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DEVELOPMENT AND DETERMINATION OF HAIRS AND BRISTLES IN THE MILKWEED BUG, *ONCOPELTUS FASCIATUS* (LYGAEIDAE, HEMIPTERA)

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SUMMARY

The term 'organule' is proposed as an English equivalent for the German word 'Kleinorgan'. The different organules on the third sternite of *Oncopeltus* are described: larvae possess innervated bristles and special sensilla termed 'chemosensilla', whereas the adult develops, in addition, a dense population of non-innervated hairs. The hairs, bristles and 'chemosensilla' each develop from mother cells which undergo a particular series of differentiative divisions. The course of events is described for each type of organule.

Electron-microscopic studies of the fine structure of the outgrowing hairs are described. As in the outgrowth of a scale, longitudinal arrays of microtubules and bundles of fibres are found in the first cytoplasmic process of the trichogen cell. Experiments show that bristle determination occurs between the onset of the moult and the proliferative cell divisions in the epidermis. The period of hair determination is found to occur later than that of the bristles and to be later than the proliferative cell divisions in the last larval stage.

A discussion of the results includes a review of the knowledge of differentiative divisions concerned in the formation of organules in other groups of insects, and a consideration of Wigglesworth's theory of bristle determination.

INTRODUCTION

The origin of the cells which form the scales of Lepidoptera has long been a subject of detailed study, and more recently the development of the cells which form the hairs in a caddis-fly, *Limnophilus*, the scales in the silverfish, *Lepisma* and the bristles in the blowfly *Calliphora*, have been examined. Within the Hemiptera, although the four cells which form a bristle of *Rhodnius* have been assumed to arise by division of a single mother cell (Wigglesworth, 1953), the course of the presumed differentiative divisions remains unknown. The sternites of the adult *Oncopeltus* are covered with a dense mat of non-innervated hairs, whilst the larvae bear more sparsely distributed, innervated bristles. In the present study the development of the hairs has been followed in detail, and an attempt made to analyse the ontogeny of the more widely spaced bristles. For the sake of simplicity, the description deals only with the third abdominal sternite.

The German word Kleinorgan (Henke, 1953) which is applied to small integumental structures such as scales, bristles, hairs, chemosensilla, campaniform sensilla,

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dermal glands, oenocytes and the like, has no equivalent in English and there is a clear need for a comparable term. Accordingly, the term *organule* is proposed. This term will cover all associations of small numbers of integumental cells which perform some specific function different from that of the general population of epidermal cells. All organules so far studied arise by differentiative divisions from epidermal cells.

In the description that follows, and for the rest of this account, the term *bristle* is used to designate an innervated trichoid sensillum, and the term *hair* to designate a non-innervated, but similar, organule.

MATERIALS AND METHODS

The milkweed bugs were kept in a closed incubator at 29·5 ± 0·5 °C, fed with milkweed seed, and provided with water. Under these conditions the 5th larval stage lasts 150 h. Insects were selected at ecdysis and kept for appropriate periods before fixation. As the transparent nature of the cuticle allows detailed microscopical study of the attached epidermis, whole mounts of the cuticle were used for routine examination. The integument was separated from the viscera and fat body and, after fixation for 1 h in Carnoy's fluid, was hydrated and stained for 1 min in Hansen's trioxyhaematein. Silver staining of the nervous system was performed according to Romanes's method (1950).

For electron microscopy the integument was usually fixed in 2·5% glutaraldehyde in phosphate buffer, washed in buffer, and post-fixed with 1% osmium tetroxide in veronal-acetate buffer at pH 7·5. Some material was fixed in osmium tetroxide alone. Sucrose and calcium chloride were added to the fixatives to produce final concentrations of 0·23 M and 8 mM, respectively. The material was dehydrated, embedded in Araldite, and sectioned on a Huxley microtome. Optimal staining was achieved by immersing the sections for 30 min in a saturated solution of uranyl acetate in 50% ethanol, followed by treatment for 3 min in lead citrate (Reynolds, 1963). The stained sections were examined in a Philips EM 200 electron microscope. Whole mounts of integument were stained in Feulgen, and the DNA content of nuclei estimated using the microspectrophotometer designed by Mendelsohn (1957) and at present located in the Department of Radiotherapeutics, University of Cambridge (for a test of the validity of this method see Mendelsohn & Richards, 1958).

RESULTS

General description

Apart from some very large bristles and occasional campaniform sensilla (each comprising four cells)—with which this paper will not be concerned—the third sternite of larvae of *Oncopeltus* bears more or less evenly distributed bristles. These are standard trichoid sensilla (Hsü, 1938) comprising four cells: the trichogen, which forms the bristle shaft itself, the tormogen, which secretes the cuticular socket, the sense cell, which has both a peripheral prolongation to the base of the bristle and an axon leading to the central nervous system, and a neurilemma cell (Figs. 1A, 12).

Electron microscopy has confirmed that the neurilemma cell forms a typical tunicated sheath around both the axon and the peripheral prolongation of the sense cell. Adjacent bristles are separated by about 10 epidermal cells.

There are also some 8-celled organules which, in addition to the tormogen, trichogen and neurilemma cells, contain 4 sense cells and one other cell (Fig. 1 B). Peters (1965) described a fifth sense cell in marginal bristles of *Calliphora*, which was probably

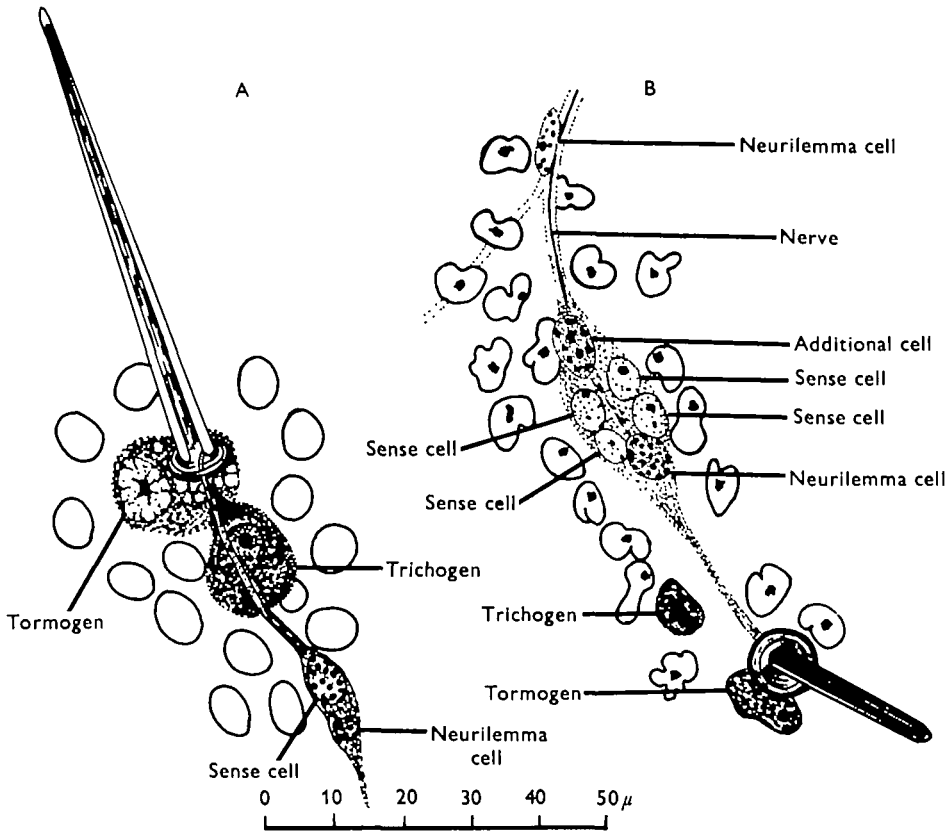

Fig. 1. Drawings of a bristle (A) and a chemosensillum (B). The chemosensillum was stained with silver, and the bristle in Hansen's trioxyhaematein.

mechanical in function, and it seems likely that the additional cell is homologous with Peters's fifth sense cell. These organules may respond to chemical stimuli, and they will be referred to as *chemosensilla*.

As the larvae grow at each moult, the number of bristles and chemosensilla increases. Just as in *Rhodnius* (Wigglesworth, 1933, 1940), and in *Galleria* (Krumiņš, 1952), all the cuticular structures associated with the organules extant at any one larval stage are reformed in the succeeding stage by the same cells. The third sternite of adults differs considerably from that of larvae, in that it bears a population of hairs which are so densely packed, in some areas, that there is a ratio of one hair to every 3 or 4 epidermal cells. In the older adult each of these cuticular hairs has only a single epidermal cell associated with it.

It has been mentioned previously that in a larval moult all the bristles are reformed. In the development of the adult, however, some of the ventral bristles, and most of the tergal ones, are lost. Such a reduction of bristles also occurs at metamorphosis in *Rhodnius*. Owing to the difficulty of distinguishing between bristles and hairs in the adult, it remains uncertain whether new bristles develop during the 5th stage. Differentiative divisions and small groups of cells similar to those known to be early stages in the genesis of bristles have, however, been seen in the 5th stage; and it is probable, but not certain, that some new bristles do develop during the last stage. Chemosensilla certainly develop during the 5th stage.

Stossberg (1938) noted that for each type of scale of *Ephestia* there is a constant ratio between scale length and socket diameter. Similar observations on the bristles of *Oncopeltus* have shown that the adult and larval bristles differ in this ratio (Lawrence, 1966*a*), due to the proportionately larger sockets in the larval bristles, but no other differences were evident. The adult hairs, however, do not show any precise relationship between the shaft length and socket diameter; and although the socket tends to be small in this organule, bristles and hairs must be distinguished on the basis of presence or absence of innervation.

Differentiative divisions

Adult hairs. During the 5th larval stage the proliferative cell divisions of the epidermal cells begin approximately 30 h after ecdysis, and decline in frequency after 65 h, when the first differentiative divisions of hair initials can be seen. The mother cell (which, before division, appears to be identical with other epidermal cells) swells slightly and bulges inwards and over the adjacent epidermal cells (Fig. 2A). As the mother cell reaches prophase of mitosis, the cuticular surface of the surrounding epidermal cells appears to encroach slightly on the cuticular territory of the mother cell. The mother cell then divides, in a more or less horizontal plane (parallel to the

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Fig. 2. *The development of hairs, I.* Drawn with a camera lucida, all from the same individual.

A. The hair mother cell (*hmI*) swells and begins to overlap the epidermal cells. The epidermis of *Oncopeltus* is peculiar in that an extra layer of large vacuolated cells intervenes between the epidermal cells themselves and the basement membrane. These cells, quite unlike oenocytes, are termed *basal cells* (*bc*). A nucleus of one of these cells is drawn. Unlabelled nuclei are those of epidermal cells.

B. The first division; the mitotic spindle is oriented parallel to the epidermal surface.

C. The two daughter cells.

D. Two separate stages: (i) on the left, the accessory mother cell (*am*) is in metaphase of a vertical division and the hair mother cell II (*hmII*) is in early prophase; (ii) on the right, the hair mother cell is in horizontal metaphase, and the accessory mother cell is in telophase.

E. The two accessory cells (a_1, a_2). The smaller cell (a_2) is distal from the cuticle. The hair mother cell is in late anaphase.

F. The 4 resultant cells: tormogen (*to*), trichogen (*tr*) and the two accessory cells (a_1, a_2).

(a_1, accessory cell 1; a_2, accessory cell 2; *am*, accessory mother cell; *bc*, basal cell; *hmI*, hair mother cell I; *hmII*, hair mother cell II; *to*, tormogen cell; *tr*, trichogen cell.)

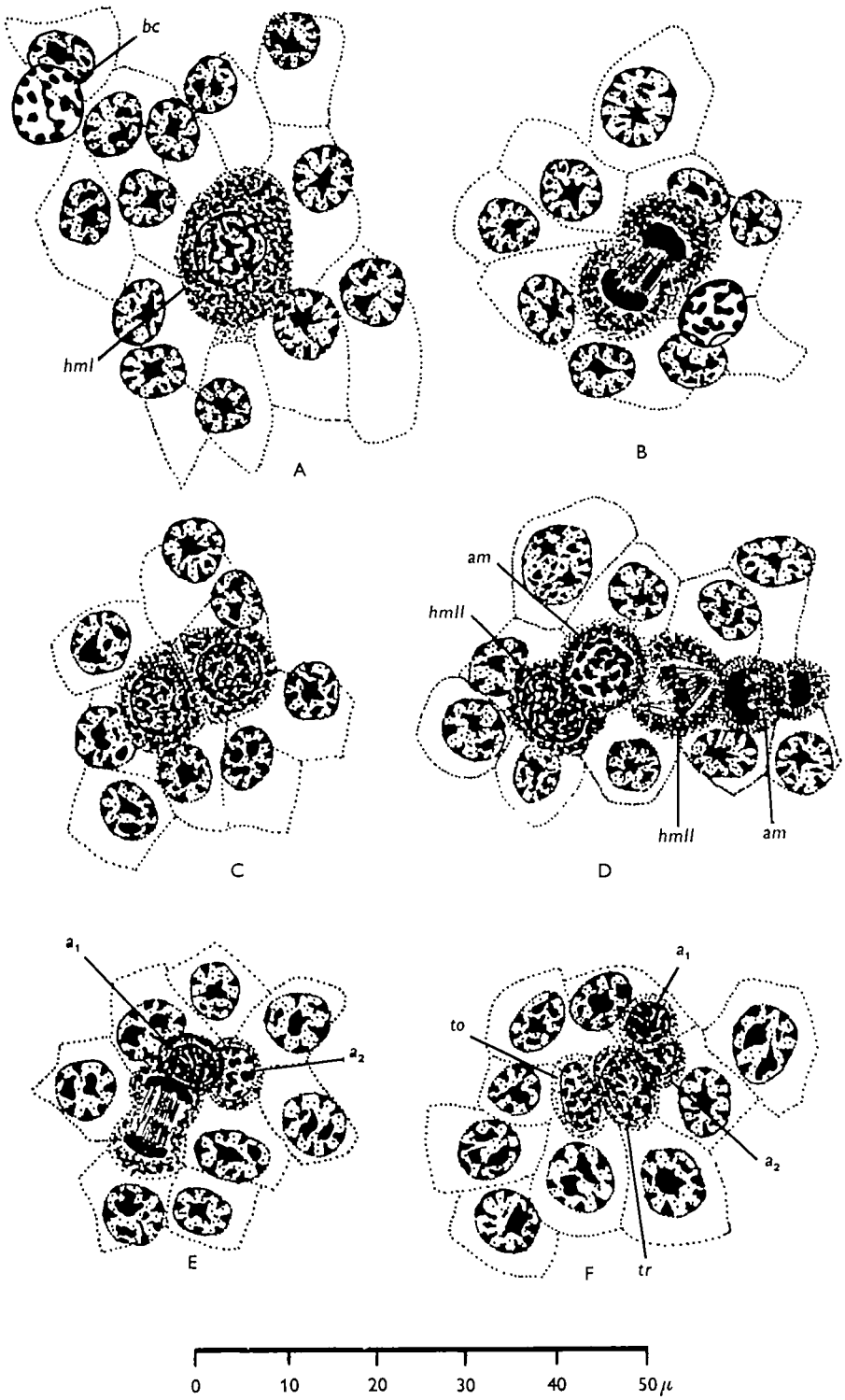

Fig. 2. For legend see facing page.

surface of the cuticle), and only one of the resulting two daughter cells maintains any cuticular territory. After a short interphase, the other daughter cell starts to divide, this time in the vertical plane (normal to the surface of the cuticle). The division is unequal, the cell nearer the cuticle receiving the greater amount of cytoplasm. The resulting two daughter cells will be referred to as the *accessory cells*. The vertical orientation of this division is merely temporary, as the daughter cells soon tilt. This movement may result from crowding between the cuticle and the basement membrane. (Richter (1962) chose to explain the similar situation in *Lepisma* in this way.) As this division reaches telophase, one of the cells (*hmII* in Fig. 2D) enters prophase of a horizontal division, which produces the tormogen and trichogen cells (Fig. 2F). Thus, four cells result, only one of which (the tormogen) maintains cuticular territory (for diagrammatic summary of this and other cell lineages see Fig. 9). A few hours after this 4-celled stage, the accessory cell nearest the cuticle degenerates to a chromatic droplet; the more permanent 3-celled stage is thus reached (Fig. 6H). Electron micrographs indicate that the nucleo-cytoplasmic ratio of these cells is high. The cytoplasm is rather featureless.

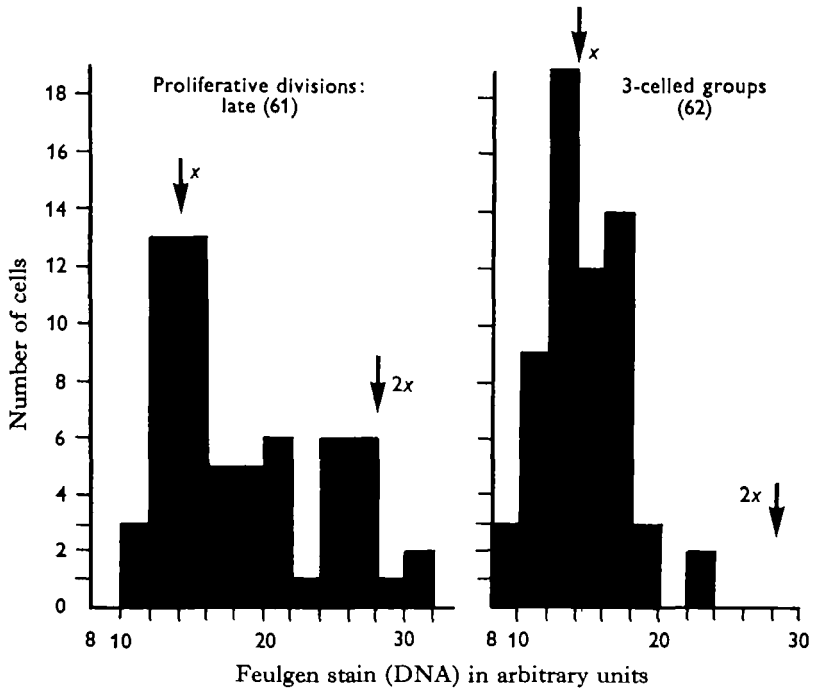

Fig. 3. *DNA values for epidermal cell nuclei at two stages of development.* The arrows indicate the peaks for unduplicated (x) and duplicated amounts of DNA ($2x$). Note that the histogram of the 3-celled groups has a single peak at the unduplicated level. Figures in parentheses denote number of nuclei examined.

These divisions occur asynchronously over the epidermis, and the various stages already described can be found occurring simultaneously in one specimen. For a time the epidermis includes such a multitude of differentiative divisions that in some regions there may be more hair initial cells than epidermal cells. Development from mother

cell to 3-celled stage is estimated to require about 12 h. As the insect ages, the proportion of 3-celled stages out of all cell groups rises to 100%, and during this time the cell groups become aligned in the antero-posterior axis. This observation raises questions discussed in a separate paper (Lawrence, 1966*b*).

Measurements of the Feulgen stain in nuclei of epidermal and hair-initial cells from an individual fixed at the 3-celled stage showed one pronounced peak when plotted as a distribution histogram. This peak coincided with the unduplicated peak measured from a specimen fixed during the proliferative cell divisions (Fig. 3). Thus there would seem to be neither polyploidy nor polyteny, nor does any substantial reduction in chromatin seem to be involved in the formation of hair initial cells in *Oncopeltus*.

Larval bristles. Soon after proliferative cell divisions begin at 25 h in 4th-stage larvae the first differentiative divisions of the bristle mother cells can be seen. Occasionally these can be positively distinguished from proliferative divisions because one division occurs in the vertical plane, and such mitotic figures are conspicuous in metaphase (Fig. 4C). Fig. 4 shows stages in the development of the bristle initial cell. It is evident that until the 4-celled stage is attained, divisions proceed just as they do during hair development. However, these 4-celled groups occasionally have been seen to include a further division (Fig. 4H suggests that the dividing cell is equivalent to the accessory cell nearest the cuticle), and indeed for a period most groups comprise 5 cells (Fig. 4I, J). The source of this fifth cell has not been conclusively determined, but it could arise by this last division, which seems its most probable source. However, there remains the possibility that the fifth cell is a neurilemma cell which wandered in along the axon from the central nervous system, and, indeed, neurilemma cells of *Oncopeltus* occur scattered along the axons within the epidermis (Fig. 1B). Moreover, Kim (1961) has demonstrated such migrations in the developing labial palps of *Pieris*. However, the chromatic droplets sometimes found near 4-celled stages may possibly result from breakdown of the fifth cell. In any case, the final result is that all groups comprise 4 cells. The differentiative divisions do not occur synchronously, but by 40 h in the 4th-stage larva all such divisions have terminated, and groups of 4 or 5 nuclei are scattered amongst the epidermal cells. The axons from both the chemosensilla and these bristles grow out from the nerve cells at about 50 h in the 4th-stage larva; at this stage all groups comprise 4 cells, which then survive throughout the life of the bristle.

The chemosensilla. These organules are easily distinguishable from the others because of their complement of 8 cells and the short, stubby bristle. There is only a small number of chemosensilla on the third sternite, but in a newly emerged 5th-stage larva about 15 sensilla can be seen on the anterior margin of the 5th abdominal sternite, whereas in the adult there are at least 50 in the corresponding region. Thus, this area is rich in differentiating cell groups in the 5th-stage larva, and it has therefore been studied in an attempt to follow the cell lineage.

From the first few hours divisions are present in the epidermis of the anterior margin of the fifth abdominal sternite; mitosis has been observed as early as 4 h after ecdysis. By 20 h in the 5th stage, the initials of the new chemosensilla can be clearly seen to comprise groups of 4–8 small nuclei; the smaller groups still include mitotic

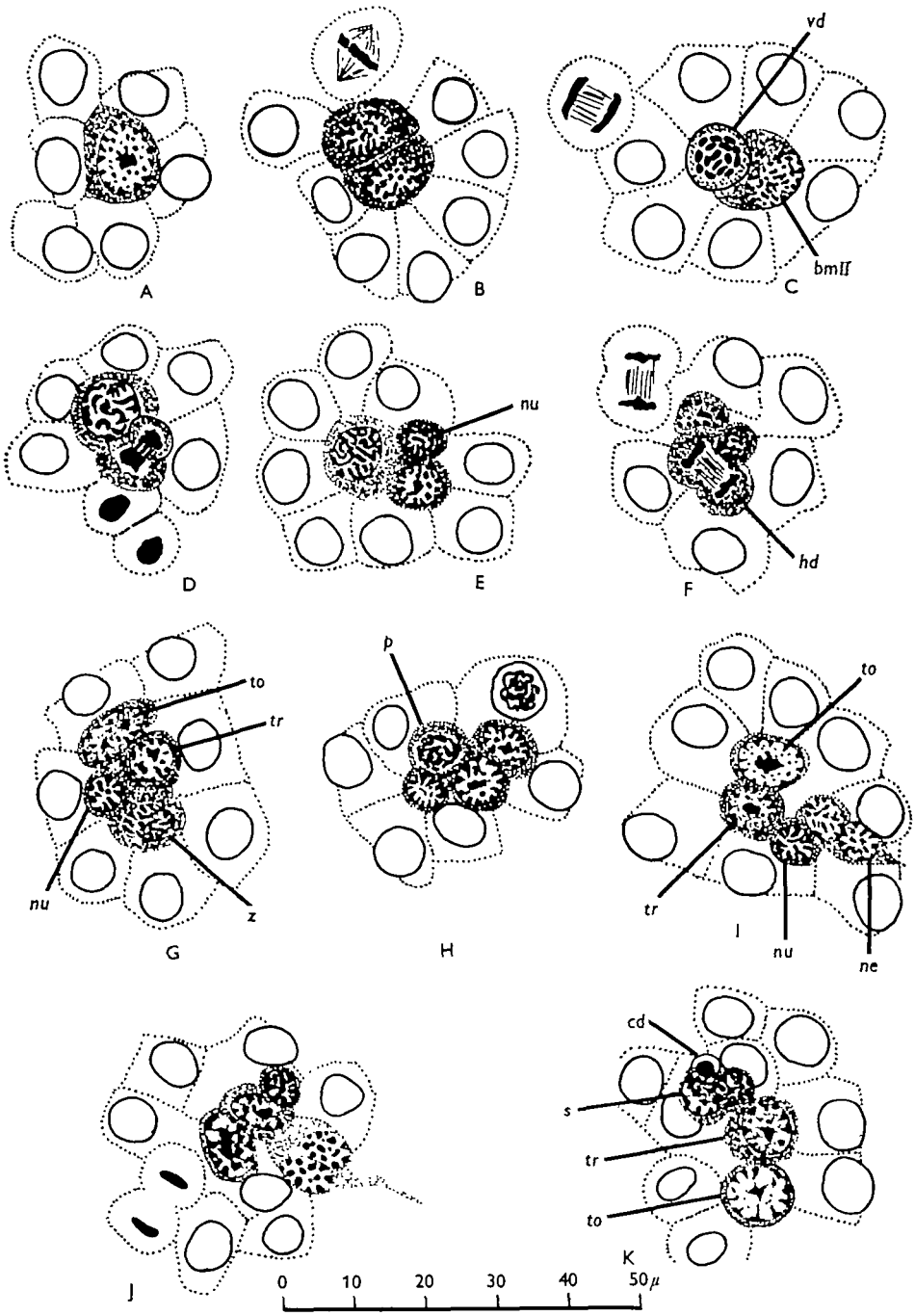

Fig. 4. *Development of bristles.* Drawn with a camera lucida from 4th-stage larvae of 26 h (A, B), 33 h (C–G), and 46 h (H–K). The development is very similar to that of the hairs, and includes a vertical division (*vd* in C). The bristle mother cell II divides in the horizontal plane (*hd* in F) to form the trichogen (*tr*) and the tormogen (*to*). The cell equivalent to the accessory cell 1 is the neurilemma cell (*nu*). H shows the cell equivalent to accessory cell 2 (*z* in G) in prophase (*p*) and I shows the 5 cells which appear to result. In both I and J the two presumed daughter cells of this division are associated with the outgrowing nerves (*ne*). K shows the chromatic droplet (*cd*) which probably arises from breakdown of 1 of these 2 daughter cells, leaving 4 cells; tormogen (*to*), trichogen (*tr*), sense (*s*) and neurilemma cell. (*bmII*, bristle mother cell II; *cd*, chromatic droplet; *hd*, horizontal division; *ne*, nerve; *nu*, neurilemma cell; *p*, prophase; *s*, sense cell; *to*, tormogen; *tr*, trichogen; *vd*, vertical division; *z*, cell equivalent to accessory cell 2.)

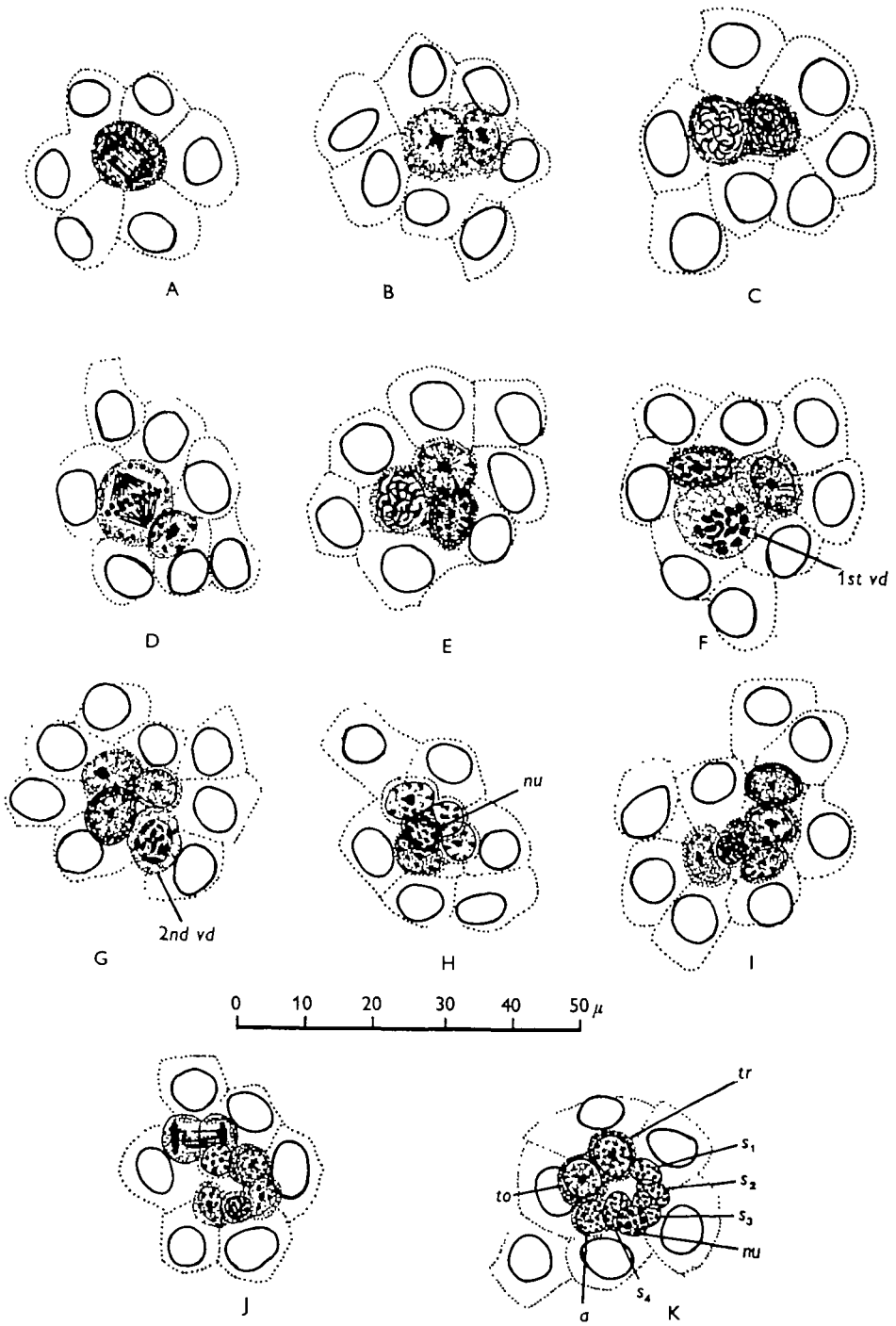

Fig. 5. *Development of the chemosensilla.* Drawn with a camera lucida from the fifth sternites of young 5th-stage larvae aged 4·5 h (A), 7 h (B), 25 h (C–J) and 56 h (K). These sketches show the probable sequence in the formation of the 8 cells comprising a chemosensillum. A mother cell, originally similar to an epidermal cell, divides in the horizontal plane (A) to produce 2 daughter cells (B). One of these daughter cells then undergoes a horizontal division (C, D) and the other a vertical division (*1st vd* in F). Four cells result. One of these probably divides again (*2nd vd* in G) to produce 5 cells. One of these cells, which is situated above the others (*nu* in H), does not seem to divide again and eventually becomes a neurilemma cell. The second vertical division is followed by three more cell divisions, which are probably all oriented horizontally in the epidermis (J) and produce 4 sense cells (s_1–s_4). Eight cells finally result, the nuclei of which are sufficiently distinct for each to be identified (K). (*a*, additional cell; *nu*, neurilemma cell; *to*, tormogen cell; *tr*, trichogen; s_1–s_4, 4 sense cells.)

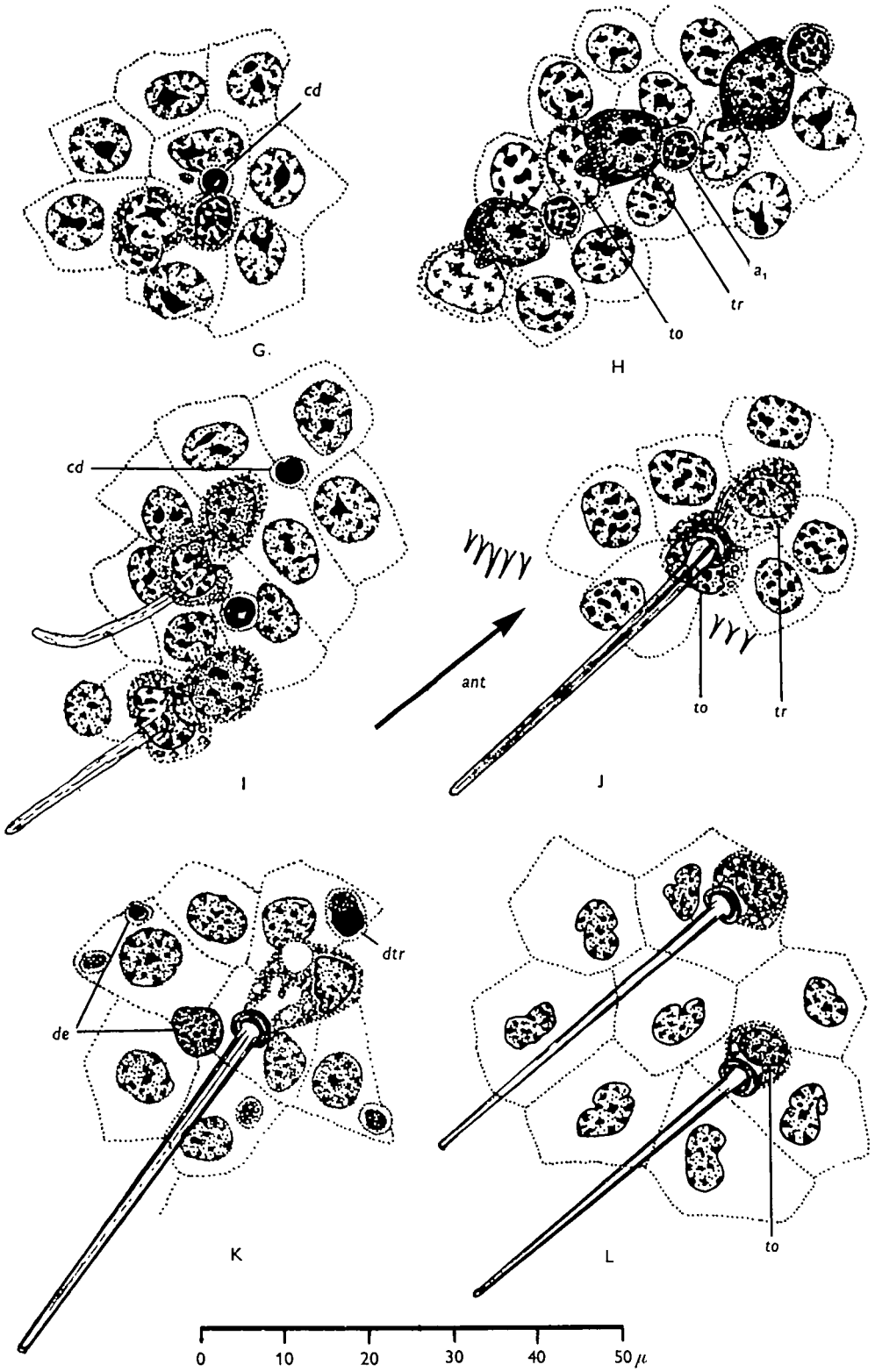

Fig. 6. For legend see facing page.

figures (Fig. 5 D, F, J), and soon all cell groups comprise 8 cells. These 8-celled groups remain dormant until the processes of the axons appear at about 50 h. The detailed procession of these differentiative divisions has not been determined, but the general sequence of events is represented in Fig. 5. As with the other organules, chemosensilla do not all develop synchronously; cell groups in different stages of development exist simultaneously.

Later development

Hairs. About 10 h after all differentiative divisions have ceased, the hair outgrowth begins as a small process of the trichogen cell (Fig. 6 H). This hair rudiment does not pierce the cytoplasm of the overlying tormogen cell, but is embraced by it as if by a collar (Lees & Waddington, 1942; Noble-Nesbitt, 1963). As seen in electron micrographs the outline of the hair outgrowth is ragged; initially both its contents and the main mass of the trichogen cell are electron-dense. In material fixed in glutaraldehyde the rudiment is evidently packed with longitudinally oriented microtubules. The cuticulin layer (the term is used in the restricted sense of Locke, 1961) develops at about this time, at first as short duplex structures (*cu* in Figs. 10, 15), which later extend and meet. The remnant accessory cells degenerate following this original phase of outgrowth and form chromatic droplets, which soon completely disappear (Fig. 6 J).

Later, the situation differs strikingly (Figs. 10, 11): trichogen cells enlarge and become much less electron-dense; the hair outgrowths extend, increase in diameter, and display a smooth boundary. Microtubules (about 200 Å in diameter) are more distinct, lying in dense array (*mt*, Figs. 10, 11), and about 20 longitudinal bundles of fine fibrils lie under the surface membrane, arranged around the circumference (Fig. 11). Some sections reveal that these fibrils are straight and lie parallel to one another; they are about 30 Å thick, and their centre-to-centre spacing is 80 Å (Fig. 14). Other sections reveal a cross-striation normal to the long axis of the bundle, with a periodicity of approximately 120 Å. These different appearances suggest that the fibrils may be beaded structures aligned in phase.

The homogeneous layer of the epicuticle is deposited over epidermal cells and hairs

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Fig. 6. *Development of hairs, II.*

G. Accessory cell 2 has degenerated to form a chromatic droplet (*cd*), thus leaving 3 cells. Drawn from 5th stage, 78 h.

H. The 3-celled groups are aligned in the antero-posterior axis (marked by arrow, *ant*) and the outgrowth of the trichogen cell has begun. Drawn from 5th stage, 90 h. (a_1, accessory cell 1; *to*, tormogen; *tr*, trichogen.)

I. At about half the final length the accessory cell 1 degenerates to form a chromatic droplet (*cd*). Drawn from 5th stage, 92 h.

J. Hairs are fully formed and their sockets secreted prior to ecdysis at 150 h. Drawn from 5th stage, 140 h. (*to*, tormogen; *tr*, trichogen.)

K. At about 8 h after the final ecdysis the trichogen cell degenerates (*dtr*). At this time the density of the epidermal cells is falling due to degeneration of many epidermal cells (*de*). Drawn from adult, 8 h.

L. The mature adult; each hair has only one associated cell (tormogen, *to*), the epidermal cells having become widely spaced. Drawn from adult, 40 h. (a_1, accessory cell 1; *ant*, antero-posterior axis; *cd*, chromatic droplet; *de*, degenerating epidermal cell; *dtr*, degenerating trichogen; *to*, tormogen; *tr*, trichogen.)

alike, the cuticulin layer becoming visibly continuous and the epidermal surface folded. The hair also develops longitudinal ridges at this time, and the tormogen cell secretes a large quantity of cuticular material to form the socket. Thus, as in *Drosophila* (Lees & Waddington, 1942), the socket is not secreted until the hair is almost full length. At this time the cytoplasm of the tormogen cells appears to be very active and possesses many granular vesicles; these resemble lysosomes and may well derive from the recently degenerated accessory cell. The Golgi apparatus is conspicuous at this time. At about 130 h the cytoplasm becomes strongly basophilic; and soon afterwards, the bristle outgrowth, now consisting mainly of cuticle, becomes amber-coloured as tanning proceeds. The cytoplasm of the hair-forming cells begins to regress and the membranes between tormogen and trichogen cells become deeply convoluted after ecdysis, the cytoplasm of the trichogen cells degenerating and eventually disappearing. A large vacuole remains, which joins the lumen of the hair and is surrounded by the microvillous membrane of the tormogen cell. The nucleus of the trichogen cell forms a chromatic droplet about 8 h after ecdysis (Fig. 6K). Finally, in the older adult, the cytoplasm of both the tormogen and epidermal cells shrinks and only one cell, the tormogen cell, remains per hair (Fig. 6L).

Adult bristles. Outgrowth and cuticularization of adult innervated bristles (and also, presumably, of larval bristles, which have not been studied with the electron microscope) are almost identical to that of the hairs, except for complications arising from the innervation of the bristles (Fig. 12). When the moulting fluid forms, a resistant continuation of the nerve fibre extends through it and innervates the base of the old bristle. Feuerborn (1927) proposed that this process maintains the sensory input from the old bristle when, at the time moulting fluid is produced, direct cytoplasmic contact between the bristle and the epidermis is relinquished. Vogel (1923) suggested that the small granule seen with the light microscope at the base of the bristle was a centriole-like structure, and later electron-microscopic observations (Gray & Pumphrey, 1958) have confirmed his suggestion. Fig. 13 shows this structure in the bristle of *Oncopeltus*.

Measurements of Feulgen-staining of the nuclei of bristle initial cells suggest that, unlike the situation in Lepidoptera and *Calliphora* (Peters, 1965), the DNA content is not increased above the normal amount, even in those cells which are to form exceptionally large bristles.

Determination of bristles and hairs

Larval bristles. The size of individuals in the 5th stage varies considerably. The density of bristles on the third abdominal sternite of specimens of different size is more constant than the number or position of such bristles. This suggests that some regulative capacity which is sensitive to density functions in the epidermis.

In 1940 Wigglesworth suggested the following model to explain this phenomenon: a substance required for the differentiation and growth of bristles is distributed homogeneously throughout the epidermal cells; extant bristles absorb this growth substance and thereby inhibit the differentiation of new bristles in their vicinity. The epidermal cells intervening between bristles respond to stretching at each moult by proliferating; and the resulting cells, now more widely separated from neighbouring bristles, are

freed from the inhibition. All these cells would tend to become bristles, but one cell becomes determined and at once absorbs the growth substance from the adjacent cells.

Wigglesworth's experiments showed that in *Rhodnius* stretching the 3rd-stage larva increased the number of proliferative cell divisions during that stage. However, the 4th-stage larva displayed no increase in the number of bristles, and extra bristles were not visible until the 5th stage. This observation suggested that the bristles which developed during the 3rd larval stage and became visible in the 4th stage had been determined prior to the proliferative cell divisions in the 3rd stage. The stretching was therefore too late to influence this determination. However, the increased cell population affected determination during the 4th stage so that the extra bristles could be counted on the 5th-stage larva. Wigglesworth further observed that determination of

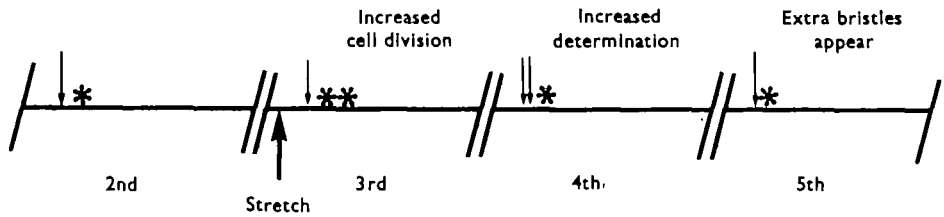

Fig. 7. Diagram showing the effect of Wigglesworth's experiment on the determination of bristles in *Rhodnius*. (Small arrows mark time of determination, asterisks mark cell division.)

bristles in a particular larval stage could be influenced by the specific hormonal milieu of that stage, thus eliminating the possibility that the bristles developing during a particular larval stage are determined during the *preceding* stage. These two observations limited the possible period during which determination occurs to the time elapsing between the initiation of moulting and shortly after the beginning of cell divisions (Fig. 7).

A comparable study has been made of *Oncopeltus*. Novak (1951) observed that nymphs of *Oncopeltus*, when starved but watered, became cannibalistic and ate little by volume, so that nymphs surviving to the next moult were in many cases abnormally small. Normal 4th-stage larvae were therefore starved so as to produce small 5th-stage larvae; these were found to have a typical number of organules, although the number of epidermal cells per organule was abnormally low (see Table 1). When 3rd-stage larvae were so treated and 4th stages fed normally, the 5th-stage larvae bore considerably fewer bristles, although they were larger and had more cells per organule than the 5th stages in the previous group.

A 6th-stage larva produced by the implantation of a corpus allatum from an adult female cockroach was examined for bristle number and compared with its 5th-stage progenitor; many extra bristles were formed in this supernumerary moult. Professor Wigglesworth kindly gave the author some 6th-stage larvae of *Rhodnius*, which also showed an increase in number of bristles compared to their respective exuviae.

Adult hairs. The larval bristles are determined before proliferative cell divisions occur. Were the adult hairs determined at an equivalent time in the moult, we would

Table 1. *Organules on the central part of the third sternite*

Condition of bug	Stage	Number of organules	Total area (mm^2)	Cells/ organule
Normal	4th	73 (4)	0·53 (4)	66 (2)
Normal	5th	122 (11)	0·96 (11)	81 (7)
Starved at 4th stage	5th	128 (3)	0·59 (3)	53 (2)
Starved at 3rd stage, fed at 4th	5th	102 (3)	0·76 (3)	72 (3)
Starved throughout development	5th	84 (3)	0·49 (3)	64 (3)
Experimentally produced	6th	147 (1)	1·24 (1)	109 (1)

Figures in parentheses represent the number of individuals in each class.

not expect the number of hairs in the adult to vary with the amount of growth occurring within the last stage. There would be a variation of hair density. However, when 5th-stage larvae of normal size were reared together without food, the adults were small and had a reduced number of hairs. In fact, among adults of different size, whatever their past history, the average density of hairs in a particular region (the central part of the 3rd abdominal sternite) was approximately equal (Fig. 8). It would therefore appear that determination of adult hairs occurs after the proliferative cell divisions in the 5th stage.

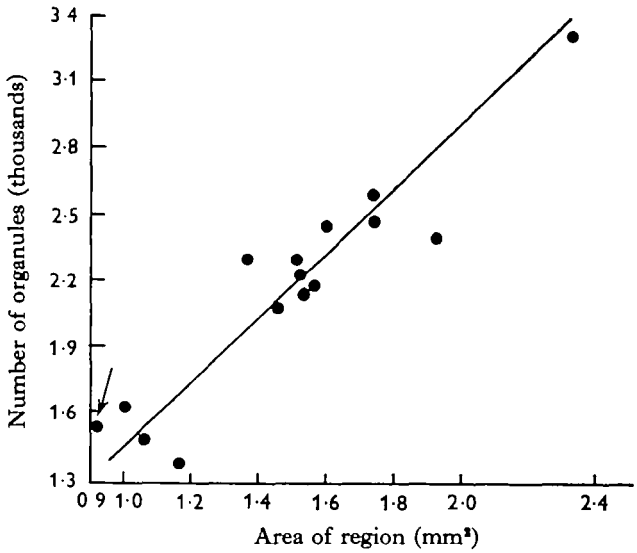

Fig. 8. The relationship between hair number and the area of the central portion of the third abdominal sternite in adult males. The density of organules on the third abdominal sternite is highly constant in spite of differences in size and past history. The arrow marks a 5th-stage larva of normal size, which produced an exceptionally small adult when starved.

Over the sternal surface there is a much wider range in the density of hairs (1–22 per oil-immersion field) than in the density of the larval bristles. The presumptive capacity to form a high or low density of adult hairs (as with other cell characteristics) is passed to progeny cells. Wigglesworth (1940) showed that the number of dense marginal bristles in *Rhodnius* could be increased substantially if wounding caused those cells which were their presumptive progenitors to divide and migrate. Similarly, in *Oncopeltus* the absolute number of hairs could be increased by enlarging the area populated by cells from a region with a high presumptive hair density. When the central part of the second and fourth sternites in the 3rd-stage larva was burned, the central area of the third sternite expanded considerably as the cells migrated and divided, healing the wound. Although then much larger, this area in adults bore hairs of normal density.

The adult bristles fit into the pattern of the adult hairs; each of the adult bristles has a small bald patch encircling it which is larger than that encircling an individual hair.

DISCUSSION

Differentiative divisions

Semper (1857) was the first worker to show that hairs and scales grow from formative cells, which, although different from the epidermis, were nonetheless part of it. Schäffer (1889) further clarified, and Mayer (1896) confirmed, the epidermal origin of scales.

Freiling (1909), studying Lepidoptera, described the cellular complement of a sensory bristle as comprising four cells. Hsü (1938) confirmed Freiling's observation and described two 'building' cells, one of which secreted the bristle shaft (trichogen cell) and the other the socket (subsequently termed 'the tormogen cell' by Wigglesworth). There were, of course, many exceptions to this generalization, and in other instances it was found that (apart from the nerve and neurilemma cells) there were 3 cells which formed chitin; the additional cell, of undetermined function, may well be referred to as the 'neighbour' cell (from 'Nebenzelle', see Barth, 1948). Such neighbour cells are found in the bristles of the larvae of the scorpion-fly, *Panorpa* (Schwink, 1951) and the scales of the peacock butterfly, *Vanessa* (Lipp, 1957). Von Schuckmann (1909) noted that vertical and horizontal divisions were associated with genesis of scale-forming cells in *Vanessa*, but to Köhler (1932) we owe the observation that those cells which constitute organules arise by means of a series of differentiative divisions from a mother cell which itself develops from an apparently typical epidermal cell. His description of this process was amplified by Stossberg (1937, 1938) and Süffert (1937), who respectively gave detailed descriptions of the series of differentiative divisions involved in the development of scales in *Ephestia* and *Araschnia*. The scheme is represented diagrammatically in Fig. 9.

These observations have been repeated within the Lepidoptera and confirmed by Lipp (1957) in *Pieris*. Identical schemes of differentiative divisions have also been described for the hairs of *Limnophilus* (Rönsch, 1954). Even in the development of the

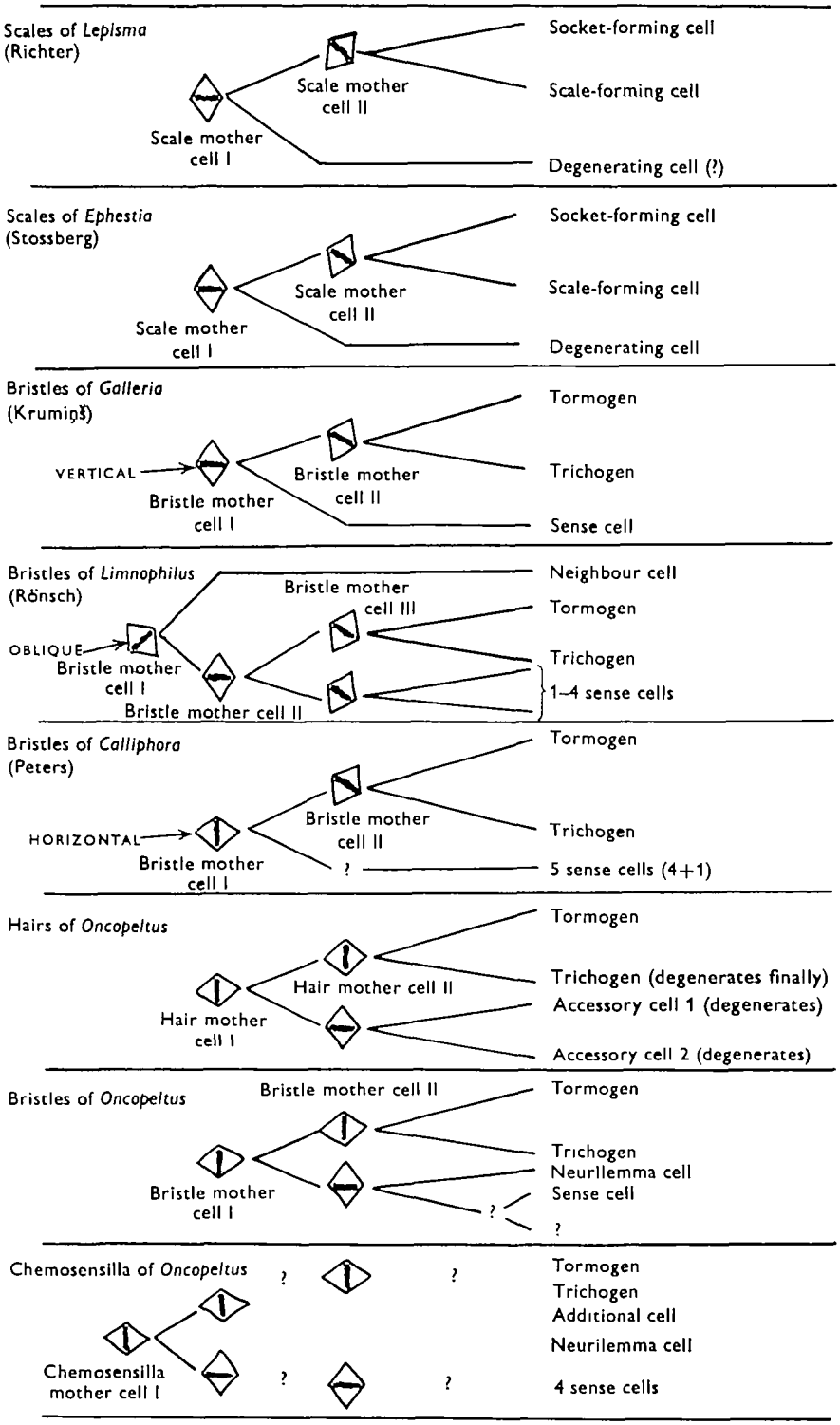

Fig. 9 Summary of the cell lineages of different organules in the insects (after Peters, 1965).

scales of the silverfish, *Lepisma* (Richter, 1962), the same process occurs, the only differences being that the second division has a more variably oblique orientation and that the fate of the 'degenerating' cell has not been fully clarified. An almost identical sequence has been reported for the development of dermal glands in *Bombyx* (Stabenau, 1952), but in this case two successive vertical divisions are involved. Little more is known about the differentiative divisions concerned in the formation of any other non-innervated organules.

The sensory bristles of *Galleria* comprise three cells: a trichogen, a tormogen, and a single sense cell. Krumiņš (1952) studied the development of these bristles and found that the cell lineage was essentially similar to that of scales in Lepidoptera, except that the degenerating cell of the latter survived as a sense cell (Fig. 9). Many authors have suggested that when the organule investigated comprises more than 3 cells, the additional cells develop by means of extra differentiative divisions. Indeed, Rönsch (1954) discovered that the neighbour cell found in the bristles of *Limnophilus* develops from a further differentiative division which is interpolated before the first vertical division (Fig. 9).

In a study of the sensilla on the wing of *Ephestia*, Clever (1960) investigated 3 types of organule: the campaniform sensilla (Sinneskuppeln), sensory scales, and sensory bristles. The campaniform sensilla comprised 4 cells, just as in *Rhodnius* (Wigglesworth, 1959) and *Oncopeltus*, these being the two cells which formed the cuticle and the nerve and neurilemma cells. The sensory scales comprised 5 cells: scale and socket cells; the neurilemma (linsenförmige Zelle); and two similar sense cells, one of which (nervöse Zelle) was more distal from the scale than the other. In the sensory bristles there are 4 sense cells: a neurilemma, polyploid trichogen and tormogen, and a third polyploid cell termed a 'kappenförmige Zelle'. Clever observed vertical and oblique divisions, which allowed him to suggest that the development of the sensory bristles in *Ephestia* was similar to that in *Limnophilus*. Clever further proposed that in *Ephestia* the neighbour cell in Rönsch's scheme divided to produce the kappenförmige Zelle and the neurilemma cell.

Clever and Rönsch both suggested that when 4 sensory cells were present they were derived from further divisions of the sensory cell. Because the divisions follow each other closely and the cells are very similar to each other, the construction of detailed schemes for the differentiation of all 8 cells concerned in the more complex sensilla has never been attempted. Peters (1965) has recently studied the marginal (chemosensory) bristles of *Calliphora*, which he found to comprise tormogen, trichogen and 5 sense cells, one of which was larger and had different staining properties from the other four. Peters concluded that this cell responded to mechanical stimuli, whereas the other cells were responsible for the chemical sensitivity of the organule. The division scheme begins with a horizontal division as do the schemes for the 3 organules in *Oncopeltus* (Fig. 9).

The cell lineage described by Peters and the lineages of organules of *Oncopeltus* thus do not conform with the general picture of organule development. Clever's supposition that the cell lineage of bristles of *Galleria* could be expected to conform to that of the bristles of *Limnophilus* may now be of doubtful validity.

There are no equivalent accounts of any hemipteran. Wigglesworth (1953), studying *Rhodnius*, found that bristles were derived from groups of 4 similar cells which he found situated in the epidermis. He suggested that these 4 cells (tormogen, trichogen, nerve and neurilemma cells) were all descended from one mother cell. Lipp (1953) described the bristles of *Corixa* as comprising 3 cells: trichogen, tormogen, and a sense cell.

Although the cell lineages of organules of *Lepisma*, *Limnophilus*, *Ephestia* and *Pieris* are similar, the schemes for the 3 types of organules in *Oncopeltus* differ in that the first division is in each case in the horizontal plane; a distinction which is shared with the bristles on the margin of the labellum of *Calliphora* (Peters, 1965). The development of all organules seems to include a vertical or oblique division, which may involve an unequal partitioning of the available cytoplasm (e.g. in *Oncopeltus*, *Ephestia* and *Lepisma*). These divisions are similar to those seen in the dividing neuroblast cells of the insect embryo, and curiously, the vertical division in the epidermis is connected with the production of nerve cells. The hairs and bristles develop identically until the 4-celled stage is reached, following which the latter would seem to pass through a 5-celled stage.

Henke (1953) provides an earlier comparison of organule development, but it is no longer possible to attach much significance to these similarities and differences between the development of organules among the insects.

The cellular differences between the daughter cells of these differentiative divisions may well be determined by cytoplasmic, rather than nuclear, factors. In a study of a similar developmental system, Carlson (1952) rotated the nuclear spindle of dividing neuroblasts of *Chortophaga* through 180°, thereby interchanging the nuclear complement of the presumptive neuroblast and ganglion cell. Such an operation produced no alteration in the cells resulting from the divisions. How the cytoplasm of the bristle mother cell becomes appropriately heterogeneous is unknown, but possibly contact with the surface in one cell causes that region to differentiate into a tormogen cell. Indeed, in the 'Hairless' mutant of *Drosophila*, two tormogen cells result when both cells are in contact with the surface (Lees & Waddington, 1942). It is not known whether this unusual spatial relationship between the two cells results from an abnormal plane of division, but this seems likely (for a criticism of this view see Nash, 1965). However, Richter (1962) observed that the scale and socket cells of *Lepisma* develop independently of the plane of orientation of their parental cell division.

Interrelations between cells

The scale, bristle and socket cells of the Lepidoptera are generally polyploid. Clever (1960) found that when the sense cells in developing bristles of *Galleria* were selectively eliminated with methylene blue, the tormogen and trichogen failed to become polyploid. It is possible that the maintenance of the trichogen and tormogen cells in *Oncopeltus* is likewise dependent on the presence of the sense cells, the absence of the sense cells in the hairs being associated with the eventual degeneration of the trichogen cells.

In this connexion it is of interest that both the tormogen and the trichogen can

function independently. It frequently happens in larval–pupal intermediates of *Galleria*, which result from the implantation of corpora allata, that the socket of the bristle is preserved when the bristle shaft is lost (Piepho, 1940). In wounded *Oncopeltus* the bristle shaft is often secreted alone; no tormogen cell can be seen, and it presumably has degenerated. Normally the two cells function together, the diameter of the socket bearing a certain relation to the length of the bristle. This ratio, however, may merely reflect that the socket is secreted later than the bristle, its size being determined by the diameter of the outgrowth and not by some more intricate relationship between the cells, as was suggested by Henke (1947).

Mechanism of outgrowth of hairs

Lees & Picken (1945) described the development of the bristles of *Drosophila* and noted that the minute initial process of the trichogen cell already showed birefringence. Electron micrographs indicate that this birefringence is caused by bundles of microtubules which fill the early outgrowth. It is clear that the outgrowth is completely formed (as first observed by Semper in 1857) before any substantial layer of the epicuticle is deposited: the cuticle only *appears* to consolidate the shape formed by the cytoplasmic outgrowth of the trichogen cell.

This outgrowth occupies two phases. Firstly, the mass of cytoplasm filled with microtubules extends from the trichogen cell. Microtubules are similar in size and structure to spindle fibres, and it is generally thought that they are identical (de Thé, 1963). While in some cases some spindle fibres apparently shorten during anaphase, in other organisms the central spindle definitely elongates, and among flagellates, for example, this elongation would appear to be active (Cleveland, 1953). Were such an extension of the microtubular bundle to occur in the trichogen cell, such extension might be responsible for the lengthening observed during this first stage of hair outgrowth. In fact, microtubules are associated with the development of cellular asymmetry in other organisms (compare the developing lens of the chick; Byers & Porter, 1964) and are responsible for extension and maintenance of axopods in the heliozoan, *Actinosphaerium* (Tilney, 1965). The second phase in the growth of the hair appears to involve inflation of the cytoplasm of the trichogen cell. As the hair becomes turgid it loses its ragged outline; and, although thickening somewhat, the hair mainly elongates.

The function, if any, of the longitudinal fibrous bundles remains unknown; but very similar structures have been found in the rod-shaped olfactory receptor cells of the fish *Phoxinus* (Bannister, 1965). A portion of these receptor cells extends from the surface of the olfactory epithelium, contains microtubules, and closely resembles a hair outgrowth. The presence of such structures in the fish, where cuticle is absent, indicates that the longitudinal fibrous bundles are structural elements, rather than cuticle precursors as suggested by Paweletz & Schlote (1964). The bundles are possibly identical to the birefringent ridges observed by Lees & Picken, who noted that these were present before deposition of that type of cuticle which is resistant to saturated solutions of potassium hydroxide.

The early events in the outgrowth of hairs are exactly similar to outgrowth of scales described in *Ephestia* (Paweletz & Schlote, 1964). Microtubules and longitudinal

fibrous bundles near the surface of the rudiment were evident. The cuticulin also develops identically. Undoubtedly, electron-microscopic evidence supports the traditional view that scales and hairs are homologous.

The furrows and ridges on the hair develop at the precise moment when the homogeneous layer of the epicuticle begins to be deposited, the general surface of the epidermis folds, and the tracheal walls undergo expansive buckling (Locke, 1958). Stellate epicuticular folds in the larvae of *Rhodnius* probably form due to the endocuticle restricting expansion of the epicuticle (Wigglesworth, 1933); the furrows in the cuticle of the hair could have a similar basis, possibly being formed by expansion of cuticulin against the longitudinal fibrous bundles (Locke, 1966).

Determination

The relation between bristle determination and the degree of stretching of the cuticle of the 3rd-stage larva, and development of extra bristles in the 6th-stage nymphs, indicate that the genesis of bristles and hairs is not a predetermined process, but is rather controlled by contemporaneous conditions. This relation supports the notion that no particular cells are predetermined to form bristles or hairs. More critical evidence for this important hypothesis derives from wounding or burning experiments (Wigglesworth, 1940). Burned areas of *Rhodnius* epidermis regenerate evenly spaced bristles after two moults. Similar experiments conducted on *Oncopeltus* have produced identical results. Transplantation experiments demonstrate that under certain conditions epidermal cells remaining after the development of a population of hairs in the adult will differentiate a second population of hairs (Lawrence, 1966*a*). It is therefore certain that bristles and hairs can be formed abnormally by cells which would not ordinarily do so.

It should be realized that according to the hypothesis of Wigglesworth, the even spacing of bristles is promoted by the *graded* introduction of new bristle centres. Possibly this relation provides the basis for the rationale of the asynchronous differentiation of hair centres in *Oncopeltus*; because if, as seems likely, this differentiation reflects asynchronous determination, then hair genesis of *Oncopeltus* consists of systematic and continuous introduction of new centres. The determination and differentiation of the marginal bristles on the wing of *Ephestia* also occurs asynchronously (Clever, 1958).

Stern's studies of bristles of *Drosophila* furnish further evidence of an influence pervading epidermal cells. Stern (1956) used flies which were a mosaic of areas of wild-type and achaete integument in order to show that when a bristle is absent from its normal situation, a neighbouring piece of wild-type tissue may develop a bristle abnormally. According to Wigglesworth's hypothesis, the absence of a normally adjacent bristle has either stimulated, or allowed, this development. As the newly formed, abnormally situated bristle is located nearer to the other bristles than it is in the wild type, the experiment suggests that *competition* for a postulated bristle-forming substance, rather than *active inhibition* by extant bristles, determines genesis of bristles. Also using integumental mosaics, Stern (1954) has inferred the existence of a more pervasive precursor—a 'prepattern'—of the specific pattern of bristles. Spickett

(1963), analysing factors causing the development of extra bristles on the sternopleurite of *Drosophila*, found one such factor which increased the size of the insect by increasing the *number* of cells. There was also another factor which affected the size of the insect; but it achieved this modification by varying the *size* of cells and did not affect the number of bristles. These results are all consistent with Wigglesworth's model.

The origin of the pervasive growth substance is subject to speculation. Because the capacity to form a particular density of bristles is transmitted to daughter cells, it must be concluded that this capacity, and therefore the concentration of the determinative substance, is an intrinsic property of the epidermal cells: the epidermal cells themselves must produce the bristle-forming substance. It may be assumed that for each cell there exists a threshold concentration of the substance to which that cell responds, becoming determined as an organule and thereafter absorbing the substance from its vicinity. This threshold may vary randomly. As the concentration of the substance rises, those cells with the lowest threshold will become determined and rapidly absorb the substance from nearby cells. For a period the whole population of cells will produce the substance faster than it is absorbed by extant or newly determined bristles, and it will therefore accumulate between these absorptive centres, thereby initiating further determinations until an equilibrium is established. At the next moult, the number of cells will increase, thereby upsetting the equilibrium and initiating still another sequence of determinations.

The experiments described here involving starvation of *Oncopeltus* have defined the limits of the periods of determination of hairs and bristles. The differentiative divisions of the hairs and bristles occur within these limits. It has thus been deduced that larval bristles are determined *before* cell divisions have much increased the number of cells. Differentiative divisions of the bristles are in fact seen amongst early proliferative divisions. Observations suggest that determination of the adult hairs takes place *after* proliferative cell divisions have occurred and indeed the differentiative divisions of the hairs are found at this time. It is therefore probable that determination occurs just before the mother cell appears.

It emerges from this study that different types of organule develop at different times. This asynchrony may be of wide occurrence among insects: Wigglesworth noted that in *Rhodnius* the dermal gland initial cells appeared later than the bristle-forming cells; Nash (1965) observed that the large bristles on the dorsal surface of the head of *Drosophila* developed in a particular sequence; and Spickett (1964), studying the sternopleural chaetae of *Drosophila*, found that the absence of one large bristle resulted in the delayed local development of several small bristles.

There may possibly be successive waves of comparable differentiations, the only definitive difference between which is their timing. If this is the case, the presence of centres formed by the earlier waves of differentiation should cause the later waves of differentiation to induce a larger number of centres in amongst them. Hair follicles of sheep develop in this way (Fraser & Short, 1960), and in all insects so far studied later differentiations seem to produce the more numerous organules. During the moult cycle, development of cells which form sparse bristles is followed by the formation of

more numerous hairs in *Oncopeltus* and dermal glands in *Rhodnius* (Wigglesworth, 1953). Distribution patterns of adult hairs, adult bristles and other sensilla are all mutually and completely integrated. This integration suggests that the same precursor substance affects both the maintenance of the bristles and the determination of the hairs. When two kinds of organule are found within the same area, this integration is not always present. Smolka (1958) noted that on the abdominal integument of *Ephestia* the distribution patterns of hairs and of scales were totally unrelated. He accordingly suggested that two growth substances might be involved in the determination of hairs and scales.

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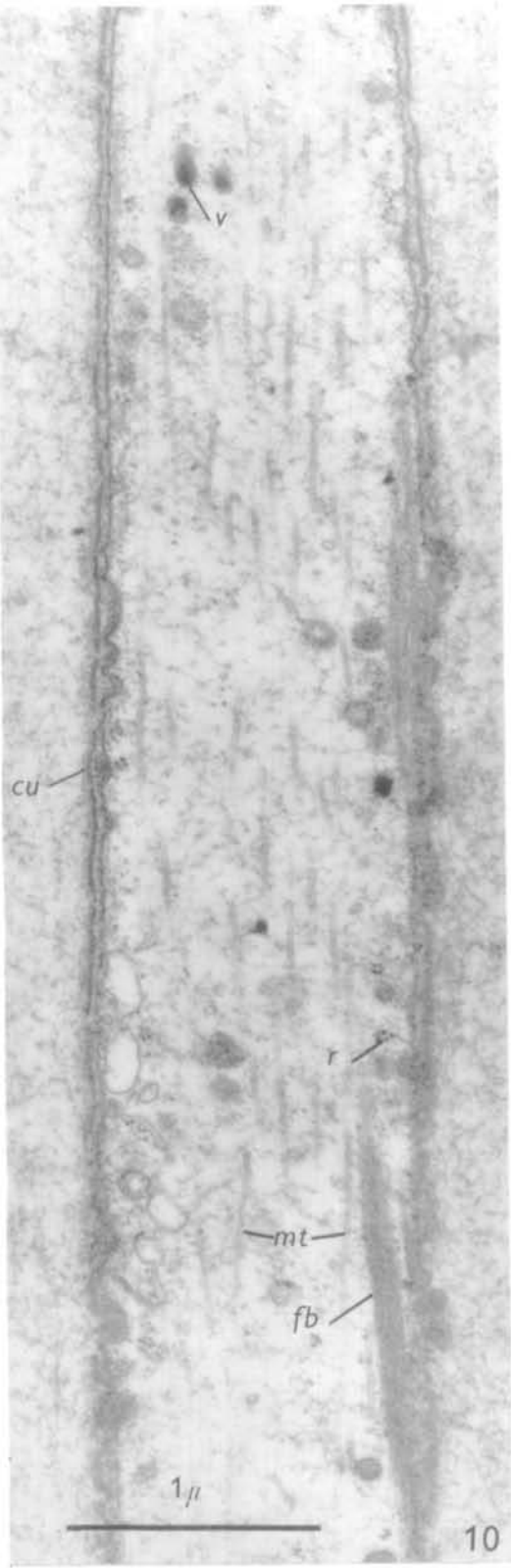
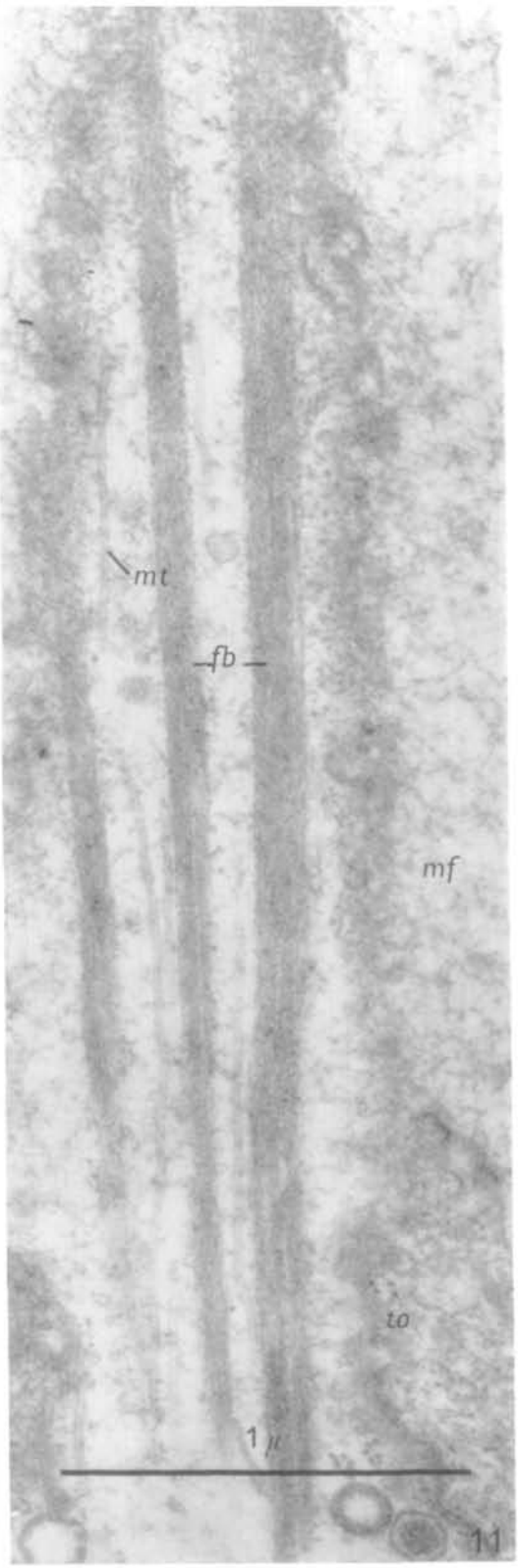
Fig. 10. Longitudinal section through the centre of a hair outgrowth of a 5th-stage larva of 95 h. The cytoplasmic contents are very electron-transparent and include longitudinally oriented microtubules (*mt*), fibrous bundles (*fb*), free ribosomes (*r*), and vesicles (*v*) about 0·1 μ in diameter. The first layer of the cuticle is visible as small sections of cuticulin (*cu*); for detail see Fig. 15. × 34000.

Fig. 11. Longitudinal section through the surface of a hair outgrowth of a 5th-stage larva of 95 h, showing considerable length of the longitudinal fibrous bundles (*fb*). Microtubules (*mt*) are visible. The tormogen cell (*to*) lies adjacent to the trichogen cell. Moulting fluid (*mf*) surrounds the outgrowth. × 54000.





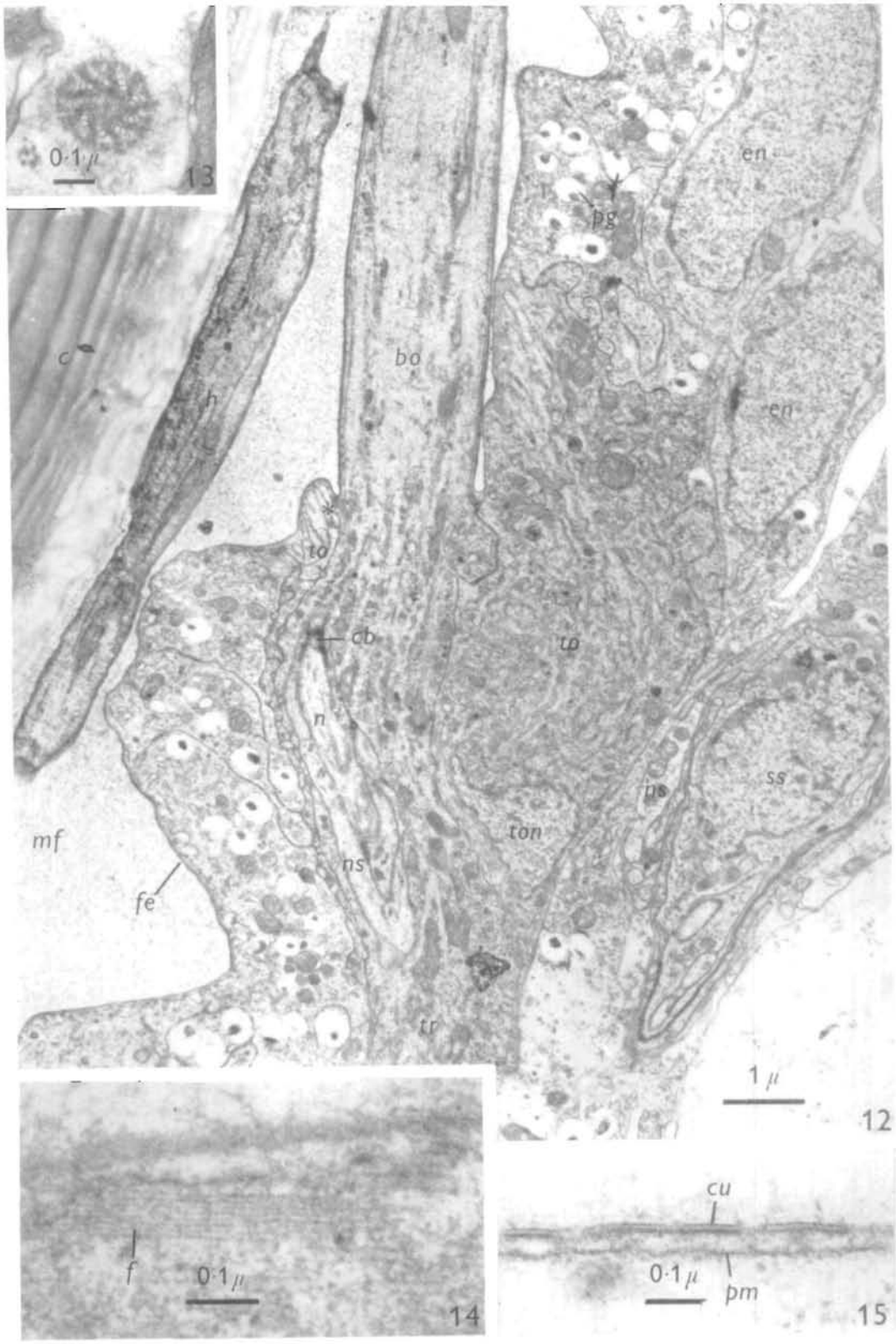
(*Facing p.* 498)

Fig. 12. Low-power micrograph of developing adult bristle of a 5th-stage larva of 98 h. The homogeneous layer of the epicuticle is being deposited, and the epidermis displays folds (*fe*). The bristle outgrowth (*bo*) extends into the moulting fluid (*mf*). The socket is not yet secreted by the tormogen cell (*to*). The nerve and neurilemma sheath (*ns*) are enclosed by the trichogen cell (*tr*). The nerve includes a centriole-like body (*cb*) and sends (at asterisk) a peripheral prolongation (not visible in this section) out between trichogen and tormogen cell through the moulting fluid to the old bristle. (*bo*, bristle outgrowth; *c*, old cuticle; *cb*, ciliary body; *en*, nucleus of epidermal cell; *fe*, folded epidermis; *h*, hair outgrowth; *mf*, moulting fluid; *n*, nerve; *ns*, neurilemma sheath; *pg*, pigment granules; *ss*, sense cell; *to*, tormogen; *ton*, tormogen cell nucleus; *tr*, trichogen.)

Fig. 13. Transverse section of centriole-like body. There are 9 groups of double profiles in a cartwheel array, which are surrounded by electron-dense material. The existence of central fibres is uncertain. × 65 000.

Fig. 14. Detail of longitudinal fibrous bundle to show fibrils (*f*) of which it is composed. × 113 000.

Fig. 15. Detail of the surface of a hair outgrowth of a 5th-stage larva of 95 h. The discontinuous duplex structure of the cuticulin (*cu*) is clearly visible, and this layer is evidently distinct from the plasma membrane (*pm*). × 99 000.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