

LOCAL AND GENERAL FACTORS IN THE DEVELOPMENT OF "PATTERN" IN *RHODNIUS PROLIXUS* (HEMIPTERA)

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(Received 22 January 1940)

(With One Plate and Thirteen Text-figures)

MOST studies on morphogenesis have been made upon composite structures, such as eyes or appendages, whose form is controlled by the co-operation of a great number of cells. In insects, however, the tiny fragment of cuticle laid down by a single cell may possess morphological characters controlled by the activities of that cell alone. The cuticle of insects provides, therefore, an unusually simple medium for the study of morphogenesis and the nature of those processes which "determine" the activity of a given cell.

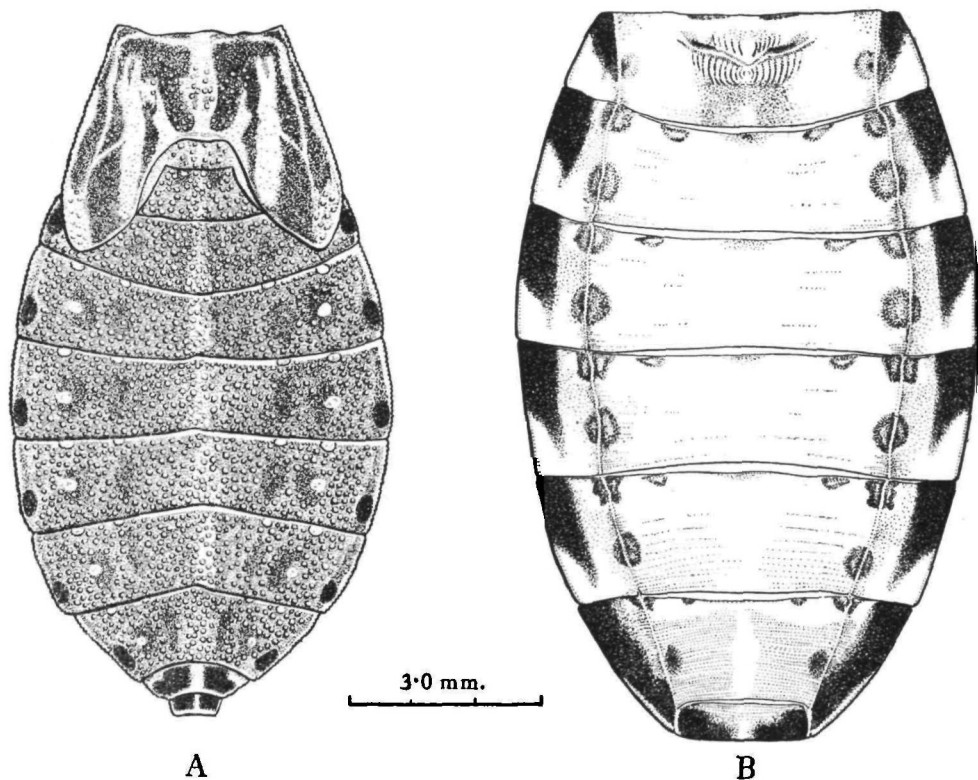
As material for the present work the epidermis of the abdomen in the blood-sucking bug *Rhodnius* has been employed. This consists of a single layer of cells which lays down an extracellular cuticle. The cuticle has a "pattern", since it varies in structure and in coloration in different regions. As we shall see, the individual cells in each region are "determined" for the formation of different elements in this pattern. Certain elements in the pattern, however, are capable of regeneration. In other words, certain of the cells retain potencies which are normally suppressed. The material therefore affords an opportunity of studying the process by which the realization of such latent potencies is brought about. Finally, the pattern shows well marked changes at metamorphosis, and these changes are controlled by hormones (Wigglesworth, 1934, 1936). The material can therefore be used to study the mode of action of these general humoral factors upon local changes in development; to study, that is, the interaction between the circulating hormones on the one hand and the competent tissue on the other.

DESCRIPTION OF THE ABDOMINAL CUTICLE IN *RHODNIUS*

Cuticle of the nymphal stages

The structure and chemistry of the cuticle in *Rhodnius* has been described in an earlier paper (Wigglesworth, 1933); but in order to make what follows intelligible it will be necessary to describe the structure again from a slightly different point of view.

There are five nymphal stages in *Rhodnius*, throughout which the pattern of the abdomen changes comparatively little. Text-fig. 1 A shows the dorsal surface of a 5th stage nymph. Nine segments are visible from above. These are separated by transverse folds in the cuticle—the *intersegmental membranes*. The cuticle is chiefly composed of a relatively thick endocuticle which is exceedingly elastic and enables the insect to ingest up to 12 times its own weight of blood at a single meal. This endocuticle is overlaid by a very thin epicuticle thrown into *stellate folds* (Pl. I, fig. 1) which become partially smoothed out as the endocuticle stretches. The epicuticle is diffusely pigmented with melanin.



Text-fig. 1. A, dorsal view of meso- and metathorax and abdomen of 5th stage nymph of *Rhodnius*. B, dorsal view of abdomen of adult female.

Scattered over the surface are *bristles*, which arise from little mounds of smooth cuticle termed *plaques*. In the region of each plaque an exocuticle is present, the outer part of the cuticle being impregnated with the so-called "cuticulin", and the epicuticle is without folds (Pl. I, fig. 1). Around the bristles the cuticle is therefore inextensible. The bristles and the plaques are unpigmented and thus appear as pale points over the surface of the abdomen. Along its borders the abdomen bears a double or treble row of *marginal plaques and bristles* set very close together (Text-fig. 3 A).

On either side of each abdominal segment there are three *muscle insertions* in the cuticle, two at its anterior margin and one in the middle, which appear as round or oval unpigmented areas. And near the posterior angle of each segment there is an oval *pigment spot* in which the outer layers of the cuticle are deeply impregnated with melanin.

A final element in the pattern is produced by local differences in the numbers of plaques. They are absent from the intersegmental membranes and from a narrow zone on either side of these; there is a zone just internal to the marginal plaques and the pigment spot which is free from them; the areas overlying and immediately around the muscle insertions are devoid of them; and there is always a patch half way between the central muscle insertion and the mid-line of the body which contains a campaniform sensillum and is relatively free from plaques. These areas in which the plaques are scanty are rather more deeply pigmented.

Beneath the cuticle is a single layer of epidermal cells by which it is laid down during moulting. Pl. I, fig. 3, shows the relation between these cells and the plaques. In addition there are numerous little dermal glands opening through the cuticle, and oenocytes scattered below the epidermal cells (Wigglesworth, 1933); with these we shall not be concerned.

Cuticle of the adult

Text-fig. 1 B shows the corresponding pattern in the adult *Rhodnius*. The segments are separated as before by *intersegmental membranes*, but the structure of each segment is markedly changed. Except at the intersegmental membranes, the outer half of the cuticle is impregnated with "cuticulin" to form an exocuticle. It is therefore inextensible. The epicuticle is less markedly folded. The *type of folding* varies in different regions but over most of the surface it consists of little parallel transverse ridges, with minute branched folds running from them (Pl. I, fig. 2). The plaques have disappeared and, except on the 7th and to a less extent on the 6th segments, *bristles* are very scanty over the central area of the tergites. The lateral region of each segment, on the other hand, bears abundant bristles, without plaques, and the double row of *marginal bristles* is again conspicuous, though their form is changed (Text-fig. 6 C).

The *muscle insertions* are present as in the nymph, though they are now covered by pigmented cuticle contrasting with the cuticle elsewhere which is unpigmented. A pigment spot is again conspicuous in each segment; but it lies now at the anterior angle and reaches more than half way across the segment, tapering to end in a point behind.

Somewhat internal to the pigment spot, and passing through the two outer muscle insertions, a line runs parallel with the margin which will be termed the *hinge line*. As previously shown, the exocuticle is wanting along this line (see Text-fig. 8 B, of the next paper (Wigglesworth, 1940)); the cuticle is therefore enabled to bend here after the ingestion of a large meal (Wigglesworth, 1933). In mounted cuticles the hinge line appears colourless against the grey or amber-coloured cuticle

on either side of it. On segments 1 and 2 the cuticle in the central region is pigmented and thrown into little curved parallel ridges.

On the ventral surface of the abdomen abundant bristles, without plaques, are retained. It is not necessary to describe the local differences these show. But close to the lateral margin on the ventral surface there is a longitudinal band of cuticle in which the exocuticle is wanting and the epicuticle is thrown into elaborate longitudinal folds. This is termed the *lateral pleat*; as already described (Wigglesworth, 1933) it is highly elastic and, working in conjunction with the hinge line, it enables the abdomen to expand and receive the enormous meals of blood.

THE PART PLAYED BY INDIVIDUAL CELLS IN THE DETERMINATION OF NYMPHAL PATTERN; THE EFFECT OF WOUND HEALING ON PATTERN

In an earlier paper it was shown that during the healing of wounds in the epidermis of *Rhodnius* the cells around the injury may migrate inwards, without dividing, for considerable distances to make good the defect. In the healing of burns this migration of existing cells is not so great as it is when the tissues are cut; after a burn the dead epidermis is replaced by the cells immediately outside the injury dividing repeatedly and spreading inwards until continuity is restored (Wigglesworth, 1937).

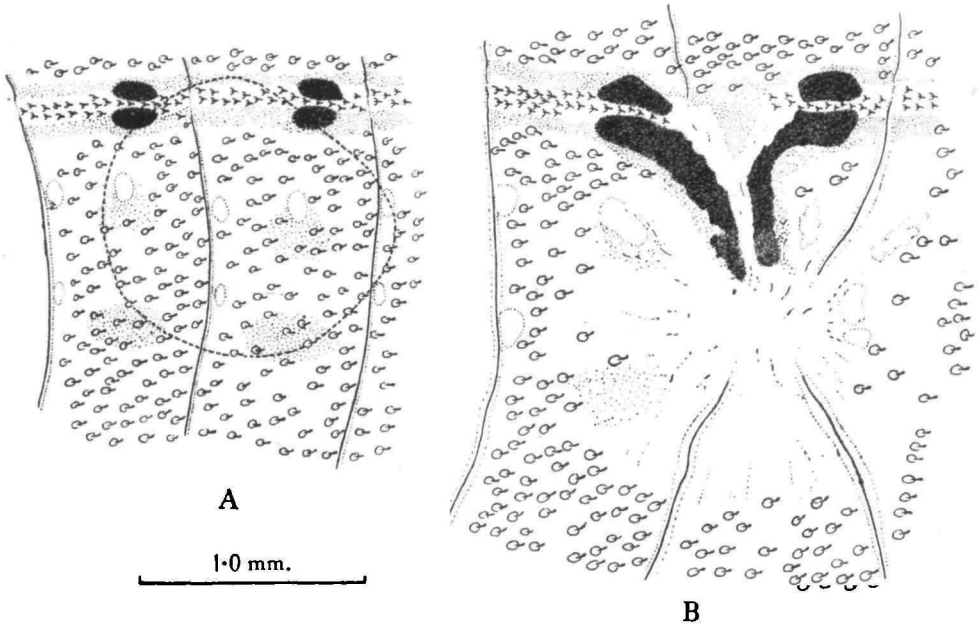
It was interesting to see whether these cells, as they multiply and spread inwards, retain their original capacities to lay down particular elements in the pattern of the cuticle; or whether their potencies are lost and they become "de-differentiated"; or whether they can develop other characters determined by the region into which they migrate.

This question was investigated by burning areas of variable position and extent in the dorsal cuticle of nymphs in the second or third instars by means of melted paraffin wax applied by a heated wire. The burns were inflicted one day after feeding. The insects were then allowed to moult, and the effects on the abdominal pattern noted in later instars.

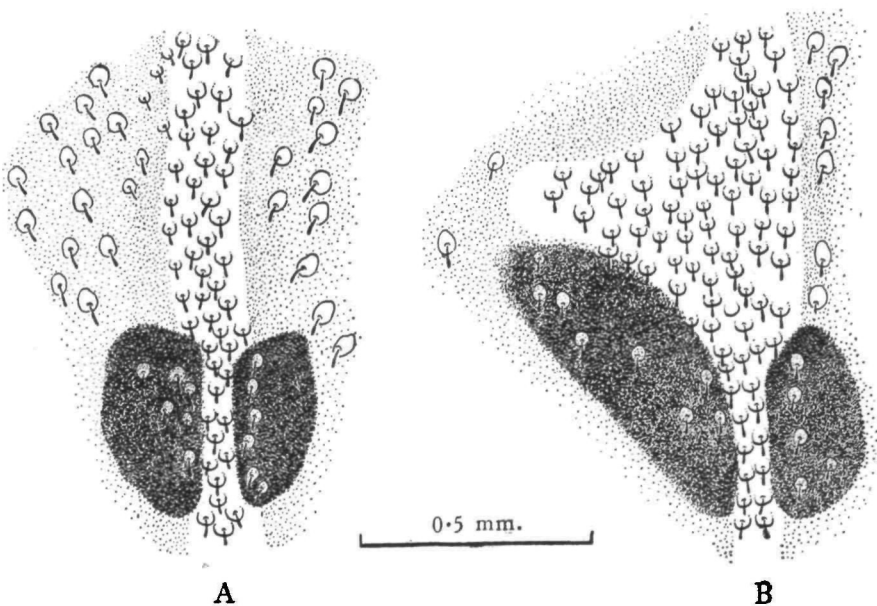
Text-fig. 2 represents a typical example of the results obtained. In Text-fig. 2 A the approximate extent over which the epidermis in the 3rd stage nymph was killed is indicated by the broken line. In Text-fig. 2 B the resulting pattern in the 4th stage nymph is shown. It is evident that as the cells which were destined to form pigmented cuticle divided and migrated towards the centre of the wound they brought with them the capacity to lay down cuticle of this type; and the cells from the intersegmental regions behaved similarly.

Text-fig. 3 B shows another example. The burn in the 3rd stage nymph extended exactly into the line of marginal plaques. Consequently, in the 5th stage nymph, the marginal plaques as well as the pigment spot have spread far inwards. Text-fig. 3 A shows the corresponding region in a normal 5th stage nymph for comparison. Other experiments have shown that when the edge of the burn extends into regions normally devoid of plaques these regions become similarly enlarged.

It is evident that the cells which form a given element in the pattern retain their property of forming this element in spite of repeated division and migration.



Text-fig. 2. A, lateral region of the dorsal surface of two segments in a 3rd instar nymph, showing the double row of marginal plaques above, and beyond this a small piece of the ventral surface. The broken line shows the extent of the epidermis killed by burning. B, the corresponding region in the 4th instar after healing and moulting.

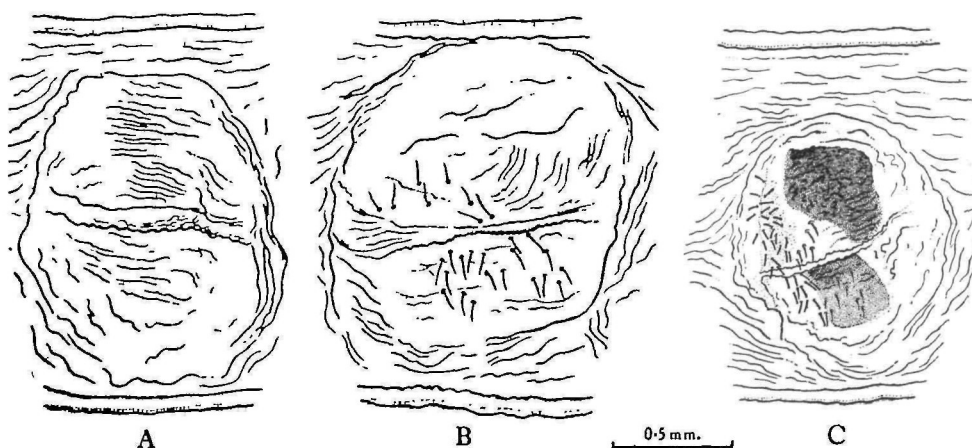


Text-fig. 3. A, marginal plaques and adjacent tergal and sternal black spots in normal 5th instar nymph. B, corresponding region in a 5th instar nymph burned on the tergites in the 3rd instar, showing extension of the marginal plaques and of the tergal black spot.

EFFECT OF WOUND HEALING IN THE NYMPH ON
THE PATTERN IN THE ADULT

It seems probable that the imaginal pattern is already "determined" in the quite young nymph and only awaits the proper hormones to become manifest. For example, first stage nymphs can be caused to undergo a precocious metamorphosis, and assume adult characters when they moult, simply by decapitating them (that is, depriving them of the secretion of their corpus allatum) at a particular critical period in the moulting process (Wigglesworth, 1934).

This question has been further tested by excising small pieces of epidermis plus cuticle (about 1-1.5 mm. square) from different regions of the abdomen of 3rd or 4th instar nymphs and implanting them into the central area of the back in



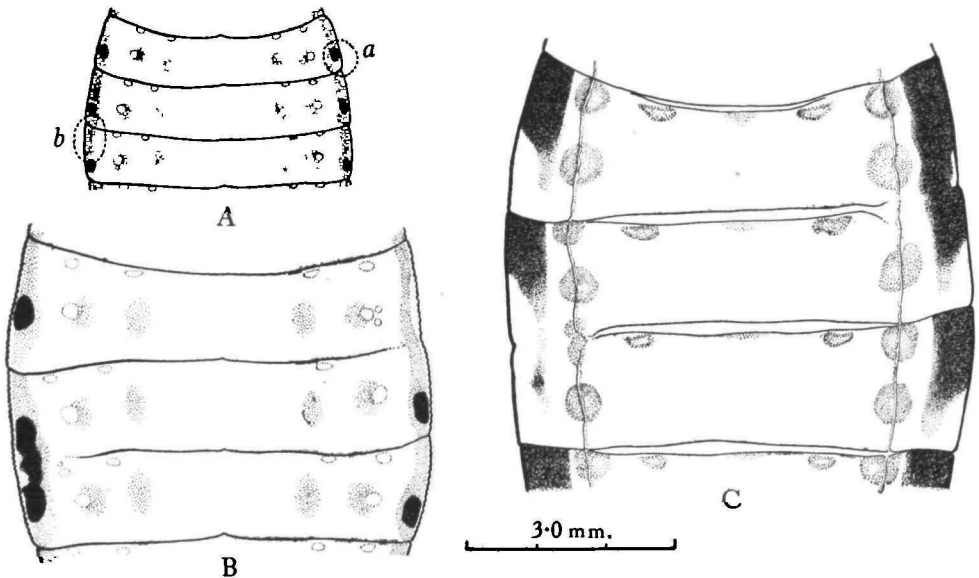
Text-fig. 4. Small pieces of cuticle from 4th instar nymphs implanted into the tergites of 5th instar nymphs and seen after moulting. Above and below in each case are the intersegmental membranes of the adult host. A, mid-dorsal cuticle with intersegmental membrane implanted. B, ventral cuticle with intersegmental membrane implanted. C, dorsal and lateral cuticle implanted, showing intersegmental membrane, pigment spots and marginal spines.

5th instar nymphs, after an excision of a suitable size had been made to receive them; the margins of the wound being sealed with paraffin of low melting point. In each case the cuticle was removed from the 3rd or 4th instar nymph 24 hr. after moulting; that is, at a stage when it was not capable of autonomous moulting. It was implanted into 5th stage nymphs a day or two after they had fed.

When the 5th stage nymphs moulted they became adults; and the implanted fragments of cuticle were also caused to undergo metamorphosis and develop imaginal characters. Then it was found that the implants developed imaginal cuticle of the type corresponding to the region from which they had been excised. Text-fig. 4 shows three examples. In A, the cuticle was removed from the central area of the back; in B, it came from the sternites, which are clothed with bristles in the adult; and in C, it came from the side of the tergites, including the zone of marginal spines.

It appears therefore that although they are not manifested until metamorphosis, the imaginal characters are determined at an early stage. That being so, if this "determination" is again localized in the individual cells, it should be possible to produce changes in the imaginal pattern by injuries inflicted on the young nymphs, of the same kind as those described for the nymphal pattern in the last section.

The experiments were carried out in the same way except that the insects were allowed to transform into adults. Text-fig. 5 A shows the position in which the epidermis was killed by burning in a 3rd stage nymph. The burn *a* completely destroyed the lateral black spot on segment 3. Consequently this spot was absent in the 5th stage nymph (Text-fig. 5 B). But the wound had been healed by means of epidermal cells from the region of the prospective imaginal black spot. Con-



Text-fig. 5. A, third, fourth and fifth tergites of a normal 3rd instar nymph. The broken lines at *a* and *b* show the regions burned. B, corresponding segments in the 5th instar nymph resulting. C, corresponding segments in the adult resulting.

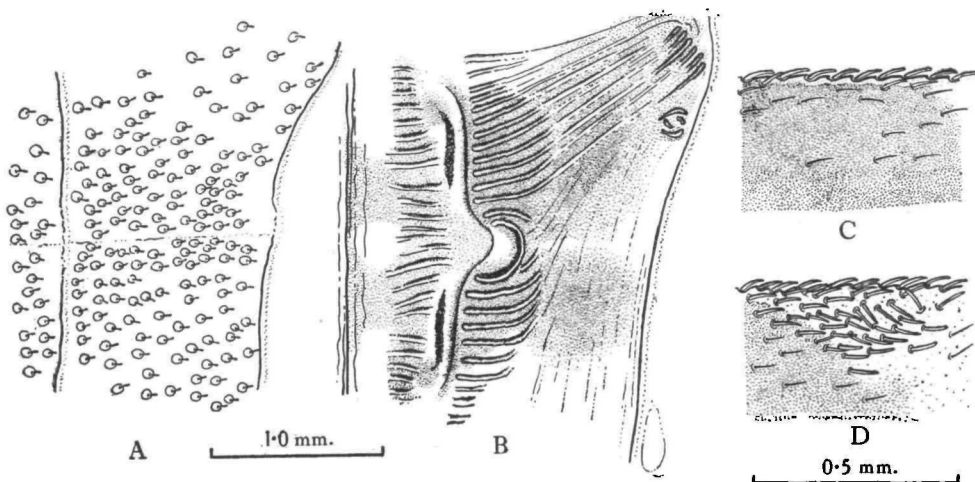
sequently, in the adult (Text-fig. 5 C) the spot on the 3rd segment has become continuous with that on the 4th.

The burn *b*, on the other hand, extended from the margin of the black spot on segment 4 to the margin of that on segment 5, destroying the region of the prospective imaginal black spot. Consequently, in the 5th stage nymph (Text-fig. 5 B) the black spots in this region have become united; whereas the black spot of the adult (Text-fig. 5 C) has been almost eliminated.

Text-fig. 6 illustrates two further examples. B shows the extension backwards and outwards in the adult of the ridged cuticle on the 2nd segment. In the nymph this region of the abdomen does not differ from other parts. Hence, although the change is very obvious in the adult, there was no sign of it in the 5th stage nymph

(Text-fig. 6 A). D shows the inward extension of marginal spines to replace a part of the imaginal black spot; and C shows the corresponding region in the normal insect.

Thus it is evident that the "imaginal determination", like the determination of nymphal characters, is a property of the individual cells already present in the nymph. When the cells divide and migrate, the imaginal properties migrate with them; but these properties become manifest only under the influence of the appropriate hormones at metamorphosis.



Text-fig. 6. A, part of mid-dorsal region of first and second segments in a 5th instar nymph which had been burned on the right side in the 3rd instar. B, corresponding segments in the adult resulting. C, marginal spines and part of one of the black spots of a normal adult. D, corresponding region showing marginal spines displacing the black spot in an adult that had been burned in the 3rd instar.

REGENERATION OF BRISTLES AND PLAQUES

The foregoing experiments prove that when all the cells possessing a given property (determined, that is, for a given purpose) are destroyed, there is no regeneration of the structure they were destined to produce. Development of those elements in the abdominal pattern so far considered is possible only so long as some of the cells which would normally contribute to them remain.¹ This is not what is usually meant by regeneration.

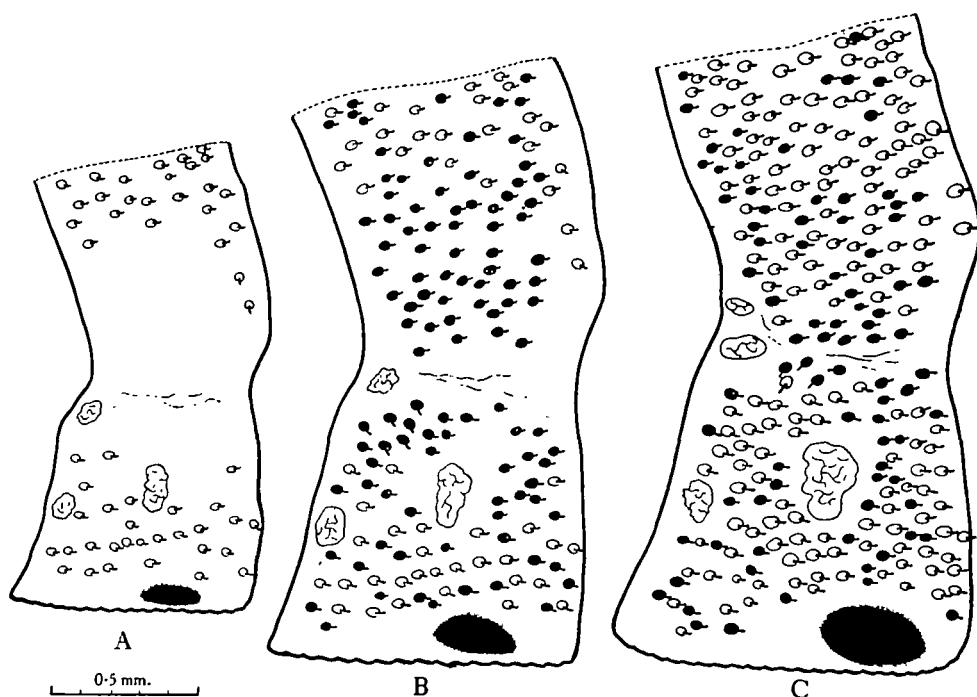
In true regeneration, when cells which would normally produce a given structure are destroyed, adjacent cells become determined for this purpose and realize potentialities which would ordinarily remain permanently latent. This phenomenon is shown by the bristles and plaques in the abdominal cuticle.

Text-fig. 7 shows the result in succeeding instars, of a burn in the general epidermis of a 2nd instar nymph. At the first moult after the burn, that is, in the 3rd instar, the burned area is covered by cuticle with stellate folding of the usual

¹ From the experiments described it cannot be stated definitely that *no* cells outside the pigment spots, for example, retain the power of forming pigmented cuticle; but it seems probable that this is so.

nymphal type, but no new plaques arise—or at most two or three new ones at the extreme margin (Text-fig. 7 A). At the next moult, plaques have appeared all over the cuticle with the exception of a small central region which is most remote from the margins of the burn (Text-fig. 7 B). At the next moult, that is, in the 5th instar, new plaques have appeared in this central region also (Text-fig. 7 C).

Clearly, groups of cells at intervals from one another have become transformed into bristle- and plaque-forming units. The number and regular distribution of these units precludes the possibility that they have been produced by cells already



Text-fig. 7. A, left half of third tergite of 3rd instar nymph after a burn in the 2nd instar. B, the same in the 4th instar. C, the same in the 5th instar. In B and C the newly formed plaques are shaded, those appearing at previously existing sites plain.

determined for this purpose. It is in fact evident that in appropriate circumstances cells which would normally contribute to the ordinary stellate intervening cuticle may become determined for the production of bristles or to contribute to the plaques around them.

When the burn is very extensive, a large central region may not only be devoid of plaques, but colourless and without stellate folding at the first moult after the burn. If the cuticle and epidermis are stained, the reason for this is apparent: the cells in this region are rather sparse and very irregular, with giant cells and other abnormalities previously described (Wigglesworth, 1937). Clearly, moulting has occurred before the epidermis in the central region has had time to reorganize itself properly. That is doubtless the reason why in Text-fig. 7 the central area of

the wound failed to develop plaques until the third moult after burning. This same figure shows another area (posterior to the centre of the burn) in which plaques failed to appear. That is evidently because this area has been regenerated by cells from the region of the intersegmental membrane where plaques are always absent.

It is worth noting that, with few exceptions, the regenerated plaques and bristles show the normal orientation. This raises a new problem which has not been further investigated.

Before discussing the mechanism by which these regenerating plaques are determined, we must consider the factors which control the appearance of plaques during the normal course of development.

DETERMINATION OF BRISTLES AND PLAQUES DURING NORMAL DEVELOPMENT

We have seen that there are certain areas in the abdominal cuticle which are devoid of plaques (p. 182). These deficiencies are doubtless caused by local differences in the properties of the cells in these regions, of the type described in foregoing sections. We have indeed seen that during the migration of cells in the healing of wounds, this property of not laying down plaques is retained by the cells from these regions. Over the greater part of the abdominal segments, however, the plaques are pretty evenly distributed. In this section we shall consider the factors which control this distribution.

Throughout this section of the work the dorsal part of the third abdominal segment has been used as the standard object of study. During moulting the old cuticle splits down the mid-line of the thorax, and the split extends into the first three or four segments of the abdomen. The old cuticle as cast at moulting, or the new cuticle formed, have been mounted, and the plaques in one entire half of this tergite, excluding the marginal plaques, have been traced by means of a drawing apparatus¹ and counted.

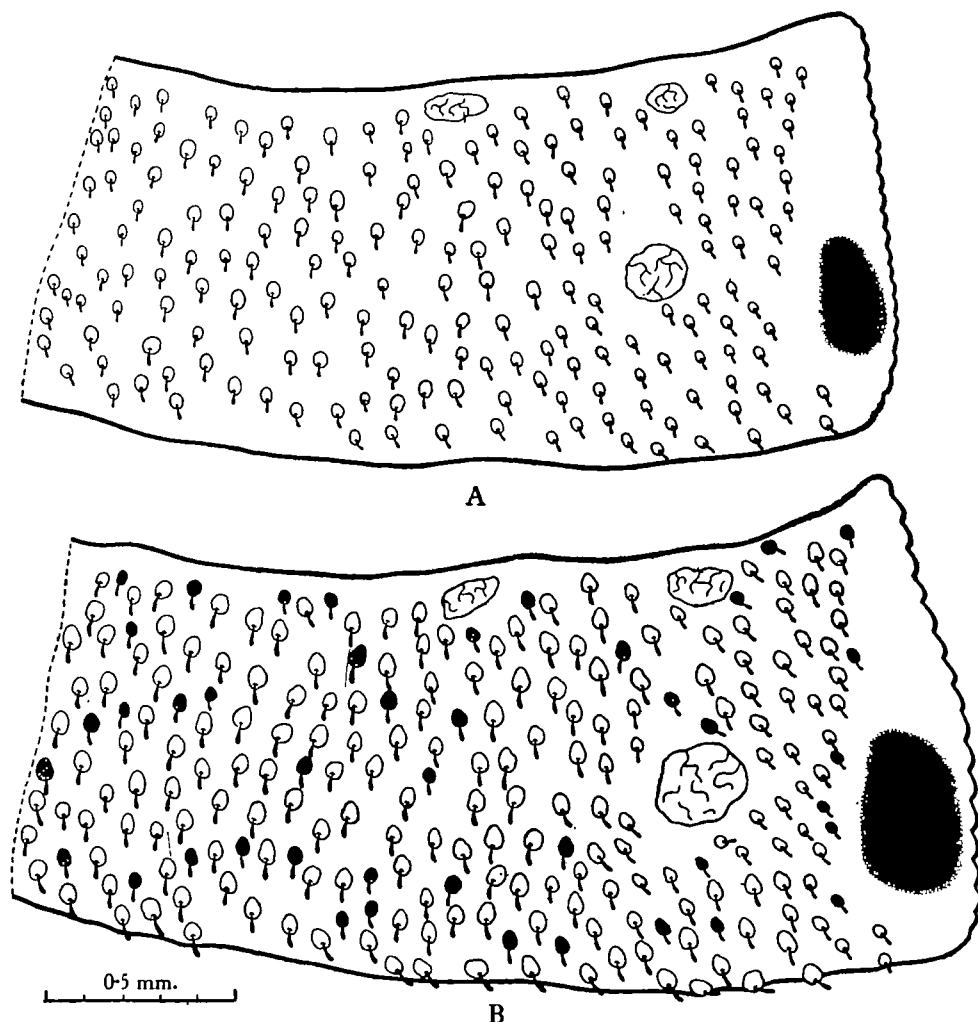
Text-fig. 8 shows a typical result obtained during the moulting of a 4th stage nymph. As pointed out in an earlier paper (Wigglesworth, 1933), the bristle-forming cells persist from one instar to the next; so that most of the plaques arise at the site of the previous plaques. But a certain number of new plaques are developed at each moult between the existing ones. In Text-fig. 8 B, the new plaques are shown shaded, the old plaques plain.

The first question that arises is whether these new plaques are "predetermined", or whether their appearance is the result of the local conditions to which the cells are exposed—for we have seen that the potentiality to form plaques is apparently retained by all the ordinary epidermal cells.

It is evident in Text-fig. 8 that, apart from regions where the plaques are naturally sparse, new plaques make their appearance at those points where the old plaques were most widely separated. That suggests that the distance from existing plaques may be the factor which leads to their determination. This question was tested by increasing artificially the mutual separation of the plaques by allowing

¹ The 'Moscon Macrograph' was used.

the insect to ingest a full meal of blood and then immediately occluding the anus with paraffin wax so that the excess fluid could not be excreted in the usual way and the abdomen remained enormously stretched.

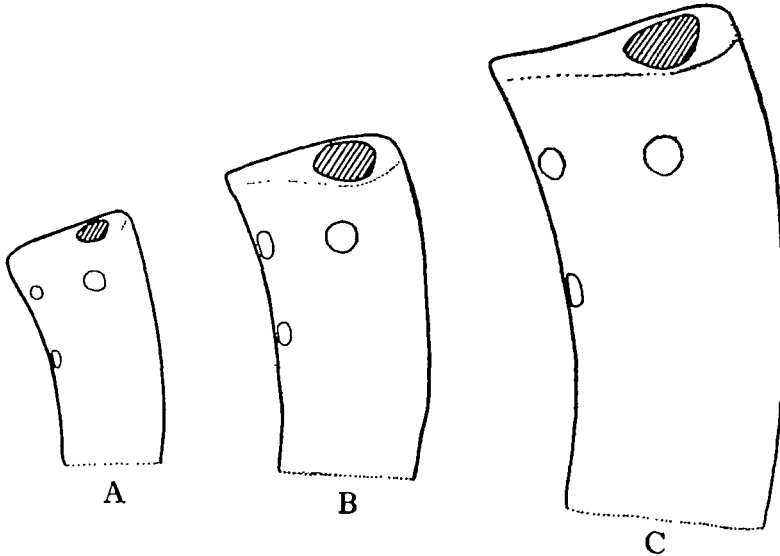


Text-fig. 8. A, right half of third tergite in normal 4th instar nymph. B, the same in 5th instar, the newly differentiated plaques shaded.

Table I shows the results obtained. The number of plaques on half of the third segment of six 4th stage nymphs ranged from 174 to 217 with an average of 191. After moulting, in the normal insect, to become a 5th stage nymph, they ranged from 226 to 287 with an average of 244. The increase thus ranged from 19.3 to 32.1% with a mean of 26.9%. In five 4th stage nymphs in which the anus was occluded, the number of plaques showed an average increase of 26.7%.

Table I. *The number of plaques on half the third abdominal tergite in 4th and 5th stage nymphs, showing the percentage increase when the 4th stage nymphs were normal, excessively stretched, or unfed*

	1	2	3	4	5	6	Mean
4th nymph (normal)	174	179	192	204	181	217	191
5th nymph	229	229	237	260	226	287	244
Increase %	31.5	27.9	23.4	27.4	19.3	32.1	26.9
4th nymph (stretched)	190	193	202	211	—	—	199
5th nymph	238	237	261	274	—	—	252
Increase %	25.2	22.8	29.2	29.8	—	—	26.7
4th nymph (unfed)	168	182	175	—	—	—	175
5th nymph	203	239	228	—	—	—	223
Increase %	20.8	31.3	30.3	—	—	—	27.4



Text-fig. 9. Right half of third tergite in 5th instar nymph. A, when 4th instar was unfed. B, when 4th instar was fed normally. C, when anus of 4th instar was occluded.

The converse experiment, of testing the effects of a minimal amount of separation between the plaques, was performed by inducing moulting in unfed 4th stage nymphs by decapitating them and joining them, in the manner already described (Wigglesworth, 1934), to 4th stage nymphs in which the anterior part of the head was removed, leaving the corpus allatum intact, at six or seven days after feeding. In three such experiments the mean increase in the number of plaques was 27.4% (Table I).

Text-fig. 9 shows the relative area of the half segment under consideration in these three cases—unfed, normal and stretched. The area which the plaques occupy

is in the ratio of about 5.4, 10.0, 21.7 in the three cases; and yet this difference has no effect on the number of new plaques formed.

These results appear to disprove the influence of mutual separation in determining the appearance of new plaques. But the possibility remains that it is the degree of separation at the *preceding* moult which is important. This was tested by occluding the anus immediately after feeding in 3rd instar nymphs; allowing them to moult to the 4th instar; and then feeding these again without occlusion.

Table II. *The number of plaques on half the third abdominal tergite in 3rd, 4th and 5th stage nymphs, showing the percentage increase when the 3rd stage nymphs were normal or excessively stretched*

	1	2	3	4	5	6	7	Mean
3rd nymph (normal)	126	112	143	119	139	128	147	131
4th nymph	186	172	204	181	217	202 ¹	211 ¹	196
Increase %	47.6	53.5	42.6	52.1	56.1	57.8	43.6	50.4
5th nymph	—	—	260	226	287	261	274	261
Increase %	—	—	27.4	19.3	32.1	29.2	29.8	27.5
3rd nymph (stretched)	132	130	137	128	138	—	—	135
4th nymph	195	195	190 ¹	186 ¹	206 ¹	193	—	194
Increase %	48.5	50.0	38.7	45.3	49.3	—	—	46.3
5th nymph	265	274	286	271	289	292	—	280
Increase %	35.8	40.5	50.5	45.7	40.0	51.3	—	43.8

¹ These insects, in addition to the 3rd stage nymphs, were excessively stretched by occlusion of the anus.

Table II shows the results obtained. The normal 3rd stage nymph has an average of 131 plaques on the standard half segment. On moulting to the 4th stage the plaques show a mean increase of 50.4 % (42.6–57.8). As in the case of the moulting 4th stage, as already described, occlusion of the anus in the 3rd stage nymph caused no increase in the number of plaques formed: 46.3 % (38.7–50.0).

On the other hand, excessive stretching in the 3rd instar causes a great increase in the number of plaques formed when the 4th instar moults to become a 5th. The mean increase in plaques during this moult was 27.5 % when the 3rd stage had been treated normally. But when the anus had been occluded in the 3rd stage this increase amounted to 43.8 % (35.8–51.3) (Text-fig. 10).

In some of these experiments the anus was occluded again in the 4th instar immediately after feeding; but this caused no further increase in the number of plaques as compared with those nymphs which were stretched only in the 3rd instar.

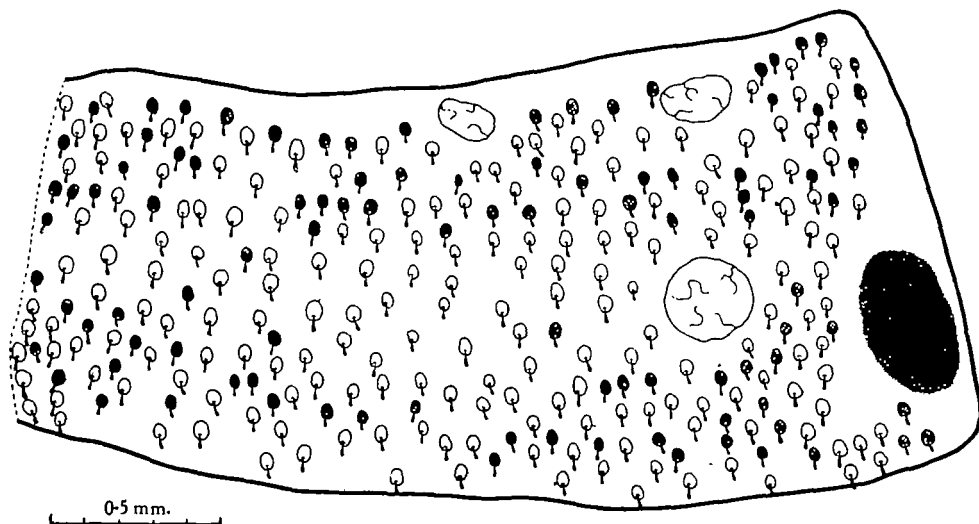
An attempt was made to rear *Rhodnius* from the first instar, occluding the anus at every meal. Most of them died, and only one reached the 4th instar, dying in the attempt to escape from the old skin. This insect gave the following counts on half the third abdominal tergite, the figures in brackets showing the percentage of new plaques at each moult:

1st, 38; 2nd, 79 (108 %); 3rd, 153 (94 %); 4th, 248 (62 %).

The corresponding counts on a normal control were:

1st, 39; 2nd, 84 (115 %); 3rd, 128 (52 %); 4th, 191 (49 %).

As before, the effect of stretching is not apparent until the second moult.



Text-fig. 10. Right half of third tergite in 5th instar nymph, the 3rd instar of which had been excessively stretched by occlusion of the anus. Compare with Text-fig. 8 B.

EFFECT OF PROXIMITY ON THE SIZE OF PLAQUES AND BRISTLES

Although the mutual separation of the plaques does not influence the determination of new plaques until the succeeding instar, it has an immediate effect on the *size* of the plaques and bristles both old and new. There are considerable differences in the size of plaques in different regions of the segment; for the purpose of comparison, therefore, the group situated at the centre of the triangle formed by the three muscle insertions has been chosen. Text-fig. 11 shows the plaques in this region in a normal 5th instar nymph (A), in a 5th instar nymph derived from a 4th stage in which the anus was occluded after feeding (B), and in a 5th instar nymph derived from an unfed 4th stage (C). It can be seen that both plaques and bristles are enlarged when they are well separated, greatly reduced when they lie close together.

INFLUENCE OF HORMONES ON THE NUMBER AND SIZE OF BRISTLES AND PLAQUES

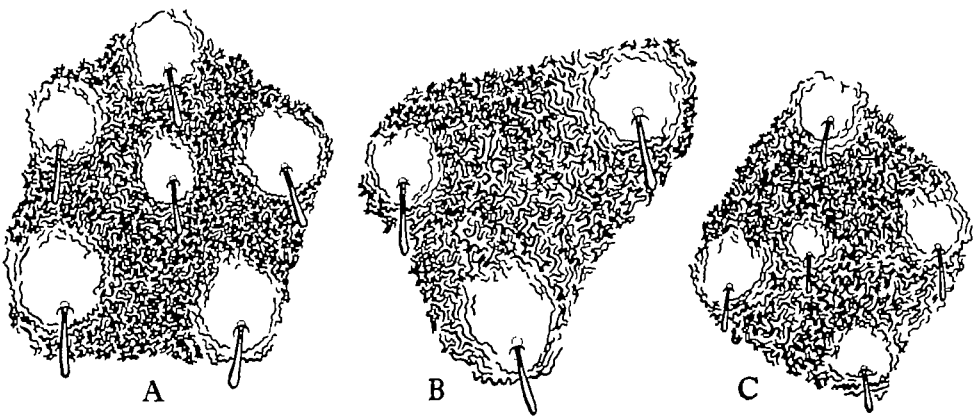
Since the number of new plaques appearing is decided by the mutual separation of the plaques at the preceding moult, must one conclude that at the commencement of a given moult the new plaques are already predetermined?

This question has been answered as follows. We have seen that during the moult from the 3rd to the 4th instar, the plaques show an increase of 50.4 % (Table II).

Whereas in moulting from the 4th instar to the 5th, the increase is only 27.5% (Table II). Now in an earlier paper (Wigglesworth, 1936) it was shown that the characters of the various nymphal instars are controlled by the hormone or hormones secreted by the corpus allatum. For example, a 4th instar normally develops 5th instar characters when it moults; but if it is decapitated and exposed to the secretion of the corpus allatum of a 3rd instar it develops 4th instar characters again. If, therefore, the new plaques are not predetermined before moulting, a 4th stage

Table III. *The number of plaques on half the third abdominal tergite in 4th stage nymphs and in the instar derived from these by joining them to 3rd stage nymphs with the corpus allatum present*

	1	2	3	Mean
4th nymph	192	178	191	187
New instar	257	246	262	255
Increase %	33.8	38.2	37.1	36.3



Text-fig. 11. Corresponding groups of plaques in 5th instar nymphs. A, after 4th instar had been fed normally. B, after 4th instar had anus occluded after feeding. C, after 4th instar had been unfed.

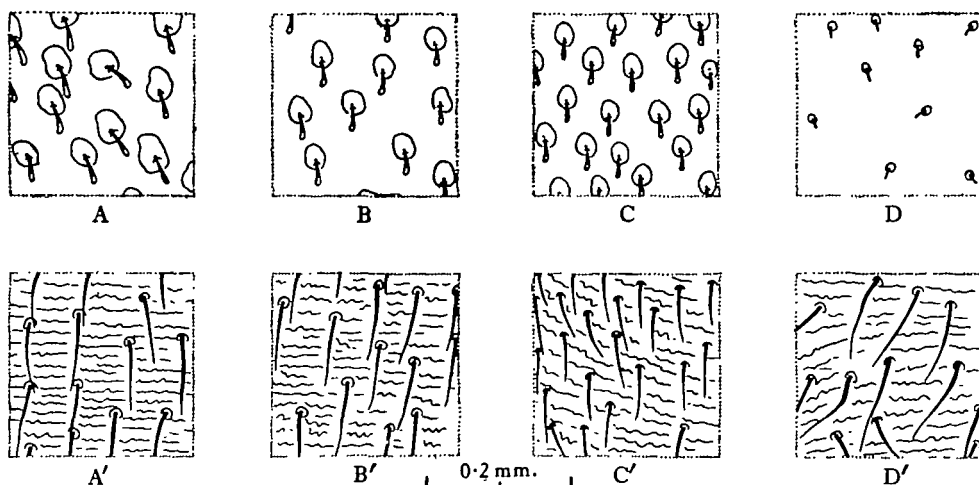
nymph caused to moult by decapitating and joining to a 3rd stage nymph with its corpus allatum intact should produce more new plaques than a normal 4th stage nymph.

Table III gives the results of three such experiments. Instead of the normal increase of 27.5%, the plaques show an increase of 36.3%.

It is evident from these results that the number of plaques developed at a given moult is influenced by the hormones to which the epidermis is exposed during moulting. The plaques are clearly not predetermined at the commencement of the moult.

The size of the plaques and bristles is similarly influenced. A group of plaques

internal to the central muscle insertion in a 5th stage nymph ranged in width from 40 to 50μ (average 45μ) with bristles ranging from 36 to 46μ (average 42μ). A corresponding group in an instar produced from a 4th stage nymph by exposure to the corpus allatum of a 3rd stage, ranged in width from 27 to 40μ (average 37μ) with bristles of 33 to 40μ (average 37μ). This reduction in size may be a direct effect of the hormone or it may perhaps be secondary to the increased number of plaques; for we have seen that when the plaques are closer together their size is reduced (Text-fig. 11).



Text-fig. 12. Approximately corresponding areas of cuticle in the sternites of nymphs above, and adults derived directly from them below. A, A', normally fed 5th stage nymph. B, B', normally fed 4th stage nymph. C, C', unfed 4th stage nymph. D, D', normally fed 1st stage nymph.

EFFECT OF PROXIMITY ON THE SIZE OF BRISTLES IN THE ADULT INSECT

In the adult *Rhodnius* bristles are confined chiefly to the sternites and the lateral region of the tergites. These bristles are derived from the bristles already present in the 5th stage nymph, and the effect of mutual proximity on their size has been studied by inducing metamorphosis in nymphs in which the plaques were separated to a variable extent.

For example, Text-fig. 12 A, A', shows the changes in the ventral bristles during the moulting of a normal 5th stage nymph. Text-fig. 12 B, B', shows the corresponding changes when an imaginal moult is induced in a 4th stage nymph (by decapitating it and joining it to a moulting 5th stage). The plaques are about equally separated in the 4th and 5th stages and the imaginal spines developed are about the same size in the two cases. But if the same experiment is performed on an unfed 4th stage nymph, in which the plaques are much closer together, the imaginal spines are very much smaller (Text-fig. 12 C, C'). Finally, Text-fig. 12 D, D' shows that even when metamorphosis is induced in a 1st stage nymph, the imaginal spines are equal in size to those in the normal adult.

DISCUSSION

The effect of proximity on the size of plaques and bristles is perhaps to be explained quite simply. When the cuticle is stretched the cells which build the plaques are stretched also. They occupy a larger area and therefore lay down a larger plaque. At the same time the cells are more freely exposed to the circulating blood. Perhaps they are better nourished, and perhaps it is merely for this reason that a larger bristle is formed. Conversely, when the plaques are close together, as in the unstretched insect, conditions for the cells are perhaps less favourable and smaller bristles are laid down.

The effect of proximity on the number of plaques is a more important matter. Here the closeness of existing plaques affects the determination of new plaques; and the explanation of this phenomenon may throw light on the whole question of determination. Apart from other factors, dependent on the regional differences in the pattern of the cuticle, each plaque appears to exert an inhibitory influence around it and to prevent the development of new plaques within a certain radius.

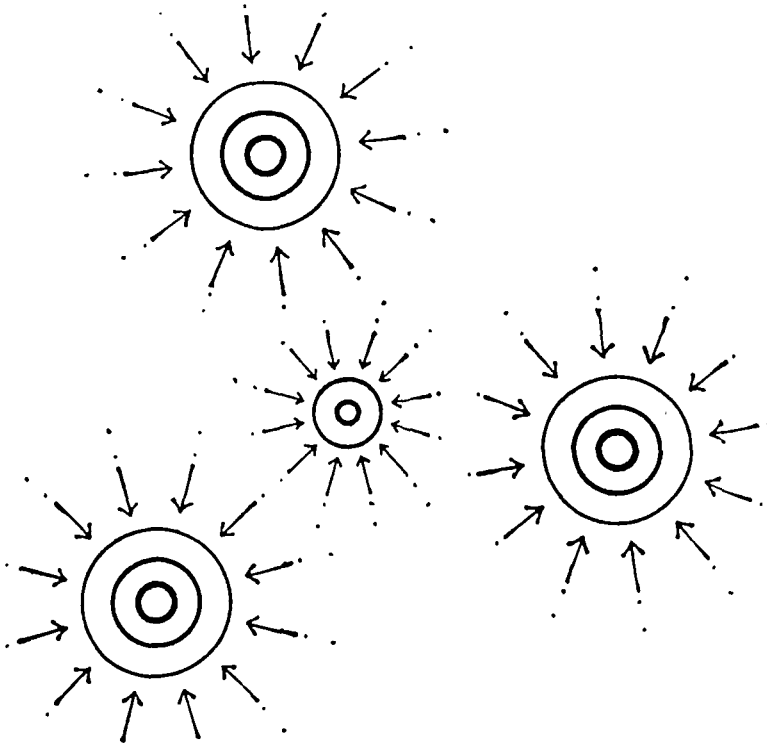
It is not, however, the absolute separation of the plaques which determines the radius over which this influence acts. For the number of new plaques that appear during the moulting is the same whether the insect is unfed and the cuticle not stretched at all, or whether it is stretched to the utmost by occlusion of the anus after a full meal. This suggests that it may be the number of cells intervening between plaques which is important; for in the early stages of moulting, this number is of course the same whether the insect has been fed or not.

This idea is supported by the effect of distension at one moult upon the number of new plaques appearing at the next. For when the cuticle has been stretched by blocking the anus, there is a compensatory increase, during moulting, in the number of epidermal cells. For example, in one 4th instar nymph in which the anus had been occluded after feeding in the 3rd stage, a square in the epidermis with a side of 75μ contained 87 nuclei; and an equal square in a corresponding part of the epidermis of a normal 4th instar nymph contained 84 nuclei. Thus the number of cells per unit area is the same. Consequently, since the plaques are more widely separated, the number of cells which lie between them must be greater in the insect that had been stretched. For example, in these same two insects, the number of nuclei lying within the square formed by four plaques just internal to the central muscle insertion was 148 in the nymph which had been stretched, 119 in the control. If the *effective distance* between plaques is to be measured by the number of cells intervening, we should expect an increase in the number of new plaques appearing when these stretched insects moult again. That is, in fact, what happens (p. 192).

At what stage of development does the determination of plaques occur? The number of new plaques arising at a given moult is influenced by the nymphal stage from which the moulting hormones are provided (p. 194). Hence the new plaques cannot already be determined before moulting begins. Their number is probably

influenced, as we have seen, by the number of cells intervening between existing plaques at the commencement of moulting. Now the number of cells increases rapidly after the critical period when moulting is well established. The determination of new plaques therefore occurs probably during the early days of the moult or soon after the critical period.

What is the nature of the inhibitory influence exerted by the plaques upon the surrounding cells? Many physical and chemical possibilities come to mind; but as a working hypothesis the following has been adopted (Text-fig. 13). It is supposed



Text-fig. 13. Diagram to illustrate hypothesis of determination of plaques. Explanation in text.

that as soon as moulting is initiated by the moulting hormones, the existing plaque-forming and bristle-forming centres become active, and draw from the surrounding cells some factor essential for their activity. (As pointed out in an earlier paper (Wigglesworth, 1937), protoplasmic bridges connect the epidermal cells with one another.) The adjacent cells, drained of this essential element, are therefore incapable of realizing their potentialities and forming bristles or plaques. But some cell sufficiently remote from existing plaques is able to become active; it thus forms the centre of a new bristle- and plaque-forming system which likewise draws the "essential element" from the zone immediately around it.

There are many phenomena, such as the appearance of new plaques in the un-

occupied areas, the inward spreading of plaques across a healed wound, the occasional appearance of contiguous plaques, which can be explained along these lines. But it is scarcely necessary to point out that the mechanism is at present a pure hypothesis, the purpose of which is merely to provide a mechanical analogy for the explanation of the observed phenomena.

It may be pointed out that an inhibitory influence of some kind, preventing the appearance of like organs within a certain limiting radius, has been invoked to explain the appearance of new zooids in colonial organisms such as hydroids. Similarly, a limb disk of a *Urodele* larva grafted on to the flank of the same animal will develop into an independent limb only if it is sufficiently remote from its original position (Huxley & De Beer, 1934); and Goldschmidt (1921) describes duplication arising in the valves of the external genital organs of certain intersexes of *Lymantria* without injury, which he attributes to an abnormal degree of separation of parts which ought to be in contact.

The nature of the determination process. It is obvious that the epidermal cells must contain the intracellular metabolic systems which are responsible for the types of cuticle they lay down. At an early stage of embryonic development the cells must contain the systems, or the precursors of systems, necessary for the production of all types of cuticle. During development they lose the potency to form particular types and become progressively more specialized. This progressive loss of potency is often called "determination".

But certain cells retain the faculty of laying down several types of cuticle. Thus the general epidermal cells in the *Rhodnius* nymph may lay down ordinary stellate cuticle, or form bristles, sockets or the smooth plaques around them. The process by which one or other of these latent systems is activated, or its mode of action altered in such a way that it leads to the deposition of cuticle of a given type, is again called "determination".

Finally, when the cell is committed to form a bristle, for example, the type of cuticle it will lay down is very different at a nymphal moult and at metamorphosis. The intracellular system is thus subject to a further modification through the action of the hormones controlling metamorphosis. The nature of the action of these hormones will be considered in the next paper, but we may provisionally adopt the hypothesis that by chemical means they modify the action of the intracellular metabolic systems to which we have referred.

To sum up; the following steps are recognized in the development of an animal such as *Rhodnius*. (i) A progressive loss of potency, or a restriction of the range of structures that may be formed by a given group of cells. (ii) An activation of one of the remaining potencies leading to the development of a particular structure. (iii) Metamorphosis, or the modification to a greater or lesser extent of the course of development following upon this activation. Different authors have used the word "determination" to describe the processes by which, at each of these steps, the cells become committed to a particular activity. But in the light of observations to be described in the next paper it seems possible that the change which takes place at the third step is of a different nature from the other two.

Relation of the cells to determination. It was interesting to use the material studied in order to show the relation of the individual cells to the determination process. The experiments on wound healing have shown that when the cells divide repeatedly, and migrate long distances over a wound surface, they still retain their existing state of determination (p. 183). Their special character is transmitted, apparently indefinitely, to the daughter cells. And this applies not only to those characters which are manifested at that time (for example, nymphal characters in a nymphal insect) but to those characters which will not become apparent until metamorphosis (imaginal characters are equally carried and displaced by the migration of cells across a healing wound in a nymph) (p. 186). In both cases the individual cell and its progeny are all-important in determination. It is therefore possible to look upon the first process of determination mentioned above (the progressive limitation of potency among certain groups of cells) as a process of orderly "somatic mutation" resulting in strains of cells with specific characters, and to regard the organism as a mosaic of such mutant strains.

SUMMARY

The pattern of the dorsal abdominal cuticle in the nymph and adult of *Rhodnius* is described.

If a patch of epidermis is killed by burning, the cells at the margin of the burn divide and migrate towards the centre until the defect is repaired. During this process the cells, and the daughter cells derived from them, retain the power of laying down the type of cuticle for which they were originally determined. Consequently a centripetal displacement of the pattern results at the next moult.

Cells migrating in this way in the early nymphal stages carry with them also the prospective imaginal characters. Similar derangements of the imaginal pattern therefore result; but these do not become manifest until metamorphosis.

Thus the individual cells appear to be all-important in the determination of the abdominal pattern.

The nymphal cuticle bears bristles arising from little plaques distributed regularly over the surface. At successive moults new plaques appear in the spaces between the old ones. If at a given moult the separation of the plaques is artificially increased or diminished this has no influence on the number of new plaques developed. But if the separation is increased at one moult, the number of new plaques may be nearly twice the normal at the succeeding moult.

This result is explained by supposing that each plaque exerts an inhibitory influence around it which prevents the development of new plaques; and that the radius over which this influence is exerted is determined not by the absolute distance but by the number of epidermal cells intervening. The nature of the inhibitory influence is discussed.

The number of new plaques is not predetermined at a given moult; for it may be increased if moulting is induced by exposure to the hormones of a younger nymphal stage.

If the plaques or bristles are brought close together during the moult, those of the next instar are reduced in size; if widely separated they are enlarged.

At the first moult after a burn the nymphal cuticle at the site of injury is devoid of plaques and bristles. Plaques of normal orientation and distribution are developed at subsequent moults. This regeneration is an exaggeration of the normal process of plaque formation that takes place at each moult.

REFERENCES

- GOLDSCHMIDT, R. (1921). *Arch. EntwMech. Org.* **47**, 654-67.
HUXLEY, J. S. & DE BEER, G. R. (1934). *The Elements of Experimental Embryology*. Cambridge.
WIGGLESWORTH, V. B. (1933). *Quart. J. micr. Sci.* **76**, 269-318.
—— (1934). *Quart. J. micr. Sci.* **77**, 191-222.
—— (1936). *Quart. J. micr. Sci.* **79**, 91-121.
—— (1937). *J. exp. Biol.* **14**, 364-81.
—— (1940). *J. exp. Biol.* **17**, 201-22.

EXPLANATION OF PLATE I

Fig. 1. Plaques, bristles, and folds in the epicuticle of a 5th stage nymph.

Fig. 2. Folds in the cuticle and a bristle in the tergites of an adult.

Fig. 3. Epidermis and cuticle of 5th stage nymph mounted whole after staining, to show the distribution of the individual cells between the plaques. The plaques are convex; hence the cells below them are out of focus.



Fig. 1.

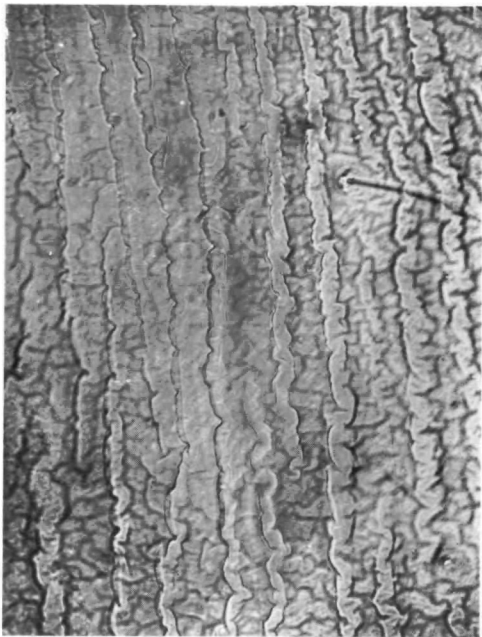


Fig. 2.

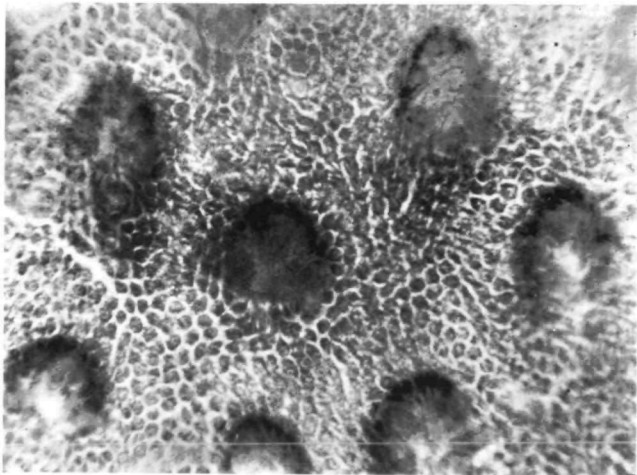


Fig. 3.

WIGGLESWORTH—LOCAL AND GENERAL FACTORS IN THE DEVELOPMENT OF
“PATTERN” IN *RHODNIUS PROLIXUS* (HEMIPTERA) (pp. 180–200)

