LOCAL RESISTANCE TO SPONTANEOUS MOUSE CANCER INDUCED BY X-RAYS.

By JAMES B. MURPHY, M.D., JOSEPH MAISIN, M.D., and ERNEST STURM.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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Recent experimentation has tended to show that cancer is not so sensitive to radiation as earlier observers believed.[1] Although there may be some variation in the amount of x-rays required to kill various transplanted mouse[2] and rat tumors, the more careful experiments indicate that the lethal dose is rarely, if ever, within the limits of a therapeutic dose for man. On the other hand, there is no doubt that certain forms of cancer are cured by x-rays.

The recent revival of interest in x-ray therapy, due largely to the development of apparatus for generating more penetrating rays, opens up anew the question of the mode of action of this agent. If cancer is more sensitive to x-rays than normal tissue, as is generally believed to be the case, this new development is unquestionably a move in the right direction; but there is no substantial experimental basis to uphold this belief, and very good evidence to the contrary. Obviously the two facts—that cancer cells are not easily killed by x-rays, and yet that cancer may be cured by this agent—require examination if x-ray therapy is to be put on a rational basis and to be developed into a more effective form of treatment.

It has already been shown that x-rays, given over an area of skin in an erythema dose, render this area highly resistant to a subsequent inoculation with a transplantable cancer.[3] It is our opinion that this increased resistance is due to the fact that x-rays induce in this

[1] Hill, E., Morton, J. J., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 89.

[2] Wood, F. C., and Prime, F., *J. Am. Med. Assn.*, 1920, lxxiv, 308.

[3] Murphy, Jas. B., Hussey, R. G., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 299.

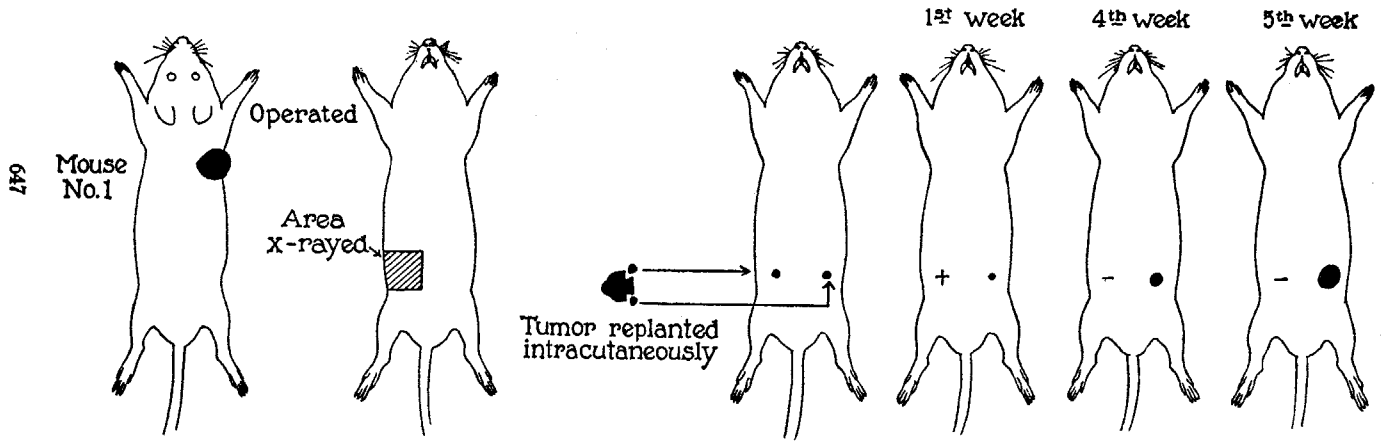
exposed area a pronounced cellular reaction of the type which under other conditions is associated with resistance to cancer. As a transplantable tumor was used for these experiments, no general deduction can be drawn as to the behavior of the spontaneous disease, and therefore it seemed advisable to determine whether the same principle was operative under the same experimental conditions when autografts of spontaneous cancer were used, thus reproducing conditions more nearly comparable to those which exist for the disease as it occurs in man.

Experiment 1.—A mouse with a spontaneous mammary cancer was operated upon with removal of the tumor. With the tumor out, an area on the left flank, 12 x 15 mm. was exposed to an erythema dose of x-rays, governed by the following facts: spark-gap 3 inches; milliamperes 10; distance 6 inches; time 2½ minutes. Immediately after this treatment a small bit of the original cancer was reinoculated intracutaneously in the x-rayed area, and a like graft in the right flank, which had been protected from the x-rays (Text-fig. 1).

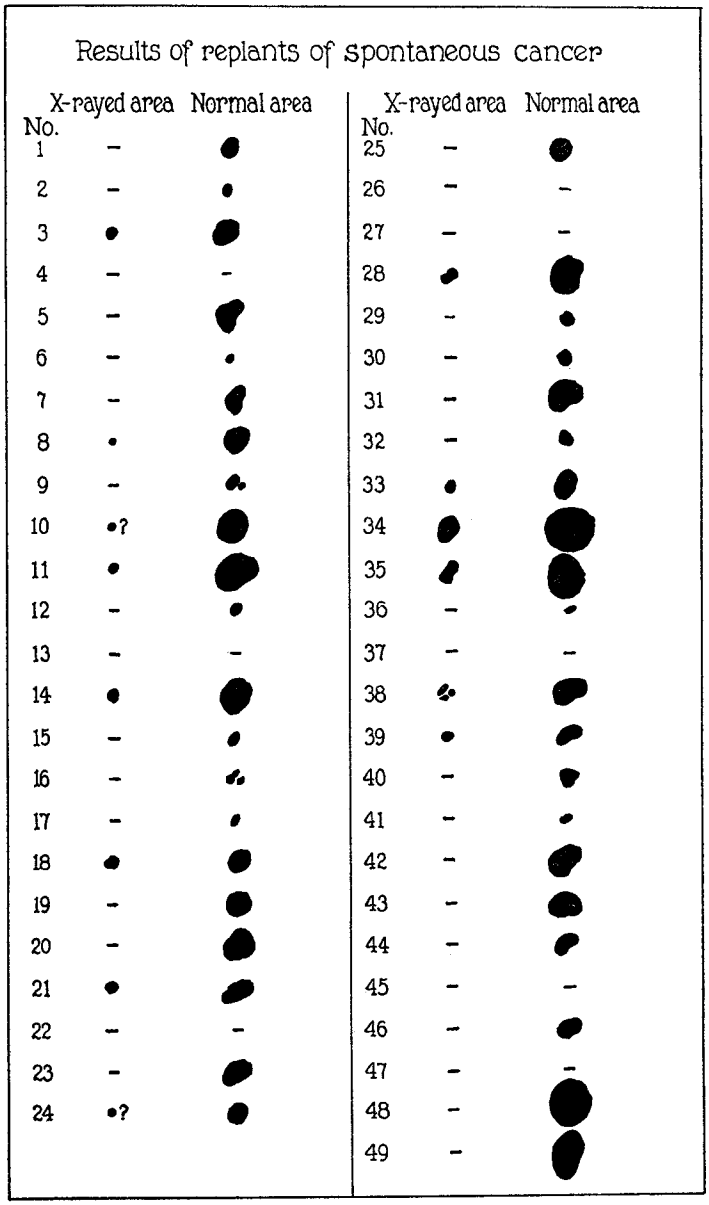
Among forty-nine mice with various types and stages of mammary tumors, subjected to this treatment, the graft inoculated in the x-rayed area failed to grow in thirty-five of the animals (71.4 per cent), while the graft in the untreated-area failed in only eight (16.4 per cent). When the graft in the x-rayed area grew, it invariably progressed at a much slower rate than the corresponding graft in the normal skin, so that at the time of death of the animal it was never more than a fraction of the size of the other tumors (Text-fig. 2).

From these experiments it is evident that the local immunizing power of x-rays is just as effective against autografts of spontaneous cancers as it is against implants of a transplantable tumor. In this experiment, however, as well as in the earlier ones dealing with the transplantable tumor, the x-ray treatment at best has prevented a take or retarded the subsequent growth of the graft. It is conceivable that slightly unfavorable environmental conditions, insufficient to influence an established tumor, might be sufficient to prevent the take of a graft in which presumably the tumor cells are at a disadvantage. Will the conditions induced by x-rays be sufficiently unfavorable to influence an established tumor in the skin? The following experiment was outlined to answer this question, and also, by way of comparison, to test the direct action of x-rays on the cancer cells.

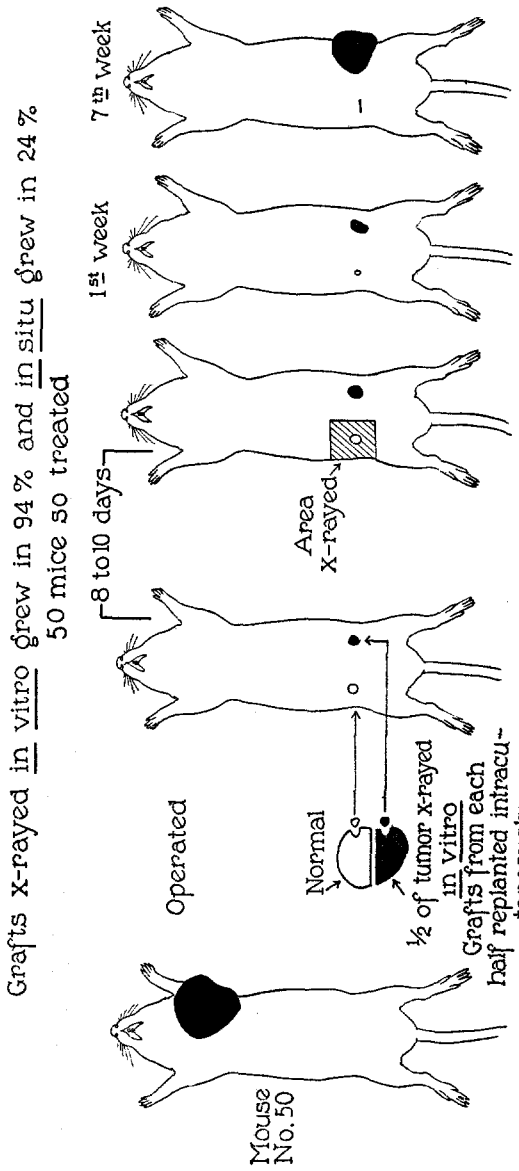
Experiment 2.—A mouse with spontaneous cancer was operated on with removal of the tumor. The tumor was then divided into two parts, and one of these subjected to an erythema dose of x-rays *in vitro*. (Spark-gap 3 inches; mili-


Spontaneous tumor mice
Autografts grew in x-rayed area in 30.6%, in normal area in 83.7%
50 mice so treated
Mouse No.1
Operated
Area x-rayed
Tumor replanted intracutaneously
1st week
4th week
5th week
+
–
–

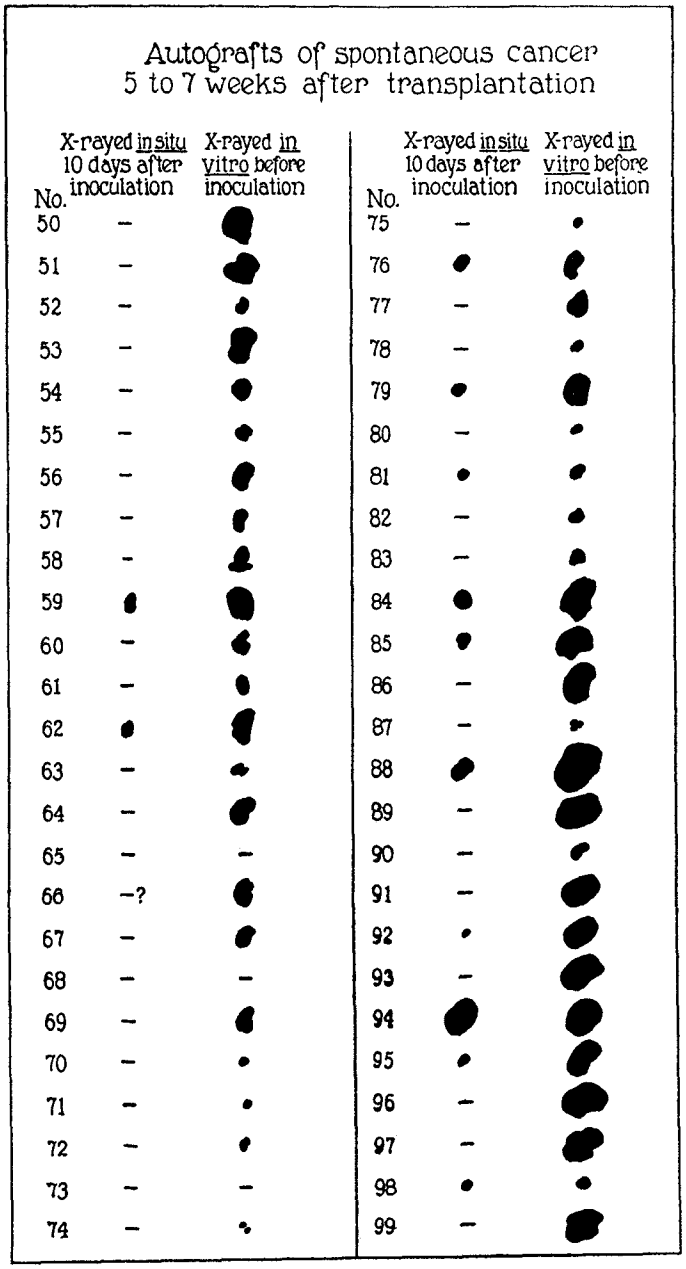
TEXT-FIG. 1.


Results of replants of spontaneous cancer
No.
X-rayed area
Normal area
1 – 2 – 3 • 4 – – 5 – 6 – 7 – 8 • 9 – 10 •? 11 • 12 – 13 – – 14 • 15 – 16 – 17 – 18 • 19 – 20 – 21 • 22 – – 23 – 24 •?
No.
X-rayed area
Normal area
25 – 26 – – 27 – – 28 • 29 – 30 – 31 – 32 – 33 • 34 • 35 • 36 – 37 – – 38 • 39 • 40 – 41 – 42 – 43 – 44 – 45 – – 46 – 47 – – 48 – 49 –

TEXT-FIG. 2.


Grafts x-rayed in vitro grew in 94 % and in situ grew in 24 %
50 mice so treated
7th week
1st week
8 to 10 days
Area x-rayed
Operated
Normal
½ of tumor x-rayed in vitro
Grafts from each half replanted intracutaneously
Mouse No. 50

TEXT-FIG. 3.


Autografts of spontaneous cancer
5 to 7 weeks after transplantation
No.
X-rayed in situ 10 days after inoculation
X-rayed in vitro before inoculation
50 – 51 – 52 – 53 – 54 – 55 – 56 – 57 – 58 – 59 60 – 61 – 62 63 – 64 – 65 – 66 –? 67 – 68 – 69 – 70 – 71 – 72 – 73 – 74 –
75 – 76 77 – 78 – 79 80 – 81 82 – 83 – 84 85 86 – 87 – 88 89 – 90 – 91 – 92 93 – 94 95 96 – 97 – 98 99 –

TEXT-FIG. 4.

amperes 10; distance 6 inches; time 2½ minutes; no filter.) A small bit of this portion of the tumor, taken from the surface nearest the x-ray tube, was inoculated intracutaneously in the right flank of the original animal.[4] A graft of similar size from the untreated portion of the tumor was inoculated in the same way in the left flank (Text-fig. 3).

In the fifty mice subjected to this experiment, the grafts from the cancer x-rayed outside the body, with perhaps two exceptions, grew as rapidly as the untreated cancer, and in many cases more rapidly. After about 10 days, sometimes longer, when the new tumors had become established, the one which originated from the untreated graft was given the same dose of x-rays *in situ*, which the other tumor had received *in vitro*, the treatment including the surrounding normal skin as well as the tumor. This was followed by a prompt disappearance of the tumor in thirty-eight of the fifty animals (76 per cent) so treated, whereas the grafts from the portion of tumor x-rayed *in vitro* continued to grow in forty-seven of the fifty mice, failing in only three (6 per cent). In the twelve instances in which the tumor x-rayed *in situ* did not disappear after treatment, without exception it grew at a slower rate than the other tumor arising from the graft which had been x-rayed *in vitro* (Text-fig. 4).

There seems little doubt from the results of this experiment that a treatment dose of x-rays fails to have any appreciable direct effect on the cancer cells, yet the same dose given to a growing cancer together with the surrounding normal tissue brings about healing in a majority of cases.

There is still another point to be considered. Is it possible that tumors x-rayed *in situ* are more sensitive than those exposed *in vitro?*

Experiment 3.—As in the previous experiments, a spontaneous mouse tumor was removed at operation, and without treatment either to the tumor or the animal, small bits of the tumor were reinoculated intradermally in both flanks. After the resultant tumors were well established and growing actively, one of them was exposed *in situ* to the same dose of x-rays as that used in the preceding experiment. After the treatment this tumor was removed and again reinoculated into an unrayed area of the same animal. Forty-seven mice with spontaneous cancer received this treatment and in thirty-seven (78.8 per cent) instances the x-rayed tumor grew well in its new location.

It would seem therefore that there is no increased susceptibility of tumor cells to x-rays when treated *in situ*, and that tumor treated in

[4] With the quality of x-rays used here the increased dosage due to scattering would be theoretically as great in the locality from which the graft was taken as in a tumor of the surface layers of an animal exposed to the same initial dosage.

such a fashion, when removed from the unfavorable environment induced by the x-rays, will grow actively when replanted in a new location on the same animal.

DISCUSSION.

The fact that a large proportion of certain forms of skin cancer yield to x-ray and radium treatment is one of the chief supports for the belief that the malignant cell is more susceptible to radiation than normal tissue. An attempt has been made in the experiments reported here to analyze the mechanism by which x-rays affect the tumor lying within the skin layers. The extent to which one is justified in assuming similarities between the behavior of tissue in man and lower forms of animals is still a question, but there is little doubt that spontaneous cancer as it occurs in animals closely resembles the disease in man. It is evident from our experiments that, as far as mouse cancer is concerned, the beneficial result from x-ray therapy is due to the reaction in the normal tissues induced by the rays, not to any direct effect on the cancer cells. That this point, first brought out with a transplantable tumor, and now confirmed for the spontaneous disease, may hold true for human cancer is not improbable. Statements by Ewing,[5] based on a careful study of human material, indicate that the reaction induced in the surrounding normal tissues by x-rays or radium is of as great importance as we have shown it to be in animals. In a recent address he makes the following statements. "It is clear that the reaction of the tissues is an essential factor in the curative process. Under some circumstances, when this reaction fails, no amount of radiation succeeds in killing the tumor cells . . . . the most detailed knowledge we possess indicates clearly that the curative action is not the result of a direct effect exclusively upon the tumor cells, but involves especially a peculiar reaction of the normal or invaded tissues."

Whether the beneficial results from the use of high frequency x-rays depend on the same factors is a point as yet undetermined; but this seems not improbable, since the maximum amount of x-rays supposedly delivered to the deep tumors in such treatment is well below the

[5] Ewing, J., *Am. J. Roentgenol.*, 1922, ix, 331.

experimentally established lethal dose for cancer cells. The wave length of the rays used in deep therapy is shorter than that of those previously used, yet longer than the gamma rays of radium. Since both the relatively long x-ray waves and the short waves of the gamma ray in all probability influence cancer through the reaction induced in the normal tissue, it is not unreasonable to expect that the high frequency x-rays will eventually be found to act in the same way.

SUMMARY.

Autografts from spontaneous cancers of mice when replanted into areas previously exposed to an erythema dose of x-rays, failed to grow in the majority of instances (71.4 per cent), while similar grafts inoculated into untreated areas grew in a large proportion of the animals (83.6 per cent).

Autografts of spontaneous cancer, established and growing in the skin, disappeared in 76 per cent of animals after the tumor and surrounding tissues had been exposed to an erythema dose of x-rays, whereas other autografts of similar derivation that had been given a like dose of x-rays outside of the body and had been implanted in the same animals grew progressively in 96 per cent of instances. That this result was not due to a greater susceptibility of the cancer cells x-rayed *in situ* was shown by the fact that tumors treated *in situ* with x-rays and then replanted in an unrayed location on the same animal grew actively. Evidently the ray had done no direct damage to the cancer cells.