LITERATURE CITED

1. M. S. Zedgenidze, B. V. Nilonenko, I. V. Spirande, et al., Byull. Éksp. Biol. Med., No. 4, 82 (1982).
2. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, et al., Immunologiya, No. 3, 88 (1981).
3. A. G. Sterlina, B. V. Nikonenko, and B. B. Fuks, Immunologiya, No. 1, 52 (1982).
4. B. B. Fuks, M. S. Zedgenidze, I. V. Spirande, et al., Immunologiya, No. 9, 342 (1981).
5. J. Ortaldo, W. Phillips, K. Wasserman, et al., J. Immunol., 125, 1838 (1980).
6. R. Schwartz, Immunobiology, 159, 156 (1981).
7. R. Welsh and L. Kunkel, Int. J. Cancer, 27, 73 (1981).

MORPHOLOGY OF GROWTH OF HETEROGRAFTS OF HUMAN OSTEOGENIC SARCOMA IN DIFFUSION CHAMBERS

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Several reports of growth of human osteogenic sarcomas in tissue culture have now been published, but there is no information in the accessible literature on heterografts of such sarcomas in diffusion chambers. This paper gives the results of observations on heterografts of this kind from seven osteogenic sarcomas. The material for transplantation was obtained from the Operating Department for General Oncology, All-Union Oncologic Scientific Center, directed by Academician of the Academy of Medical Sciences of the USSR, N. N. Trapeznikov. The experimental technique was described previously [1]. Synpor filters with a mean pore diameter of 0.23-0.3 μ were used for making the chambers.

In the early stages (3-5 days after transplantation) outgrowths of elongated cells with oval nuclei, with more or less well-marked features of fibroblast-like cells (Fig. 1a), could be seen at the periphery of the graft. Some of the outgrowths were arranged in a radial direction, but some had lost this regular orientation and formed a reticular structure. In some cases, mainly in areas of more compact arrangement, groups of two or three cells lying in different planes and superposed one above the other could be seen.

A compact zone of growth, extending for a distance of up to 1.5-2 mm from the graft, was observed 1-2 weeks after transplantation around the graft or part of its circumference (Fig. 1b). Cells with oval nuclei distributed along the long axis or irregularly were oriented not quite regularly in the radial direction, and frequently were superposed one above another. Mitotic figures were found. At the periphery the compact zone gave way to a less dense reticular structure with small concentrations of flattened compact cells. Some of the cells were no longer fusiform in shape but had acquired angular outlines or had become branched.

Later the greater part of the filter or nearly the whole of its surface was covered with fields sometimes of compactly arranged cells, sometimes of less densely packed cells of different shapes: fusiform, round, triangular, irregularly polygonal, or branching (Fig. 2a). Their nuclei were round, oval, irregularly oval with invaginations of their membrane in some areas, or bean-shaped. Binuclear and, occasionally, multinuclear cells were found. In experiment 1L after 20 days, and in experiment 6D after 26 days, a distinct pattern was formed at sites of the most compact distribution of the cells grouped in different orientations (Fig. 2b). If neighboring areas of the specimen are examined consecutively a gradual transition can be seen toward regions of less compact arrangement of the cells and, finally, fields of scattered branching cells (Fig. 3a).

In experiment 2L the filter in the later stages was covered by haphazardly oriented cells with irregularly round or oval nuclei, containing course grains of chromatin, lying in different

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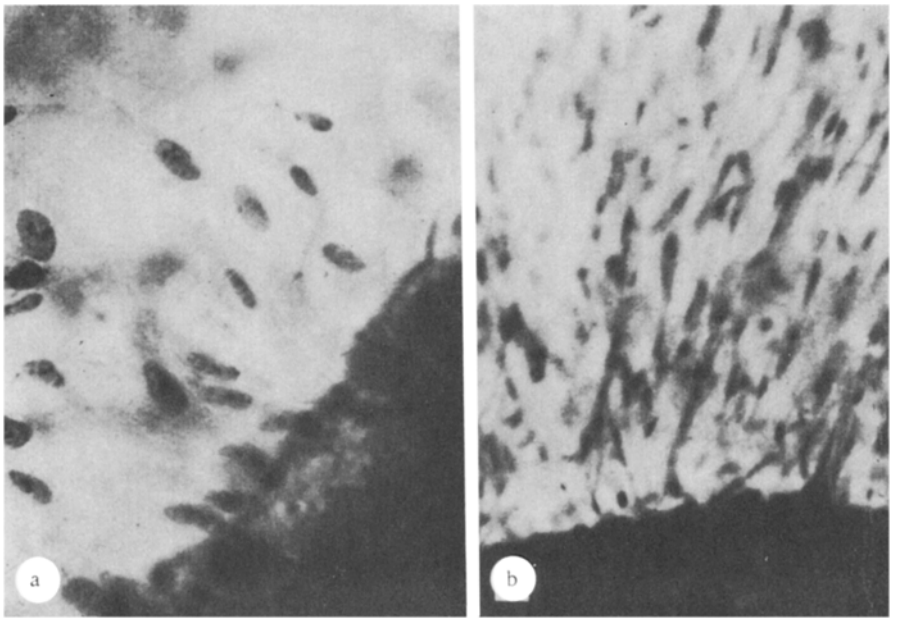

Fig. 1. Cell outgrowth around graft in the early stages: a) initial stage of cell outgrowth. Experiment 6D, 5 days. 390×; b) compact zone of growth. Experiment 1L, 12 days. 180 ×.

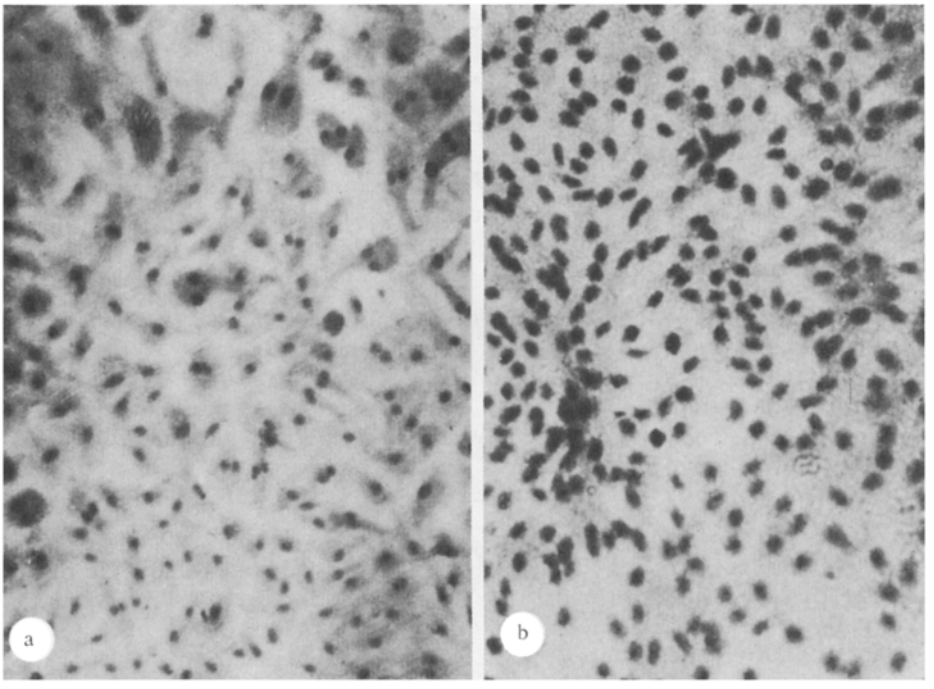

Fig. 2. Field of cells covering filter at later stages. a) Part of field of polymorphic cells, covering nearly all of filter surface. Bottom right — transition to less dense distribution of cells. Experiment 1L, 30 days. 180 ×; b) pattern-like compact arrangement. Experiment 6D, 26 days. 270 ×.

planes. In less compact regions these cells were branching in character, and at the edge of the layer they became fusiform and (not quite regularly) oriented away from the edge. Some very large cells were found (40-50 μ or even 100-180 μ in diameter). Many of them were binuclear or multinuclear. Sometimes they lay singly, sometimes they formed groups occupying a considerable part of the field of vision under low power (objective 10, ocular 10) and more extensive regions (Fig. 3b). In this same experiment, in one preparation on the 27th day a

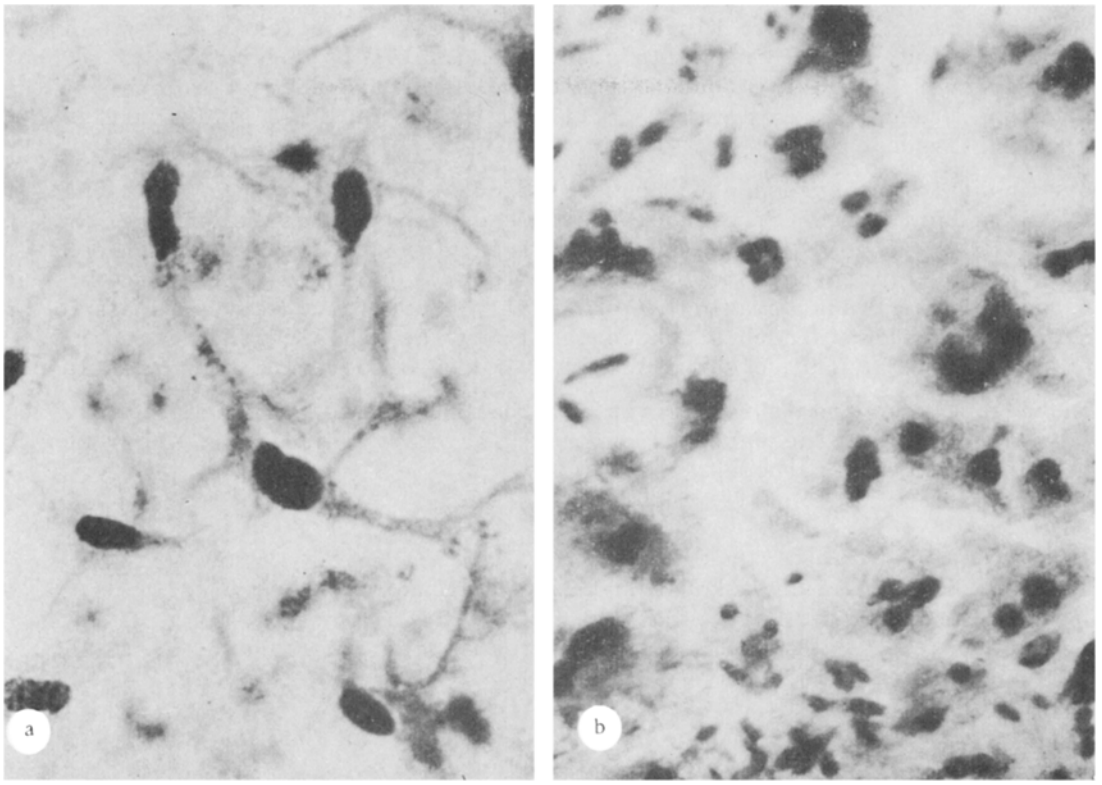

Fig. 3. Branching and multinuclear cells in late stages. a) Branching cells. Experiment 6D, 26 days. 500 ×; b) multinuclear cells. Experiment 6D, 26 days. 180 ×.

compact outgrowth of strictly parallel fibroblast-like cells could be seen in a limited region. This was evidently an outgrowth of normal fibroblasts and not of tumor cells. In experiment 10L the observations were limited to a period of 13 days. Toward this time a zone of growth up to two diameters of the field of vision under low power formed around the graft. It was not as compact as in the other experiments. Its cells were larger. Their oval nuclei measured 4 × 13 to 6 × 15 μ, or sometimes even 5 × 20 μ or, conversely, they were a little smaller. Even here, however, many cells and their nuclei were irregularly oriented. The cells were arranged in layers one above the other and formed compact groups of three to six nuclei. At the periphery of the zone of growth sometimes cells with varied orientation were incorrectly oriented. The cells were superposed one above another and formed compact groups of three to six nuclei. At the periphery of the zonal growth sometimes cells with varied orientation were sparsely distributed, but mainly fields of compactly arranged polygonal cells with pale cytoplasm and large nuclei, measuring from 6 × 6 to 10 × 14 μ or sometimes 18 × 18 μ, could be seen.

When the results of the morphological observations on heterografts in diffusion chambers are assessed, just as in the case of tissue cultures, it must be recalled that growth of normal fibroblasts is inclined to precede growth of tumor cells. This applied both to epithelial tumors, which we proved when studying heterografts of lung cancer [2], and sarcomas [4]. It is important to decide whether the patterns observed are due to growth of tumor cells or not. With regard to heterografts which we studied it can be asserted confidently that it was indeed proliferation of tumor cells that took place in them. This conclusion is supported by the irregular orientation of the cells in the zone of growth, their tendency toward a stratified arrangement, indicating absence of contact inhibition, their considerable polymorphism, and the haphazard orientation of cells in the field which formed in the late stages. Only in one preparation in experiment 2L after 27 days was an outgrowth evidently of normal fibroblasts found in a limited area, as was mentioned above.

The patterns of growth of the heterografts which we observed were similar to those described by other workers during growth of osteogenic sarcomas in tissue cultures [5-10]. There also, besides fusiform cells, sometimes closely resembling fibroblasts, sometimes differing from them substantially, fields of cells of polygonal shape characterized by the authors

cited as "epithelioid cells," were histologically formed. Binuclear, and sometimes multinuclear cells were found. Branching cells also were observed. Outgrowths at the periphery of the graft were very similar to outgrowths of cells at the edge of a layer of tumor cells formed in regions freed from explant of cartilage, which inhibits proliferation of osteogenic sarcoma cells in tissue culture [7].

We have insufficient evidence to answer the question whether the giant cells found in experiment 2L are derivatives of osteoclasts, present in the graft, or whether they were formed from tumor cells. However, their appearance only in the late stages suggests that they are not cells characterized by a multinuclear structure in their original form, but cells appearing as a result of transformation of originally nonmultinuclear cells of the growing graft, and evidently tumor cells. In this connection it may be recalled that Shchuklinov [3] observed the formation of multinuclear cells in cultures of normal connective tissue also.

It can be concluded from this description that growth of tumor cells truly took place in these experiments and that the patterns of growth were very similar to those observed in tissue cultures of osteogenic sarcomas.

LITERATURE CITED

1. M. V. Svyatukhin and É. N. Malenkova, Arkh. Patol., No. 1, 36 (1974).
2. M. V. Svyatukhin, E. M. Lysenkova, and É. N. Malenkova, Éksp. Onkol., No. 3, 17 (1980).
3. V. A. Shchuklinov, Arkh. Anat., No. 8, 35 (1977).
4. S. A. Aaronson, G. J. Todaro, and A. E. Freeman, Exp. Cell Res., 6, 1 (1970).
5. J. Fogh and G. Tremp, in: Human Tumor Cells *in Vitro*, J. Fogh, ed., New York (1975), pp. 115-159.
6. G. Jiraldo, E. Beth, Y. Hirshaut, et al., J. Exp. Med., 133, 454 (1971).
7. K. E. Kuettner, B. U. Pauli, and L. Soble, Cancer Res., 38, 277 (1978).
8. R. M. McAllister, M. B. Gardner, A. E. Green, et al., Cancer (Philadelphia), 27, 397 (1971).
9. R. Manoury, J. Arnoldt, and C. Vedrenne, Pathol. Biol., 20, 369 (1972).
10. J. Portent and E. Saksela, Int. J. Cancer. 2, 434 (1967).