cells and megakaryocytes, and they are found only rarely in the liver cells [14]. It can be tentatively suggested that the absence of a response of DNase II in the spleen cells is connected with the fact that the virus acts in a certain manner on the lysosomal apparatus of the infected cells. An alternative possibility cannot be ruled out, namely that Friend virus, which has many antigens common with the cytoplasmic membrane of cells sensitive to the virus on its surface, is not recognized as "foreign" and, consequently, escapes the action of lysosomal DNase.

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CONTACT INTERACTION BETWEEN ASCITES HEPATOMA 22a CELLS AND SOLID SUBSTRATE

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Under certain conditions, widely different types of cells can adhere to the surface of any solid substrate such as glass, certain polymers, metals, and so on. Adhesion is the initial phase of a complex reaction of contact interaction between cell and solid substrate. The study of the particular features of this reaction in tumor cells is not only of general biological, but also of medical, interest: Processes such as metastasization and invasive growth may be based on a disturbance of contact interaction with the substrate [1]. Recent investigations have shown that the character of contact interaction with the substrate is considerably modified in cells subjected to tumor transformation, compared with their normal analogs [2-6].

Cells which evidently stand at the highest level of tumor progression, namely ascites tumor cells, are of great interest. According to some features these cells possess the highest degree of transformation. It was considered important to discover to what extent the reaction of contact interaction with a solid substrate is disturbed in ascites cells.

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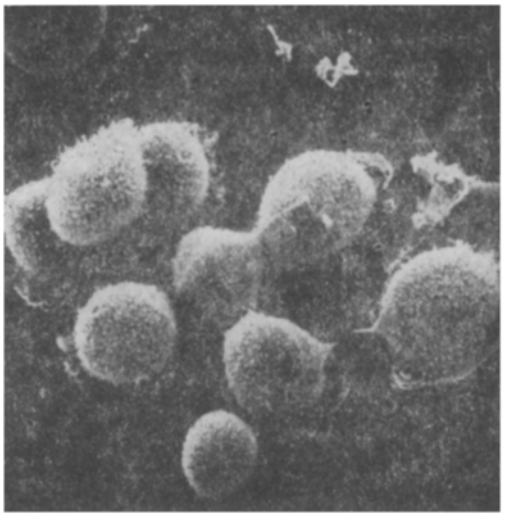

Fig. 1

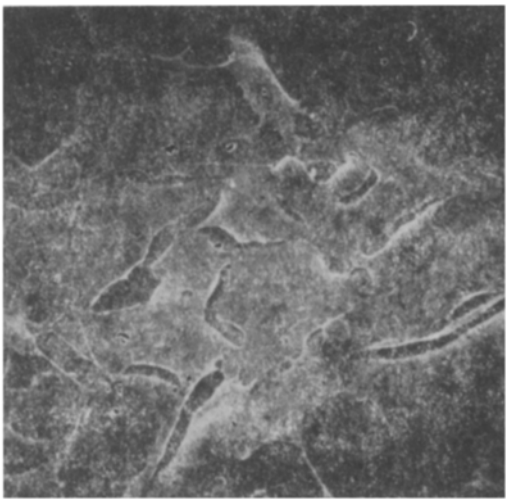

Fig. 2

Fig. 1. AH-22a cells adherent to surface of glass (incubation for 24 h), hemispherical in shape, with thin concentrically arranged lamelloplasm at their base (1050 ×).

Fig. 2. Cells of line AH-22a K (24-h culture), widely spread out and considerably flattened (525 ×).

EXPERIMENTAL METHOD

Ascites hepatoma 22a (AH-22a) was transplanted intraperitoneally into C3HA mice. The cells were separated from the ascites fluid by centrifugation and were washed once with warm Hanks' solution or with medium No. 199.

The washed cells were resuspended in culture medium (50% medium No. 199, 30% lactalbumin hydrolysate, 20% embryonic calf serum) and poured into wells on plastic plates (diameter of well 15 mm, height 17 mm), in the bottom of which the solid substrates were placed: pieces of ordinary coverslips, coverslips coated with a layer of carbon (by vacuum spraying) or of collagen. Incubation continued for between 2 h and 3 days at 37°C, after which substrates with adherent cells were carefully washed, fixed with formalin, and stained with methylene blue. Individual preparations were fixed with warm 2% glutaraldehyde solution and then processed for scanning electron microscopy by the standard method [6].

Quantitative assessment of adhesion of AH-22a cells to solid substrate was carried out on stained preparations by counting the number of cells to a definite area (4.6 mm^2) of the surface of the substrate. The results were compared with the expected number of cells on the same area of the floor of the well in the plastic plates, provided that all cells placed in the well were adherent to the bottom and they were distributed uniformly. This expected number of cells was taken to correspond to 100% adhesion. On the basis of these data the observed degree of adhesion of the AH-22a cells to the surface of the solid substrate was calculated.

To study a monomer culture of AH-22a cells, the ascites tumor cells, after washing, were resuspended in culture medium and introduced into glass flasks (5×10^5 cells/ml medium). The cells were cultured at 37°C, the medium was changed every 48-72 h, and once a week the cells were subcultured. By now, the monolayer culture (called AH-22a K) has passed through more than 17 passages.

EXPERIMENTAL RESULTS

Quantitative analysis showed that after incubation for 2 h between about 3 and 10% of the AH-22a cells adhered to the surface of the glass; during subsequent incubation (up to 72 h) the percentage of adherent cells showed no significant change. Adherence was independent of the initial concentration of the cell suspension. Most adherent cells remained spherical or hemispherical in shape; at the base of the cells a more or less concentrically arranged lamelloplasm was observed (Fig. 1).

To determine whether AH-22a cells remaining nonadherent to the glass after incubation are still capable of adhering, the following tests were carried out. After incubation for 24 h the coverslips with adherent cells

were removed from the suspension and replaced by clean coverslips. Calculations showed that about 2-7% of cells from the "old" suspension again adhered to these "new" coverslips after reincubation for 24 and 48 h.

The possibility of an inhibitory influence of the tumor cells themselves, preventing them from adhering to the glass, was ruled out by special experiments. These showed that the ability of AH-22a cells to adhere to coverslips: a) preincubated with cell-free fluid obtained from "conditioned" medium (after incubation of AH-22a cells for 24 h), b) with AH-22a cells previously adherent on their surface, and c) with a surface cleaned by chemical or mechanical methods to remove previously adherent cells, – was not reduced compared with the control (untreated coverslips).

Comparison of adherence to the coverslip of AH-22 cells obtained from ascites fluid and of cells taken from a coverslip after incubation for 24 h showed that the ability of the latter to adhere was considerably increased (up to 40%).

Adherence of AH-22a cells to other solid substrates (carbon, collagen) was approximately the same as to the surface of glass. The ability of AH-22a cells to undergo passage in syngeneic mice was the same whether they were adherent or nonadherent to the substrate.

The study of the adherence of AH-22a K cells to glass showed that adherence increased in the course of culture with an increase in the number of passages, to reach 70-78% by the 12th passage. Most adherent cells appeared considerably spread out and flattened, although many were still hemispherical in shape (Fig. 2).

After intraperitoneal injection of AH-22a K cells into syngeneic mice the recipients developed ascites tumors; cytological analysis of films confirmed a high percentage (85-93%) of tumor cells in the ascites which was formed. The resulting ascites tumor was named AH-22a K-A; the tumor was maintained by intraperitoneal passage in syngeneic mice.

The study of adherence to glass of AH-22a K-A cells from a third-generation ascites tumor showed that the percentage of adherent cells was similar to that observed for AH-22a cells (5-7%).

These results show that cells of ascites hepatoma AH-22a have very low powers of adhesion to the surface of various solid substrates during incubation in vitro. Low ability to establish firm contact with the substrate is not a feature of any particular part of the AH-22a cell population: All members of this population evidently adhere to a substrate more or less equally poorly. The low power of adherence, which is a feature of AH-22a cells, is evidently associated with certain properties of the cell surface. These properties can probably be sharply modified by establishment of firm contact between cell and substrate: After the first adherence to glass the AH-22a cells acquired increased ability of adherence to the substrate. Subsequent repeated establishments of contact between cells and substrate (in the course of passage of cell culture AH-22aK) enhanced this property even more. However, it is a very interesting fact that the changes mentioned above are reversible: After intraperitoneal inoculation with AH-22a K cells the mice developed an ascites tumor, whose cells (AH-22a K-A) quickly lost their previously acquired high ability to adhere to a solid substrate. The conditions of multiplication of AH-22a K cells in the suspended state evidently led to rapid structural changes in the cell surface and a consequent loss of ability to establish firm contacts with the solid substrate.

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