WOUND HEALING IN AN INSECT (*RHODNIUS PROLIXUS* HEMIPTERA)

By V. B. WIGGLESWORTH
London School of Hygiene and Tropical Medicine

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(With Nine Text-figures)

If the epidermis of an animal is injured so that its continuity is destroyed, the surrounding cells multiply and spread over the wound until the gap is filled. Then their growth ceases. The factors which initiate this renewed growth of "repair", and the factors by which growth is arrested when the defect has been made good, are very incompletely known. The object of the present work was to re-investigate this old problem upon new material.

The blood-sucking bug *Rhodnius* is particularly suited to this purpose for several reasons. Once the cuticle of the adult insect has been laid down, the single layer of epidermal cells beneath it shows no further growth; there is no growth of "maintenance" such as there is in the epidermis of vertebrates. Hence any cell divisions that are seen after an injury represent growth of "repair". The same is true of the nymphal stages if they are given only small feeds of blood (growth is brought about only by a large distending meal (Wigglesworth, 1934)), or if they are decapitated soon after feeding (that is, before the hormone which initiates growth and moulting and which is probably secreted in the head has been produced (Wigglesworth, 1934, 1936)). Lastly, the experimental wounds are conveniently made on the dorsal surface of the abdomen; and it is easy to dissect off the cuticle of the abdomen and mount it entire, so that the behaviour of every epidermal cell around a given injury can be observed in a single preparation.

METHODS

The procedure has been to injure the epidermis of the abdominal tergites of adult insects, or of 5th-stage nymphs decapitated 24 hours after feeding, by punctures with a needle, incisions with a minute lancet, excisions of small areas or by burning with a heated wire; the wound in each case being sealed with paraffin wax of low melting point. At intervals thereafter the tergites have been removed, fixed with Carnoy's fixative, and either stained with haematoxylin and mounted whole or embedded in celloidin and paraffin and sectioned. The insects have been kept at 24° C.

DESCRIPTION OF THE EPIDERMIS

The structure of the cuticle and epidermis of the abdomen in *Rhodnius* has already been described (Wigglesworth, 1933) but there are some further details that require mention here. In the unfed 5th-stage nymph the cells are densely

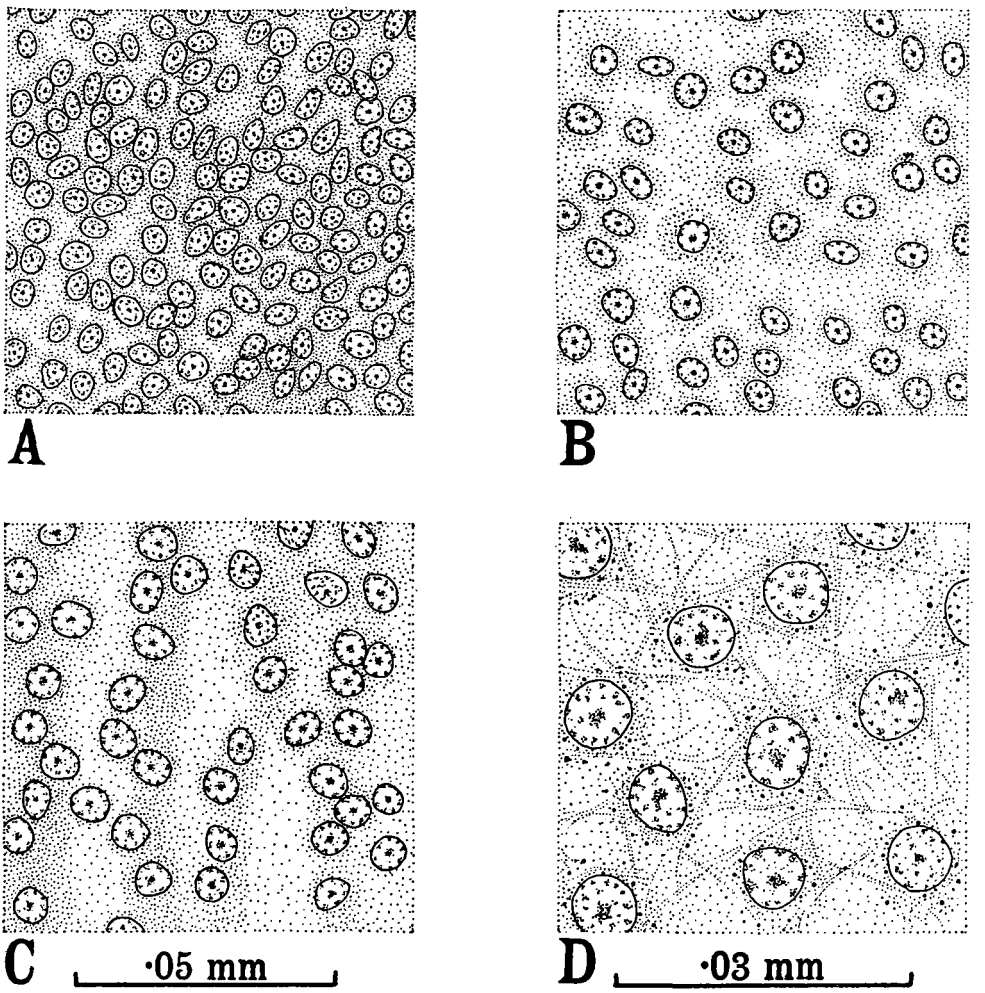

Fig. 1. Epidermis of *Rhodnius* in surface view. A, unfed 5th-stage nymph; B, the same 24 hours after feeding; C, adult; D, 5th-stage nymph immediately after feeding, higher magnification showing plasma fibrils connecting the cells.

packed so that the nuclei are close together (fig. 1 A). After feeding, when the cuticle is stretched, they are more widely separated (Fig. 1 B). In the adult they collect in the transverse furrows in the cuticle leaving the intervening spaces thinly covered (Fig. 1 C). In both nymphs and adults there are cytoplasmic bridges connecting the cells on all sides; and within these bridges there appear to be fine fibrillae which run towards the centre of the cell and interlace around the nucleus.

These structures can be brought out by Unna's orcein stain (Unna, 1904); but they show almost equally well in preparations stained deeply with Ehrlich's haematoxylin. When the cells are crowded the fibrillae are difficult to make out; they are most easily seen in 5th-stage nymphs fixed in the distended state immediately after feeding (Fig. 1 D).

HEALING OF A MECHANICAL INJURY

Incision or excision of cuticle and epidermis causes the following responses:

(i) *Activation* of the surrounding cells.
(ii) *Migration* of the epidermal cells and their crowding round the site of injury.
(iii) *Accumulation of haematocytes.*
(iv) *Spreading* of the epidermal cells over the defect.
(v) *Cell division* in the surrounding zone to replace the emigrated cells.
(vi) *Secretion of a new cuticle.*
(vii) *Formation of a new basement membrane.*
(viii) *Return to the resting state.*

(i) The *activation* of the surrounding cells is described by Lazarenko (1928) in *Oryctes*. The cell bodies become elongated and columnar, the cytoplasm containing much fibrillar basophil material; the nuclei are swollen and show a great increase in visible chromatin which may be collected in irregular masses or in a single central nucleolus (Figs. 2 A, 5 A). This change begins within 6 hours in the cells at the site of injury and then spreads outwards, reaching its maximum extent of about 0·2 mm. from the wound in 2 or 3 days.

(ii) *Migration* of epidermal cells is well advanced 12 hours after the injury; by 48 hours the cells have collected in a dense crowd along the injured margin (Fig. 2 A). The surrounding cells often lie in rows radiating outwards; an arrangement which is probably a mechanical effect of the cytoplasmic connexions between them (Fig. 2 B). A like appearance was described by L. Loeb (1898) in the rabbit.

In the 5th-stage nymphs the occurrence of migration is proved by the fact that the cells beneath the bristles, which contain opaque white spheres of uric acid (Wigglesworth, 1933), can be seen in unstained preparations streaming away from their normal position towards the cut margin. In the adult, in which the epidermal cells are more sparse than in the nymph, the migration is very obvious because it results in the creation of a zone usually about 0·1 mm. from the wound in which the cuticle is almost devoid of cells (Fig. 2 A). The same can be seen in 5th-stage nymphs if the anus is waxed immediately after a large meal of blood so that the epithelium is abnormally stretched. There is no difference in the amount of migration and aggregation of cells in response to a simple incision or an excision of several square millimetres.

(iii) *Haematocytes accumulate* along the cut margin of the incision within a few hours. In the course of a day or two they may form a solid plug over the perforation and they accumulate more sparsely, spread out on the basement membrane, over approximately the same area as the zone of activated epidermal cells (Fig. 4). They

apply themselves also to the lower surface of the epidermal cells as these spread over an excision. Mitoses are plentiful among them. It is important to note, however, that in very superficial incisions which do not penetrate the basement membrane, there may be no accumulation of haematocytes although the epidermal cells show the usual reactions.

(iv) *Spreading* of the accumulated epidermal cells over the wound begins in about 48 hours. Under favourable circumstances, if the excised area is not too

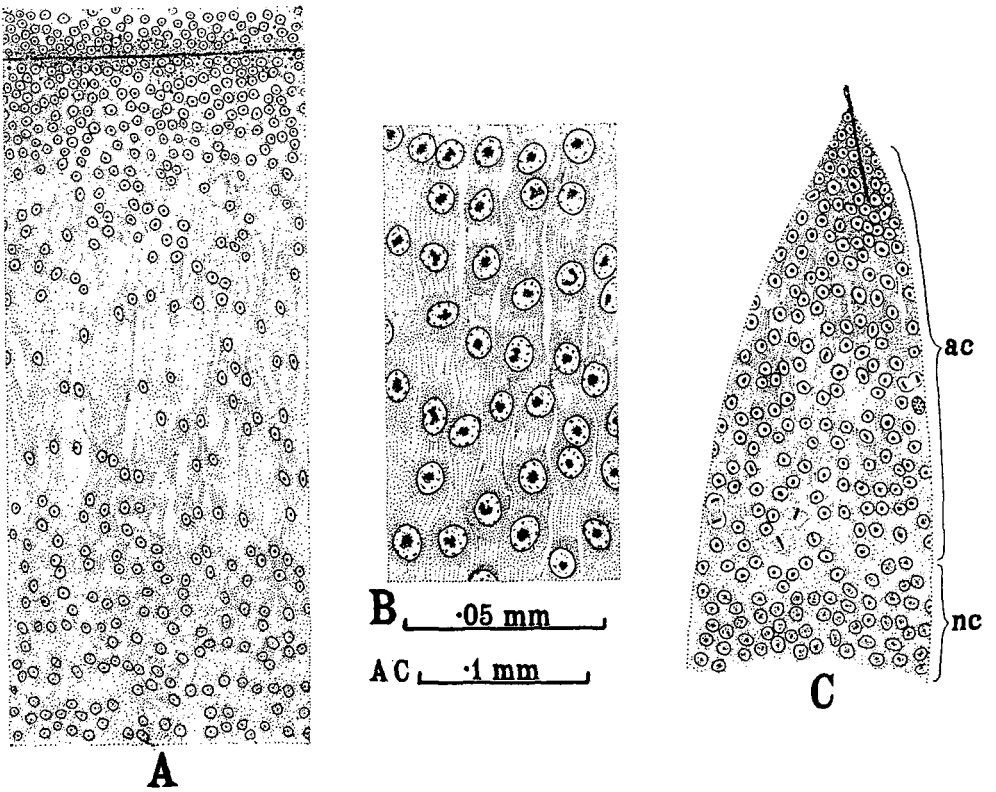

Fig. 2. A, epidermis of adult 24 hours after a simple incision showing migration of epidermal cells. Note the chromatin droplets derived from injured cells among the congested cells along the cut; B, epidermis of 5th-stage nymph 3 days after incision (which lies beyond the upper part of the figure) showing the tandem arrangement of the activated cells; C, epidermis of 5th-stage nymph 6 days after simple incision showing mitoses chiefly in the sparse zone.

large (say 1 × 1·5 mm.) and there is no injured tissue to obstruct the process, and especially if the basement membrane is intact, the wound is quickly covered by a few cells spread excessively thinly but still connected by their cytoplasmic processes (Fig. 3). As more cells follow these the nuclei become more and more crowded until the normal density is reached; spreading then ceases. More often, however, there are irregularities in the process of spreading and the normal single-celled layer of the epidermis is not restored immediately. These complications will be described later.

(v) *Cell division* begins after the processes of activation and migration are finished. Sometimes it may begin 2 days after the injury; more often no mitoses occur until the fourth or fifth day. Occasional mitotic figures are to be seen among

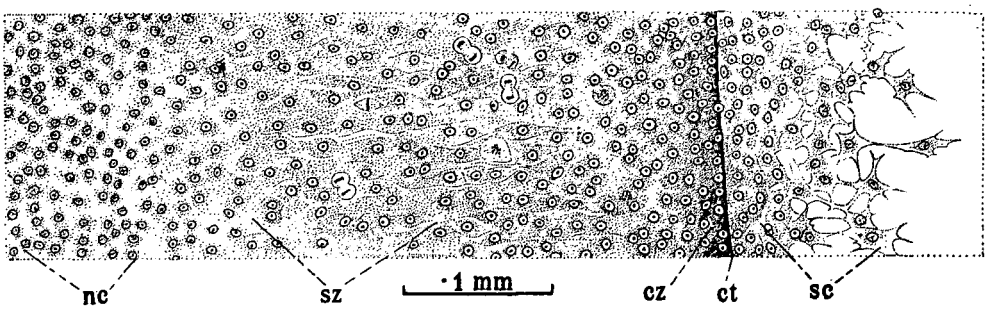

Fig. 3. Epidermis of adult 4 days after an excision of about 1 mm. square. *ct*, margin of excised area; *cz*, zone of congested cells along the cut margin; *nc*, normal unchanged cells; *sc*, cells spreading over the excised area; *sz*, zone of sparse activated cells, many undergoing division.

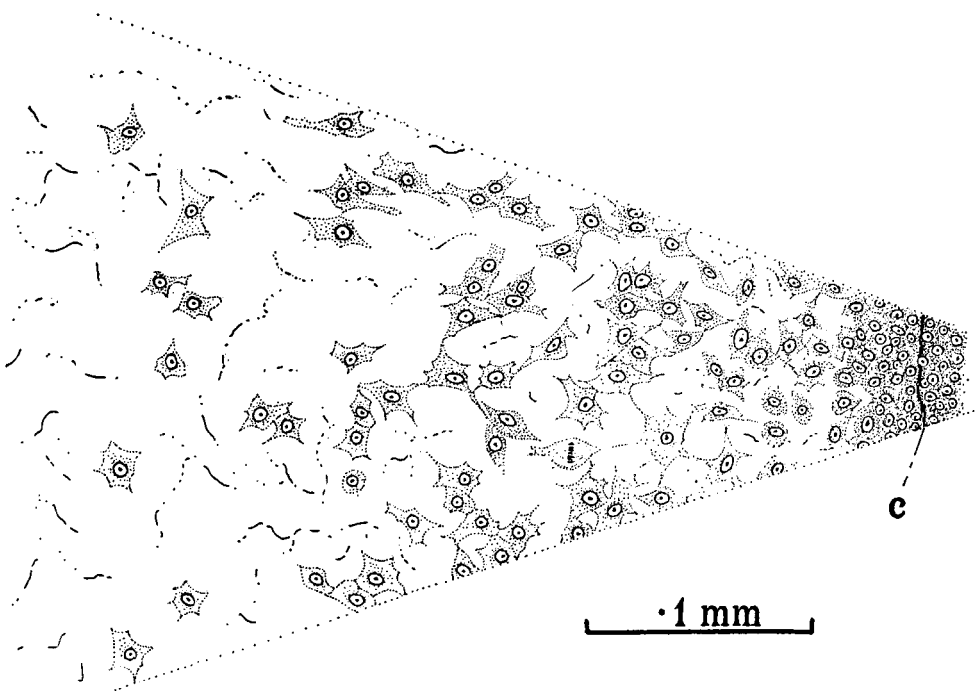

Fig. 4. Haematocytes below the basement membrane crowding round a cut (*c*) in a 5th-stage nymph 6 days after the operation.

the cells which have migrated to the margin of the wound; a few occur among the cells spreading over the defect; but by far the greater number are to be seen towards the periphery of the activated zone. Figs. 2 C and 3 show the typical distribution of mitotic figures during the height of cell division. Although there are many exceptions, the majority of the mitotic spindles are disposed radially from the

wound (cf. Lazarenko, 1928); that is, in the long axis of the cells. As usual in growing tissues they tend to occur in pairs or in groups. Cell division reaches its peak 6 or 7 days after the injury and then gradually ceases.

(vi) *Secretion of cuticle* takes place in several stages. The blood of *Rhodnius* does not clot, but any which escapes on to the surface of the wound dries into a brownish scab which is slowly converted into an amber-coloured, highly insoluble substance which has the properties of the cuticulin fraction of the normal cuticle (Wigglesworth, 1933). That is, it is insoluble in strong hydrochloric acid, highly resistant to solution even in hot concentrated nitric acid, but dissolving at once with the

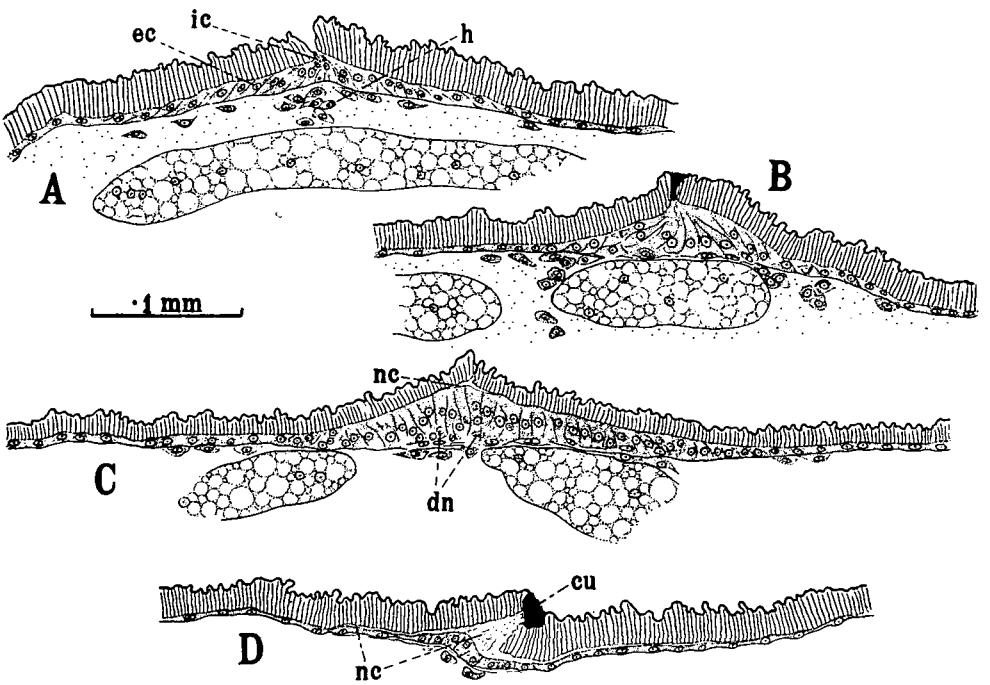

Fig. 5. Sections of epidermis and cuticle of 5th-stage nymph showing healing of simple incision. A, 2 days after cutting; B, 6 days; C, 14 days; D, 1 month. *cu*, plug of dark amber-coloured material (cuticulin); *dn*, degenerating nuclei among the crowded epidermal cells; *ec*, enlarged (activated) epidermal cells migrating towards the cut, their cuticular pole leading; *h*, haematocytes; *ic*, chromatin droplets derived from the injured cells; *nc*, new chitinous cuticle.

formation of oily droplets in hot nitric acid saturated with potassium chlorate (cerinic-acid test). It contains no chitin and dissolves slowly in saturated caustic potash at 160° C. The formation of this substance is described by Blunck & Speyer (1924); indeed, this was the only type of healing they observed in their insects (Fig. 5 D). Over the main surface of the excision where this is covered with paraffin wax, a thin convoluted membrane of cuticulin is likewise formed, often remote from the cells. Below this, in both nymphs and adults, a thick layer composed of chitin and protein is then laid down; so that in 3 or 4 weeks after the injury a new cuticle of the normal composition has been formed. This cuticle lacks the usual bristles. It extends outwards beneath the pre-existing cuticle, becoming gradually thinner

peripherally, approximately to the limit of the zone of "activated" cells (Figs. 5 D, 6 C).

(vii) *Formation of basement membrane.* As the epidermal cells migrate to the margin of the wound they become heaped upon one another; and as they spread over the defect the deeper cells form an irregular layer, their connecting processes interlacing in all directions, the whole being often several cells thick (Fig. 6 B). The uppermost layer of all becomes organized to form the new epidermis and lays down the cuticle as described; the nuclei in the deeper layers eventually undergo

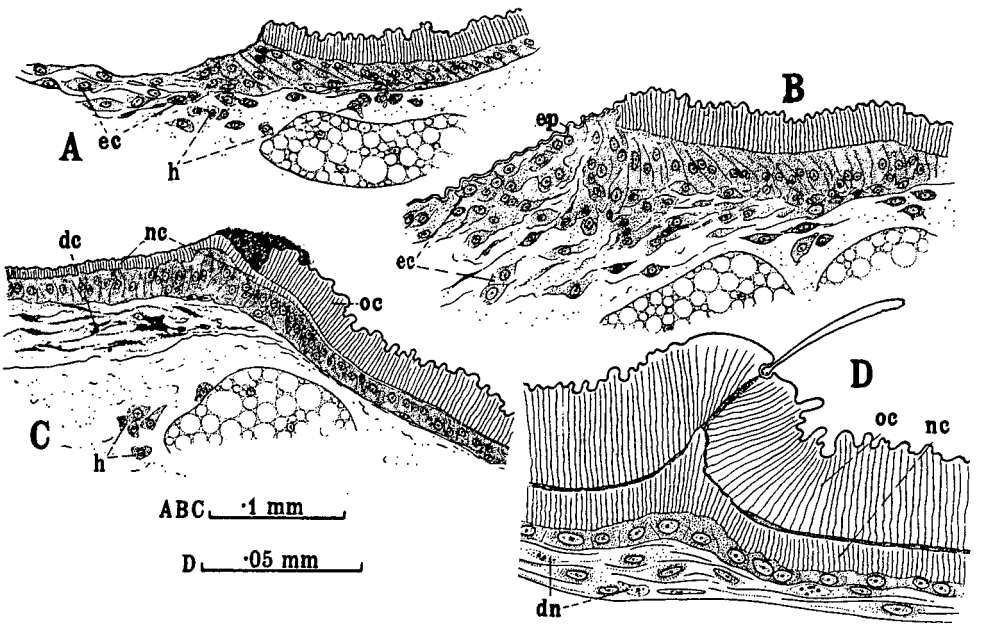

Fig. 6. A–C, sections of epidermis and cuticle of 5th-stage nymph showing healing of excision. A, 6 days after excision, showing epidermal cells spreading over the excised area; B, 14 days, new cuticle just appearing over the excised area; C, 1 month, showing thick layer of a new cuticle extending under the margin of the old cuticle; unwanted epidermal cells and nuclei are breaking down; D, epidermis and cuticle of 5th-stage nymph 6 weeks after burning, showing new cuticle laid down beneath the old, but bristle has not been re-formed. *dc*, degenerating cells and nuclei; *dn*, degenerating nuclei; *ec*, epidermal cells spreading below wound; *ep*, epicuticle; *h*, haematocytes; *nc*, new cuticle; *oc*, old cuticle.

chromatolysis and disappear (Fig. 6 C), and nothing remains but a stout basement membrane. Haematocytes occur scattered over the deep surface of these interlacing cells and in the later stages it is impossible to distinguish them from cells derived from the epidermis; but (contrary to Lazarenko (1925) who believes that the basement membrane is formed from blood cells) after following the migration of these basement membrane cells in sections and in whole mounts, I am of the opinion that they are derived from the epidermis; as indeed Ost (1906), Emmel (1910), Friedrich (1930) and others have found that all the new-formed tissues (with the exception of the nerves) that arise during regeneration in arthropods are derived from the epidermis.

(viii) *Return to the resting state.* The spreading of the epidermal cells over an excised wound may relieve the congestion of cells along the margins, the result of the initial migration. But in the case of simple incisions the cells remain excessively crowded: there is an over-reaction to the injury. A month after such an incision the cells have become small, they have almost reverted to their resting appearance, and they are no longer overcrowded. Arey (1932) has suggested that the overcrowding is relieved by a migration of cells away from the wound again. This would result in a diffuse zone of overcrowding around, which in *Rhodnius* does not exist. There is no doubt that in *Rhodnius* the superfluous cells are dissolved and disappear; pycnotic and disintegrating nuclei may be seen among these overcrowded cells 3 weeks after the injury (Fig. 5 C), as among the underlying cells which are going to form the basement membrane.

ABNORMALITIES OF HEALING

As the epidermal cells spread inwards over a wound, it sometimes happens that the layer advancing from one side overlies that advancing from another. In such cases, it is not always the outermost sheet which becomes organized to form the new epidermis. We have seen how the basement membrane is formed from the

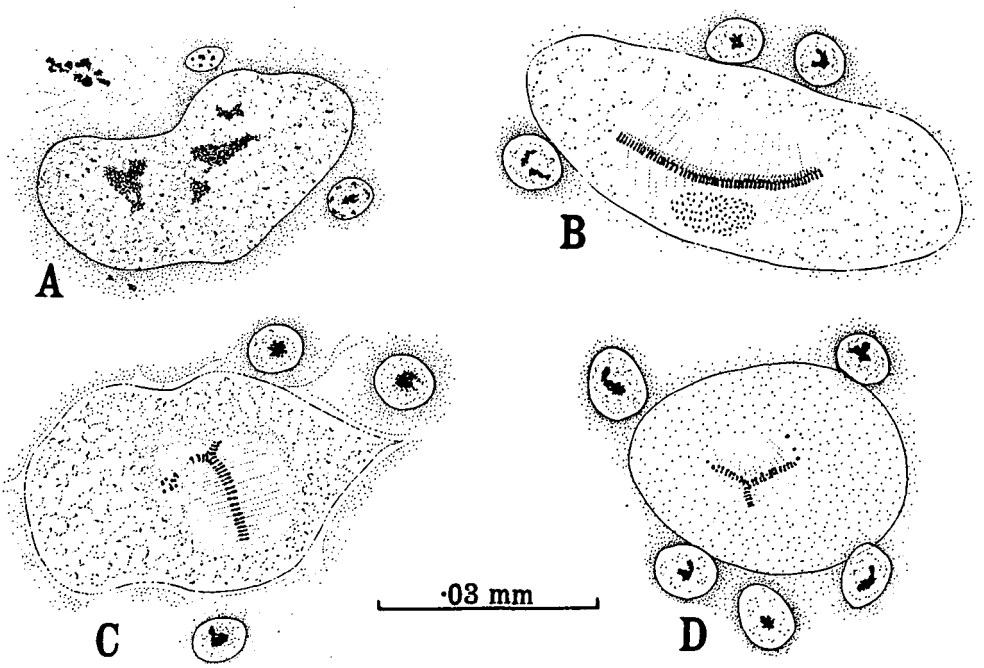

Fig. 7. Abnormal giant cells and nuclei from late stages of healing burns and excisions. A, nucleus of giant cell in adult 1 month after excision; B, giant cell in 5th-stage nymph 3 weeks after excision showing polyploidy and an accessory spindle seen endways; C, accessory spindle in dividing giant cell in 5th-stage nymph 1 month after excision; D, multipolar mitosis in giant cell in 5th-stage nymph 14 days after burn.

innermost layers. Sometimes there is a similar layer of more or less disorganized cells *above* the epidermis, and even after the new cuticle has been laid down this layer of straggling cells (protected as they are by the coating of paraffin wax) can still be detected upon its outer surface. This is the nearest approach that I can find to the state of affairs described by Lazarenko (1928) in *Oryctes*, in which regeneration of the epidermis is said always to take place between two connective-tissue capsules. But whereas Lazarenko states that these capsules are derived from syncytia of wandering blood cells, there is no doubt that in *Rhodnius* (*a*) they are derived from inwandering epidermal cells, and (*b*) the outer cellular membrane at least is generally absent and the most superficial layer forms the epidermis.

Another common abnormality is the occurrence of irregular mitoses, polyploidy, and giant cell formation among the epidermal cells covering the wound. This is particularly common in wounds in starved insects or in decapitated insects (in which metabolism is so reduced that the tissues suffer as in starvation) or in the disorganized layers above or below the definitive epidermis. The nuclei of these cells may attain enormous proportions (Fig. 7 A), multipolar mitotic figures are frequent (Fig. 7 C, D), or the dividing cells may show equatorial plates containing a hundred or more chromosomes (Fig. 7 B). Similar degenerative changes have been described in the pupa of *Culex* during metamorphosis (Holt, 1917) and in insects invaded by parasites (Kowalski, 1919). Eventually all these abnormal nuclei disintegrate and disappear, and a uniform epidermis with evenly distributed cells remains. Likewise in the healing of wounds in mammals multinucleate giant cells appear principally among cells cut off by connective tissue from the overlying epithelium. As healing becomes complete the nuclei of these cells dissolve and vanish (Loeb, 1898).

HEALING OF BURNS

Burns have been inflicted by placing a minute drop of paraffin wax on the cuticle and melting this with a red hot wire. The cuticle is not injured but the underlying cells are killed.

The reactions of the surrounding epidermis to a burn are different from the reactions to a cut. This is readily demonstrated by making a radial incision through the margin of a burn. Three days after the injury (Fig. 8 A) there is a wide zone of activated and migrating cells around the incision, but along the margin of the burn only the cells in the immediate vicinity are activated, and there is very little migration or crowding of epidermal cells. Soon the cells at the margin of the burn begin to spread inwards, often forming a sheet between the dead bodies of the burned cells and the cuticle. Seven days after the injury mitoses are abundant; but whereas around the incision they occur chiefly in the peripheral zone far removed from the cut, they occur only in the spreading margin of the healing burn (Fig. 8 B). These differences have an important bearing on the mechanism of wound healing (p. 375).

When the new epithelium is reformed over the burned area, a new layer of chitin is laid down beneath the old (Fig. 6 D). In severe burns the basement

membrane is reformed as already described by a deeper layer of epidermal cells (Fig. 6 D).

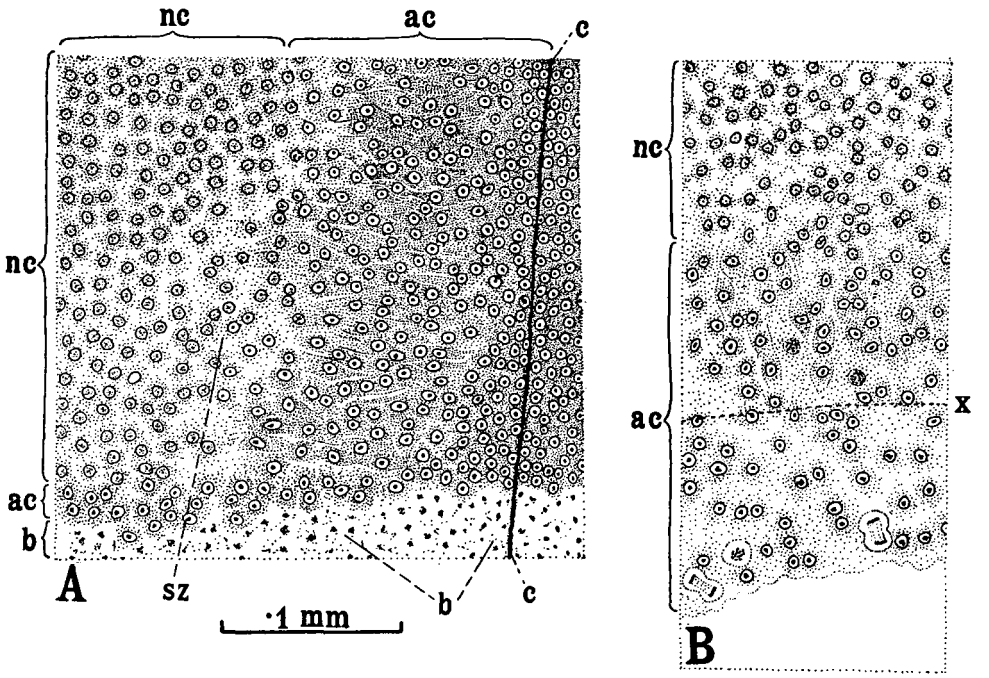

Fig. 8. A, incision through the margin of a burn in 5th-stage nymph, mounted 3 days after operation; B, healing margin of a similar burn mounted at 7 days. *ac*, zone of activated cells; *b*, burned area, cuticle intact; *c*, cut through cuticle; *nc*, normal cells, *sz*, sparse zone; *x*, marks the margin of the area where the cells were killed by burning.

FACTORS REGULATING HEALING

The cause of activation and migration

Since activation and migration of epidermal cells take place at the same time and to the same extent around the wound, they are probably induced by the same stimulus. In many preparations it is evident that migration is most pronounced towards points where the cells have suffered most injury, such as the corners of a rectangular excision, or where there are conspicuous fragments of dead tissue or chromatin droplets derived from the dead cells. This suggests that activation is brought about by chemical substances produced by the injured cells and that migration is a chemotactic response to these substances. But the possibility exists that the interruption of the epidermal syncytium is a necessary stimulus for the movement. This has been tested in three ways.

(i) Epidermal cells scraped from the cuticle and suspended in Ringer's solution were injected into the abdomen of an adult through a small incision. Six days later the tergites were removed and stained. In many places the blackened necrotic remains of the injected cells could be found adherent to the lower surface of the

basement membrane. At these points the overlying epidermal cells had congregated and in the sparse area around many cell divisions were in progress. Thus the healing reaction had been produced without injury to the epidermis or the basement membrane.

(ii) A fragment of cuticle with the epidermis intact, about 1·5 mm. square, was cut from the abdomen of an unfed 5th-stage nymph and inserted through an incision in the abdomen of an adult so that it lay with the cells uppermost against the basement membrane of the adult epidermis. Six days later the tergites of the adult were stained and mounted. Where the basement membrane had been in contact with

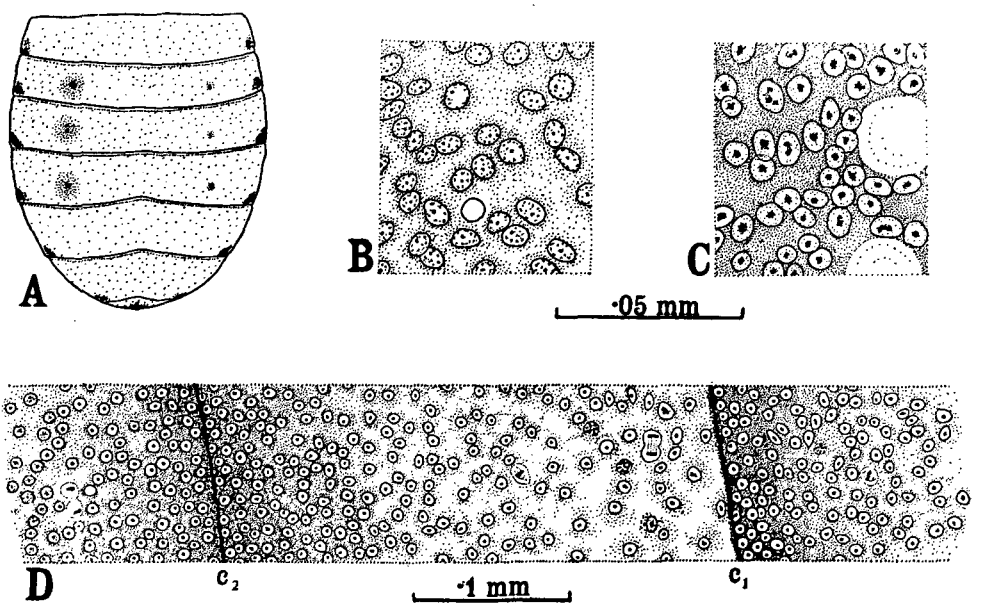

Fig. 9. A, abdominal tergites of 5th-stage nymph showing three control punctures on right, on left three punctures treated with 10 per cent Witte's peptone, mounted at 6 days; B, normal epidermal cells at side of tergites of adult; C, cells from corresponding area on opposite side of body 4 days after the application of crushed tissues to the intact cuticle: vesicles of dermal glands are distended and the cells are activated; D, epidermis of adult mounted 3 days after the second incision (c_2), 6 days after the first incision (c_1).

the cut margin of the implanted fragment, the epidermal cells had congregated and all around were numerous mitoses.

(iii) A still simpler procedure has been merely to apply fresh epithelial scrapings to the outer surface of the cuticle and cover over the mass with paraffin wax. The cuticle is pierced by the ducts of numerous glands particularly abundant at the sides of the abdomen in the adult (Wigglesworth, 1933). When the tergites treated in this way are mounted 4 or 5 days later the intracellular vesicles of the glands are found to be distended and around each a zone of activated cells has collected (Fig. 9 B, C).

The importance of chemotaxis in the movements of the epidermal cells can be shown also as follows. An incision is made in the abdomen of an adult, and 3 days later, when the migration of cells is complete, a second incision is made parallel

to the first, about 0·2 mm. distant. When the cuticle is mounted 3 days after the second incision it is found in many cases that the epidermal cells have left the near margin of the first incision, leaving the cells sparse along the margin, and have migrated away to the second incision (Fig. 9 D). This experiment shows that the chemical stimulus resulting from these slight injuries is short lived, being over in about 3 days.

The nature of the chemical factor

To throw light on the nature of this chemical factor the following procedure has been adopted. On 5th-stage nymphs decapitated 24 hours after feeding a row of minute punctures is made with a finely pointed entomological pin at corresponding positions on either side of the abdominal tergites. The punctures on one side serve as controls, those on the other are dressed with the material to be tested. When this material provides the chemical factor the zone of activated cells and of subsequent mitosis extends much further out from the puncture. In stained preparations 6 or 7 days after the injury the massed cells with nuclei rich in chromatin can be seen with the naked eye as a deep blue zone around the wound; this zone is greatly enlarged in successful experiments (Fig. 9 A). But a more exact comparison is obtained by measuring the radius of the activated zone with a micrometer eyepiece and $\frac{1}{8}$th in. objective. The activated nuclei merge gradually into the resting nuclei; the outermost nuclei showing undoubted change are taken as the limit and measured to the nearest 5 micrometer divisions (100 divisions = 0·33 mm.). The number of mitoses and their distance from the wound can also be estimated, but these afford a less reliable measure of stimulation.

One example may be quoted in detail (Table I). The four punctures on the control side were sealed with paraffin wax; those on the experimental side had an emulsion of fat body applied to them and were then waxed over. The tergites were mounted 6 days later.

Table I

Radius of activated zone in micrometer divisions				
Control punctures	75	70	80	75
Experimental punctures	130	115	120	105
Number of mitotic figures				
Control punctures	4	8	5	1
Experimental punctures	24	35	19	27

Results similar to those in Table I have been obtained with autolysing tissue from other parts of the body: epidermal cells 110–130; muscle 120–140; controls 65–85.

On the other hand, if tissues are taken from insects which have been heated at 100° C. for 5 min. the effect is much less: emulsion of fresh muscle and epidermal cells 115–135; heated muscle and epidermal cells 95–110; controls 65–85. This experiment bears out the observations recorded above on the healing of burns (p. 372, Fig. 8 A): the epidermal cells show far less attraction to cells killed by heat even over a considerable area, than to a small incision.

These results support the idea that the chemical factor is a product of autolysis. As the following results show, it is not specific either for tissue or animal. Dried calf's liver 115–140; desiccated thyroid (B.H.D.) 110–170; acetone dried anterior pituitary (B.H.D.) 120–150; controls 60–80.

Proteins alone also provide the chemical stimulus: 20 per cent casein gel 110–130; emulsion of powdered blood albumen 100–120; controls 70–95.

Still more effective are digested proteins (boiled, applied on minute squares of blotting paper and waxed over): 10 per cent Witte's peptone 150–210; 10 per cent Savory and Moore's peptone 110–125; 10 per cent B.D.H. peptone 120–180.

It was found by Carrel & Baker (1926) that for the stimulation of the growth of fibroblasts in tissue culture the proteose fraction of partially digested proteins was the most active; and Willmer & Kendal (1932) found that the "heteroproteose" fraction of Witte's peptone (the fraction precipitated from a 5 per cent solution in distilled water) was the most effective. The epidermal cells around a wound in *Rhodnius* seem to react differently. Whole Witte's peptone moistened with Ringer's solution and applied to punctures gave values of 130–200; the fraction of Witte's peptone precipitated from a 5 per cent solution in distilled water (amounting to about 3 per cent of the whole) gave values of 95–120. And "Difco" bacto-peptone which contains little proteose gave the same values (120–200) as "Difco" proteose-peptone which is rich in proteoses.

Dipeptide (glycyl-glycine) moistened with Ringer's solution, and the amino-acids glycine, alanine, cystine, tyrosine, and trytophane similarly treated gave the same amount of activation, 75–95, as the controls. The only amino-acid with a stimulating effect was free cystein (110–140). The tripeptide, reduced glutathione, gave the same result (110–160). In both cases perhaps the effect could be ascribed to the known action of sulphydryl compounds in stimulating autolysis (cf. v. Gaza & Gissel, 1932). A strong solution of trypsin gave values of 110–200; the same solution inactivated by boiling gave 75–110.

One can conclude from these experiments that the substances responsible for activation and chemotaxis are protein degradation products: polypeptides and peptones.

The cause of haematocyte accumulation

The accumulation of haematocytes runs more or less parallel with that of epidermal cells, and is doubtless due to the same causes. It is important to note, however, that in very superficial cuts without injury to the basement membrane, the epidermal cells may migrate to the wound and yet no haematocytes accumulate. From this it is clear that the epidermal cells and haematocytes are responding to the same stimulus: the epidermal cells are not dependent for their activation upon substances produced by the congregated blood cells. This is evident, also, from such a section as Fig. 5 A, in which the epidermal cells are crowding around their injured fellows without any regard to the haematocytes below the basement membrane.

The cause of cell division

So far we have considered only activation and migration. What is the relation of these to the subsequent cell division? Cell division occurs only in activated cells (Friedrich (1930) refers to what are here called activated cells as "wachstumsfähiges Gewebe"); but we have seen that cell divisions occur not among the cells crowded round the wound where the chemical stimulus to activation and migration is the greatest, but in the peripheral zones remote from the wound. This suggests that sparseness among activated cells is the factor which determines cell division.

This idea is supported by the fact that in the healing of a burn, in which (perhaps because of the failure of rapid autolysis) there is much less migration to the margin of the wound, it is in the diffuse growing border that mitoses occur (p. 372 and Fig. 8 B). Further support is given by the experiment described (p. 374) in which the epidermal cells are attracted away from one incision by a second incision made 3 days later. Under these circumstances mitoses may become plentiful close to the first incision, the cells there being now very sparse (Fig. 9 D). But the most conclusive evidence is given by a comparison between fed and unfed 5th-stage nymphs. In the unfed nymphs the nuclei are so crowded together (Fig. 1 A) that even after massing round a wound there is no perceptible sparseness in the surrounding zone. Associated with this, mitoses are extremely infrequent after incisions or excisions in unfed nymphs: I have never found more than two or three around a wound—in spite of the fact that the closeness of the cells must result in more of them being injured by the cut. Nor are mitoses produced by applying autolysing tissues to the wounds. Yet there is no inherent incapacity among the cells in the unfed nymph to divide, for abundant divisions occur among the diffuse cells at the margins of healing burns. Conversely, mitoses are extremely plentiful around incisions in the highly stretched epidermis of nymphs in which the anus is occluded immediately after feeding.

Exactly how "sparseness" of nuclei brings about division in the activated cells is, of course, not known. Many hypotheses could be suggested. But it is just possible that here may be one of the factors responsible for simultaneous mitoses among groups of adjacent cells; for obviously sparseness will be felt by all the cells in one spot.

The cause of spreading

The spreading of epithelial cells over a wound is generally regarded as an example of thigmotaxis: the cells, while retaining connexion with one another by their cytoplasmic filaments, apply themselves as closely as possible to flat surfaces. Good preparations showing this can be obtained by excising an area of cuticle and sealing over the opening a fragment of coverglass which is fixed and mounted a few days later. A striking example of spreading was described in an earlier paper (Wigglesworth, 1936): if cylindrical fragments of the head or of a leg are implanted in the abdomen the epidermal cells spread from the cut ends along the outer surface of the cuticle until they unite with the cells spreading from the opposite end. In

this way two superimposed sleeves of epidermal cells continuous with one another at the margins are formed. In the same way the epidermal cells of cut tracheae may spread down the lumen.

The cause of the cessation of new growth

Since the epidermal cells are activated and their growth initiated by the products of autolysis in the injured cells, the removal of these products must be one factor in the cessation of growth. But it is evident from the way in which the cells will continue to grow and spread over a large burned area (in which, as we have seen, activating substances are not abundantly produced) or over a large excision (in which the autolytic products will be little more than in a simple incision of the same size) that the lack of continuity in the epidermis must be responsible for the continuance of growth. Growth (cell movement and cell division) does not cease until this continuity has been restored and a normal density of normal nuclei has been established. Cell division is perhaps self-limited by overcrowding. All abnormal polyploid nuclei and all nuclei which as a result of misdirected growth or over-reaction have come to lie above or below the continuous epidermis then degenerate and disappear.

Obviously there are regulative factors at work here which are not to be described by the simple chemotactic and thigmotactic responses dealt with in this paper. There seems to be a unification within the epidermis that rests perhaps upon the cytoplasmic bridges by which the whole epidermis is bound together. According to the hypothesis of A. Fischer (1925) unification among cells is secured through the interchange of chemical substances ("desmones") by way of these cytoplasmic connexions.

DISCUSSION

The mechanism here described accords in most respects with the general picture of wound healing developed in recent years and reviewed by Arey (1936). As shown particularly by L. Loeb (1898, 1919) and confirmed by many others, migration of the adjacent epidermal cells over the wound is the primary response to injury. Mitoses in the surrounding zone occur later, often when the epithelialization of the wound is complete. Modern explanations of the factors responsible for repair have been derived mainly from a study of tissues cultured *in vitro*. The "formative stimulus" which evokes the renewed growth of wounded tissues is compared by Carrel (1930) to the growth-promoting factor present in embryonic tissue juices. The migration and subsequent division of fibroblasts provided with a minimal quantity of embryo extract is enormously stimulated by the higher degradation products of proteins, especially proteoses (Carrel & Baker, 1926; Baker & Carrel, 1928*a*, *b*; Willmer & Kendal, 1932). Similar degradation products ("trephones") arising from the autolysis of the damaged cells themselves in superficial wounds (Carrel, 1930) or liberated by the accumulated leucocytes in more severe injuries, (Carrel, 1923), are believed to provide the "formative stimulus" in wound healing.

Witte's peptone will accelerate the healing of wounds in man and animals (Wallich, 1926; Kiær, 1927). The so-called "wound hormones" of plants, which stimulate cell division, likewise seem to be products of autolysis in the injured cells. Their formation is prevented by boiling (Haberlandt, 1922). Fischer (1930) recognizes two factors in the initiation of repair. (i) A chemical stimulant produced from the injured cells: saline extracts of wounded tissue cultures will revive growth in colonies which have become latent from lack of embryo extract. (ii) A disturbance in the state of mutual equilibrium among the cells. This equilibrium (its exact nature is not known, though possibly the interchange of "desmones" through the cytoplasmic bridges may provide the basis for it) is upset, through the want of neighbouring cells, at the margin of a cell colony or at the margin of a cut. Therefore, in the presence of the growth-promoting stimulant cell divisions occur in these regions. This idea is expanded by Mayer (1933) who considers that the density of the cells plays a large part as a regulator of growth and form; excessive density causing outward migration of cells, excessive sparseness causing cell division. Arey (1932) also has suggested that mitoses in the zone around an injury may be due to the depletion of cells resulting from migration. And Willmer (1933) has shown that in tissue cultures there is an optimal density of cells for divisions to occur: the cells divide most frequently when they are neither too crowded nor too widely separated.

The present account of wound healing in *Rhodnius* links together these theories. Products of the partial autolysis of proteins in the damaged cells activate the surrounding cells and provide the chemotactic stimulus to migration. They do not evoke cell division unless the mutual relation of the cells (the sparseness of their nuclei) demands an increase in their number. Growth ceases when the products of autolysis have been removed and the epidermal cells have recovered their equilibrium by spreading over the wound, by mitosis in the sparse zones, by degeneration in the dense.

The relation of wound healing to normal growth and diapause

These are problems which will form the subject of future work, but a few points may be noted here. The histological changes in cells and nuclei activated around a wound are precisely those shown by cells during moulting (Wigglesworth, 1933, 1934); and as in moulting, this process is followed by cell division and the secretion of cuticle. It is possible that here may be the key to normal growth. We have seen that the tissues of the adult are capable of repair. This suggests that they should also be capable of moulting. In fact, recent experiments have shown that the previous statement (Wigglesworth, 1936) that the adult bug will not moult in response to the moulting hormone was mistaken: adult *Cimex* have been caused to moult by the hormones of *Rhodnius* nymphs.

It is well known that insects in a state of arrested development or diapause may be caused to renew their growth if pricked or burned or invaded by endophagous parasites. These are stimuli which will cause much cellular breakdown; perhaps that is why they will "break" the diapause and bring about renewed growth.

SUMMARY

Incised or excised wounds in the epidermis of *Rhodnius* nymphs or adults are healed by the following mechanisms: (i) "activation" or enlargement of the surrounding cells; (ii) migration of these epidermal cells and their crowding round the wound; (iii) a simultaneous accumulation of haematocytes; (iv) mitosis in the peripheral zone depleted of cells by this inward migration; (v) spreading of cells over the wound; (vi) the restoration of continuity in the epithelium, the secretion of new cuticle where needed and the degeneration of overcrowded or abnormal nuclei or nuclei that have come to lie outside the epidermis.

In the healing of burns the migration of surrounding cells is much less marked and cell divisions occur chiefly among the cells spreading over the burned area.

Evidence is given that migration of activated cells is a chemotactic response to the products of autolysis of proteins in the injured cells. Cells killed by heat are much less attractive.

Proteins, especially hydrolysed proteins (peptones) from any source will stimulate this process. Dipeptide (glycyl-glycine) and free amino-acids have no effect. Glutathione and free cystein stimulate migration (perhaps by favouring autolysis).

The occurrence of mitoses seems to be determined by sparseness among activated cells. Healing may occur without mitosis if the cells are sufficiently crowded, as in unfed nymphs.

The healing reaction can be produced experimentally without interrupting the continuity of the epidermis. Thus destruction of continuity is not responsible for the initial reaction to injury; but the restoration of continuity appears necessary to bring about the cessation of new growth and the elimination of misplaced cells.

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