

## THE EFFECT OF ROENTGEN RAYS ON THE RATE OF GROWTH OF SPONTANEOUS TUMORS IN MICE.

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(Received for publication, September 1, 1915.)

In a previous communication we reported the existence of a relationship between the resistant state to transplantable tumors in mice and a lymphocytic crisis in the circulating blood. We further demonstrated that by a previous destruction of the lymphoid tissue of these animals with x-ray a potentially resistant animal was rendered susceptible to cancer inoculation.<sup>1</sup>

In the course of some experiments on x-ray we have noted that the lymphoid elements, after extensive depletion by x-ray,<sup>2</sup> will soon start to regenerate actively. This process will continue, as has been noted before, to a period of overproduction of the lymphoid elements. The rapidity with which this occurs depends somewhat on the amount of original destruction and somewhat on the general condition of the animal. We have further noted that by one small dose of x-ray we could obtain in a certain proportion of animals a stimulation of the lymphoid elements, preceded by a comparatively short period in which the lymphocytes were below normal. This suggested an explanation of certain therapeutic effects of x-ray.

Our first problem was to determine whether or not x-rays in a small dose administered to an animal as a whole would produce an effect on the subsequent growth of a cancer, different from that produced by a similar dose applied directly to the cancer outside the body. For this purpose it was necessary in one set of animals to confine the x-ray effect to the animal alone, ruling out any possible action on the cancer, and, in a second set to confine the x-ray effect to the cancer, preventing an indirect effect on the animal. Spontaneous tumors of the mouse were selected for this work as a more

<sup>1</sup> Murphy, Jas. B., and Morton, J. J., *Jour. Exper. Med.*, 1915, xxii, 204.

<sup>2</sup> Heineke, H., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, xiv, 21.

suitable material than the transplanted tumors, for reasons that will be explained later. The results are given here in a brief summary. A complete analysis of the size and characters of the cancers with autopsy findings will be given in a subsequent report.

*Series I.*—There were 52 mice with various stages and types of spontaneous cancers in this group. The tumors were removed as completely as possible by operation, and, with the cancer out, the whole animal was exposed to a stimulating dose of x-ray (Coolidge tube). Immediately afterwards a graft of the original cancer was replaced in the groin of the animal. In 26 of the 52 animals treated in this fashion, there resulted a complete immunity to the recurrence of the disease. Only those animals were included in this number that lived and remained in good physical condition for at least five weeks after the treatment. The majority lived from two to four months, some to eight months, and some are still living. There has been no evidence of a local recurrence at the site of operation, nor where the graft was implanted, or of metastasis in those that have died. Among the remaining 26 animals of the series the average time for the appearance of the graft was five weeks and four days, a figure which contrasts strongly with the figure for the control animals. The number of recurrences at the original location of the tumor was 11 among the 52 animals, all occurring in the latter 26.

*Series II.*—For a control series we had 29 mice with spontaneous tumors of various sorts. These were operated on in the same manner as the animals in the first series, but a graft of the cancer was returned without treatment to either the animal or the cancer. The tumors were kept outside the body for the same length of time as in the first series. In 28 of the 29 the grafts grew progressively. In one the graft grew for a period and then retrogressed to complete absorption. The average time for the grafts to become palpable was one week and five days. This is about the same figure obtained by Rous.<sup>3</sup> Local recurrences of the cancer occurred in 14 of the 29 animals.

*Series III.*—The cancers in these animals were removed in the same manner as in the first two series, but in this group the cancers were subjected to the same amount of treatment that the animals had

<sup>3</sup> Rous, P., *Jour. Exper. Med.*, 1914, xx, 433.

received in the first series. A graft from the cancer, after this treatment outside of the body, was returned to the groin of the original host, as in the other experiments. 10 mice with spontaneous tumors were used for this series, and in all 10 the returned grafts grew. The average time for these to become palpable was one week and three days. There was a local recurrence of the tumor in 4 of the 10 animals.

A tabulation of the figures for the three experiments is given for comparison in Table I.

TABLE I.

	Immune.	Susceptible.	Local recurrence of tumor.	Average time for ap- pearance of graft.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Series I*.....	50.0	50.0	21.2	5 wks., 4 days.
" II.....	3.4	96.6	48.3	1 wk., 5 "
" III.....	0.0	100	40.0	1 " 3 "

\* Series I, animals treated by x-ray, while cancer was out. Later a graft of the tumor was returned. Series II, control animals in which cancer was removed and a graft returned without treatment to either animal or tumor. Series III, cancers removed and subjected direct to x-ray treatment and a graft returned to the original host.

It will be seen from the figures in Table I that x-ray administered directly to the cancer outside of the body is insufficient to prevent the growth of a graft returned to the original host. On the other hand, the same small dose of x-ray given to the animal with the cancer removed was sufficient to render 50 per cent of the mice so treated immune to returned untreated grafts of their own tumors and greatly retard the growth in the other 50 per cent.

The contrast between the control animals with 1 immune out of 29, and the x-rayed series with 26 immune out of 52 is striking, as is also the comparison of local recurrence in the two series. The delayed appearance of the graft in the treated series is important and this period could perhaps have been prolonged or a recurrence prevented altogether by a second exposure to x-ray after a suitable interval.

Total white counts and differentials were done on all of these mice

before operation<sup>4</sup> or treatment and on part of them at intervals after. So far in the limited number counted systematically afterward, our treatment has given in the successful cases a definite increase in the lymphocytes. Whether or not this increase is vitally concerned in the immunity process to spontaneous tumors is a point to which at present we are unwilling to commit ourselves. The results in the light of our previous experiments are strongly suggestive of this, however.

We have demonstrated a direct effect of x-rays on the animal, which renders it more highly resistant to replants of its own cancer than would normally be the case. There is also an absence of any demonstrable effect of this small dose of x-ray when administered to the cancer direct. Grafts of such tumors when returned to their original host grow as well as do the controls.<sup>5</sup>

<sup>4</sup> Counts made on over 100 untreated mice with spontaneous tumors have failed to show an abnormally low lymphocytic content in the circulating blood. This result is contrary to that obtained by Baeslack (Baeslack, F. W., *Ztschr. f. Immunitätsforsch., Orig.*, 1913-14, xx, 421) in counts on two mice with spontaneous tumors. We were also unable to confirm his reported decline in the numbers of lymphocytes in animals with growing transplantable cancers (Murphy and Morton, *loc. cit.*). He also gives differential counts on four mice with natural immunity to transplanted tumors, but as no total white cell counts are given it is impossible to tell whether his percentage variations are due to fluctuations in the polymorphonuclear cell or in the lymphocyte.

<sup>5</sup> We have avoided in this communication any discussion of the massive and contradictory literature on direct x-ray effects. We are unaware of any experiments that bring out the above points.