A Hamster Fibrohemangiosarcoma: Influence of Host Sex on Tumor Mass[1] (36806)

LUCIAN J. CUPRAK[2] AND WALTER F. LEVER
(Introduced by W. W. Fishman)

Department of Dermatology, Tufts University School of Medicine, Boston, Massachusetts 02111

The ready development of neoplasias in the Golden Syrian hamster, *Mesocricetus auratus,* is attributable to the high sensitivity of the animal tissues to hormonal imbalances (1, 2). However, the incidence of spontaneous connective tissue tumors is reportedly no greater than in other commonly used laboratory animals (3). The present report concerns a fibrohemangiosarcoma that had developed from a spontaneously transformed hamster embryo cell culture, HE-44, in its 69th passage. Tumors induced from this culture were the kind gift of Dr. Leila Diamond of the Wistar Institute, Philadelphia, PA. These tumors upon receipt were dense, white, hard fibromas which in a few areas showed differentiation into bone and cartilage. The selection and repassage of cells from primary monolayer cultures resulted in rapidly growing cultures of pleomorphic cells. Subcutaneous injection of cells from these cultures produced tumors composed of moderately anaplastic, spindle-shaped cells and endothelial cells. Histochemical methods were used to demonstrate the presence of certain lysosomal enzymes not only in cells of the animal tumor but their continued presence in monolayer cell cultures established from the tumor. The dependence of tumor size upon the sex of the animal was subsequently observed and is herein reported. A report of the characteristics of these cells in culture will be published elsewhere.

Materials and Methods. Animals. All animals used in these experiments were the result of inbreeding from one pair of Golden Syrian hamsters obtained from the Woodman Animal farms, Wayland, MA. Mature animals less than 1 yr old were used, except where noted in the text. They were maintained on standard laboratory pellets in polycarbonate cages. All animals were sacrificed by etherization 6–7 wk after the injection of cells. Tumors were cleanly extirpated without skin and the wet weight was determined to the nearest 0.1 g.

Monolayer cell cultures. Single cell suspensions were obtained by mincing the tumor tissue and disaggregating the cells with 0.1% Viokase (Viobin Corp., Monticello, NY) in phosphate buffered saline as described previously (4). The growth medium consisted of a basal medium having the amino acid and salt composition of Ham's F10 medium and the vitamin composition and supplements of Ham's F12 medium (5, 6). The basal medium for the long-term propagation of cultures was supplemented with 10% horse serum obtained from the Grand Island Biological Co., Grand Island, NY. Prepared medium was sterilized by Millipore filtration and stored at $-10°$. Cells were grown on 60×15 mm Falcon plastic culture dishes in a water-saturated atmosphere of 5% CO_2 at 37°. Medium changes of 3 ml were made every 3 days. Cell number was estimated using the method of Sanford *et al.* (7) for the enumeration of cell nuclei. The method of Puck, Marcus and Cieciura (8) was used for clonal selection.

Animal inoculations. Cells in culture were first rinsed with 2 ml physiological saline three times and then harvested with a rubber policeman in an appropriate volume of physiological saline to give a final concentra-

[1] Aided by Grant No. 1326-C-1 from the Massachusetts Division of the American Cancer Society.

[2] To whom inquiries should be forwarded: Present address: V.A. Hospital, Leech Farm Road, Pittsburgh, PA 15206.

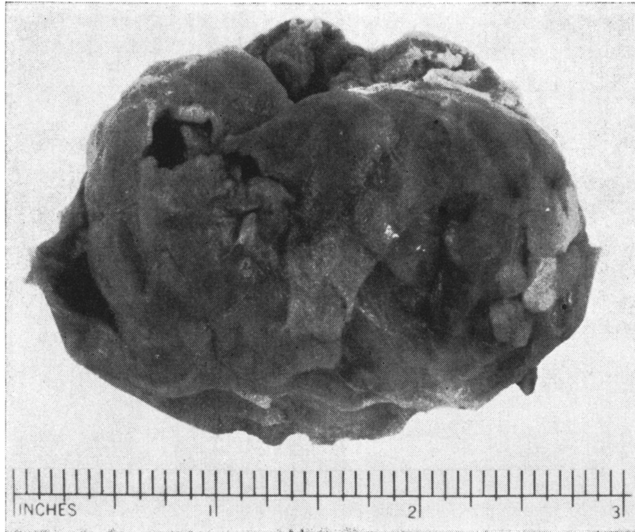

FIG. 1. Tumor removed from a lactating female Syrian hamster. The tumor was the result of a single dorsal subcutaneous injection into a pregnant female of 1×10^6 cells of clonal culture, hamster fibrohemangiosarcoma-13, in 0.2 ml of physiological saline. Period of growth was 5 wk. Weight of tumor was 40 g. Total weight of animal was 160 g.

tion of about $0.5–1.0 \times 10^6$ cells/0.1 ml. Each animal received 0.1 ml of the above cell suspension subcutaneously dorsally, in either the right or left lateral lumbar region. Cell suspensions obtained from tumor tissue consisted of aliquots of single cell suspensions prepared for cell culture as described above.

Histology. Portions of nonnecrotized tumor tissue were fixed in either 10% formalin or Zenker's solution and embedded in paraffin for sectioning. Sections of 7 μm were deparaffinized, stained with hematoxylin and eosin, dehydrated and mounted in Permount (Fisher Scientific Co.).

Histochemistry. Cells from full monolayer cultures were grown on 22×22 mm glass cover slips, fixed and stained for the following enzymes: nonspecific acid phosphatase according to the method of Goldberg and Barka (9); nonspecific alkaline phosphatase according to Rutenberg, Rosales and Bennett (10); β-glucuronidase as described by Lorbacher, Yam and Mitus (11); and nonspecific esterase by the method of Yam, Li and Crosby (12). Cryostat sections of tumor tissue were cut at 7 μm and were fixed and stained generally according to the method cited above for each enzyme. Controls were duplicates without substrate.

Viral extracts. Spent media from full monolayers of cultures were sterilized by passage through a 0.22 μm Millipore filter. Newborn hamsters less than 3 days old each received 0.25 ml of the sterile spent medium subcutaneously in the dorsal lumbar region. Viral extracts of tumor tissue were prepared according to the method of Moloney (13). Pellets were reconstituted in physiological saline to an equivalence of about 1 g of tumor tissue/ml. An aliquot of the prepared extract was further filtered through a 0.22 μm Millipore membrane. Animals were injected subcutaneously with 0.1 ml of either preparation as previously described.

Results. Primary monolayer cultures were grown from hamster tumors which were induced by cells from a transformed embryo cell culture HE-44 in its 69th passage. These primary cultures were then subjected to three alternate animal and culture repassages. Cultures obtained from tumors induced in the third animal repassage grew readily and were the source of stock cultures maintained by periodic subculture. Animal passage of about 10^6 cells from these cultures in their third and subsequent *in vitro* passages produced large, encapsulated multinodular and noninvasive tumors in inbred pregnant fe-

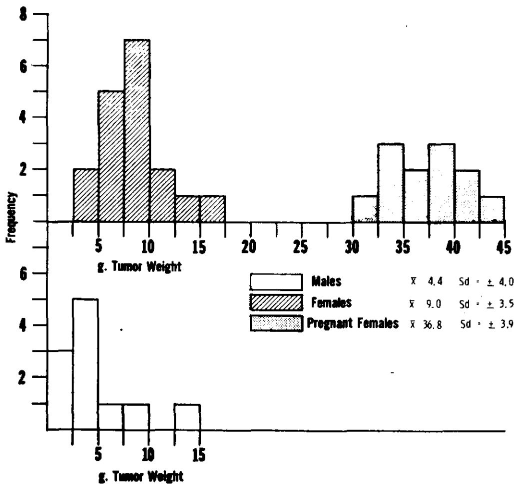

FIG. 2. Distribution of wet tumor weight among males, pregnant and nonpregnant females.

males (Fig. 1). Similar but smaller tumors were produced in males and nonpregnant females. The tumors were soft, friable and spongy in consistency and had areas that varied from black to purple to brown in color. Their gross appearance and consistency differed greatly from the original tumors which were characterized by dense, firm, white nodules of a hard to rubbery consistency. The distribution of wet tumor weight is shown in Fig. 2. Pregnant, lactating females consistently produced tumors with a wet weight above the range of the wet tumor weight obtained in either mature males or nonpregnant females. On the average they were 4.1 times larger than in nonpregnant females and 8.3 times larger than in mature males.

Histopathology. The observed alterations in gross pathology were accompanied by parallel histopathological changes.

Sections of the tumors induced by the original cell culture revealed fibroblasts arranged in varying degrees of density. In areas of least density the stroma appeared edematous. Capillaries were few in number. The fibroblasts as well as the endothelial cells lining the few capillaries present were normal in appearance showing no evidence of anaplasia.

Sections of the tumors obtained in pregnant and nonpregnant females and in male hamsters through inoculation of cultures of pleomorphic cells showed a variable histologic picture. Predominantly, the tumors consisted of dense aggregates of fibroblasts that produced only very little collagen and had rather anaplastic nuclei. Variable numbers of vascular lumina and slits were present. Some of the tumors contained areas of hemorrhage. Also present were areas of necrosis as can be expected in a rapidly growing tumor.

The anaplastic fibroblasts had either cuboidal or spindle-shaped nuclei. Both types of nuclei were seen in all tumors, although in most tumors one type predominated by far. In a few tumors some of these cells were multinucleated.

The vascular lumina were variable in size. Some of them were lined entirely by normal appearing thin endothelial cells. Others were lined either partially or entirely by anaplastic endothelial cells. The vascular slits were lined by cells resembling fibroblasts rather

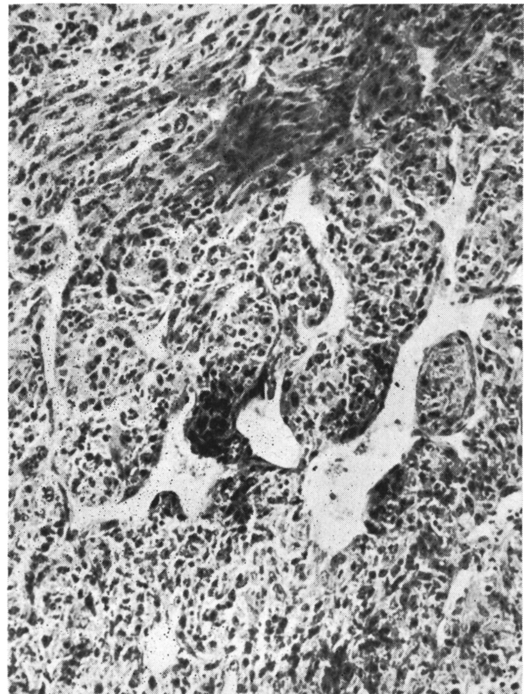

FIG. 3. Hamster fibrohemangiosarcoma. The tumor contains irregularly shaped vascular lumina lined partly by typical endothelial cells and partly by atypical cells that resembe fibroblastic cells more than endothelial cells (×149).

than endothelial cells although it was not always possible to decide whether an individual lining cell was a fibroblast or an endothelial cell (Fig. 3).

Histochemistry. It was possible to demonstrate strong activities of nonspecific acid phosphatase, nonspecific esterase and β-glucuronidase in cryostat sections of tumor tissue as well as in cells grown in culture up to the 16th passage. Nonspecific alkaline phosphatase activity was not present in cultured cells and was either lacking or very weakly present in some endothelial cells of the tumor.

Viruses. Six newborn hamsters, three males and three females, were inoculated with sterile spent medium. At the end of 6 mo none of the animals had any gross evidence of tumor formation upon necropsy. Viral extracts of tumor tissue prepared according to Moloney also failed to produce tumors at the site of inoculation into one adult male and one adult female hamster after 4 mo.

Discussion. The first animal repassage of HE-44 cells in their 69th passage had produced dense, avascular fibromas which, upon repassage, yielded tumors with proliferating spindle-cell and vasoformative elements. Clonal selection, as well as alternate cell culture and animal repassage into inbred Syrian hamsters may have been selective factors responsible for the emergence of the present tumor. It is also possible that the He-44 culture, since it originated from a hamster embryo culture that had undergone spontaneous transformation, had retained some capacity to differentiate into the cellular elements now observed. Even at present, cultures that had been obtained by using the conventional ring cloning procedure still produce this characteristic tumor after 16 passages. It is estimated that this represents nearly 100 cell generations since approximately six population doublings occur from a single passage of 1×10^5 cells to a confluent monolayer of about 4×10^6 cells. In order to explain the presence of two proliferating cell types in the tumor one could assume that either (a) the cultures represent a mixture of cell types with identical growth characteristics, or (b) the cells in culture have a degree of pluripotentiality, or (c) the vasoformative elements represent a proliferation of the vascular support for the tumor. Of these three possibilities the first seems least likely because of the length of time that the cells have been in continuous culture. The latter two assumptions have also been considered as possibilities in the human hemorrhagic sarcoma of Kaposi which, like the tumor described here, shows a proliferation of endothelial and fibroblastic cells (14). Hashimoto and Lever (15) have proposed that Kaposi's sarcoma arises from pluripotential vascular cells. The histochemical demonstration of lysosomal enzymes in both the hamster tumor described in this paper and in human Kaposi's sarcoma is an additional similarity. The method of alternating culture with animal repassage of the hamster tumor offers an opportunity to determine the histogenesis of the tumor with extrapolation of the results possibly to the human tumor.

The wet tumor weight observed in pregnant female hamsters is significantly greater than that noted in either males or nonpregnant females. This difference appears to indicate a hormonal control of the tumor growth. Lipkin (16) has observed in hamsters that melanotic and amelanotic malignant melanomas grew more rapidly in nonpregnant females than in males and were lethal in females at an earlier age. However, in pregnant females he noted the growth of these tumors to be inhibited. Thus, the hormonal influences on growth in malignant melanomas are different than in the tumor described here. For this reason it is premature at this time to ascribe the growth control of this fibrohemangiosarcoma to a particular hormone.

Summary. A fibrohemangiosarcoma was produced in hamsters following the injection of cell inocula. These tumors in pregnant, lactating female hamsters attained a wet tumor weight 4–8 times greater than that noted in either adult male or female hamsters within 6–7 wk.

Monolayer cell cultures obtained from a fibroma produced by the injection of cells from a Wistar Institute HE-44 culture in its 69th passage was alternately repassaged through inbred Syrian hamsters. Following the fourth animal repassage a culture line was obtained which consistently through the

16th subculture produced tumors composed of spindle cells, endothelial cells and cells intermediate between these two types of cells. It is considered likely that the culture line consisted of mesenchymal cells that have retained some degree of pluripotentiality. Strong acid phosphatase, β-glucuronidase and nonspecific esterase activity was present in the cells of the tumor tissue as well as in the cells grown in culture for 16 passages. It is probable that hormonal influences associated with growth are responsible for the observed differences in wet tumor weight in pregnant and nonpregnant hamsters. This tumor showed histologic resemblance to the human Kaposi sarcoma.

1. Kirkman, H., Nat. Cancer Inst. Monogr. n **1**, (1959).

2. Fortner, J. G., Mahy, A. G., and Cotran, R. S., Cancer. Res. **21**, 199 (1961).

3. Kirkman, H., and Algard, F., *in* "Spontaneous and Non-viral Induced Neoplasma in the Golden Hamster, Its Biology and Use in Medical Research" (R. A. Hoffman, P. F. Robinson and H. Magalhaes, eds.). Iowa Univ. Press, Ames (1968).

4. Cuprak, L. J., and Sato, G. H., Exp. Cell Res. **52**, 632 (1968).

5. Ham, R. G., Exp. Cell Res. **29**, 515 (1963).

6. Ham, R. G., Proc. Nat. Acad. Sci. U.S.A. **53**, 288 (1965).

7. Sanford, K. H., Earle, W. R., Evans, V. J., and Shannon, J. E., J. Nat. Cancer Inst. **11**, 773 (1951).

8. Puck, T. T., Marcus, P. I., and Cieciura, S. J., J. Exp. Med. **103**, 273 (1956).

9. Goldberg, A. F., and Barka, T., Nature (London) **195**, 297 (1962).

10. Rutenberg, A. M., Rosales, C. L., and Bennett, J. M., J. Lab. Clin. Med. **65**, 698 (1965).

11. Lorbacher, P., Yam, L. T., and Mitus, W. J., J. Histochem. Cytochem. **15**, 680 (1967).

12. Yam, L. T., Li, C. Y., and Crosby, W. H., Amer. J. Clin. Pathol. **55**, 283 (1971).

13. Moloney, J. B., J. Nat. Canc. Inst. **24**, 933 (1960).

14. Cox, F. H., Cancer **12**, 289 (1959).

15. Hashimoto, K., and Lever, W. F., J. Invest. Dermatol. **43**, 539 (1964).

16. Lipkin, G., Cancer Res. **30**, 1928 (1970).

Received Jan. 14, 1972 P.S.E.B.M., 1972, Vol. 141.