Cellular Adhesiveness in Relation to the Invasiveness of Cancer: Electron Microscopy of Liver Perfused with a Chelating Agent

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Cancer cells are characterized by reduction in, or loss of, their adhesiveness to one another (3, 8). The consequent ease with which they become separate ameboid units is believed to be an important factor in the invasiveness of malignant tumors (4). In previous investigations (5, 10), it has been shown that this loss of cellular adhesiveness is correlated with calcium deficiency of the cancer cell, presumably at its surface. It is not known as yet, however, why the cancer cell is unable to bind a normal amount of calcium.

It seemed possible that inability to bind calcium is related to some structural peculiarity that might be made visible if viewed under sufficiently high magnification. In approaching this problem, normal cells have been observed, and then cells from which calcium has been removed by a chelating agent. Comparison of the ultramicroscopic appearance under these two conditions would, it was hoped, demonstrate differences in the cell surface according to whether calcium was present or not at the cell interfaces.

The liver was selected for this preliminary study because it has been shown that hepatic cells are very tightly adherent to each other, it being impossible to separate them by violent mechanical shaking (8). However, it has also been demonstrated (1) that, after perfusion with a chelating agent, the individual liver cells can be shaken out, indicating a loss of their adhesiveness.

MATERIALS AND METHODS

Tiny pieces of liver, approximately 1-mm. cubes, were removed from anesthetized rats and quickly immersed in 1 per cent osmic acid buffered at pH 7.4. The osmic acid fixative was prepared with 0.2 mg calcium/liter of diluting fluid to prevent possible removal of calcium from the tissues that might occur by diffusion in a calcium-free medium. The tissues were fixed for varying periods from ½ hour to 2 hours, and were then dehydrated with alcohol and imbedded in 100 per cent butyl methacrylate according to the method of Palade (9). Sections were cut with a Krug thermal-advance microtome and then mounted on Formvar-coated copper grids and examined with a Phillips EM 100 microscope.

In other rats the livers were perfused under anesthesia, with 0.11 M Di-sodium versenate (di-sodium ethylenediamine tetraacetate), through the hepatic artery and portal vein, until the livers were well blanched. This well known chelating agent has a strong affinity for calcium and has been demonstrated to destroy the adhesiveness of liver cells (1). Thereafter, these livers were treated as above, except that the osmic acid fixative did not contain calcium.

RESULTS

Normal liver.—The intimacy of contact between adjacent liver cells is remarkable. The cell surfaces are usually so tightly appressed as to appear as very fine lines (Fig. 1). The thickness of these linear structures in our sections approximates 150 A. At higher magnifications it is possible, under appropriate conditions, to see that these boundary lines between cells consist of two elements—the surface membranes of the apposed cells (Fig. 2). Measurements upon the projected images of photographic negatives indicate that each of the cell membranes is approximately 50 A in thickness and that the membranes of two opposed cells are separated by a space about 50 A wide. The thickness of the membrane is the same as that calculated for the erythrocyte membrane by Hillier and Hoffman (7). There is, then, no evidence of the existence of a “cement substance”
between these cell surfaces; whatever the nature of the adhesive bond between the cells, it must be upon a molecular level.

There are, however, two types of structures between apposed cells:

a) The bile canaliculi (Fig. 8) appear as clefts having villiform ultramicroscopic processes extruding from the cell surfaces as described by Fawcett (6).

b) Near the bile canaliculi are the interlocking structures (Fig. 4) first described by Fawcett (6). These interesting structures were not conspicuous in our material, only an occasional one being found by diligent search. It might be that these “joints” help to hold cells in apposition, as suggested by Fawcett, though it is difficult to imagine that they would serve very efficiently in this capacity because of their relative scarcity and in view of the soft gel character of living cells. It may be that their importance lies in the somewhat increased surface area they provide, though again their scarcity would seem to contradict such an interpretation.

**Livers perfused with disodium versenate.—**Generally after livers were perfused with versenate, the cells became separated (Figs. 5, 6), either slightly or widely. This effect is consistent with the observation that, after perfusion with versenate, cells are readily dislodged by mechanical shaking.

However, in some parts of the tissue, cells remained tightly apposed, presumably because in these places the chelating agent had failed to penetrate.

A further change due to versenate was observed in some cells: the membranes became more or less disrupted. They became detached from the subjacent cytoplasm, and here and there their continuity was lost (Figs. 7, 8).

That such structural changes were not due to mechanical effects of perfusion was shown by perfusing livers with balanced salt solution instead of versenate: the sinusoids became dilated, but the cells did not separate.

**DISCUSSION**

Disodium versenate, while not specific for calcium as a chelating agent, may well be presumed to have removed calcium along with other cations from the liver cells in the experiments reported here. Except for calcium and magnesium, however, it is doubtful if the other cations are important in maintaining adhesiveness (10).

The effect of chelation in the present experiments is what would be expected to follow upon withdrawal of calcium and magnesium, i.e., the loss of adhesiveness of the cells. It was not expected, however, that the integrity of the membrane would thereby be destroyed; presumably its existence as an entity is dependent in part upon the presence of these cations.

From these observations it is inferred that the adhesiveness of liver cells is largely dependent upon the arrangement of molecules at the cell surface. As no intercellular cement was demonstrated, and as the thin surface membranes are very closely apposed, it is difficult to imagine an alternative explanation.

It is also apparent that removal of the cations from the cell membranes, by chelation, destroys the adhesiveness of the cells, allowing them to separate from one another, and may also eventually lead to loss of the integrity of the membranes and to their disappearance as structures visible by electron microscopy.

Danielli (2), in his analysis of the chemical structure of the cell surface, conceives of it as composed of lipoid molecules with an adsorbed protein layer. The outermost lipoid layers are so orientated that the hydrated polar groups are in the interfaces, with the protein molecules adsorbed on the inner and outer surfaces. Calcium is presumably bound to the carboxyl groups of the proteins and to the phosphate groups of the lipoids. Inability to bind calcium, then, as in cancer cells might depend upon lack of available carboxyl and phosphate groups on the cancer cell surfaces.

Such a fault in the protein-lipid structure of the cancer cell has yet to be demonstrated. This might be done if it were possible to reveal the cancer cell surface in the same detail that Hillier and Hoffman (7) attained with the erythrocyte surface in which the actual molecular structure was visualized. Although such a project presents dis-

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**Fig. 1.**—Normal rat liver showing intimate contact of cell surfaces. The cell interfaces appear as fine linear structures indicated by arrows. Portions of two nuclei are present. The dark spots represent fat droplets. The oval reticulated structures are mitochondria. The black markers in the right lower corner indicate approximately 1 μ in this photograph and in the other pictures when present. ×7,500.
Fig. 2.—Normal rat liver showing double nature of liver structures seen in preceding figure. Arrows point to areas where the two apposed cell membranes can be distinguished. Tip of nucleus at top of picture. X 2,500.
Fig. 3.—Normal rat liver. Bile canaliculus showing villiform projections from the cell surfaces. × 92,500.

Fig. 4.—Normal rat liver. Interlocking “joint” structure between apposed liver cells. × 15,000.
FIG. 5.—Versene-perfused liver showing beginning separation of cells along interfaces. ×11,250.
FIG. 6.—Versene-perfused rat liver. Separation at the interface between two hepatic cells as a result of the activity of the chelating agent. X30,000.

Fig. 7.—Versene-perfused rat liver showing absence of cell membranes. X15,000.
FIG. 8.—Versene-perfused rat liver. Separation of the hepatic cells is apparent. The cell membranes are absent in some places, present in others, and appear to be partially disintegrated in still other areas. ×11,250.
maying technical obstacles when applied to more complex cells, it seems worth while to make concerted efforts in this direction.

SUMMARY

In view of previous work it was anticipated that lack of calcium on the cell surface might be correlated with ultramicroscopic structural changes. Such changes were looked for in rat livers perfused with a chelating agent (disodium ethylenediamine tetraacetate) to remove calcium from the cell surfaces. Livers so treated were compared by electron microscopy with normal livers.

In normal livers the cells were tightly apposed, with no visible intercellular cement substance. The surface membranes were calculated to be about 50 A in thickness, and the membranes of adjacent cells were separated from one another by 50 A.

In liver perfused with versenate, cells were no longer closely apposed but were separated to greater or lesser degree. In some instances the membranes were completely detached from the underlying cytoplasm, and even tended to disappear entirely.

These findings suggest a molecular bond of calcium between apposed cells, presumably by linkage of the calcium to the carboxyl groups of the proteins and to the phosphate groups of lipoids, as the basis of cellular adhesiveness.

REFERENCES

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