A Transplantable 9,10-Dimethyl-1,2-Benzanthracene Sarcoma in the Syrian Hamster*

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Comparatively little has been published on the use of the hamster in cancer research and only two reports, including some 15 instances, of spontaneous tumors are available (1, 9). The earliest experimental work appears to be Gye and Fould's (9) production of a mixed-cell sarcoma in two males by injecting 3,4-benz-pyrene, in 1939. A year later Halberstaedter (11) reported the production of a similar sarcoma in 29 hamsters by repeated injections of benzpyrene and in 1944 extracts of a sarcoma produced by benzpyrene were used to study the rate of cell growth in tissue cultures (6).

No work on the use of 9,10-dimethyl-1,2-benzanthracene as a carcinogen in hamsters has been found. However, results of several investigations with mice (2, 4, 14, 15), with rabbits (3) and by other standards (12) show that this is a very potent carcinogen.

This work was undertaken to determine if the hamster is a suitable animal to use in studying the histologic structure of a tumor induced by injecting 9,10-dimethyl-1,2-benzanthracene and perpetuated by transplantation.

MATERIALS AND METHODS

Male and female Syrian hamsters (Cricetus auratus, or Mesocricetus auratus) from two geographically widely separated colonies were used in these experiments. The stock from the University of Colorado was inbred, but not consistently brother-sister mated, from two males and four females obtained from the Fairfield Rabbit Farms, Caldwell, N. J., and the other stock was from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The JAX hamsters ranged from 39 to 74 days old at time of implantation, but the Colorado animals varied from 36 to 335 days in age and included sexually immature young, adults and senescent breeding animals.

The original tumors for this work were obtained by injecting four female hamsters with 9,10-dimethyl-1,2-benzanthracene. The tumors thus derived, as well as those obtained by transplantation, were polymorphous, or mixed-cell, sarcomas.

The carcinogenic solution was made by dissolving 20 mgm. of 9,10-dimethyl-1,2-benzanthracene (Eastman Kodak Co.) in 5 cc. of olive oil and 5 cc. of benzene (Merck, thiophene freed, A. C. S.). This solution was injected into the areolar tissue under the cutaneous maximus (panniculus carnosus) muscle in the right lateral lumbar region. In the hamster, as well as in other animals having a well developed cutaneous maximus muscle, the areolar tissue in the lumbar area is an excellent site for injection because the iliolumbar artery and vein anastomose freely with 5 other important arteries and veins in this muscle and the long thoracic and internal mammary anastomose with the inferior epigastric and other arteries and veins in the body wall and thus form a high vascularity for this extensive, areolar area (here designated as the subpannicular area to avoid incorrect use of the term subcutaneous).

Each of the 4 original hamsters received 4 injections of this solution over a period of 41 days. Three injections of 0.10 cc. were made 17 and 8 days apart and one of 0.15 cc., 16 days later. Three of these animals supplied the original tumors for all the transplantations; while the fourth was allowed to develop an extensive lung metastasis.

The transplantations were effected either by grafting, in which a wedge-shaped piece of solid tumor or a mass of soft material from a necrotic growth was inserted subpannicularly with concave forceps or by injection, in which fluid and solid material from an incision in the tumor were drawn through an 18-gauge needle into warm salt solution and injected into one or both lateral lumbar regions. Usually, transplantations, were made to one side of the mid-dorsal line, but in some cases a single graft was inserted subpannicularly into the mid-lumbar region; while in others transplants were made by grafting into one side and injecting into the other (Fig. 2).

Transplantable metastases to lymph nodes, lungs and kidneys were obtained by subtotally removing the sarcoma once or twice and allowing the new growth to develop until the animal became moribund before it was killed. At each removal a small piece of the neoplasm was left in situ to insure early recurrence.

The 6 principal lines of this sarcoma, all derived from one benzanthracene-induced tumor (Fig. 1, * Aided by grants from the Research Council of the Graduate School of the University of Colorado.
Fig. 1.—Generations of transplants from a benzanthracene-induced tumor to show main lines and lines derived from metastases to lymph nodes, lungs and kidneys. Transplants of organs made purely as tests for metastasis are not included. Each of the boxes for the 234 transplants includes the symbol of a single host and the date it was implanted, except that the numbers preceding the letters JAX denote the total number of individuals receiving transplants from a single donor; thus, the symbols 2-, 13-, and 54 JAX represent transplants to 110 JAX hamsters from 5 different donors. The letter J denotes JAX stock, all the others represent Colorado stock. Failure of the 7 JAX hosts to take was the result of an accident.
A4:1F), that were transplanted to both Colorado and JAX hamsters included the following lines: (a) Main line (Fig. 1, A4:1F through J3F), 17th passage; (b) second donation of A4:1F (Fig. 1, B5:1F to J18F), 13th passage; (c) Metastatic kidney (Fig. 1, A15:1F through C30:1F), 12th passage; (d) Metastatic lymph node (Fig. 1, 19-F through E21:2F), 12th passage; (e) First metastatic lung (Fig. 1, BS:IF to J18F), seventh passage; and (f) Second metastatic lung (Fig. 1, 10-F through 25 JAX hosts), 4th passage.

All transplantations, including injections, were done under nembutal anesthesia in which a 2 per cent solution was injected subcutaneously in dosages carefully estimated at 0.04 ml. to 10 gm. of body weight for males and females weighing 80 to 115 gm.

BENZANTHRACENE-INDUCED TUMORS

No symptoms of pain were apparent in any of the 4 animals following the first three injections of the carcinogenic solutions, but 41 days after the first injection three of the hamsters had the skin adherent at the site of the injections. The fourth injection in each animal was, therefore, made immediately cranial to the adhesion. Eleven days after the last injection (52 days from the first) two of the hamsters had developed lesions at the site of the last injection. Another developed a similar lesion 2 or 3 days later, but there was no lesion in the fourth animal and the tumor appeared on the side opposite the site of injection. Another animal not only developed a lesion and a tumor at the site of the injection, but a second one about 15 mm. in diameter was found on the abdomen 25 days after the first appeared.

Tumors were first noted in each animal 77, 81, 92, and 95 days following the first injection of the carcinogen. These tumors grew rapidly and attained a diameter of about 5 cm. before showing signs of necrosis.

All four of the original 9,10-dimethyl-1,2-benzanthracene-produced tumors became enormous in size without affecting the general physical condition of the hamster. One animal evidenced no symptoms of pain and ate heartily 127 days after the first injection, but became definitely moribund and was killed 6 days later. At this time the original tumor extended, in the form of an exaggerated oval, from low on the left ischium across the back to the right scapula; while a second tumor adhered to and protruded fully 12 mm. from the abdominal wall.

Two hamsters that had the primary tumors removed 89 and 98 days following the first injection of benzanthracene and were killed 10 and 13 days after the operation had no macroscopic metastases. Although growth had been very rapid, the tumors apparently had not had time to metastasize.

The tumor of one of these hamsters was transplanted at the time of subtotal extirpation into two hosts one of which died in anesthesia and the other, a sterile female, was negative after 21 days when she was again grafted with the regenerated tumor of the same donor with two other hosts which developed normal takes. The tumor in this sterile female was first noticed 10 days after transplantation. It was very small, attached to the body wall, and continued to develop much more slowly than those in the other 2 hosts. It was transplanted at 77 days and developed normally during the next 5 passages, with a minimum of 16 and a maximum of 26 days between transplantations. The tumor in a male, which was grafted at the same time as the sterile female, was passed through 5 transplantations with a minimum of 17 and a maximum of 44 days between passages, thus indicating that environmental conditions, rather than a different strain, was the cause of the delay in growth of the tumor in the sterile female.

The other two hamsters were allowed to live unmolested until one was found dying of its tumor and the other was moribund, when they were killed. The first hamster, which had lived 125 days from the first injection, showed gross macroscopic metastases to the lungs. The other animal, which lived 135 days from the first injection, showed macroscopic metastases to the exterior of the abdominal musculature, retroperitoneum, broad ligament, left superficial inguinal lymph node and lungs. The microscopic structure of the metastases is essentially the same as that of the primary tumors.

TRANSPLANTED TUMORS

Injections of the viscous white or red, and of aqueous liquid material from soft tumors were approximately as capable of transferring the tumors as grafts of solid tissue and had about the same period of latency. Of 23 hamsters doubly implanted with injections of liquid necrotic tumors all were takes on one or both sides. Complete records of the interval between implantation and definite take, although available for only 7 of these animals, ranged from 7 to 8 days in both sides, except that in one animal the right side was negative.

Latency.—The time required for development of the tumor was found to differ according to the method of transplantation used. Transplants made by injecting blood from solid tumors commonly were about 10 days later in developing than those made by grafting tissue (Table I). However, if the walls of the incision in a solid tumor were gently scraped with a scalpel and particles of the tumor withdrawn and injected with the blood a noticeably higher percentage of takes was obtained and the resulting tumor developed about as
rapidly as if a piece of solid tissue had been grafted. This condition indicates an insufficient dosage (5), rather than an infection or delay of the tumor cells in reaching healthy tissues, as Haddow (10) suggests.

The time interval between implantation and take, as indicated by palpable, solid enlargement of the implant, is more closely related to the volume than to the physiological condition of the implanted tumor or to the method of implanting it. Thus, if the volume of the transplant were optimal in size, takes were obtained about as readily with injections or grafts of necrotic as with grafts of solid, actively proliferating tissue. Injections of blood taken from incisions in solid tumors invariably gave delayed takes with an appreciable number of failures (Table I), but after the period of latency these tumors often grew more rapidly than those derived from grafts. If a number of particles of the tumor were included in the blood injection the latent period of takes and the number of failures were reduced to about the level of those obtained by grafting. Unusually large quantities of tumor failed to shorten the latent period for takes over those obtained by optimal quantities of tissue, but did affect the host adversely, especially when necrotic material was im-

<table>
<thead>
<tr>
<th>Group and number of hamsters</th>
<th>Implantation to termination of experiment, days</th>
<th>Grafted tumor, maximum diameters, in mm.</th>
<th>Injected tumor, maximum diameters, in mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 12</td>
<td>9-19 (av. 15.0)</td>
<td>25 × 27—35 × 55 (av. 36.8)</td>
<td>3 × 3—25 × 35 (av. 15.9)</td>
</tr>
<tr>
<td>B, 10</td>
<td>22-47 (av. 28.9)</td>
<td>23 × 35—40 × 43 (av. 30.9 × 49.2)</td>
<td>5 × 5—35 × 43 (av. 15.9 × 20.1)</td>
</tr>
<tr>
<td>C, 12</td>
<td>13-77 (av. 29.7)</td>
<td>20 × 15—43 × 45 (av. 29.0 × 36.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

![Fig. 2](image1.png) Fig. 2.—A male hamster that died 25 days after being inoculated with tumor-blood (left side) and grafted (right side). The injection site is unusually far forward.

![Fig. 3](image2.png) Fig. 3.—An anesthetized female hamster 56 days after being injected with tumor-blood, showing a palpably solid sarcoma that had metastasized to the lungs and formed deep cell-aggregates and thrombi. The tumor weighed 16.5 gm. more than the host, and sections showed many necrotic areas.

planted. However, only 10 of the total of 311 implanted animals died of tumors.

**Ulcration.**—Tumors derived by grafting the transplant frequently ruptured at the site of the graft before any considerable amount of necrotic fluid had accumulated; while tumors derived by injecting blood or necrotic fluid often did not rupture throughout the entire experiment (Fig. 3). When cut open, each tumor 25 mm. or more in maximum diameter was found to contain a quantity of necrotic fluid which...
was surrounded by a thin layer of solid, growing tissue in some instances.

Grafted tumors sometimes covered more surface of the host's body than injected tumors but seldom attained as great thickness because of the readiness with which they ruptured at the site of the graft. When a tumor ruptured, the host commonly removed the necrotic material and overlying skin thus leaving a large crater in the foul-smelling tumor.

**Tumor weight.**—The respective weights of the tumors and of the carcass after the tumors had been removed in 7 hamsters that had been implanted by injections were 23.3, 36.5; 65.5, 85.5; 40.5, 62.5; 33.5, 36.0; 61.8, 62.0; 68.5, 52.0; and 75.0, 46.5 gm. Six of these animals had definite metastases.

**Biopsy and recurrence.**—It was noted that in those instances in which total extirpation of the two transplanted tumors was attempted, the tumor from which transplants had been taken or the one that had been ruptured during removal commonly recurred; while the one that had been removed intact did not recur. Although this observation is confirmed by records on only 9 hamsters, it indicates that in this particular sarcoma biopsy will usually assure recurrence of the tumor after it has been removed. Recurrence does not appear to be a characteristic of this tumor as has been reported for the Flexner-Jobling carcinoma (7).

The escape of necrotic fluid during removal of a soft tumor was usually followed by a rapidly spreading, confluent growth over most, if not the entire, surgical area and often covered most of the back and sides of the animal. Flushing the surgical area with salt solution failed to reduce or localize this condition appreciably.

**Specificity**

When this tumor was transplanted from hamsters into mice of different strains, sex and ages the implant persisted and simulated a take for several days, but in each instance it was resorbed within 6 to 57 days. The 35 hosts comprised 14 heterozygous white, 4 young adult dba, 4 nursing, 2 old adult and 11 young adult C3H high-tumor, homozygous mice.

This sarcoma grew as readily when transplanted from Colorado hamsters to JAX hamsters and vice versa as it did when transplanted within either colony, that is, when meeting Loeb's (16) requirements for a strictly syngenesioplastic transplantation. Thus, each of the 34 JAX hamsters (30 of which were doubly injected and 4 singly grafted) that received transplants directly from the Colorado donors of the 6 lines of tumors developed the transplants.

In one experiment with 100 JAX hamsters (44 males and 56 females, comprising 12 entire litters and 2 individuals from another) 25 received transplants of a metastatic lung line tumor directly from a Colorado donor (Fig. 1, B21:2F) and 75 received transplants of the main line tumor from 2 JAX hamsters (Fig. 1, J2F and J3F) which had received the tumor directly from a Colorado donor (Fig. 1, I24:1F).

In this experiment 65 of the hosts received double injections containing fragments of the tumor and the remaining 35 received single, central grafts. The experiment was terminated 23 to 26 days after the transplantations were completed with the following results:

99 of the 100 hosts had definite takes
39 males and 49 females (34 singly grafted and 54 doubly injected) developed definite tumors within 9 days after implantation
35 singly grafted hosts had definite takes in each animal
1 of the doubly injected hosts was negative 37 days after implantation, when it was killed and examined
2 female hosts had tumors removed 6 days after being inoculated (one had been doubly injected), the other singly grafted
5 had died of their tumors
7 had solid tumors less than 25 mm. in maximum diameter
(5 doubly injected and 2 singly grafted hosts)
8 doubly injected hosts had one side negative
61 of the hosts had necrotic tumors more than 25 mm. in maximum diameter
26 of the 61 hosts had one or more enlarged deep axillary or superficial inguinal lymph nodes
4 of the 61 hosts had metastases to the lungs
31 of the 61 hosts (15 males and 16 females) had no macroscopic metastases to deep axillary or superficial inguinal lymph nodes, lungs, kidneys, liver or spleen, although the condition of the tumors was favorable for metastasis to one or more of these organs
28 of the 99 hosts had solid tumors more than 25 to 55 mm. in maximum diameter, but no metastasis, as was expected.

There was no sexual difference in response to implantation in either colony of hamsters. Of 10 litter mates in the group of 100 JAX hamsters, all implanted at the same time and with the same tumor, 2 males and 1 female had well formed tumors 9 days later; while 3 other males and 4 females were negative, as judged by palpation, on the ninth and again on the 13th day after implantation, but all 7 had definite tumors when killed 26 days after implantation. In the remaining 90 hamsters of this group 39 males and 49 females had definite tumors 9 days after implantation; while 1 male was negative 13 days after implantation, but had a well defined tumor when killed 26 days after inoculation and one female did not take at all.

**Metastases**

As a test for metastasis some of the suspected organs were transplanted. Thus, pieces of 4 different livers with areas having macroscopic appearance of early metastasis were transplanted, but all 4 failed to take. A single graft from each of 3 kidney metastases was a definite take and one (Fig. 1, F15:2M) is now in the
fifteenth passage. Of 10 enlarged deep axillary lymph nodes from donors having subpannicular implants 8 produced definite sarcomas when transplanted and one of these (Fig. 1, 22-F) was transplanted through 13 passages and is still going. A single graft from each of 9 lung metastases was a definite take and one of these (Fig. 1, 27-F) was transplanted through 5 passages before being lost by accident. Another line of metastasis to the lungs (obtained from A30:1M, Fig. 1) is now in the sixth passage.

It was early apparent that in most instances necrosis of the tumor would be followed by metastasis unless the toxicity of the necrotic material was sufficient to kill the host before the metastatic material had time to become organized into a demonstrable neoplasm. In this work the tumor was considered as being necrotic if it was open or if it was found to be soft or to have a soft spot when palpated. Thus, when a palpably solid sarcoma having a maximum diameter of 25 mm. or more was cut open macroscopic necrotic spots were usually found within it; while much smaller solid tumors commonly showed milky ulceration when sectioned. Other conditions, such as those inhibiting, or at least not favoring, entrance of metastasis-producing material into the circulatory system, are probably responsible for the prolonged interval between implantation.

**Table II: Relation of Condition and Duration of Tumor to Metastasis in Hamsters Not Subjected to Subtotal Removal of Sarcomas**

<table>
<thead>
<tr>
<th>Group and number of hamsters</th>
<th>Implantation to death, days</th>
<th>No. hamsters 1 or all tumors necrotic</th>
<th>No. hamsters all tumors solid</th>
<th>No. hams. node enlargements</th>
<th>Lung metastasis</th>
<th>Liver metastasis</th>
<th>Kidney metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 10</td>
<td>15-21 (av. 18.5)</td>
<td>10</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aa, 15</td>
<td>14-21 (av. 18.0)</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B, 37</td>
<td>23-26 (av. 24.3)</td>
<td>32</td>
<td>5</td>
<td>33</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bb, 34</td>
<td>23-26 (av. 24.1)</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C, 7</td>
<td>27-32 (av. 28.6)</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ce, 7</td>
<td>27-35 (av. 30.7)</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>D, 9</td>
<td>36-81 (av. 47.1)</td>
<td>8</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Dd, 8</td>
<td>37-74 (av. 52.1)</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>E, 5</td>
<td>18-77 (av. 36.2)</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ec, 2</td>
<td>17-34 (av. 25.5)</td>
<td>2</td>
<td>0</td>
<td>0</td>
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**Table III: Effect of a Single Subtotal Removal on the Relation of Duration and Condition of the Tumor to Metastasis**

<table>
<thead>
<tr>
<th>Group and number of hamsters</th>
<th>No.Implantation to first subtotal removal</th>
<th>No. Implantation to death, days</th>
<th>No. hamsters 1 or all tumors necrotic</th>
<th>No. hams. all tumors solid</th>
<th>No. hams. node enlargements</th>
<th>Lung metastasis</th>
<th>Liver metastasis</th>
<th>Kidney metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F, 9</td>
<td>7-16 (av. 11.8)</td>
<td>7</td>
<td>2</td>
<td>19-42 (av. 33.7)</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Ff, 10</td>
<td>6-17 (av. 12.6)</td>
<td>5</td>
<td>5</td>
<td>18-50 (av. 32.9)</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G, 17</td>
<td>17-40 (av. 26.5)</td>
<td>14</td>
<td>3</td>
<td>30-88 (av. 58.7)</td>
<td>16</td>
<td>1</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Gg, 4</td>
<td>25-49 (av. 31.7)</td>
<td>3</td>
<td>1</td>
<td>37-71 (av. 53.0)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H, 5</td>
<td>10-15 (av. 13.8)</td>
<td>5</td>
<td>0</td>
<td>26-43 (av. 30.8)</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**Description of Figures 4 to 9**

The external side of the growth is toward the left in each figure.

**Fig. 4.**—A benzanthracene-induced sarcoma, second donation of hamster A4:1F (Fig. 1). Mag. X 400.

**Fig. 5.**—Secondary sarcoma from the mediastinal wall, fifth passage, hamster A17:1F (Fig. 1). Mag. X 400.

**Fig. 6.**—Left kidney (from which Fig. 8 was developed), hamster A17:1F, 39 days from the second subtotal removal of the tumor. The neoplasm extended from the periphery of the organ through an area of atrophied glomeruli and tubules into the medulla. A deeply stained, nearly normal glomerulus has survived within the dense part of the growth. Mag. X 35.

**Fig. 7.**—Sarcoma in A21:1F produced by a graft from the right kidney of A17:1F. Mag. X 400.

**Fig. 8.**—Sarcoma produced by a graft from the left kidney of A17:1F (Fig. 6) into A21:1F. Note persistence of renal tubules in the graft. Mag. X 47.

**Fig. 9.**—Area infiltrated by small lymphocytes and cancer cells in a spotted liver 18 days after the host (fifth passage in another benzanthracene-produced tumor line) was grafted. No other metastasis was found. Mag. X 400.
tation and death in those hosts in which the neoplasm was definitely necrotic, but metastasis could not be found.

The material in Tables II and III indicates that a relationship exists between the duration and extent of necrosis and the duration of the tumor to the formation of metastases, but the data do not support this tenet sufficiently to enable one to draw conclusions. However, Haddow (10) found a significant correlation between the size and duration of the primary mammary tumor and occurrence of metastases in mice. In groups A and Aa the interval between implantation and death is too short (14 to 21 days) to be significant.

In groups B and Bb, although the interval is short (23 to 26 days), 32 of the 37 hosts having enlarged lymph nodes or metastases (86.5 per cent) had definitely necrotic growths; while in the 54 non-metastatic animals of group Bb, that had the same interval, only 27 (50 per cent) had necrotic tumors when killed.

Although the number of instances is not significant, the results shown by groups E, Ee and H indicate that grafts of metastatic organs are more potent as producers of necrotic tumors than are the implants from transplanted tumors and at least as potent as producers of metastases.

Extending the interval between implantation and death by performing two subtotal removals of tumor not only produced metastasis in each of the 7 animals so treated but also resulted in multiple metastases in 5 of them. Two of these 5 had metastases to the lungs and both kidneys and the other 3 had metastases to the lungs, and enlarged lymph nodes. One pair of kidneys was not sectioned, but histologic examination showed definite metastasis to all the other organs. The intervals for these 7 hamsters were as follows: Implantation to the first subtotal removal of the sarcomas, 9 to 44 (average 24.0) days; first to second subtotal removal, 7 to 45 (average 17.6) days and implantation to death, 46 to 134 (average 80.1) days. In every instance the tumors were definitely necrotic at each of the 2 subtotal removals and at death of the animal. Thus, each hamster was exposed 3 times to actively necrotic conditions superimposed upon the potential duration of the neoplasm.

Histology of Metastases

Histological variation from the benzanthracene-induced type of sarcoma through the various passages and various metastases was no greater than could be demonstrated in a single section of this sarcoma, with the possible exception of the metastases to the liver and kidneys.

Lymph nodes.—All the enlarged lymph nodes that were sectioned varied from a condition in which the distended subcapsular and medullary sinuses contained an unusual number of monocytes and small lymphocytes to more advanced conditions in which tumor cells had infiltrated the node (verified by Dr. Cloudman and Miss Fekete) as described for mouse sarcoma 37 (17, 19) and spontaneous mammary carcinoma (17). Later, definitely sarcomatous changes including stages of obliteration of all characteristics, as described in nodes of mice implanted with sarcoma 37 and Mal. sarcoma (17) and hamsters implanted with strain H. M. of a benzpyrene-induced sarcoma (11), appeared. In very advanced stages the metastasis was indistinguishable from the primary sarcoma, and all characteristics of the lymph node had been eliminated. During the stages of altered nodal tissue and loss of the capsule, characteristic streams of large spindle cells appeared.

The number of instances of metastases to lymph nodes in which the implant had failed to take on one side or had been made on one side only, rather than in the middle or on both sides of the lumbar region, is too small to be significant. However, in an animal that died 47 days after implantation, the right side (tumor-blood injected) was negative but the right superficial inguinal lymph node was so much enlarged that sections measured 6 mm. in diameter. This node had been converted into a neoplasm without a trace of lymphoid tissue or of the capsule remaining; while on the left (the tumor-bearing side) the superficial inguinal and deep axillary nodes were enlarged only about twice, but each contained tumor cells. None of these lymph nodes was adjacent to the sarcoma.

In an effort to gain information on the route of dissemination, tumor substance was injected into the anterior jejunum with the result that a female died 14 days later of a sarcoma that completely encircled and strangled the intestine at the site of injection, and one of the three lymph nodes that comprised the pancreas Aselli contained tumor cells. A male became moribund and when killed, 27 days after implantation, two sarcomas, each about 35 mm. in diameter, were found. One of these was at the site of injection and encircled the jejunum; while the other was on the ileum 10 mm. from the cecum. One lymph node of the pancreas Aselli had been almost entirely converted into a sarcoma (verified by Dr. Mugrage) and another contained numerous large and small tumor cells.

Since lymphatic drainage of the jejunum is through the pancreas Aselli; while the venous drainage is via the hepatic portal system, it appears that these two are clear-cut instances in which a sarcoma was disseminated via the lymphatics and produced a true metastasis in a regional lymph node.

Lungs.—In every instance of metastasis to the lungs histological examination verified the macroscopic determination. In early stages the metastatic neoplasms...
were commonly of four types: (a) pointed, (b) fungiform (both protruding through the pleura of the lung, the latter spreading over but not adhering to the pleura), (c) thrombi, and (d) deep cell-aggregates. All four types were readily detected and were often concurrent in the same lobe. In advanced stages all four types had been obliterated by fusion.

Liver.—Only early stages of dissemination to the liver were obtained. In these the surface of the organ appeared to be speckled or spotted with lighter color, but when pieces of the mottled livers were sectioned, they showed deep and superficial areas densely infiltrated by small lymphocytes, with a few large and small round tumor cells, as may be expected from interpretation of other works on the relation of lymphocytes to the development of neoplasms (13). In some livers there were instances of fusion of the lymphocyte-aggregates to form irregular patches (Fig. 9).

Kidneys.—Although takes were obtained from each of the 3 kidneys from which transplants were made, histologic structure of the metastasis was not clearly defined. In each instance the metastasis arose in the caudal end of the organ and extended cranially along its ventrolateral margin.

In animal A17:1F (Fig. 1), which was killed 46 days after the second subtotal removal, both kidneys showed macroscopic lesions and a piece of the right and of the left organ was transplanted to the corresponding side of the host (A21:1F, Fig. 1) where each graft produced a sarcoma. When the host was killed, 26 days after implantation, the left, a solid tumor (Fig. 8), was 15 mm. and the right, a necrotic, open tumor (Fig. 7), was 22 mm. in diameter.

The renal tubules in both the living kidney (Fig. 6) and in the left transplant (Fig. 8) had resisted destruction and contained viable nuclei, but the right graft, including the adjacent, necrotic tissue, had been removed by the host.

Subserous structures.—Metastases to the body wall, mesenteric stroma and other structures covered by serous membranes were fairly common and were invariably mixed-cell sarcomas (Fig. 5).

**SUMMARY**

1. The hamster is capable of 100 per cent susceptibility to the carcinogen used, as indicated by 4 animals, and to implants of the resulting tumors, as indicated by transplants to 311 individuals of various ages and both sexes.

2. The percentage of takes was as high in reciprocal transplants between JAX and Colorado hamsters as within either colony, but transplants from hamsters to 35 mice failed to take.

3. Transplants of growing and of necrotic tumors and of injections of liquid necrotic material usually developed definite takes in 4 to 10 days, but this latent period was commonly increased 8 to 10 days and the number of takes reduced about 50 per cent by injecting blood from incised solid tumors. Scraping the walls of the incision so that particles were aspirated and injected with the blood brought the latent period and percentage of takes to nearly the same level as that of the grafts.

4. Variation in latency of takes, rate and volume of growth, toxicity and metastatic potency in subperitoneal transplants could not be maintained in a series of passages.

5. Tumors induced by benzanthracene, those that had been transplanted several generations and those resulting from grafts of metastases to lymph nodes, lungs and kidneys were about equally potent in producing metastases.

6. Metastases occurred in hamsters which had not been operated upon, but subtotal removal of the sarcoma 1 or 2 times greatly favored metastasis by prolonging the life of the host and thereby increasing the duration of the effects of necrosis of both the primary and recurrent tumors.

7. In 9 animals having similarly injected implants on either side complete removal of the sarcoma from which transplants were taken immediately before removal of the tumor was followed by recurrence, whereas the sarcoma of the opposite side, which had been removed intact, did not recur.

8. No permanent histologic changes were observed in 12 lines of transplants, including 8 of metastases to lymph nodes, kidneys and lungs, which were carried through 4 to 16 passages.

9. Histologic differences found in some of the newly formed metastases in the lungs, kidneys and subserosa appeared to be transient, for all well-developed primary tumors and advanced metastases had reverted to the mixed-cell type of the benzanthracene-induced sarcoma.

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