*On the Local and Generalised Action of Radium and X-Rays upon Tumour Growth.**

By S. Russ, D.Sc., Helen Chambers, M.D., and Gladwys M. Scott, Cancer Research Laboratories, Middlesex Hospital.

(Communicated by Sir Walter Fletcher, F.R.S. Received December 31, 1920.)

There are many considerations in the successful treatment of a tumour in the animal body by radiation. Broadly speaking, the subject may be divided into two main divisions: (1) the action of X-rays and the beta- and gamma-rays from radium on the tumour cells, and (2) the effect of these rays upon the animal itself.

In both cases a gradual change of effects is observed according to the dose of radiation. Actively growing malignant cells, given a large dose of radiation, degenerate and die when re-inoculated into a living animal; a dose short of this quantity causes the tumour cells to grow at a slower rate than they would do normally, but if given a very small dose, the cells appear to be stimulated rather than hindered in their subsequent growth.

Prolonged exposure of the animal to the rays results in severe wasting and death; with a reduction in the exposure a growing animal will retain its health, but with a diminished rate of increase of body weight. As the dose of radiation becomes less, a stage is reached when the rate of increase of body weight exceeds that of the normal animal; and, when the animal is given these very small doses of X-rays, it is found to develop a state of increased resistance to an implanted tumour.

Such briefly are the chief effects to be considered when devising methods by which a lethal dose of radiation may be given to a tumour without reducing the resistance of the animal.

The experimental data in this paper will be dealt with as follows:—

(A) The effect of the rays in various doses upon malignant cells, before inoculation.

(B) The effect of the rays in various doses upon normal animals—(1) body growth, and (2) subsequent inoculations of malignant cells.

(C) The effects of the rays in various doses upon animals which are bearing tumours.

This investigation has been carried out upon rats, and the tumours have been of three distinct types: Jensen's rat sarcoma; a very slowly growing

* This investigation includes, though is not restricted to, experimental work undertaken at the request of the Medical Research Council upon the general biological effects of small doses of X-rays.

rat sarcoma (F. 16), for which we are indebted to Dr. J. A. Murray, the Director of the Imperial Cancer Research Fund; and a rat carcinoma, which occurred as a spontaneous tumour in these laboratories in January, 1919, and which has been described (1) to the Pathological Society of Great Britain and Ireland, March, 1920. Most of the work has been done with Jensen's rat sarcoma, and, except where otherwise stated, the observations refer to this tumour.

(A) *The Effect of Rays in Various Doses upon Malignant Cells.*

A dose of X-rays or radium rays can be given most accurately to malignant cells when the tumour is not in the animal body. The method we have adopted since 1912 (Wedd and Russ, 2) has been to excise a tumour, cut a slice of it, and expose this aseptically to radiation for any length of time desired; the tumour cells are then implanted into normal rats, and the size of the subsequent growth is accurately recorded. It is more convenient in this type of experiment to use the beta-rays from radium than X-rays, for, however carefully the X-rays may be controlled, there is much more likely to be a change in the radiating power of the source than if radium is used.

Table I shows the results which are obtained when the cells of Jensen's rat sarcoma, before being inoculated into normal animals, receives exposures ranging from forty minutes down to twelve seconds. The last column compares the size of the irradiated with the non-irradiated tumour in the same animal; the inoculations were made in the right and left axillæ. The source of the rays used was a capsule containing 20 mgrm. of radium bromide ($Ra.Br_2.2H_2O$), spread over an area of 4 sq. cm., and covered with a thin layer of varnish; the varnish absorbs alpha- and soft beta-rays.

Table I.

Time of exposure to radium.	Number of rats in which the irradiated tumours did *not* grow.	Number of rats in which the irradiated tumours *did* grow.	Volume of growth compared with control 3–4 weeks after inoculation.
40 minutes	12	—	—
35 „	11	1	0·07
30 „	13	5	0·29
10 „	—	6	0·44
5 „	—	6	0·85
1 minute	—	8	0·82
12 seconds	—	8	1·35

The conclusion drawn from this series is that, as the dose of rays given to the sarcoma cells diminishes from a lethal dose, a gradual change is observed.

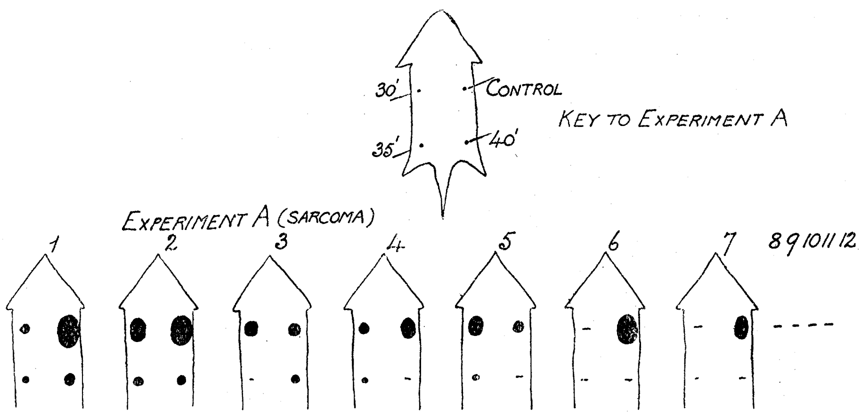
A reduced rate of growth changes into a rate which appears slightly quicker than that of the untreated tumour. This latter phenomenon will be referred to again in Section C.

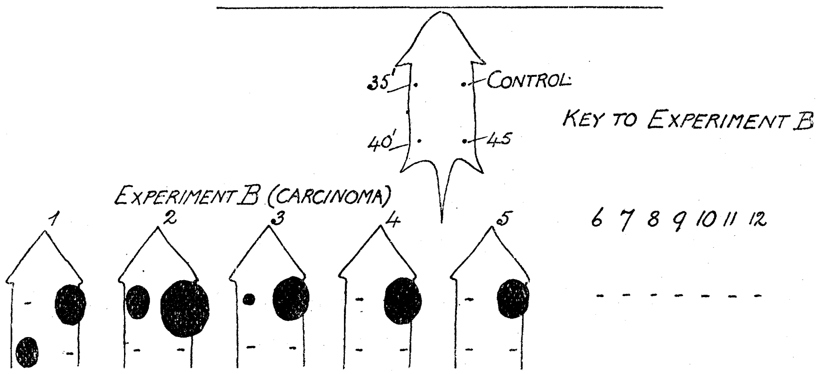
It will be seen from the data in Table I that the lethal dose is about thirty-five minutes, only one animal out of twelve bearing a tumour, when the cells had previously been irradiated; normal tumour inoculated into the left axillæ of the same animals grew in every case.

The lethal dose for the two other varieties of tumour already referred to has been found by following a similar method to that just outlined.

It is not possible to express the results of these experiments in quite the same way as for the Jensen's tumour, because they do not give 100 per cent. of growing tumours when inoculated. The rat carcinoma is very difficult to propagate, rarely as many as 50 per cent. of the inoculations into normal animals resulting in tumours.

The subjoined two charts illustrate the results obtained upon two series of animals. The tumours depicted in the charts are drawn to scale, the time being fifty-six days and twenty-seven days respectively after inoculation.





In the case of the slowly growing sarcoma, Experiment A, it will be seen that, in three animals out of seven, grafts of the tumour, irradiated for forty minutes, formed very slowly growing tumours; the lethal dose thus appears to be rather larger than for the Jensen tumour or for the carcinoma, Experiment B.

In this connection we may recall the results of Wood and Prime (3); these authors observed only small differences in the dose of beta- and gamma-rays required to prevent growth of four different types of tumours in the mouse and rat.

The dose of radiation needed to prevent any particular tumour growing depends, therefore, not only upon the type of tumour, but also upon the susceptibility of the animal into which the irradiated cells are implanted. The figure made use of, in referring to the lethal dose, is the upper limit given by the highly susceptible animal.

(B) *The Effect of the Rays in Various Doses upon Normal Animals.*

(1) Body weight.
(2) Resistance to subsequent inoculations of malignant cells.

(1) *Body Weight.*—The rays used were X-rays from a Coolidge tube run at an alternative spark gap of 4·5 cm. between spheres 5 cm. diameter. The animals were placed 20 cm. below the anti-cathode, the rays being unscreened except for a thin mica sheet below the tube and a perforated cardboard cover of the animal box. By slightly tilting the tube, a lateral beam of the X-rays used passed from the exposure room through a hole in the wall across a passage into an adjoining room, where its intensity was measured by a gold-leaf electroscope 6 metres distant. By adjusting the radiation to give a standard rate of movement of the gold-leaf, before, and where possible during the exposures, a useful check was kept on the constancy of the radiation used. Under these experimental conditions, daily doses of X-rays were given to batches of animals for a number of weeks, and their body weights compared with those of an equal number of normal animals of the same initial weight. Exposures of five minutes per day were found to be injurious to the animals, so the daily doses were reduced to one minute, in the next batch to twelve seconds, then to two seconds; the effects of this repeated radiation are summarised in Table II.

It will be seen that a daily exposure of one minute is deterrent to increase of body weight; with a diminished dose, there is a more rapid increase than in normal animals.

Table II.

Number of X-rayed animals.	Exposure to X-rays.	Effect upon body weight.
16	1 minute daily for 9 weeks	*Result*: 25 per cent. diminution below normal rate of growth in 63 days. (All but 4 died.)
22	12 seconds daily for 8 weeks	*Result*: 15 per cent. increase above normal rate of growth in 62 days.
21	12 seconds daily for 4 weeks	*Result*: 7·5 per cent. increase above normal rate of growth in 50 days.
23	2 seconds daily for 9 weeks	*Result*: 10 per cent. increase above normal rate of growth in 69 days.

(2) *Resistance to Subsequent Inoculations of Malignant Cells.*—It has been recorded (4) that rats exposed to small daily doses of X-rays before inoculation were found to have a greater resistance to the growth of sarcoma than normal rats. This immunity was not absolute, for many of the rats grew tumours, though smaller ones than the controls. Further experiments have been carried out to determine whether complete immunity could be produced by varying the period over which the irradiation was continued, the actual daily dose of 12 seconds being unaltered.

The data in Table III show the rate of growth of Jensen's rat sarcoma in animals treated in this way. In all cases in which rats have been previously submitted to X-rays the tumours grow at a slower average rate than in the normal animal. It has not been found possible to confer an absolute degree of immunity in this way, but it should be mentioned that we are dealing with a very rapidly growing tumour, one that doubles its volume in less than four days.

It appears from these results that the most prolonged time of irradiation does not give the highest degree of immunity.

In contrast with this increase in the resistance of the susceptible animal we have the experiments of Murphy and Morton (5), of Mottram and Russ (6), and of Prime (7), who showed that, after a very large dose of X-rays, an immune animal becomes temporarily susceptible to carcinoma and sarcoma inoculations.

The effect of a single rather large dose of X-rays in increasing the susceptibility of the animal to inoculation has also been shown by the authors (1), in the propagation of a spontaneous rat carcinoma.

Table III.

Period of irradiation.	Interval before inoculation.	Number of animals.	Volume of tumour compared with that of controls. Time after inoculation. 2 weeks.	3 weeks.	4 weeks.	Remarks on tumour growth.
4 weeks	28 days	18 X-rayed 26 controls	0·77	0·46	0·40	X-rayed animals grew 4 progressive tumours. 14 disappearing tumours. Control animals grew 12 progressive tumours. 14 disappearing tumours.
6 weeks	13 days	9 X-rayed 10 controls	0·65	0·44	—	X-rayed animals grew 3 progressive tumours. 6 disappearing tumours. Control animals grew 9 progressive tumours. 1 disappearing tumour.
8 weeks	11 days	21 X-rayed 20 controls	0·42	0·40	0·35	X-rayed animals grew 7 progressive tumours. 14 disappearing tumours. Control animals grew 14 progressive tumours. 6 disappearing tumours.
12 weeks	13 days	29 X-rayed 24 controls	0·84	0·76	0·65	X-rayed animals grew 19 progressive tumours. 10 disappearing tumours. Control animals grew 17 progressive tumours. 7 disappearing tumours.

(C) *The Effect of the Rays in Various Doses upon Animals which are Bearing Tumours.*

Here we have to distinguish between three varieties of exposure :—

(1) Exposures in which the animal does not share to an appreciable extent in the radiation that the tumour receives, *i.e.*, a localised exposure to beta-rays.

(2) Exposures in which the tumour does not share in the irradiation of the animal, *i.e.*, tumour screened from the rays.

(3) Exposures in which the animal shares in the radiation that the tumour receives, *i.e.*, a generalised exposure of X-rays to the whole body.

(1) *Localised Exposures.*—If a small superficial tumour exists in the animal body, it is possible, by the use of easily absorbed rays, such as beta-rays, to expose the cells of the tumour without the rest of the animal sharing to an appreciable degree in the radiation.

A series of experiments upon a number of animals was carried out on the

following lines. About the fourth day after inoculation of a graft, when the tumour was palpable, the radium capsule was held on it for a certain length of time. The minimum time of exposure necessary to prevent growth of the tumour was found; it was not appreciably different from the exposure required if the tumour material was removed from the body, given its dose of radiation and re-inoculated into susceptible animals in the manner described in Section A. The results were analogous indeed to those given in Table I to the extent of showing that a small dose of radiation given to the tumour, increased rather than hindered its growth.

With the tumour in the animal, these exposures to small quantities of radiation may be repeated in a way not possible when the tumour is irradiated outside the body.

In the next series of experiments, the rats were inoculated on each side, four days later all the tumours on the right sides were exposed to the radium for times varying from ten minutes to twelve seconds; these doses were given once a week, their effect upon the growth of the tumour is seen in Table IV.

Table IV.

Conditions of irradiation of tumour in animal.	Volume of irradiated tumours compared with the volume of controls in the same animal.				Remarks.
	1 week.	2 weeks.	3 weeks.	4 weeks.	
15 rats, 10 minutes' exposure to beta-rays once a week	0·59	0·43	0·42	0·44	In 13 rats the control tumour was the larger.
15 rats, 5 minutes' exposure to beta-rays once a week	0·92	0·76	0·76	0·85	In 13 rats the control tumour was the larger.
19 rats, 1 minute's exposure to beta-rays once a week	0·98	1·07	1·25	1·41	In 14 rats the irradiated tumour was the larger.
17 rats, 12 seconds' exposure to beta-rays once a week	1·14	1·28	1·33	1·46	In 10 rats the irradiated tumour was the larger; in 5 rats the control; and in 2, both tumours were the same size.

The general trend of these observations is that, while a weekly exposure of ten or five minutes checks the tumour growth, a reduction to one minute or less has an opposite tendency. The beta-rays used were those from the capsule employed in the experiment detailed under Section A (Table I). The results show that a dose of radiation of about one-fiftieth of the lethal dose (thirty-five or forty minutes, *vide* Table I), has, if anything, an accelerating effect upon tumour growth.

(2) *Generalised Radiation of the Animal, the Tumour being Screened.*—A number of animals (twenty-four) were inoculated with the sarcoma, in the middle of the back. The next day twelve of the animals were submitted to a daily 12-second dose of X-rays; the graft was covered with a piece of lead 2 mm. thick, to screen it from the X-rays. These exposures were continued daily for a month, measurements of the tumours being made twice a week. A comparison with the tumours in the twelve control animals is made in Table V.

Table V.

Number of animals.	Time after generalised radiation began.	Volume of X-rayed tumours compared with controls.	Remarks.
12 X-rayed	1 week 2 weeks	0·80 0·74	X-rayed animals grew 6 progressive tumours. 6 disappearing tumours.
12 controls	3 weeks 4 weeks	0·56 0·55	Controls grew 10 progressive tumours. 2 disappearing tumours.

The result is similar to those in Section B (2), Table III, in which a decreased susceptibility to tumour growth follows a generalised irradiation.

(3) *Generalised Exposures.*—From the preceding observations, it will be clear that we have to deal with a positive and a negative factor; for if, on the one hand, we have shown that a repeated exposure of the animal to small doses of X-rays *increases* its resistance to tumour growth, we have to recognise, on the other hand, that the effect of small doses of radiation upon the tumour cells is rather to accelerate their rate of growth. It remained for experiment to decide which process would outweigh the other when a tumour-bearing animal was given a generalised radiation repeated at frequent intervals.

Two batches of animals were inoculated in the right axilla; one batch received a daily dose of X-rays, the other no radiation. The dose of X-rays, in different experiments, was varied from five minutes to two seconds. The growth of the tumour in the two batches was then recorded for a period of about one month; the results obtained will be seen in Table VI.

The effect of five minutes' exposure to X-rays daily for one week is to retard the tumour growth, probably by direct action upon the cells, but it has at the same time a profound effect upon the animal, causing death in nearly every case. A reduction of the exposure to one minute again results in reduced rate of tumour growth, but the body weight suffers in comparison with the non-irradiated animal. Further reduction of the exposure has less effect in slowing up the tumour, though the body weight has

Table VI.

Number of animals.	Time of exposure to X-rays.	Growth of tumour in X-rayed animals compared with controls. Weeks after inoculation. 1	2	3	4	General Remarks. Tumour growth.	General condition.
12 X-rayed 12 controls	5 minutes daily for 1 week	0·13	0·03	0·01	—	No progressive growth of tumour in the X-rayed animals	All X-rayed animals except 4 died within 4 weeks, 1 survived 3 months.
23 X-rayed 19 controls	1 minute daily for 4 weeks	1·0	0·76	0·72	0·36	X-rayed animals grew 13 progressive tumours 10 disappearing tumours Controls grew 15 progressive tumours 4 disappearing tumours	X-rayed animals well, but body weight increased 19 per cent. less than the normals.
14 X-rayed 15 controls	12 seconds daily for 4 weeks	0·81	0·73	0·82	0·73	X-rayed animals grew 9 progressive tumours 5 disappearing tumours Controls grew 11 progressive tumours 4 disappearing tumours	X-rayed animals well, no record of body weight, but *vide* Table II, rows 2 and 3.
36 X-rayed 33 controls	2 seconds daily for 4 weeks	1·25	1·23	1·05	0·79	X-rayed animals grew 17 progressive tumours 19 disappearing tumours Controls grew 20 progressive tumours 13 disappearing tumours	Animals well, body weight increase same as normals.

increased more than normally. When the tumour growth figure 0·73 at the fourth week is compared with the figure 0·55 at the fourth week in Table V, it seems rational to attribute this difference to the slightly stimulating effect which small doses have been shown to have upon the malignant cells (*vide* Tables I and IV).

Discussion of Results.

Since the attempt has been made throughout these investigations to express the results quantitatively, it remains to consider to what extent the observed differences in the rate of growth of the animals and of the tumours are significant. In Section A, the matter which calls for comment is that the rate of growth of tumour was 1·35 times that of the normal when the tumour cells had previously received a small dose of beta-rays. The number of animals used, viz., eight, is not large enough to consider the matter established, but, when the experiment is done under slightly different conditions with the same dose of rays, a similar increase occurs in the rate of

growth of tumour, viz., 1·46 times the normal, the number of animals being seventeen (*vide* Table IV).

The data in Section B (Table II) show that the body weight of the rat increases 15 per cent. more rapidly under small dose X-ray treatment than that of untreated animals of the same initial body weight; the number of animals used is enough to preclude the effect being accidental.

The increased resistance to tumour inoculations of an animal which has previously been given small daily doses of X-rays is a fairly constant feature of Table III. Considering the number of animals used (viz., 77, and an equal number of controls), it is very improbable that the reduction in tumour growth, amounting on the average to about 40 per cent., can be attributed to anything but the X-ray treatment.

In Section C, Table V, the quantitative reduction in the rate of tumour growth appears to be sufficiently large to preclude the element of chance. There is less restraint upon the growth of the tumour when the tumour itself is allowed to share in the small-dose radiation treatment (*vide* data in Table VI).

The bearing of these investigations upon the radiological treatment of malignant disease in man appears to be two-fold. In the first instance it must be recognised that the uniform irradiation of a large tumour in the human subject is hardly possible whether X-rays or radium be used. Small variations from the lethal dose would appear unimportant, but should the quantity of radiation reaching outlying portions of the growth be so diminished in intensity as to be a *small dose* (such as a few per cent. of the lethal dose) it might have a stimulating instead of a destructive effect on the malignant cells. Turning now to the body as a whole, there seems ample evidence to show that large generalised doses of radiation lower the normal resistance to tumour growth. This result is completely reversed when the normal animal is given very small generalised doses of X-rays, repeated at frequent intervals, and it would therefore seem a rational measure to supplement the local intensive irradiation of a tumour by a feeble generalised irradiation of the patient, care being taken wherever possible not to expose the tumour cells to this radiation.

REFERENCES.

1. Chambers, Scott and Russ, 'Journal of Pathology and Bacteriology,' vol. 23, 1920.
2. Wedd and Russ, 'Journal of Pathology and Bacteriology,' vol. 17 (1912).
3. Wood and Prime, 'Annals of Surgery,' vol. 62, p. 751 (1915).
4. Russ, Chambers, Scott and Mottram, 'Lancet,' April 28, 1919.
5. Murphy and Morton, 'Journal of Experimental Medicine,' vol. 22 (1915).
6. Mottram and Russ, 'Roy. Soc. Proc.,' B, vol. 90 (1917).
7. Prime, 'Journal of Cancer Research,' p. 115, April, 1920.