Growth of Human Tumors in Cortisone-treated Laboratory Animals: The Possibility of Obtaining Permanently Transplantable Human Tumors*†

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A preliminary paper reported that human neoplasms, implanted as either a suspension of cells or a fine mince, often proliferated extensively for 8–10 days in the subcutaneous tissues of adult, x-radiated heterologous hosts before changes associated with regression occurred; they did not survive in nonirradiated animals (2). Subsequently, it was noted that human tissues, similarly implanted in x-radiated rats of weanling age, could grow progressively for 12–14 days or longer (3, 4), a period which allowed the more vigorously growing tumors to be of practical value for the testing of chemotherapeutic agents (6). More recently, it was learned that human cancer cells showed a higher percentage of “takes” and proliferated considerably longer in x-radiated weanling rats than were hamsters (3). Transfers of tumor into rats were by implantation of a mince obtained from material of the previous generation whether grown in rats or hamsters. (Prior experiments had also indicated that the subcutaneous implantation of a tumor mince into the rats gave more favorable results than did trocar pieces or sieved cell suspensions.) Transfers into hamsters, which had been anesthetized with nembutal, were by trocar pieces implanted through the cheek pouches, according to the method of Lutz et al. (1). Each group of animals reported as positive or negative for tumor growth consisted of at least two animals and usually three or more.

Rats treated with cortisone were given four subcutaneous injections of the drug as distant as possible from the tumor: 5 mg/dose for the x-radiated, 6 mg. for the nonirradiated animals. The initial dose was on the day of, and immediately after, tumor implantation, with subsequent injections on alternate days following. The 5-mg. dose in the x-radiated hosts produced results comparable to those obtained with the 6-mg. dose in nonirradiated hosts. Daily injections or increases in the doses cited often proved to be toxic to the animals, and less beneficial to the growth of the tumors which failed to become stromatized. Hamsters were more sensitive to cortisone than the rats, and an optimal dosage has not been established as yet. For the work reported in Charts 1 and 2, the hamsters were treated with one injection of cortisone given on the day of implantation (5 mg. if x-radiated, 6 mg. if nonirradiated) and a second similar injection 1 week later.

The term “normal” has been used to designate a nonirradiated animal.
RESULTS AND DISCUSSION

One hundred and one human tumors were implanted in weanling rats. With the exception of melanomas and mammary adenocarcinomas, which will be reported as a group in another paper, they were consecutively obtained. The first 27 neoplasms that were received were implanted in x-radiated rats only; these rats were killed on the 10th–13th days after implantation (Table 1, Group 1). Slightly over 50 per cent (fourteen) of the 27 tumors grew in the x-radiated control animals, whereas almost 90 per cent (24) were positive in similarly x-radiated animals treated with cortisone (Figs. 1–3). The epidermoid carcinomas grew in 56 per cent of the x-radiated control rats; this was comparable to our previous report that almost 60 per cent of this type tumor had a favorable growth response in x-radiated weanling rats (3). The next eighteen neoplasms were implanted in both x-radiated and normal rats. Half the animals in each group were treated with cortisone (Table 1, Group 2). Again, a greater number of tumors grew in the x-radiated hosts treated with cortisone than in the x-radiated controls: seventeen out of eighteen, as compared to ten out of eighteen. Of even more interest, however, was the finding that seventeen of the eighteen tumors implanted in nonirradiated hosts receiving cortisone also grew (Fig. 4), while none survived in the normal control rats. Of the total of 45 tumors implanted in x-radiated hosts (Table 1, Group 1 plus Group 2) 50 per cent grew in the control x-radiated hosts, as compared to 90 per cent in the cortisone-treated x-radiated hosts, since 24 tumors were positive in the former and 41 in the latter.

It was apparent from microscopical examination that the tumors in the cortisone-treated hosts were uniformly larger and more vigorous than those in the animals x-radiated only. It thus seemed likely that they would survive longer than...
TABLE 1

GROWTH OF HUMAN TUMORS IN X-IRRADIATED AND NORMAL RATS TREATED WITH CORTISONE

Animals killed 10-13 days after implantation

<table>
<thead>
<tr>
<th>Group I (Implanted in x-irradiated rats only):</th>
<th>Group II (Implanted in both x-irradiated and normal rats):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermoid carcinomas</td>
<td>Epidermoid carcinomas</td>
</tr>
<tr>
<td>Oat-cell carcinoma of lung</td>
<td>Mammary tumor of undifferentiated and reticulum carcinoma</td>
</tr>
<tr>
<td>Giant- and spindle-cell carcinoma of thyroid</td>
<td>Adenocystic carcinoma of parotid</td>
</tr>
<tr>
<td>Adeno-cystic carcinoma of parotid</td>
<td>Adenocarcinoma of rectum</td>
</tr>
<tr>
<td>Mixed tumor of parotid</td>
<td>Synovioma</td>
</tr>
<tr>
<td>Adenocarcinoma of pancreas</td>
<td>Reticulum-cell carcinoma</td>
</tr>
<tr>
<td>Synovioma</td>
<td>Adenocarcinoma of pancreas</td>
</tr>
</tbody>
</table>

**TOTALS (Groups I and II)**

| 18 | 1 |
| 1 | 1 |
| 1 | 1 |
| 2 | 1 |
| 1 | 1 |
| 2 | 1 |
| 1 | 1 |
| 2 | 1 |
| 14 | 7 |
| 1 | 1 |
| 0 | 1 |
| 0 | 1 |
| 0 | 1 |
| 10 | 24 |

**Research.**
the 12–14-day period which had been considered optimal for maximal growth in the x-radiated hosts. Accordingly, the next 56 tumors which were received were allowed to remain in the rats for 14–20 days, or longer, before the animals were killed and the implanted material examined microscopically. The findings for this group are given in Table 2. As expected, only a comparatively small per cent of the tumors were still in good condition in the x-radiated control hosts at this later period (eleven out of 56). In the cortisone-treated animals, however, whether x-radiated or not, 49 of the two types of cortisone-treated hosts (x-radiated and nonirradiated) indicated that, in general, they were the same, with a slight balance in favor of the x-radiated animals.

As a result of the findings just noted, it seemed likely that the use of cortisone would facilitate serial transfer of the human tumors in laboratory animals. One of the problems associated with previous work on the transfer of human neoplasms in x-radiated hosts was the relatively short period that the tumors remained viable in the animals. Since a certain number of cells were always lost

### TABLE 2

GROWTH OF HUMAN TUMORS IN X-RADIATED AND NORMAL RATS TREATED WITH CORTISONE

<table>
<thead>
<tr>
<th>Animals killed 14–20 days after implantation</th>
<th>No. positive in x-radiated control rats</th>
<th>No. positive in x-radiated plus cortisone</th>
<th>No. positive in normal control rats</th>
<th>No. positive in normal rats plus cortisone</th>
<th>No. failed to grow in any rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermoid carcinomas</td>
<td>39</td>
<td>6</td>
<td>36</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Adenocarcinoma of cervix</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>0</td>
<td>1 (28 days)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Papillary and follicular carcinoma of thyroid</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Giant- and spindle-cell carcinoma of thyroid</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma of kidney</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Wilms tumor (kidney)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Wartiness tumor (parotid)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid adenoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Papillary adenocarcinoma of ovary</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma of rectum</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thymoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hemangiopericytoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hypernephroma</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosarcoma (patient aged 91)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Reticulum-cell sarcoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>56</td>
<td>11</td>
<td>49</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

(88 per cent) were growing progressively. No tumor cells were seen in normal control hosts.

In total, approximately 90 per cent (90) of the 101 human tumors implanted in cortisone-treated x-radiated hosts and 89 per cent (66) of the 74 tumors implanted in cortisone-treated nonirradiated hosts were growing 12–20 days after implantation of the tumors.

Six of the epidermoid carcinomas had been implanted in enough rats so that some animals were available for examination at a still later period. It was found that, if an additional injection of cortisone was given to the rat host 10–14 days after the final injection in the initial series of four doses, the tumor borne by this animal not only stayed alive but was still growing 28–80 days after implantation. Regressive changes or death of the tumor cells occurred where this supplementary injection was not given.

A comparison of the extent of tumor growth in with each transfer, it was necessary for the tumor to multiply sufficiently in any one generation to make up for this deficiency. This did not often occur. A further difficulty encountered was the hyalinization of old stromal material which, in x-radiated rats, was transferred from generation to generation along with the tumor cells. Instead of dying, to be replaced with the new host stroma, as is the usual case in transplantable tumors, it survived in poor condition from one x-radiated rat to another and eventually, in the third or fourth generation, tended to choke off the tumor cells and allow no penetration of new supporting tissue or blood vessels. It was hoped that use of cortisone would enhance and prolong the growth of the tumors so that the number of cells lost during transfer would not determine the fate of the transplant. Furthermore, it was thought that cross transfers between animals of two species, such as rats and hamsters, might eliminate retention of
the old stroma and therefore allow new stromal
tissue to support the tumor cells.

It is not at all certain that this reasoning is
correct. However, it was found that if the more
vigorous tumors were selected, serial transplants
were accomplished with comparative ease in
cortisone-treated hosts. One adenocarcinoma
(Figs. 5–8) has been transferred for four genera-
tions at the present writing, and an epidermoid
carcinoma, originally obtained on two separate
occasions as small biopsy specimens, each approxi-
mately 4 × 4 × 5 mm., now gives promise of be-
coming a permanently transplantable tumor
(Figs. 9–11). It has increased in amount, so that
in the fourth generation ten rats and 38 hamsters
are carrying excellent tumors, all derived from one
of the small biopsy specimens, minced and im-
planted in a single cortisone-treated rat. Details of
the transfer records of the two biopsies are given in
Charts 1 and 2.

The first biopsy (Fig. 12) contained some
necrosis and, possibly, a low grade infection which
has appeared in some of the animals of the later
generations. In spite of the discard of certain ani-
mals because of this infection, there has been a
notable consistent increase in material from the first
(Figs. 13, 14) to the fifth generations (Fig. 16).
In order to determine whether the tumor had main-
tained its human characteristics in spite of its so-
journ in animals (a matter which is of the utmost
importance in determining the future value of trans-
plantable human tumors carried in the laboratory),
two back-transfers of this tumor (trocar-size pieces)
to the original donor patient were made. One was
done in the second generation, another in the third
(Chart 1). When removed, approximately 3 weeks
after their subcutaneous insertion, they were in ex-
cellent condition, with every evidence of acclimati-
ze (Fig. 15). Naturally, such an experiment is
strictly limited and was only permissible in this
instance because of the completely inoperable and
terminal condition of the patient.

The second biopsy from the same patient
(Chart 2) was composed of solid tumor with no
evidence of necrosis or infection. It has proliferated
rapidly and transferred very well. Large solid
tumors, free from necrosis, have been produced
(Figs. 9–11) from every transplant. At the present
time, it is being carried in x-rayed rats and non-
irradiated hamsters, all treated with cortisone.
Normal, untreated animals, which, in our experi-
ence, have never supported proliferative and
progressive tumor growth, have not as yet re-
ceived implants. This procedure has been followed
in order that no tumor might be lost and that a
uniform base-line might be established for an in-
crease in material from one generation to another
in the cortisone-treated hosts. We have now ob-
tained enough tumor so that untreated animals
also be used as hosts. It will be desirable, of
course, to establish a tumor in such animals, if
possible.

It should be noted that even though 90 per cent
of human tumors implanted in the cortisone-
treated hosts survived and proliferated, only a
comparatively small per cent appear vigorous
enough for permanent cultivation (about one of
every fifteen to twenty tumors implanted in our
series). This is not too unfavorable when compared
to the transfer record of spontaneous tumors in
laboratory animals.

The importance of the cross transfers between
rats and hamsters in promoting tumor growth has
not as yet been determined. There appears to be
some tendency in the rat-to-rat transfers for the
presence of an increasing proportion of stroma to
tumor—some of the stroma being apparently re-
tained from the previous generations. This is not
true if cross transfers are made, and at the present
writing does not appear to occur in the hamster-to-
hamster transfers. In a number of tests (not tabulated),
the cortisone-treated rats were more
susceptible hosts to the original human tumors
than were the hamsters. After the first generation
the cortisone-treated hamsters supported the
tumor growth as well as did the rat hosts. In none
of the tumors which grew was there much evidence
of host reactive tissue (Fig. 4), unless they were
stored longer than the periods cited. Vasculariza-
tion was excellent (Fig. 14), and mitosis was most
extensive and always greater than in the original
specimen (Fig. 7).

Four tumors were implanted in adrenalecto-
ized x-rayed and nonirradiated rats, as well as
in the usual hosts. Growth in this series occurred in
all the cortisone-treated animals, whether ad-
renalectomized or not, and in none of the other
rats. Occasionally, some slight cortisone-like effect
has been obtained with serum injections of ACTH,
but, in general, this substance appears to promote
reactive tissue. Certainly this last study is not
complete, for the amount of drug to be given, as
well as the problems involved in the inter-relation-
ship of ACTH with cortisone, desoxycorticosterone,
and other adrenal steroids, remains as yet
undetermined. There is also no certainty that the
doses of cortisone employed in the experiments of
this paper are optimal for establishing and main-
taining human tumor growth in the heterologous
hosts. Considerably more work is being planned on
this problem. The value of pretreatment of an-
imals with the cortisone, as well as the mixing of
cortisone and tumor mince at the time of implantation, are being investigated.

SUMMARY

Approximately 90 per cent of 101 human tumors implanted in cortisone-treated x-irradiated rats survived and proliferated for 12-20 days. In some instances they were maintained as long as 30 days. A similar percentage of tumors implanted in cortisone-treated nonirradiated rats were also positive for growth. None survived in normal control animals.

One biopsy specimen of a human epidermoid carcinoma, implanted originally in a single cortisone-treated rat, has so increased in the fourth transfer generation that tumors are being carried by ten rats and 38 hamsters, all cortisone-treated. It is hoped that this neoplasm can be maintained permanently as a transplantable tumor in laboratory animals.

ACKNOWLEDGMENTS

The author wishes to express her special gratitude to Miss Alice Gale and Miss Joan Livoti for their technical assistance and to Dr. Chester Southam for his valuable aid in arranging for the biopsy material and back-transplants noted in Charts 1 and 2.

ADDENDUM

Since this paper was written, the small biopsy specimen of Chart 2, originally implanted in one rat, is in its eighth and ninth transfer generations. It is now being carried by 150 laboratory animals (rats, hamsters, and mice).

REFERENCES


Fig. 1.—Human epidermoid carcinoma (original specimen from lung), 12 days after implantation as a fine mince in the subcutaneous tissues (flank) of an x-irradiated rat treated with cortisone. H and E (Hematoxylin and Eosin) stain. X160.

Fig. 2.—Higher magnification of Figure 1. The numerous mitoses are indicated by arrows. H and E stain. X450.

Fig. 3.—Human metastatic squamous-cell carcinoma (primary in floor of mouth), 12 days after implantation in the flank of an x-irradiated rat treated with cortisone. Arrow points to blood vessel. H and E stain. X140.

Fig. 4.—Human metastatic ovarian carcinoma, 11 days after implantation in the flank of a nonirradiated rat treated with cortisone. Note the lack of reactive cells in the rat tissue (arrows) about the tumor. H and E stain. X160.
Fig. 5.—Original human tumor: metastatic adenocarcinoma in a small nodule removed at operation from the liver of a 60-year-old female (primary, unknown; probably in large bowel). There were comparatively few of these tumor foci per c. cm. of the specimen. H and E stain. ×140.

Fig. 6.—Tumor from Figure 5, 12 days after implantation in the subcutaneous tissues of a nonirradiated rat treated with cortisone. The tumor is now very compact with very little stroma. H and E stain. ×160.

Fig. 7.—As Figure 6. Another view at higher magnification. Arrows point to numerous mitoses. H and E stain. ×360.

Fig. 8.—Tumor of Figures 5–7 at the end of four generations (60 days) in nonirradiated animals treated with cortisone. First two generations in the subcutaneous tissues of rats; second two generations in the cheek pouches of hamsters. H and E stain. ×160.
FIG. 9.—Third generation of human epidermoid carcinoma grown in the subcutaneous tissues of weanling rats. (Chart 2, x-radiated rat No. 1, text.) For the first two generations the tumor was grown in nonirradiated rats and during the third, in an x-radiated rat; all were treated with cortisone. This generation was 17 days old; the total number of days since removal from the patient was 52.

FIG. 10.—As Figure 9, third generation, x-radiated rat No. 2 (Chart 2, text). The tumor has been pried away somewhat from the rat supporting tissue for photographic purposes.

FIG. 11.—As Figure 9, third generation, cheek pouch of cortisone-treated hamster No. 8 (Chart 2, text). First two generations in cortisone-treated rats.

FIG. 12.—Original biopsy specimen of the human epidermoid carcinoma used for transplants of Chart 1 (text). The biopsy was obtained from metastatic tumor in an inguinal node. Primary tumor was in cervix. H and E stain. X140.
Fig. 13.—The human epidermoid carcinoma of Figure 12, 12 days after implantation in a nonirradiated rat treated with cortisone. (Chart 1, text.) H and E stain. ×140.

Fig. 14.—Higher magnification of Figure 13 showing good vascularization (arrow). H and E stain. ×400.

Fig. 15.—Tumor of Figure 12 at the end of the third generation which was a back-transfer (21 days duration) to the patient (Chart 1, text). The first generation (12 days) was in the rat of Figures 13 and 14, the second generation (22 days) in the cheek pouch of a cortisone-treated hamster. The tumor does not appear to have been altered in relation to the patient by its sojourn in the laboratory animals. H and E stain. ×90.

Fig. 16.—Tumor of Figure 12 at end of the fourth generation in cortisone-treated animals. The first two generations were in rats; the second two in hamsters. Total number of days since removal from patient was 61. H and E stain. ×140.
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