Studies on the Solid Form of Mouse Sarcoma 37 Grown in Tissue Culture*

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Mouse Sarcoma 37 has been extensively used in research problems ever since its isolation about 40 years ago. In 1928, Drew (2), while working with the problem of the growth of embryonic and adult tissues in tissue cultures, reported the successful culturing of mouse Sarcoma 37 in a balanced salt solution. He pointed out that in tissue cultures the sarcoma begins to degenerate in 48 hours and, unless subcultured, dies within 2-3 days. However, with repeated subculturing, “vigorous growth” could be maintained for approximately 1 month, after which interval the cultures usually died. He included two drawings showing the general shape of the culture and its tumor cells. In 1952, Diller (1) published a careful cytological study of Sarcoma 37 based on fixed and stained preparations of the tumor. She discussed cytoplasmic components, mitotic abnormalities, aberrant behavior of the chromosomes, and spontaneous regression. Among her numerous illustrations were two photomicrographs of Sarcoma 37 cells growing in tissue culture (her Figs. 6, 18).

However, when a study of the effects of various hexoses upon tissue cultures of the solid (subcutaneous) form of Sarcoma 37 was contemplated in these laboratories (5), it was found that descriptions of the tumor’s cytology and growth characteristics in tissue cultures were inadequate for such an investigation. Hence, the present work was undertaken.

MATERIALS AND METHODS

The solid (subcutaneous) form of Sarcoma 37 was implanted by a trocar in the axillary region of adult male albino CWF mice (Webster strain). Four to 10 days after inoculation, the tumors were removed and aliquots made into hanging-drop tissue cultures. Portions of the tumor were made into paraffin sections and stained with hematoxylin and eosin. The medium for the tissue cultures consisted of 6 ml. of Gey’s solution (3) and 6 ml. of chick embryo extract (9-10-day-old embryos) made up with Gey’s solution in the proportion of 1 part of chick embryo to 2 parts of Gey’s solution. To this mixture were added 6-8 drops of human placental serum and 0.001 gm. of penicillin G. potassium (4). The tumor tissue in this medium was added to a drop of chicken plasma. A total of 800 cultures was prepared on twelve different occasions over a 7-month period, with tumors obtained from 88 mice. The cultures were incubated at 37° C. They were observed for 6-8 days. At 48-hour intervals, the medium was removed by aspiration, and fresh medium was added. Some cultures were fixed in 10 per cent formalin and stained with toluidin blue and hematoxylin and eosin. Wright’s stain, sudan III, and neutral red were used with a few preparations. Cultures of subcutaneous tissue removed from the uninoculated axilla were studied as controls. Tissue cultures of mouse thymus also were prepared and stained for comparison with certain small cells observed in the cultures of the Sarcoma 37.

RESULTS

Living cells.—A good migration of tumor cells was observed in about 75 per cent of the cultures after 24 hours’ incubation.

Initially, two morphologic types of cells were observed to migrate radially from the explanted tissue (Figs. 1, 2). One type consisted of large

All photomicrographs were made from 24-hour-old tissue cultures of the solid form of mouse Sarcoma 37 (4-6-day-old tumors).

Figs. 1-6.—Living tissue cultures.

Fig. 1.—Spindle-shaped cells and small cells migrating from explant. X250.

Fig. 2.—Spindle-shaped cells showing processes and nuclei. X1940.

Fig. 3.—Small cells enlarged. X1940.

Fig. 4.—“Fern-like” cells with finger-like processes. X1940.

Fig. 5.—“Fern-like” cells enlarged. X1940.

Fig. 6.—Spindle-shaped cells and small cells showing fat globules. X1940.

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FIGS. 7-12.—Tissue cultures fixed in formalin and stained with iron hematoxylin.

Fig. 7.—Spindle-shaped cells showing mitosis (m) and nucleoli (L).

Fig. 8.—“Fern-like” cell with irregular chromatin masses.

Fig. 9.—Spindle-shaped cell with many nuclei.

Fig. 10.—Spindle-shaped cell with nucleus and a dark staining body with halo-like structure.

Fig. 11.—Group of small cells with irregularly shaped nuclei.

Fig. 12.—Small cells showing one or more nuclei.
spindle-shaped cells which differed from fibroblasts in having only a few fine processes (Fig. 2). The advancing ends of the cell were usually blunt. The nucleus was oval, with one or two nucleoli (Fig. 7). The cells contained many fat globules which stained a brilliant red with sudan III (Fig. 6). These globules were concentrated about the ends of the elongated nucleus (Fig. 6). When neutral red was added to the cultures, it stained a mass of fine granules distributed fairly evenly in the cytoplasm around the nucleus.

The other type of cells resembled fern fronds migrating from the explanted tissue (Figs. 4, 5). In many cases the culture contained cells with finger-like processes. The nucleus of these living cells could not be discerned, perhaps owing to the apparently irregular surface of the "fern-like" structure. After 48 hours' incubation these "fern-like" cells appeared to flatten out and resemble the first type.

Numerous small cells were observed surrounding both types of advancing tumor cells (Figs. 1, 5). These small cells rapidly changed their shape and position. Under high magnification they usually appeared irregular in outline (Figs. 3, 6) and contained fat globules and a few granules which took neutral red stain. A nucleus could not be definitely seen in the living cell.

The tumor cells continued to migrate from the explant for the first 48 hours of incubation. After this time, unless the nutrient medium was renewed, the cells usually began to contract, and their cytoplasm became increasingly granular. No attempt was made to determine how long the cells could be maintained in tissue culture.

Fixed and stained cells.—In fixed preparations of tissue cultures stained with hematoxylin and eosin, the nuclei of both types of cells showed great variation in shape, size, and number (Figs. 7—9). The shape of the nucleus varied from oval to an irregular mass of nucleoplasm. The oval form predominated. The nuclei in a cell varied in number from one to eight (Fig. 9). Within a single cell there was much individual variation in the size and shape of these chromatin masses. One or two nucleoli could usually be seen.

In some of the cells a small, dark-staining body was observed near the nucleus (Fig. 10). This body was sometimes surrounded by a halo-like structure. Mitosis was occasionally seen (Fig. 7).

In these stained preparations the small cells usually had one nucleus (Fig. 11). This was sometimes elongated or dumbbell-shaped, as though it were dividing (Fig. 12). Some objects were present which looked like small cells but had no nuclei (Fig. 7).

DISCUSSION

Many small cells were observed in the cultures. They were larger than mouse lymphocytes, which they resembled when packed in among the large tumor cells. They were of irregular, amoeboid form when they migrated out beyond the large tumor cells. Diller1 also has seen small cells in stained sections of the ascitic form of Sarcoma 37. At present, the origin of these cells is unknown, but they may represent variants of the tumor cells.

The "fern-like" growth of tumor cells often seen after 24 hours' incubation is probably the same cell type as the larger spindle-shaped tumor cells, since the "fern-like" cells appear to flatten out and develop into the spindle-shaped cells. The density of the plasma clot may be a factor in determining the type of growth observed.

SUMMARY

The solid form of Sarcoma 37 was readily maintained in hanging-drop tissue cultures. Two types of cells predominated after 24 hours' incubation: (a) spindle-shaped cells with blunt processes and (b) cells with numerous, irregular, tortuous processes which, under low magnification, resembled fern fronds. In 2—3-day-old cultures the "fern-like" cells apparently developed into the spindle-shaped cells. A much smaller, active, amoeboid cell was present in all cultures. It may be a variant of the tumor cells.

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