J. Exp. Biol. (1966), 44, 507–522
With 5 text-figures
Printed in Great Britain

THE HORMONAL CONTROL OF THE DEVELOPMENT OF HAIRS AND BRISTLES IN THE MILKWEED BUG, *ONCOPELTUS FASCIATUS*, DALL.

BY PETER A. LAWRENCE*
Department of Zoology, University of Cambridge

(*Received* 28 *November* 1965)

INTRODUCTION

The insect cuticle has long been appreciated as excellent material for the study of cellular responses to hormones. Not only is the insect epidermis a single layer of cells, but also 'the tiny fragment of cuticle laid down by a single cell may possess morphological characters controlled by the activities of that cell alone' (Wigglesworth, 1940*a*) and cell differences will thus be rendered visible in the types of cuticle they secrete. The hairs and bristles formed by the integument are also indicators of certain otherwise invisible properties of cells. For instance, the scales of moths develop even on small pieces of implanted integument provided that the epidermal cells are in an appropriate hormonal milieu (Piepho, 1938*a*). When the types of scales or bristles differ in subsequent stages in the development of the insect, then the form of the scale or the bristle may indicate the developmental state of a piece of experimental integument.

The larval *Oncopeltus* is furnished with bristles which are sparsely distributed on the abdomen, being on the average ten epidermal cells apart. The mature bristle is an innervated sensillum and comprises four cells: the trichogen, the tormogen, nerve, and neurilemma cell; at every moult subsequent to its formation the trichogen and tormogen cells secrete a new bristle shaft and socket. At each moult, just as in *Rhodnius* (Wigglesworth, 1940*a*) new bristles appear amongst the old ones, so that the density of their distribution remains more or less constant throughout growth. During the final moult some of the bristle cell groups degenerate and others form cuticular bristles once again. These adult bristles, as in *Rhodnius*, differ in form from their larval predecessors; in this case the ratio of the diameter of the socket to the length of the bristle (length/socket ratio) is smaller than in the larval type (Fig. 1).

In addition to these adult *bristles* there is, during metamorphosis, the development of a large new population of densely distributed adult *hairs*. The spacing of these adult hairs varies, but in some areas they are only one or two epidermal cells apart. These hairs are not innervated and in the mature form consist of but one cell. The hairs, as the bristles, arise from mother cells, which undergo differentiative divisions to produce the four distinct cells which together form the complete hair. Three of these cells degenerate during the maturation of the hair. A cytological account of the development of hairs and bristles in *Oncopeltus* is in preparation.

* Present address: Department of Biology, University of Virginia, Charlottesville, Virginia, U.S.A.

PRELIMINARY EXPERIMENTS

One might expect the genesis of adult hairs to be one facet of the general metamorphosis occurring during the last moult. As metamorphosis is initiated by the presence of the moulting hormone, coupled with the absence of the juvenile hormone (JH) it is this hormonal milieu which might be expected to stimulate the epidermis

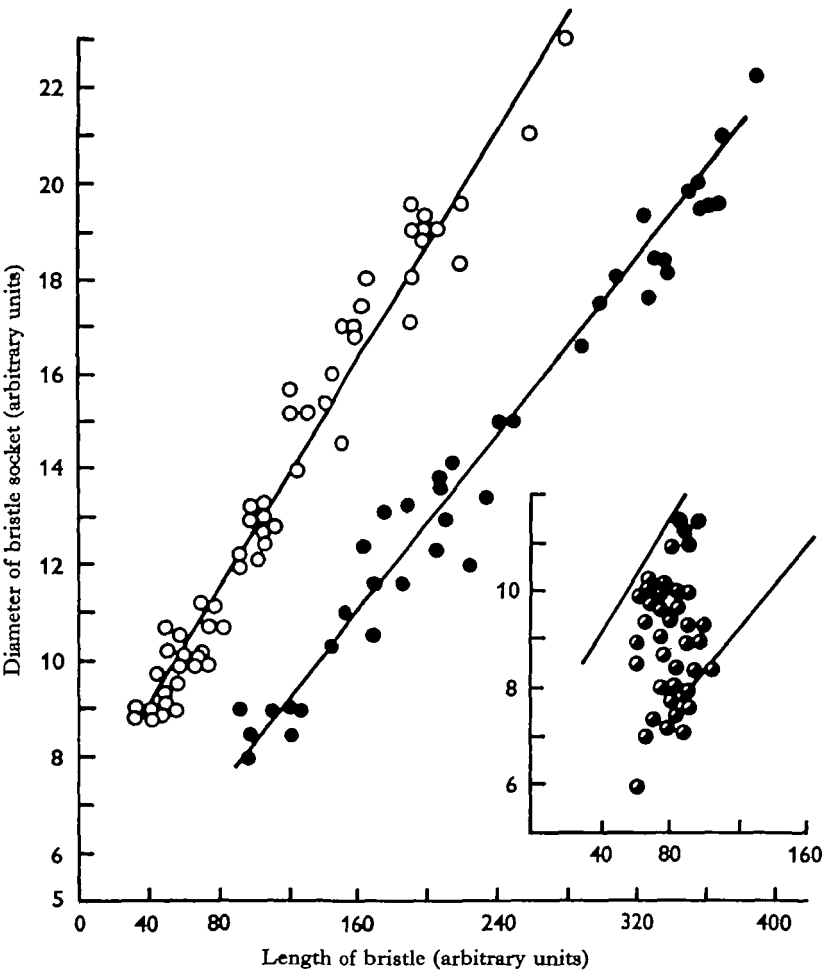

Fig. 1. The relationship between the length of the bristle and the socket diameter, in larval (empty circles), adult (full circles) and intermediate (half-filled circles) bristles.

to differentiate adult hairs. To check on this hypothesis cockroach corpora allata, known to be a source of JH, were implanted into 5th-stage larvae of *Oncopeltus*.

The results were similar to those reported by Wigglesworth (1936) who did equivalent experiments on *Rhodnius*. Following upon the implantations, some 6th-stage larvae were produced which bore a normally distributed population of larval bristles including ones which had developed during the supernumerary moult. There were also adult individuals with only a patch of larval cuticle and larval bristles over the

implanted organ. In the latter case, in between the normal adult cuticle and the fully larval cuticle there was a region which had intermediate characteristics. In this area there was a mosaic of cells some of which were covered by cuticle of the adult type and others by cuticle of the larval type. Some cells, however, produced a cuticle with a surface pattern intermediate between the larval and adult types. In the whole of this region there were innervated bristles which not only had an intermediate length/socket ratio, but occurred in a density between those of the larval bristles and the adult hairs. These bristles will be referred to as adult/larval bristles. The results of these implantation experiments suggested that the system of hairs and bristles in *Oncopeltus* could be made useful in an exploration of the reaction of the integument to hormones. Accordingly grafting experiments were designed to pursue the effects of the moulting and the juvenile hormones on the epidermis of certain larval stages and more particularly, of the adult.

METHODS

Pieces of integument from 200 μ–1 mm. square were cut from a donor animal. Sternal tissue was always used and the grafts were transplanted into a hole in the tergites of the host, which was prepared with pieces of new razor blade and iridectomy scissors. The host insect was anaesthetized by drowning for 10–20 min. in water (Novák 1951). No antibiotics or sealing material were found to be necessary. The rate of survival from these operations depended far more on the state of the culture than the damage inflicted on the host. It was found that isolating each generation of eggs from the parents and rearing the larval insects in separate sterilized containers kept the infestation of intestinal parasites (such as *Leptomonas* species) down to a low level. Survival rates were 95% from transplantations in which 5th-stage larvae, reared in this way, were used as hosts. When younger hosts were employed survivals were lower (4th, 70%; 3rd, 30%; 2nd, 15%) and this was due mostly to the mechanical difficulties which beset these small insects at ecdysis as a result of the relatively large size of the grafts.

All transplantations involved wounding which was localized to a minimum depth of five cells along the boundary of the hole in the host and the peripheral area of the graft. All the reactions to wounding which Wigglesworth (1937) observed in *Rhodnius* have been seen on this border; activation spreads further and usually affects all the cells of the graft, and cell division and migrations are, at best, localized around a fairly narrow region. Cells which are severely affected by wounding do not produce normal cuticle and instead form a thin wound cuticle. As reported by Wigglesworth this wound cuticle is quite distinct from either adult or larval cuticle. It is uneven, thin, and often, when produced by cells which normally form black cuticle, unpigmented. Recent work has shown that, unlike normal cuticle, wound cuticle has no lamellae (Lai-Fook, personal communication). Usually no hairs or bristles are found in wound cuticle and no new bristles or hairs differentiate from amongst cells which have been severely affected by wounding.

Sternal integument is pigmented in the adult, is furnished with a high density of hairs and is distinguishable from the hosts' unpigmented tergal cuticle in both larvae and adults. Following upon wound healing the source of each cell can be deduced from the type of cuticle it secretes. It had been earlier shown (Wigglesworth, 1940*a*;

Kühn, 1949) that transplanted cells, or cells caused to migrate by wounding, retained their individual pattern characteristics. The grafted cells could thus be identified; examination ruled out the possibility that the cells of the transplant had been infiltrated or replaced by host cells from the neighbouring epidermis.

When the experimental animal underwent more than one ecdysis, records of the intervening moults were provided by the exuviae which had all the cuticular vestiges of those hairs and bristles which had been present in the previous instar.

Unless otherwise stated, more than ten successful experiments of each type were completed.

RESULTS

1. *Timing experiments*

Studies have not previously been undertaken to discover when exactly in the moulting cycle a piece of donor tissue becomes too old to be synchronized with the moulting cycle of the host. In order to determine this, experiments were conducted in which grafts were taken from 5th-stage larvae (whose integumental remnants were fixed in order to determine the exact physiological age of the donor) and transplanted on to young 5th-stage individuals which were at the very beginning of their moult cycle (less than 10 hr. old). The moulting cycle lasts 150 hr. from ecdysis to ecdysis in the 5th-stage larva at 29·5° C.

It was found that when donor animals were at any stage up until the production of moulting fluid (90 hr.) no further development occurred in the donor tissue, until the host animal reached a similar stage; from this point development proceeded exactly synchronously in the host and graft tissues. The process of hair outgrowth, which lasts but 4 or 5 hr., was always at an identical stage in host and graft even when the original age difference had been as much as 80 hr.

On the other hand, donor animals in which the epicuticle had been completed always produced transplants which moulted autonomously. After this first autonomous ecdysis the transplants went on to moult again with the host, so that the adult which resulted had two cuticles over the grafted area. The period between the production of the moulting fluid and the completion of the epicuticle is about 15–20 hr. and when grafts were taken from donors during this physiological period, a partial cuticle was cast off by the transplant before it went on to moult with the host. When either a partial or a complete cuticle had been formed by the transplant, it then behaved in its second ecdysis as if it were the next instar. In the present experiments the first cuticle formed bore partial or complete hairs as in an adult, but the second cuticle formed bore no hairs, as in a piece of adult integument caused to moult again (Expt. I; p. 511). These experiments showed that minor differences in the age of host and transplant were not likely to affect the results of other grafting experiments.

2. *Grafts between larval stages*

When transplants were taken from 5th-stage individuals and placed on either 4th-, 3rd-, or 2nd-stage hosts, the transplant never underwent its presumptive metamorphosis. Instead it responded to the hormonal stimuli current in the host and produced another larval cuticle with the host. Some new bristles developed on the graft and these were always larval in character. When the host underwent its metamorphosis

the graft did also and formed a typical area of sternal adult cuticle on the tergite of the host.

When the transplant was taken from 1st-, 2nd-, 3rd-, or 4th-stage donors and transplanted on to 5th-stage hosts the integument always metamorphosed with the host. The metamorphosis was complete in the grafted tissues; the adult cuticle formed had all the characteristics of normal adult sternal cuticle and the hairs which differentiated from the epidermal cells were typically adult in form and density. Any bristles had the adult length/socket relationship.

3. *Grafts in which adult tissue was employed*

Expt. I. Adult integument onto 5th-stage larvae (Fig. 2).

When young adult tissue was transplanted onto a 5th-stage larva the epidermis became activated and formed a new cuticle. At the periphery of the graft wound cuticle was secreted. The form of the central integument differed from typical adult cuticle being pigmented but rather uneven in thickness, with no adult hairs. Some of the bristles were secreted once again, and in this case the bristles formed had an adult length/socket ratio.

This grafted cuticle was removed from the host and then regrafted onto another 5th-stage larva, when the transplant was found to moult once again with the host and produced the same kind of cuticle as previously. The number of persistent bristles decreased and in no case were new bristles or hairs developed. This transplantation experiment has been extended to a third supernumerary moult and in one case to a fourth supernumerary moult. No bristles persisted on the transplants which had moulted three times and the area of wound cuticle increased at each successive transplantation.

Expt. II. Adult integument onto 4th-stage larvae (Fig. 2).

After a single moult (host moulted from 4th to 5th) the appearance of the integument differed in no major way from that after a moult in the 5th-stage host although pigmentation was possibly more reduced at the periphery of the transplant.

After a second moult, however, the transplanted epidermis secreted a typical adult cuticle, which bore adult hairs in the normal density as well as some adult bristles. Except at the margin, where signs of wounding were evident, the cuticle was pigmented. Since all but one of the cellular complement normally required to form each old hair had degenerated, the new hairs had probably arisen by means of a new wave of differentiative divisions. Indeed, such divisions have been observed in material fixed at the appropriate moment in the moult cycle.

This experiment was extended by grafting the transplanted integument from the adult onto another 4th-stage host. Once again a new population of adult hairs was formed during the metamorphosis of host. *This was the third time that these epidermal cells had produced hairs by differentiation.*

Expt. III. Control (Fig. 2).

This development of a new population of adult hairs may depend either upon the tissue passing through two moult cycles free from transplantation or may in some way stem from the moult in the 4th stage. To distinguish between these two possibilities a control experiment was devised in which the previous experiment was repeated, except that an additional transplantation was interpolated. After the first moult of the

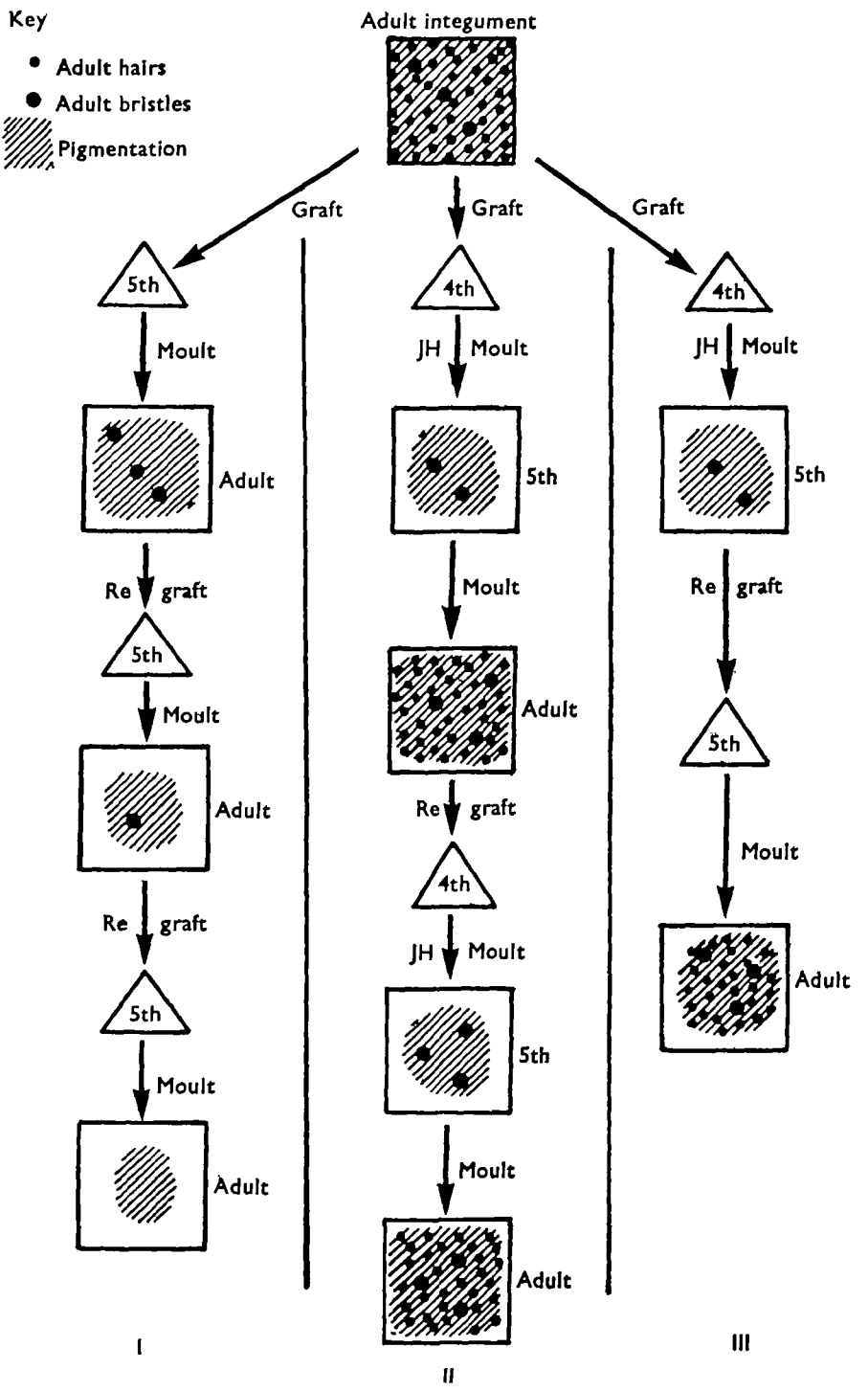

Fig. 2. Diagram to summarize the results of transplantation Expts. I, II, and III. JH = juvenile hormone.

host to the 5th-stage, the transplant was removed and grafted on to another 5th-stage individual. The results of this experiment were identical to the results of the former, except that, consequent upon the damage done by the extra transplantation, the area of wound cuticle was larger.

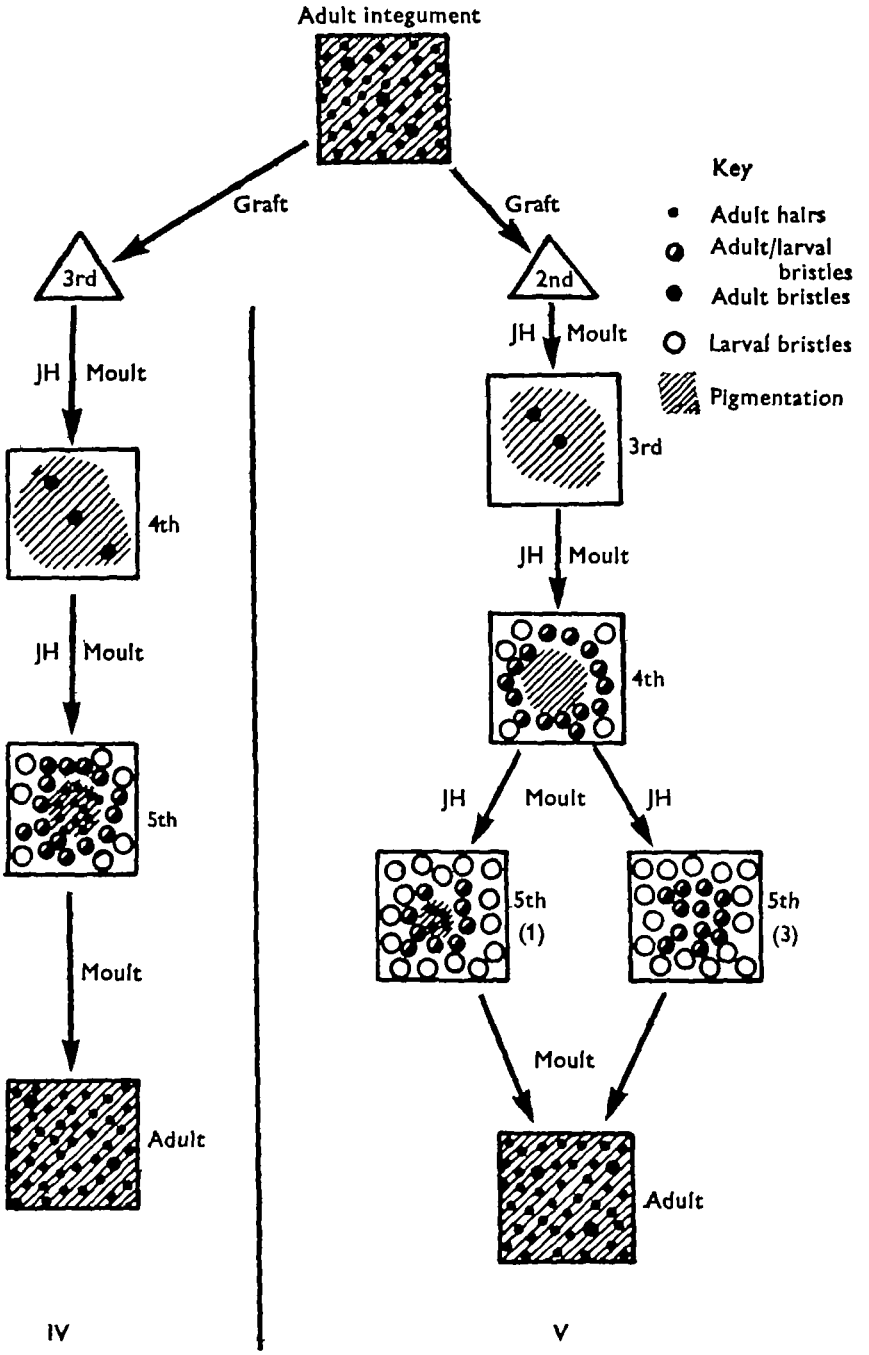

Fig. 3. Diagram to summarize the results of transplantation Expts. IV. and V. JH = juvenile hormone.

Expt. IV. Adult integument onto 3rd-stage larva (Fig. 3).

After the first moult (host moulted from 3rd to 4th) the integument was as in Expt. II.

After the second moult, in which the host reached the 5th stage, the grafted integument had a very complex appearance (Fig. 4). In a central area of pigmented cuticle there was a dense population of *adult hairs*. Just inside the thin peripheral boundary

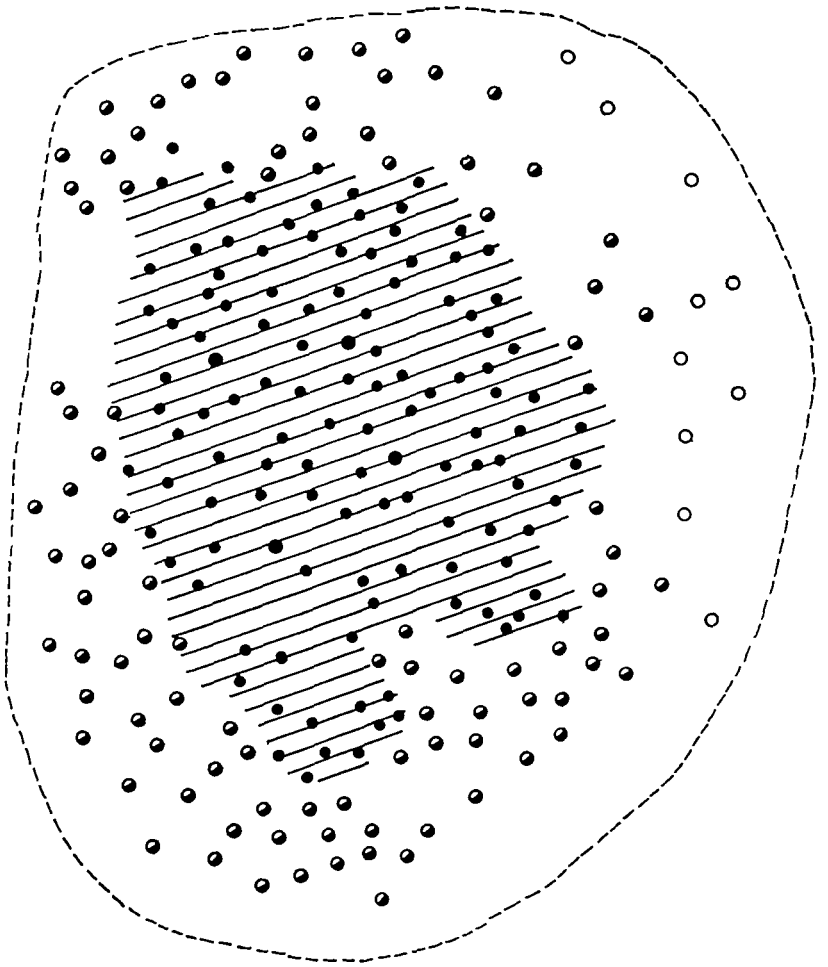

Fig. 4. Camera lucida drawing of transplant in the 5th-stage host (Expt. IV): empty circles, larval bristles; half-filled circles, adult/larval bristles; small filled circles, adult hairs; larger filled circles, adult bristles. The dotted line marks the boundary of the graft. The pigmented area is cross-hatched.

of wound cuticle there were a few widely spaced bristles which were larval in respect to their length/socket ratio. In the zone which intervened between the two above regions, bristles were present in a density that was intermediate between those of adult hairs and larval bristles. The length/socket ratio of these bristles was also intermediate between the ratios for adult and larval bristles (Fig. 1). The more peripheral the

bristle the more larval was it in its form and the further was it situated from its neighbours. There were occasional adult bristles amongst the adult hairs.

The final moult of the host produced a metamorphosis in the transplanted integument and the whole graft became pigmented and bore a population of adult hairs. These hairs were slightly more sparse in the central region; nevertheless, the epidermis of this area had apparently differentiated hairs during two successive moult cycles. Those bristles which persisted were found to have the adult length/socket ratio.

Expt. V. Adult integument onto 2nd-stage larvae (Fig. 2).

The small size of the host animals made these operations difficult; for while 15 % of the insects survived to complete the first moult, more died from mechanical troubles at later ecdyses. However, four individuals were reared to the adult stage.

After the first moult (host moulted from 2nd to 3rd) as before there was a central pigmented area and one or two adult bristles were reformed.

After the second ecdysis, in which the host reached the 4th stage, there was a peripheral population of adult/larval bristles and a central pigmented area. *There were, however, no adult hairs in this area.*

In the 5th-stage larva, in three instances, the grafted integument bore no adult hairs, and the whole area was covered with larval and adult/larval bristles, the former being nearer the periphery than the latter. In one individual there was, situated in the centre, a small pigmented patch and here and there were some unmistakeable adult hairs in high density.

When the host underwent metamorphosis the transplant did also and produced a typical adult cuticle with adult hairs.

DISCUSSION

The formation of types of cuticle and bristles intermediate between larval and adult has been noted before (Wigglesworth, 1936). It is not known whether the formation of such structures indicates that the cells are intermediate between larval and adult at the time when the structures were formed or whether it means that during the time of formation of the bristles or the cuticle, the cells were for part of the time 'adult' and for part of the time 'larval'. Electron microscopic observations have shown that the folding of the epicuticle is primarily responsible for the microsculpture of the cuticle, and although numerous individuals fixed at about this stage have been examined, no instances of intermediate folding have been discovered. Accordingly, the process is assumed to occur suddenly, and if this is so then the intermediate type of cuticle is produced because the cells are in an *intermediate condition* at the time of secretion of the epicuticle.

The timing experiments reveal that the degree of regulation possible between host and transplant is considerable. To achieve this, powerful feed-back mechanisms must obtain between the endocrine organs and the cells and between the cells themselves. Yet there would appear to be an important turning point at the time of moulting fluid secretion. After this moment the transplanted integument can no longer be directly regulated to moult with the host; it is a further 'critical period' for the epidermal cell.

The grafting experiments between larval stages parallel similar experiments performed with implants or transplants by Piepho (1938*b*, *c*) and Bodenstein (1953). In all their results, when young donors were used, the metamorphosis of the graft only

occurred with that of the host, and never autonomously. Many supernumerary larval moults could be undergone by the integument, or conversely premature metamorphosis was possible even with 1st instar material (Wigglesworth, 1934). In the present experiments differentiation of the adult hairs appears to be but one character associated with metamorphosis.

In the following section of the discussion the main points emerging from those transplantation experiments in which adult donor material is used will be discussed in turn, each point being recapitulated before it is considered.

1. (The epidermal cells can, by means of differentiative divisions, develop a second population of adult hairs.)

The differentiation of a second population of adult hairs provides conclusive evidence that they develop from cells which are homogeneous in respect to their capacities to form hairs, and that the cells which remain retain the capacity not only to differentiate into hairs but to form them in the correct density. This result thus lends support to Wigglesworth's (1940*a*) hypothesis that in *Rhodnius* any epidermal cell sufficiently distant from an extant bristle can become determined as a bristle mother cell, absorb some essential growth substance from the cells around and thereby inhibit the development of new bristles in its vicinity. Every cell thus has the potency to develop into a bristle. Whether this kind of system applies to scale development is still in doubt for it has been suggested that a system of restricted potency, rather than such a determination by distance, does indeed operate in scale formation in the Lepidoptera (Henke, 1947). Henke formulated his 'law of compensation' (*Kompensationsprinzip*) to explain certain inverse correlations he found in *Ephestia* between the size of the scales and the number of epidermal cells which intervened between those scales. He found that counts agreed with the assumption that at an early stage of wing development all cells are scale-epidermal mother cells which undergo a division in which a growth factor is partitioned amongst the two daughter cells. This growth factor is required for the endomitosis and the cell divisions of the scale mother cells and the epidermal cells respectively. At the division of the scale-epidermal mother cell the growth factor can be shared out in different proportions between the two daughter cells, so that where scales are large (with a ploidy of $32n$) they are interspersed with a few epidermal cells (2 of $2n$) and where scales are small ($4n$) they are interspersed with many epidermal cells (16 of $2n$). It is still disputed whether Henke's law of compensation adequately explains the scale pattern in *Ephestia*. The theory has been criticized by Smolka (1958) who found that his counts of scales and cells were not properly in accord with it. Lipp (1959) defended the theory but conceded that there are epidermal cells (*Zwischenepithelzellen*) distributed amongst the others to which the theory does not apply; this concession, it seems to the writer, undermines the basic evidence for the theory she is attempting to defend. Certainly no such system exists in *Oncopeltus*, where for the moment one can hold a much simpler view of determination. Microspectrophotometric measurements of the DNA have shown that in *Oncopeltus* there is no polyploidy in the nuclei of the bristle-forming cells.

Piepho & Meyer (1951) studied implants of integument of *Galleria* which had undergone one metamorphosis and produced one population of scales and found that, after moulting in a second larval host, they could produce a second population of scales when that host underwent metamorphosis. As they could see no differentiative

divisions they concluded that the scale cells remained inactive during the supernumerary larval and pupal moults but resecreted scales during the second metamorphosis. Piepho (1947) had earlier investigated the development of integumental implants of *Galleria* in older last instar hosts. He found that, after a certain critical stage in the hosts' moulting process, the implants could no longer be induced to produce scales. Wiedbrauck (Meyer) (1953) took integumental implants which had already undergone a metamorphosis, and implanted them into older larvae which were past this critical stage; unlike normal implants, these implants produced a second population of scales. This experiment provided further evidence that these secondary scales developed from scale initial cells which had persisted, but not functioned, throughout the supernumerary larval and pupal moults.

It seems that the situation is quite different in *Oncopeltus* where at each succeeding metamorphosis new hairs develop as a result of fresh differentiative divisions.

2. (A redevelopment of adult hairs does not depend on there being two successive moults free from transplantation (Expt. III) but does depend on the moult in which the host moulted from the 4th to the 5th stage.)

Expt. I in which adult integument was grafted onto a 5th-stage larva, and then after the moult transplanted again onto another 5th-stage larva, differed only in one respect from Expt. III. In the latter case the adult integument passed through one moult in the hormonal milieu of the 4th stage, while in the former, two moults were passed in the hormonal milieu of the 5th stage. That adult hairs develop in Expt. III and not in Expt. I shows that this development depends on the moult in the 4th stage. We may assume that the difference between the moults of the 4th and 5th stages is that in the former the JH is present. 'Reversal of metamorphosis' is a term introduced by Wigglesworth in 1940(*b*) when he discovered that adult epidermis of *Rhodnius*, when stimulated by the moulting hormone in the presence of the JH, could show at least partial recovery of the larval condition. We may tentatively adopt the hypothesis that the *capacity* to produce adult hairs is a 'larval' character and its retrieval during a moult in the JH is an example of reversal of metamorphosis.

Clearly, reversal of metamorphosis has not been completed; for Expt. IV has shown that it can go so far, that typical larval bristles can be produced. We have seen that cells may occupy intermediate states between those of the larval condition and the adult condition; it now seems that this intermediate condition may result either from partial inhibition of metamorphosis, such as has been achieved by the implantation of corpora allata into 5th-stage *Oncopeltus*, or from incomplete reversal of metamorphosis.

The interesting results obtained by Krishnakumaran & Schneiderman (1964) may be discussed in connection with this point. They found that an adult male cynthia moth which had been in parabiosis with a diapausing pupa of cecropia for a long time (120 days), when caused to moult by the eventual production of ecdysone by the pupa, produced a perfect adult cuticle with scales. On the other hand, an adult male of the same species when caused directly to moult in parabiosis with a chilled polyphemus pupa, produced a scaleless adult cuticle. Normally there is JH present in the adult male cynthia moth and this, in the second experiment, caused the pupal partner to moult to a second pupa. In the first experiment Krishnakumaran and Schneiderman noted that the pupal partner metamorphosed normally and pointed out that this meant that effective levels of JH were no longer present. Although there was no JH

still present in the haemolymph of the moulting adult in the first experiment, the adult tissues had probably been exposed to it for longer than in the second experiment. We can here make the suggestion that this long exposure to JH had worked a restricted reversal of metamorphosis sufficient to retrieve the capacity of those adult cells to form adult scales by redifferentiation. Krishnakumaran and Schneiderman indeed pointed out that a change must have occurred, but did not consider whether *this* change could have been a result of prolonged exposure to the JH itself. It would be essential to discover whether or not the second population of scales developed as a result of differentiative divisions before this hypothesis could be adopted seriously.

3. (Central areas of integument which remain adult according to the criterion of pigmentation can develop adult hairs even during a moult in which JH is present (Expt. IV)).

The development of adult hairs during the 4th stage demonstrates clearly that hair genesis is not itself a response to the absence of the JH. It must be a property of the 'adult' cells (originally caused to become adult by the absence of the JH) which, when they respond to the moult cycle which is sustained by the moulting hormone, produce adult hairs. As already pointed out, the capacity to produce adult hairs is in one respect a 'larval' character, and is retrieved by the action of JH on adult cells. How can this capacity be both an 'adult' and a 'larval' character? This apparently paradoxical situation can be resolved when it is realized that a character may be 'larval' when it is compared to the fully adult condition and yet 'adult' when compared to the fully larval condition.

4. (After two moults in a larval environment larval bristles are formed at the periphery of the transplant, and adult/larval bristles are produced near the centre (Expts. IV and V).)

The marginal cells after the second moult in Expt. IV are larval both by the criterion of pigmentation and because they produce bristles of the larval type in the typical larval density. Yet the central areas are much more adult as they produce adult hairs and pigmented cuticle. The intermediate density of the adult/larval bristles formed in the intervening areas indicates that this local population of cells was actually in a condition between the adult and the larval states at the time of bristle determination. Since the whole graft will have received the same hormonal stimuli, some factor which has sponsored the reversal of metamorphosis has evidently varied over the surface of the graft. This factor has been most potent at the periphery of the graft and has diminished in effectiveness centripetally. It is possible that this factor is connected with wound healing. We have already noted that wounding is most severe at the edge of the graft. An examination of the cells during the 3rd stage in Expt. IV reveals that, by cytological indicators, wounding extends from the periphery inwards and diminishes towards the centre, where at the middle of the transplant the nuclei show only activation, but no divisions or migration. Could it be that the cells undergoing divisions, and other changes associated with wounding, are more responsive to the JH, so that when they settle down to a new equilibrium condition they are more larval in character than their unwounded, undisturbed neighbours? Increased sensitivity of wounded cells to JH has been suggested before. Piepho (1939), in his classical work with *Galleria*, noted that reversal of metamorphosis was more immediate in those areas of integument formed by that epidermis which, undergoing considerable cell divisions,

grows out from the implant and migrates around to produce a continuous vesicle (*Umwachsungshypodermis*). Schneiderman & Gilbert (1958) in their search for a very sensitive indicator for the JH, found that the regenerating cells in the epidermis of the pupal *Hyalophora* were the most responsive to minute quantities of JH. Piepho (1950) and Piepho & Heims (1952) studied this effect of wounding in greater detail, and showed that wounding with its concomitant cell divisions was indeed associated with sensitivity to the JH.

It seems very unlikely that this graded reversal of metamorphosis achieved over the surface of the transplants is due to any influence spreading slowly into the graft from the host epidermis because Piepho & Meyer's results with disconnected implants were essentially similar to those reported here.

FURTHER DISCUSSION: THE MODE OF ACTION OF THE JUVENILE HORMONE

There are two main views as to the ways in which the JH functions. Williams (1956, 1961) suggested that the JH is an inhibitor, a *status quo* hormone which restricts an intrinsic tendency in the cells to develop towards the adult condition. Wigglesworth on the other hand conceives the JH as the key agent which causes the cell to adopt one of two (or three) equivalent metabolic states; transformations from the larval state to the adult state, or the reverse being changes of comparable type, and degree of difficulty. He suggested (1964) that there is an 'inertia' which retards cells undergoing both reversal of metamorphosis and premature metamorphosis.

The *status quo* hypothesis, taken simply, cannot survive the observation that cells in the adult can be caused to revert to the larval state by the presence of the JH during one or two moult cycles. Observations of such a change, dependent on the JH, have now been made with the epidermis of *Rhodnius* (Wigglesworth, 1939, 1940*b*), *Anisolabis* (Ozeki, 1959), *Oncopeltus*, and with the epidermis (Piepho & Meyer, 1951) and the midgut (Piepho & Holz, 1959) of *Galleria*.

On the other hand, studies of the integument of *Periplaneta* (Bodenstein, 1953) showed that the JH of the larval stages seemed to work no reversal of metamorphosis on the grafted adult epidermis even over as many as three moults. Williams (e.g. 1963) had never seen any examples of this phenomenon in his extensive studies of the silkmoths.

The nature of the evidence at hand thus clearly depends on the type of animal used; one nevertheless suspects that reversal of metamorphosis is such a well-documented phenomenon that if there is to be any universal theory of mode of action of the JH, it must form a part of it.

One striking point emerges from the experiments of Piepho & Meyer and those on *Oncopeltus* described above: reversal of metamorphosis takes several moults and is dependent for completion on the effects of wounding. On the other hand, metamorphosis or a second metamorphosis, is a rapid process. Reversal of metamorphosis and the second metamorphosis are strictly comparable changes since they occur in grafted tissue which has undergone the same experimental treatment. It seems that, at least in *Oncopeltus*, larval or adult/larval cells will, in the absence of the JH, develop rapidly towards the adult condition but adult cells will exhibit resistance to reversal.

There are two major ways in which differentiation may be recognized. There is first the development of morphological and chemical characteristics within cells which distinguishes them from their progenitors. Second, there is the acquisition of

a resistance to revert to the condition of their forebears or of their earlier selves; such a change of itself involves a loss of potency to develop in one direction and often in other directions also. For instance, when a hair mother cell divides and produces hair initial cells, these cells are, firstly, cytologically different from their epidermal forebears, and, secondly, they can never (apparently) revert to the parental condition. Wigglesworth (1940*a*) has shown that the epidermal cell has the potency to develop into a dermal

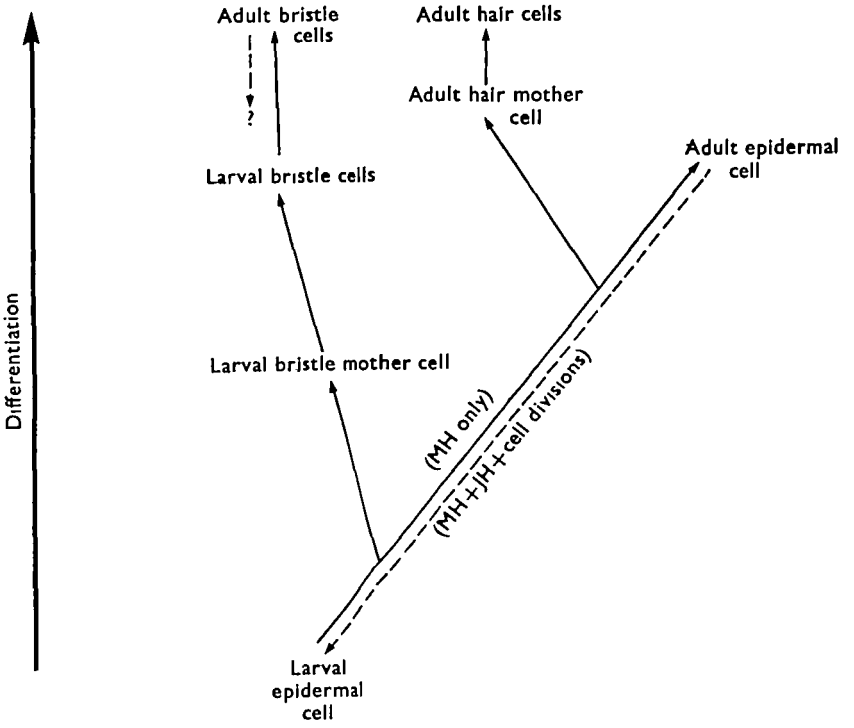

Fig. 5. Diagram to summarize the ontogeny of the epidermal cell of *Oncopeltus*. JH = juvenile hormone; MH = moulting hormone.

gland. However, when it becomes a hair mother cell it must lose this potency. By both the above criteria a hair mother cell is more differentiated than an epidermal cell (Fig. 5).

In both structure and function adult and larval epidermal cells would seem to be of comparable cytological complexity; according to the first criterion, they are similarly differentiated. The second criterion of differentiation is more amenable to experimental analysis, and as it has been shown that, at least in *Oncopeltus*, the adult-to-larval change is more difficult than the reverse transformation; by this criterion adult cells of *Oncopeltus* are more differentiated than larval cells. Within this context we may regard the adult-to-larval change as 'dedifferentiative'. It has also been noted that this change is hastened by wounding reactions which include cell divisions. Grobstein (1959) has remarked that 'There has long been an impression that differentiation and propagation are reciprocal in some degree, that increase of one implies decrease of the other'. If this generalization applies in this case these cell divisions may also be 'dedifferentiative' in effect. Whether the JH itself brings about the juvenilization associated with the cell divisions or whether it acts only upon 'de-

differentiated' cells rendered so by cell divisions is a moot point at this time. In either case we may postulate that the JH favours the larval stage but that its effectiveness is limited not by its mode of action, but by the state, or threshold of responsiveness, of the target cells. This threshold is, in this case, considerably lowered by cell divisions. If this threshold were to vary in different insects as it has been shown to vary in different parts of the same species (Kühn & Piepho, 1936) we would have a temporary rationale for the failure of some insects to exhibit reversal of metamorphosis in conditions which elicit the phenomenon in others.

Thus the picture which emerges draws on the viewpoints of both Williams and Wigglesworth. It is here suggested that in the ontogeny of the epidermal cell, as indeed in the ontogeny of cells generally, development to a state of 'higher' differentiation occurs progressively, each step depending on local and general conditions. Development in the reverse direction is akin to 'dedifferentiation' and intervening cell divisions may be obligatory (Fig. 5).

We may finally conclude that the experiments described above reveal more about the state of the epidermal cells than about the mode of action of the JH.

SUMMARY

1. As in *Rhodnius*, the larval *Oncopeltus* has bristles which are supplemented at each moult. However, at metamorphosis a dense population of non-innervated hairs develops.

2. Implantation of corpora allata into 5th-stage larvae showed that the development of these hairs can be inhibited universally or locally by the juvenile hormone (JH).

3. Transplantations of integument between 5th-stage larvae of different stages in the moult cycle gave some information about the power of the host to synchronize the graft to its own moult cycle.

4. Transplantations between different larval stages showed that the grafted integument responded to the hormonal milieu of the host.

5. Adult integument was transplanted onto larvae to study the reversal of metamorphosis. It was found that the development of a supernumerary population of hairs depended on the integument passing through a moult cycle in the presence of JH.

After two moults in the presence of JH, reversal of metamorphosis was found to vary over the surface of the transplant, being further advanced at the margin. At the edge of the graft properly formed larval bristles developed, while at the centre adult hairs were formed in adult cuticle. Intermediately formed bristles were found in the intervening areas. It is suggested that reactions associated with wounding are the cause of this heterogeneous result.

6. The significance of these results in relation to other work and to theories concerning the mode of action of the juvenile hormone is discussed.

This work was done while I was a recipient of an Agricultural Research Council Studentship. I am indebted to the whole of the Insect Physiology Unit at Cambridge and in particular to Professor Sir Vincent Wigglesworth, C.B.E., F.R.S. whose writings initiated my interest in this field, and who supervised the work, and to Dr A. D. Lees who spent much of his time discussing it with me. Una Smith kindly read the manuscript.

REFERENCES

BODENSTEIN, D. (1953). Studies on the humoral mechanism in growth and metamorphosis of the cockroach *Periplaneta americana*. I. Transplantations of integumental structures and experimental parabioses. *J. Exp. Zool.* **123**, 189–232.

GROBSTEIN, C. (1959) Differentiation of vertebrate cells. In *The Cell*, vol 1, edited by J. Brachet & A. E. Mirsky. New York and London (Academic Press)

HENKE, K. (1947). Einfache Grundvorgänge in der tierischen Entwicklung. I. Über Zellteilung, Wachstum und Formbildung in der Organentwicklung der Insekten. *Naturwissenschaften*, **34**, 149–57, 180–6.

KRISHNAKUMARAN, A. & SCHNEIDERMAN, H A. (1964). Developmental capacities of the cells of an adult moth. *J. Exp. Zool.* **157**, 293–305.

KÜHN, A. (1949). Wundheilung und Riesenzellenbildung bei *Ptychopoda seriata*. *Z. Naturf.* 4*b*, 104–8.

KÜHN, A. & PIEPHO, H. (1936). Über hormonale Wirkungen bei der Verpuppung der Schmetterlinge. *Ges. Wiss. Göttingen, Nachrichten Biol.* **2**, 141–54.

LAI-FOOK, J. (1965). Personal communication.

LIPP, C. (1959). Cytologische Untersuchungen zum Kompensationsprinzip nach HENKE am Flügel von *Ephestia kühniella* Z. *Biol. Zbl.* **78**, 1–21.

NOVÁK, V. J. A. (1951). The metamorphosis hormones and morphogenesis in *Oncopeltus fasciatus* Dall. *Mém. Soc. zool. tchécosl.* **15**, 1–47.

OZEKI, K. (1959). Secretion of molting hormone from the ventral glands. *Sci. Papers Coll. gen. Educ. Univ. Tokyo*, **9**, 256–62.

PIEPHO, H. (1938*a*). Wachstum und totale Metamorphose an Hautimplantaten bei der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **58**, 356–66.

PIEPHO, H. (1938*b*). Über die Auslösung der Raupenhäutung, Verpuppung und Imaginalentwicklung an Hautimplantaten von Schmetterlingen. *Biol. Zbl.* **58**, 481–95.

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

PIEPHO, H. (1939). Raupenhäutungen bereits verpuppter Hautstücke bei der Wachsmotte *Galleria mellonella* L. *Naturwissenschaften*, **27**, 301–2.

PIEPHO, H. (1947). Determinationsvorgänge in der Entwicklung der Schmetterlingshaut. *Nachr. Akad. Wiss. Göttingen Maths.-phys. Klasse*, 27–9.

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

PIEPHO, H. & HEIMS, A. (1952). Das Kutikulamuster der Schmetterlingslarve und die hormonale Grundlage seiner Entstehung. Untersuchungen an der Wachsmotte *Galleria mellonella* L. *Z. Naturf.* 7*b*, 231–7.

PIEPHO, H. & HOLZ, I. (1959). Verjüngung des Mitteldarms von Schmetterlingen. *Biol. Zbl.* **78**, 417–24.

PIEPHO, H. & MEYER, H. (1951). Reaktionen der Schmetterlingshaut auf Häutungshormone. *Biol. Zbl.* **70**, 252–60.

SCHNEIDERMAN, H. A. & GILBERT, L. I. (1958). Substances with juvenile hormone activity in Crustacea and invertebrates. *Biol. Bull., Woods Hole*, **115**, 530–5.

SMOLKA, H. (1958). Untersuchungen an Kleinorganen im Integument der Mehlmotte. *Biol. Zbl.* **77**, 437–78.

WIEDBRAUCK (MEYER), H. (1953). Wiederholung der Metamorphose von Schmetterlingshaut. Versuche an der Wachsmotte *Galleria mellonella* L. *Biol. Zbl.* **72**, 530–62.

WIGGLESWORTH, V. B. (1934). The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and 'metamorphosis'. *Quart. J. Micr. Sci.* **77**, 191–222.

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. (1937). Wound healing in an insect (*Rhodnius prolixus* Hemiptera). *J. Exp. Biol.* **14**, 364–81.

WIGGLESWORTH, V. B. (1939). Häutung bei Imagines von Wanzen. *Naturwissenschaften*, **27**, 301.

WIGGLESWORTH, V. B. (1940*a*). Local and general factors in the development of 'pattern' in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17**, 180–200.

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

WIGGLESWORTH, V. B. (1964). The hormonal regulation of growth and reproduction in insects. *Adv. Ins. Physiol.* **2**, 247–336.

WILLIAMS, C. M. (1956). Insect metamorphosis. *Proc. R. Ent. Soc. Lond.*, C, **21**, 20; 27–8.

WILLIAMS, C. M. (1961). Present status of the juvenile hormone. *Science*, **133**, 1370.

WILLIAMS, C. M. (1963). The juvenile hormone. III. Its accumulation and storage in the abdomen of certain male moths. *Biol. Bull., Woods Hole*, **124**, 355–67.