THE CUTICULAR PATTERN IN AN INSECT— THE INTERSEGMENTAL MEMBRANES

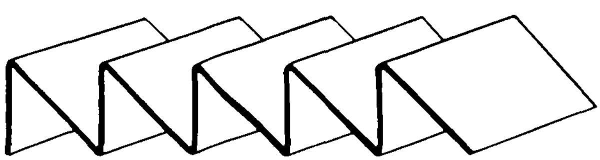
By M. LOCKE*
Department of Zoology, University of Cambridge

(*Received* 9 *January* 1960)

(With Plate 6)

INTRODUCTION

On the abdomen of adult *Rhodnius prolixus* there is a segmentally repeating pattern of transverse ripples. In grafting operations within a segment it has been found that the pattern is only disturbed when grafts are interchanged in the axis, showing that the cells responsible for the pattern resemble one another from side to side but differ from head to tail (Locke, 1959*a*, *b*). This axial difference was described as a gradient of incompatibility, for the host cells recognize the level of a graft and respond by maintaining the side-to-side continuity of the ripple pattern anterior or posterior to it. The anterior region of the segment was said to be high in the gradient because the anterior ripples join up with one another when there is competition between anterior and posterior ripples to maintain continuity. It is convenient to picture the segmentally repeating gradient as in Text-fig. 1.



Text-fig. 1. Diagram of the segmentally repeating gradient referred to in the text.

The operations which gave rise to this description were all performed within the area of integument destined to form the ripple pattern in the adult. In this way complications involving other patterns were avoided, but the results only apply to this area. It was therefore important to see if the incompatibility gradient included other patterns as part of the segmental organization as is implied in Text-fig. 1. In this connexion the intersegmental membranes are of particular interest for they separate the tail end of the gradient in one segment from the top end in the next. This problem, although one of cell behaviour, has again been studied by way of the cuticle secreted by the cells, ignoring for the moment the more fundamental problem of the relation between the cells and the cuticle pattern.

* On leave from the Department of Zoology, University College of the West Indies.

METHODS

The techniques have already been described (Locke, 1959*a*). Operations were performed in 4th- or 5th-instar *Rhodnius* larvae and the effects observed in the adult. The results of many experiments were confirmed by taking grafts from the sternites which have a darker pigmentation and retain the plaques in the adult.

RESULTS

(1) *The segmental nature of the gradient*

At the margins of the intersegmental membranes the ripple pattern becomes an irregular reticulum. This region was frequently included with the ripple pattern in the earlier grafting experiments in which it appeared to react in the same way, suggesting that the gradient occupies the whole area between the intersegmental membranes regardless of minor variations in pattern. It remained to be seen whether this also applied to other segments with a different type of pattern.

The nature of the ripple pattern makes it ideal for detecting displacements in the axis. It is relatively easy to interpret the way in which the ripple lines are displaced and link up in response to grafting operations. This advantage is lost when experiments are performed upon other segments, for example the thorax, where the pattern is more complicated. However, there is a simple way round the difficulty which shows at the same time the extent of compatibility between different segments. The reaction of the ripple lines can be observed in the abdominal segments when grafts of different pattern are implanted, the changes in the graft itself being ignored because of the difficulty of interpretation. The technique adopted has been to excise a square of integument from the test site and divide it transversely, implanting each rectangle in adjacent abdominal segments.

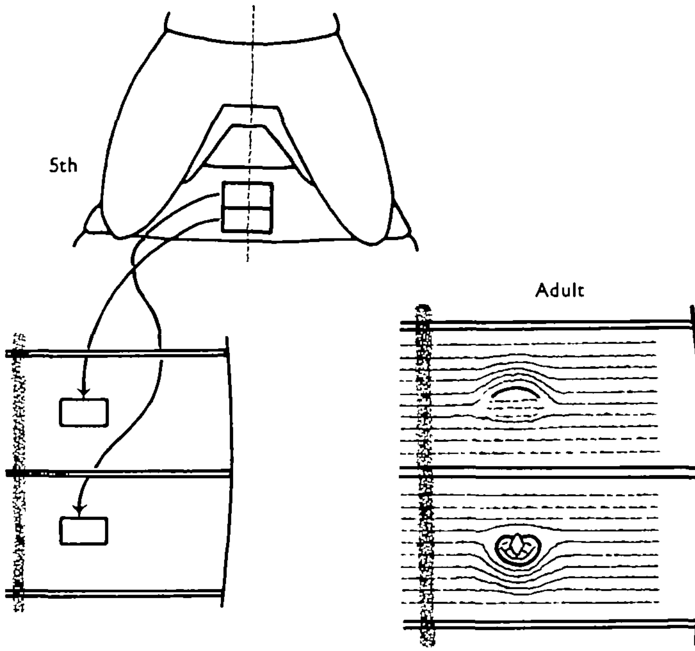
On the second abdominal tergite the ripple pattern is replaced in the midline by one of curved ridges (Pl. 6, fig. 1). Transplants were made as in Text-fig. 2. The grafts survived, and the host responded by restoring the continuity of the ripples anteriorly or posteriorly according to the origin of the graft (Pl. 6, figs. 2, 3). Thus the gradient in the 2nd abdominal segment resembles that in the 4th and 5th, although the cuticular pattern is very different. The gradient, then, is a segmental phenomenon not restricted to the ripple pattern.

(2) *The intersegmental membranes*

The segmental nature of the gradient makes the intersegmental membranes of interest. If they behave in the same way as the ripple cuticle it could be as part of the beginning or end of the gradient.

Squares of integument with an intersegmental membrane were cut and interchanged from side to side. The intersegmental membranes always linked up with one another. Squares of integument containing the intersegmental membrane were also cut and interchanged between segments. There was no distortion in the adult even when host and graft intersegmental membranes were morphologically distinguishable as in Pl. 6, fig. 4. Thus the intersegmental membranes are similar from side to side and in different segments, and in these ways resemble the ripple cuticle, but this is not sufficient to establish their position within the gradient.

This point has been resolved by studying the effects upon the ripple pattern of a cut edge of the intersegmental membrane. If a small square of integument with an intersegmental membrane is excised there may be complete regeneration. Thus



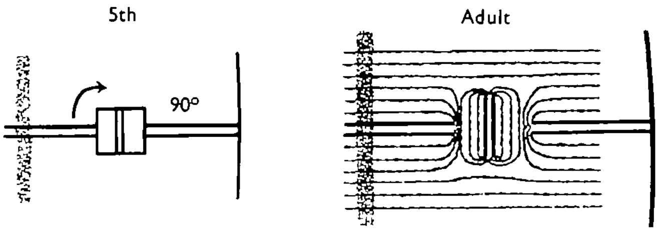
Text-fig. 2. The effect upon the adult ripple pattern of implanting grafts of another pattern from two different levels in a segment (cf. Pl. 6, figs. 1–3).

the intersegmental membrane can regenerate in an ordered fashion. When larger squares of integument are excised the ripple pattern is displaced into the wound so that the ripples end at the intersegmental membranes as if they were attracted there (Pl. 6, fig. 5). This effect is also noticeable on grafts. A square of integument with intersegmental membrane was rotated through 90° as in Text-fig. 3. In the adult the ripples of the graft had curved round on each side to meet the intersegmental membrane. On the host, too, ripples from each segment had united with the intersegmental membrane. From this experiment it is clear that during regeneration the intersegmental membrane does not select or reject ripple cuticle of a particular level.

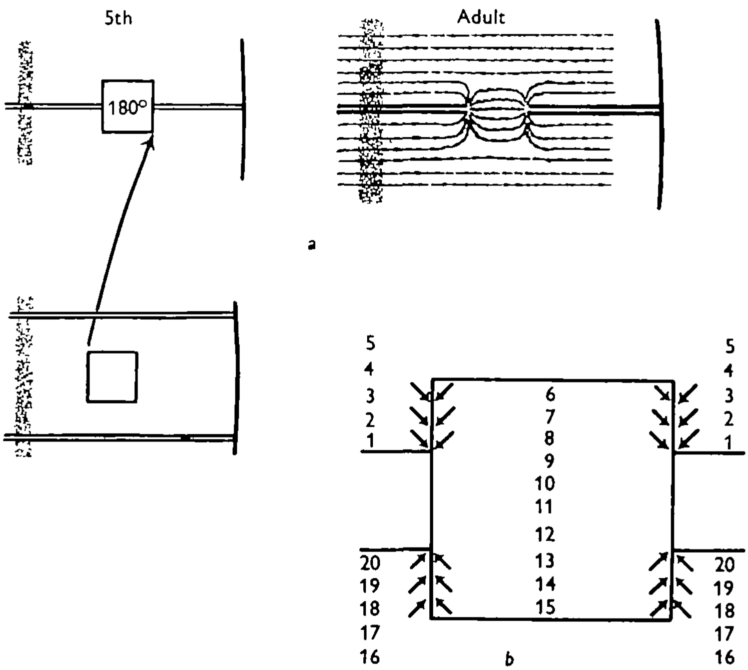
A square of integument was excised from the centre of a tergite, rotated through 180°, and implanted in a hole in the intersegmental membrane as in Text-fig. 4*a*. The ripples on the graft were deflected at each side to unite with the intersegmental membrane (Pl. 6, fig. 6). Thus even though the ripples are disoriented by 180°

to the host they are not rejected by the intersegmental membrane. This behaviour is quite different from the rejection of disoriented grafts within the ripple pattern.

A square of integument containing an intersegmental membrane was excised, rotated through 180° and implanted in the centre of a tergite (Text-fig. 5*a*). In the adult the host ripples point to the cut ends of the intersegmental membranes of the graft although they are oriented at 180° to one another (Pl. 6, fig. 7).

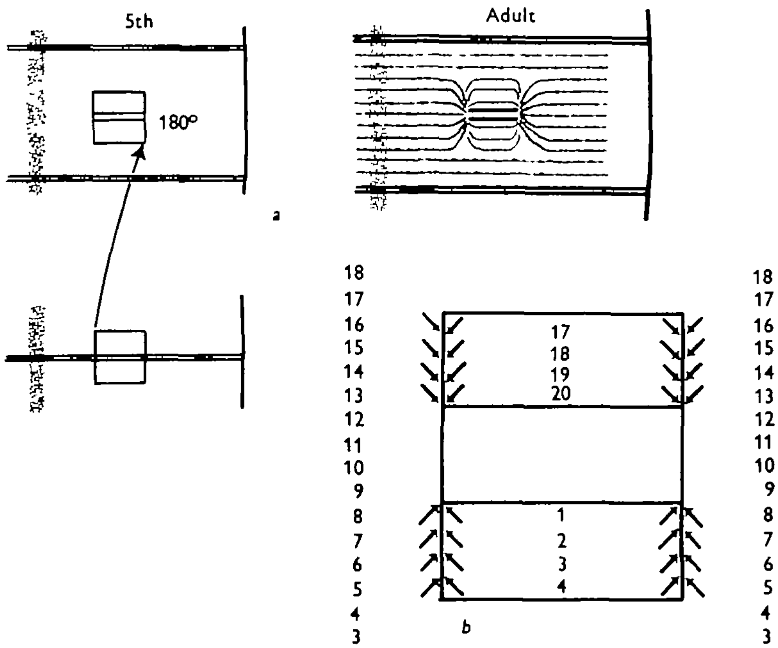


Text-fig. 3. The effect upon the adult cuticle of rotating a graft containing an intersegmental membrane through 90°.



Text-fig. 4. (*a*) The effect of implanting a graft from the centre of the ripple pattern into a hole cut in the intersegmental membrane after rotating it through 180° (cf. Pl. 6, fig. 6). (*b*) A diagram of this graft in which the gradients are represented by numbers and arrows indicate the predicted direction for the regeneration of the ripple pattern.

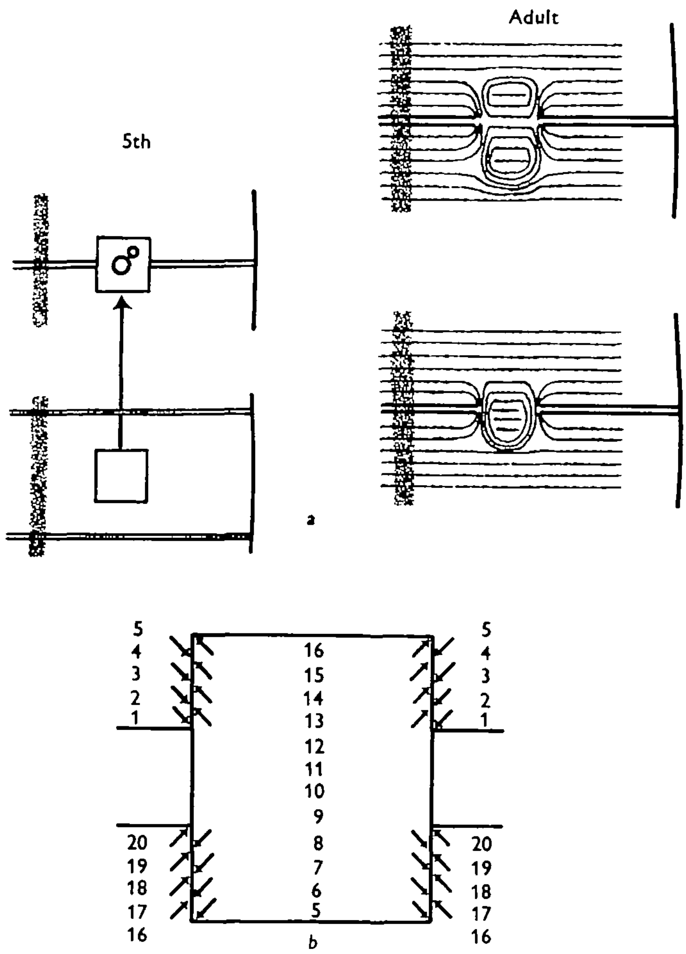
This movement of the cut ends of the ripples to the intersegmental membranes could be the result of a general attraction of the intersegmental membrane for the ripple ends, or it might result from the interaction of ripple gradients if the intersegmental membrane were neutral. A square of integument was excised from the centre of a tergite and implanted with normal orientation in a hole cut in the intersegmental membrane (Text-fig. 6*a*). If the intersegmental membrane has any general attraction for free-ending ripples the pattern in the adult should be similar to that in Text-fig. 4*a* above. The result is shown in Pl. 6, fig. 8. The intersegmental membrane can have no such attraction, for the graft was invariably isolated in a discontinuity pattern of its own. This was occasionally double as in the upper diagram, but more often single, when sometimes the intersegmental membrane had reunited anterior to it.



Text-fig. 5. (*a*) The effect of implanting a graft containing an intersegmental membrane into the centre of the ripple area after rotating it through 180° (cf. Pl. 6, fig. 7). (*b*) A diagram of this graft in which the gradients are represented by numbers and arrows indicate the predicted direction for the regeneration of the ripple pattern.

The results of the experiments described in Text-figs. 4*a*, 5*a* and 6*a* are readily understood if the intersegmental membranes are neutral with respect to the gradient behaviour. The movement of the ripples can then be ascribed to the interaction of host and graft cells, their recognition and reaction to one anothers level. In Text-figs. 4*b*, 5*b* and 6*b* numbers have been used to indicate the gradient. The arrows indicate the direction of ripple movement expected from an interaction of the gradients. The host ripples move anterior or posterior to the graft relative to the whole animal. The distortion of the host ripples is precisely that expected from a consideration of the gradients. The graft ripples move anterior or posterior relative to the original orientation of the graft in a manner which can also be predicted (except for the single pattern in Text-fig. 6*a* for which see below.) In general, when cut edges of ripples are opposite the intersegmental membranes or are forced opposite by the host-graft interaction they stay put.

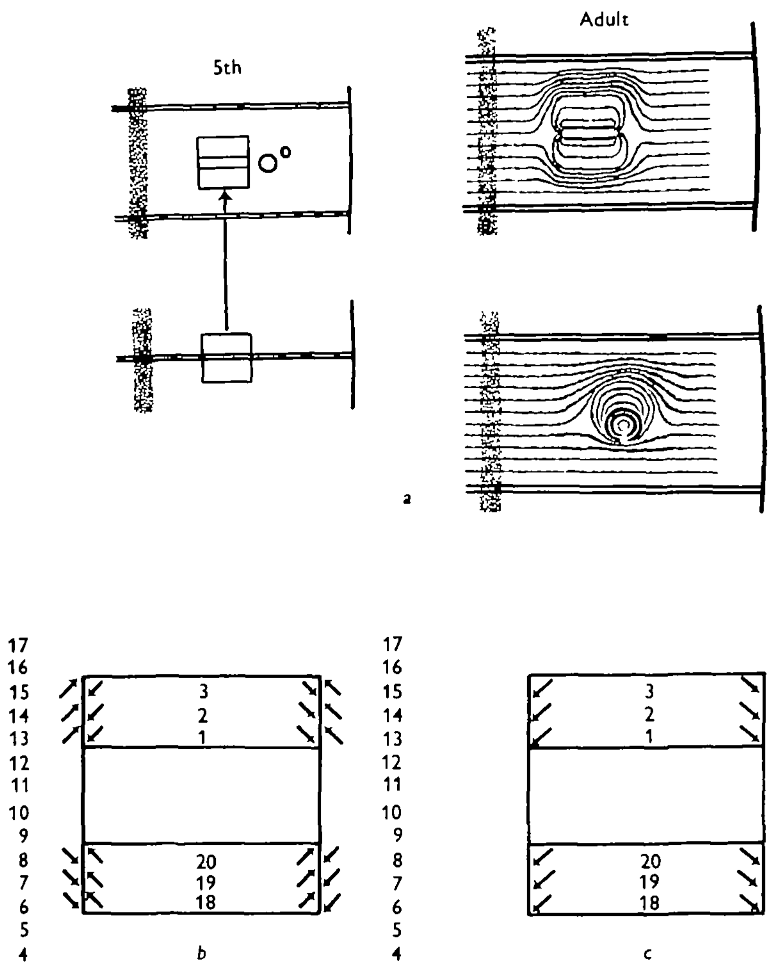
From these experiments we may conclude that the intersegmental membranes are not part of the segment exhibiting the gradient behaviour.



Text-fig. 6. (*a*) The effect of implanting a graft, cut with normal orientation from the centre of the ripple pattern, into a hole in an intersegmental membrane. The predicted pattern in the upper diagram of the adult was less common than the other (cf. Pl. 6, fig. 8). (*b*) A diagram of this graft in which the gradients are represented by numbers and arrows indicate the predicted direction for the regeneration of the ripple pattern.

(3) *The regeneration of the intersegmental membrane*

When a square of integument containing an intersegmental membrane is excised and implanted with normal orientation into the centre of a tergite, the gradients are apposed as in Text-fig. 7*b*. If the graft came from a tergite the double discontinuity pattern predicted from Text-fig. 7*b* usually resulted in the adult. But when the graft came from a sternite the pattern described in Text-fig. 7*a* and Pl. 6, fig. 9, commonly occurred. The host pattern is distorted as predicted but the graft forms a single concentric pattern. It is as if the graft intersegmental membrane had



Text-fig. 7. (*a*) The effect of implanting a graft containing an intersegmental membrane into the centre of the ripple area. When the graft was from a tergite the predicted pattern was usual. When the graft was from a sternite the single concentric discontinuity pattern resulted (cf. Pl. 6, fig. 9). (*b*) A diagram of this graft in which the gradients are represented by numbers and arrows indicate the predicted direction for the regeneration of the ripple pattern. (*c*) A diagram showing the gradients in an isolated graft and the direction expected for regeneration uninfluenced by the host.

its own powers of regeneration to restore continuity. When the interaction is between tergite and tergite the graft is strongly influenced by the host and the predicted double pattern results. When the interaction is between tergite and sternite the host influence is weaker and the graft restores its pattern as if it were in isolation (Text-fig. 7*c*). In isolation the dominance of the anterior regions of the segment in regeneration play a part in the formation of the pattern. On each side of the intersegmental membrane the anterior parts would begin to enclose the posterior. If the intersegmental membrane regenerates between them the concentric pattern may be accounted for.

Thus the pattern formed by the graft depends very much upon the influence of the host. A variable influence of the host may also account for the anomalous results given by some preparations described in Text-fig. 6. From the apposition of host and graft gradients described in Text-fig. 6*b* the graft would be expected to form a double discontinuity pattern, but more frequently a single concentric pattern developed. The single concentric pattern has the form expected for regeneration in isolation, the anterior ripples uniting round the posterior part of the graft. This could be interpreted to mean that when the host ripples have migrated in to end at the intersegmental membrane they cease to influence the graft.

The capacity of the intersegmental membrane for regeneration may be satisfied in one of two ways. It may join up with itself following the track laid down for it on either side by the segmental pattern, itself kept in place by the gradient behaviour (this occurs after burns or excisions and in the isolated graft described in Text-fig. 7*a*). Or it may join up with the ripple pattern at any level in the segment as long as the ripples are not directed away by the gradient on each side of the intersegmental membrane.

DISCUSSION

The first impression gained from grafts involving intersegmental membranes is one of confusion because the regeneration in the graft does not always seem to follow the pattern expected from the gradient concept. The host reaction is consistent, and provides a valuable confirmation of the gradient description, but the regeneration in many grafts behaves as if it is but little influenced by the host. The effect is analogous with the single discontinuity pattern resulting when a graft of ripple cuticle is rotated through 180° (Locke, 1959*a*). Here also a double pattern is expected but only occurs in large grafts. This might be explained if the host only exerts its effect on the graft while its own pattern is being re-ordered. Once this is completed the graft is left with only its own orientation as a guide for regeneration. This interpretation is confirmed by many details. Small grafts, in which host repair would be quickest, most commonly result in single discontinuity patterns. In a graft from an anterior region implanted posteriorly, the host should influence the graft ripples to regenerate in the anterior direction, that is, opposite to the direction of regeneration in isolation. The result is frequently intermediate (Locke, 1959*a*, text-fig. 8; pl. 9, fig. 14). Tergites influence grafts from tergites more strongly than grafts from sternites. Bearing this interpretation of the apparent anomalies in mind, the gradient concept is still useful in interpreting the behaviour of grafts involving the intersegmental membranes.

Grafts from the non-ripple pattern on the 2nd abdominal segment react with the host in the same way as grafts from other abdominal segments, showing that the gradient is not restricted to the areas with a ripple pattern. But this does not mean that all integument is ordered on this common plan. Indeed it has been found that grafts from the thorax and from the sides of the segment are consistently and completely suppressed. Grafts may be of three sorts, those in which the regeneration pattern is influenced by the host, those in which the pattern regenerates solely in relation to the graft, and those which although apparently healthy are completely suppressed by the host in one moult.

SUMMARY

1. Grafts including intersegmental membrane have confirmed the existence of a segmentally repeating gradient of incompatibility in the integument of *Rhodnius*.

2. When host and graft influence one another the pattern can be predicted from the interaction of the gradients. When a graft is uninfluenced by the host the anterior part of the graft is dominant in restoring pattern continuity.

3. The intersegmental membranes are neutral with respect to the gradient behaviour.

4. The gradient is not restricted to the area forming the ripple pattern but includes other patterns in the abdomen.

I am grateful to Prof. V. B. Wigglesworth for the hospitality of his department during my 6 months leave.

REFERENCES

LOCKE, M. (1959*a*). The cuticular pattern in an insect, *Rhodnius prolixus* Stal. *J. Exp. Biol.* **36** 459–78.

LOCKE, M. (1959*b*). Skin grafting in insects. *New Scientist*, **5**, 1337–9.

EXPLANATION OF PLATE 6

All figures are unstained whole mounts of the abdominal tergites of adult *Rhodnius* taken with a phase-contrast microscope and oriented so that the head end is at the top of each figure.

Fig 1. Abdominal segment 2 and part of abdominal segment 1 in the midline.

Fig. 2. The effect upon the ripple pattern of implanting a graft from the anterior half of segment 2 (cf. Text-fig. 2).

Fig. 3. The effect upon the ripple pattern of implanting a graft from the posterior half of segment 2 (cf. Text-fig. 2).

Fig. 4. The intersegmental membrane from segments 2–3 can be grafted to replace that between segments 4–5 although it has a different structure.

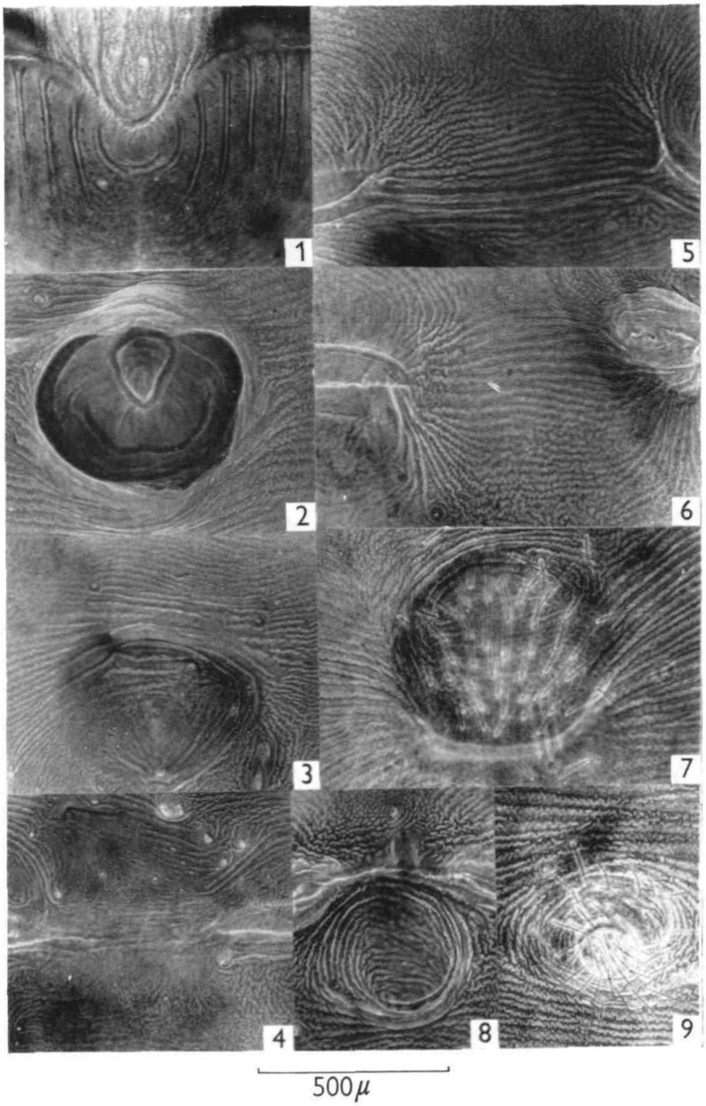
Fig. 5. Partial regeneration of an intersegmental membrane which had been burned in the 5th instar.

Fig. 6. A graft from the centre of the ripple pattern has been rotated through 180° and placed in a hole in the intersegmental membrane (cf. Text-fig 4).

Fig. 7. A graft with an intersegmental membrane from a sternite has been rotated through 180° and placed in a hole in the centre of the ripple pattern (cf. Text-fig. 5).

Fig. 8. A graft from the centre of the ripple pattern has been placed in a hole in an intersegmental membrane with normal orientation. In the preparation illustrated the intersegmental membrane has begun to regenerate anterior to the single concentric discontinuity pattern (cf. Text-fig. 6)

Fig. 9. A graft with an intersegmental membrane from a sternite has been implanted in the centre of a tergite with normal orientation (cf Text-fig. 7).



M. LOCKE—THE CUTICULAR PATTERN IN AN INSECT—THE INTERSEGMENTAL MEMBRANES

(*Facing p.* 406)