult organisation is achieved.

12. In embryos of L.paradalina, the presence of neurosecretory cells of the brain and a pair of glands in the head has been confirmed. It was significant that the glands were well formed at the time of the moult.

REFERENCES.

Auerbach, C. (1935-36). "The development of the legs, wings and halteres in the wild strains of *Drosophila malanogaster*". Transactions of the Royal Society of Edinburgh, *58*; Part III, page 787-817.

Bodenstein, D. (1953). "Endocrine control of metamorphosis" Proc. sixth Int.Congress Entom. Amsterdam. (After Wigglesworth,1953).

Boelitz,E. (1933). "Beiträge zur Anatomie und Histologie der Collembolen. Darmkanal und Mitteldarmepithelregeneration bei *Tomocerus vulgaria Tullberg* und *Sinella Coeca.*" Schott.Zool Jahrb. Abt. Anat. U, Ont.,*57*, (3), page 375-432.

Durken, B. (1907). "Die Tracheenkiemenmuskulatur der Ephe meriden unter Berücksichtigung der Morphologie des Insektenflügels". Z. Wiss. Zool., 87, page 435-550.

Ewest, A. (1937). "Struktur und erste Differenzuring im des Mehlkäfers *Tenebrio molitor*." Areh.Entwickl.Mech.Organ.*135*, page 689 - 752.

Fukuda, S. (1940). "Induction pupation in silk-worm by transplanting the prothoracic glands." Proc. Imp. Academy, Japan, 16, page 414-416.

Fukuda, S. (1941). "Role of prothoracic glands in differentiation of the imagical character in silk-worm pupa." Annot. Zool. Jap., 20 page 9-13.

Fukuda, S. (1944). "The hormonal mechanism of larval moulting and metamorphosis in the silk-worm" Jour.Fac.Sci.Tokyo Univ. Section IV, 6, page 477-532.

Ganin, M. (1876). (After C.W.Robertson, 1936).

Geigy,R. and Ochse,W.(1940). "Versuche über die innern Faktoren der verpupung bei Sialis lutaria." L.Rev. Suisse. Zool., 47,page 225-241.

Hanstrom, B. (1938). "Zwei Probleme betreffs der hormonalen Lokalization im Insektenkopf." K. Fysiogr. Sällsk. Handl. Lund, N.F., 49, page 3-17

Hare (1936). (After V.B.Wigglesworth, 1952).

Henneguy, F. (1906). "Les modes d'insertion des muscles /

Henneguy, F. (1906). "Les modes d'insertion des muscles sur la Cuticle chez les Arthropods." C.R. Assoc. Anat. (8th reunion, Bordeaux), page 133-140.

Hinton, H.E. (1946b). "Concealed phases in the Metamorphosis of Insects." Nature, *157*, page 552-553.

Hinton, H.E. (1947). "On the function, Origin and Classification of Pupae". Proceedings of the South London Entomological and Natural history Society. Page 111-154.

Jones, B.M. (1950). "Acarine growth; a new ecdysial mechanism." Nature,*166*, page 908-911.

Jones, B.M. (1951b). "The growth of the harvest mite, *Trombicula autumnalis* Shaw." Parasitology, *41*, page 229-248.

Jones, B.M. (1951). "Cyclic growth in mites, does it suggest new approach to such growth in insects". Jour. Soc. Brit.Entom. *4*, (1), page 20-21.

Jones, B.M. (1953). "Activity of the incretory centres of *Locustana paradalina*, during /

embryogenesis: Function of the prothoracic glands". Nature, <u>172</u>, page 551-552.

Kopec', S. (1922). "Studies on the necessity of the brain for the inception of the insect metamorphosis." Biol. Bull. Woods Hole, <u>42</u>, page 322-342.

Koller, G. (1938). "Hormone bei Wirbellosen Tieren. Akademische Verlagsgesellschaft m. b. h." Leipzig. Page viii+143.

Kowalevsky, (1887). "Beiträge zur Kenntniss der nachembryonalen Entwicklung der Musciden." Zeits.Wiss. Zool.<u>45</u>, (After A General Text Book of Entomology, A.D. Imms,1938).

Lison, L. (1936). "Histochimie Animale," Paris, (Gauthier Villars).

Mansour, K. (1934). "The developmentof the adukt mid-gut of Coleopterus insects and its bearing on systematics and embryology." Egypt University Bulletin, Faculty of Science <u>2</u>, page 1-34.

Matthee, J.J. (1951). "The Structure and Physiology of the /

Egg of *Locustana paradalina* (Walk)." Deptt. of Agriculture, Locust Research Series No.13. Union of South Africa.

Nelsen, O.E. (1934). "The segregation of the germ cells in the Grasshopper *Melanoplus differentia* ". Jour. Morph. *55*, page 545-574.

Pflugfelder, C.F.A. (1947). "Uber die ventraldrüsen und einige andere inkretorische Organe des Insektenkopfes." Biol. Zbl. *66*,page 211-235.

Piepho. H. (1942). "Untersuchungen Zur Entwicklungsphysiologie der Insektenmetamorphose." Roux . Arch. Entw. Mech. Organ., *141*, page 500-583.

Possompes, B. (1950). "Roled de Cerveau au Cours de la Metamorphose de Calliphora erythrocephalia. Meig. C.R.Acad. Sci., Paris, *251*, page 594-596.

Poyarkoff, E. (1914). "Essai d'une theorie de la nymphe des insectes holometabolous." Arch. Zool. Paris, *54*, page 221-265.

Pratt, H.S. (1900). The embryonic history of imaginal discs in Melanophagus ovinus, togather with an account of earlier stages in the development of the insects." Proc. Boston Soc. Natural History, 29, (13), page 241-272.

Pryor, M.G.M. (1940). "On the hardening of the ootheca of *Blatta orientalis*." Proc. Royal Society of London, Series B, No.852, 128, page 378-392.

Robertson, C.W. (1937). "The Metamorphosis of *Drosophila melanogaster* including an accurately timed account of the principal morphological changes." Jour. Morph. 59, (2), page 351-399.

Roonwal, M.L. (1936). "Studies on the embryology of the African migratory locust, *Locusta migratoria migratorides*. The early development with a new theory of multi phased gastrulation among insects." Phil. Trans. Roy. Soc. Sec. B. 226, page 391-421.

Roonwal, M.L. (1937). "Studies on the embryology of the African migratory locust, *Locusta migratoria migratorides*. Organogeny." Phil. Trans. Roy. Soc. Series B 227, page 175-244.

Scharrer, B. (1948a). "The prothoracic glands of *Leucophaea maderae* (orthoptera)." Biol. bull. Woods Hole,95,page 186-198.

Sellier, R. (1951). "La Glande prothorcique des Gryllides." Arch. Zool. Exp. Gen. 88, (Notes et Rev.), page 61-72.

Strasburger, E.H.(1935). "*Drosophila melanogaster* Meig. Eine Einführung in den Bau und die Entwicklung." 60pp. Julius Springer, Berlin.

Stuart, R.R. (1935). "The development of the mid-intestine in *Melanoplus differentialis*". Jour. Morphology, 58,page 419-438.

Thomsen, E. (1941). "Ringdrüse und Corpusallatum bei Musciden". Naturwissenschaften, 29, page 605-606.

Tower, W.L. (1906). "Observations on the changes in the hypodermis and cuticula of Coleoptera during ecdysis". Biol. Bull., 10, page 176-192.

Vaney, C. (1902). "Contributions a'l'e' tude des larves et des me'tamorphoses des Dipteres". Ann. Univ Lyon.n.s.1. Fase.9. (After A.D.Imms.).

Van Rees, (1888). "Beitrage zur Kenntniss der innern metamorphose von *Musca vomitoria*". Zool. Jahrb. Anat., 3, (After A.D.Imms).

Vogt, M. (1943). "Zur produktion und Bedutung metamorphosefordernder Hormone Wahrend der Larvenentwicklung Von *Drosophila*". Biol. Zbl., 63, page 395-446.

Weismann, C.M. (1864). "Die nachembryonale Entwicklung der Musciden". Zeits. Wiss. Zool., 14. (After A.D.Imms.).

Wigglesworth, V.B.(1933b). "The physiology of the cuticle and of ecdysis in *Rhodnius prolixus*; with special reference to the function of the oenocytes and of the dermal glands". Quart. Jour. Micro. Science. 76, page 269-318.

Wigglesworth, V.B.(1936). "The function of the Corpus-allatumin the growth and reproduction of *Rhodnius prolixus*."Quart. Jour. Micros. Sci. 79, page 91-121.

Wigglesworth, V.B.(1940). "The determination of character at metamorphosis in Rhodnius prolixus (Hemiptera)". Jour.Exp. Biol. 17, page 201-222.

Wigglesworth, V.B.(1947). "The Epicuticula of an insect *Rhodnius prolixus*, (Hemiptera)". Proc. Roy. Society of London.(B), 134, page 153-181.

Wigglesworth, V.B.(1948). "The structure and deposition of the cuticle in the adult mealworm, *Tenebrio molitor*,L.(Coleoptera)". Quart. Journ. Micro. Sci. 89, page 197-217.

Wigglesworth, V.B.(1950). "A TEXT BOOK OF INSECT PHYSIOLOGY" METHUEN AND CO. LTD., LONDON.

Wigglesworth, V.B.(1951). "Source of moulting hormone in Rhodnius". Nature, 168, page 558.

Wigglesworth, V.B.(1952). "The thracic glands in *Rhodnius prolixus*,(Hemiptera), and its role in moulting". Journ. Exp.Biol. 29, page 561-570.

Wigglesworth, V.B.(1952). "Hormone balance and control of metamorphosis in *Rhodnius prolixus*, (Hemiptera)". Journ. Exp. Biol. 29, page 620-631.

Williams, C.M. (1947). "Physiology of Insect diapause. II Interaction between the pupal brain/

and prothoracic glands in the metamorphosis of giant silk-worm *Platysemia cecropia*". Biol. Bull. Woods Hole, *93*, page 89-98.

Williams, C.M. (1948b) "Physiology of Insect diapause; III The prothoracic glands in the Cecropia silk-worm". Biol. Bull. Woods Hole, *94*, page 60-65.

Williams, C.M. (1949) "The prothoracic glands of Insects in retrospect and prospect". Biol. Bull. Woods Hole, *97*, page 111-114.

Williams, C.M. (1952) "Physiology of Insect diapause:IV. The brain and prothoracic glands as an endocrine system in the Cecropia silk-worm". Biol. Bull. Woods Hole, *103*, page 120-138.