# INVESTIGATION OF INTERCELLULAR CONTACTS IN ISLETS OF ZAJDELA'S ASCITES HEPATOMA BY THE DISPERSION METHOD

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Investigation of contacts between cells of Zajdela's ascites hepatoma revealed their great similarity with contacts in the normal liver. An essential difference in ascites hepatoma is the presence of a powerful supramembranous layer, uniting large groups of cells, resulting in a stronger linking of the cells together than in the normal liver.

By means of the tissue dispersion method, definite information can be obtained regarding the chemical nature of intercellular contacts. This method has shown that in most epithelial tissues calcium is essential for maintenance of a strong link between cells, and its removal leads to the rupture of intercellular contacts [1, 4, 5].

When differences between the contacts of ascites hepatomas and those of the normal liver are investigated, the dispersion method with different calcium concentrations has shown differences not disclosed by any other methods: neither by measurement of mechanical linking, nor by determination of the electrical bond between the cells. It was therefore decided to use this method to study contacts in Zajdela's ascites hepatoma (AH), which are indistinguishable from contacts in normal liver as regards both their linking [2] and their electrical properties (personal observations).

## EXPERIMENTAL METHOD

On the sixth day after grafting to the tumor, ascites fluid was taken from the peritoneal cavity of the rat. To prevent it from clotting, the vessel was rinsed with 1% sodium citrate solution. The liquid was diluted with physiological saline and the tumor cells were separated from erythrocytes by centrifugation at 1000 rpm for 2 min several times until all erythrocytes had been removed. Physiological saline was added to the cell residue, and the suspension was poured in volumes of 5 ml into tubes 0.8 cm in diameter and 10 cm long. To obtain better dispersion, between five and seven glass beads 0.3 cm in diameter were placed in each tube. The tubes were sealed and placed on a mixer, on which they were aggitated at 240/min for 30 min at 20°. In the course of treatment with several agents in succession, dispersion was carried out only in the last substance, and ordinary incubation for 30 min in the remaining substances. After treatment with each agent, the cells were washed three or four times with pure medium.

## RESULTS

The action of two substances—trypsin and EDTA (versene)—was most effective and interesting. The results of experiments with these agents are given in Table 1.

After dispersion in calcium-free medium, the distribution of the cells in complexes was indistinguishable from that before the experiment. No lysis was found.

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TABLE 1. Distribution of Cells by Complexes (in %) Following Action of Various Agents

| Agent | No. of cells in complex | | | | | | No. of cells remaining after exp. (in %) |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5–10 | over 10 | |
| Calcium-free medium (control) | 2,2 | 4 | 5,3 | 5,4 | 17,5 | 66 | 100 |
| EDTA, 0.5% | 5 | 6 | 8 | 11 | 37 | 39 | 90 |
| Trypsin, 0.5% | 12 | 3 | 6 | 8 | 16 | 50 | 30 |
| Trypsin, 0.5%; EDTA 0.5% | 40 | 28 | 6 | 16 | 7 | — | 20 |
| EDTA 0.5%, trypsin 0.5% | 52 | 20 | 19 | 7,6 | 1,4 | — | 85 |

Dispersion in EDTA leads to destruction of large complexes containing more than ten cells. Lysis was slight. After dispersion for more than 30 min, the intensity of lysis was increased, but no further breakdown of the complexes took place.

Trypsin caused severe damage to the cells. Lysis reached 70%.

After treatment first with trypsin, then with EDTA, the action of these agents was complementary. Lysis was increased to 80%, the large complexes disappeared completely, and they were replaced by small complexes and single cells.

The action of these same agents in the reverse order was more effective as regards increasing the number of single cells and reducing lysis. After dispersion for more than 30 min, appreciable damage was done to the cells, but no further destruction of small complexes took place.

These results show that contact between cells in AH differs from contact between normal liver cells in two respects. First, normal contact is destroyed in calcium-free medium, but rupture of the contact in AH requires the use of EDTA. Second, after removal of calcium, before AH cells can be separated, additional treatment with trypsin must be given, which is not necessary for normal liver cells. Both these facts can be explained on the assumption that islets of AH cells are surrounded by a polysaccharide membrane. This was confirmed by staining the complexes by Mowry's method. This membrane may prevent elution of calcium, and may also hold the cells together in a complex after removal of calcium or its inactivation. Incubation of the cells in EDTA destroys the intercellular contacts, while dispersion in trypsin evidently destroys the supramembranous layer of polysaccharides. The fact that trypsin in this case does not destroy the cells themselves, although in treatment in the reverse order lysis takes place strongly can be explained on the basis of Malenkov and Modyanova [3]. When normal liver cells are incubated in a saline medium they become resistant to the lytic action of trypsin. If, however, cells are treated with trypsin immediately after isolation, they undergo lysis. This phenomenon is explained by reconstruction of the cell membrane under the action of prolonged incubation so that it becomes impermeable to trypsin. In the case of AH, an analogous reconstruction of the cell membrane evidently also takes place at the points of contact.

The essential difference between AH cells and normal liver cells is thus that complexes of AH cells are surrounded by a mucopolysaccharide layer which gives additional strength of linkage. Otherwise, the properties of the contacts in AH and in normal liver are similar.

## LITERATURE CITED


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