The Cheek Pouch of the Hamster as a Site for the Transplantation of a Methylcholanthrene-induced Sarcoma*

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INTRODUCTION

A method by which neoplastic tissue may be grown in a physiological environment and at the same time be readily accessible for periodic experimentation and observation is described in this paper. The membranous cheek pouch of the hamster (Mesocricetus auratus), used by Fulton, Jackson, and Lutz (5) for the cinephotomicrographic study of small blood vessels, was selected as the site for the transplantation of a methylcholanthrene-induced hamster sarcoma. The hamster has been used relatively little in tumor work. Few inbred strains have been developed, and the only report of a spontaneous tumor in the hamster has been by Ashbel (3), who found thirteen cases in 1,000 animals over a period of 7 years. Gye and Foulds (6) and Halberstaedter (7) have produced benzpyrene-induced tumors in hamsters. Crabb (4) described a transplantable sarcoma, induced in hamsters with 9,10-dimethyl-1,2-benzanthracene, and in 1949 reported the relation of duration of a transplanted sarcoma to metastasis. Kelsall (8) described hematopoiesis in the spleen of tumor-bearing hamsters.

Subpannicular induction and transplantation have been commonly practiced. Crabb (4) obtained 100 per cent takes when he transferred his benzanthracene-induced sarcoma by means of a hypodermic needle to this site. When the object is to study the effect of the tumor on the animal, this site is useful. When the object is to study the tumor per se, or the local tissue responses, subpannicular growth has obvious disadvantages. The mouse skin-flap preparation described by Algire (1) and modified by Algire and Legallais (2) has become virtually a tissue culture in vivo preparation. It has, however, definite limitations, imposed by the necessary operative procedures and by the physical confines of the chamber.

The advantages realized by using the membranous cheek pouch of the hamster as a site for transplantation are (a) the tumor grows freely without physical hindrance and in a normal physiological environment; (b) the same tumor can be observed at successive stages (measured, photographed); (c) the tumor may be subjected to various experimental procedures (chemical and physical) with no undesirable disturbance beyond that produced by anesthesia; and (d) transillumination for microscopic study of early stages of growth and vascularization is practicable.

METHOD

Hamsters of both sexes, 8–10 weeks old and weighing 90–100 gm., were used throughout this work. The original tumor was induced in hamsters by a series of six weekly subpannicular injections of 0.25 cc. of 0.4 per cent methylcholanthrene in oil of lard. This tumor is tentatively classified as a type of spindle-cell sarcoma, histologically somewhat different from the benzanthracene-induced sarcoma described by Crabb (4), although no pictures of his original tumor were published. The methylcholanthrene-induced tumor was propagated by transplantation in the flank of other hamsters and in cheek pouches.

This material was the source of the 35 cheek pouch transplants, the growth characteristics of which are reported here. Pieces of hamster tissue such as liver, kidney, adrenal cortex, cheek pouch, epidermis, and skeletal muscle were used to compare the effect of normal tissue per se with that of the neoplastic tissue. A few minutes before making a cheek pouch transplant, the hamster was anesthetized by an intraperitoneal injection of veterinary nembutal (Abbott) in a dose of 0.1 cc/100 gm body weight. The animal was placed in a spun-steel preparation dish, developed for blood...
vessel work (9), the cheek pouch gently everted with forceps, and held with "bank" pins over the optical glass block. With fine scissors, an incision about 1 mm. long was made through the epidermal layer. A piece of tumor or control tissue about 1 c. mm. was inserted with forceps into the loose connective tissue beneath the epidermis and then massaged gently 5 mm. beyond the edge of the incision. When the pins were removed the incision closed by itself. Instruments were clean, but no special surgical asepsis was necessary. No infection occurred either at the incision or at the points of pinning.

The three dimensions of the tumor transplant were measured periodically by means of an ocular micrometer and a vertical scale attached to a binocular microscope (X9). The symmetrical form assumed by the free-growing tumor permitted a reasonably accurate determination of its volume.

Tissues from the original induced tumor and from cheek pouch transplants taken for microscopic study were fixed in 4 per cent formol or in Zenker's fluid and stained with hematoxylin and eosin, or with phloxine.

RESULTS

Subpannicular injections of methylcholanthrene into nine hamsters resulted in the induction of tumors in five. Two tumors were palpable in 8 weeks, one in 16 weeks, one in 23 weeks, and one in 28 weeks. The tumor-bearing animals were used as donors, and no data were obtained concerning the malignancy of their tumors at this stage. Thirty-five transplants of the methylcholanthrene-induced tumor were made, and all became successfully established. Twenty-nine of these were derived from the same original induced tumor, at the same time and under the same conditions. These were used to compare the effects of the number of passages on the rate of growth of transplants. Six were first generation transplants (directly from the original tumor), nineteen were second generation (one passage), and four were third generation. The average length of time for all transplants from transplantation to the first measurable increase in size was 4 days. During this time vasculanization appeared to be established. In the next 10 days the average transplant increased gradually from 2.6 c. mm. to 60 c. mm. After the fourteenth day growth was exceedingly rapid. Thus, the average tumor had grown to 445 c. mm. in the next 10 days. Some tumors became ulcerated at this time. Examination of these tumors showed a large central necrotic region and a thin cortical layer of active growth. Others continued to increase in size at a uniform rate, the largest becoming 2,380 c. mm. at the end of 43 days. The time at which ulceration occurred varied between 24 and 43 days from transplantation. After ulceration occurred, the size was difficult to determine, but the total volume increase was definitely retarded.

The data indicate that each passage was followed by an increased rate of growth of the transplant (Chart 1). After 18 days the average size of first generation transplants in six animals was 105 c. mm. (Chart 1, line A). At the same time the average size of second generation transplants in nineteen animals was 222 c. mm. (line B), and the average size of third generation transplants in four animals was 316 c. mm. (line C).

Microscopically, the original methylcholanthrene-induced tumor appears to be a spindle-cell sarcoma type with areas of pleomorphism (Figs. 1–3). In the spindle-cell regions the cells are close together with little if any collagen, being arranged typically in bundles of different sizes, which run through the tumor in various directions. The nuclei are elongated and vesicular. The tumor is richly vascular, with small arteries and capillaries as well as typical vascular slits. Often nodules pro-
ject into the vascular sinuses. In the pleomorphic regions many types of cells are found, including multinucleated giant cells. Little if any supporting substance is evident. The nuclei show a great range of shape, size, and density, and mitotic figures are numerous. Naked vascular sinuses are abundant. Foci of necrosis are seen in deeper regions of the tumor.

The cheek pouch transplants were generally symmetrical in shape, oval, or spherical (Figs. 5–6). As they increased in size, active growth was confined to a definite cortical region, while the entire central portion became necrotic. The histology of the cheek pouch transplants is similar to the original tumor (Fig. 4). The tumor mass seems to be composed largely of anaplastic cells of pleomorphic nature. Those on the peripheral growth area, contiguous with the connective tissue, tend to be spindle-shaped, with abundant cytoplasm and large nuclei. They are arranged loosely, in bundles at places, although no definite capsule is present. In some peripheral portions no spindle-cells are evident. In these actively growing regions the cells tend to become spherical, and mitotic figures are numerous. Small blood vessels with a definite vascular lining are numerous but largely confined to the outer region of the tumor, while others more deeply situated toward the necrotic region are less numerous and irregularly sinusoidal in nature, with and without vascular lining.

Metastases from the methylcholanthrene tumor were found in the liver, lung, spleen, mesentery, and intestinal wall (Figs. 7–10). Microscopically, the spleen metastasis resembles the original tumor more clearly than it does the cheek pouch transplant, showing a preponderance of spindle-cells, arranged in bundles. It is moderately well vascularized, but no vascular slits are found. Some round cells are highly anaplastic, and mitotic figures are common. The cells of the liver metastasis show considerable pleomorphism, with little tendency for them to be arranged in bundles. They are exceedingly anaplastic with many multinucleated cells, some of giant size. Mitotic figures are common. A few small foci of necrosis are found. The same variety of cells is present as occurs in the original tumor. A few small blood vessels are present. The periphery shows invasion of the tumor cells among the cords of the liver (Fig. 7). The intestinal metastasis was spherical, with central cells, which were anaplastic and pleomorphic, and a cortical layer of spindle cells (Fig. 10).

**SUMMARY**

The cheek pouch of the hamster has been used as a site for the transplantation of a new methylcholanthrene-induced malignant sarcoma. The advantages of this site are (a) the tumor grows freely without physical hindrance; (b) the same tumor can be observed frequently at successive stages (measured, photographed); (c) the tumor may be subjected to various experimental procedures with little disturbance of the physiological environment; (d) a high percentage of "takes" is obtained; and (e) transillumination for microscopic study of early stages of growth and vascularization is practicable.

The original tumor was induced in five hamsters out of nine. Thirty-five cheek pouch transplants were made, and all became successfully established, measurable, on the average, in 4 days. The tumors were either spheroid or ovoid. Growth was rapid and, after the fourteenth day, essentially exponential. Ulceration usually occurred between the twenty-fourth and the forty-third day. Each successive transplant increased in rate of growth.

Microscopically, the original methylcholanthrene-induced tumor appeared to be a spindle-cell sarcoma with areas of pleomorphism. The cheek pouch transplants were similar but with more anaplasia and pleomorphism. Metastases were found in the liver, lung, spleen, mesentery, and intestinal wall. Similar anaplastic and pleomorphic characteristics were found in the metastases. While that in the spleen showed some tendency for spindle cells to be arranged in bundles, the liver metastasis was extremely anaplastic and pleomorphic.

**REFERENCES**

FIG. 1.—Methylcholanthrene-induced tumor produced in the hamster by subpannicular injection. Zenker's solution, phloxine, and methylene blue. Mag. ×150.

FIG. 2.—Original induced tumor showing spindle-cell nature. Mag. ×645.

FIG. 3.—Original induced tumor invading skeletal muscle. Mag. ×150.

FIG. 4.—Methylcholanthrene cheek pouch transplant, outer growth zone showing pleomorphism and anaplasia. Zenker's solution, phloxine and methylene blue. Mag. ×645.

FIG. 5.—Cheek pouch transplant of methylcholanthrene tumor, 40 days old, volume 1,125 c. mm.

FIG. 6.—Methylcholanthrene cheek pouch transplant, 16 days old, volume 90 c. mm. Note vascularization.

FIG. 7.—Metastasis in liver from methylcholanthrene cheek pouch transplant after 107 days. Note remaining cords of liver cells. Mag. ×150.

FIG. 8.—Portion of liver metastasis shown in Figure 8. One or two liver cells remaining. Note resemblance to cheek pouch transplant shown in Figure 9. Mag. ×645.

FIG. 9.—Metastasis in spleen from cheek pouch transplant after 107 days. Hematoxylin and eosin. Mag. ×480.

FIG. 10.—Metastasis in wall of small intestines from cheek pouch transplant after 107 days. Note the pleomorphism. One of several hundred pearl-like nodules in the peritoneum and mesentery. Mag. ×645.
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