THE CUTICULAR PATTERN IN AN INSECT, *RHODNIUS PROLIXUS* STÅL

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(With Plates 8–10)

INTRODUCTION

In the larval stages of *Rhodnius* the abdominal cuticle between the intersegmental membranes is covered with a pattern of stellate pleats among which are set the bristle-bearing plaques (Pl. 8, fig. 1). This stellate pattern results when the newly formed cuticulin layer is 'thrown into folds'. Wigglesworth (1933) says: 'The folding must be a spontaneous change occurring in the membrane itself, for the folds bear no relation to the arrangement of the underlying cells. The secretion of the cells evidently coagulates at first to form a smooth membrane, which then expands (perhaps by the inhibition of water from the moulting fluid outside it) and becomes folded, falling naturally into stellate folds like those in the skin which appears at the surface of hot milk.' In adult *Rhodnius* the plaques are lost upon the tergites and the stellate pattern is replaced by transverse ridges (Wigglesworth, 1940). The ridges or ripples resemble stellate pleats which have been stretched transversely (Pl. 8, fig. 2) and merge at the margins into the more randomly pleated pattern (Pl. 8, fig. 3). In tracheae the form of the cuticle has also been ascribed to the expansion and buckling of the first formed cuticulin layer (Wigglesworth, 1954a; Locke, 1957, 1958a). A very simple model shows how expansion and buckling can cause a stellate pattern. The epithelium was represented by a flat sheet of 25% gelatin and the cuticulin layer by a thin film of rubber latex. The latex was then caused to swell by immersion in xylene. The expanding rubber took a form resembling the larval stellate pattern, distorting the gelatin beneath it (Pl. 8, fig. 4). It seemed probable that the ripples of the adult cuticle could arise similarly with the addition of an axial or transverse orientating stress. Rubber and gelatin models were made to swell after the block had been slightly stretched or compressed. This distortion caused ripples not unlike the cuticle in adult *Rhodnius* (Pl. 8, fig. 5). Tension resulted in the elongation of the pleats in the direction of the force and compression caused the formation of ripples at right angles. Thus the adult pattern could result from that in the larva with the addition of axial compression or lateral tension. The experiments to be described resulted from attempts to verify this naive hypothesis.
METHODS

All experiments were performed upon larvae of *Rhodnius prolixus* Stål. Recently moulted 5th-instar larvae are remarkably resistant to rough surgical treatment and no special antiseptic precautions were necessary. The operations were carried out under a binocular microscope with a mounted fragment of razor blade. With practice, squares down to 100 μ across could be cut and manipulated with a needle in a drop of blood on the tip of the blade. No wax was used to seal the wounds, which hardened with congealed blood in a few hours. The dark rim of blood along the line of cuts was useful in assessing the extent of wounding which when severe might cause anomalous results. Histological preparations showed that after a week at room temperature (25—32° C.) the cells had completed their repairs. The larvae were then fed to initiate moulting and metamorphosis. After most operations it was found necessary to give the larvae two small blood meals separated by several days, rather than the customary single meal causing severe distension and the risk of hernia. The results were observed on the adult cuticle mounted unstained side by side with the exuvium, which provided a perfect record of the operation performed. Pl. 8, fig. 6 shows the exuvium from a typical operation. Between six and twelve larvae were used for each experiment, but conclusions were frequently verified by repeating the operation in a slightly different way. In experiments in which the adult pattern showed no change, the site of the operation could be made out by the remains of tracheae trapped between the host cuticle and the transplant. The tracheal lining resisted digestion by the epithelium and haemocytes and caused small tubercles to outline the cut edges (Pl. 8, fig. 9). Transplanted cuticle could also be distinguished by its different level, since it seldom happened that the graft could be made to fit the hole exactly. Some experiments were performed in earlier instars than the 5th. In transplants there was no indication of incompatibility due to the origin of graft and host from different animals.

RESULTS

The regeneration of the ripple pattern

A simple physical explanation of the adult cuticle pattern is favoured by the results of some wounding experiments. If the ripple pattern is determined by the disposition of individual cells each with a fixed potency, it might be expected that the cell migration which takes place into a wounded region should cause a distortion of the pattern. Wigglesworth (1937, 1940), found pigment patterns disturbed in this way. If, on the other hand, the pattern is caused by the operation of some simple physical force, like expansion and buckling operating over a large area, then migration of cells might have little effect upon the later pattern.

The centre of a tergite of one side was burned by a small drop of stearic acid (melting-point 69° C.). The drop was kept molten for 60 sec. with a hot wire and flattened out to a disk about 0.05 cm. in diameter, well within the intersegmental membranes. The larvae were left for a week for the wound to heal before being fed.
The pattern in the adult showed no distortion, although the burned region could be recognized by its shininess (the dermal glands secreting cement had probably been destroyed) and a slightly greater separation of the ripples.

To confirm this result a square of cuticle was excised from the centre of the tergite of one side and the wound covered with a glass plate to promote healing. The larvae were fed a week later when the cells could be seen covering the glass. The pattern in the adults showed no centripetal displacement.

A different result was observed when the burn or excision was large enough to affect the boundary region of the segment or the intersegmental membrane itself. The cuticular patterns from these regions were displaced towards the centre of the disturbance, showing that migration of cells had taken place (Wigglesworth, 1940).

Thus it seemed that the ripple pattern was not the result of the disposition of cells each with the potentiality for forming only a particular part of a ripple.

Possible factors in ripple orientation

These results suggested that the ripple pattern might be caused by expansion opposed by an axial restraint over a large area, perhaps over the whole of each segment. Many structures, for example the evanescent segmental muscles described by Wigglesworth (1956), could cause such coarse axial restraints.

To test this a square was cut from the tergite of one side, rotated through 90°, and replaced (Text-fig. 1). The wound healed and the larvae were fed a week later. Now, if the ripple pattern is the result of some simple physical force operating upon the epithelium as a whole, the adult pattern should be unchanged except for the results of injury in the operation. If the adult pattern is determined in the larva at the cellular level the square of cuticle should show traces of the original orientation. Pl. 8, fig. 7 shows that in the adults the ripples on the rotated cuticle remained approximately at right angles to the remainder of the pattern. Thus the orientation of the pattern is determined at the cellular level.

Cuticle symmetry and epithelial asymmetry

The adult ripple pattern has two axes of symmetry, anterior-posterior and side to side. The shape of the ripples in the centre of a segment does not allow differentia-
tion between the anterior and posterior directions, or left and right. A force acting along either axis could produce the pattern—axial compression or lateral tension. The simplicity of the cuticular pattern suggested that there might be an equal simplicity in the cellular mechanism responsible for its orientation.

A small square was cut from the centre of a tergite on one side, rotated through 180°, and replaced. The ripple pattern on such a square should be parallel to that of the surrounding cuticle. If the pattern is oriented by an axial or transverse force or even by both together no discontinuity would be expected at the edges of the square. The result obtained is shown in Pl. 8, fig. 8 and diagrammatically in Text-fig. 2. The ripples in the surrounding cuticle make no contact with the rotated square but are deflected to unite with one another anteriorly and posteriorly. The ripples in the centre of the square retain their orientation parallel to the surrounding cuticle, but those on the periphery take a circular course linking up with themselves. This discontinuity of the pattern at the edges of the square suggested that the epithelium underlying the pattern is not symmetrical about the two axes. This does not preclude the possibility that the ripples in the cuticle may be formed by some simple physical force acting in one direction, but there is no similar simplicity in the epithelium.

Text-fig. 2. The effect upon the adult pattern of rotating a square of larval integument through 180° (cf. Pl. 8, fig. 8; Pl. 10, figs. 19, 22).

The causes of asymmetry

The asymmetry could be ascribed to a polarity or a gradient. The simplest description would suppose that the epithelium is polarized, with a directional property in one or both axes. A more complex theory would suppose that one or two gradients existed. These would have polarity but would differ quantitatively along the gradient. Thus there are several possible descriptions of the asymmetry:

1. A polarity in the axis;
2. a transverse polarity;
3. a compound axial polarity being reversed or interrupted at certain points, for example, the intersegmental membranes;
4. a compound transverse polarity split, for example, at the sides, mid-dorsally or mid-ventrally;
5. an axial gradient;
6. a compound axial gradient perhaps varying segmentally;
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(7) a transverse gradient;
(8) a compound transverse gradient perhaps varying from the sides, mid-dorsally and mid-ventrally.

This being so, in a square rotated through $180^\circ$ four features will have been altered. (a) The relative position of all parts except the dead centre of the square will have been altered in the transverse direction with respect to the rest of the animal, and (b) in the anterior-posterior direction. (c) The orientation will have been reversed in the transverse direction, and (d) in the anterior-posterior direction. One or all of these features might be responsible for the discontinuity.

Text-fig. 3. The effect of interchanging squares of integument in symmetrical positions upon either side of the mid-line. The adult pattern is undisturbed (cf. Pl. 8, fig. 9).

Transverse mechanisms. Two squares were cut from the same tergite in symmetrical positions on each side of the mid-line (Text-fig. 3). They were removed and replaced in opposite holes. A handle of soft wax fixed to each square facilitated this operation. In this way two squares were obtained in which the mesial-lateral direction alone was reversed. The pattern in the adult remained unblemished (Pl. 8, fig. 9). Thus reversal of the mesial-lateral direction does not induce the discontinuity seen in squares rotated through $180^\circ$. There is, therefore, no evidence for a compound transverse polarity reversing in the mid-line (alternative (4) above). This experiment also negates the possibility of any transverse gradient (alternatives (7) and (8)), for the relative positions have been altered in the transverse direction without inducing discontinuity. Thus all mechanisms operating transversely have been eliminated except a simple transverse polarity.
This result has been confirmed in other ways. Two squares were cut from the same tergite in symmetrical positions on either side of the mid-line as before. The squares were exchanged and rotated through $180^\circ$ (Text-fig. 4). In this way two squares were obtained reversed except for the mesial-lateral direction. PI. 9, fig. 10 shows the typical discontinuity pattern produced in the adult.

Text-fig. 4. The effect upon the adult cuticle of reversing the anterior-posterior direction in the larva while leaving the mesial-lateral direction unchanged (cf. PI. 9, fig. 10).

The importance of changes in the axis as causes of the discontinuity pattern has also been confirmed by operations in the mid-line. A square of cuticle was cut in the centre of a tergite in the mid-line. It was removed, rotated through $180^\circ$, and replaced (Text-fig. 5). Thus the direction and relative position were altered only in the axis and with respect to a simple transverse polarity and a simple transverse gradient. The typical discontinuity pattern resulted in the adult (Pl. 9, fig. 11).

From these experiments it was thought that disturbances in the axis interfered with the normal orientation of the ripple pattern although a simple transverse polarity had not been excluded.

Mechanisms in the axis. If the asymmetry is due to any form of axial polarity then it should not be possible to induce the discontinuity pattern by changes in relative position in the axis. Two rectangles were cut from the centre of a tergite upon one side as in Text-fig. 6. The position of the rectangles was interchanged without altering their orientation. The result was somewhat variable. The most usual result is shown in Pl. 9, fig. 12. The ripple pattern is deflected to unite with the rectangle from the anterior margin of the segment. The other shows the discontinuity pattern. In some preparations both rectangles showed discontinuity
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patterns. In either event the asymmetry is not the result of axial polarity (alternative (1) above). Also the discontinuity has been induced without any change in transverse orientation and a transverse polarity cannot be concerned (alternative (2)). The asymmetry is therefore due to axial displacement alone, either in a simple axial gradient or an axial gradient varying segmentally.

Text-fig. 5. The effect upon the adult cuticle of reversing the anterior-posterior direction in the mid-line where mesial-lateral effects should be symmetrical (cf. Pl. 9, fig. 11).

Text-fig. 6. The effect upon the adult cuticle of interchanging rectangles of integument in the axis (cf. Pl. 8, fig. 6; Pl. 9, fig. 12).

If the gradient varies quantitatively in the length of the animal a disturbance of the pattern might be expected in grafts exchanged between segments. If, however, there is a serial repetition of the gradient in each segment, no disturbance might result. A square was cut from the centre of the 4th and the centre of the 5th tergite of one side. The squares were interchanged without altering their orientation (Text-fig. 7). The pattern in the adult showed no trace of discontinuity. This was
not because the epithelium from different segments was incapable of stimulating the discontinuity pattern. When the experiment was repeated, cutting squares towards the anterior and posterior margins of their respective segments as in Text-fig. 8, discontinuity patterns resulted (Pl. 9, figs. 13, 14). Thus the gradient is within each segment (alternative (6)) and not in the length of the animal (alternative (5)).

Text-fig. 7. The effect upon the adult cuticle of interchanging squares of integument from similar positions in adjacent segments.

Text-fig. 8. The effect upon the adult cuticle of interchanging squares of integument from adjacent segments when one square is close to the posterior intersegmental membrane and the other is close to the anterior intersegmental membrane (cf. Pl. 9, figs. 13, 14).

The 'handedness' of the orienting mechanism

The preceding experiments show that within the area of a tergite occupied by the transverse ripples a mechanism of orientation exists in the axis. Within this area cells cannot be interchanged in the axis without affecting the pattern. The
description of this as an axial gradient introduces the possibility of a 'handedness', a distinction of right and left at each of the transverse levels. This possibility is explored below.

Text-fig. 9. The effect upon the adult cuticle of rotating a square of integument through 90° either clockwise or anti-clockwise (cf. Pl. 8, fig. 7; Pl. 9, fig. 15).

Text-fig. 10. The pattern which might have been expected in a square rotated through 90° if the cuticular ripples had been repaired by joining up the cut ends closest to one another.

A small square was cut in the centre of a tergite of one side, rotated through 90°, and replaced. The pattern formed in the adult depended upon the direction of rotation of the square, resembling the letter S or its mirror image (Text-fig. 9; Pl. 8, fig. 7; Pl. 9, fig. 15). The ripples upon the square and the surrounding cuticle joined up so that those of the square were continuous with the transverse pattern in the same direction as they would have been without rotation. Without the 'handedness' implicit in a gradient, a pattern of the form seen in Text-fig. 10 might have been expected. It is as if the cut edges of the square and hole retained an affinity for one another, the repair of the pattern tending to minimize the 90° displacement. This effect is not connected with a mesial-lateral gradient. A similar
result is obtained if the square is cut and rotated in the mid-line (Text-fig. 11; Pl. 9, figs. 16, 17). A ‘handedness’ does not necessarily imply a transverse quality. It can result from any axial gradient.

Text-fig. 11. The effect upon the adult cuticle of rotating squares of integument through 90° clockwise and anti-clockwise in the mid-line where mesial-lateral effects should be symmetrical (cf. Pl. 9, figs. 16, 17).

Text-fig. 12. The forms which the gradient might take within each segment: (a) an axial gradient of greatest intensity either anteriorly or posteriorly, (b) a contoured gradient with the lateral limits set close to the margins and not necessarily symmetrical from the centre as drawn here, (c) specific lateral affinity without quantitative form.

The form of the gradient

The gradient could take one of several forms, the only necessary constant being the similarity of properties in the transverse direction. For example, it could be a quantitative change from the anterior to the posterior (Text-fig. 12a), or the segment might be contoured (Text-fig. 12b) simulating an axial gradient from the centre. It need not involve any quantitative change. Each level in the axis might have a specific qualitative affinity for its lateral neighbours (Text-fig. 12c). Equally well a quantitative change from anterior to posterior could itself result in a specific lateral affinity.

If the gradient is contoured as in Text-fig. 12b, the mesial-lateral effects at the sides would not have been noticed because experiments have only been performed...
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in the centre of the segment. Two squares were cut and interchanged as in Text-fig. 13. The pattern in the adult was not disturbed. Thus if the gradient is contoured the lateral limits must be very close to the edge of the segment. The possibility of a gradient from the centre of the segment has been tested in another way. If there is such a centre then a square rotated through 180° about it should not disturb the symmetry of the pattern. A number of squares were cut at slightly different levels in the axis and rotated through 180° in an attempt to confirm this. A discontinuity pattern always resulted. Thus there is no evidence for a gradient from the centre of the segment.

This conclusion has been confirmed in another way. A quantitative difference from the centre might be expected to affect the facility with which continuity is established in axially displaced cuticle. Pairs of rectangles were cut and interchanged as in Text-fig. 14. With a greater lateral affinity in the centre, the rectangle farthest from the intersegmental membrane might be expected to be the one isolated in the discontinuity pattern. If the converse is true, with the greatest lateral affinity away from the centre, the rectangle towards the edge should be isolated. In a head-to-tail axial gradient the isolated rectangle should be consistently anterior or posterior independently of its position relative to the intersegmental membrane.

Pl. 10, figs. 18, 21 show the result. Except in a few instances where double discontinuity patterns formed, the rectangle from the posterior part of the segment is the one isolated. The anterior region of the segment shows dominance over the posterior region in its ability to restore pattern continuity after displacement. The double discontinuity patterns are probably caused by the migration of host cells across the wounded cut surface between the two rectangles, producing a result similar to the experiment described in Text-fig. 15.

These experiments suggested that the gradient might be an axial one as in Text-fig. 12a, one of its properties being the greater facility with which pattern continuity is restored anteriorly. This being so the transverse line of cells in the centre of a square rotated through 180° remains in the same position relative to the surrounding cuticle. A pair of discontinuity patterns would therefore be expected upon each side of this transverse level. In the earlier experiments small squares
were cut and this double pattern escaped notice, but in larger squares or in rectangles with the long axis in the axis of the animal this double pattern does indeed occur (Pl. 10, fig. 19; Pl. 9, figs. 10, 11), but the two patterns are never of equal size, the posterior one always being difficult to distinguish. This could be interpreted to mean that some of the cells of the square which are more anterior in origin tend to establish continuity with the edges of the hole, and in doing so isolate the major part of the square, forcing it into a discontinuity pattern. The remainder of the anterior cells are lost in small squares, or in large squares go to make the much smaller and more irregular posterior discontinuity pattern. This interpretation would be in agreement with the conclusion above that the most anterior parts of the segment have the greatest capacity for establishing pattern continuity. This description in terms of a segmentally repeated axial gradient is taken further in the discussion.

An interpretation of the discontinuity pattern

When a piece of cuticle is rotated through 90° the ripples in the adult do not end blindly but unite with the undisturbed transverse pattern. The cells responsible for the pattern have a capacity for restoring their relative positions to one another as far as the disturbance of the operation allows. The same effect is noticeable in the cuticle surrounding a square which has been rotated through 180°. The ripples at the same level on each side of the square are joined to one another by ripples deflected round the disturbed region. In experiments interchanging two pieces of cuticle in the axis transverse continuity is maintained through one of the pieces.
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The ripples from the cut edges of the hole are deflected to unite with the cuticle which used to be at the same transverse level. It is evident from all these experiments that the cells behave in such a way that transverse continuity is maintained with cells similar in position in the axis. Now in two of the experiments mentioned above a piece of cuticle has been prevented from establishing this continuity. In the squares rotated through 180° the surrounding cuticle has maintained continuity round the square. In the axially displaced cuticle continuity could only be maintained with both rectangles if ripples could cross one another. The absence of such a pattern indicates that the epithelium cannot have a dual potentiality, it has no capacity for joining up with two levels in the axis. These pieces of cuticle isolated from the normal transverse continuity presumably retain an unsatisfied capacity for joining up with the appropriate level. Continuity will be satisfied least at the cut edges of the square. It has been suggested that the most anterior levels satisfy their capacity for continuity by uniting with one another round the square. A detailed examination of the patterns gives this impression. In the patterns induced by rotating through 180° the continuity is restored anteriorly. The posterior part of the square, anterior in origin, remains least disturbed but the edges extend in narrow ridges anteriorly, sealing off that part of the square which is posterior in origin (Pl. 10, figs. 19, 22). In the patterns induced by axial displacement the ridges on the anterior part of the square are least disturbed and continuity is restored posteriorly (Pl. 10, fig. 23). If this is the correct interpretation it might be expected that if the operation were performed in an earlier instar the adult would show a more concentric type of pattern with fewer transverse ripples ending freely. Squares were cut in 3rd-, 4th- and 5th-instar nymphs and rotated through 180°. The patterns in the adult showed a progressive trend from transverse to concentric type with the number of instars which had elapsed since the operation (Pl. 10, fig. 25).

It may be concluded that the discontinuity pattern is the result of the capacity of the epithelium for uniting cells of the same level in the axis.

Ripple orientation and the control of growth

It has been assumed that the ripple pattern of the cuticle is caused by, and reflects, some similarly oriented mechanism within the cells of the epithelium. The question arises whether any other property of the cells obeys similar rules. In the tracheal system it has been shown that epithelial continuity is necessary for the quantitative control of growth but not for the normal sequence of events at moulting (Locke, 1958b). The tracheal epithelium is polarized with respect to this control: a trachea cut from its tissue connexions moult but does not grow normally in diameter. If the gradient in the axis of the tergites is homologous with the polarity of growth control in the tracheal system, the cuticle isolated in a discontinuity pattern might be expected to behave like a trachea isolated from its tissue connexions, moulting normally but without the usual increase in size.

The effects of isolation in a discontinuity pattern upon growth in area have been followed making use of the darkly pigmented sternal cuticle as a marker. The sternal cuticle also differs from the tergal in retaining bristles in the adult, but it
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has similar transverse ripple marks, and grafts to the tergites take satisfactorily. Squares were cut from the centre of the sternites in 3rd-instar larvae and implanted in similar positions in the tergites. Some were rotated through $180^\circ$ to induce the discontinuity pattern, others were implanted with normal orientation as controls. The changes in area could be followed by examining the exuviae. The difference between the isolated cuticle and the normally oriented transplant were most marked in the adult (Pl. 10, figs. 24, 26). In the rotated squares the transplant was reduced to a few bristles with no pigmented cuticle. In those with normal orientation the transplant was marked by an oval of pigment with normally spaced and oriented bristles. These results are in agreement with the hypothesis derived from a study of growth in tracheae that epithelial continuity is necessary for the quantitative control of growth. The epithelial continuity must be of a particular kind, being part of an axial gradient in the normal tergite.

Further properties of the orienting mechanism

It has been shown that the tergal epithelium responds to various disturbances by restoring the transverse continuity of the cuticular pattern. This could be described as an affinity between the cells at each level in the axis, the strength of the affinity being greatest anteriorly. The patterns obtained in some experiments point also to an interaction between cells of different axial levels as well as to the affinity between cells of the same level.

Text-fig. 15. The effect upon the adult cuticle of interchanging non-adjacent rectangles of integument in the axis (cf. Pl. 10, fig. 20).

Two rectangles were cut from the same tergite, one towards the anterior margin, and one towards the posterior margin, and interchanged as in Text-fig. 15. In the adult each showed a discontinuity pattern, being isolated from its own level by the undisturbed central strip of cuticle. The surrounding ripples were not evenly distributed anteriorly and posteriorly to the rectangle as in squares rotated through $180^\circ$, but almost entirely to the side between the transplant and the intersegmental membrane (Pl. 10, fig. 20). A similar effect was noticeable in earlier experiments (Pl. 9, figs. 13, 14). The pattern is easily explained if there is a reaction between the graft and the host varying with the difference in level in the axis. In Text-fig. 15 the rectangle close to the anterior intersegmental membrane has come from a posterior region of the segment and the surrounding pattern is stimulated to move
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The cuticular pattern gives no indication of the gradient in the epithelium inferred from the experiments. It has an axis of symmetry requiring at most a polarity for its orientation. The experiments neither confirm nor deny the hypothesis of expansion with an orienting stress and ripple-like buckling; they only show that any such postulated stress must operate at the cellular level. This could be due to non-uniform restraint as in tracheae or to unequal expansion. The behaviour of the epithelium—the facility with which lateral continuity of the pattern is restored—might well reflect a difference in mechanical properties in different directions. Put in its crudest way the cell membranes might be stretched by the side-to-side continuity, making them more resistant to buckling in that direction. This approach will have to await future work upon the cellular basis of the pattern.
The gradient concept

The term axial gradient has been introduced to describe the mechanism of orientation of the adult pattern of cuticular ridges. It is merely a shorthand description of the results of the experiments, representing no advance in itself. Its usefulness is more easily seen if the gradient is given numerical values (Text-fig. 16).

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Text-fig. 16. A linear gradient is indicated by the numbers 10–1. The differences in value between the square and the host gradient are shown in small numbers. The direction in which the host ripples maintain continuity is indicated by arrows. Three typical experiments are shown in the diagram. In each the negative or positive value of the gradient difference agrees precisely with the direction in which continuity is maintained (cf. Text-figs. 2, 8, 9).

In a square displaced axially from an anterior region there is a consistent difference in level along the lateral margins of the square, and the host ripple lines all unite posteriorly to it. In a square rotated through 180° the difference in level varies with opposite sign from a neutral region at the centre of each lateral margin. In agreement with this the host pattern divides and unites equally anteriorly and posteriorly. In a square rotated through 90° there are two corners opposite to one another (the corners varying with the direction of rotation) where there is no difference in level. The S-shaped pattern results by uniting the ripples along the sides of the square in the direction determined by the sign of the difference in levels. In all three examples the host epithelium reacts to that of the square according to the level of the latter, moving anterior or posterior to it until the levels are similar and the pattern is reunited. Evidence will be presented in a future paper that this is due to the migration of cells. If this were all, the term gradient might be inapplicable, since the results could be accounted for without any quantitative axial change. There is no evidence that the degree of interaction varies quantitatively as the numbers in Text-fig. 16 might suggest. Qualitative recognition of difference of level, + or −, between cells would suffice to account for the capacity of similar levels to unite. But a gradient is suggested in another way. The anterior cells show dominance over the posterior cells in restoring pattern continuity. The concentric pattern in any piece of cuticle showing discontinuity is formed by the
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anterior cells uniting posteriorly however the discontinuity has been induced. In adjacent rectangles interchanged in the axis continuity is established through the cuticle which is anterior in origin. The recognition of level and the anterior dominance could be considered as separate properties of the epithelium. For the moment it is convenient to consider both as being due to a gradient, but this may not prove a satisfactory description for long.

The most important property of the gradient then is the behaviour of the cells resulting in continuity of the ripples of the same level.

The gradient and the control of growth

Isolated pieces of cuticle may have satisfied their capacity for uniting with cells of the same axial level by forming a concentric pattern, but they fail to grow harmoniously with the rest of the animal. Satisfaction of the capacity for completing the pattern is alone insufficient to induce normal growth, the cuticle must also be in the gradient of the whole segment. The quantitative control of growth is therefore not coincident with the behaviour of the cells described as a gradient. If the amount of growth depended upon a blood-borne factor alone, the converse might have been expected. That this is not so implies that in the normal animal the cells receive information about the growth to be made in some other way—presumably through the epithelium as in tracheae. The cuticle in a discontinuity pattern differs from normal cuticle in that the ridges ‘do not go anywhere’, they unite with themselves. If this is the cause of the failure of controlled growth it could mean that in the continuity of the normal segment the cells receive information from somewhere in the direction of the gradient. This may be the real significance of the cells maintaining contact with their own level. As revealed by the abnormal conditions of the experiments, the gradient appears as a curiosity. To the animal it may be a mechanism for maintaining the cells in a preferred order for the transport of growth stimuli. The source of these growth stimuli will be explored in a future paper.

SUMMARY

1. The cuticle on the abdominal tergites of Rhodnius larvae has a uniform pattern of stellate pleats. In the adult this pattern is replaced by an oriented one of transverse ridges resembling greatly elongated pleats.

2. Neither pattern results from the disposition of cells each with the potentiality for forming only part of it. The simplicity of the patterns suggests some simple mechanical cause operating over a large area.

3. The effect upon the adult of altering the orientation and relative position of pieces of larval integument has been used to study the mechanism of orientation of the pattern.

4. The orientation of the adult pattern is mediated through the epithelium, since rotation of the larval integument causes corresponding disturbances in the adult pattern.

5. The epithelium has a capacity for maintaining transverse continuity within
similar levels in the axis. There is an axial gradient within each segment, the anterior showing greatest facility in maintaining continuity.

6. Pieces of transplanted integument unable to restore their continuity with the whole animal show a concentric pattern of ridges, as if the capacity for continuity had been satisfied by each level joining up with itself.

7. Such isolated pieces of integument moult but fail to grow normally, suggesting that continuity in the axial gradient of the whole animal is necessary for the quantitative control of growth.

8. Pieces of integument from different axial levels within a segment when juxtaposed influence the direction of displacement of the pattern according to their level. A transplant from the anterior stimulates the host ridges to maintain continuity posterior to it, and conversely.

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REFERENCES


EXPLANATION OF PLATES

PLATE 8

All figures except 4 and 5 are unstained whole mounts of *Rhodnius* cuticle taken with a phase contrast microscope and oriented so that the head end is at the top of each figure.

Fig. 1. 5th-instar larval cuticle showing plaques and unorientated stellate pleats.

Fig. 2. The centre of abdominal segment 2 of one side in an adult showing the transverse ripples.

Fig. 3. The intersegmental membrane between segments 2 and 3.

Fig. 4. Random buckling in a rubber and gelatin model of larval cuticle.

Fig. 5. Oriented buckling produced in another model by stretching from side to side.

Fig. 6. The 5th-instar exuvium, showing an operation in which the two rectangles of cuticle have been interchanged (cf. Text-fig. 6).

Fig. 7. The effect upon the adult of rotating a square of integument in the larva 90° anti-clockwise (cf. Text-figs. 1, 9).
LOCKE—THE CUTICULAR PATTERN IN AN INSECT, *RHODNIUS PROLIXUS* STAL

(Facing p. 476)
LOCKE—THE CUTICULAR PATTERN IN AN INSECT, RHODNIUS PROLIXUS STAL
LOCKE—THE CUTICULAR PATTERN IN AN INSECT, RHODNIUS PROLIXUS STÅL
Cuticular pattern in an insect, R. prolixus

Fig. 8. The adult pattern when a square of cuticle has been rotated through 180° in the larva (cf. Text-fig. 2).

Fig. 9. The effect of interchanging squares of cuticle in symmetrical positions upon either side of the mid-line. The transplant of one side only is shown (cf. Text-fig. 3).

PLATE 9

All the figures are phase contrast photomicrographs of the cuticular pattern in adult Rhodnius showing the effects of operations upon the larvae.

Fig. 10. The effect of reversing the anterior-posterior direction of a square of cuticle in the larva while leaving the mesial-lateral direction unchanged (cf. Text-fig. 4).

Fig. 11. The effect of reversing the anterior-posterior direction in the mid-line where mesial-lateral effects should be symmetrical (cf. Text-fig. 5).

Fig. 12. The effect of interchanging adjacent rectangles of integument in the axis (cf. Text-fig. 6).

Fig. 13. A square of cuticle has been transplanted from the posterior part of one segment to the anterior part of the segment shown (cf. Text-fig. 8).

Fig. 14. A square of cuticle has been transplanted from the anterior part of one segment to the posterior part of the segment shown (cf. Text-fig. 8).

Fig. 15. The effect of rotating a square of larval integument 90° clockwise (cf. Text-figs. 1, 9).

Fig. 16. The effect of rotating squares of larval integument clockwise through 90° in the mid-line where mesial-lateral effects should be symmetrical (cf. Text-fig. 11).

Fig. 17. The effect of rotating a square of larval integument through 90° anti-clockwise in the mid-line where mesial-lateral effects should be symmetrical (cf. Text-fig. 11).

PLATE 10

All the figures are phase contrast photomicrographs of the cuticular pattern in adult Rhodmus showing the effects of operations upon the larvae.

Fig. 18. Two adjacent rectangles have been interchanged in the axis in the anterior half of a segment. The rectangle posterior in origin has formed a discontinuity pattern (cf. Text-fig. 14).

Fig. 19. The effect of rotating through 180° a rectangle with its long axis in the axis of the animal. A partial double discontinuity pattern has formed.

Fig. 20. Two rectangles separated by a strip of untouched cuticle have been interchanged (cf. Text-fig. 15).

Fig. 21. Two adjacent rectangles in the posterior half of a segment have been interchanged in the axis. The rectangle posterior in origin has formed a discontinuity pattern (cf. Text-fig. 14).

Fig. 22. A discontinuity pattern obtained by rotating a square through 180° (cf. Text-fig. 2).

Fig. 23. A discontinuity pattern obtained by displacing a square from a more posterior position in the axis to its present one in the anterior half of a segment (cf. Text-fig. 8).

Fig. 24. The remains of a square of sternal cuticle with normal orientation taken from the centre of a segment in the 3rd instar. The sternal cuticle is distinguished by its darker pigmentation and bristles (cf. Fig. 26).

Fig. 25. A more concentric discontinuity pattern obtained by rotating a square of integument through 180° in the 4th instar.

Fig. 26. The remains of a square of sternal cuticle taken from the centre of a segment in the 3rd instar and rotated through 180°. No pigmented cuticle and few bristles remain (cf. Fig. 24).