by rotation however, since this could not explain how the ridge of the transplant joins up with that of the host. The generation of intermediate gradient values by intercalary regeneration is one possible explanation.

Though it is only the pattern on the cuticle whose rotation can be observed in *Leucophaea* there is no doubt that the epidermis of the transplant, too, rotates back since the cuticle is only a secretion product of the epidermis. More direct evidence for the movement of the epidermis is presented in the following contributions[9,10]. So pattern reconstitution in the tergites of *Leucophaea* is, at least mainly, brought about by a rerotation of the transplant tissues; it is not necessary to assume a resetting of the cells. In this respect the epidermis of the tergites is like the legs in which a resetting to new levels seems to be possible only in proliferating tissues[5].

The mechanics of the rerotation has not been studied in detail up to now. Muscle contraction as a means for movement can be excluded since the transplant region is completely free of muscles. Most likely the rotation is achieved by an active movement of the border cells, while the more central parts are carried along with them passively as can be judged from the unchanged pattern of large central parts (Fig. 1*g–k*; see also ref. 10). Pushing the transplant by intercalary regeneration should also be taken into account.

This work was supported by Deutsche Forschungsgemeinschaft.

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Received November 1, 1973.

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Cell movement during pattern regulation in *Oncopeltus*

THE cuticle of adult *Rhodnius* (Hemiptera) is marked by mediolaterally oriented surface ripples. From transplantation and rotation experiments on the epidermis Locke[1] demonstrated a segmentally reiterated gradient. We[2] have proposed a model of the gradient which is considered as a concentration of some diffusible substance, the direction of steepest slope of concentration specifying cellular polarity and thereby the orientation of the ripples. We suggested that the cells might contribute to the stability of the gradient and attempt to maintain their original or 'set' level when transposed.

Rotation of a larval square of epidermis through 90° results in an S-shaped pattern of ripples in the adult cuticle When such an adult is made to moult again to produce a second cuticle the S-shaped pattern flattens, so that the ripples are now aligned nearer to the mediolateral axis. This change of pattern was associated with the presence of cell divisions in the artificial moult cycle; when the moult cycle was truncated and cell divisions omitted, the first and second

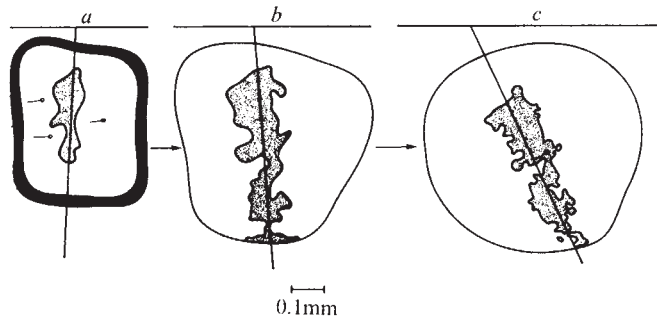

FIG. 1 Camera lucida drawings showing the result of transplanting a piece of orange epidermis containing a transparent clone (shaded) on to a white fourth-stage host. The graft was rotated through 90° clockwise. *a*, The situation immediately on grafting (clone rotated c. 93°); *b*, the young fifth-stage (angle of clone now c. 85°); *c*, the adult (angle of clone now about 65°).

cuticular patterns were almost identical. We therefore suggested that the cells' set level was re-established at some stage in the cell division cycle allowing further diffusion and consequent changed polarity. An alternative cause of pattern change could be bodily rotation of the entire graft in the opposite direction to that of the original experiment[9]. This paper and the two accompanying contributions[3,4] show that in fact there is bodily rotation of the graft.

Pieces of epidermis were exchanged between wild type (orange pigmented cells) and a mutant, *wb* (white pigmented cells) of *Oncopeltus fasciatus*[5]. The adult hairs and cuticular tubercles were used as polarity indicators, both normally

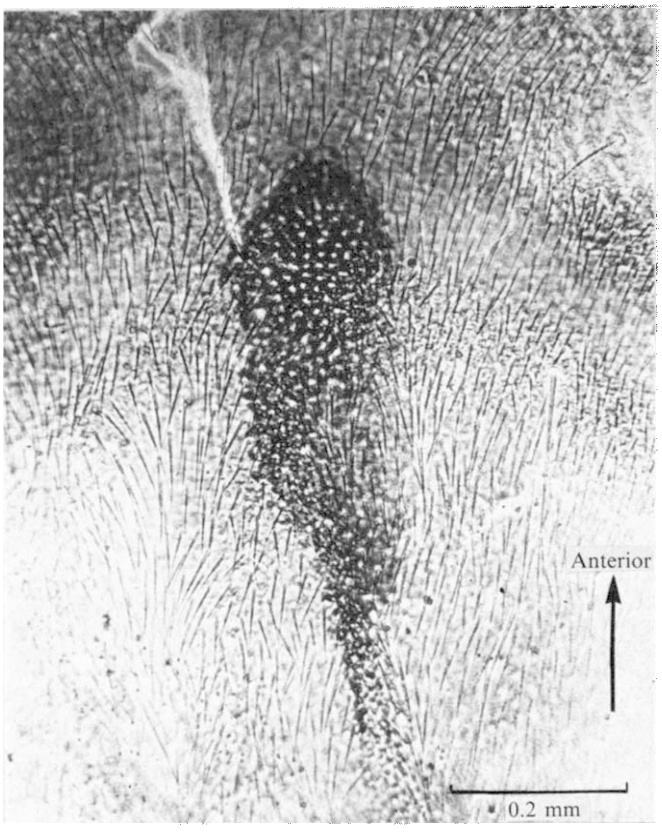

FIG. 2 A strip of orange epidermis (long axis mediolateral) was rotated through 90° clockwise and transplanted on to a white fourth-stage larva. In the adult the strip of orange cells is still clearly visible and still retains the same orientation as it had on grafting (long axis antero–posterior). As in the host, the hairs in the graft point posteriorly.

pointing posteriorly. Following X-irradiation as eggs[6], fourth-stage larvae were screened for long thin clones which were a different colour than orange and had their long axes parallel to the mediolateral axis of the body. Square pieces of epidermis containing such clones were excised, rotated through 90° and implanted on a white host in the centre of the segment. The graft border and shape and orientation of the clone were drawn by camera lucida in the two succeeding instars (Fig. 1). In some cases the clone was damaged by the operation but in all five cases where the clone survived as a coloured strip, there was clear evidence for the rotation of the clone and therefore for bodily rotation of the graft; in no case did the central hairs become reorientated while the clone remained in the original orientation. In one case the clone itself became S shaped suggesting more cell movement at the edges of the graft.

My unpublished experiments on *Dysdercus fasciatus*, and those of Nübler–Jung[4] on *D. intermedius* suggest that the rotation of grafts is restricted to a relatively short period (about 20 h[4]) when the epidermis separates from the cuticle; that is, after cell divisions have ceased.

In a second series of experiments (suggested to me by Dr H. Bohn) thin strips of epidermis, with their long axes oriented mediolaterally were taken from orange *Oncopeltus* larvae, rotated through 90° and transplanted on to white larvae. When the graft survived as a strip, it remained oriented close to the anteroposterior axis, but the orientation of the hairs, and the small epidermal tubercles turned so that they pointed posteriorly (Fig. 2).

These two classes of experiments show that the situation is complex; the first shows that cell movement is an important component of pattern regulation, and the second that regulative changes of polarity can occur without bodily rotation of the entire graft. It is still possible that regulation of polarity is achieved by smaller parts of the graft rotating independently. Rotation back of grafts has been demonstrated in other systems (the amphibian ear[7] and eye[8], and the insect leg[9]), while polarity changes that are not dependent on rotation are found after juxtapositions of regions from different levels in the axis of both the abdominal segments[2] and the legs of insects[9]. These changes are associated with cell division.

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Received November 1, 1973.

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Cell migration during pattern reconstitution in the insect segment (*Dysdercus intermedius* Dist., Heteroptera)

IF in the insect segment a piece of epidermis is rotated through 90° the changed pattern tends to return to normal after several moults.[1] This observation can be explained by a rotation of the whole transplant (ref. 2 and H. Bohn, personal communication) or by a resetting of the transposed cells.[3] Until now it was not possible to decide between these two mechanisms since pattern reconstitution in the leg or body segment was studied only by the observation of cuticular structures such as bristles and ripples[4]. These structures reflect the physiological status of the epidermal cells only during the short periods of epicuticle formation but not during the intervals in between. Moreover, by studying the cuticle it is rarely possible to determine the exact boundary between host and transplanted epidermis. These disadvantages can be overcome by using a colour mutant[5] which enables continuous observation of the transplanted cells.

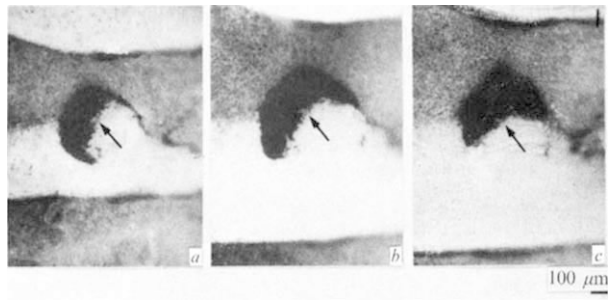

FIG. 1 Development of a transplant rotated anticlockwise through 90° during the third larval instar. *a*, Aspect early in the fourth instar; *b*, late fourth to early fifth instar; *c*, shortly before metamorphosis. Posterior segment border at the bottom. Scale, 100 μm.

In the wild type of *Dysdercus intermedius* from the third larval instar onwards the anterior zone of the third ventral abdominal segment is red and the posterior zone white. The mutant lacks the red pigment and therefore the anterior zone looks grey. It should be noted that the pigment is located inside the epidermal cells. The cuticle is transparent and permits direct observation of the location and possible movements of the transplanted cells. Transplantations were carried out on third instar larvae as described by Lawrence *et al.*[3]. The transplants always included the border between the red and white zones. The hosts were of the grey mutant type. The segments carrying transplants were filmed by time-lapse cinematography (1 frame per 3 min) or photographed at daily intervals until after metamorphosis.

Three stages in the development of a transplant which had been rotated anticlockwise through 90° are illustrated in Fig. 1. After the first moult following the operation (Fig. 1*a*) some red cells are found between the white cells of the graft and

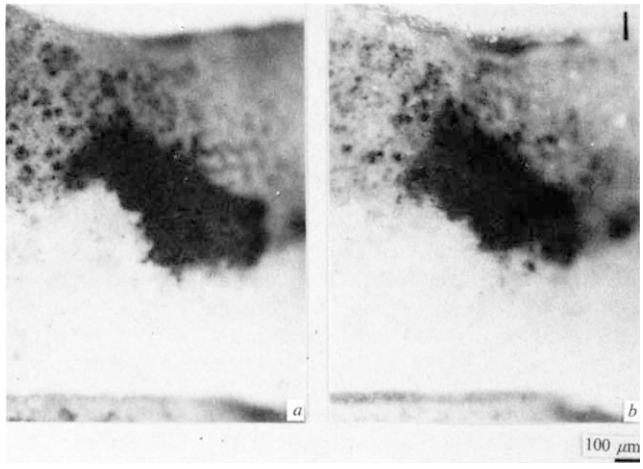

FIG. 2 Time-lapse film which was taken towards the end of the fifth larval instar. *a*, Aspect at the beginning of film (0 h); *b*, at the end (54 h) of the film. Scale, 100 μm.