Morphology and Histochemical Features of Human Sarcomas in Tissue Culture*

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SUMMARY

Morphologic and some histochemical aspects of six human sarcomas were studied in tissue culture. The explants were incubated with polysaccharide-containing heparin only, heparin and/or hyaluronic acid, and ACTH nutrient media.

Cytoplasmic granular metachromasia, with toluidine blue stain, was found in fibrosarcoma cells after 72 hours' incubation with the mixed polysaccharide media. The metachromasia has been attributed to the capacity of fibrosarcoma cells to ingest and store heparin, hyaluronic acid, or both. The significance of this process is discussed in relation to the synthesis and breakdown of ground substance mucopolysaccharide.

Previous studies of human sarcomas in tissue culture were related to the development of permanent strains and morphologic aspects of growth (3, 4, 12, 13, 15-17).

The experiments reported here were primarily designed to investigate micellophagosis in fibrosarcoma cells in vitro.

MATERIALS AND METHODS

Sterile tissues from three fibrosarcomas and two malignant hemangiopericytomas were obtained at, or shortly after, operative removal; tissue from one fibrosarcoma was removed shortly after the patient's death.

The portions of neoplasm used for explants were cut into 1- to 2-mm. fragments. The clot was formed by mixing 3 drops of chicken blood plasma, one drop of embryo extract, and 1 per cent thrombin. Three explants were placed on separate slides. Sixteen to 24 double slides (back to back) were incubated in roller tubes with either human ascitic or serum nutrient fluid.

1. Ascitic fluid medium, 50 per cent Gey's BSS without glucose, 45 per cent ascitic fluid, 2 per cent embryo extract, and 3 per cent antibiotics (penicillin, streptomycin, mycostatin).

2. Human serum medium, 79 per cent Gey's BSS without glucose, 20 per cent human blood serum, 1 per cent embryo extract, and antibiotics (penicillin, streptomycin, mycostatin).

Phenol red was used as an indicator. After at least ten days' incubation in roller tubes, the explants were transferred to perfusion chambers in the same nutrient medium. On the 3d day one of the following mixtures was added:

a) Heparin fluid, 1 ml. (10 mg.) heparin, 9 ml. nutrient medium.1

b) Heparin with ACTH, 1 ml. (10 mg.) heparin, 2 ml. ACTH in BSS, 7 ml. nutrient medium.

c) Hyaluronic acid with ACTH, 1 ml. (10 mg.) hyaluronic acid, 2 ml. ACTH in BSS, 7 ml. nutrient medium.

d) Hyaluronic acid and heparin with ACTH, 1 ml. (10 mg.) hyaluronic acid, 1 ml. (10 mg.) heparin, 2 ml. ACTH in BSS, 6 ml. nutrient medium.

e) Control—nutrient only.

At the beginning of the experiment the cultures were kept in the above media for 24, 48, and 72 hours. Following the initial results mixture (a), heparin without ACTH, was excluded, and the cultures were incubated with the other nutrient fluids for 3-5 days. After each period of incubation

1 Upjohn Co., Kalamazoo, Mich.
2 Number (1) ascitic fluid medium or (2) human serum medium.
3 1 ml. (40 units) ACTH in 100 ml. of BSS.
4 Bovine vitreous humor. Nutritional Biochemical Co., Cleveland, Ohio.
the explants were placed in Lillie's formol-alcohol-nitrate fixative (11) and stained with toluidine blue (14). Slides from roller tubes were fixed in 4 per cent formaldehyde and prepared with Masson trichrome (5) and Wilder (20) reticulum stains. The supernatant medium fluid was treated with 0.2 ml. 1 N acetic acid for a qualitative determination of mucopolysaccharide (6, 7).

RESULTS

Morphologic findings of cells in the cultures.— Tissues from the six sarcomas showed active growth beginning 24–48 hours after incubation. The fibrosarcoma cells were spindle-shaped and rounded at one or both ends. An occasional polygonal or slightly stellate cell was seen with a large globular nucleus and small nucleolus. The cytoplasm was finely granular. In older cultures the cells formed chains in which they were arranged end-to-end, and some were connected to form elongated loops. The individual cells measured 75–250 µ in length, and the nuclei 30–50 µ in diameter (Figs. 1, 3). The tissue from the malignant hemangiopericytoma showed two types of cellular growth—i.e., slender, spindle-shaped cells and small groups of round or oval cells. The former had oviod nuclei with nucleoli. The cells were smaller and more slender than the cells from the fibrosarcoma. The spindle cells measured 50–150 µ in length, and the nuclei varied from 20 to 50 µ in diameter. The round cells measuring 25–50 µ in diameter contained large nuclei but had little cytoplasm. The nuclei ranged from 10 to 35 µ in diameter. After a few days' incubation some of the slender spindle cells became stellate and grew in a netlike pattern, forming small loops. At this time the round cells became more stellate (Fig. 2).

The growth patterns and cytologic features of both types of sarcomas in tissue culture were distinctive but, when observed casually, might be difficult to differentiate. The morphologic features seen in the tissue cultures of sarcomas were similar to those described by Fisher et al. (3), although the latter reported a benign hemangiopericytoma. The cells of the malignant hemangiopericytoma were larger than the ones described in the benign tumor. The spindle cells appeared to be more active than the round cells.

Heparin and hyaluronic acid uptake.—The cells of the cultures incubated in the perfusion chambers with fluid (a), heparin without ACTH, showed no granular metachromasia in the cytoplasm when stained with toluidine blue (Fig. 3). However, when grown with solutions containing heparin or hyaluronic acid with ACTH (b), (c), or (d), the cells contained metachromatic granules, pinkish-red with toluidine blue, after 72 hours’ incubation. The granules were distributed throughout the cytoplasm with a somewhat greater concentration near the nucleus (Fig. 4). No definite granular metachromasia was seen after incubation for 24–48 hours, except for a slight diffuse pink cytoplasmic stain. The cells of the culture treated with heparin showed a stronger reaction and contained larger granules than those treated with hyaluronic acid. The cells in the control cultures showed no cytoplasmic granular metachromasia. The uptake of heparin and hyaluronic acid by the cells in the culture from the malignant hemangiopericytoma was evident (after 72 hours' incubation) in both types of cells. The granular (pink-red) metachromasia was present in these cells, although to a lesser degree than in the cells from fibrosarcomas. The cytoplasmic granular metachromasia in all the cultures of sarcoma decreased after prolonged incubation (5 days).

The qualitative evidence of mucopolysaccharide in nutrient media was found when cultures were injected with mixtures containing heparin or hyaluronic acid. The supernatant fluid formed a mucous clot when treated with 0.2 ml. 1 N acetic acid.

Other staining reactions.—The reticulum pattern in the cultures of sarcoma cells was similar to that found in the original histologic sections. The fibrosarcoma cultures showed a fibrillar network which surrounded the cells. The malignant hemangiopericytoma cells displayed a mixed fibrillar pattern; a few fibers were within but most were outside the neoplastic cells.

With the Masson trichrome stain the fibrosarcoma cells in culture were stained green, whereas those from the malignant hemangiopericytoma took the red stain.

DISCUSSION

The phenomenon of micellophagosis was defined by Dougherty and Higginbotham (2, 8). This capacity of fibroblasts to ingest and temporarily store various substances—i.e., polysaccharides—was observed by Schneebeli, Dougherty, and Loewe (18) and extensively studied by Dougherty and Higginbotham (9, 10).

The cytoplasmic granular metachromasia in fibrosarcoma cells, and to a slight degree in malignant hemangiopericytoma cells, following incubation with heparin or hyaluronic acid and ACTH media, indicates that the cells are able to ingest and store polysaccharides as do normal fibroblasts. However, it seems that in vitro this is only possible when a mucopolysaccharide complex is formed by adding a basic protein such as ACTH to the solu-
tion. Subsequent decrease in granular cytoplasmic metachromasia suggests that storage is temporary.

Heparin is polysulfated polysaccharide, and therefore the histochemical observations in tissue culture of human sarcomas may be correlated to some extent with the observations of Curran (1), who studied S-35 uptake autoradiographically in tissues. According to Curran, fibroblasts, fibrosarcomas, chondrosarcomas, and capillary endothelium in normal and pathologic tissues show increased uptake of S-35. Our study suggests that there is a difference in heparin and hyaluronic acid ingestion and storage between cells of fibrosarcoma and malignant hemangiopericytoma. The difference in polysaccharide uptake, as well as the two types of cell growth, favor the diagnosis of malignant hemangiopericytoma, rather than hemangiosarcoma. This observation might be useful in differential diagnosis of sarcomas.

Recent studies of mucopolysaccharides in non-human fibroblasts and fibrosarcoma cells by Teysie et al. (19) suggest that fibrosarcoma does not produce mucopolysaccharides in vitro detectable by metachromasia staining; however, small quantities were found in the nutrient media by turbidimetric methods. They concluded that the sarcoma cells have a low capacity to synthesize mucopolysaccharides. The ability of human fibrosarcoma to ingest, and temporarily store, heparin or hyaluronic acid complex suggests that these cells might more efficiently catabolize than synthesize the ground substance mucopolysaccharides. If this is the behavior of fibrosarcoma in vivo, the growth characteristics and invasiveness could be explained partly on cellular inability to maintain the balance between synthesis and breakdown of mucopolysaccharides; this process might be enzymatic in nature. The question must also be raised whether the affinity of mucopolysaccharides in fibroblast cells for certain cationic substances is different from that in normal fibroblasts. Further in vitro and in vivo study of fibrosarcoma cell function may be helpful in the therapeutic approach to this neoplasm.

REFERENCES
16. ———. The Isolation of Pure Strains of Cells from Human Tumors; Growth Characteristics of Sarcoma and Two Brain Tumors in Tissue Culture; Conclusions. Ibid., 29:25-46, 1937.
Fig. 1.—Fibrosarcoma—8-week-old culture. Spindle-shaped cells, some slightly stellate. Approx. mag. × 400.

Fig. 2.—Malignant hemangiopericytoma—8-week-old culture. Abundant growth of two cell types. The spindle cells form a netlike pattern and many small loops. The round cells are in clusters; many become stellate. Approx. mag. × 350.

Fig. 3.—Fibrosarcoma—15-day-old culture; incubated with heparin-containing medium for 7½ hours. Spindle-shaped cells (arranged end to end) formed chains. Granular cytoplasmic metachromasia absent. Approx. mag. × 900.

Fig. 4.—Fibrosarcoma—15-day-old culture; incubated with heparin and ACTH-containing medium. Cytoplasmic granular metachromasia with somewhat greater concentration near the nuclei. Approx. mag. × 1200.
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