An Induced Transmissible Sarcoma in Hamsters: Eleven-Year Observation through 288 Passages

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SUMMARY

A tumor was induced in hamsters by subcutaneous injection of 20-methylcholanthrene. It proved to be 100% transmissible and 100% fatal. It has been passed through 288 generations over an 11-year period. The tumor is a sarcoma, initially pleomorphic, passing through a phase resembling fibrosarcoma, and eventually showing the cellular structure of a primitive reticulum cell sarcoma that has not changed since the 20th generation. After transfer, palpable tumor appears in the host after about 4 days, grows locally with great rapidity, metastasizes chiefly through lymphatic pathways, and kills the animal usually between the 20th and 35th days. The weight of the tumor at death is predictable within limits. The tumor is useful in assay of cancerocidal agents, as well as in other aspects of experimental oncology. It is available to any interested investigator.

The golden Syrian hamster provides an excellent medium for the study of malignant disease in a broad spectrum of both spontaneous tumors and those induced by a variety of means (2, 7—9, 21—27, 29, 31, 33, 36, 39—41). To refer briefly to the spontaneous tumors that have been described in hamsters, the papers of Ashbel (1) and Crabb and Kelsall (5, 6) are mentioned. Fortner et al. (12—14) provided important data in this field, particularly with respect to transmissibility of the spontaneous tumors. Greene (18, 19) and Greene and Harvey (20) added instances of melanoma and lymphoma. Staibli and Haemmerli (38) and Brindley and Banfield (3) reported lymphomas, Garcia et al. (17) plasmocytoma, Friedell et al. (15) myxofibrosarcoma studied in transplant to the cheek-pouch, and Sherman et al. (37) an undifferentiated carcinoma with hemolytic anemia interpreted as secondary hypersplenism.

Sarcomas induced by Rivière et al. (34, 35) and by Nettleship (32) were perhaps similar to the one described here. However, the former was carried through only 13 passages, and the latter was observed over a period of 68 days with emphasis on the initial cellular changes after injection of the carcinogen. Lutz et al. (30) employed the same carcinogen to produce a similar tumor which could be transplanted in the cheek pouches of hamsters. Crabb (4) obtained much the same type of tumor with another carcinogenic hydrocarbon, and followed its course through 16 transfers.

Most of the tumors, both spontaneous and induced have proved transmissible, suggesting a low level of host resistance unmodified by adrenocorticosteroids, exposure to radiant energy, or other measures (12). Inbreeding does not produce strains of high or low incidence of tumors, as it does in mice, for example, so that the results of studies on hamsters are comparable regardless of the source of the animals. Perhaps of greatest significance, however, is the close resemblance of both spontaneous and induced tumors in hamsters to their counterparts in humans with respect to cellular structure and behavior (10, 11).

The present report describes the induction of a sarcoma in hamsters with 20-methylcholanthrene, the changing cytologic characters through the earlier passages, the ultimate stabilization as a primitive reticulum cell sarcoma, as well as the increasing degree of clinical aggressiveness to a final pattern of behavior in which longevity, size of tumor, and metastatic spread can be predicted within reasonable limits. Thus, this tumor may prove useful in evaluation of cancerocidal agents.

MATERIALS AND METHODS

A group of young male golden Syrian hamsters received S.C. injections of a carcinogenic hydrocarbon, each developing a tumor thereafter. Details relate to the hamster from which the long-range transfer program was derived. This animal was given 0.1 ml of a 10% solution of 20-methylcholanthrene in olive oil on March 27, 1953; the same dose was repeated at the same site on June 24, 1953. On August 5, 1953, a small nodule was noted at the site of injection, reaching a size of 2.5 × 1.5 cm by September 9, 1953. The mass was removed surgically under sterile conditions, minced, and approximately 0.4 ml transferred
by trochar into the subcutaneous tissue of unselected young male and female hamsters, generally in the right subscapular region. This procedure was followed throughout the subsequent 288 passages.

Initially and at each transfer, tumor tissue was fixed in formalin, sectioned, and stained with hematoxylin and eosin; silver impregnation and PAS staining were also used through the first 20 generations. A complete autopsy was performed on each animal, the majority being sacrificed when they appeared terminal to avoid week-end deaths and consequent autolysis. Tumor weight was obtained by the water-displacement method with respect to the main mass, metastasis being recorded likewise when possible; otherwise, metastatic foci were recorded by description.

A collateral line of tumor was begun on February 17, 1959, using tissue from an axillary lymph node metastasis. This has now been carried through 115 transfers, differing in some respects from the parent tumor, as will be noted later.

From time to time, tumor tissue was frozen, and transfer was delayed to evaluate viability of the cells. Early in the course of the study, an aliquot of tumor tissue was homogenized, subjected to ultrasonic treatment, passed through microfilters, and injected into young hamsters in an effort to determine the possibility of a virus factor.

The animals for the most part were obtained from the Lakeview Hamstery, Newfield, New Jersey. Both male and female animals were used, generally 6 to 8 weeks old, although younger and much older animals were employed at times. They were fed Purina chow pellets and given water ad lib.

RESULTS

From the very outset of the experiment no transfer failed to induce a tumor in the recipient animal when intact cells were used, without regard to freezing and maintenance of cells at —15° to —18°C. for a maximum of 30 days. A period of 138 days elapsed between the 2d injection of 20-methylcholanthrene and removal of the first tumor, the 2d generation requiring 71 days to attain significant size, i.e., 1—2 cm in diameter. Thereafter, this sort of growth time varied from 26 to 23 days, tending to lessen in later generations so that a 1-cm nodule required an average of 4—5 days, with metastasis occurring as early as the 9th day. The tumor grew equally well in animals of either sex at any age. At present viral studies are in progress.

Clinical behavior.—Once established at the site of inoculation, the tumor grew with striking rapidity, occasionally virtually immobilizing the animal because of its sheer bulk (Fig. 1). Generally, however, the hamsters remained active until the day of their death, maintaining fairly good nutrition throughout. Surgical excision of a tumor was followed in a few days by speedy regrowth at the site (Fig. 2).

Chart 1 illustrates the general scatter of longevity of the animals and weights of the tumors as observed in recent years. This demonstrates the striking growth found in certain animals, the tumor in 1 attaining a weight of 60 gm in 28 days.

In contrast to the initial tumor line displayed in Chart 1, the collateral, so-called axillary node line mentioned earlier killed most of the animals within 20 days and metastasized...
to the lungs much more frequently and extensively, as verified microscopically.

**Gross findings.**—There was little or no metastasis in the early generations, and the primary tumor was nearly always confined by a capsule (or compression pseudocapsule) (Fig. 3), but occasionally invading subjacent perirenal fat by contiguity. The major tumor invariably showed extensive central necrosis and hemorrhage. As time went on, the tendency to spread increased, mostly along lymphatic pathways. Table 1 lists the findings in 3 unselected groups of 25 animals observed during the past 2 years, and shows fairly consistent behavior. Of the involved lymph nodes, the axillary ones were nearly always the largest (Fig. 3), and the degree of extension elsewhere was somewhat variable. Beyond the sites of metastasis shown in Table 1, there has been a rare instance of cardiac and splenic involvement. Spleens have been uniformly enlarged, often greatly so, to the extent that the organ may curl around in the lower abdomen (Fig. 3).

**Cytologic characters.**—The first tumor to be removed presented a marked pleomorphism, the multiplicity of cell types including spindled, stellate, polyhedral, and rounded forms, with a scattering of multinucleated giant cells, some of which resembled the so-called ‘strap cells’ of rhabdomyosarcoma. Reticulin stains emphasized concentric whorls around small vessels, reminiscent of hemangiopericytoma. Reticulin fibers were rather richly dispersed throughout the tumor in a more diffuse pattern in contrast to later developments.

During the 3 succeeding generations the cellular components became predominantly spindled, their appearances becoming rather typically fibrosarcomatous. Subsequently, the spindle cells became plumper, and a progressively increasing number of stellate to polyhedral forms were interspersed, so that by generation 10 the tumor resembled reticulum cell sarcoma of man. Later, the cells adopted a more primitive appearance, the membranes becoming less distinct and the growth acquiring an almost syncytial pattern. By this time reticulin fibers were hardly detectable. This is similar to primitive human reticulum cell sarcomas. This structure has remained constant throughout the hundreds of transplants that followed. (Figs. 4–9)

The splenomegaly was due in part to lymphocytic hyperplasia, but to a much greater extent it was the result of reactive plasmocytosis. Some spleens were so tightly packed with these cells that they resembled a plasmocytoma. This is regarded by some (Friedell et al.) (16) as an autoimmune reaction, necrotic tumor serving as an antigen. Kelsall (28) suggested that the enlarged spleens in tumor-bearing hamsters were also produced by extramedullary hematopoiesis.

**Tissue culture.**—Explants from the tumor during early generations grew well in tissue culture, forming sheets of large polyhedral and multipointed cells, with interspersed multinucleated giant forms. More recently the growths have been more difficult to establish and maintain, the cell population being much less rich. Various means of enrichment of the culture medium are being investigated.

### DISCUSSION

The importance of the hamster in experimental oncology has been emphasized in the early part of this article, and a listing has been given of certain of the spontaneous and induced tumors reported in this species. None of the articles that has appeared thus far describes the natural history of induced transmissible neoplasms over any great period of time. Thus, our experience with a 20-methylcholanthrene sarcoma through 288 passages over a span of 11 years seems unique.

Our sarcoma has established a pattern of reliability over this long period of observation, being invariably transmissible, growing at a fairly uniform rate, metastasizing in not too diverse a fashion, and causing death of the host within well established limits. After an initial period of cellular variability, a histologic picture evolved that resembles a poorly differentiated reticulum cell sarcoma of humans, a picture that has not altered in more than 260 transfers. The tissue remains viable through at least a 1-month period of freezing and can be grown in culture. All of these properties would serve to recommend the tumor as a standard one for basic studies on malignant growth, particularly in the testing of potential cancerocidal agents.

In our own laboratories certain chemicals have already been tested in such tumor-bearing animals; details are outside of the scope of the present communication. Suffice it to say that styryl quinolines, for example, will inhibit the take of an implant and restrain progress of established growths. The tumor is rather resistant, however, and can not be eradicated by chemicals as can certain lymphomas in rats and mice, behaving rather as do the more aggressive reticulum cell sarcomas in man.

Experiments relative to a possible viral background are planned in the hope that another bit of evidence can be added in the field of oncogenic viruses.

It is hoped that other investigators will be interested in employing this tumor in their studies. Material will be furnished on request.

### ACKNOWLEDGMENTS

The author wishes to acknowledge the valuable services of Mr. Joseph E. Sunner for the medical illustrations and the valu-
able technical services of Mr. Robert C. Bell and Mr. Norman H. Greenberg.

REFERENCES

Fig. 4.—Section through original tumor showing the marked pleomorphism of cellular structure. H. & E., X 350.

Fig. 5.—Section through tumor of 3d generation, the tendency toward spindling becoming pronounced. A giant cell resembling those of rhabdomyosarcoma is present. H. & E., X 350.

Fig. 6.—Section through tumor of 4th generation. The tumor is exclusively spindle-celled, having the appearance of a typical fibrosarcoma. H. & E., X 350.

Fig. 7.—Section through tumor of 6th generation. The spindle cells are larger, plumper and have a more vesicular nucleus, evidences of de-differentiation. H. & E., X 350.
FIG. 8.—Appearance of the tumor past the 20th generation, forming a diffuse sheet of primitive reticulum cells. H. & E., X 100.

FIG. 9.—Detail of Fig. 8, showing the virtually syncytial type of growth; cell outlines are poorly defined. H. & E., X 500.


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