HORMONE BALANCE AND THE CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS* (HEMIPTERA)

By V. B. WIGGLESWORTH
Department of Zoology, University of Cambridge

(*Received* 19 *May* 1952)

(With Plates 22 and 23 and Two Text-figures)

The body cells of the developing insect carry the potentialities for both larval and imaginal differentiation. During larval life imaginal differentiation is suppressed because in the presence of the juvenile hormone (sometimes referred to as the 'inhibitory hormone'), secreted by the corpus allatum, the intracellular system which leads to the formation of larval structures takes precedence over the system which leads to the formation of adult structures (Wigglesworth, 1940).

If the corpus allatum in *Rhodnius* is removed by decapitation at varying times in the moulting cycle of a young larva, when varying amounts of juvenile hormone have been secreted, all intermediate grades of metamorphosis between larva and adult can be produced (Wigglesworth, 1934). In the normal insect there is a nicely adjusted balance between the juvenile hormone and the moulting hormone;* in these experiments the balance has been grossly upset. The operation of this hormone balance has been well illustrated by the work of Piepho (1940, 1950*a*) on *Galleria*.

In the course of normal development in *Rhodnius* there is a small amount of differentiation towards the adult form in each larval instar. This is most striking at the moult from the 4th to the 5th instar, where the differentiation of the external genitalia, the enlargement of the wing pads, the sharp fall in the rate of increase in the number of abdominal plaques and bristles (Wigglesworth, 1940) are all indications of limited imaginal differentiation.

These fine adjustments during growth are also the result of controlled hormone balance. In the present paper some of the ways in which this balance may be upset are described—with the object of throwing light upon the mechanisms by which the correct balance is normally maintained.

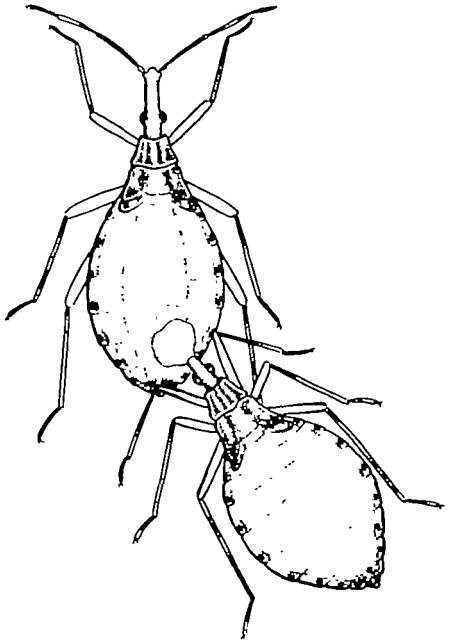
(i) *Transfusion from an earlier larval stage*

It has been shown already that if the 4th-stage larva of *Rhodnius* is decapitated 1 day after feeding and joined to a 3rd-stage larva, retaining its corpus allatum, which has just passed the critical period, the 4th-stage larva does not develop the

* As pointed out elsewhere (Wigglesworth, 1952), the 'moulting hormone' is composite and consists of a factor secreted in the brain that activates the thoracic gland which then secretes the hormone which initiates growth and moulting.

characters of a 5th stage, but develops again those of the 4th. It was from this experiment that it was inferred that 'the characters of the various instars are controlled by the corpus allatum in the same way as metamorphosis is controlled' (Wigglesworth, 1936).

In these experiments the insects were joined neck to neck and were therefore unable to escape from the cuticle at moulting. Consequently, it was not possible to detect small differences in the differentiation of the crumpled wing lobes; all the conclusions were based on the minute growth changes in the rudiments of the external genitalia.



Text-fig. 1. 4th-stage larva of *Rhodnius* with a second 4th-stage larva joined to the abdomen.

A new technique has now been adopted in which a small hole is cut in the abdomen of the insect that is to be subject to experiment; the head of the insect that is to be used for transfusion, after cutting off the tip, is inserted into this hole and the margin sealed with paraffin wax (Text-fig. 1). It is advisable also to secure the legs of the second insect with paraffin. Under these conditions the first insect is often able to free the head and thorax from the old cuticle at moulting and to expand the wing lobes, etc., in a normal manner.

Pl. 22, fig. 1, shows the normal 4th-stage larva and Pl. 22, fig. 2, the normal 5th stage. Pl. 22, fig. 3, shows a larva produced from a 4th stage to which had been joined by the above technique, at 1 day after feeding, a 3rd-stage larva at 5 days after feeding. In this experiment the two insects moulted simultaneously 14 days after the union. The 4th-stage larva has developed into a 5th stage with wing lobes which have differentiated little more than in the normal 4th stage.

It seems probable from this experiment that the corpus allatum secretes into

the blood a higher concentration of juvenile hormone during the 3rd stage than during the 4th. It might be argued that this results from a simple relationship between the relative volumes of the gland and the total body fluid (cf. Novak, 1951). But if the 4th-stage larva in the above experiment is decapitated, it produces characters (in the genitalia and the crumpled wing lobes) which show just as little change towards those of the 5th instar as does the 4th-stage larva which retains its corpus allatum. Thus a single 3rd-stage corpus allatum acting upon combined larvae of 3rd and 4th stages is just as effective as the combined corpora allata of the two larvae.

One must conclude that the corpus allatum of the 3rd-stage larva is adapted to raise and maintain the concentration of juvenile hormone in the blood at a level characteristic of that instar. But there is another factor to be considered: the timing of the secretion.

(ii) *Transfusion from the same larval stage at different periods in the moulting process*

There is evidence that in the normal process of moulting the juvenile hormone is not secreted into the blood until after the critical period (Wigglesworth, 1934). The time of exposure of the tissues to this hormone has been varied experimentally by transfusing 4th-stage larvae with the blood of other larvae, also in the 4th stage, which had been fed some days earlier. The same technique was used: batches of 4th-stage larvae at 24 hr. after feeding had connected to them, towards the hind end of the abdomen, other 4th-stage larvae fed 2, 3, 4, 6, 7 and 8 days previously.

Those joined to 2- and 3-day larvae gave rise to normal 5th-stage larvae (Pl. 22, fig. 4). Of those joined to 4-day larvae, one (out of six) developed genital rudiments which had not differentiated quite so much as they should. Of those joined to the 6-day larvae, all gave rise to slightly juvenile forms in which the wing lobes did not extend quite so far backwards as in the normal 5th stage.

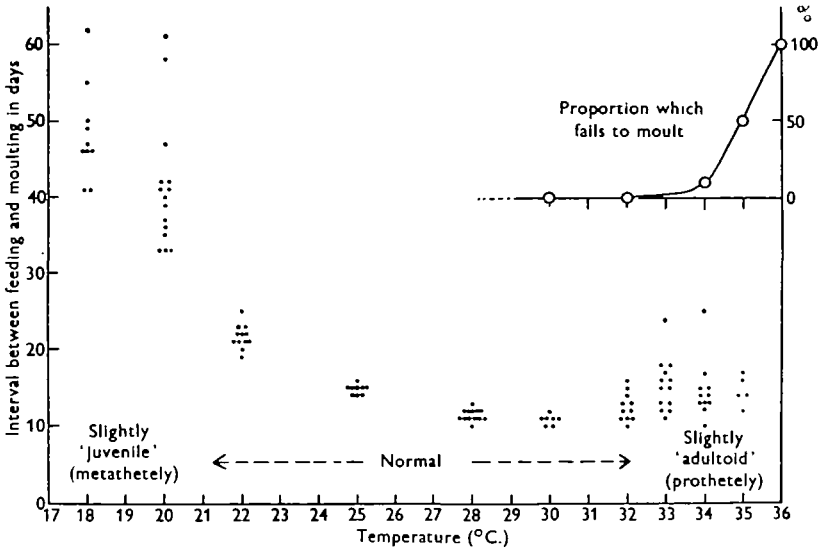
In those joined to larvae fed 7 and 8 days earlier, the juvenile characters were more striking. Pl. 22, figs. 5 and 6, shows two of these larvae: the wing lobes are not so juvenile in form as those produced by transfusion from a 3rd-stage larva (cf. Pl. 22, fig. 3), but they are far smaller than those of the normal 5th-stage larva (cf. Pl. 22, fig. 4). The genitalia show corresponding changes.

In these experiments there can be no question of the juvenile hormone being present in higher concentration than usual. But it has been introduced into the system too early in the moulting process. Instead of being first produced in notable quantities when growth has already been initiated by the 'moulting hormone', it is present in the blood from the commencement of the moulting process.

(iii) *Effect of abnormal temperatures*

Batches of 4th-stage larvae of *Rhodnius* were placed, immediately after feeding, in a humid atmosphere at temperatures ranging from 18 to 37° C. Text-fig. 2 shows the number of days after feeding at which moulting took place. At 28–30° C. moulting requires about 11 days. Below this temperature moulting is progressively retarded and the individual variation becomes very great. At 18° C. the time

required varies from 40 to 60 days. Above 30° C., also, moulting is slightly retarded and there is a large amount of scatter. At 34° C. some larvae (about 10 %) fail to moult altogether; at 35° C. about 50 % fail to moult; and at 36° C., and above none moults.* If they are fed again and placed at the optimum temperature these larvae moult normally.



Text-fig. 2. Effect of temperature on the moulting of 4th-stage larvae of *Rhodnius*.

There are no very striking morphological differences in the 5th-stage larvae produced at these different temperatures; those at the higher temperatures are slightly paler, but all have unmistakable 5th-instar characters. When examined closely, however, slight differences are detectable. Some of the females moulting at the lowest temperatures have the genital lobes slightly less differentiated than normally; and of the females moulting at the higher temperatures an increasing proportion shows the first pair of valvulae slightly separated (as shown in Wigglesworth, 1948, text-fig. 2B). The numbers of females concerned were very small but the proportion showing this change was as follows: 32° C., 50 %; 33° C., 60 %; 34° C., 85 %; 35° C., 100 %. This slight separation is an 'adultoid' character.

A better character for detecting these very slight changes is afforded by the anterior wing lobes. Pl. 23, figs. 1, 3 and 5, shows the form of these in 5th-stage larvae that had moulted at 18, 25 and 34° C. At the low temperature (Pl. 23, fig. 1) the distal half of the wing (the 'membrane') is much less clearly marked off from the basal half (the 'corium'). At the high temperature (Pl. 23, fig. 5) the 'membrane' is relatively smooth and is thus sharply differentiated from the 'corium' which is roughened by prominent plaques.

* The lethal temperature, defined here as the temperature at which 50 % of larvae 1 day after feeding are killed within 24 hr. is about 40° C.

Microscopically the difference is well seen in the type of cuticle that occurs in the proximal region of the 'membrane' (Pl. 23, figs. 2, 4 and 6). In the normal 5th-stage larva there are well-developed plaques bearing bristles in the basal half of the wing pad. In the distal half many of the plaques and bristles have disappeared, although the sites at which they occurred in the previous instar can still be seen in the sculpturing of the surface (Pl. 23, fig. 4). In 5th-stage larvae produced at the lowest temperatures, many more plaques have persisted and the bristles which they bear are larger than in the normal 5th stage (Pl. 23, fig. 2). In 5th-stage larvae produced at the highest temperatures, the reduction of plaques has proceeded further than in the normal insect, and the wing lobes, particularly the distal parts, are relatively smooth, and the few remaining bristles much reduced in size (Pl. 23, fig. 6).

At low temperatures the wing lobes are clearly more 'juvenile' in character than those of the normal 5th-stage larva, at high temperatures they are more 'adultoid'. Those at the low temperatures may be described as exhibiting 'metathetely', those at the high temperatures exhibit 'prothetely'.

Clearly, low temperature upsets the hormone balance very slightly in favour of the juvenile hormone, while the high temperature upsets the balance very slightly in favour of the 'moulting hormone'. But as the temperature rises above 35–36° C. the secretion of the moulting hormone fails, although the other metabolic processes in the insect are not visibly affected. Whether it is the 'activating' component from the brain, or the 'moulting' factor from the thoracic gland (Wigglesworth, 1952) which is lacking has not been determined.

(iv) *Effect of deficient oxygen*

Groups of four 4th-stage larvae, 1 day after feeding, were placed at 25° C. in 250 c.c. conical flasks containing different concentrations of oxygen. The gas mixture was changed each day.

In 99 % oxygen the larvae were all dead within 5 days. In 75 and 50 % oxygen normal 5th-stage larvae were produced. In 5 % oxygen moulting was delayed; it required 19–32 days compared with the normal 14–16 days in air. The resulting 5th-stage larvae, as judged by the genitalia and the plaques on the wing lobes, were very slightly 'adultoid'; that is, like the larvae exposed to high temperature they show very slight prothetely. In 2·5 % oxygen all died within 11 days without any sign of moulting beginning.

(v) *Implantation of the corpus allatum of* Rhodnius *and* Periplaneta

We have seen that 4th-stage larvae transfused with blood from intact 3rd-stage larvae develop 4th-instar characters again when they moult. But the implantation of corpora allata from 3rd-instar larvae, at a week or so after feeding, into 4th-stage larvae 1 day after feeding, does not have that effect: perfectly normal 5th-instar larvae are produced. Presumably the interference with the tracheal supply of the implanted gland causes a temporary suppression of hormone secretion; or, as

suggested previously (Wigglesworth, 1948), the corpus allatum of the host may control the level of concentration of the juvenile hormone.

The corpus allatum of the adult insect again secretes juvenile hormone which is necessary for yolk production (Wigglesworth, 1948). It was shown by Novak (1951) that the corpus allatum of the adult *Periplaneta* will cause the development of larval characters at moulting in the Lygaeid *Oncopeltus*. This has been confirmed in *Rhodnius*: of ten 5th-stage larvae, each of which received implants of a pair of corpora allata from adult *Periplaneta*, all gave rise to 6th-stage larvae or intermediates; of those which moulted again, six gave rise to 7th-stage larvae and one to a 7th-stage adult. The gland therefore continues to secrete juvenile hormone for a long time after implantation. In histological sections the implanted corpus allatum of *Periplaneta* appears normal and healthy. (The apparent identity of the juvenile hormone in both hemimetabolous and holometabolous insects has been described by Piepho (1950*b*).)

Corpora allata from mature adult *Periplaneta* were implanted into 4th-stage larvae of *Rhodnius* 24 hr. after feeding; but, as in the case of larvae receiving implants of corpora allata from 3rd-stage larvae of *Rhodnius*, they developed normal 5th-instar characters.

(vi) *Change in function of the corpus allatum in the last larval stage*

Metamorphosis results from the failure of the corpus allatum in the last larval stage to secrete the juvenile hormone. In order to see whether the gland plays a more active part in bringing about metamorphosis, corpora allata from 5th-stage larvae or from newly moulted adults were implanted into 2nd-, 3rd- and 4th-stage larvae. In many of these there was a partial metamorphosis at the moulting of the 4th stage (Wigglesworth, 1948).

This result signifies that the implanted corpus allatum is acting upon the corpus allatum of the host and weakening its capacity to secrete the juvenile hormone, or that it is actively removing juvenile hormone from the blood, or that it is now secreting a hypothetical 'metamorphosis hormone' which favours the differentiation of adult characters.

In the earlier paper the explanation preferred was an active removal of juvenile hormone. The grounds for this preference were that if the 5th-stage larva of *Rhodnius* had the brain removed and was then caused to moult by joining it to another 5th-stage larva which had just passed the critical period, then the imaginal differentiation, particularly of the wing lobes, was more complete when the insects retained their corpora allata than when these were removed. It was therefore inferred that in the normal 5th-stage larva some juvenile hormone is still present in the blood and that it is removed by the altered activity of the corpus allatum during the final moult.

These experiments have been repeated in modified form. A number of 4th-stage and 5th-stage larvae were fed within 2–5 days after moulting (so that they might be expected to have the maximum amount of juvenile hormone persisting). They were decapitated at 24 hr. after feeding and caused to moult by implanting

into the abdomen the thoracic glands from 5th-stage larvae at 10 days after feeding (Wigglesworth, 1952). Half of the decapitated larvae received, in addition, an implant of the corpus allatum from the 5th-stage larvae.

In this way nineteen 4th-stage larvae moulted without the corpus allatum, twenty moulted with the corpus allatum of a 5th-stage larva. All developed adult characters; and there was no detectable difference in the differentiation of the wings, abdominal cuticle or genitalia between the two groups.

Among the 5th-stage larvae, eleven without the corpus allatum moulted and nine with the corpus allatum. All developed adult characters. In many of them the folding of the wing lobes was deficient, and in some the wings were like those shown in Wigglesworth (1948, pl. 1, fig. 2). But these deficiencies occurred with equal frequency in the two groups. They appear to be the result of decapitation, which may perhaps interfere with the circulation of blood in the wing lobes.

These results, therefore, give no indication of any notable persistence of juvenile hormone from one instar to the next, and they invalidate the evidence on which it was concluded that there is an active removal of juvenile hormone by the corpus allatum at the final moult. Such an active removal may still be the explanation of the results recalled at the beginning of this section; but it is equally possible that the hormone balance is being slightly upset in some other way. It is worth recalling that Pflugfelder (1939) observed that the implantation of corpora allata into *Dixippus* leads to a partial suppression of growth in the corpora allata of the host insect.

DISCUSSION

Prothetely and metathetely

The new experiments described and the old experiments recalled in this paper illustrate the various ways in which the hormone balance may be upset and abnormalities in metamorphosis produced. In the unoperated insect such abnormalities take the form of prothetely, metathetely or neoteny. These abnormal forms were quoted by Goldschmidt (1923) as examples of his principle of the control of morphological characters by differential reaction velocities; they were regarded as the result of an upset in the time relations of two processes going forward simultaneously.

According to Goldschmidt the two processes were the 'evagination of wing buds' and 'metamorphosis'. This same explanation was adopted by v. Lengerken (1924*a*, *b*) who considered one of the processes in question to be the formation of the oxidase which Dewitz (1902) had supposed necessary for metamorphosis: deficient production of this enzyme resulting in delayed or incomplete metamorphosis (metathetely), excessive production leading to prothetely.

The recognition of the control of metamorphosis by the balanced action of two hormones provided a more precise description of prothetely and metathetely (Wigglesworth, 1934; Piepho, 1942). In a later paper it was suggested that the two competing processes were (i) 'differentiation towards the adult form' brought about by the moulting hormone, and (ii) the deposition of the new cuticle brought about by the juvenile or inhibitory hormone. If the second process was accelerated

metathetely or partial restraint of metamorphosis was the result; if this process was delayed precocious metamorphosis or prothetely supervened (Wigglesworth, 1936).

But a comparative study of the epidermis during larval moulting and during the metamorphic moult showed that this explanation is too crude: the process of growth is different from the outset if the juvenile hormone is present. Moreover, if the adult is caused to moult in the presence of the juvenile hormone it suffers a partial *reversal* of metamorphosis, which cannot be explained by an early deposition of cuticle. The description was therefore elaborated by supposing that the intracellular system which is responsible for the formation of larval characters, and which is activated by the juvenile hormone in the presence of the moulting hormone, works more rapidly than the system responsible for the formation of adult characters. It is for this reason, it was suggested, that larval characters predominate in the presence of the juvenile hormone (Wigglesworth, 1940).

But, as was pointed out by Pfeiffer (1945), and as has become apparent in the course of further experiments on *Rhodnius*, the length of time required for moulting shows no constant relation to the characters, larval or adult, that are eventually produced. The characters produced are determined by the balance between two opposing hormone formulae: moulting hormone alone, and moulting hormone plus a variable amount of juvenile hormone. The final result is controlled by the relative concentration of the juvenile hormone and by the stage in moulting at which it is introduced into the system. The parallel with sex determination and intersex formation has been pointed out before.

The best-known environmental agencies which may bring about abnormalities of metamorphosis are temperature and asphyxiation. As long ago as 1813 Majoli (quoted by Pruthi, 1924) observed prothetely in silkworms under the influence of high temperature. Pruthi (1924) obtained *Tenebrio* larvae with wing rudiments on exposure to high temperature (29·5° C.) and Arendsen-Hein (quoted by v. Lengerken, 1932) observed metathetely in the same insect after transfer to abnormally cold conditions. Nagel (1934) obtained the same result in *Tribolium*. Other examples are quoted by Thomas (1932). (Cf. Radtke, 1942.)

There are fewer observations on the effect of asphyxiation, but Dewitz (1902) obtained intermediates between larvae and pupae of *Pieris* when the larvae had been placed in sealed tubes.

In the present work high temperature is shown slightly to depress the action of the juvenile hormone in *Rhodnius* so that the resulting insect shows a mild degree of prothetely; low temperature slightly enhances the action of the juvenile hormone so that a mild degree of metathetely results. A low partial pressure of oxygen acts like high temperature and slightly depresses the action of the juvenile hormone. The experiments do not reveal the site of action of these environmental factors.

Cause of metamorphosis

There is much that is still obscure about the causation of metamorphosis. It is clear that the absence of the juvenile hormone at the final larval moult leaves the larval system unactivated so that the adult system may differentiate and meta-

morphosis occur. But there is much uncertainty as to the part played by the developing tissues themselves—that is, by their responsiveness or competence to differentiate.

The 1st-stage larva of *Rhodnius* deprived of its corpus allatum and transfused with moulting hormone from the 5th-stage larva undergoes metamorphosis (Wigglesworth, 1934). Larvae of Lepidoptera in the 2nd instar transform into diminutive pupae and moths if the corpora allata are removed (Bounhiol, 1938); and isolated fragments of integument from caterpillars, even in the 1st instar newly hatched from the egg, can be induced to pupate (Piepho, 1938).

In these insects the tissues are clearly capable of metamorphosis at any stage of larval growth. But there are some insects in which this is less certain. If the corpora allata are removed from larvae of *Dixippus* in the 3rd stage they make two more moults before they begin to lay eggs (Pflugfelder, 1937). Similarly, the cockroach *Leucophaea* (which has an average of eight larval instars) deprived of its corpora allata in the 5th stage, first moults to give rise to an intermediate 'pre-adultoid form' and then moults a second time to produce a diminutive adult (Scharrer, 1946).

Scharrer (1946, 1948) attributes this delay in the appearance of adult characters to the tissues being as yet incapable of metamorphosis. That certainly appears to be the case in the experiments of Bodenstein on isolated imaginal disks from *Drosophila*: salivary glands transplanted into mature larvae will not undergo metamorphosis unless they have reached a fairly advanced stage of growth (Bodenstein, 1943*a*); and the other organ disks vary in their responsiveness to the ring gland hormone in a definite order (Bodenstein, 1943*b*). 'Whether the organ disks respond...with growth or differentiation depends on a definite relation between hormone concentration and organ responsiveness' (Bodenstein, 1943*b*).

It may well be that in the higher Diptera in which the germs of the imaginal organs are completely independent of the larval integument, these imaginal disks must reach an advanced stage of growth before they can undergo differentiation. But in view of the ready metamorphosis of Hemiptera and Lepidoptera at a very early stage of larval growth it is unlikely that the delay in metamorphosis after removal of the corpus allatum in the Orthoptera *Dixippus* (Pflugfelder, 1937) and *Leucophaea* (Scharrer, 1946) is due to a lack of responsiveness in the tissues. All these are insects in which the imaginal potencies are latent in cells which play a functional part in the larval organism.

It was on these grounds that it was suggested (Wigglesworth, 1948) that this delay might be due to the persistence of juvenile hormone in the blood or tissues from one instar to the next. And it was further suggested that the active removal of such persistent juvenile hormone might be a necessary step in the process of metamorphosis.

In the present work no evidence has been obtained for the persistence of any juvenile hormone in *Rhodnius*, and it has therefore not been possible to test the hypothesis that the corpus allatum, when it ceases to secrete the juvenile hormone

at the last moult, also actively removes or inactivates the traces of that hormone persisting in the blood. But this hypothesis is still worthy of being tested on other insects. In *Rhodnius* the removal of the corpus allatum by decapitation has been carried out before any juvenile hormone has been secreted; but it may well be that at the time of this removal in *Dixippus* (Pflugfelder, 1937) and *Leucophaea* (Scharrer, 1946) the blood already contains some juvenile hormone. In that case it is not surprising that the ensuing moult should give rise to a larval form. In the silkworm the corpus allatum in the last or 5th-stage larva appears to change its function during the instar: early in the instar it secretes juvenile hormone, but after the fourth day it no longer does so (Fukuda, 1944).

There remains the problem of what causes the corpus allatum to change its function at the last larval moult so as to allow metamorphosis to occur. It has been found again in the course of the present work (cf. Wigglesworth, 1948) that the isolated corpus allatum implanted into the abdomen no longer regulates its function in this way. One must conclude that the normal regulation is dependent on its connexion with the central nervous system and that the signal to the corpus allatum to change its secretory activity comes from the brain.

SUMMARY

A technique is described by which the intact larva of *Rhodnius* can be transfused with blood from another larva without interfering with ecdysis.

If the 4th-stage larva receives blood from a 3rd-stage larva it develops characters little different from those of the 4th instar. This is attributed to the 3rd-stage larva producing juvenile hormone at a higher concentration.

If the 4th-stage larva at 24 hr. after feeding receives blood from another 4th-stage larva at 8 days after feeding it develops characters intermediate between those of the 4th and 5th instars. This is attributed to the juvenile hormone being introduced too early in the moulting cycle.

The hormone balance is upset by abnormal temperatures. The 4th-stage larva will not moult at a temperature of 36° C. although the larvae can survive up to about 40° C.

At temperatures a little below 36° C. moulting is somewhat delayed and the characters developed are slightly 'adultoid' (prothetely). This is attributed to slightly reduced activity of the corpus allatum.

At temperatures below 20° C. moulting is greatly delayed and the characters developed are slightly 'juvenile' (metathetely). This is attributed to relatively increased activity of the corpus allatum.

Low concentrations of oxygen (less than 5 %) have an effect similar to that of high temperature.

If 5th-stage larvae of *Rhodnius* receive implants of corpora allata from mature adults of *Periplaneta* they develop into 6th-stage larvae and many of these subsequently into 7th-stage larvae. The 'juvenile hormone' appears to be the same in the two insects.

No evidence could be obtained for the persistence of juvenile hormone in the

blood from one instar of *Rhodnius* to the next. The hypothesis of an active elimination of juvenile hormone by the corpus allatum at the time of metamorphosis remains therefore unproven.

REFERENCES

BODENSTEIN, D. (1943*a*). Factors influencing growth and metamorphosis of the salivary gland in *Drosophila*. *Biol. Bull. Woods Hole*, **84**, 13–33.

BODENSTEIN, D. (1943*b*). Hormones and tissue competence in the development of *Drosophila*. *Biol. Bull. Woods Hole*, **84**, 35–58.

BOUNHIOL, J. (1938). Recherches expérimentales sur le déterminisme de la métamorphose chez les Lépidoptères. *Bull. Biol.* (Suppl.), **24**, 1–199.

DEWITZ, J. (1902). Untersuchungen über die Verwandlung der Insektenlarven. *Arch. Anat. Physiol., Lpz.* (*Abt. Physiol.*), pp. 327–40.

FUKUDA, S. (1944). The hormonal mechanism of larval moulting and metamorphosis in the silkworm. *J. Fac. Sci. Tokyo Univ.* Ser. IV, **6**, 477–532.

GOLDSCHMIDT, R. (1923). Einige Materialien zur Theorie der abgestimmten Reaktionsgeschwindigkeiten. *Arch. mikr. Anat.* **98**, 292–313.

v. LENGERKEN, H. (1924*a*). Prothetelie bei Coleopterenlarven. *Zool. Anz.* **58**, 179–85.

v. LENGERKEN, H. (1924*b*). Prothetelie bei Coleopterenlarven (Metathelie). *Zool. Anz.* **59**, 323–80.

v. LENGERKEN, H. (1932). Nachhinkende Entwicklung und ihre Folgeerscheinungen beim Mehlkäfer. *Jena. Z. Naturw.* **67**, 260–73.

NAGEL, R. H. (1934). Metathetely in larvae of the confused flour beetle (*Tribolium confusum*, Duval). *Ann. Ent. Soc. Amer.* **27**, 425–8.

NOVAK, V. (1951). New aspects of the metamorphosis of insects. *Nature, Lond.*, **167**, 132–3.

PFEIFFER, I. W. (1945). The influence of the corpora allata over the development of nymphal characters in the grasshopper *Melanoplus differentialis*. *Trans. Conn. Acad. Arts Sci.* **36**, 489–515.

PFLUGFELDER, O. (1937). Bau, Entwicklung und Funktion der corpora allata und cardiaca von *Dixippus morosus* Br. *Z. wiss. Zool.* **149**, 477–512.

PFLUGFELDER, O. (1939). Wechselwirkungen von Drüsen innerer Sekretion bei *Dixippus morosus* Br. *Z. wiss. Zool.* **152**, 384–408.

PIEPHO, H. (1938). Über die experimentelle Auslösbarkeit überzähliger Häutungen und vorzeitiger Verpuppung an Hautstücken bei Kleinschmetterlingen. *Naturwissenschaften*, **26**, 841–2.

PIEPHO, H. (1940). Über die Hemmung der Verpuppung durch corpora allata. Untersuchungen an der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **60**, 367–93.

PIEPHO, H. (1942). Untersuchungen zur Entwicklungsphysiologie der Insektenmetamorphose. Über die Puppenhäutung der Wachsmotte *Galleria mellonella* L. *Roux Arch. Entw. Mech. Organ.* **141**, 500–83.

PIEPHO, H. (1950*a*). Über die Hemmung der Falterhäutung durch corpora allata. Untersuchungen an der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **69**, 261–71.

PIEPHO, H. (1950*b*). Über die Ausmass der Artunspezifität von Metamorphosehormonen bei Insekten. *Biol. Zbl.* **69**, 1–10.

PRUTHI, H. S. (1924). Studies in insect metamorphosis. I. Prothetely in mealworms (*Tenebrio molitor*) and other insects. *Proc. Camb. Phil. Soc.* (*Biol.*), **1**, 139–47.

RADTKE, A. (1942). Hemmung der Verpuppung beim Mehlkäfer *Tenebrio molitor* L. *Naturwissenschaften*, **30**, 451–2.

SCHARRER, B. (1946). The role of the corpora allata in the development of *Leucophaea maderae* (Orthoptera). *Endocrinology*, **38**, 35–45.

SCHARRER, B. (1948). Hormones in insects. *The Hormones*, ed. by S. Pincus and K. V. Thimann, **1**, 121–52.

THOMAS, C. A. (1932). Prothetely in an Elaterid larva (Coloptera). *Ent. News*, **44**, 91–6.

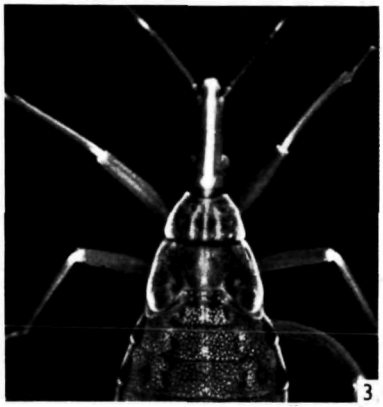
WIGGLESWORTH, V. B. (1934). Physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and metamorphosis. *Quart. J. Micr. Sci.* **76**, 269–318.

WIGGLESWORTH, V. B. (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. Micr. Sci.* **79**, 91–121.

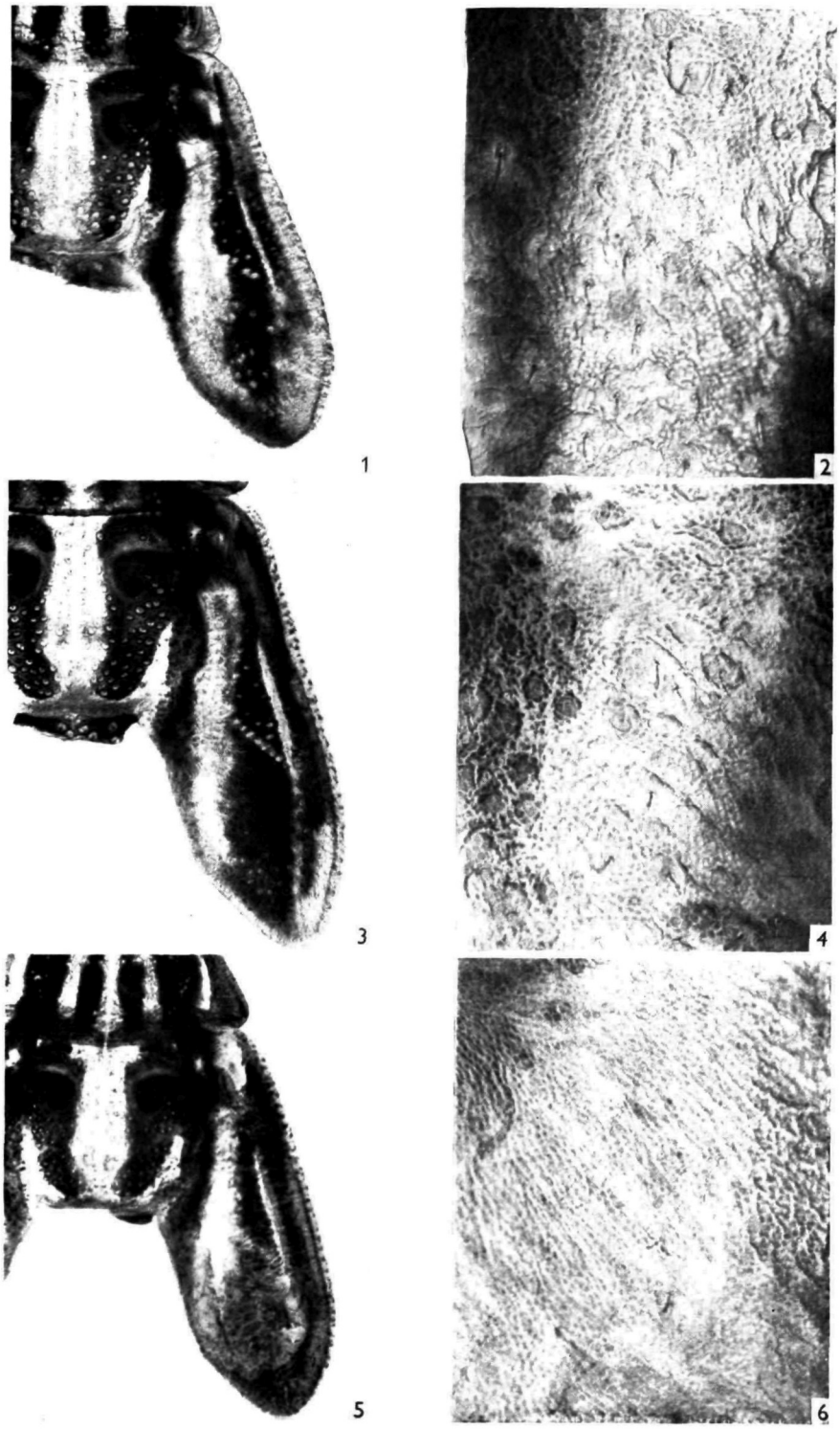
WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17**, 201–22.

WIGGLESWORTH, V. B. (1948). The functions of the corpus allatum in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **25**, 1–14.

WIGGLESWORTH, V. B. (1952). The thoracic gland in *Rhodnius prolixus* (Hemiptera) and its role in moulting. *J. Exp. Biol.* **29**, 561–70.



WIGGLESWORTH—CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS*



WIGGLESWORTH—CONTROL OF METAMORPHOSIS IN *RHODNIUS PROLIXUS*

EXPLANATION OF PLATES

Plate 22

Fig. 1. Normal 4th-stage larva of *Rhodnius*.
Fig. 2. Normal 5th-stage larva.
Fig. 3. 5th-stage larva produced from 4th stage to which a 3rd-stage larva had been joined.
Fig. 4. 5th-stage larva produced from 4th stage to which another 4th-stage larva 2 days after feeding had been joined.
Fig. 5. As fig. 4, but second 4th-stage larva joined at 7 days after feeding.
Fig. 6. As fig. 4, but second 4th-stage larva joined at 8 days after feeding.

Plate 23

Fig. 1. Anterior wing lobe of 5th-stage larva produced at 18° C.
Fig. 2. Detail of proximal region of membrane from the same (18° C.).
Fig. 3. Wing lobe of 5th-stage larva produced at 25° C.
Fig. 4. Detail of membrane in the same region as fig. 2 (25° C.).
Fig. 5. Wing lobe of 5th-stage larva produced at 34° C.
Fig. 6. Detail of membrane in the same region as fig. 2 (34° C.).