Determination of Cell Function in an Insect

by V. B. WIGGLESWORTH[1]

From the Department of Zoology, University of Cambridge

I PROPOSE to consider two kinds of determination and differentiation which have been studied in the hemipteron *Rhodnius prolixus*. (i) The determination of the cell or group of cells, with their subsequent differentiation to produce a given part of the body. (ii) The determination or control of the characters of that part—whether these are to be juvenile (larval) or adult (imaginal). Discussion of this second type of determination will require consideration of the role of hormones in controlling differentiation in insects.

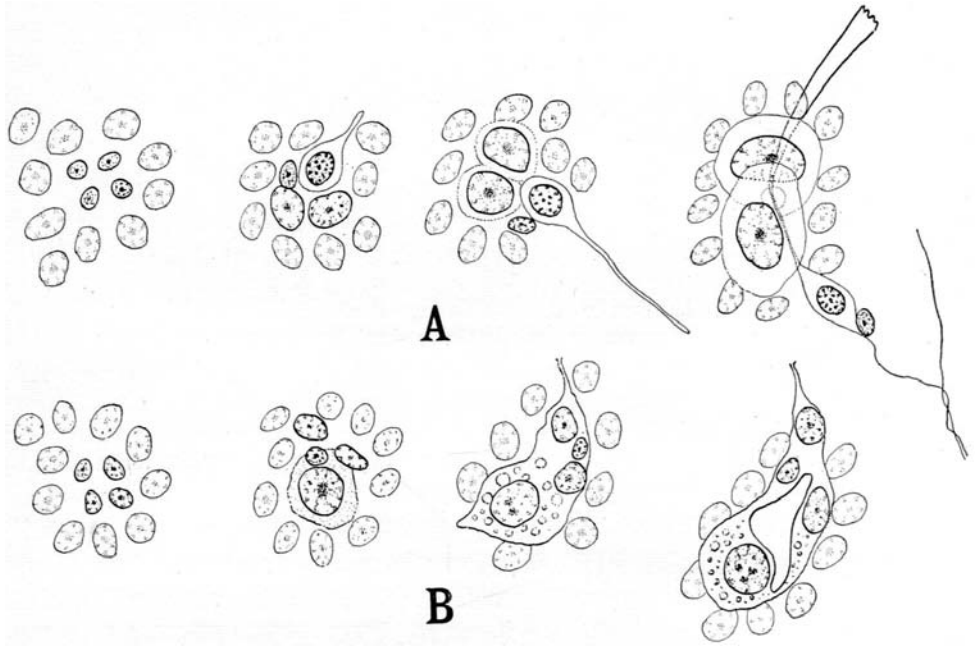

FIG. 1. A, four stages in the development of a sensory hair showing four small cells becoming differentiated into the trichogen, tormogen, sense-cell with inwardly growing axon, and neurilemma cell. B, four stages in the development of a dermal gland by the differentiation of four small cells.

DETERMINATION AND DIFFERENTIATION OF NEW STRUCTURES

The integument of the abdomen in the *Rhodnius* larva consists of a single layer of epidermal cells and the overlying cuticle. At regular intervals the cuticle

[1] *Author's address*: Department of Zoology, Downing Street, Cambridge, England.

[J. Embryol. exp. Morph. Vol. 1, Part 3, pp. 269–277, September 1953]

is modified to form little plaques each of which bears an innervated bristle (Wigglesworth, 1933). The cuticle is pierced at intervals by the ducts of dermal glands: these form a cluster of 4 or 5 around each plaque, with occasional single glands in the clear space between (Wigglesworth, 1947) (Fig. 3, A).

At each moult bristles and plaques are re-formed at the existing sites and new ones are developed in the spaces between (Wigglesworth, 1940*a*). The new bristle first appears as a group of four apparently identical cells with small nuclei, doubtless the product of rapid division of a single cell which has been determined

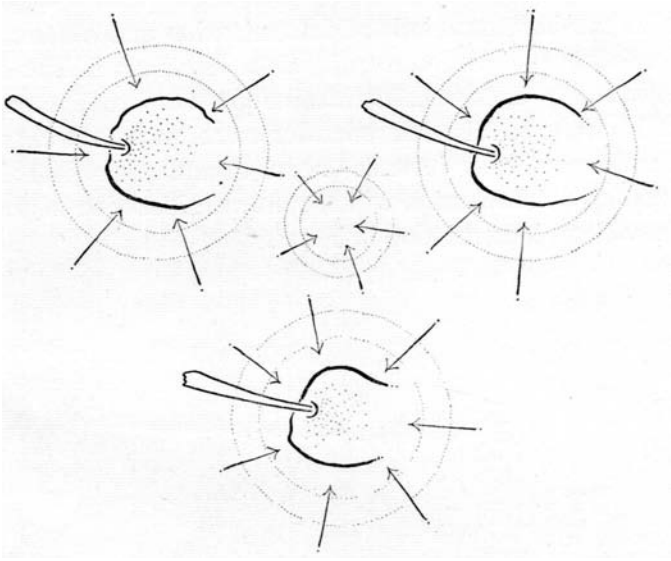

FIG. 2. Diagram to illustrate the hypothesis of determination of a new sensory hair in the interval between existing hairs.

as a bristle-forming centre (Fig. 1, A). These four cells become the trichogen, the tormogen, the sense-cell which sends an axon inwards to join with neighbouring axons, and the neurilemma cell (Wigglesworth, 1953).

At their earliest appearance the dermal glands likewise arise as four apparently identical cells with small nuclei which later become differentiated into a large glandular cell, two cells with smaller nuclei, and a cell with a very small nucleus resembling the neurilemma cell (Wigglesworth, 1933, 1953) (Fig. 1, B).

The determination of these new structures is controlled by their distance from the existing structures. New bristles appear where the existing bristles are most widely separated. If the epidermis is destroyed by burning over a wide area, new bristles are regenerated at the normal intervals. If the existing bristles are abnormally separated by stretching the integument experimentally (for example, by blocking the anus after a large meal of blood), an unusually large number of new bristles appear between them (Wigglesworth, 1940*a*).

It would seem that existing bristles inhibit the emergence of new bristle-form-

ing centres for a certain distance around them. The epidermal cells are intimately connected together by means of anastomosing processes below the basement membrane so that they form in effect a syncytium (Wigglesworth, 1937, 1953). As a working hypothesis (Fig. 2) it is considered that some substance present in the epidermis is necessary for the determination of a bristle-forming centre. Each centre drains this substance from the surrounding cells. Hence it is at a point as far removed as possible from existing centres that the new centre will arise; and this in its turn will inhibit the appearance of further centres in its vicinity by drawing off the hypothetical substance (Wigglesworth, 1940*a*, 1948*b*).

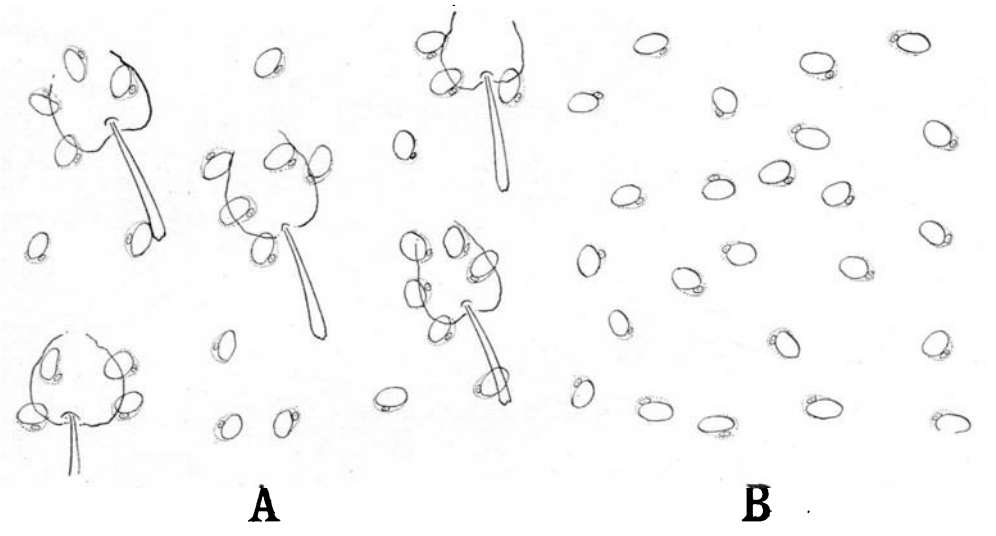

FIG. 3. A, normal integument showing distribution of sensory hairs and dermal glands. B, integument at the first moult after the repair of a burn; only dermal glands have been regenerated.

In the first moult after burning no new bristles are formed in the regenerated area; they appear at the second moult. But examination of the regenerated epidermis after a burn shows that new dermal glands *have* been differentiated and at regular intervals, much closer together than the bristles (Wigglesworth, 1953) (Fig. 3, B).

There is no obvious reason why new bristles should not be regenerated at the first moult after the repair of an extensive burn—for during the early stages in their differentiation the bristles are indistinguishable from the developing dermal glands (Fig. 1), and we have seen that dermal glands with normal intervals between them are developed even at this stage. One might put forward the hypothesis that the same substance is necessary to evoke the differentiation of sensory bristles and dermal glands but that it is required in higher concentration for the production of sensory bristles. One might then suppose that the concentration of this substance in the epidermis of a recently healed burn is insufficient

for the formation of bristles but adequate for the differentiation of glands. An hypothesis of this kind would also explain the fact that glands develop closer together than bristles.

New bristles arising over a healed burn are normally orientated; that is, they are directed posteriorly; whereas if a piece of the integument is excised, rotated through 90° or 180° and reimplanted, the bristles show a corresponding change in orientation (Wigglesworth, 1940*b*). One must therefore conclude that some sort of orientation already exists within the cytoplasm of the undifferentiated epidermal cells, with the result that when they divide to produce the tormogen

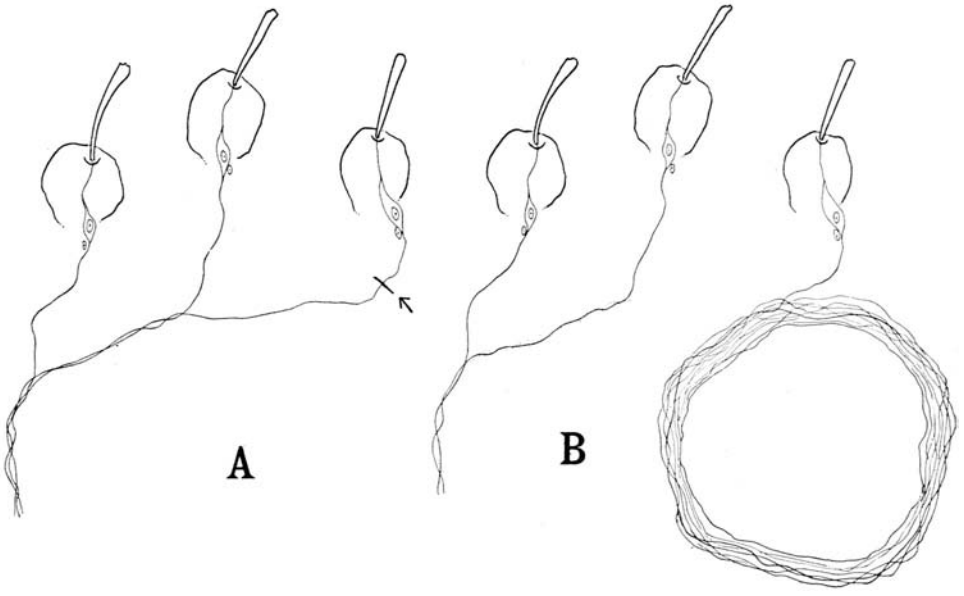

FIG. 4. A, three sensory hairs with their axons forming a small nerve. The arrow shows point of interruption of one axon by a burn. B, the same after repair. The outgrowing axon has formed an annular nerve with no connexion with the central nervous system.

and trichogen cells these are so placed in respect to one another that the bristle grows out in the predetermined direction.

These observations point to the existence of some kind of 'cytoskeleton' within the undetermined cell, which defines the antero-posterior axis and controls the mutual relations of the daughter cells (themselves perhaps still forming a syncytium) and in this way controls the orientation of the resultant structures—just as a unicellular protozoan may have a highly differentiated structure.

The new sensory neurones arise by differentiation from the ordinary ectodermal cells throughout post-embryonic life. Their inwardly growing axons illustrate another common phenomenon in the relations between differentiated cells: the mutual affinity of the same cell types. The new axon may grow in any direction, between the epidermal cells and the basement membrane, until it makes contact with an existing nerve or axon. It then proceeds to accompany this

nerve, which it follows through the basement membrane and to the central nervous system. If an existing sensory nerve is cut or interrupted by a burn, the axons grow out during the healing process in the same manner and accompany any nerve they chance to meet. Sometimes, however, they will grow round and make contact with themselves and will then proceed to grow round and round in circles until a stout annular nerve is produced which has no connexion with the central nervous system (Wigglesworth, 1953) (Fig. 4).

DETERMINATION AND DIFFERENTIATION OF JUVENILE AND ADULT CHARACTERS

Some insects, notably Diptera, have the imaginal cells set aside at an early stage of development. But in *Rhodnius* the same epidermal cells are responsible

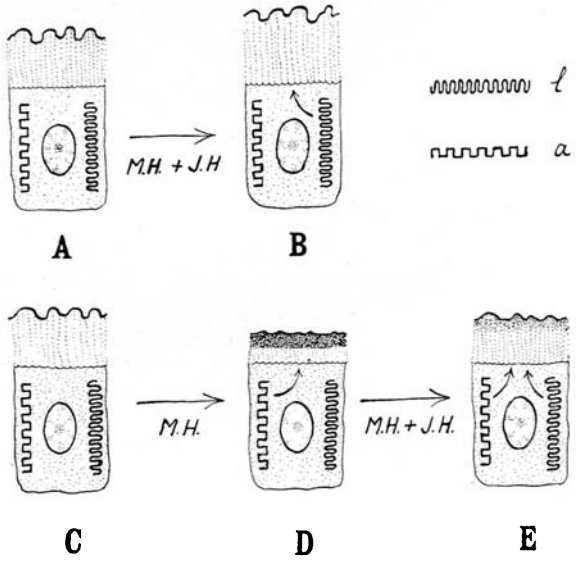

FIG. 5. Diagram to illustrate the control of metamorphosis by hormones. *a*, intracellular system producing adult characters; *l*, intracellular system producing larval characters. Epidermal cell with larval cuticle (A) in presence of moulting hormone (M.H.) and juvenile hormone (J.H.) lays down larval cuticle (B). Similar epidermal cell (C) in presence of moulting hormone alone (M.H.) lays down adult cuticle (D). In presence of moulting hormone and juvenile hormone it now lays down cuticle intermediate between adult and larva (E).

for forming both larval and adult structures. When the cells are determined to form some particular region of the body in the larva they are committed to form the corresponding region of the adult.

The morphological characters of the cuticle may be totally different in larval and adult stages; which characters appear is determined by the hormones in the

circulating blood. Growth and moulting are initiated by hormones from the brain and thoracic gland. In the presence of these hormones (the 'moulting hormone') alone the intracellular system which lays down adult structures takes precedence over that laying down larval structures (Fig. 5, D). But if the juvenile hormone secreted by the corpus allatum of the young larval stages is present in addition, the system which lays down larval structures is preferentially activated and metamorphosis is therefore suppressed (Fig. 5, B) (Wigglesworth, 1934, 1936, 1940*b*).

The change in function of one and the same cell is seen most strikingly in the

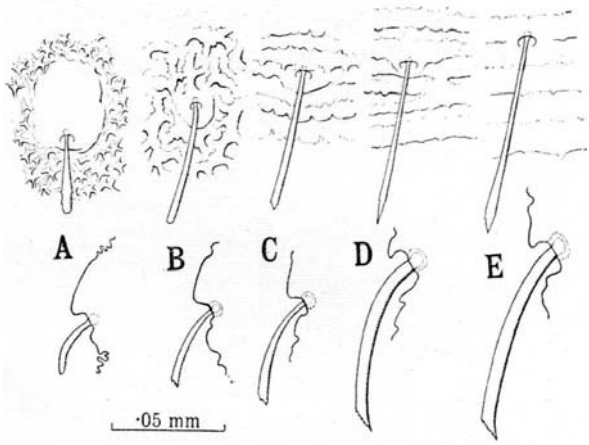

FIG. 6. Bristles from dorsal surface of abdomen (upper row) and from margin of abdomen (lower row). A, normal fourth-stage larva; B, C, and D, insects showing varying degrees of metamorphosis following removal of corpus allatum from third-stage larva; E, normal adult.

form of the bristles. These are produced by outgrowths from a single trichogen cell which persists from the larva to the adult. By appropriate manipulation of the growth-controlling hormones it is possible to produce *Rhodnius* larvae showing all intermediate degrees of metamorphosis between larva and adult. The corresponding changes are manifested in the individual bristles (Wigglesworth, 1934) (Fig. 6).

Even in the adult *Rhodnius* the system which produces larval structures is still latent within the cells. If the adult is caused to moult again by exposure to the 'moulting hormone', and if at the same time it is provided with a source of juvenile hormone, it shows a partial reversion to larval characters (Fig. 5, E). In the light of these results it is not wholly satisfactory to refer to the juvenile hormone as the 'inhibitory hormone', restraining the differentiation of imaginal characters by sustaining the differentiation of larval characters (Wigglesworth, 1934) or as the *status quo* hormone (Williams, 1952) maintaining the larval characters by restraining imaginal differentiation. This hormone must be pic-

tured as having a more *positive* action on the latent morphological system in the cells, and as actively favouring differentiation by the larval system even after metamorphosis has taken place (Fig. 5, E). For that reason the term 'juvenile hormone' (Wigglesworth, 1940*b*) is perhaps to be preferred.

It may be pointed out that the 'moulting hormone' (which consists of a tropic

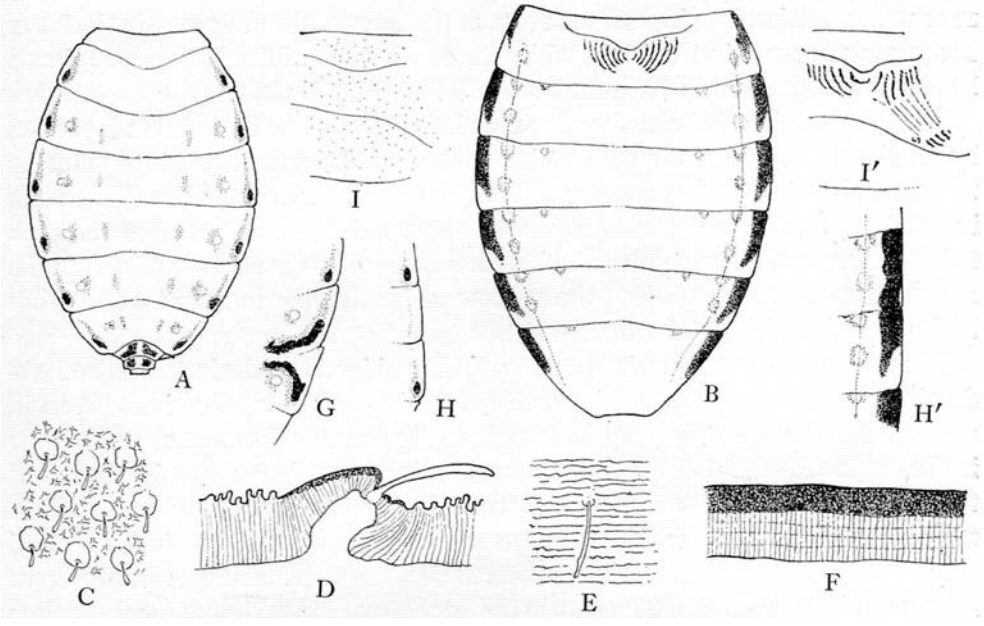

FIG. 7. A, dorsal abdominal pattern of *Rhodnius* larva; B, the same, *Rhodnius* adult; C, detail of surface of cuticle in larva, showing plaques and bristles; D, section through cuticle of larva; E, detail of surface of cuticle in adult showing transverse folding of epicuticle and few bristles; F, section through cuticle of adult; G, margin of abdomen of fifth-stage larva after repair of a burn involving two marginal black spots (note extension inwards of the black spots concerned); H, elimination of one marginal black spot in fifth-stage larva as result of a burn in this area during third stage; H′, the corresponding area when this insect became adult (the marginal black spot of that segment in the adult has extended over the burned area); I, first three segments in fifth-stage larva following an extensive burn on the right side in the third stage (apart from some distortion of the second segment there is no visible effect); I′, corresponding area when this insect became adult (there is a striking displacement of the ridged cuticle on the second segment).

factor from the brain activating an endocrine thoracic gland which then secretes the hormone acting on the tissues) (Wigglesworth, 1952*a*; Williams, 1948, 1952) has been alternatively named a 'growth and differentiation factor' (Scharrer, 1946). But the results outlined above make it clear that differentiation may take place in the larval direction or in the imaginal direction. The 'moulting hormone' merely initiates and maintains the process of growth which terminates in moulting. The capacity for dimorphic differentiation is a property of the growing body cells, the *direction* of differentiation being controlled by the concentration (Wigglesworth, 1934) and by the timing (Wigglesworth, 1952*b*) of the secretion

of the juvenile hormone. There is, therefore, no justification for ascribing to the 'moulting hormone' the property of *inducing differentiation* which is what the term 'differentiation factor' implies.

The structural pattern of the abdomen is quite different in larva and adult. An area on the first and second abdominal segments, indistinguishable in the larva from the rest of the surface of the abdomen, forms in the adult a characteristic zone with semicircular ridges. Pale areas at the sides of the abdomen in the larva become black spots in the adult; while the black spots of the larva are replaced by pale areas in the adult (Wigglesworth, 1940*a*) (Fig. 7, A, B).

That the imaginal pattern already exists in invisible form in the larva is proved by eliminating areas of the epidermis by burning. Regeneration takes place by the multiplication and spreading of cells from the adjacent areas. When the insect finally undergoes metamorphosis it is found that the power of the cells to lay down particular elements in the adult pattern has been transmitted to the daughter cells and that during the process of healing the imaginal pattern has been displaced (Wigglesworth, 1940*a*).

These results are summarized in Fig. 7. The ridged cuticle in the second abdominal segment may be strikingly displaced in the adult following a burn in an early larval stage (Fig. 7, I′), although no sign of this displacement is visible in the larva (Fig. 7, I). Likewise a burn eliminating a black spot in the larval tergites (Fig. 7, H) may be repaired by cells destined to produce a black area in the adult so that two black spots in the resulting adult are found to have fused (Fig. 7, H′). Whereas a burn in the pale area between two black spots in the larva may result in the fusion of the spots in the later larval stages, but at the same time it has eliminated the area destined to form a black spot in the adult so that this spot is absent.

It is a matter for discussion whether the simultaneous inheritance of the dual potentialities for larval and adult differentiation within these societies of cells is by way of the nucleus or cytoplasm or both.

SUMMARY

This paper reviews briefly a wide range of published observations on growth and differentiation in *Rhodnius*. Subjects mentioned include:

i. The differentiation of sensory hairs and dermal glands.
ii. The effect of mutual separation in controlling the formation of new hairs and glands.
iii. The differentiation of primary sense-cells and the behaviour of the sensory axon during growth and regeneration.
iv. The existence of larval and imaginal potencies in single epidermal cells and the role of hormones in controlling their differentiation.
v. The reversal of metamorphosis in the adult insect.
vi. The persistence of the latent potencies for particular elements in the imaginal pattern during wound healing in the larval stages.

REFERENCES

SCHARRER, B. (1946). *Endocrinology*, **38**, 35.
WIGGLESWORTH, V. B. (1933). *Quart. J. micr. Sci.* **76**, 269.
—— (1934). *Quart. J. micr. Sci.* **77**, 191.
—— (1936). *Quart. J. micr. Sci.* **79**, 91.
—— (1937). *J. exp. Biol.* **14**, 364.
—— (1940*a*). *J. exp. Biol.* **17**, 180.
—— (1940*b*). *J. exp. Biol.* **17**, 201.
—— (1947). *Proc. roy. Soc.* B, **134**, 163.
—— (1948*a*). *J. exp. Biol.* **25**, 1.
—— (1948*b*). *Symposia Soc. Exp. Biol.* **2**, 1.
—— (1952*a*). *J. exp. Biol.* **29**, 561.
—— (1952*b*). *J. exp. Biol.* **29**, 620.
—— (1953). *Quart. J. micr. Sci.* **94**, 93.
WILLIAMS, C. M. (1948). *Growth Symposium*, **12**, 61.
—— (1952). *Biol. Bull.* **103**, 120.