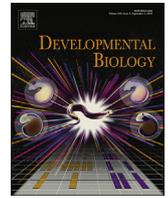


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## Commentary

### Commentary on Fristrom (1976)



Dianne Fristrom's 1976 paper was a pioneering contribution in developmental and cell biology, and one that spawned and informed a number of papers emerging from the morphogenesis group at the University of California, Berkeley in the years following, and many more thereafter and elsewhere. Until this study, epithelial participation in morphogenesis was thought of in terms of cell division, growth, spreading, and bending of sheets of cells, cells linked by circum-apical junctions into a contiguous arrays much like a grouted mosaic of tiles on floors and serving as mechanical and physiological barriers, properties that seemed to rule out any planar deformations due to cell rearrangements. Based on previous work (Fristrom and Fristrom, 1975), the 1976 paper laid out two alternative ways that epithelial tubes, in this case, the *Drosophila* imaginal leg disc, could become narrower and longer with a given number of cells: change in cell shape, or change in cell arrangement. Fristrom tested these alternatives with a difficult morphometric analysis of scanning electron micrographs of the basitarsal region of leg discs at several intervals during their evagination, an analysis that revealed halving of the number of cells in its circumference and doubling the number in its length. The conclusion was clear; epithelial cells, tightly bound into a "pavement" by apical septate junctions could rearrange and potentially play an active role in the tissue shape changes of morphogenesis!

More papers followed in the 1980s (see Fristrom, 1982, 1988), and this work contributed to an exciting time in morphogenesis at Berkeley over this period. It stimulated a number of studies there and elsewhere on epithelial cell rearrangement in many systems, including invagination and archenteron elongation in echinoderms (Hardin and Cheng, 1986; Hardin, 1989), in axis extension of *Xenopus laevis* (Keller, 1978), and in epiboly of the fish, *Fundulus heteroclitus* (Keller and Trinkaus, 1987). Computer modeling studies of cell rearrangement and other aspects of epithelial morphogenesis were done in several systems (Odell et al., 1981), Oster and Welicky (1990), Weliky and Oster (1990), and Weliky et al., (1991). The mechanisms and morphogenic functions of cell rearrangement were topics of vigorous discussion in the very popular "Embryology Club", a Wednesday evening colloquium (and graduate class) at Berkeley. Many unsuccessful attempts were made in the 1980s to live image cell rearrangement during the complex unfolding and elongation of the imaginal disc. Then Condic et al. (1991) found that a novel mechanism of apical cell shape change also contributes to elongation of the imaginal leg disc, a contribution larger than that of cell rearrangement in a particular region, which took some attention away from cell rearrangement. Finally, thirty-some years after Dianne Fristrom's 1976 paper, Taylor and Adler (2008) used time-lapse confocal imaging of GFP expression, driven in defined domains of the leg and wing discs, to show directly that cell rearrangement and cell division occur throughout the imaginal wing and leg discs and contribute to their elongation during evagination. Fristrom (1976) is the beginning of long story of how cell rearrangement has come to be known as one of the most widely distributed, most important, and most studied mechanisms of morphogenesis, a "household" word in developmental biology.

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# The Mechanism of Evagination of Imaginal Discs of *Drosophila melanogaster*

## III. Evidence for Cell Rearrangement

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The shape and arrangement of cells in leg discs of *Drosophila melanogaster* at different stages of evagination were examined by scanning electron microscopy. The observations indicate that the change in shape of the disc during evagination is largely a result of cell rearrangement. This process involves small movements of many cells within the disc epithelium while close associations between neighboring cells are maintained.

### INTRODUCTION

Imaginal discs are small packets of cells which occur in the haemocoel of the *Drosophila* larva and are the precursors of most of the external structures of the adult fly. Each disc forms a specific region, e.g., a leg disc gives rise to a leg and part of the thorax, an eye disc gives rise to an eye and surrounding head structures. The first step in the metamorphosis of appendage-forming discs (legs, wings, halteres, and antennae) involves a dramatic change in the shape of the tissue, termed evagination. Within 15 hr after exposure to  $\beta$ -ecdysone *in vitro*, the disc takes on the approximate shape of the adult structure it is destined to form. In the case of the leg discs, which are described here, evagination involves the conversion of a concentrically folded epithelial disc into a long, narrow, segmented cylinder (Fig. 1). In order to account for this observed change in morphology it was previously proposed that the cells of the disc re "rearranged" during evagination (Fristrom and Fristrom, 1975). This report provides evidence for cell rearrangement from scanning electron microscope (SEM) observations of the shape and arrangement of cells in the leg disc at different stages of evagination.

### MATERIALS AND METHODS

Discs from late third instar larvae were prepared for SEM by allowing them to settle onto small squares of glass coated with poly-L-lysine (Mazia *et al.*, 1975), to which they remained attached. They were then fixed and dehydrated as described previously (Fristrom and Fristrom, 1975), critical point-dried from Freon, coated with palladium/gold, and observed in a Coates and Welter microscope. Partly and fully evaginated discs were obtained by incubating discs from late third instar larvae for 8 hr or overnight in Robb's culture medium (Robb, 1969) with 1  $\mu$ g/ml of  $\beta$ -ecdysone and then by processing as for unevaginated discs. This high concentration of  $\beta$ -ecdysone was used since it inhibits cuticle deposition (Logan *et al.*, 1975), which otherwise obscures cell boundaries. Under the conditions used here, the proximal leg segments are not well formed in the evaginated disc, so observations were confined to the five tarsal segments and the tibia. It can be seen from Fig. 1 that the corresponding regions of unevaginated discs are concealed from surface view by the overlying peripodial layer and precursors of the proximal leg segments. This overlying tissue was dis-

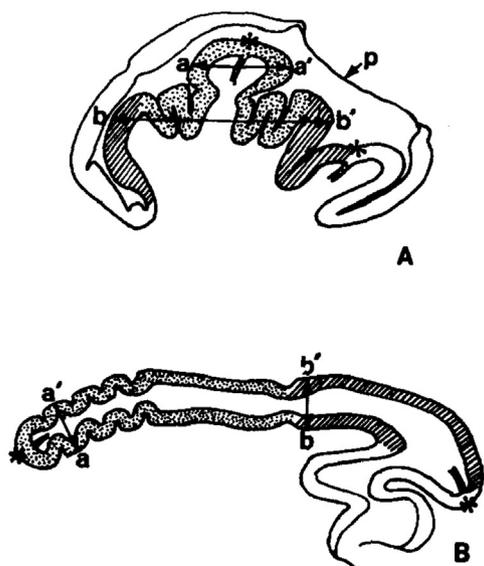


FIG. 1. Diagram of a median longitudinal section of (A) an unevagnated and (B) an evagnated leg disc. (Not to scale.) Stippled area shows approximate extent of the prospective tarsus, shaded area shows the tibia, and unshaded area shows the proximal segments (femur, coxa, and trochanter). Peripodial layer (p). Note the reduction in the distances  $a$ — $a'$  and  $b$ — $b'$  in (B) cf. (A), asterisks, points of invagination of apodemes. [N.B. The labeling of this figure with respect to the extent of the tibia and femur has been revised from Fig. 1 in Fristrom and Fristrom (1975).]

sected off with fine tungsten needles after fixation. Estimates of cell numbers were made from SEM photographs of discs at different stages of evagination.

#### RESULTS

Throughout evagination, the disc epithelium consists of a single layer of cells connected along all adjacent surfaces by a series of specialized junctions (Poodry and Schneiderman, 1970; Fristrom and Fristrom, 1975). We know from previous studies that cell division is not necessary for evagination *in vitro* since evagination can occur in the absence of DNA synthesis (Fristrom *et al.*, 1973) and in the presence of colchicine (Fristrom and Fristrom, 1975). Similarly, cell death has been eliminated as a morphogenetic factor in disc evagination (Fristrom and Fristrom, 1975). Thus, we conclude that evagination can occur *in vitro* without a significant

change in cell number. Theoretically, there are two ways to change the overall shape of a given set of cells in a single layer: (1) by changing the shape of the component cells or (2) by moving cells into new positions. These alternatives are illustrated diagrammatically in Fig. 2 for the conversion of a short, wide cylinder to a long, narrow one. This particular example is used because it illustrates the actual change in shape observed in the basitarsus of the disc during evagination (cf. Fig. 6). It can be seen that a change in shape of this magnitude will require either a substantial change in cell shape (Fig. 2A) or a rearrangement of the cells (Fig. 2B).

SEM observations of unevagnated, partly evagnated, and fully evagnated discs (Figs. 3, 4, and 5) show that the cells remain approximately hexagonally shaped and hexagonally packed throughout. There is a slight increase in the surface area of cells in the evagnated disc, which can be attributed to cell flattening, a process which has been well documented from sectioned material (Auerbach, 1936;

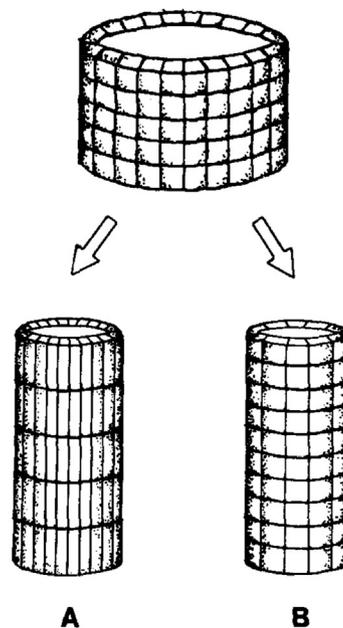


FIG. 2. Diagram of alternative ways to change the shape of a cylindrical piece of tissue with a given number of cells. (A) By changing cell shape. (B) By rearranging the cells.

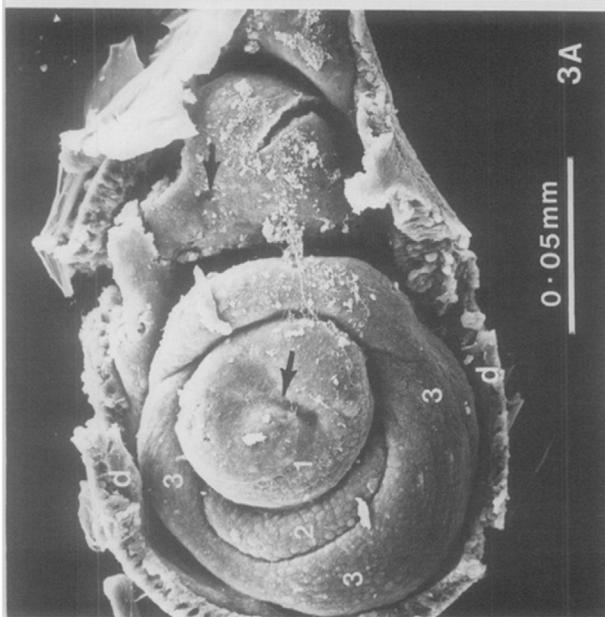
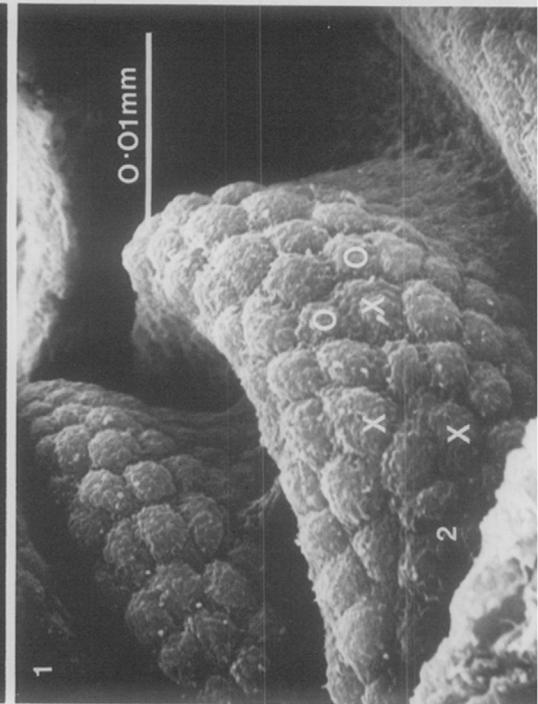
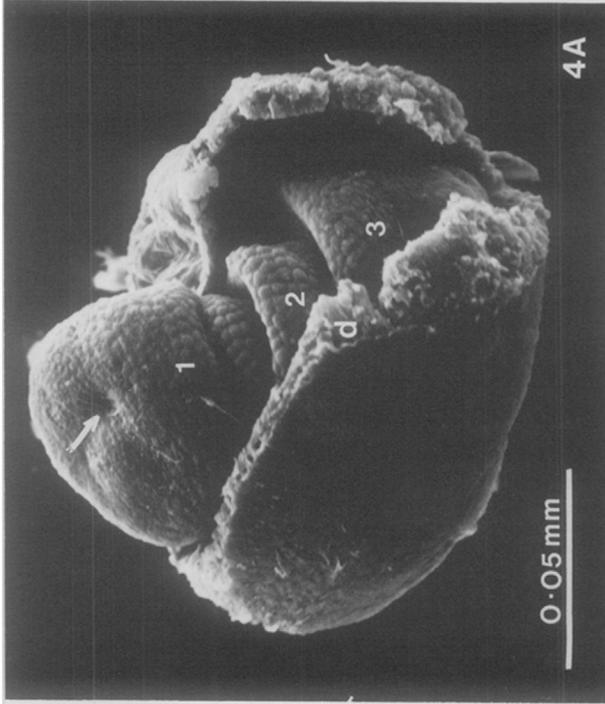
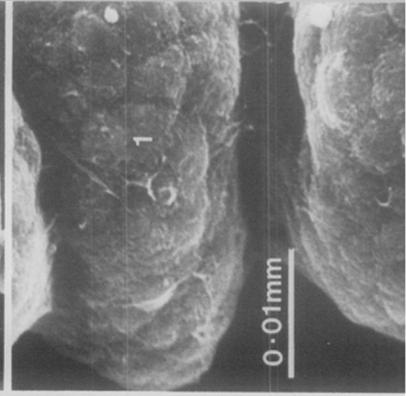
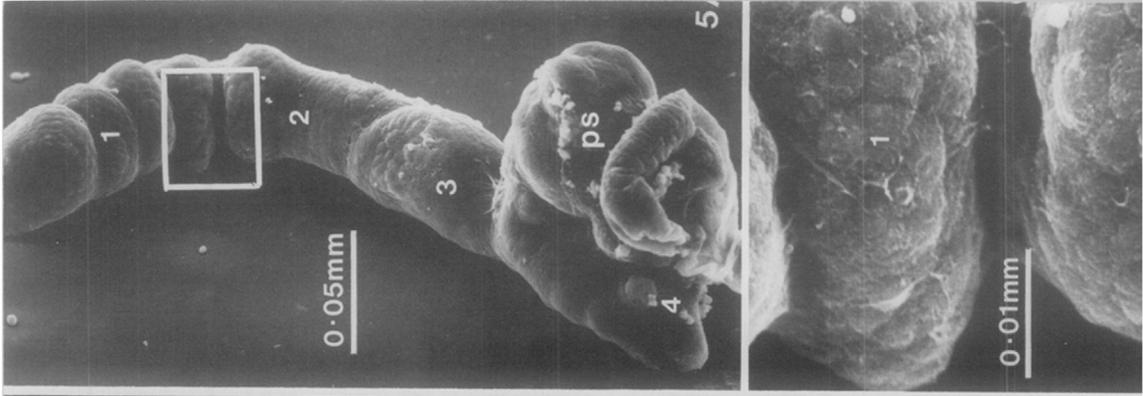
Fristrom *et al.*, 1969; Poodry and Schneiderman, 1970, 1971; Fristrom and Fristrom, 1975). However, cell flattening is not particularly pronounced in leg discs at the stages observed here and results in only a slight increase in surface area. Most importantly, cell flattening does not alter the ratio of length to width of the cells, which would be necessary to effect a change in tissue shape (Fig. 2A). The remaining alternative, then, is that cells move into new positions during evagination. Support for this idea is obtained from cell counts. Referring again to Fig. 2, one would expect an increase in the number of cells in the long axis and a decrease in the number of cells encircling the disc during evagination. The number of cells in the long axis of unevaginated discs cannot be accurately estimated because of the large amount of tissue hidden in the folds. However, it is possible to compare the number of cells encircling unevaginated and evaginated discs in the regions corresponding to the prospective tarsus and tibia (Figs. 3A and 5A). The results are given in Table 1. The decrease in number of cells encircling the evaginated disc is dramatic, especially in the tibia. A greater reduction in the number of cells encircling the tibia (as compared with the tarsus) is expected, since the tibia is peripheral to the tarsus in the disc and so must undergo a greater degree of constriction as the disc is converted to a cylinder (see Fig. 1). It should be pointed out that identification of corresponding regions for cell counts in unevaginated and evaginated discs is only approximate. However, the number of cells encircling the evaginated appendage is fairly uniform from the tip of the tarsus to the base of the tibia (Fig. 5A) and is substantially lower than the number for *any region* of the unevaginated disc.

Perhaps the most useful comparison of cell numbers can be made in the basitarsal regions in partly and fully evaginated discs (Figs. 6A and 6B) (the basitarsus is largely hidden in the unevaginated disc).

Figures 7A and 7B show scanning micrographs of the same region at corresponding stages of evagination. In this case it is possible to estimate the number of cells both along the length and around the circumference of the segment and to make comparisons between different stages of evagination (Table 2). These results demonstrate two points. First, the total number of cells in the basitarsus does not change significantly during evagination. Second, a decrease in the number of cells encircling the segment is concomitant with an increase in the number of cells along the length of the segment. Recalling that cell division and cell death have been eliminated as causes of evagination, this observed change in the distribution of cells in the disc can only result from movements of cells into new positions.

#### DISCUSSION

A number of schemes involving a systematic displacement of cells which results in the observed change in cell distribution can be devised. The model for cell rearrangement originally proposed (Fristrom and Fristrom, 1975) had alternate cells move into new rows, thereby doubling the number of cells in the long axis and halving the number in the short axis (Fig. 8). Although hypothetical, this model can account for the observed change in distribution of cells in the basitarsal region and variations of such a scheme can provide for greater or lesser changes in tissue shape. One restriction on any model for cell rearrangement in discs is that any individual cell cannot move far from its original neighbors. This is dictated by the well-known observations on somatic mosaics in *Drosophila* (Stern, 1940; Bryant and Schneiderman, 1969; Postlethwait and Schneiderman, 1971) which show that clonally related cells remain together during development to form patches. Extensive translocations of individual cells or groups of cells would disrupt patch formation. The scheme illustrated in Fig. 8 in-



volves very slight movements of individual cells. In fact, each cell retains three of its original six neighbors. This type of model is actually supported by the mosaic data referred to above. It can be seen from Fig. 8 that a hypothetical mosaic patch would elongate and narrow along with the rest of the tissue. Thus, the final shape of a mosaic patch would be influenced by the morphogenetic movements involved in evagination and reflects, to some extent, the shape of the tissue in which it occurs. Legs, which are very long and narrow, have correspondingly long, narrow mosaic patches, whereas eyes, which undergo little change in shape during development, have wedge-shaped patches. Finally, one can predict from the cell rearrangement model that marked cells occasionally

might be separated from the main body of the mosaic patch by short distances. Such separations have been observed in wing mosaics (Bryant, 1970) and leg mosaics (Tokunaga, personal communication).

Although observations of the disc surface provide evidence that disc cells are rearranged during evagination, they give no information about the direction or extent of movement of individual cells. However, the actual arrangement of cells suggests a system in a state of flux. In regions of the adult insect epidermis where individual cells can be observed, they form a precise hexagonal array (Dobzhansky, 1929; Whitten, 1973). The disc, in contrast, shows numerous departures from regular hexagonal packing. Many cells are bordered by seven or five cells instead of six, and the cells themselves are slightly asymmetrical (Fig. 4B). Distortions in cell shape and departures from hexagonal packing would be expected in a system where cell associations are changing, since it is impossible to rearrange cells and maintain a perfectly hexagonal array. This is seen in Fig. 8, where intermediate steps in the transition from one hypothetical hexagonal array to another are shown. Although this does not imply that cells which show departures from hexagonal packing are necessarily undergoing rearrangement, it demonstrates that departures from hexagonal packing must occur in the course of cell rearrangement.

Time-lapse films of evaginating discs have been made in an attempt to obtain

TABLE 1

COMPARISON OF THE NUMBER OF CELLS ENCIRCLING UNEVAGINATED AND EVAGINATED LEG DISCS<sup>a</sup>

	Unevaginated	Evaginated
Tarsus	55.16 ± 7.16	34.5 ± 3.9
Tibia	105 ± 16.07	45.16 ± 3.125

<sup>a</sup> Means were determined from at least six specimens ± standard deviation. Regions where cell counts were made are indicated in Figs. 3A and 5A by the numbers 1 and 3. Estimates of cell number were made by counting cells over a measured distance at right angles to the viewer in an area where the cells are clearly defined. Then the circumference of that region was determined either by direct measurement (in unevaginated discs) or from measurements of the diameter (evaginated discs). Total cell number around the circumference was then determined.

FIG. 3. Scanning electron micrographs of an unevaginated disc. (A) Low magnification shows the relative positions of the prospective leg segments: (1) tarsal segments 2-5; (2) basitarsus; (3) tibia; and (4) femur. The peripodial layer and most of the proximal segments have been dissected off. (d) Dissected edge of tissue. Arrows indicate points of invagination of apodemes. (B) A higher magnification of the same disc shows the shape and arrangement of cells.

FIG. 4. Scanning electron micrographs of a partly evaginated disc. (A) Low magnification. Labels as for Fig. 3A. (B) A higher magnification of the same disc shows cell boundaries clearly. Note that the cells are approximately hexagonally shaped but that departures from regular hexagonal packing occur; e.g., some cells (X) have seven neighbors, others have only five neighbors (O).

FIG. 5. Scanning electron micrographs of a fully evaginated disc. (A) Low magnification. (ps) Proximal segments; otherwise as for Fig. 3A. (B) A higher magnification of the region enclosed in the box in (A). Note similarity of cell shape in Figs. 3B, 4B, and 5B.

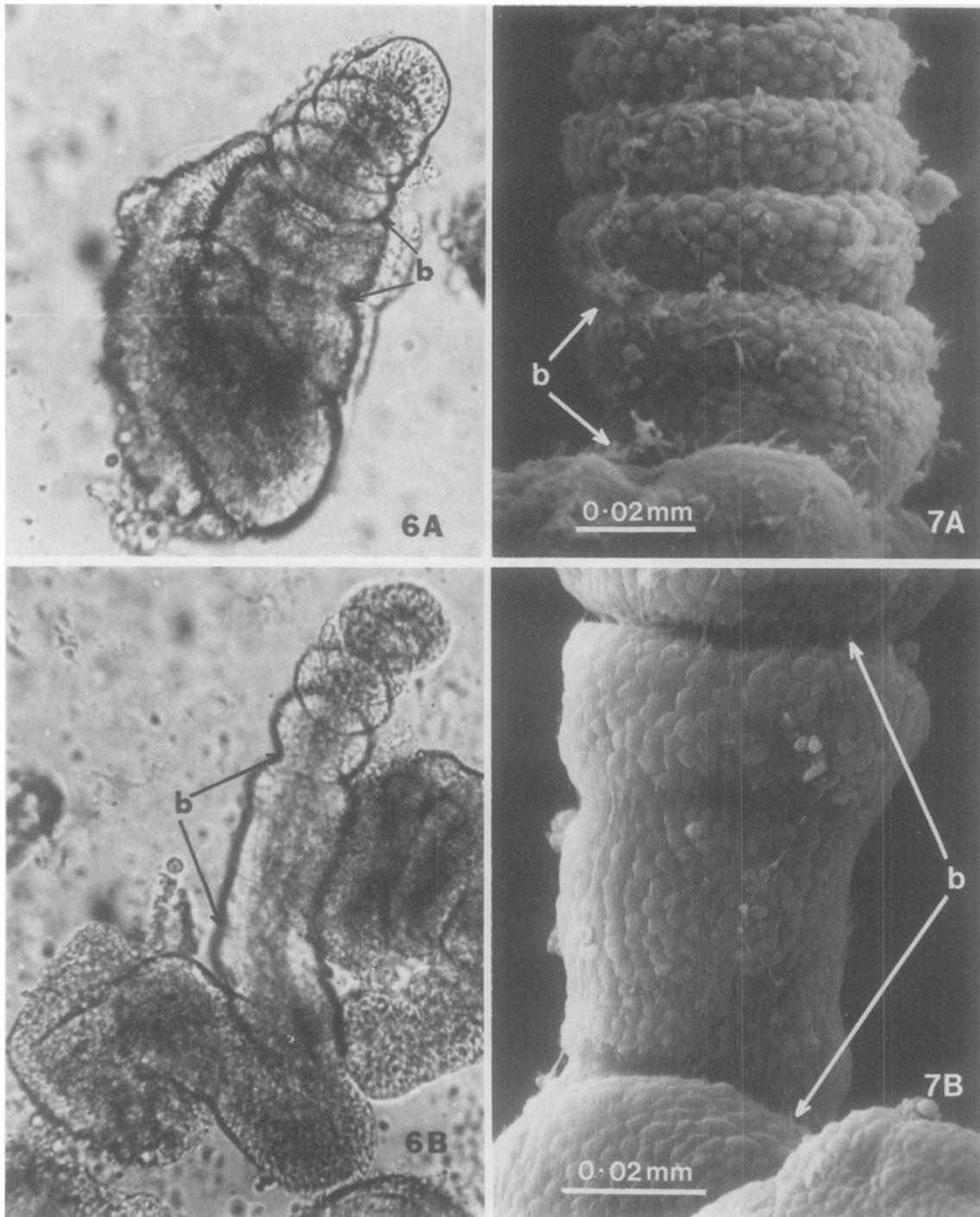


FIG. 6. Light micrographs of a disc in the process of evagination. (A) Approximately half evaginated. (B) The same disc, fully evaginated. Note the dimensions of the basitarsus (b).

FIG. 7. Scanning electron micrographs of the basitarsal region (b). (A) In a half-evaginated disc. The estimated number of cells in the basitarsus of this specimen was 16 (length) and 63 (circumference). (B) In a fully evaginated disc. The estimated number of cells in the basitarsus was 30 (length) and 38 (circumference).

TABLE 2  
CHANGES IN CELL DISTRIBUTION IN THE BASITARSUS  
DURING THE FINAL STAGES OF EVAGINATION<sup>a</sup>

Degree of evag- ination	Mean cell number ( $\pm 1$ SD) <sup>b</sup>		
	Height	Circum- ference	Total
Partial ( $n = 3$ ) <sup>c</sup>	13 $\pm$ 2.6	69 $\pm$ 6.0	888 $\pm$ 101
Intermediate ( $n = 5$ )	20 $\pm$ 2.3	45 $\pm$ 6.6	884 $\pm$ 74
Full ( $n = 5$ )	30 $\pm$ 1.2	31 $\pm$ 4.8	921 $\pm$ 130

<sup>a</sup> Cell number circling the basitarsus was estimated as described in Table 1. Cell number in the proximal-distal direction was determined by counting the longest stretch available. Total cell number was calculated by multiplying height  $\times$  circumference for each specimen.

<sup>b</sup> *t* tests: For total cell number, differences between classes are not significant. For circumference and height, respectively, all differences between classes are significant at the 0.05 level or better.

<sup>c</sup>  $n$  = number of specimens examined.

direct evidence for cell rearrangement. It has not been possible to identify individual cells in these films, but continuous pulsating movements of the disc surface are seen. Pulsating movements are very slight in the unevaginated disc and become increasingly pronounced during evagination. These movements are promptly and completely inhibited by the addition of cytochalasin B (as is evagination, Mandaron and Sengel, 1973; Fristrom and Fristrom, 1975). Thus, pronounced surface activity of cells appears to be a feature of evaginating discs but is not necessarily related to cell rearrangement per se.

The mechanism by which cell rearrangement occurs presents a new problem in cell movement. Cells move without any apparent organ of locomotion, such as pseudopodia or leading lamellae characteristic of the movements of single cells. Indeed, there are no intercellular spaces to permit the extension of such processes. Analogous observations have been made on cultures of fibroblastic (3T3) and epitheloid (embryonic chick liver) cells, where individual cells in confluent monolayers are able to translocate while entirely sur-

rounded by other cells (Steinberg, 1973). In order to distinguish this limited type of cell movement from the migratory movements of single cells, we suggest the use of the term "cell rearrangement" to refer to small movements of many cells within a contiguous sheet of cells where close associations are maintained between cells. In discs, the associations between neighboring cells take the form of specialized junctions (including Zonulae adherentes and gap and septate junctions). These junctions occur along all adjacent cell surfaces. Thus, in spite of their role in cell adhesion, cell junctions must be capable of breaking down and reforming continuously as cells move past each other. The need for junctional fluidity is particularly apparent when evagination is accelerated by mild trypsinization to take place within 10 min

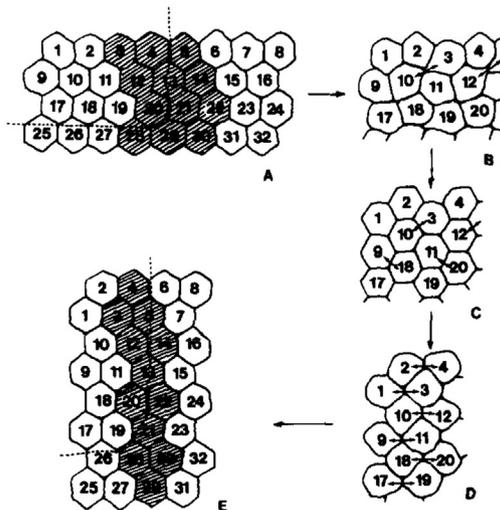


FIG. 8. A hypothetical scheme for cell rearrangement. (A) Cells from an unevaginated disc. The shaded region represents a mosaic patch. (E) The same cells after evagination with every second cell displaced to the upper left. Both the whole tissue and the mosaic patch become longer and narrower and the continuity of the patch is preserved. (B), (C), and (D) Intermediate steps in the rearrangement of the group of cells to the upper left of the dotted line indicate formation of new cell associations. In (B), departures from hexagonal packing are shown; e.g., cell 10 has seven neighbors and cell 11 has five neighbors (cf. Fig. 4B). Note that in (C) a regular hexagonal array is again produced.

(Poody and Schneiderman, 1971). There is no decrease in the extent of junctional contacts seen in thin sections or freeze-fractured preparations of "trypsin-accelerated" discs (Fristrom and Boyles, in preparation) and the process itself appears to be identical to normal evagination (Fekete *et al.*, 1975). Thus, cell rearrangement can occur very rapidly under these conditions.

Cell rearrangement as defined here may well be a widespread morphogenetic process. The fact that it has not, apparently, been described for a developing tissue before, is not altogether surprising. The well-known morphogenetic processes such as cell migration and changes in cell shape (e.g., Gustafson and Wolpert, 1967; Baker and Schroeder, 1967; Spooner and Wessels, 1972; Trinkaus, 1973) are much more readily observed than the cell rearrangement described here. Furthermore, most tissues undergoing morphogenetic changes also contain dividing cells. It would be very difficult to determine whether a change in the distribution of cells is due to cell division or to rearrangement of existing cells. Indeed, cell rearrangement was only recognized in discs after the systematic elimination of cell division, migration, and cell shape changes as possible morphogenetic processes leading to evagination.

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