HOW DO CELLS KNOW WHERE THEY ARE?*

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THE TOPIC of this paper is the organization of animal development. One of the most fascinating aspects of this development is precision; in each species a highly complex process very rarely fails to produce a new individual perfect in characteristic detail. This reliability implies so much spatial and temporal organization that it is easier to take it for granted than to study it.

Living things are extremely complicated, and the organizational problems are analogous to ours when, for instance we make a complex building, or a space rocket. We start with two-dimensional blueprints and make sure that we manufacture and assemble parts in the correct sequence. We must be certain that when constructing, say the space capsule, we consult only the appropriate section of the plans, even though the main engines may require identical components. Our use of the blueprints must be well organized in time and in space.

In living things the majority of the blueprints are written in a simple language encoded in one-dimensional form on the DNA. We need to know how this information is used in the right places at the right times.

I want to talk particularly about the organization of cells in space. It is the spatial organization of cells which is responsible for the characteristic construction of parts of the body. The limbs for instance are made of the same cell types—bone, muscle and connective tissue—but it is their arrangement that makes an arm different from a leg. While a great deal is known about the development and maturation of individual muscle cells almost nothing is known about how they are organized into co-ordinated populations. A typical experiment on this type of problem is to transplant cells from one place, A, to another place, B. Generally there are two kinds of results: if the operation is performed very early in development the cells mature according to their new position, B, and if late they continue to develop as if they had not been moved and were still in place A. At a certain time the cells become 'determined'; they have found out where they are, what their developmental fate is to be, and do not forget it. For example, if two young mice embryos are fused a perfectly normal sized mouse made up of cells descending from both embryos results (Tarkowski, 1961). Later fusion and regulative development becomes impossible. Even at the 8-cell stage in rabbit development, a single cell can compensate for the loss of the remaining seven, and grow into a perfect animal (Moore, Adams and Rowson, 1968). Perfectly formed humans may also result from either the fusion of two different embryos (see Stern, 1968) or from the splitting of one. These cases show that development is not precisely programmed; the situation of the whole developing organism is monitored and adjustments are made to ensure the perfect end result.

I want to take a closer look at determination with the help of some experiments on amphibians (for references see Jacobson, 1968). In these animals as in humans, the visual image on the retina is translated into a pattern of nerve impulses which projects the image on to the brain, where it is analysed. Long before the optic nerve grows from the retina to the brain, the developing eye can be rotated and reimplanted. When this is done at embryonic stage 31 in the frog, *Xenopus* the nerves grow out and make connexions with the same part of the brain as they would have done. Consequently the animal, as can be shown by its behaviour, sees an inverted world, where right and left are interchanged. A fly offered top right will be snapped at bottom left. We can conclude that at stage 31, the

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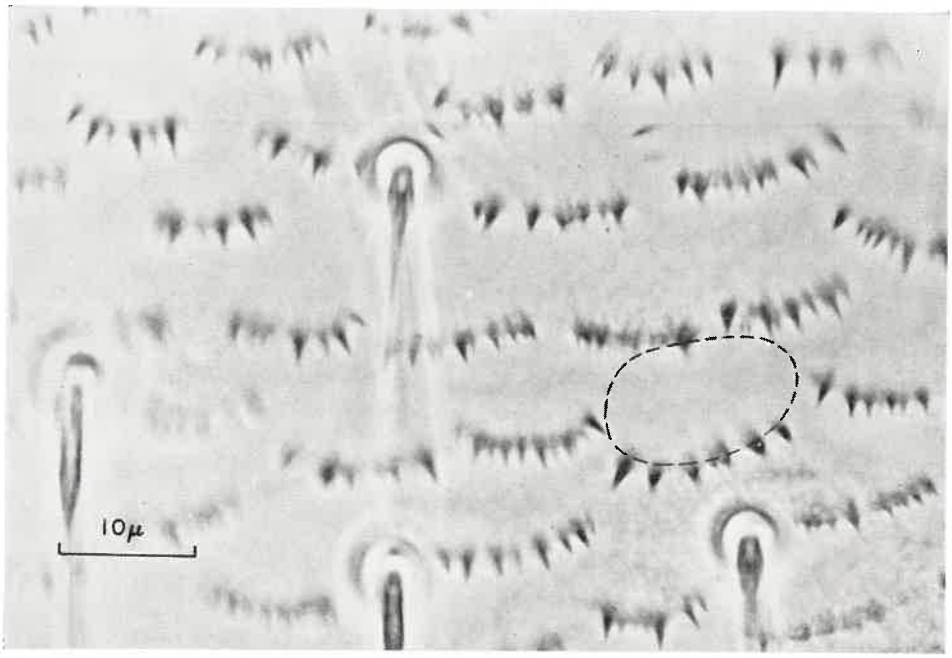


FIG. 1. A region of cuticle from *Oncopeltus*. The cuticle secreted by a single cell is demarcated by the dotted line and includes small tubercles which indicate the polarity of that cell and point posterially. Phase contrast. Scale = 10μ.

retina was mapped out, the cells were already committed to send axons to particular parts of the brain.

When the same operation is performed earlier, at stage 29, normal development occurs, so that the cells make a complete adjustment to their new situation. At stage 29 therefore the retina was not spatially determined. A most interesting result is obtained when the same operation is performed between the two previous ones at stage 30. Then it is found that the cells of the retina were determined in the antero-posterior axis, but not in the dorso-ventral axis. In this case when the frog seeks a fly offered in the top right corner of its view, it attacks in the top left.

These exciting experiments indicate an axial system of determination, in which each axis is independent, and suggest that the retinal cell measures its position on a biaxial grid. Carrying this grid reference the cell then forms an axon which seeks out the equivalent reference on the optic tectum in the brain.

To find out more about the establishment of these axes, we need an animal in which they are still plastic, so that they can react to interference. We also need the cells to express their polarity and position more directly. The insect epidermis seems to fulfil these requirements: it is a single layer of cells and each cell secretes its own piece of cuticle. Each individual piece of cuticle thus tells us about the cell which made it, and may for instance include a number of small tubercles which point in a particular direction, and indicate the orientation of the cell (Fig. 1). In many insects a special minority of cells divide to form bristle-forming cells. These bristles grow out in a particular direction in relation to the axes of the insect. It is also helpful that, because the cuticles are made successively by the growing and moulting epidermis, the effect of an operation can be recorded in several stages from one insect.

Locke (review, 1964) made use of these advantages in an important study of the bloodsucking bug *Rhodnius*. The cuticle on each segment of the adult is marked by folds or ripples which run at right angles to the antero-posterior axis (Fig. 2). Locke's experiments consisted of cutting out pieces of

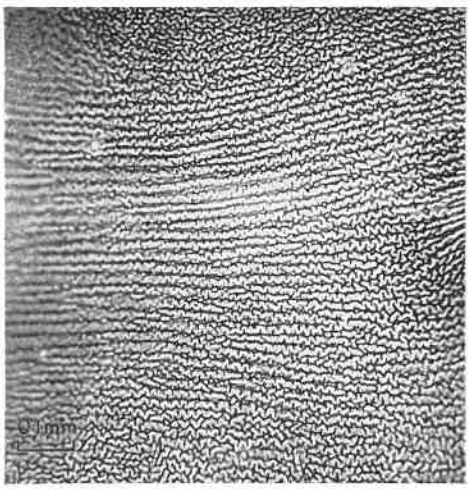


FIG. 2. Part of a tergite of adult *Rhodnius* to show the oriented ripples. Phase contrast. Scale = 0·1mm.

epidermis and cuticle and reimplanting them in a different place or in different orientations. He performed these operations on the 5th-stage larva; the epidermis healed and then made the adult cuticle. He soon found that the ripples expressed the polarity of the cells, for when he took out a piece and reimplanted it in the same orientation, the adult was normal, but rotation of the piece through 90 or 180 degrees resulted in a change in the ripple pattern (Fig. 3). Locke showed that there was no effect when he transplanted pieces from side to side and therefore deduced that he only studied a single axis. However transposition of pieces within the segment but *without change in their orientation* (Fig. 3) had strong effects on the adult. Thus the reaction between graft and host which led to the altered pattern could not be explained by a difference in polarity only. Locke deduced that there was a gradient of something down the segment which controlled the orientation of the ripples, and when cells of different gradient levels were placed together they interacted to result in a change in ripple orientation. Because transplantation between segments at the same level produced no effect he showed that this gradient was repeated in each segment.

These experiments drew attention to the intersegmental membrane which intervenes between two adjacent gradients and constitutes a 'precipice'. In *Oncopeltus* the milkweed bug, the indicators of polarity are not ripples, but oriented hairs. Occasionally this bug lacks a section of intersegmental membrane and here the polarity of the hairs is strikingly altered (Fig. 4). These interesting insects suggested something else about Locke's gradient. They suggested that the gradient itself was in dynamic equilibrium, and that Locke's operations upset that equilibrium. I visualized this in terms of a model, in which the gradient was represented by a slope of sand (Lawrence, 1966). A sand gradient has a maximum stable slope which results from an equilibrium between gravity and the friction between the sand grains. Figure 6 shows how the disposition of sand near an interruption in glass plates which separate two adjacent gradients has the same patterns as the hair pattern in the insect (Fig. 4).

The insect segment is not full of sand. The gradient was therefore postulated to consist of something which behaved in the same way as a sand gradient, such as a concentration gradient of a diffusible substance. It was suggested that the gradient was maintained by an equilibrium between diffusion (equivalent to gravity in the sand model) and some cellular pumping of the substance (equivalent to friction between the sand grains). This diffusion gradient could, for example, depend on action by the two margins. The anterior margin of the segment might maintain a high concentration of the gradient substance and the posterior, a low concentration. How well does this model predict the results produced by Locke? We have recently been repeating and extending Locke's experiments and comparing the results with computed patterns, assuming a diffusion gradient (Lawrence, Crick and Munro, in preparation). The result of an operation in which a certain time period intervenes between operation and cuticle deposition is compared with a computed pattern in Fig. 7. There is a good fit, so it is likely but not certain, that a diffusion gradient specifies polarity in the axis of the *Rhodnius* segment.

A gradient contains two kinds of information, a direction of slope (polarity) and a concentration (position). We have suggested that the cells use the polarity, do they also use the positional information? Some important experiments by Marcus (1962) on another insect, the Waxmoth *Galleria*, have suggested that they do. The adult segment of *Galleria* is conveniently divided into strips of different kinds of cuticle, and scales indicate the

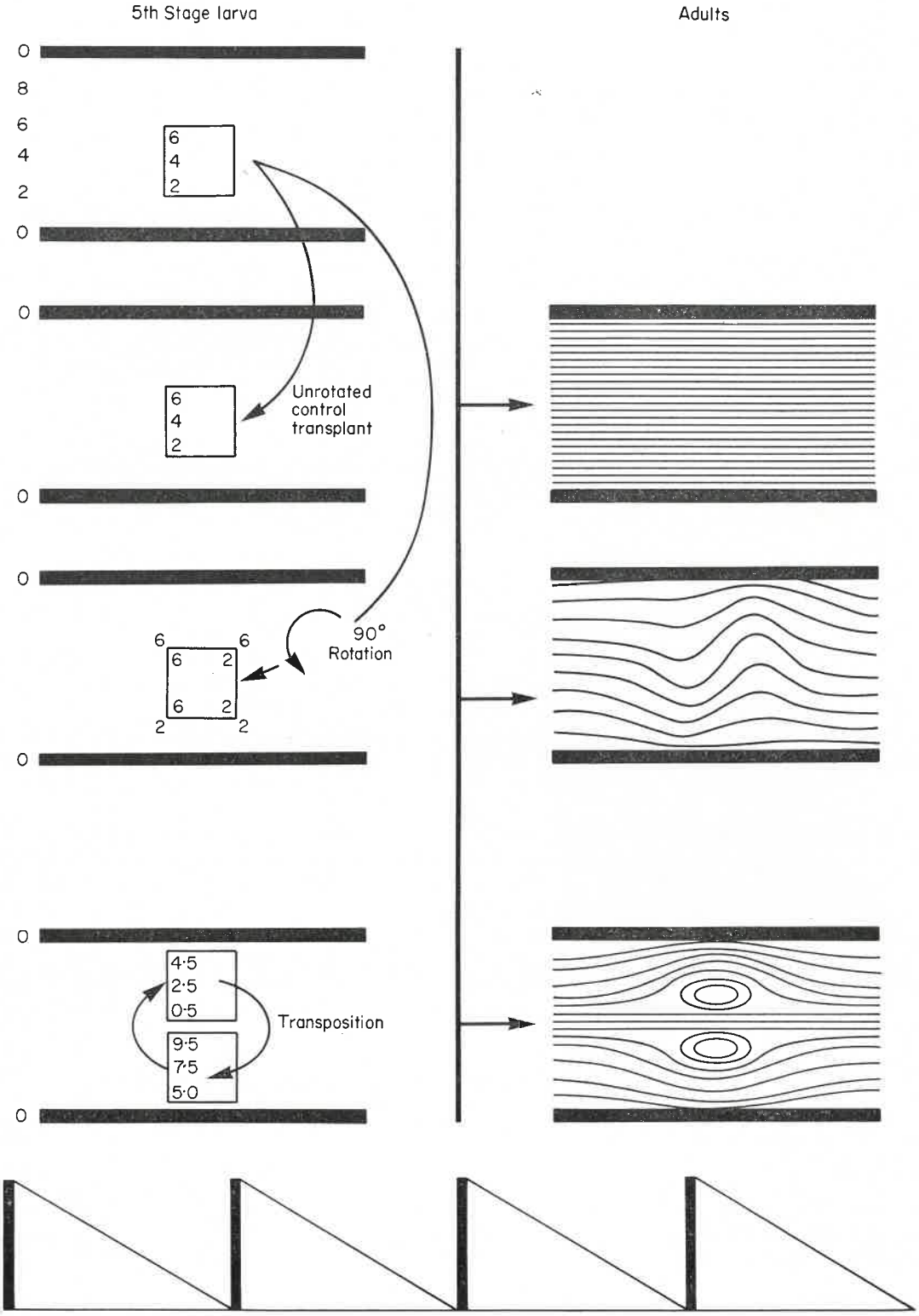
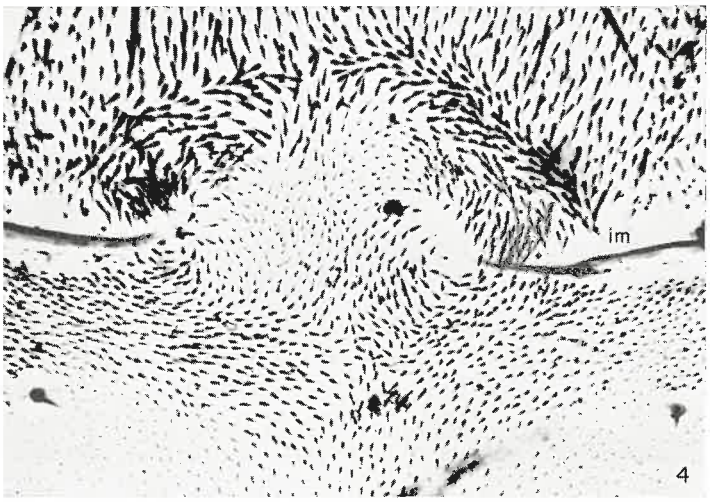


FIG. 3. Summary diagram of Locke's experiments on *Rhodnius*. The operation is indicated on the left and the effect on the adult ripple pattern on the right. Below the repeating segmental gradient is shown.



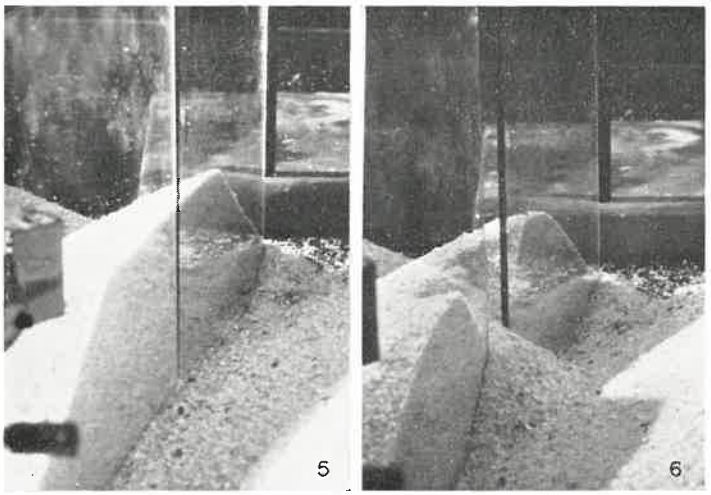


FIG. 4. *Oncopeltus* sternite near a discontinuity in the intersegmental membrane. Note pattern of altered hair orientation (from Lawrence, 1966).

FIGS. 5 and 6. The sand model: the segmental gradient is represented by sand and the intersegmental membrane by intervening glass plates. When these glass plates are opened the sand flows until it comes to rest as shown in Fig. 6. The new pattern of slopes is very similar to the hair orientation pattern (from Lawrence, 1966).

local polarity. These structures are not present in the caterpillar epidermis. Marcus cut out a piece of integument in the caterpillar including cells which would make a special kind of cuticle found at the posterior margin of the adult segment (say gradient height 0). He implanted this graft into the central part of the segment (say gradient height 2). After moulting and metamorphosis the orientation of scales in the adult showed there was a pit in the gradient, and in the centre there was cuticle of type 0 made by the grafted cells. In the general area around there was of course cuticle and scales of type 2. The most surprising fact was that in between the type 0 and type 2 cuticle there was a region of type 1 cuticle (Fig. 8). Here the cells had responded according to their position in the gradient, and *not* to their position in the segment nor to their developmental history. It seemed that the position in the gradient, the concentration of the postulated diffusible substance, determined their development.

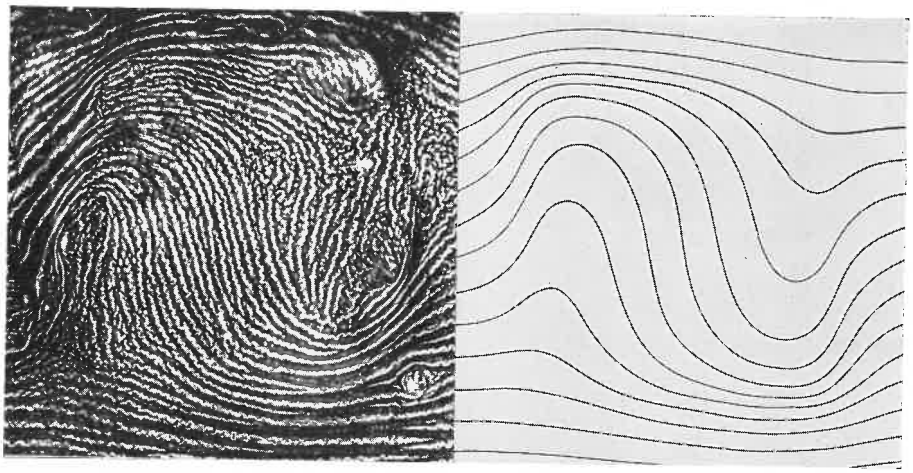


FIG. 7. The pattern produced in adult *Rhodnius* after 90 degree rotation in the 5th-stage larvae compared with the computed pattern assuming a concentration gradient and allowing diffusion for the appropriate period of time.

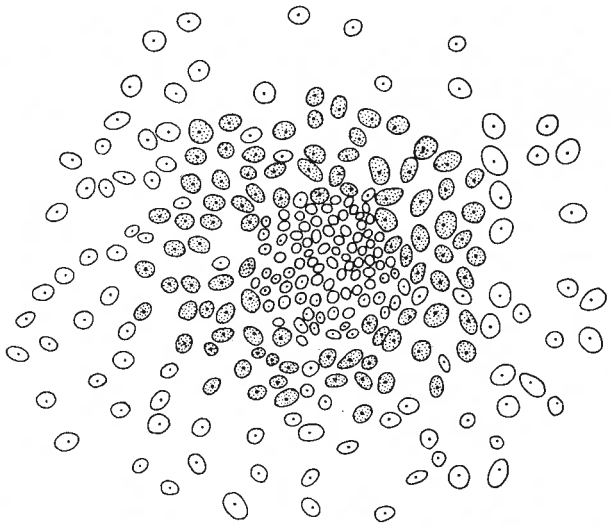


FIG. 8. Marcus' experiment. A detail of the adult after transplantation in the caterpillar of presumptive region 0 into presumptive region 2. The small nuclei in the centre are forming region 0 cuticle and are mostly from the graft (undotted nuclei). The stippled nuclei are from the host (all host nuclei have a central dot) and are forming scales of type 1.

We may conclude that in the insect segment there is a gradient which behaves as if it is a diffusible substance, and determines both the polarity and the position of the cells within it. According to this hypothesis the cells know where they are by measuring the concentration of diffusible substance.

It is characteristic of biologists to generalize from the most particular to the universal. In this case we would like to know whether similar gradients are present in other developing animals. In *Hydra* morphogenetic gradients have long been postulated, and recent work (Webster, 1971) has shown that in certain respects they too behave as a concentration gradient of diffusible substance. Some experiments performed on amphibians have also been interpreted as indicating a diffusion gradient (see Dalcq and Pasteels, 1938). In these organisms however, because of the lack of polarity indicators, the local direction of slope cannot be determined, and the nature of the gradient remains a more open question.

There are a number of experiments in insects which suggest that the pattern-forming process has two components: a general gradient-like system, and a more specific response to it. I shall illustrate these experiments mostly performed by Stern and his school (Stern, 1968) with one example: Roberts (1964) was able to construct *Drosophila* individuals which had a mosaic limb growing on an antennal base. The mosaic was made up of marked patches of leg and patches of antennal tissue, and was an organized integrated structure. It seemed that the response of the leg cells was always to make the appropriate leg structure characteristic for that position in the limb. Similarly the antennal cells reacted appropriately to their position. Both these cell types were reading their position in the same way, but interpreting it differently. Wolpert (1968) has produced a helpful analogy: he pointed out that the French Flag and the Stars and Stripes are patterns which are invariant regardless of the size of the flags. Size invariance of pattern is also a characteristic of many organisms (a familiar example is *Hydra*). Wolpert suggested that a gradient could be set up from the end and along each axis of the flag (which for our purposes can consist of a mosaic of coloured cells). This gradient would run between two maximum limits (say 0 and 10) so that the mean value (5) would always specify the middle of the flag regardless of absolute size. The two gradient values would specify the positional information to the cell in the developing pattern, which provided it knows which 'country' it belonged to, and had an appropriate information store, it could use to determine its colour. These gradients would be the same in the different flags, so that if a small portion of French tissue were transplanted to the American flag it would react appropriately to its new position in the whole flag but in a French way. An identical mapping system could be used to make two different patterns.

How does a cell learn its 'nationality'? It could be that this has been learnt from a similar gradient system which was set up much earlier, when there were far fewer cells. During cell division this information is then inherited by all the daughter cells. In this view, the acquisition of a cell's identity —determination—is a progressive process each step restricting the cell's developmental repertoire. These ideas can be regarded in this way: suppose the information in the DNA is like an encyclopaedia consisting of 10 volumes; and suppose the pattern-forming system can spatially subdivide a population of cells into 10 parts. Each group of cells then closes those 9 volumes which are inappropriate to it. The cells in a group then divide, but remember their volume. After growth an identical pattern-forming system then subdivides the group into ten further groups according to chapter. After further growth and subdivision the cells are limited to a particular page. It is not clear how far the information is really organized in this way: if it were exactly in this form functions common to differentiated cells in many regions of the body would have to be repeatedly written. However the analogy makes the point that a simple pattern-forming process could be used repeatedly, to achieve immense diversity of spatial organization. Indeed, some evidence that determination is progressive (to leg, then to tarsus, then to a particular bristle) has come from studies on imaginal discs of *Drosophila* (review see Lawrence, 1970).

These very generalized views of development hardly begin to explain the phenomenon we started out with, the characteristic and precise development of each species. However they do point to the possibility that mechanisms which organize cells in space may be far more general that the diverse end results would appear to indicate. This view speaks strongly for the careful study of

particularly amenable systems—such as the insect segment and the amphibian retina—which might lead to more fundamental insight. Most strongly of course, the diffusible gradient hypothesis demands identification of the substances involved.

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