Injection of thymalin caused a greater increase than injection of hemalin in the size of the REC nuclei, indirect evidence of the higher intensity of function of the epithelial cells of the thymus in animals receiving thymalin.

The impression was gained that thymalin and hemalin, as early as on the 4th day of the experiment, prevented the reaction to stress connected with the actual injection of the drugs and of physiological saline. Meanwhile the action of thymalin and hemalin continued to be reflected in the structure of the thymus even on the 21st day, after 10 injections: Thickening of the cortex of the lobules and an increase in size of the REC nuclei, in both cortex and medulla, were observed.

Thus, thymalin and hemalin act both on the lymphoid and on the reticuloepithelial component of the thymus. The action of the drugs is aimed at restoring the structure of the thymus through proliferation and differentiation of lymphoid cells; the intensity of the process is higher with thymalin than with hemalin.

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ROLE OF THE CYTOSKELETON IN RESTORATION OF NORMAL MORPHOLOGY OF TRANSFORMED CELLS IN CULTURE

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An important feature distinguishing tumor cells is their weakened adhesion to cellular and noncellular structures. The degree of spreading of tumor cells grown in culture is usually lower than that of their normal precursors [4]. It was shown previously [3] that artificial formation of multinuclear tumor cells leads to the appearance of features in them that are absent in mononuclear transformed cells, and by which they closely resemble in phenotype untransformed normal fibroblasts, i.e., to partial morphologic normalization.

It is generally accepted that the shape of cells is largely determined by cytoskeletal structures. In fact, agents which modify the structure of the cytoskeleton, such as cytochalasin B and colcemid, also affect the shape of cells in culture [1, 9],

The effect of changes in the cytoskeleton on the increase in the degree of spreading of transformed cells, caused by the multinuclear state, was investigated. The degree of spread (the area of the cell expressed per single nucleus) was chosen as criterion for evaluating the degree of the changes, because it can be estimated quantitatively and accurately. The sensitivity of the process of restoration of normal morphology of multinuclear tumor cells to colcemid, an alkaloid which destroys microtubules, was investigated. We also know that colcemid causes condensation of the system of intermediate filaments in the perinuclear zone of the cell [6, 7]. We showed that an increase in spreading of the cells in the multinuclear state is connected with reorganization of their microtubules and intermediate filaments.

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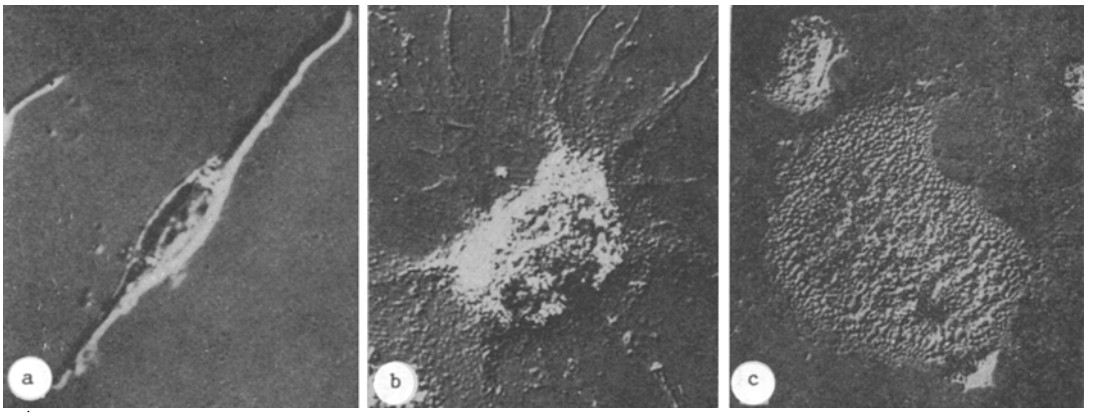

Fig. 1. Cells of line L197/6: a) mononuclear cell, b) multinuclear cell, c) multinuclear cell after incubation for 24 h in presence of colcemid. Nomarskii's optical system. Magnification: a) 600; b, c) 300.

EXPERIMENTAL METHOD

Clonal line L197/6 was obtained by A. A. Stavrovskaya (All-Union Oncologic Scientific Center, Adademy of Medical Sciences of the USSR) from mouse cells of line L. The cells were cultured in basic Eagle's medium, diluted with a 0.5% solution of lactalbumin hydrolysate in the ratio of 1:1 and supplemented with 10% bovine serum. Cells grown on plastic dishes were washed in serum-free medium and treated for 70 sec with 50% polyethylene-glycol (PEG), mol. wt. 1000 (from Loba Chemie, Austria), heated to 37°C. After 2-3 h the cells were transplanted on to cover slips. The area and immunofluorescent staining were measured 24 h after transplantation. Colcemid (from Sigma, USA) was added to the medium in a concentration of 0.1-0.3 μg/ml. To measure the area of the cells the cultures were fixed with 4% formaldehyde and photographed on an Opton III microscope (West Germany), using the phase contrast optical system. Pictures of the cells (30-100 in each series) were projected on paper, cut out, and weighed. The significance of differences between the series was determined by Student's test. Immunofluorescent staining of the cultures with antibodies against tubulin was carried out as described previously [2]. To stain the intermediate filament, S47D9 monoclonal antibodies, generously provided by E. B. Mechetner (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR), were used.

EXPERIMENTAL RESULTS

Cells of line L197/6 as a rule are spindle-shaped (Fig. 1a). As a result of treatment with PEG polykarya were formed: these are giant cells with long radially diverging processes, between which lie extensive lamellar regions (Fig. 1b). Comparison of the areas of mononuclear and multinuclear cells showed that the area per nucleus is 2-3 times greater in the polykarya (Table 1). The increase in area of the multinuclear cells is not a direct result of the action of PEG: Cells remaining mononuclear after fusion had virtually the same area as the control mononuclear cells (Table 1).

Addition of colcemid to the multinuclear cells led to disappearance of their processes; the lamella of these cells was located as a narrow ring around the central part containing the nuclei (Fig. 1c). The area of these cells was considerably reduced (Table 1). The effect of an increase in spreading of the cells in the multinuclear state was inhibited by colcemid by about 70%. Colcemid acted when added at the time of transplantation and also by cells already spread out. At the same time, it virtually left unchanged or actually increased the degree of spreading of mononuclear cells. Since colcemid acts on microtubules and intermediate fialments, we studied the organization of these structures in mononuclear and multinuclear cells. In mononuclear cells the microtubules and intermediate filaments have a similar type of distribution and they constitute a dense, twisting network, occupying the whole of the cytoplasm (Fig. 2a, b). In multinuclear cells the endoplasm contains a dense network of these cytoskeletal structures. From it powerful bands consisting of parallel microtubules and intermediate filaments leave it to enter the lamella (Fig. 2d, e). Treatment of these cells with colcemid, reducing their area, was accompanied by destruction of their microtubules and complete disappearance of the bands of intermediate filaments from the medella (Fig. 2c).

TABLE 1. Area (in μ^2) of Mononuclear and Multinuclear Cells of Line L197/6 per Single Nucleus, Action of Colcemid

Type of cells	Control	Conditions of treatment: colcemid added during transplantation	Conditions of treatment: colcemid added 6 h after transplantation
Mononuclear (control)	754±51	1044±80	948±46
Mononuclear after PEG	735±58	903±89	916±62
Multinuclear:			
2—3 nuclei	1839±342	1213±181	1316±83
4—5 nuclei	1710±161	1284±68	1245±154
over 6 nuclei	1813±212	1070±117	1355±63
mean for all multinuclear cells	1784±145 $P_1<0,001$	1191±101 $P_1<0,05$ $P_2<0,002$	1310±68 $P_1<0,001$ $P_2<0,002$

Legend. Mean values ± standard deviations (σ) indicated for each series: P_1) level of significance of differences in degree of spreading between multinuclear cells and mononuclear cells after treatment with PEG. P_2) Level of significance in degrees of spreading between multinuclear cells treated with colcemid and control multinuclear cells.

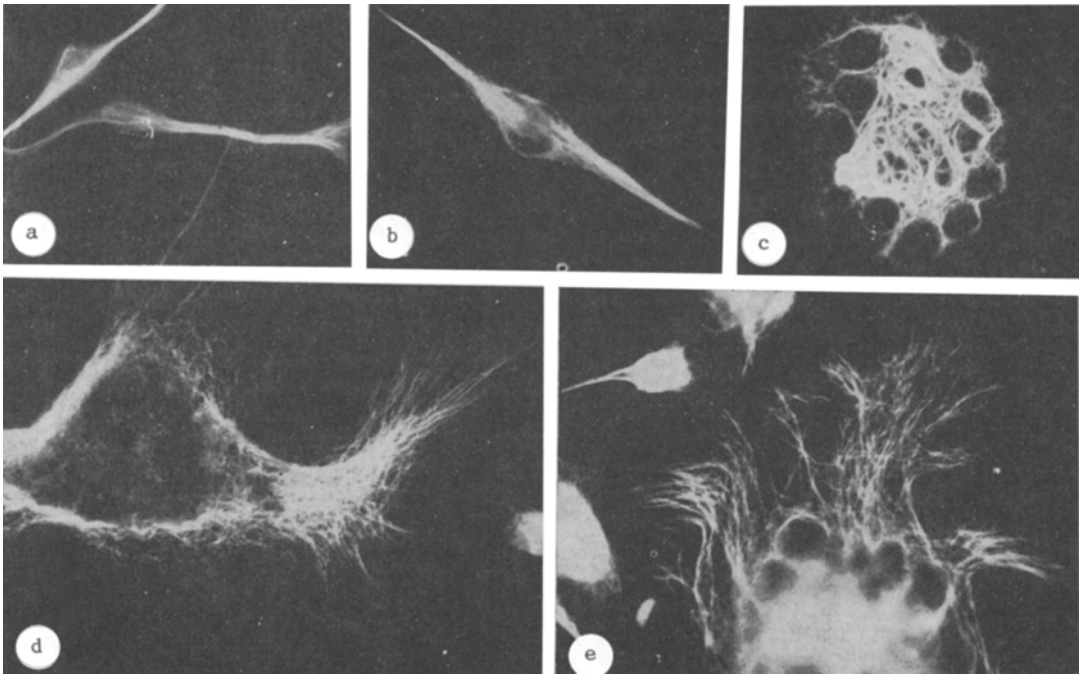

Fig. 2. Immunofluorescent staining of L197/6 cells (magnification 600). a) Mononuclear cell, microtubules; b) mononuclear cell, intermediate filaments; c) multinuclear cell after incubation for 24 h with colcemid, intermediate filaments; d) multinuclear cell, microtubules; e) multinuclear cell, intermediate filaments.

The process of morphologic normalization of tumor cells during their fusion into polykarya, described previously [3], is thus accompanied by the formation of numerous bands of microtubules and intermediate filaments, radiating from the center of the cell. Unlike the polarized spindle-shaped mononuclear cells, multinuclear cells are star-shaped, and the rays of the star contain cytoskeletal bands.

Growth of microtubules is known to begin in the so-called centers of organization [11]. There is also evidence that polymerization of intermediate filaments begins in regions located on the nuclear membrane [5]. The number of centers of organization of the microtubules usually corresponds to the number of nuclei [10]. It can be tentatively suggested that the large number of cytoskeletal bands in the polykarya is due to an increase in the number of centers of organization per cell.

Colcemid, which destroys these bands, reduces the area of the polykarya. In our opinion the degree of spreading of multinuclear cells is greater than that of mononuclear cells because of the formation of extensive lamellar regions, located between the cytoskeletal bands. Bands of microtubules and intermediate filaments may, because of their own growth, stretch the cytoplasm of the multinuclear cells or serve as guidelines for the cells to throw out pseudopodia [8]. Whatever the case, it is clear that the system of microtubules and intermediate filaments plays an important role in the spreading of cells on the substrate.

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