COMMENTS ON REPORTED OBSERVATIONS OF CELLS SPREADING ON THE UPPER SURFACES OF OTHER CELLS IN CULTURE

Although we state that "the inability of the upper cell surface to support spreading *may be* a general phenomenon," we point out in the text (reference 4, p. 219) that "we limit our conclusions only to the cell types used in this study and only to in vitro conditions." In fact, we cite one case where this may not apply.

In his letter, Dr. Prop cites several cases where cells in culture are apparently spreading on the upper surfaces of other cells. Elsdale and Bard (5) report that epithelial cells "adhere to and spread out upon fibroblastic lawns." Visser et al. (16) state that primary mouse mammary fibroblastic cells move actively on a mouse mammary epithelial sheet.

We have examained these papers with care and are not entirely convinced that spreading on the upper surfaces of cells in vitro actually occurred in the cases cited.

Our reservations concerning the spreading of epithelial cells on a "lawn" of fibroblasts (5) are the following. (*a*) A spreading epithelial sheet adheres to a plastic substratum predominantly at the margin of the sheet, and it is the marginal cells that play the major role in epithelial spreading (3, 14). Since the area of adhesion to the substratum is relatively small compared to the area of the entire spreading cell sheet (3), it is conceivable that marginal cells send locomotory processes (lamellipodia) to the plastic or collagenous substratum through the gaps which occur frequently in a fibroblast sheet. (*b*) The possibility that the fibroblasts have secreted a collagen carpet or that the epithelial cells and fibroblasts have produced a "basement membrane" between them has not been explored (8). (*c*) Translocation of a given cell over the upper surface of another cell is in no instance shown in any detail. This is inferred by the authors from films in which, unfortunately, the magnification is too low and the cultures too dense to tell whether cells spread by lamellipodia adhering to the plastic substratum or to the upper cell surface. In order to observe in detail the contact behavior of cells on top of a sheet, they must be observed as individuals and followed continuously, as we did (4).

In passing, we also question the general conclusion of Elsdale and Bard (5) that the inability of epithelial sheets to support cell spreading in culture fits them for their in vivo role at free surfaces in epithelial-mesenchymal systems. In light of our results (4), this statement is not valid. As we reported, the upper surfaces of fibroblasts in culture are likewise inhospitable to spreading by other cells; fibroblasts, of course, are not found at free surfaces in vivo.

The conclusions of the paper by Visser et al. (16) are likewise open to question. First of all, it is not clear from their Fig. 1 that there is a "monolayer of epithelial-like cells in mosaic arrangement covered by a layer of loosely arranged fibroblast-like cells." The magnification is too low. There may be a great deal of undetected underlapping (1, 2, 7). Second, they state that after the addition of hormones, "these layers rearrange themselves—part of the epithelial-like and all of the fibroblast-like cells concentrate in interconnected multilayered ridges leaving more or less circular spaces, *bare* or covered with a thinly spread monolayer of cells in epithelial configuration." The *process* of this rearrangement is not described, only the end result. It is possible (indeed probable) that the sheet of cells in a monolayered region detached spontaneously and retracted as a sheet, forming ridges at its

detached edges, with only a few localized areas at the edge remaining attached to the plastic substratum. Cells that remain adherent to the substratum and are therefore not members of the retracted sheet could spread under the retracted sheet at the frequent loci on the margin where such formations are typically not attached to the substratum. Moreover, the existence of "bare" areas (which are not distinguished from "thinly covered areas" in their Fig. 2) suggests that the sheets are indeed not continuous and that areas of plastic substratum are available for spreading. Third, the possibility that the fibroblasts have secreted a layer of collagen has not been tested. There are several reasons for suspecting that such an extracellular matrix has been secreted. (*a*) Visser et al. (16) report that at lower cell densities the epithelial and fibroblastic cell populations remain separate and do not become superimposed on one another. It is precisely at lower cell densities that collagen production is minimal (6, 9, 10). (*b*) The changes in cell distribution which Visser et al. observed with hormone treatment took 36 h to materialize, a sufficient time to establish a substantial extracellular maxtrix. (*c*) The metabolism of collagen and other extracellular material is quite sensitive to a variety of hormones (13, 15). Objection (*c*) to the spreading of epithelial cells on a "lawn" of fibroblasts is also applicable here.

Dr. Prop provides some interesting ideas on the varying tendency of different cells to attach to a variety of substrata (see 17, 18) and its implication for morphogenesis. We know of only one study wherein cells display varying "tendencies" to adhere to what might be termed an "upper" cell surface. Roth and Weston (12) and Roth (11) showed that under the same conditions, the number of cells of one type adhering to the surfaces of aggregates of cells of their own type is significantly greater than the number adhering to aggregates of a different cell type. Unfortunately, however, since it is difficult to observe directly and in detail the surface behavior of cells on the surface of an opaque aggregate, we are in no position to compare these cells with those adhering to and spreading on a plane substratum. Nor do we know what percentage of colliding cells adhered.

The cells used in our study certainly display what Dr. Prop refers to as "tendencies for attachment." However, with respect to cells spreading on a plane substratum, such tendencies are exhibited only in *lateral* intercellular adhesions and adhesions to the plane noncellular substratum, and not in adhesions to the *upper* cell surface. Chick heart fibroblasts completely fail to spread on the upper surface of an epithelial sheet in culture. But, moving on a plane substratum, they do adhere laterally to the marginal cells of an epithelial sheet. Moreover, the duration of lateral adhesions between chick heart fibroblasts on a plane substratum is significantly greater than that between chick heart fibroblasts and chick epithelial cells (19.2 ± 12.3 min for the former, 6.4 ± 3.1 min for the latter; df = 46, t = 3.0202) (DiPasquale, unpublished observations). This suggests a specificity for lateral adhesion but not for adhesion to the upper cell surface. We observed no direct adhesion of particles or cells to the upper surface of cells in culture. Particles come to adhere to the upper cell surface only after first adhering to the leading edge and subsequently moving backward toward the nucleus. Even when cells come to lie on the upper cell surface in this fashion, they still fail to spread and remain rounded.

In sum, we believe that there is still no direct, hard and fast evidence that cells can actually adhere directly to and spread on the upper surfaces of cells in culture on a plane substratum without the intervention of some extracellular material such as collagen.

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