IS THE UPPER CELL SURFACE UNABLE TO SUPPORT ACTIVE CELL MOVEMENT IN CULTURE?

Recently, in an extensively documented article, DiPasquale and Bell (2) came to the conclusion that the upper surfaces of monolayered cultured cells—epithelia as well as fibroblasts—are unsuitable surfaces for other cells to move or to spread on. Thus, an extra dimension was added to the concept of "contact inhibition of motion" (1). In this paper by DiPasquale and Bell (2), it is suggested that "the inability of the upper cell surface to support spreading may be a *general phenomenon*." This conclusion is well supported by the experimental facts presented and is attractively straightforward; so it might easily be considered to be a kind of *law of nature* and enter into fundamental biological literature as such!

Another article by Elsdale and Bard (4), also very well documented, seems to support this view, at least for the epithelial surface. In their experiments, epithelial cells adhere very well to an underlying "lawn" of fibroblasts, at least if those epithelial cells can act collaboratively as an epithelial sheet. This observation weakens the concept of the "general phenomenon" of DiPasquale and Bell. On the other hand, Elsdale and Bard found that fibroblasts do not spread on the surface of epithelial sheets, which for this aspect confirms the conclusion of the previous authors. The latter authors (4) conclude that "the free surface of an attached epithelium does not provide a suitable substratum for the attachment and locomotion of either fibroblastic or epithelial cells."

In this letter I wish to dispute the *generality* of the conclusions drawn from the observed phenomena by the authors of both papers (2, 4), *not* the validity of their results, by presenting opposite observations. An attempt will be made to reconcile the conflicting observations and opinions by presenting a unifying concept of cell interactions in vitro and in vivo.

In a paper by Visser et al. (5), experiments with primary cell cultures from mouse mammary glands are described; these are cultures of a mixed population of epithelial cells and fibroblastic cells from the mammary stroma. Cultures of high cell densities were striven after. The epithelial cells attached to the glass or plastic bottom of the culture vessel, whereas the fibroblastic cells formed a second layer on top of the epithelium; this is the always occurring feature of these cell-dense mammary gland cell cultures. An occasional fibroblast (unpublished observations) may be attached between the bottom and the epithelial sheet without disturbing the latter's continuity. If, by seeding smaller amounts of cells, a lower cell density is obtained, there is a tendency in the cultures for the cells to grow in separate epithelial and fibroblastic areas (unpublished observations), such as those observed by Ebner et al. (3) in cultures of bovine mammary glands.

In the cell-dense cultures the fibroblasts in the upper layer show active movements resulting in the formation of multilayered "ridges" if hormones are added to the culture medium, and also active movements when, after withdrawal of the hormones, the "ridge structure" is pulled down; all this on top of an epithelial sheet (5)! This example shows that the upper surface of epithelium in vitro is not by definition unsuitable for supporting attachment or movement of other cells.

The following concept may reconcile the conflicting observations. Different cells of different origins, fibroblastic as well as epithelial, and the latter differently if from different organs, have different *tendencies for attachment*. And attachment means attachment to substrata like glass, plastic, and collagen, and also attachment to other cells, cells of the same nature or cells of a different nature. The final pattern of attachment is based on an equilibrium between those different tendencies plus the space available for attachment. The well-known mosaic aspect of epithelium in vitro is the product of a strong tendency of the cells to adhere to each other and a tendency to extend on a substratum. In mixed cultures of epithelial and fibroblastic cells, the stronger tendency of cells of the same nature to stick to each other over the weak tendency to adhere to cells of the other group results in separate groups of each, unless there is insufficient space for such a separate existence. In high-density cultures, the cells with the higher affinity to the substratum will attach to the substratum, and the cells with the lower affinity will have to content themselves with attachment onto the layer of the former cells. Whether the lower layer will be fibroblastic or epithelial in nature may vary with the organ from which the cells originate. Certain cells may refuse to attach to certain other cells or to certain substrata. It should be possible to express in units these different tendencies for attachment; such measurements were made by Weiss (6-8) for the adhesion of cells to different substrata.

Morphogenesis and morphogenetic alterations, e.g., under the influence of hormones, can be explained under the concept given. Hormones, then, act on morphogenesis by altering the equilibrium between the different tendencies for attachment. This does not mean that this

should be the only factor in morphogenesis. Formation of structural reticulin and collagen is another important feature. And so also is control of mitosis.

It is clear that the *nature* of the attachment between cells is different from that of the attachment between cells and a noncellular substratum. Nevertheless both can be expressed in the same units for tendency for attachment.

It is possible that in a more elaborate concept there should be a differentiation between "tendency for attachment" and "force of attachment," the latter being the force required to sever a once-established attachment.

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