Factors Affecting Hamster Sarcoma Growth in the Cheek Pouch*

H. M. LEMON AND E. SMAKULA

(Department of Medicine, Boston University School of Medicine and the Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, Boston, Mass.)

The cheek pouch of the golden hamster (Mesocricetus auratus) has been shown to be a favorable transplantation site for a variety of homologous and heterologous malignant tumors, including a few human epidermoid carcinomas and sarcomas (5, 10, 12). Since accurate measurements of transplant volume are possible according to the technic described by Lutz and co-workers, the kinetics of tumor growth and the influence thereupon of various conditioning factors may be closely followed.

In the present investigation our major objective was to obtain further information concerning the most favorable circumstances for the serial transplantation of tumors into the hamster, with special reference to age of the host, effect of repeated inocula of tumor cells, influence of metastases, and role of cortisone. A survey of 700 previous reports concerning the hamster failed to reveal any similar information.1

METHODS

Animals.—Hamsters were all secured from a single farm, at various ages beginning at 2 weeks, and were maintained in individual cages at constant temperature and humidity in our laboratory. During the studies a Purina Chow pellet diet with water ad libitum was provided. Tumor transplants were obtained from the third to 30th passage of a methylcholanthrene-induced hamster sarcoma, in all cases from a tumor the appearance and growth curve of which indicated that it was in the most rapid phase of proliferation. Test and control animals were usually given implants from the same tumor. A 1-c. mm. portion of viable tumor was implanted into the cheek pouch by the methods described by Lutz (6), and the sizes of the implants were measured at intervals of 2–3 days. The length, width, and depth of the implant were measured to the nearest 0.1 mm. with a dissecting microscope at 10x magnification. The product of these dimensions divided by 2 closely approximates the volume of a sphere or oblate spheroid, the shape commonly assumed by the transplants in the early stages of their growth. A constant error of 3 per cent resulting from calibration of the optical micrometer has not been deducted from the measured tumor volumes. With a highly cellular tumor containing a minimum amount of stroma, the rate of change of this volume closely parallels mitotic activity, provided that no cells are excluded from the process of proliferation by necrosis. Gross and microscopic evidence of transplant necrosis seldom appeared until after a volume of 0.1 c. mm. had been surpassed. The time in days elapsing from transplantation until this volume was reached was selected as the simplest single measure of the complicated processes of nidation, vascularization, and tumor growth through which all transplants progress; this time will hereafter be referred to as the “critical time.” The time necessary for a tumor to exceed 1 c. mm. in volume will be referred to as “nidation time.” For metastases beyond the cheek pouch, serial

1 Bibliography from Dr. J. W. Nichols, Dept. of Psychology, Univ. of Florida, Gainesville, Fla.

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estimates of size were made on the basis of careful
tumor palpation, 1,000 c. mm. being utilized as the
reference volume for purpose of comparison. Both
types of observations were controlled by dissection
of the tumor and wet weight determination. The
estimated volume usually agreed within 5 percent
of the weight of the specimen, provided measure-
ments were not carried out after the onset of ul-
ceration or necrosis.

RESULTS

General characteristics of sarcoma growth.—Vas-
cularization generally developed 8–5 days after
implantation. Measurable volume increase com-
menced within 3–12 days after implantation (nid-
ation time). A rapidly rising growth curve, which
resembled an exponential type of curve, occurred
in over 99 per cent of primary transplants. Con-
siderable individual variation in growth curves was
noted, as expected in a heterogenetic host strain
(Chart 1). Each curve showed a lag in growth on-
set, probably composed of vascularization time
and host resistance to the graft, which differed
from animal to animal. Plotting of the log volume
against time (Chart 2) showed that during the sec-
ond stage of growth tumor volume increased expo-
nentially for several days with a characteristic
"doubling time" for animals of each age group.
In the third stage of growth a continuous decelera-
tion of volume change occurred, along with exten-
sive necrosis, particularly when tumors exceeded
500 to 1,000 c. mm. In spite of necrosis, tumor
volumes beyond 1,000 c. mm. and up to 80,000 c.
mm. were observed in cervical metastases, which
increased less and less rapidly to an asymptote of
10^8 c. mm., approaching the animals’ own body
weight.

After the onset of cell death, the rate of volume
increase can no longer be expected to be linear with
the rate of cell multiplication. Beyond an approxi-
mate volume of 200 c. mm. production of viable
tissue combined with tissue necrosis, with partial
resorption of the latter, determined the rate of
volume increase. The ratio between viable and
necrotic tissue changed inversely with increasing
volume. Large-sized tumors had only a very thin
cortical layer of active growth. No 100 per cent
necrotic tumor was observed.

The balance of this volume increase resulting
from cell growth and decay is represented in the S
type curve. The graphical presentation of tumor
volume increase in stages following the onset of
necrosis does not permit conclusions whether the
rate of cell multiplication, the doubling time (9),
remains constant or decreases when the graft ages.

Factor of host age: sarcoma growth in adult vs.
suckling animals.—a) In adult hamsters 6–8 weeks
old, weighing 75–90 gm., nidation time varied
from 8 to 12 days. The period of exponential vol-
ume increase was approximately 9 days, the slope
0.28 ± 0.05, and the doubling time 30.7 ± 4.9

CHART 1.—A. Tumor growth range of nineteen hamsters
implanted at 8 weeks of age (wt., 14–23 gm.); 50 per cent
of tumor growth within range demarcated by solid lines. B.
Tumor growth range of 71 hamsters implanted at 6–8 weeks
of age (wt., 75–90 gm.); 50 per cent of tumor growth within
range demarcated by solid lines. ♂—♂ Frequency distribu-
tion of critical times for tumors implanted in young animals.
♂—♂ Frequency distribution of critical times for tumors im-
planted in adults animals.
b) In suckling animals 2 weeks old, weighing 14–22 gm., a much more rapid development of the transplanted sarcoma took place, with a significant acceleration of the critical time (Chart 1). The 200-c. mm. volume was attained between the 9th and 13th day with the maximum of frequency on the 9th–10th day. Scarcely any overlap occurred in the volumes during the later stages of tumor growth in the sucklings, with those of adult hamsters. The nidation time lasted from 2 to 6 days. The period of exponential volume increase was approximately 8 days, the slope 0.36 ± 0.04, the doubling time 20.3 ± 2.2 hours in eleven randomly selected animals. Similar observations were made when weanling animals were studied, giving intermediate values.

No abnormalities occurred in the rate of host growth during the phase of rapid enlargement of transplanted tumors. Preweanling animals gained about 200 per cent in weight during a 2-week period of tumor growth. Animals 6–8 weeks of age gained 8–10 per cent in weight during a 2–3-week period of tumor growth. Weight losses occurred only when tumors started to ulcerate. The spleen was always enlarged in tumor-bearing animals (0.8–0.7 gm.) and reverted back to normal (under 0.2 gm.) several days after tumor removal. The tumor volume attained when ulceration appeared was about 400–6,000 c. mm. for cheek pouch grafts and about 20,000–80,000 c. mm. for cervical metastases. Ulceration occurred independently of the volume or the ratio of viable to necrotic tissue. Local tissue invasion by the tumor attached it to the epidermis, where further expansion of the tumor caused secondary infection and ulceration.

**Influence of age on metastasis.**—In some hamsters cervical lymph node metastases developed following primary implantation, whether or not the primary graft was later excised. Autopsy showed no metastases in lungs, liver, spleen, peritoneum, or kidneys in 23 out of 34 animals 8 weeks after primary implantation or in twelve animals 13 weeks after implantation. In a few animals autopsied at later intervals, visceral metastases were seldom grossly discernible.

All cervical metastases exhibited rapid growth with increase of volume from 1,000 to 20,000 to 30,000 c. mm. within 3–4 weeks and up to 80,000 c. mm. in another 2 weeks. Measurements pro-
ed by an identical growth stimulus, leading to a similar response.

Repeated transplantation, following excision of a primary implant.—In a number of animals established tumors were resected within 2 and 3 weeks after implantation, by excision of that portion of the cheek pouch bearing the tumor. An even greater delay occurred in the growth of second implants in hamsters that had their first transplants between 2 and 3 weeks of age (Chart 4). While part of this delay was no greater than would be expected as a result from the aging of the host between transplants, the frequency distribution curves reveal that some of these animals also showed excessively prolonged critical times, far in excess of those seen in primary implants in adult animals.

a) Under these conditions, re-transplantation to the other cheek pouch within a time interval of 0–35 days resulted in 60 out of 63 cases in a delay in the critical time for the second implant, compared with the critical time for the primary implant in each case (Chart 4). These observations indicate a mean 30 per cent or 4-day increase in critical time for the second implant, with a range of variation in delay between 1 and 24 days. Control experiments were carried out to rule out post-operative or aging effects on animals of corresponding age and also on those with cheek pouch resections in which no tumor had been previously transplanted. Growth curves similar to other primary implants were obtained in these controls.

b) An even greater delay occurred in the growth of second implants in hamsters that had their first transplants between 2 and 3 weeks of age (Chart 4). While part of this delay was no greater than would be expected as a result from the aging of the host between transplants, the frequency distribution curves reveal that some of these animals also showed excessively prolonged critical times, far in excess of those seen in primary implants in adult animals.

c) After excision of a second implant, continued challenging of the host with 3d, 4th, and 5th temporary implants led to progressively lower rates of takes and to complete resistance in a few (Chart 5). Occasionally, spontaneous regressions developed in tumors which had begun to grow. Less than 80 per cent of second or third transplants survived and grew, and in the few animals transplanted for the fourth time, over half were resistant to further challenge with the tumor. Each animal was observed for 1 month before a graft failure was recorded.
Effect of established metastases upon second transplantation.—Excision of the primary take was followed by the appearance of cervical metastases in some hamsters. Second contralateral implants in ten animals at a time when cervical metastases were well established were followed by delayed nidation and vascularization period in five animals, during which period no demonstrable volume increase occurred. The critical time necessary for second implant development considerably exceeded that commonly required for second implants in the absence of metastases. The major portion of the delay was the result of a prolonged nidation period. In three of these, poor tumor growth occurred. In the other five animals no survival of the implant could be demonstrated. These metastases had no detectable effect on the well-being of the host, as evidenced by the weight of the animal.

Simultaneous primary grafts to both cheek pouches.—Simultaneous 1-c. mm. transplants to both cheek pouches of a litter of ten 2-week-old animals always resulted in tumors of highly unequal volume in the individual hosts. As a group, these twenty separate transplants most frequently showed a critical time exceeding by more than 1 day that previously found in single primary implants. The range of the critical times extended to the 16th day. However, adding together the volumes of each pair of transplants resulted in a mean growth curve indistinguishable from that characteristic of single primary implants into animals of this age group, as presented in Chart 1.

**EFFECT OF CORTISONE ON HAMSTER SARCOMA**

**CHART 6.—Each symbol represents the mean critical time of an experiment in which six animals were implanted**
The doubling time calculated for the total volume of each pair of transplants was 20.8 ± 4.1 hours.

The mean doubling time for the faster growing member of each pair of transplants averaged 19.5 ± 3.7, while that of the slower developing implants was 23.0 ± 3.8, which is not significantly different. A significant variation in the nidation time occurred, with an increase in the range of observed values, from a normal of 2–6 days to 3–9 days. There was no correlation observed between prolonged nidation and the doubling time. The increase in critical time observed for the slower-growing members of each tumor pair therefore was due to delay in nidation. In spite of this, these smaller tumors were able to achieve a doubling time not significantly different from that of the larger tumor, or that of single primary implants.

Cortisone therapy.—Administration of 6 mg. of cortisone at the time of transplantation and again at 10 days resulted in arrested growth of 5-week-old animals and an 8–28 per cent decrease in body weight of the 6–8-week-old hamsters (Chart 6). In all six experiments a considerable delay occurred in critical time. This delay occurred even when cortisone therapy was withheld until the 4th or 5th day, when nidation and vascularization were completed.

DISCUSSION

These observations suggest the value of utilizing 2-week-old animals for heterologous transplantation studies as a result of the increased primary tumor and metastasis growth seen in animals implanted at this age. At this time the cheek pouch is just large enough to be utilized for transplants.

The altered response to further tumor grafting into hamsters in whom growing neoplasms had been temporarily established strongly indicates the presence of acquired humoral inhibitory substances. Whatever the mechanism is, it appears to be more effective in adult than in young animals in delaying metastasis, but is inadequate to prevent ultimate death of the host from continued local growth with ulceration in every instance in which the primary lesion is not excised. If the tumor is excised, sufficient inhibition may be exerted against a second implant during its nidation period to prevent growth. Somewhat similar observations have been made for other tumors (11). It is generally accepted that in animal strains of mixed genetic composition development of humoral inhibitory mechanisms can be demonstrated following homologous tumor transplantation.

To the best of our knowledge, the duration of interphase for growing tumors has not been established. The doubling time of the hamster sarcoma represents the period necessary for a twofold increment in tumor volume to occur. At least three variables enter into this volume change: the rate of stromal production, rate of cellular hypertrophy, and rate of cell division. Since histologically the first variable appears to be more or less constant in the initial growth period and since there are also definite limitations to the mean size attainable by tumor cells, the doubling time would appear to us to reflect most closely the rate of cell division in the tumor. However, further investigations of mitotic counts would be necessary to establish this point. The doubling time reported in this study closely resembles that reported for the Krebs ascites tumor (9).

In these studies we have not investigated the influence of a massive tumor inoculum (exceeding 500–1,000 c. mm. in volume). Necrosis of portions of such large inocula, and the much larger areas of neoplastic cell surface transplanted, may contribute in some way to a response by the tissue and humoral defense mechanisms of the host different from that described herein. However, in the presence of viable metastases of such large size no "immunologic paralysis" could be detected which potentiated subsequent growth of additional tumor grafts. On the contrary, under these conditions reduced growth or graft failure occurred throughout this study.

Our investigations on this point are in essential agreement with those made by Crabb, who studied the influence of the implant upon incidence of metastases of a 9,10-dimethyl-1,2-benzanthracene-induced sarcoma in this species. Excision of the primary transplant increased the incidence of microscopically verified metastasis threefold, even when the time of observation was less than that usually required for host death from the implant (1).

The inhibitory action of cortisone against a hamster sarcoma was previously demonstrated by Crabb (2). Lutz and his co-workers have emphasized the vaso-constrictor action of this agent upon hamster vessels exposed to various types of injury (7), and Ebert has demonstrated somewhat similar vascular response in protecting rabbit arterioles from hypersensitivity damage secondary to acid-fast bacillary infection (3, 4). In addition to these vascular effects of cortisone administration, the results of transplantations of human cancer tissue to the hamster must be considered in the light of possible direct cortisone inhibition of the tumor. Most of the human cancers successfully transplanted in the hamster have been epidermoid or undifferentiated carcinomas of epidermoid origin (5, 10, 12),
which have not yet been shown to be inhibited by cortisone therapy.

However, in view of the deleterious effects on body weight resulting from cortisone in the doses used, the tumor inhibition observed may also be secondary to somatic disturbances in the host (8).

The method of observation of tumor growth used in this study appears to have considerable application for precise evaluation of factors affecting this and other transplantable solid neoplasms in this host.

SUMMARY

A methylcholanthrene-induced hamster sarcoma showed accelerated growth after transplantation to 2–8-week-old animals, in contrast to 6–8-week adult hamsters, with increased growth of metastases as well. Second transplants, after primary grafts were excised, showed delayed growth. Nidation and vascularization of second transplants were also delayed in the presence of a rapidly growing metastasis. Cortisone in the doses used showed host toxicity and delayed tumor growth.

REFERENCES


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H. M. Lemon and E. Smakula


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