THE DETERMINATION OF CHARACTERS AT METAMORPHOSIS IN *RHODNIUS PROLIXUS* (HEMIPTERA)

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(With One Plate and Twelve Text-figures)

Two factors have been recognized as regulating moulting and metamorphosis in *Rhodnius prolixus*: a "moulting hormone" and a so-called "inhibitory hormone" (Wigglesworth, 1934, 1936).

(i) *Moulting hormone.* The secretion of this factor is brought about by the stretching of the abdomen by a large meal of blood. Such distension apparently provides a nervous stimulus that is conveyed to the brain. The hormone seems to be secreted in the head, and it was suggested that the corpus allatum is the source of it. But no satisfactory experimental evidence could be produced for this view, which will be refuted in the present paper. The moulting hormone circulates freely in the blood, and after a "critical period" (some 5 days after feeding in the 4th stage, some 7 days in the 5th stage), if the blood is transfused through a capillary tube into other nymphs deprived of the source of hormone (by decapitation while unfed or shortly after feeding), these will be induced to moult. The moulting hormone is present in the blood of each of the five nymphal stages; it initiates cell division, which is followed by the deposition of a new cuticle. In the presence of this factor alone, the structural characters of this cuticle, no matter which instar has been caused to moult, are those of the adult insect.

(ii) *Inhibitory hormone.* This factor becomes operative only when growth and moulting have already been initiated by the moulting hormone. It is present in the blood of the first four nymphal stages, and has the effect of preventing the development of the imaginal characters. (It is not inhibitory in the sense of opposing the action of the moulting hormone, as some authors have assumed). In the presence of this hormone (in addition to the moulting hormone) the insect develops nymphal characters once more when it moults. The inhibitory hormone is secreted by the corpus allatum in the first four nymphal stages. This gland appears to produce it when exposed to the action of the moulting hormone; nervous stimulation is not required. Further, the inhibitory action of the corpus allatum is greater in the earlier nymphal stages than in the later: the gland in the 3rd stage will prevent a

moulting 4th stage nymph from developing 5th instar characters and cause it to develop 4th instar characters again.

The purpose of the present work is first to reinvestigate the source of the moulting hormone; and then to consider more closely the mode of action of these two factors in controlling the morphological characters; to find out, in particular, at what stage in development determination of morphological characters takes place and to what extent such determination is reversible.

SOURCE OF THE MOULTING HORMONE[1]

When the study of moulting in *Rhodnius* was first undertaken (Wigglesworth, 1934) many attempts were made to induce moulting in nymphs decapitated 24 hr. after feeding by the implantation of their head contents into the abdomen. These experiments were unsuccessful; and when it was found that moulting could be induced by transfusion of blood from decapitated insects they were abandoned. They have now been taken up again—but with the difference that the organs for implantation have been removed from insects that had just passed the critical period.

In the first series of experiments the brain, together with the suboesophageal ganglion, the sympathetic (oesophageal) ganglion and the corpus allatum, was dissected in Ringer's solution from 4th stage nymphs at 6 or 7 days after feeding or from 5th stage nymphs at about 10 days after feeding. The nerves connecting the sympathetic ganglion with the brain were cut through and the brain plus sub-oesophageal ganglion then separated from the sympathetic ganglion plus corpus allatum (Text-fig. 1 A). These fragments were then transplanted separately, through oblique cuts at the hind end of the abdomen, into 4th stage nymphs decapitated at 24 hr. after feeding. Nymphs decapitated at this stage never moult unless artificially provided with moulting hormone. Consequently, if moulting could be induced in a single one of them it would afford conclusive evidence that it had been provided with a source of this hormone.

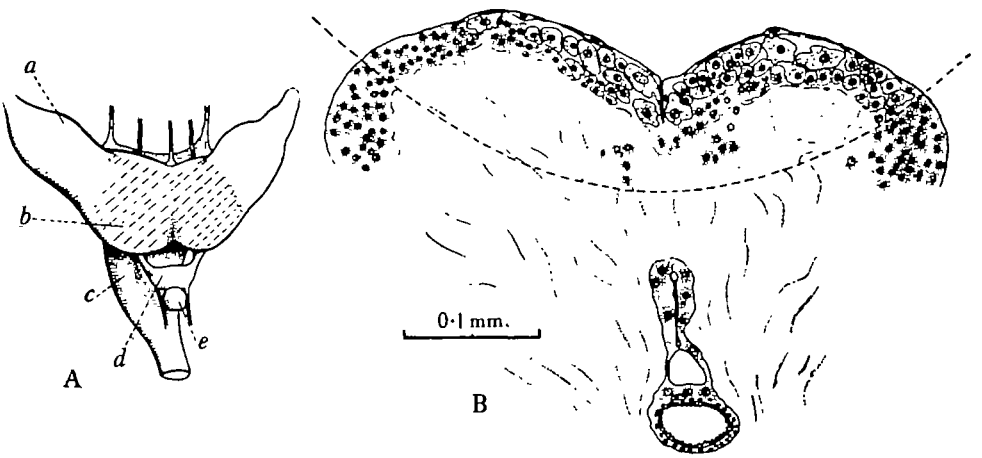
Excluding the few insects (about 10% of the whole) which died, 16 received implants of the corpus allatum and sympathetic ganglion; in two of them corpora allata from two insects were implanted; but although they survived in many cases for more than six months none of them was induced to moult.

On the other hand, of 25 insects which received implants of the brains, 21 moulted. In the absence of the inhibitory hormone of the young corpus allatum they developed imaginal characters.

The brain forms a very much larger fragment than the corpus allatum, and in order to exclude the possibility that in these experiments it served merely to transfer mechanically hormone secreted into the blood elsewhere, large pieces of fat body were transplanted from the donor insects, without washing in Ringer's solution, into decapitated 4th stage nymphs. In five such experiments no moulting was obtained, although the washed brains from the same insects induced moulting.

[1] Preliminary note published (Wigglesworth, 1939*b*).

An attempt was then made to locate the source of the hormone within the brain. In preliminary experiments the optic lobes, the dorsal and ventral halves of the central mass of the brain, and the suboesophageal ganglion were implanted separately. Positive results were obtained only with the dorsal half of the central mass. The brain was then divided into two parts—a small fragment excised from the dorsum of the protocerebrum, including the floor of the depression between the protocerebral lobes, and the remainder of the brain plus the suboesophageal ganglion. Out of nine decapitated 4th stage nymphs which received the small dorsal fragments, six were caused to moult; out of nine insects which received the rest of the brain none moulted. In this case the fragment which gave negative results was far larger than that which provided the moulting hormone.



Text-fig. 1. A, brain of 5th stage nymph. *a*, optic lobes; *b*, protocerebrum; *c*, suboesophageal ganglion; *d*, sympathetic (oesophageal) ganglion; *e*, corpus allatum. The shaded area of the protocerebrum shows the approximate extent of the excised region. B, vertical section through the posterior part of the protocerebrum. In the central region above are the large cells with fuchsinophil inclusions; laterally are ordinary ganglion cells. The broken line shows approximately the region excised.

In the adult *Rhodnius* Hanström (1938) has described peculiar large nerve cells in this dorsal region of the protocerebrum, in which the cytoplasm is often lobulated and contains rounded masses which stain with acid fuchsin. He suggests that these may be secreting cells.

In order to see whether these cells show visible changes during the secretion of the moulting hormone, brains of the 4th stage nymphs were fixed in Bouin's solution before feeding and at six days after feeding, sectioned, and stained with Ehrlich's haematoxylin and van Gieson. Text-fig. 1 B shows a section through the dorsum of the protocerebrum. The large cells with the fuchsinophil inclusions are exactly as described by Hanström; but no certain differences could be detected between the cells in the fasting insect and at the height of the secretion of the moulting hormone. Cells of this type were very evident in the disorganized brain tissue when sections were made of the implanted dorsal fragments.

Whether these cells are really the source of the hormone must be left undecided. The presence of fuchsinophil inclusions of this kind is commonly regarded as evidence of a secretory function (Scharrer, 1937); but it may be pointed out that the cells of the corpus allatum, which are almost certainly the source of the inhibitory hormone, do not stain in this way; they are either vacuolated or have a dense and homogeneous or very finely granular cytoplasm.

THE EARLY HISTOLOGICAL CHANGES PRODUCED BY THE "INHIBITORY HORMONE"

The histological changes in the epidermis and associated tissues of the abdomen during moulting have already been described in detail (Wigglesworth, 1933). But in order to throw light on the mode of action of the "inhibitory hormone" these changes have been reinvestigated.

The entire dorsum of the abdomen has been fixed with Carnoy's fixative, stained with haematoxylin, and mounted whole on successive days after feeding; three preparations being usually made for each day. Omitting the changes that occur in the oenocytes and dermal glands, which have already been described, the changes in the epidermis, at 24° C., when the 5th stage nymph moults to an adult may be summarized as follows.

1st day after feeding: no visible change. Chromatin still scattered throughout the nuclei.

2nd day: "activation" of the cells begins; i.e. the cytoplasm becomes increasingly dense and the chromatin becomes clumped at the centre of the nuclei (cf. Wigglesworth, 1937). This change occurs all over the abdomen but is most marked at the intersegmental membranes and at the margins.

5th day: mitosis begins at the margins of all the segments and over the general surface of the first segment and the two terminal segments (Text-fig. 2 A). Chromatin droplets derived from dead nuclei begin to appear.[1]

6th day: mitoses begin at the central point of the intersegmental membranes. They are now exceedingly numerous in the lateral, anterior and posterior regions of the abdomen—that is, in the regions where morphological change is greatest (Text-fig. 2 B).

7th day: mitoses very numerous at the central point of the intersegmental membranes (Text-fig. 2 C).

8th day: vacuolation appears in the apical parts of the cells all over the tergites.

9th day: mitoses now occur all along the intersegmental membranes (Text-fig. 2 D).

10th–11th day: cells appear to be detached from the cuticle all over the tergites. A very *few* mitoses occur on the general surface remote from the margins and the intersegmental membranes. Chromatin droplets begin to appear below the bristles and plaques.

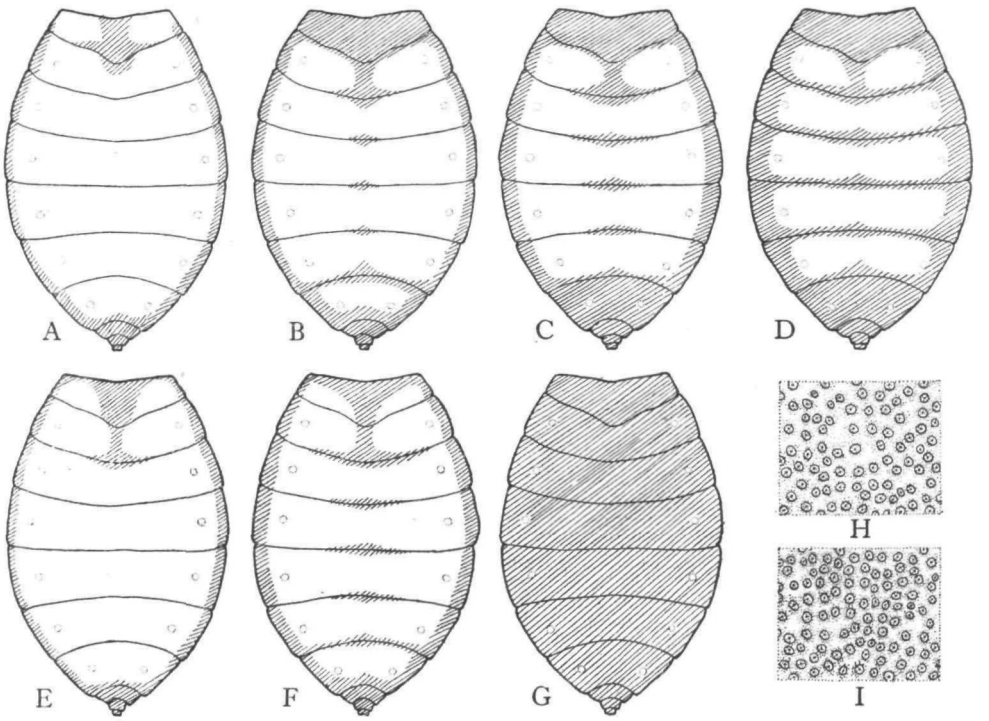
[1] The significance of these chromatin droplets and the evidence that they are derived from defunct cells will form the subject of a separate paper.

13*th day:* formation of chromatin droplets occurs over the general surface of the segments, particularly around the plaques.

16*th day:* a few mitoses still occurring. Formation of chromatin droplets still in progress.

18*th day:* deposition of new cuticle begins.

28*th day:* moulting occurs.



Text-fig. 2. Shaded areas show the approximate distribution of mitoses during the moulting of 5th stage nymph; A–D, imaginal moult. A, 5 days after feeding; B, 7 days; C, 8 days; D, 9 days. E–F nymphal moult. E, 5 days; F, 6 days; G, 7 days. H–I, density of cells in approximately corresponding areas of tergites after the completion of mitoses; H, imaginal moult; I, nymphal moult.

The first change visible in the cells is their general "activation". This occurs on the second day after feeding and affords evidence that the moulting hormone is already being secreted at this stage. The "critical period", which occurs at about the seventh day, corresponds with the stage at which mitoses are becoming general. They have already begun in the peripheral regions of the abdomen by the fifth day. In those insects which, under experimental conditions, occasionally show partial moulting, this is confined to those regions in which mitosis occurs earliest. It is worth noting that there is no sign of a "wave" of mitoses passing from before backwards over the abdomen, such as has been described by Kühn & Piepho (1938) in Lepidoptera larvae.

If the 5th stage nymphs are decapitated and caused to moult by joining them

to other 5th stage nymphs that have passed the critical period, the same changes take place in the abdomen. But they appear more rapidly—doubtless because the moulting hormone is supplied earlier in higher concentration. Within four days the normal seventh day stage is reached; by the tenth day mitoses have almost ceased and the normal fifteenth day stage is reached.

During the moulting of the 4th stage nymph to produce the 5th stage the histological changes are at first similar to those just described; but mitoses later become numerous throughout the epidermis and other differences are to be observed. In order to prove that these differences are due to the presence of the "inhibitory hormone" a number of 5th stage nymphs were decapitated 24 hours after feeding and caused to moult by joining them to 4th stage nymphs with the corpus allatum intact. The histological changes in the 5th stage nymphs, which, owing to the presence of the inhibitory hormone, now undergo another nymphal moult, were studied as before.

Cross-circulation in this way causes, as we have seen, a considerable acceleration of the moulting process. In order to allow a better comparison with the changes already described in the normal moulting of the 5th stage nymph, the times in the summary which follows have been corrected as far as possible so as to eliminate this accelerating effect.

5th day after feeding: "activation" everywhere; mitoses numerous in the marginal zone and at the anterior and posterior extremities of the abdomen (Text-fig. 2 E).

6th day: mitoses very numerous laterally and just beginning at the middle of the intersegmental membranes (Text-fig. 2 F).

7th day: mitoses numerous along the intersegmental membranes.

8th day: mitoses exceedingly numerous not only along the intersegmental membranes but all over the general surface of the segments. They are most abundant above the heart, in the mid-line of the tergites (Text-fig. 2 G).

10th day: mitosis almost complete; cells densely packed all over the surface, and readily detached from the cuticle. Chromatin droplets appear in small numbers here and there.

12th day: deposition of new cuticle begins.

These times are subject to much variation, but the chief differences between a nymphal moult and an imaginal moult are clear. At first the distribution of mitoses is similar; but soon after the critical period they occur in great numbers all over the abdominal segments during the nymphal moult, whereas during the imaginal moult they are practically confined to the intersegmental membranes and the periphery of the abdomen. Hence, when the period of cell division is complete, the number of cells per unit area is much greater at a nymphal moult (Text-fig. 2 H, I). The formation of chromatin droplets, indicating the death of existing cells, is much more evident at the imaginal moult.

In the converse experiment, when an imaginal moult is induced in a decapitated 4th stage nymph by joining to a 5th stage nymph, the histological changes are those of the imaginal moult.

SWITCH OVER FROM NYMPHAL TO IMAGINAL DEVELOPMENT

It is clear from the foregoing account that the histological changes in the epidermis of the abdomen—the universal "activation" of the cells, followed by mitoses in the marginal zone and later along the intersegmental membranes—are the same whether a nymphal or an imaginal instar is to be produced. Indeed, in the earlier papers on moulting in *Rhodnius* evidence has already been produced that at all instars growth is initiated by the same moulting hormone, and that the "inhibitory hormone", responsible for the production of nymphal characters, is secreted, or is able to exert its action, only when the process of moulting has already been started. It was shown, for example, that by decapitating the early nymphal stages around the critical period, and thus eliminating the source of the inhibitory hormone, it is possible to obtain a graded series of forms ranging from normal nymphs, through all degrees of intermediates, to precocious adults (Wigglesworth, 1934).

These intermediate forms were believed to represent insects which had begun developing nymphal characters and then, as a result of a deficiency of "inhibitory hormone", had continued with an intermediate type of development.

In these experiments there is not, of course, a complete removal of the inhibitory factor at a given stage. Any quantity of this factor present in the blood at the time of decapitation either persists in the blood, or is absorbed by the tissues, and therefore is able presumably to continue to exercise its effects throughout development. There is not a complete "switch over" in development.

SWITCH OVER FROM IMAGINAL TO NYMPHAL DEVELOPMENT

It was of interest to perform the converse experiment: to allow nymphs to start developing towards adults and then at different stages in the process to expose the tissues to the inhibitory hormone and to see to what extent they could be induced to switch over to a nymphal type of development.

The experiment was carried out as follows. A large batch of 5th instar nymphs was fed, and at daily intervals thereafter several of them were decapitated and joined to 4th instar nymphs, six or seven days after feeding, in which the anterior part of the head had been removed but the corpus allatum retained. At 24° C. the interval between feeding and moulting in the 5th stage nymph averages 28 days. The experiment was carried out at daily intervals from the time of feeding until the twenty-seventh day. As illustrating the simplicity of the technique it may be recorded that out of 160 insects joined for this purpose only one pair died before moulting took place.

It is evident that in these experiments the 5th instar nymphs developed as imagines up to the time of decapitation. Thenceforward they received inhibitory hormone. They certainly received small quantities of this hormone within the next hour or two; but it would probably be several days before the corpus allatum of the

4th stage nymphs could raise the concentration of this hormone up to the normal level in the blood of both insects. The "switch over" in development must therefore be gradual. It is also subject to considerable variation; due in part to the insects on a given day having reached slightly different stages in the moulting process, but probably due chiefly to differences in the efficiency of mixing of the blood from the two insects after joining. The time-table which follows is therefore to some extent arbitrary—it is based on the most successful effects produced at a given day. The only characters studied are those visible in the dorsal surface of the abdomen.

1st–5th day: (Pl. I, fig. 1). If the switch over occurs before the critical period the characters of the dorsum of the abdomen are entirely nymphal. Many new plaques and bristles have made their appearance, particularly in the lateral parts of the segments (Text-fig. 3 A).

7th day: (Pl. I, fig. 2). The pigment pattern of the abdomen is that of a normal nymph; except at the hinder end of the seventh segment where the pigmentation and the type of cuticle are imaginal. Almost all the plaques in the general cuticle are normal in form, but a few have disappeared or are imperfect (Text-fig. 3 B).

8th–9th day: (Pl. I, fig. 3). The general pigmentation is nymphal but the sides of the segments show traces of imaginal pigmentation at their anterior margins. The first segment and the terminal segments are becoming imaginal in form. There is no sign of the hinge line (Wigglesworth, 1940) except on the seventh segment. The cuticle of the general surface has the stellate folding characteristic of the nymph, but many of the plaques have disappeared, particularly in the central region of each segment. When plaques are present many of them have defective bristles or none at all (Text-fig. 3 C).

10th day: the imaginal area on the seventh segment is spreading further forward. The general cuticle now has very few plaques, but numerous pale areas in which the epicuticle is unpigmented are visible where they previously existed (Text-fig. 3 D).

11th day: (Pl. I, fig. 4). The nymphal pigment spots are present and at the same time a mottled pigmentation extends over the area normally occupied by the imaginal pigment spots.

12th day: (Pl. I, fig. 5). The imaginal area extends almost all over the seventh segment. The hinge line is well developed in this segment but not elsewhere. The abdominal plaques are still more scarce.

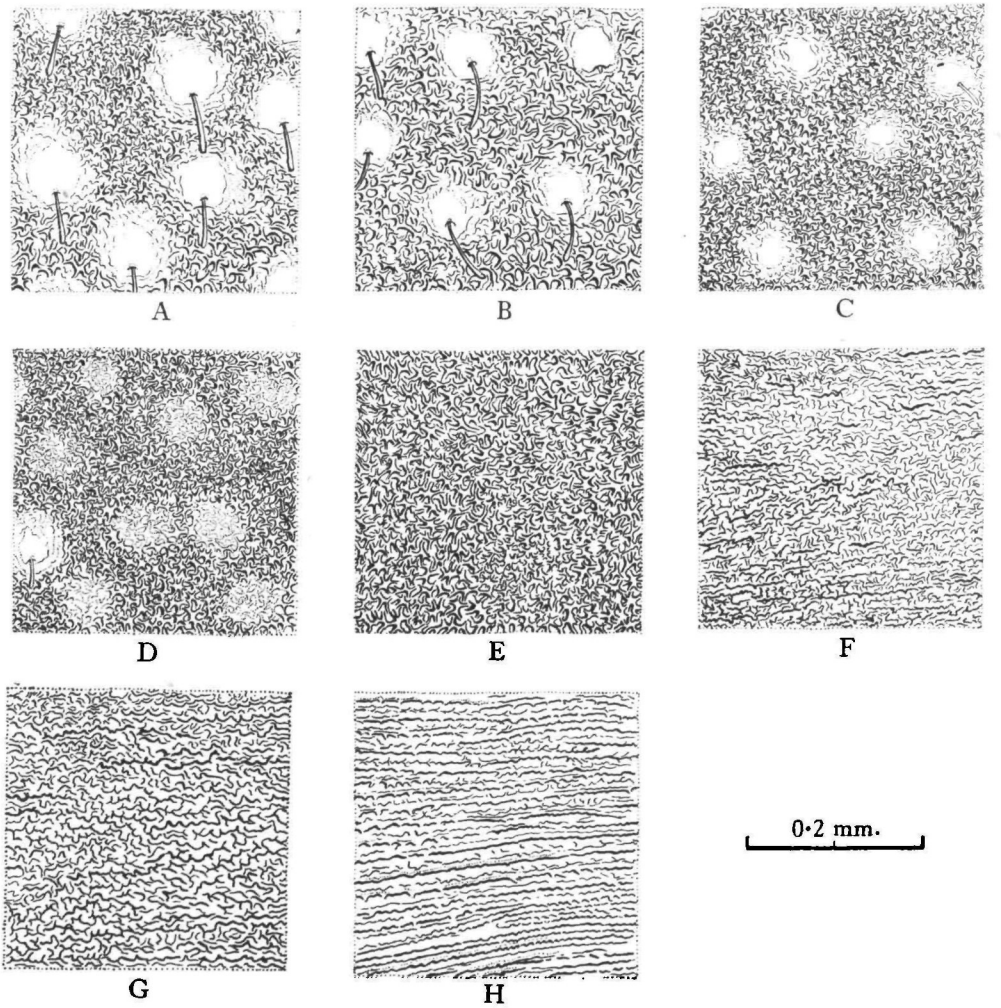
13th day: (Pl. I, fig. 6). The hinge line now extends forward to the sixth segment, and traces of it are visible on most of the remaining segments. The pigment spots combine both adult and nymphal distribution. The general cuticle is of nymphal type, but plaques are practically absent (Text-fig. 3 E).

14th day: (Pl. I, fig. 7). Imaginal cuticle present in the intersegmental regions and at the sides of the abdomen. The hinge line is present throughout, though not fully formed. Imaginal pigment spots are present and normal; nymphal pigment spots are becoming incomplete. The cuticle is of nymphal type in the central part of the segment, but plaques are absent; it becomes imaginal towards the margins (Text-fig. 3 F).

16th day: apart from a small area of intermediate cuticle in the central part of

each segment (Text-fig. 3 G), pigmentation and cuticle structure are entirely imaginal.

17th day onwards: (Pl. I, fig. 8). Pigment pattern and cuticle structure are of normal imaginal type (Text-fig. 3 H).



Text-fig. 3. Typical areas of the cuticle in tergites of insects produced from 5th stage nymphs switched over from imaginal to nymphal development at different times after feeding. A, 5 days; B, 7 days; C, 9 days; D, 10 days; E, 13 days; F, 15 days; G, 16 days; H, 17 days. All drawn over photographs and then bleached.

These experiments prove that the determination of the epidermis to form imaginal cuticle proceeds at a different rate in different parts of the abdomen. Many parts remain capable of reverting to a nymphal type of development, under the influence of the inhibitory hormone, long after the critical period; while other parts are early committed irrevocably to lay down imaginal structures.

When this sequence of changes is compared with the normal succession of histological changes in the epidermis, as described above (p. 204), the two are seen to be closely related. Activation and cell division, with the formation of chromatin droplets as the result of cell death take place earliest in the first and the terminal segments and along the margins of the abdomen. And these are the regions which first become irrevocably determined. Chromatin droplets begin to appear beneath the plaques and bristles about the tenth day. It is on the eighth or ninth day that a switch over to nymphal development results in plaques with bristles wanting or imperfect; while after a switch over on the tenth or eleventh day the plaques are almost absent.

The appearance of chromatin droplets is often due to the death of cells which had some specialized function in the nymph—for example, bristle-forming cells. When these cells die this function is necessarily lost. But it is certain that in many cases the same cell may have its function altered by the inhibitory hormone. A striking example is seen where the switch over occurs about the tenth day. Many of the bristle-forming cells have already succumbed, so that bristles are absent. In many places the cells which surrounded them are incapable of laying down the characteristic cuticle of the plaque. They can, however, lay down ordinary nymphal epicuticle with stellate folds and they retain the faculty of secreting an epicuticle that is unpigmented, as it normally is over the plaques (Text-fig. 3 D). From this it is evident that the various faculties of a given cell can be determined to some extent independently of one another.

Finally, it is interesting to note that the type of cuticle to be laid down by the cells over the central parts of the abdominal segments is still capable of reversal two or three days before its deposition is due to begin. Deposition of cuticle commences about the eighteenth day; a switch over on the fifteenth day results in nymphal cuticle on parts of the segments; a switch over on the sixteenth day results in small areas of intermediate cuticle. But once the deposition of cuticle has begun, neither its form nor its pigmentation (the pigmentation does not appear until after moulting, about the twenty-eighth day) can be influenced by the inhibitory hormone.

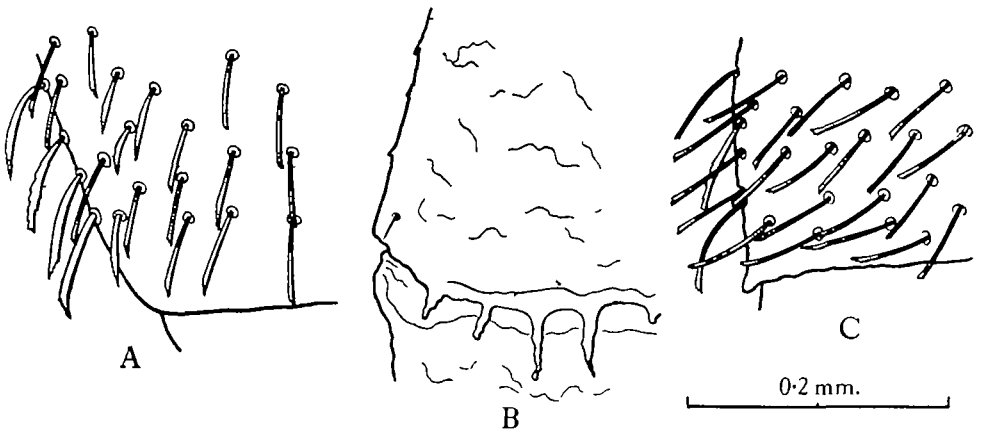
INDUCTION OF MOULTING IN THE ADULT

The question now arises as to whether the epidermis of the adult retains the faculty of laying down a new cuticle; and whether the characters of this cuticle can be influenced by the hormones present.

When studying the effect of the secretion from the corpus allatum on egg production in the adult female, 5th stage nymphs of *Rhodnius* were joined, soon after the critical period, to decapitated adults; but in no case was the adult caused to moult. From these experiments it was concluded that the adult is incapable of renewed ecdysis (Wigglesworth, 1936). But later it was found that the adult insect is able to repair wounds, with the local formation of new cuticle, and that the histological changes in the epidermis during this process are identical with those occurring during moulting (Wigglesworth, 1937). Meanwhile Furukawa (1935) had

shown that if the antenna of an adult earwig (*Anisolabis*) is transplanted on to the base of the cercus of a nymph of the same species it will moult again simultaneously with the nymph; and recently Mauser (1938) has shown that the imaginal fore-limb of *Dixippus* implanted on to the stump of a limb of a 5th or 6th stage nymph can be induced to moult again and to develop traces of the red colouring on the inner aspect of the femur, which is an imaginal character.

It seemed probable therefore, that the failure of the adult *Rhodnius* to moult in the earlier experiments was due to an insufficient quantity of moulting hormone. Acting on this notion the small adult of the bed-bug *Cimex* was joined after decapitation to the relatively large 4th or 5th stage nymph of *Rhodnius*. As stated in a preliminary note (Wigglesworth, 1937), this experiment was successful, and the *Cimex* adults were duly caused to moult.

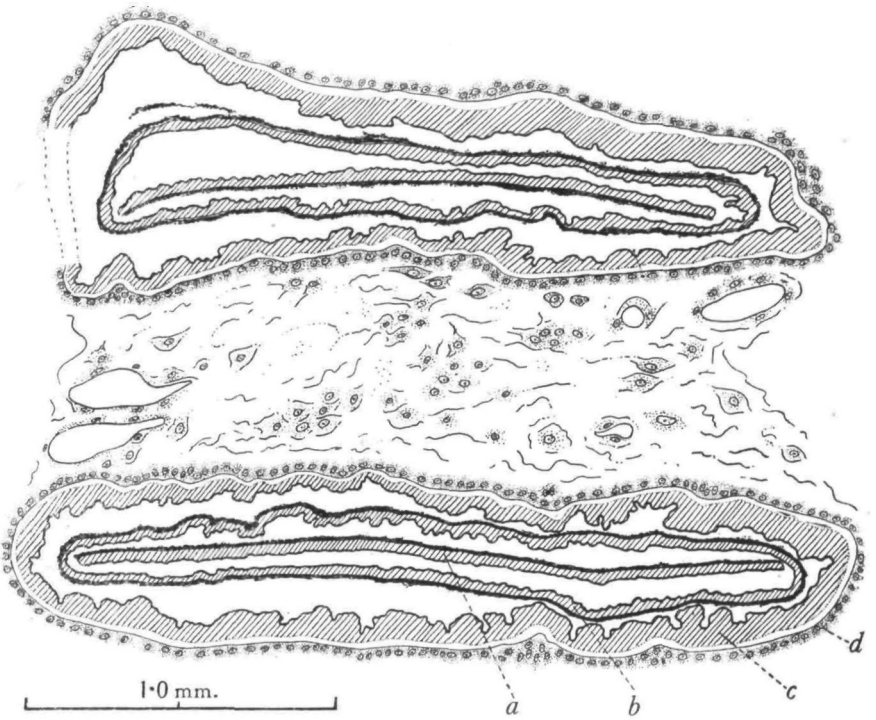


Text-fig. 4. Corresponding areas at posterior angle of third abdominal tergite in *Cimex*. A, normal adult; B, instar produced by inducing moulting in an adult 3 months old. C, instar produced by inducing moulting in an adult less than 24 hr. old.

The new cuticle laid down by the adult *Cimex* in these experiments was more or less formless. The intersegmental membranes of the abdomen were recognizable, but the cuticle was practically devoid of hairs or spines and had developed along the margins of the segments a number of curious non-articulated outgrowths of bizarre shapes (Text-fig. 4 B). The epidermis of the adult *Cimex* thus appears to have lost the faculty of laying down a cuticle of characteristic form.[1] But the adults used in these experiments were at least 3 months old and it seemed possible that in younger adults this faculty might be retained. When the experiment was repeated with adults 3 weeks old, the same result was obtained; but on joining adults within 24 hr. of moulting, at a time that is when the epidermis is still engaged in secreting the inner layers of the new cuticle, they were caused to moult again and to produce a cuticle covered with well-formed bristles (Text-fig. 4 C). *Cimex* adults 3 days old reacted similarly; the precise stage at which the faculty to form bristles is lost has not been determined.

[1] Piepho (1938*b*), has shown that a fragment of imaginal cuticle of the moth *Galleria* implanted into young pupae lays down a similar formless cuticle without scales.

It was shown in an earlier paper that if cylindrical segments of the head of *Rhodnius* nymphs are implanted in the abdomen of nymphs which are moulting, the epidermis grows outwards to restore continuity with itself and then the encapsulated cuticle proceeds to moult (Wigglesworth, 1936). This method has been employed to prove that the epidermis of the adult *Rhodnius* is capable of moulting again. Fragments of the femur or tibia, ½ to 1 mm. in length, were implanted into the abdomen of 4th stage nymphs 24 hr. after feeding. In one such experiment the



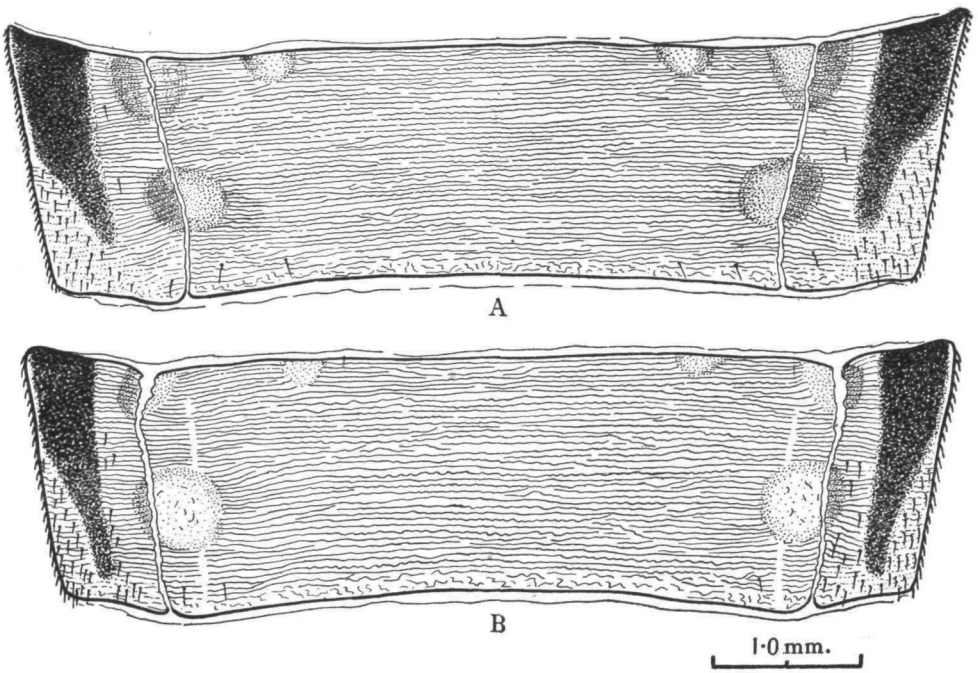
Text-fig. 5. Longitudinal section of fragment of tibia of adult *Rhodnius* implanted in 4th stage nymph and fixed when this host became adult. *a*, original cuticle; *b*, first new cuticle forming continuous capsule; *c*, second new cuticle forming continuous capsule; *d*, epidermis. The exocuticle is shaded in each case. The partially digested endocuticle beneath *a* and *b* stains deeply with haemotoxylin.

implantation was made on 19. v. 1936; the 4th stage nymph moulted to the 5th instar on 9. vi. 1936; it moulted again to become adult on 15. vii. 1936, and the implant was removed and sectioned a few days later. The result is shown in Text-fig. 5, from which it can be seen that the fragment of adult tibia has been caused to moult twice more. (This method of implanting fragments of cuticle has recently been used extensively by Piepho (1938 *a*, *b*: 1939 *a*, *b*) for studying the control of moulting and metamorphosis in Lepidoptera (see p. 216).)

Since it was evident from these experiments that the epidermis of the adult *Rhodnius* will respond to the moulting hormone, a renewed attempt was made to induce moulting in the whole insect. Adults were decapitated at a time when there

was plenty of blood in the stomach; a 5th stage nymph about 10 days after feeding, with the anterior part of its head removed, was joined to the neck of the adult; and a second 5th stage nymph was joined to the abdomen—a small window about 1 mm. square being cut in one of the sternites and the abbreviated head of the nymph inserted into this. A single adult now received blood from two 5th stage nymphs, and it was caused to moult simultaneously with these.

Some ten adults were caused to moult in this way. At the time of joining they varied in age from a few days to several months; but, unlike the *Cimex* adults, no



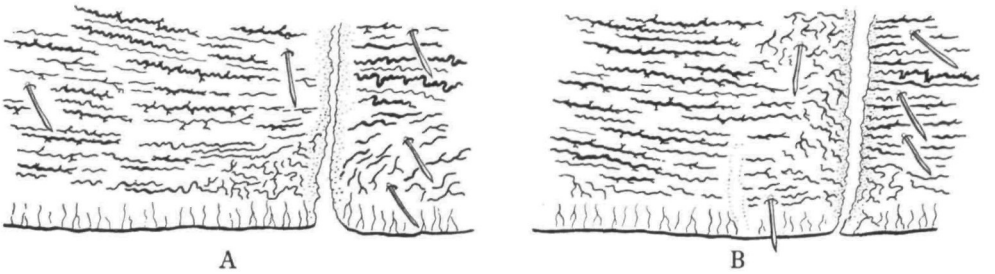
Text-fig. 6. A, tergite of fifth abdominal segment of adult *Rhodnius*. B, new cuticle of the same after moulting induced with the blood from 5th stage nymphs.

matter what their age, they laid down a new cuticle of normal form and pigmentation, furnished with bristles of the usual type—though the cuticle may occasionally show small imperfections, and some of the bristles may be ill-formed. The characters of this cuticle are purely imaginal. This is seen in the distribution of pigment in the tergites, in the presence of a "hinge line", in the form of the bristles and the type of folding in the epicuticle (Text-figs. 6, 7).

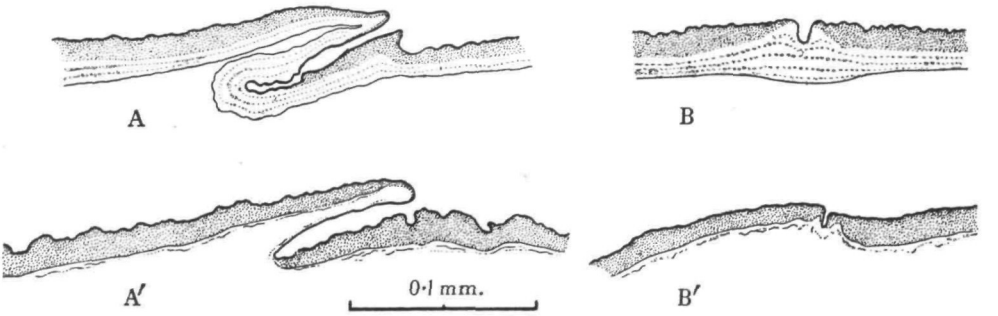
During moulting the oenocytes become enormously enlarged, as they do in moulting nymphs, and the dermal glands become active. In the new cuticle the mouths of the dermal glands often extend to form finger-like outgrowths—at first mistaken for vestigial bristles.

It is worth noting that during moulting in the adult the endocuticle is dissolved, as it is in moulting nymphs (Wigglesworth, 1933). Consequently, at points where

an exocuticle is wanting, such as the hinge line and the intersegmental membranes, the old cuticle becomes excessively fragile and breaks at a touch (Text-fig. 8). It is also worth noting that in females which were caused to moult, the development of eggs was arrested during the process. This is further evidence that the hormones necessary for moulting differ from those needed for egg production (Wigglesworth, 1936).



Text-fig. 7. A, posterior margin of fifth abdominal tergite of adult *Rhodnius* showing part of the hinge line. B, the same region of the cuticle after moulting induced with the blood from 5th stage nymphs.



Text-fig. 8. A, longitudinal section of intersegmental membrane in normal adult *Rhodnius*. A′, the same after moulting, showing digestion of endocuticle. B, transverse section of hinge line in normal adult. B′, the same after moulting.

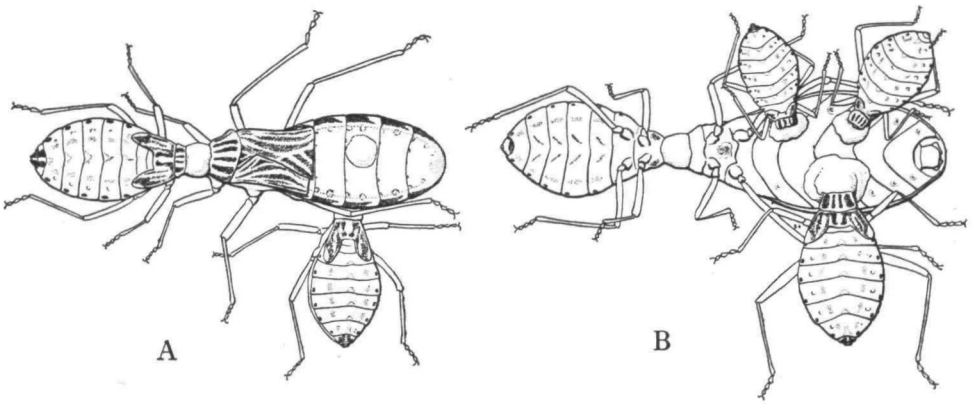
REVERSION TO NYMPHAL CHARACTERS DURING MOULTING IN THE ADULT[1]

The next question is whether the epidermis of the adult insect, exposed to the "inhibitory hormone" during moulting, can lay down again a cuticle with nymphal characters. This was first tested in 1936 by excising a piece of cuticle about 1 mm. square from one of the tergites of a 4th stage nymph soon after feeding, implanting in its place a similar fragment removed from an adult, and sealing the edges with paraffin of low melting point. When the 4th stage nymph moulted, the cuticle laid down at the site of the implant, though devoid of plaques, had the epicuticle thrown into star-shaped folds exactly like those of the nymphal cuticle elsewhere. It appeared, therefore, that under the influence of the inhibitory hormone of the 4th

[1] A preliminary note on these results has already been published (Wigglesworth, 1939*a*).

stage nymph the adult epidermis had formed a nymphal cuticle. There remained, however, the possibility that in these experiments the imaginal epidermis had died, and the new cuticle was secreted by nymphal cells that had spread in from the margins of the wound.

An attempt was therefore made to induce moulting in the whole insect by joining it to 4th stage nymphs with the corpus allatum intact. For some unknown reason these experiments failed. When moulting was not induced by two 4th stage nymphs, as many as five (one on the neck and four on the abdomen) were joined to a single adult, but without success. Whether this failure was due merely to chance (for even after joining to 5th stage nymphs, the adults occasionally fail to moult) or whether the imaginal tissues respond less readily to the moulting hormone from the 4th stage is not known.

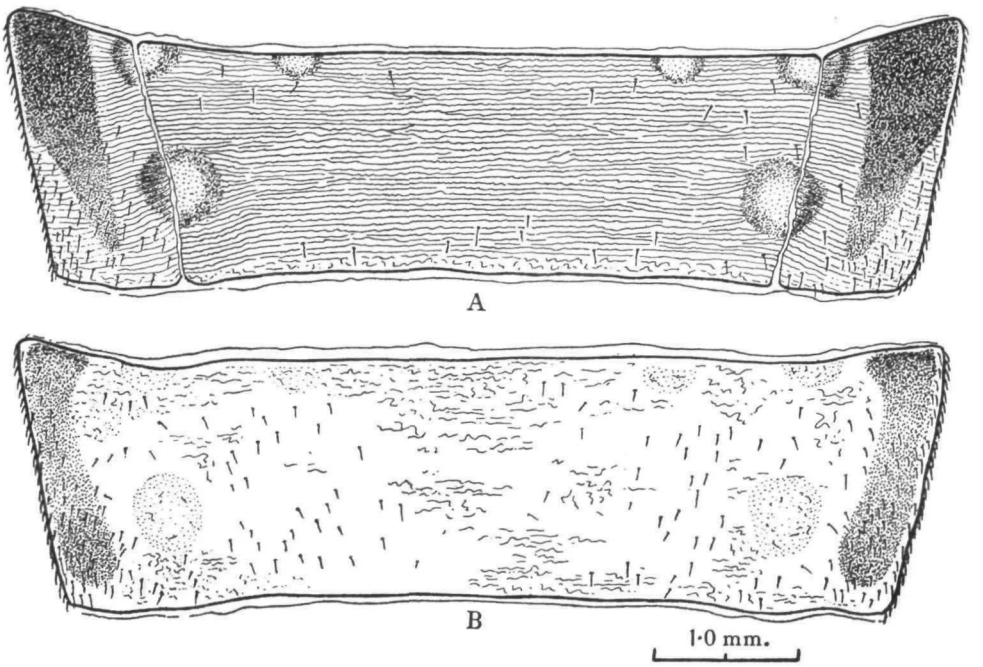


Text-fig. 9. A, adult *Rhodnius*, with wings cut short and corpora allata of 4th stage nymphs implanted in abdomen, decapitated and joined to two 5th stage nymphs. B, adult *Rhodnius*, decapitated and joined to two 5th stage nymphs and two 4th stage nymphs.

The experiment was finally carried out as follows (Text-fig. 9). The adult was caused to moult as before by joining one 5th stage nymph to the head and a second to the abdomen; and in addition it was provided with the secretion from the corpus allatum of the 4th or some earlier stage. This was done in several ways in an effort to produce the maximum effect. (i) One or two 4th or 3rd stage nymphs with the corpus allatum present were joined to the abdomen in the same way and at the same time as the 5th stage nymphs. (ii) The hind part of the head, containing the corpus allatum, was removed from five or six nymphs in the 1st, 2nd, 3rd or 4th stages and implanted through an incision in the abdomen of the adult. (iii) Brains plus corpora allata or corpora allata alone were dissected out of six 4th stage nymphs and implanted in the same way. In each case the 5th stage nymphs served as indicators for the presence of the inhibitory hormone in the circulating blood; for when this was present they developed nymphal characters again.

Altogether 24 adults were caused to moult while exposed to the inhibitory hormone by these means. When the characters developed by the 5th stage nymphs

indicated that appreciable quantities of inhibitory hormone were present, the new cuticle of the adult always showed signs of a partial reversion to nymphal characters. This change did not affect elaborate structures such as the genitalia. But in the most successful experiments it was evident in the general pattern of the abdomen (Text-fig. 10). The distribution of pigment at the sides of the segments showed a partial return to the nymphal arrangement; the hinge line was absent; the cuticle of the lateral pleat (which is highly elastic and devoid of an exocuticle in the normal adult) developed in places a pigmented exocuticle; a considerable number of new



Text-fig. 10. A, fifth abdominal tergite of adult *Rhodnius*. B, the same segment after moulting induced by two 5th stage nymphs and the implantation of brains and corpora allata from six 4th stage nymphs. The bristles lying between the hinge lines in A, and in the corresponding area in B, are copied exactly from the specimen. Compare Text-fig. 6.

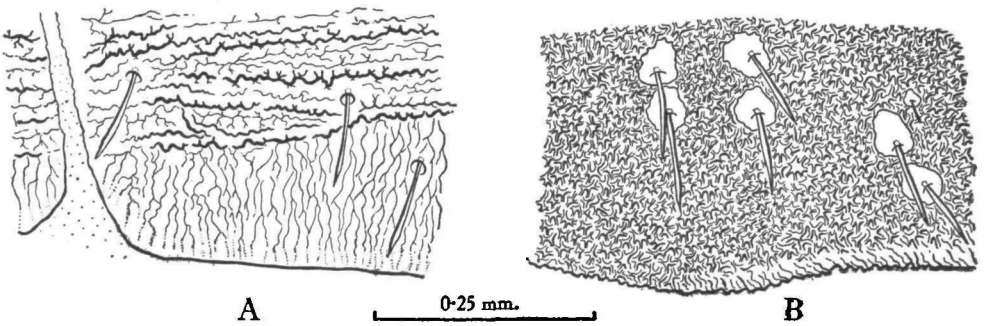
spines appeared in places where no spines existed before;[1] the form of these spines was intermediate between those of adult and nymph, and they were often surrounded by a small plaque of nymphal type; and finally the new epicuticle showed the characteristic star-shaped folding of the nymph (Text-fig. 11 B).

In experiments carried out at the same time as these, Piepho (1939 *a*, *b*) showed that when fragments of pupal cuticle from the wax-moth *Galleria* were caused to moult by implantation into young larvae, they laid down again a cuticle of larval type. This effect was most readily induced in that region of the epidermis which had grown outwards to restore continuity with itself ("Umwachsungshypodermis").

[1] The adult represented in Text-fig. 10 had more spines than usual on the segment illustrated and more new spines appeared at moulting than in any other experiment in this series.

In the case of *Rhodnius* it was observed in several cases that the nymphal form of the cuticle was most marked at the site of the implantation of the corpora allata. This result may perhaps be comparable with that obtained by Piepho—a consequence, that is, of the wound-healing process. Or it may be due simply to this region of the cuticle being closer to the implanted glands. When small burns were made in the cuticle remote from the site of the implantation they did not seem to produce this effect.

Piepho (1939*a*) also observed that the epidermis immediately under the implanted fragment of pupal cuticle ("Stammbereich") often laid down cuticle of pupal type on moulting for the first time in a young larva, but produced larval cuticle if caused to moult a second or a third time. In the hope of obtaining a more complete reversion to nymphal characters in *Rhodnius* many attempts were therefore made to induce adults to moult a second time by removing the first set of



Text-fig. 11. A, posterior margin of fifth abdominal tergite of adult *Rhodnius*, including part of hinge line. B, the same area after moulting induced by two 5th stage nymphs and the implantation of brains and corpora allata from six 4th stage nymphs. Compare Text-fig. 7.

nymphs when they had moulted and applying a new set. Decapitated adults which had moulted once (they were still enclosed, of course, in their original cuticle as well as the new one) remained alive for more than 3 weeks after the first moult; but only in one insect was a second moult induced. Unfortunately, for some reason, the cuticle and spines of both new skins had very ill-defined characters in this insect.

When the dorsum of the abdomen in these adult insects was removed and stained during the course of moulting, it was found that if moulting was induced by the moulting hormone from 5th stage nymphs alone, mitoses were confined to the periphery of the abdomen and to the intersegmental membranes; whereas if exposed also to the "inhibitory hormone", mitoses were numerous over the entire area of the segments. As a result, the epidermal cells per unit area were far more numerous in those which resumed their nymphal characters than in those in which the adult characters were retained (cf. p. 204 and Text-fig. 2 H, I).

DISCUSSION

Source of the moulting hormone

It was suggested in an earlier paper (Wigglesworth, 1934) that the moulting hormone might be secreted by the corpus allatum. This suggestion was based on the histological examination of the head contents during the early stages of moulting, in the course of which it was found that during the critical period the cells of the corpus allatum became swollen and their cytoplasm dense. This evidence was not very convincing, for the histological changes in the epidermal cells show that the moulting hormone is already being secreted within 48 hr. of feeding, several days before the critical period. Unfortunately, the corpus allatum of *Rhodnius* is so placed that it has not proved possible to remove it without damaging the brain or its blood supply. No satisfactory experimental proof of this hypothesis was therefore produced. Attempts at section of the nerves from the brain to the corpus allatum gave inconclusive results.

In a later paper (Wigglesworth, 1936) an attempt was made to prove this hypothesis by showing that nymphs with the brain removed, but with the corpus allatum intact, provide a better source of moulting hormone than those decapitated behind the corpus allatum. But in view of the results described in the present paper, it is probable that the apparent success of these experiments was due to the inclusion of a small piece of the posterior dorsal region of the brain with the corpus allatum.

In the present work we now have good evidence that the moulting hormone is secreted by the brain; probably by cells located in the dorsum of the protocerebrum. It may be well to compare this conclusion with those reached in other groups of insects.

In Lepidoptera, removal of the corpora allata does not prevent pupation (Bounhiol, 1938). It was originally suggested by Kopeć (1922) that in this group a hormone causing pupation is secreted by the brain; and Bounhiol (1938) has confirmed that removal of the brain before the critical period in the silkworm and other caterpillars arrests pupation. He is, however, inclined to attribute this, not to the production by the brain of a hormone, but to its nervous control of the function of the gut. (Bounhiol indeed does not consider that moulting or pupation hormones have been satisfactorily demonstrated in any group of insects). Attempts to induce pupation in caterpillars deprived of the brain before the critical period, by implanting the brain into the abdomen, were successful in a few cases (3·6%) in *Ephestia* (Kühn & Piepho, 1936) and in a greater proportion (15·3%) in various Sphingid larvae (Caspari & Plagge, 1935: Plagge, 1938). These implants were successful only in larvae shortly before pupation; they were ineffective in larvae 2–4 days after the last moult (Plagge, 1938).

Removal of the corpora allata in Orthoptera (*Melanophus* (Weed, 1936), *Dixippus* (Pflugfelder, 1937)) likewise does not prevent moulting. Though recently Pflugfelder (1939) has induced two extra moults in adult *Dixippus* by the implantation of five or six corpora allata from young insects.

Among Diptera, on the other hand, there is good evidence that the hormone which induces pupation in flies (*Calliphora* (Fraenkel, 1935); *Drosophila* (Bodenstein, 1936)) is secreted by the ring gland or Weismann's ring (Hadorn, 1937; Burtt, 1938), and that this gland probably contains the homologues of the corpora allata and the oesophageal ganglia (Burtt, 1937, and unpublished work).

At the present time, therefore, there seems to be little uniformity as to the source of this hormone among the few insects studied.

As regards hormones acting more or less like the "inhibitory hormone" in *Rhodnius*: these have been clearly demonstrated as coming from the corpora allata in Lepidoptera by Bounhiol (1938) and by Piepho (unpublished experiments); Pflugfelder's (1937) observations on the results of excision of the corpora allata in young *Dixippus* nymphs may be similarly interpreted; and there is some indication that the ring gland in muscid larvae has a similar effect (Burtt, unpublished work).

Mode of action of the inhibitory hormone

The main object of this paper was to study the mode of action of the hormones in controlling the morphological change at metamorphosis. For the moment it will be well to consider only those characters (the form of the cuticle, the presence or absence of melanin, etc.) which are determined by the actions of single cells, and defer discussion of complex changes such as the elaboration of wings or external genitalia which require the co-ordinated activity of many cells.

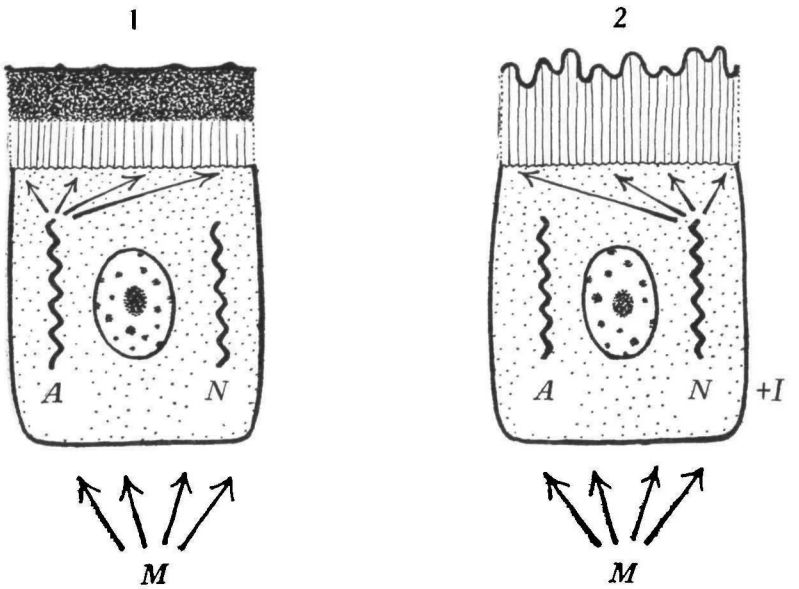
Now many of the cells persist from the nymph to the adult. The type of cuticle they lay down is dependent on the hormones present. Clearly each cell must contain the metabolic systems needed for the formation of both nymphal and imaginal cuticle. The role of the hormones is to determine which of these systems is effective. Since we have no knowledge at present about the internal organization of the living cell, we can of course only speculate about the nature of these intracellular systems which it is necessary to postulate.

If the young nymph is decapitated at the critical period, it may show a switch over in development from nymph to adult; and the cell (e.g. a bristle-forming cell) may lay down a structure which is intermediate in form between that of nymph and adult. Hence the two systems are not mutually exclusive—in many of the cells they must be very closely related chemically.

The setting in motion of these systems, the activation of the cell and the subsequent processes ending in the deposition of the new cuticle, is brought about by the moulting hormone. This may prove ultimately to be made up of more than one constituent; but for the moment it may be regarded as a single substance. When this hormone alone is present, the system which leads to the deposition of the imaginal cuticle is caused to function. If the inhibitory hormone is added in varying amounts, the system which leads to the deposition of nymphal cuticle is activated to a variable extent, and all grades of intermediate structures are produced.

Now it is characteristic of the inhibitory hormone that it causes more rapid development than the moulting hormone alone (a 5th stage nymph at 24° C. will

moult to an adult in 28 days; it will make a nymphal moult in 17 days (Wigglesworth, 1934)). We may thus formulate an hypothesis for the control of metamorphosis in *Rhodnius* as follows (Text-fig. 12). Each cell contains two enzyme systems, imaginal and nymphal. The moulting hormone initiates the process of growth and cuticle formation in the cell and when it alone is present the imaginal system is activated and leads to the secretion of imaginal cuticle (Text-fig. 12, 1). The "inhibitory hormone" activates the nymphal system; it acts perhaps as a "co-enzyme"; and since this system works more rapidly than the imaginal, the production of imaginal structures is suppressed[1] when this hormone is also present (Text-fig. 12, 2). Even after the assumption of the adult form, the nymphal system



Text-fig. 12. Diagram to illustrate hypothesis for the control of metamorphosis in *Rhodnius* by hormones. 1, formation of imaginal cuticle in presence of moulting hormone alone. 2, formation of nymphal cuticle in presence of moulting hormone and inhibitory hormone. A, imaginal system; N, nymphal system; M, moulting hormone; I, inhibitory hormone.

must be supposed to remain intact within some of the cells at least. For when the adult is caused to moult this system may be activated once more and a nymphal cuticle is laid down.

This hypothesis is an elaboration of the idea, already put forward (Wigglesworth, 1936) that the determination of characters at metamorphosis results from the interaction of two developmental processes with different velocities. Its very close analogy with Goldschmidt's well-known theory of sex determination and intersex production is obvious. In the foregoing discussion it has been considered only from the point of view of single cells which persist from one stage to the next.

[1] Suppression of the imaginal system may, of course, be brought about by other means; such as the absorption by the nymphal system of some essential factor. This would bring the hypothesis into line with that put forward to explain the suppression of new plaques around existing plaques (Wigglesworth, 1940).

But the ideas require only slight modification to cover those cases where there is extensive cell death at metamorphosis and much change in form through the co-operation of the dividing cells.

In previous papers the "inhibitory hormone" was so called because in its presence the production of imaginal characters at moulting is suppressed. But in view of its probable mode of action through the activation of the nymphal system at the expense of the imaginal, it might be preferable to refer to this hormone as the "nymphal" or "juvenile" hormone.

SUMMARY

Nymphs of *Rhodnius* decapitated 24 hr. after feeding can be induced to moult by implanting into the abdomen the dorsal region of the protocerebrum removed from other nymphs during the critical period. Implantation of other parts of the brain, of the corpus allatum, and of fat body from the same insects did not cause moulting.

The presence of large nerve cells with fuchsinophil inclusions discovered by Hanström in this region of the brain has been confirmed.

The histological changes in the epidermis of the abdomen and the distribution of mitoses at an imaginal moult and at a nymphal moult have been compared. During a nymphal moult mitoses occur all over the tergites; during an imaginal moult they are largely confined to the intersegmental membranes and the periphery of the abdomen, and there is a more extensive breakdown of existing cells.

If 5th stage nymphs in the course of moulting to become adults receive "inhibitory hormone" from young nymphs, they may be caused to "switch over" to nymphal development. Such a "switch over" soon becomes impossible for the most specialized structures of the adult; other structures follow in turn; but the general cuticle of the tergites may still be influenced up to a short time before it is due to be laid down. The various faculties of a given cell can become determined to some extent independently of one another.

Isolated fragments of cuticle and epidermis from *Rhodnius* adults may be induced to moult, more than once, by transplantation to young moulting nymphs.

Decapitated *Cimex* adults may be caused to moult again if they receive blood from moulting *Rhodnius* nymphs; but they lay down a normal cuticle with bristles only if they have become adult very recently.

Decapitated *Rhodnius* adults may be caused to moult again if they receive blood from two moulting 5th stage nymphs. They lay down a cuticle of normal adult type even when they have been adults for several months. The old skin is digested up to the level of the exocuticle.

If such moulting adults are provided with inhibitory hormone from the corpus allatum of young nymphs, they show a partial reversion to nymphal characters when they moult. This change probably does not extend to the most specialized imaginal structures; but the pigmentation and the structure of the general cuticle, and of the bristles it carries, may become partially nymphal again.

The "determination" of imaginal or nymphal characters thus takes place at different times in different organs. And for some characters, at least, such determination is not irrevocable. In the light of these results a new hypothesis is put forward to explain the action of the inhibitory hormone in controlling metamorphosis.

This work was begun during a stay of three months in Berlin-Dahlem as the guest of the Kaiser-Wilhelm Gesellschaft, to whom my thanks are due. Throughout this stay I was indebted to Prof. A. Kühn and his colleagues at the Kaiser-Wilhelm Institut für Biologie for every assistance and for much stimulating discussion.

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EXPLANATION OF PLATE I

Tergites of insects produced from 5th stage nymphs switched over from imaginal to nymphal development at different times after feeding.

Fig. 1. 5 days. Fig. 3. 9 days. Fig. 5. 12 days. Fig. 7. 15 days.
Fig. 2. 7 days. Fig. 4. 11 days. Fig. 6. 13 days. Fig. 8. 17 days.

Fig. 1.

Fig. 2.

Fig. 3.

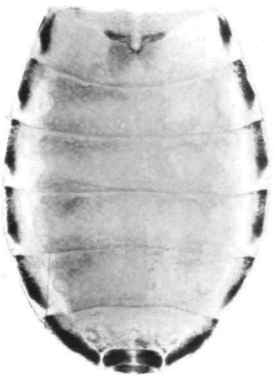
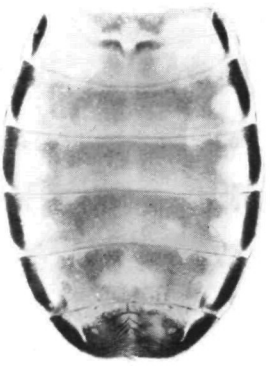


Fig. 4.



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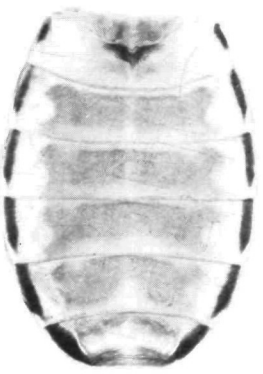

Fig. 6.

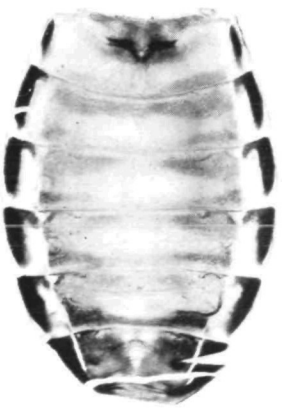

Fig. 7.

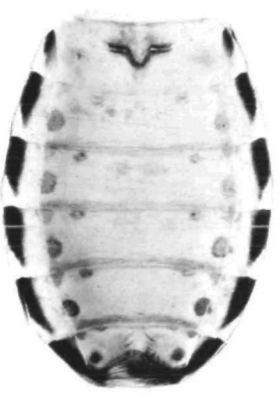

Fig. 8.

WIGGLESWORTH—THE DETERMINATION OF CHARACTERS AT METAMORPHOSIS IN *RHODNIUS PROLIXUS* (HEMIPTERA) (pp. 201–222)