Transplantable Human Neoplasms Maintained in Cortisone-treated Laboratory Animals: H.S. #1; H.Ep. #1; H.Ep. #2; H.Ep. #3; and H.Emb.Rh. #1

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In a previous paper (7) it was noted that a biopsy specimen of a human epidermoid carcinoma, originally implanted subcutaneously in a single cortisone-treated rat, had so increased by the 8th and 9th transfer generations in cortisized animals that it was being carried by 150 rats and hamsters. It was hoped that this tumor would become the first of a line of transplantable human neoplasms, a wish which has been fulfilled, since H.Ep. #1 (human epidermoid carcinoma #1, as it is now designated) is in its 18th month of quantity production in tissue culture.

Subsequent to this first report of a human tumor regularly transplantable in laboratory animals, preliminary accounts (8, 9) were given of other human cancers which had been developed to the point where, as transplantable tumors, they could be employed for experimental purposes. Two of these neoplasms, a soft part sarcoma (H.S. #1), which has now been transferred for 16 months, and an epidermoid carcinoma (H.Ep. #3), which has been transferred for more than 5 months, are growing so vigorously that they kill their animal hosts 10–15 days after implantation. Within this short period they may increase as much as 50- or even 100-fold in mass. Both tumors are being produced in large quantities and are in wide use in this Institution and in laboratories throughout the country. For this reason, they will be described in some detail. Three other cancers, the original epidermoid carcinoma already mentioned (H.Ep. #1), as well as another epidermoid carcinoma designated as H.Ep. #2 and an embryonal rhabdomyosarcoma (H.Emb.Rh. #1) have a more moderate growth rate and are in limited use at present. They will be mentioned briefly as a reference for those investigators who may work with them.

MATERIALS AND METHODS

The animals, materials, and procedures employed for this work have been described previously (7). For the reader's convenience, and since a few general changes have been made, a résumé will be given, with some emphasis placed on details which have been of particular interest to visitors to this laboratory.

The albino rats (CF Wistar, Carworth Farms, and Wistar, Charles River) and the hamsters (Mesocricetus auratus, Lakeview Hamster Colony) which were used were all females of weanling age and approximately 45 gm. in weight. They were of one sex for convenience in caging. When males were occasionally employed no difference was noted in the growth of the tumors. Rats were the animals of choice for general use in "screening" tumors and in carrying transfer material, since they could be implanted in large numbers in a minimum of time with no special skill. Hamsters, which were found to be of special value for experimental purposes as will be discussed later, were not used for routine transfer, as they required a trained technician to implant the tumor in their pouches and approximately 10 times as much time per animal as the rat.

With few exceptions, the rats received one dose of 180 r total body x-radiation 1–3 days prior to implantation of the tumor. (In several emergencies rats were implanted the same day as they were x-radiated; they were also considered suitable for use any time up to a week after x-radiation.) Two 180-kVP tubes were used, to save time. One tube (above) was satisfactorily x-rayed with proper allowance for longer radiation. Each of the tubes delivered 25 ma. filtered with ½ mm. Cu and 1.65 Al filters (not parabolic) at a TSD of 40 cm. (75 units per minute). Hamsters were not x-radiated except in rare instances which will be noted. As a general rule they were treated with cortisone only.

The tumors, when originally obtained, were implanted as soon as possible after removal from the patient. Experience acquired in "screening" over 1,000 human tumors has indicated that the morphology of the neoplasm is correlated with necessity for haste. Although all tumors grow more successfully if implanted in treated animals within such a period (as possible, some can survive a time interval better than others. In general, the epidermoid carcinomas are the "hardest," e.g., H.Ep. #3, one of the tumors to be described herein, which was over 5 hours old before it was obtained from the surgeon. On the other hand, cancers composed of cells producing autolytic enzymes such as adenocarcinomas of the stomach and bowel seldom do well unless implanted in animals within minutes of their removal from the human donors. If the tumor was to be transferred from a rat, the animal
The neoplasm was then removed as aseptically as possible. When the tumor was in a hamster pouch, the host animal was given a lethal dose of nembutal, the pouch everted, pinned on a sterilized cork board, and the cancer removed "dry," i.e., the pouch was wiped as clean as possible with a small piece of sterile gauze and no liquid of any kind applied to the area. Implantation of the tumor in hamsters was also "dry." Experience has indicated that the addition of any fluid, with or without antibiotics, added to the possibility of contamination by spread of local bacterial flora.

As soon as the tumor material was removed, it was placed in a sterile petri dish and minced finely enough with scalpels so that it could be implanted by injection with a No. 10 or No. 16 needle. The suspending medium was Ringer's solution buffered to a pH of approximately 7.5, with 6 mg. of glucose and 200 units of penicillin added/cc. This is a solution which has been in general use in this laboratory and is of special value when cells are to remain in suspension for some time. However, since most transplants are effected in 15 minutes or less, the buffered Ringer's medium is not by any means a necessity, and, for the investigator interested in transplanting an occasional tumor, ordinary sterile isotonic saline can be a most satisfactory substitute.

About 1 cc. or less of suspended tumor mince containing 1-3 gm. solid tumor/cc was implanted subcutaneously in the flank of each previously X-radiated rat. Immediately after the tumor was implanted, each rat was given a subcutaneous injection near the nape of the neck of 8 mg. of cortisone. Three subsequent injections of cortisone of 3 mg. each were given on alternate days following the date of implantation. For well established tumors such as H.S. #1 and H.Ep. #8, the fourth dose of cortisone was omitted.

Hamsters were not used as "screening" hosts for the reasons cited. When employed, these animals were given implantations in the pouch of trocar-sized pieces (about 100 mg. of tumor) with scalpels in the flank of each previously X-radiated rat. Immediately after the tumor was implanted, each rat was given a subcutaneous injection near the nape of the neck of 8 mg. of cortisone. Three subsequent injections of cortisone of 3 mg. each were given on alternate days following the date of implantation. For well established tumors such as H.S. #1 and H.Ep. #8, the fourth dose of cortisone was omitted.

DESCRIPTION OF TUMORS

H.S. #1: Human sarcoma #1 (soft part sarcoma, origin unknown).—H.S. #1 was obtained in January, 1958, as a soft, white mass approximately 1 cm. which had been removed from the calf of the leg of a 43-year-old male. It was minced and implanted in several unirradiated rats subsequently treated with four doses of 6 mg. of cortisone each (though new tumors are usually implanted in X-radiated rats, none was available at this time). Growth was excellent in all the animals, and at the end of 15 days enough neoplastic material was available for transfer to seven X-radiated rats and twenty unirradiated hamsters, all of which were treated with cortisone. From the first, H.S. #1 consistently showed at least a seven- to tenfold increase in mass in either rats or hamsters during the usual 14-day interval between implantation and transfer; at the present time, probably owing to selection of the fastest growing tumors for propagation, a 70- or 100-fold increment is common (Figs. 1, 2). Like the original, this neoplasm is still soft in consistency and solid throughout, with little necrosis (Fig. 3). The host animals are killed by the tumor within 12-15 days after implantation. Apparently death is due to the cachectic state of the emaciated animals which on autopsy have pale, anemic livers but huge blood vessels supplying the tumor.

Careful transfer records have been kept of all the animals treated. So great has been the increase of H.S. #1 from the original, small c. cm. piece that in 40 generations (16 months) it has been harvested from almost 15,000 animals. It is being transferred now to 400 rats and hamsters per week. At the same time, a supply of this tumor (Fig. 4) is being made available to laboratories at this Institution and to those of collaborating cancer centers.

The microscopic morphology of H.S. #1 is of considerable interest, since it furnishes a prime example of the variable growth pattern potentialities of some neoplasms as seen by the experienced pathologist. Because microscopic examination of a small piece of the original tumor revealed a picture closely resembling that of a hypernephroma (clear-cell tumor) of the kidney (Figs. 5, 6), it was at first so diagnosed, and an exploratory operation was performed on the donor patient in February, 1953, to locate a possible primary site in the kidneys. No tumor was found. Two months later a second abdominal operation was done, because the patient had complained of numerous bowel pains. Again the findings were negative.

As no primary tumor was found in the kidneys, the possibilities of other diagnoses, e.g., liposarcoma, were considered, but none of the stains for lipids, unmasked or masked, was effective. Stains designed to demonstrate the presence of mucin or polysaccharides in the cytoplasmic vacuoles were also negative. (Fjelde and Biesele2 stained and designed to demonstrate the presence of mucin or polysaccharides in the cytoplasmic vacuoles were also negative. (Fjelde and Biesele2 stained and studied these cells in tissue cultures derived from rat-grown tumor with similar negative results. They further stained their material for pentose nucleic acids and found none in the vacuoles.)

1 That the animals do not die from cortisone treatment per se or from necrosis of the tumors is evident from the death of hamsters which have only received 3 mg. of cortisone, with large solid tumors. Also, intensively treated rats both X-radiated and cortisone treated do not die when they are carrying small or slowly growing cancers.

2 A. Fjelde and J. Biesele, personal communication.
Meanwhile, after the tumor had grown for two generations in cortisonized and X-radiated rats (the microscopic pattern of the first generation growth was similar to that of the original tumor except that the cells, though vacuolated, were often elongated), a most interesting change occurred in the histological findings. The tumor now resembled a fibrosarcoma (Fig. 7). A number of variations of the original picture have been seen in the many generations which have ensued. Indeed, it was decided to wait at least a year before a detailed description of H.S. #1 should be given, to determine whether there would be a trend to any one over-all pattern. From the fibrosarcoma variant just noted, which is rarely seen, as well as another solid type of growth composed of closely packed polyhedral cells (Fig. 8), to the most commonly observed microscopic picture of a miscellaneous cell types which usually are separated from each other by fluid interspaces (Fig. 9), all gradations have been found. With the exception of the fibrosarcoma, different variants have been seen in the same specimen. This is mainly owing to the fact that the clear-cell type is almost always found near areas of central necrosis or regions where accidental infection has occurred. It may also be seen at any time as the sole cell type (Fig. 10), especially in hamsters and in egg-grown tumors. All the variants which have an especially rapid rate of growth, as evidenced by numerous dividing cells, are likely to have tumor giant cells and polyploid mitotic figures. A reason for the variation is not clear at present; there is some evidence that it is related to water uptake by the malignant cells, and further work is being done to determine, if possible, if this is so. The positive statement may be made that any of the types may produce any one of the others. The individual host probably plays at least some of the determinant role as well as (and possibly correlated with) local environment in areas of infection or necrosis. At the present time (May, 1954), it is of interest that the donor patient is alive, well, and without evidence of recurrence or metastases of his original growth. His case history has been cited in some detail, since his tumor is one of several we have had which, contrary to Greene's (2) theory that only the most malignant neoplasms can be grown in the heterologous host, has done very well in animals though of low-grade malignancy in its human donor. It should be noted that from the second generation to the present (40th) it has been tested at intervals for growth in the rat eye (Greene's test method) and has been propagated successfully in this site, both with and without treatment of the host with cortisone and regardless of whether the implanted material has been derived from the rat or hamster. A higher percentage of "takes" occurred in the cortisonized animals, as might be expected (11). Relation of such "takes" in the eye to vascularization will be discussed in a later paper.

H.Ep. #3: Human epidermoid carcinoma #3 (metastatic epidermoid carcinoma, grade III, primary in the buccal mucosa).—H.Ep. #3, originally a neck node comprised mainly of metastatic tumor, was removed at surgery, Dec., 1953, from a colored patient, 62 years old, who had had a 2-year history of carefully attended leukoplakia prior to the appearance of a tumor nodule in the buccal mucosa. Once the cancer was noted, progress of the disease was rapid, the patient dying in 3 months (shortly after operation) with numerous metastases. In this case the tumor has retained its original growth characteristics, since it increases at a tremendous rate and is the fastest growing and most invasive neoplasm of any of the five herein described. Though H.S. #1 produces large tumors in its animal hosts, it is usually fairly well circumscribed. H.Ep. #3, on the other hand, will invade nearby tissues and, if injected intraperitoneally, is likely to be found in all the abdominal organs, e.g., liver, spleen, and even the small and large intestine; it may also produce an ascites. It is the easiest of the tumors to grow in cortisone-treated, unirradiated mice which are poor hosts, in general, for most human neoplasms. By giving ordinary young Swiss albino mice a dose of 8 mg. of cortisone at the time of implantation of tumor and 1 mg. on 3 alternate days following, a good growth of H.Ep. #3 has been obtained; indeed, there is every indication that an ascitic form of this tumor can be developed in these hosts.

The microscopic picture has remained similar to that of the original (Fig. 11), the only variation being a quantitative difference in the amount of cell interspace present (Figs. 12, 13). The invasiveness of this tumor is illustrated in Figure 14, where muscle of the chest wall have been pushed aside by rapidly dividing cells derived from a subcutaneous implantation in the flank area. A transfer record of H.Ep. #3 for the first six generations (Chart 1) shows how steadily it has increased in volume. Material originally implanted in six animals was being carried by over 450 rats and hamsters in the sixth generation, exclusive of animals and material used for experimental purposes. Like H.S. #1, it is now (in its 14th generation, 54 months after it was first implanted) transferred to 400 or more animals a week, and large quantities are being distributed to investigators. Because H.Ep. #3 grows even faster and is more

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1 Both H.S. #1 and H.Ep. #3 are being grown on the chorioallantoic membranes of chick eggs in the laboratory of Dr. D. Karnofsky, Sloan-Kettering Institute.
invasive than H.S. #1, it is likely to kill its animal hosts as early as the 10th day after implantation. To insure longer survival of the animals, a smaller amount of this tumor is implanted in animals than any other neoplasm (about 1 gm. of tumor mass material produce typical neoplasms when re-implanted in x-radiated, cortisonized rats.

H.Ep. #1: Human epidermoid carcinoma #1 (metastatic epidermoid carcinoma primary in cervix).—H.Ep. #1, obtained from a 52-year-old female in Nov., 1952, is the metastatic epidermoid carcinoma (primary in cervix) described in some detail previously (7) as the first of the human neoplasms which gave promise of being permanently transplantable in cortisone-treated animals. Still being carried by both rats and hamsters, it is now in its 40th generation, 18 months after a node biopsy of metastatic tumor was implanted in cortisone-treated rats. No change has occurred in the microscopic picture during the 1½-year period.

This neoplasm, which is very hard and solid, grows much more slowly than either H.S. #1 or H.Ep. #3 and seldom kills its animal hosts. If im-

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### Chart 1.—Transfer record for first five generations of H.Ep. #3. Some tumor was used in almost every generation for experimental purposes.

<table>
<thead>
<tr>
<th>1st Generation</th>
<th>2nd Generation</th>
<th>3rd Generation</th>
<th>4th Generation</th>
<th>5th Generation</th>
<th>6th Generation</th>
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<tbody>
<tr>
<td>6 H</td>
<td>11 days (6)</td>
<td>18 XR</td>
<td>6 H</td>
<td>15</td>
<td>4 XR</td>
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<td>10 XR</td>
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<td>10 XR</td>
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<tr>
<td>4 H used for expt.</td>
<td>12</td>
<td>24 XR</td>
<td>10 H</td>
<td>15 XR</td>
<td>7 XR</td>
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<td>4 H</td>
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<td>4 XR</td>
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<td>10 (3)</td>
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<td>4 XR used for expt.</td>
<td>12</td>
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<td>11 (14)</td>
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<td>12 (5)</td>
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<td>42 XR</td>
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<td>12 (2)</td>
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<td>16 XR</td>
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<td>12 (8)</td>
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<td>33 XR</td>
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<td>6 H</td>
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<td>10 (3)</td>
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<td>15 XR</td>
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<td>3 XR</td>
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**HEP NO. 3**
**EPID. CA OF**
**BUCCAL MUCOSA**
R = RAT
H = HAMSTER
X = X-IRRADIATED

FROM PATIENT TO 6 XR
5 days (2)

11 days (10)
12 XR
27 XR
11 (9)

13 (3)
33 R

14 (6)
9 XR
11
planted in x-radiated rats treated with four doses of cortisone (3 mg. each) on the day of and on alternate days after implantation, it may regress on the 18th–20th days if further cortisone treatment is not given. Tumor implanted in hamsters grows best if these hosts receive 3 mg. of cortisone at the time of implantation and 1 mg. every 3d or 4th day following. It is interesting that in tissue culture, which was derived from animal-grown material, it is a most vigorous tumor in quantity production and, at this time, exceeding H.Ep. #3 in growth rate.

Of special note is the finding that in every sixth or seventh generation of continuous rat culture, there appears to be a diminution of the tumor growth, less yield of material, and an occasional failure to “take.” Microscopic examination of H.Ep. #1 at such a period will often reveal cells with “empty” cytoplasms (i.e., they fail to stain) and crenolated nuclei. If such tumors are implanted intraperitoneally in hamsters (not in rats) or sometimes subcutaneously in the hamster hosts, they will become revitalized and again do well in rats for another six or seven generations. The reason for this is not certain and can only be surmised. It is possible that the rat host is either lacking in or a poor source of some material needed for the welfare of the tumor cell which the hamster can supply. Since this premise is in itself worthy of investigation, H.Ep. #1 is considered valuable for a study of its special requirements (apparently adequate in tissue culture).

It is not available for general distribution at this time.

H.Ep. #2: Human epidermoid carcinoma #2 (metastatic epidermoid carcinoma, primary in larynx).—H.Ep. #2 actually antedates H.Ep. #1 and belongs to a group of tumors described in early publications (5, 6). The donor patient, a male aged 57 years, was first seen in Nov., 1951, at a time when optimal radiation dosages of animal hosts were not established (5). His tumor, a primary epidermoid carcinoma of the larynx, showed some good growth in x-radiated rats but was not transferred. When the patient was re-admitted with metastatic neck nodules in August, 1952, initial work had been done with x-radiated animals treated with cortisone (6), and therefore the small tumor specimen obtained at that time (Fig. 15) was implanted in rats both x-radiated and cortisonized. Slow-growing hard tumors, somewhat similar to those of H.Ep. #1, developed in the animal hosts. After they had been cultivated for two generations in rats (Sept., 1952), some of the tumor was given to Dr. A. Fjelde4 for tissue culture. It grew very well in this medium—so well, indeed, that it was transferred serially and soon in volume culture, which has been continued to the present day. Meanwhile, the rat-grown tumor was discontinued after twelve generations of transfer as not offering enough promise for large-scale production. Seventeen months after H.Ep. #2 had been started in tissue culture, tumor cells from this source were returned to x-radiated rats treated with cortisone. In these hosts, the tumor, at present in its fifth re-implant generation, has grown at the same slow rate as it did previously. The interesting finding, however, was that even after the long interval in tissue culture, the tumor cells immediately assumed and maintained a pattern similar to that of the original specimen (Fig. 16).

Because it grows so slowly in animals and so well in tissue culture, it is likely that H.Ep. #2 will be of primary importance to investigators using the latter medium.

H.Emb.Rh. #1. Human embryonal rhabdomyosarcoma #1.—H.Emb.Rh. #1 has been transplanted in treated rats and hamsters since Sept., 1953, when the original tumor was removed from the chest of a 37-year-old male. It is now in its eighteenth generation after 8 months of transfer and grows as a pink, clear, and somewhat jelly-like tumor without necrosis of any kind. Microscopically, it is composed of closely packed cells with somewhat pointed nuclei and cross striations in the cytoplasm. Usually these striations are discernible only in unstained material with the aid of a phase microscope. Resemblance of the animal-grown neoplasms (Figs. 18, 19) to the original tumor (Fig. 17) is striking.

Of the five tumors described in this paper, H.Emb.Rh. #1 has shown the most erratic growth, for in any one group of rats, implanted material from the same suspension of cells can produce either large, soft growths or no tumor at all. In contrast, the other four tumors produce remarkably uniform growths from any one cell suspension.

Since much time has been spent on the development of the fast growing tumors, H.S. #1 and H.Ep. #3, H.Emb.Rh. #1 has been neglected and merely transferred without any effort to find optimal conditions for its growth. It is hoped that in the near future this tumor can be developed into a transplantable neoplasm of more consistent growth and general use.

DISCUSSION

One of the questions of prime importance to investigators who wish to establish their own strain of human tumor in animal hosts is how many neoplasms must be screened in order to find one which can be carried as a transplantable tumor. The answer is that under the present conditions of treatment only two or three tumors out of a hundred implanted will proliferate well enough in the ani-
human hosts to warrant their continued transplantation. If a new tumor is implanted in a small group of x-radiated and cortisone-treated rats and allowed to remain in these animals for approximately 2 weeks, it is possible to predict from the results of the first implantation what the future growth pattern of this neoplasm will be. All the tumors herein described have grown either fast or slowly from the first and usually (with the exception of H.Emb.Rh. #1) in all or in the majority of the animals implanted. Since it was the goal of this laboratory to obtain human tumors which could be produced in quantity, about twenty tumors other than the five described were carried as slow-growing neoplasms for as many as ten generations before they were discarded as not yielding enough material.

Tumors which can be maintained only by continued treatment of the host are limited in their usefulness for experimental purposes except in special circumstances. It was our hope that cancers could be developed which would not alone grow in quantity but which could be carried in the animal hosts with little treatment of the latter. Both H.S. #1 and H.Ep. #8 have met this requirement. Often the only procedure needed to insure progressive growth of these tumors in normal nonirradiated hamsters is the injection of a single dose of 3 mg. of cortisone at the time of implantation (1-1.5 mg. have occasionally been given 5-7 days following). Such a minimum amount of this drug is not likely to interfere with chemotherapeutic and other cancer studies where tumors a week or more old are used. For this reason the hamster is considered the preferred host for experimental purposes and the rat mainly a reservoir animal, since it is still x-radiated and subjected to fairly vigorous cortisone treatment. There is some indication that both the rat and the mouse can be of further use to the investigator through the development and employment of an especially fast growing strain of ascitic tumor (H.Ep. #8) which requires less treatment of these hosts with x-radiation and/or cortisone than does the subcutaneously implanted material. The hamster has another attraction as an experimental animal, for the state of the tumor implanted in its pouches can be determined at any time by anesthetizing the host and pulling out the implanted pouch, which can then be reinserted without damage to the neoplasm or its host.

It is important to stress that even the fast growing tumors such as H.S. #1 and H.Ep. #3 have never been grown subcutaneously or intraperitoneally in animal hosts untreated by x-radiation or cortisone. It has been noted (8) that such growth would probably indicate a loss of human antigens which are still present in the two tumors (H.S. #1 and H.Ep. #8) that have been tested according to the agar plate method of Ouchterlony (4).

How, then, can as small a dose as 3 mg. of cortisone suppress the usual heterologous tissue incompatibility? This problem has been discussed previously (8). In brief, it is thought that the cortisone may act through abrogating the natural immunity, however that may be expressed by the hamster host (Bogden and Aptekman [1] have reported the presence of naturally occurring heteroagglutinins to human red cells in the rat). The tumor is thus able to establish itself. Subsequently, if the neoplasm quickly attains a great size, as do H.S. #1 and H.Ep. #3, it may be able to overcome, by absorption or other means, any remaining natural forces plus the acquired immunity. In short, if the tumor-host relationship is considered broadly as an “antigen-antibody” ratio, the large mass of the neoplasm (antigen) produces a balance of this ratio in its favor. Slower growing tumors are not able to do this and thus would regress if additional cortisone treatment were not given to the host. Experiments which support this theory and additional consideration of the role of the cortisone are given in the paper cited.

It is noteworthy that a number of the human tumors transplanted appeared to grow better in animals than they did in their donor. This was obvious in the case of H.S. #1 and even H.Ep. #3 with their tremendous increment in volume, but was also seen in some of the so-called “slow-growing” tumors by comparing the number of mitotic cells in the slides of the rat-grown material with that of the original tumor. Apparently, if the reactive forces of the host (whether expressed by connective tissue, phagocytic cells, humoral components, or other elements) could be restrained, at least some of the human cells flourished more vigorously in their foreign environment than in their natural host. The possibility immediately presents itself that the patient donor might have had some sort of iso-antibody restraining his own tumor from attaining its full growth potentialities. That this is not the only answer is indicated by the observation that normal human tissues such as adult epithelium or thyroid which have few or no mitotic figures in their natural site, will also proliferate extensively in treated rats. Another finding which may be related to the one just noted is that human embryonic tissues (10) or tumors consistently grow better in tissue culture after one generation in rat hosts than when they are implanted directly from the human source. In the latter case, the tissue...
tumor-bearing animals and of tumor material is that over 800 animals per week are needed for cortisone. This animal was given 3 mg. of cortisone at time of implantation of tumor and l mg. l week later. Two of these neoplasms, H.S. #1, which has been maintained for over 16 months, and H.Ep. #3, which has been in culture for a shorter period, are now being produced in such quantity as to be considered ideal for research purposes. Care must be taken not to ascribe too much significance to the heterotransplantability of any one tumor or group of tumors, for it has been our frequent experience that different biopsy specimens from the same patient may vary considerably in their ability to grow in treated rats. (It is not always the metastatic or last biopsy which grows best, either.) The type of tumor (for example, does it have autolytic or invasive enzymes?), the freshness of the specimen, freedom from infection, the specific condition of any particular piece removed, as well as the host susceptibility variation due to our still far-from-perfect preparation methods, are just a few of the many factors which operate to determine the outcome of any one implantation. Nevertheless, with these thoughts in mind, it is hoped that future work will bring clarification of this fundamental problem.

SUMMARY

Five human cancers are described which are being propagated as transplantable tumors in laboratory animals treated with x-radiation and/or cortisone. Two of these neoplasms, H.S. #1, which has been maintained for over 16 months, and H.Ep. #3, which has been in culture for a shorter period, are now being produced in such quantity that over 800 animals per week are needed for transfer of the two growths; a steady supply of the two growths; a steady supply of human connective tissue may be available also for investigators. Both tumors are growing well on the membrane of the chick egg and in tissue culture.

Because a single subcutaneous dose of cortisone injected at the time of tumor implantation is sufficient to insure the growth of H.S. #1 or H.Ep. #3 in the pouches of nonirradiated hamsters, these animals are considered ideal for research purposes. It is felt that such a small dose is not likely to interfere with experimental work on tumors 7–14 days old.

None of the human tumors has ever grown in normal, untreated control hosts.

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude to Dr. C. P. Rhoads, without whose faith and encouragement this work could not have been done, and to the Misses Joan Livoti, Alice Gale, and Karen Nielsen for their technical assistance.

REFERENCES

Fig. 5.—H.S. #1, original tumor, a soft-part sarcoma from the calf of the leg of a 44-year-old male. Note resemblance to a hypernephroma (clear-cell tumor) of the kidney. × 120.

Fig. 6.—H.S. #1. Higher magnification of Figure 5. × 440.

Fig. 7.—H.S. #1, 2nd generation, 15 days after implantation in the flank of an x-radiated rat treated with cortisone. Tumor here looks like a typical fibrosarcoma. × 120.

Fig. 8.—H.S. #1, 6th generation, 18 days after implantation in the pouch of a cortisone-treated hamster. Tumor is very compact with polyhedral cells. × 120.

Fig. 9.—H.S. #1, 29th generation, 15 days after implantation in the flank of an x-radiated rat treated with cortisone. Note cells of miscellaneous type and the active mitoses. This is the most frequent phase of the tumor now seen. × 440.

Fig. 10.—H.S. #1, 30th generation, 15 days after implantation in an x-radiated rat treated with cortisone. Note similarity to the original neoplasm (Figs. 5 and 6). The tumor of this figure was derived from material of Figure 9. × 330.
Fig. 11.—H.Ep. #8, original tumor, an epidermoid carcinoma, grade III, from the buccal mucosa of a 62-year-old male. × 180.

Fig. 12.—H.Ep. #3, 7th generation, 10 days after implantation in the flank of an x-irradiated rat treated with cortisone. This is a comparatively "solid" type of the tumor. Note excellent vascularization. × 180.

Fig. 13.—H.Ep. #3, 6th generation, 10 days after implantation in the flank of an x-irradiated rat treated with cortisone. The tumor here has a "shredded" appearance often seen in this growth and similar to the morphology of the original tumor. Many mitotic figures are present. × 180.

Fig. 14.—H.Ep. #3, 8th generation, 14 days after implantation in the flank of an x-irradiated rat treated with cortisone. The tumor has invaded muscles of the chest wall. Vascularization is excellent. × 180.
Fig. 15.—H.Ep. #2, original tumor, an epidermoid carcinoma from the larynx of a 57-year-old male. × 140.

Fig. 16.—H.Ep. #2, 21 months after original tumor was removed from the patient. After two generations in x-irradiated rats treated with cortisone, this strain of the tumor was propagated in tissue culture for 17 months before being returned to the animal hosts. This is the 4th generation, 20 days after implantation, in the new series of x-irradiated, cortisone-treated rats. Note how similar the morphology is to the original (Fig. 15). × 140.

Fig. 17.—H.Emb.Rh. #1, original tumor, an embryonal rhabdomyosarcoma from the chest wall of a 32-year-old male. × 160.

Fig. 18.—H.Emb.Rh. #1, 12th generation, 12 days after implantation in the flank of an x-irradiated rat treated with cortisone. Arrow points to small blood vessel. × 160.

Fig. 19.—H.Emb.Rh. #1. Higher magnification of Figure 18. Arrows point to mitotic figures. × 360.
Transplantable Human Neoplasms Maintained in Cortisonetreated Laboratory Animals: H.S. #1; H.Ep. #1; H.Ep. #2; H.Ep. #3; and H.Emb.Rh. #1

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