The Effect of Radioactive Phosphorus on the Viability of Mouse Sarcoma 180*

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Although artificial radioactivity was first induced but nine years ago by F. Joliot and I. Curie, at the present time about two hundred radioactive isotopes of the common elements are known. Some of these have been used satisfactorily for tracer-reaction studies and others have proved to be valuable materials for study of metabolism.

Some radioactive isotopes are selectively absorbed by certain organs and tissues. Thus radioactive iodine (1, 17-19, 22, 23) is selectively taken up by the thyroid gland; radioactive iron (12-14, 39) in the red blood cell; radioactive sodium (10, 11, 15, 16, 20) in the spinal fluid and the blood plasma; radioactive phosphorus (2-9, 21, 24-30, 32-36, 38, 40, 45-49) in the bones, liver, leukemic cells, and neoplastic tissues; and radioactive calcium and radioactive strontium (41, 42) in the skeleton. On the other hand, no detectable localization was noted following the administration of radioactive potassium and radioactive rubidium (16).

In view of the fact that the radioactive isotopes, in the course of disintegration, give off radiant energy (beta rays, gamma rays, positrons, etc.) similar to radium, the question at once arises as to what will be the effect of radioactive isotopes on tumor tissues; that is, whether the radiation of radioactive isotopes has effects on tumor tissue quantitatively different from those of x-rays or radium. This can be determined readily by exposing tumor fragments to the radioactive isotopes, and subsequently transplanting such fragments into host animals. The amount of reaction produced by a radioactive isotope is thus compared with that produced in tumors similarly treated with x-rays.

In the present study radioactive phosphorus was used because the radiations (beta rays) that are given off by it penetrate a considerable thickness of animal tissues, and P^{32} has a reasonably prolonged activity; *i.e.*, a half life time of 14.3 days.

MATERIALS, METHODS AND RESULTS

Mouse sarcoma 180 was selected on account of the regularity and high percentage of successful takes (essentially 100 per cent) obtained in transplantation experiments. The transplants showed only occasional spontaneous regression (about 4 per cent).

Subcutaneous inoculations of the tumor fragments in the lateral thoracic region of healthy young adult albino mice (Rockland albino mice) were carried out by the usual trocar method, the tumor materials being selected from rapidly growing tumors which had not ulcerated. These were from 7 to 10 days old. Aseptic precautions were taken throughout.

For the study of the effect of radioactive phosphorus upon mouse sarcoma 180 we used 8 preparations, containing respectively 286, 517, 850, 475, 265, 202, 260, and 192 microcuries (μc.) per cc. at the time they were used.

The radioactive phosphorus was prepared by bombarding red phosphorus with deuterons in the cyclotron at the Crocker Radiation Laboratory,[1] University of California, and thereafter was converted to a neutral solution of sodium phosphate.

Our previous studies with transplantable carcinoma and sarcoma (43, 44) indicated that these tumors showed marked differences in their reaction to different hydrogen-ion concentrations and salts in different concentrations. Because of possible changes in the reaction of the P^{32} solutions on shipping and standing we determined the hydrogen-ion concentration of these solutions electrometrically. It was found that the P^{32} solutions had pH values of 7.15 to 7.40. At these pH levels, the growth capacity of mouse sarcoma 180 was unaffected.

Since the P^{32} solution contained 15 mgm. of Na_2HPO_4 per cc., the extent of the deleterious action of disodium hydrogen phosphate upon the growth of mouse sarcoma 180 in mice was determined. It was found that immersion of fragments of mouse sarcoma 180 in 1.5 per cent Na_2HPO_4 solution (pH 7.4) for from 24 to 48 hours, at 4-5° C., was without effect, the tumors subsequently growing normally when implanted in animals. This study included three groups of experiments, involving a total of 40 implants.

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Effect of P^{32} on the Growth of Mouse Sarcoma 180

Various concentrations of P^{32} were prepared by diluting the original phosphate solutions with 1.5 per cent ordinary Na_2HPO_4 solution (pH 7.4). The solutions had previously been sterilized in a steam autoclave at 15 pounds pressure for 15 minutes.

Eleven pieces of fresh tumor tissue (mouse sarcoma 180), each weighing about 6 mgm. and measuring 1.5 to 2.0 mm. in thickness, were placed in 2 cc. portions of P^{32} solution. The weighing bottles (approximately 14 mm. in diameter) containing the tumor fragments were kept in a refrigerator for 24 hours at 4-5° C. with gentle shaking four times in 12 hours.

Table I: Results of Transplanting Mouse Sarcoma 180 after Immersion in Radioactive Phosphorus Solution at 4-5° C.

Experiment number	Number of tumor transplants	P^{32} (μc./cc.)	Duration of immersion (hours)	Equivalent roentgens	Growth of transplants (per cent)
1	10	40.0	24	1,720	100
2	10	49.9	24	2,140	100
3	10	57.4	24	2,460	100
4	10	61.7	24	2,650	90
5	10	63.2	24	2,710	100
6	10	65.0	24	2,790	90
7	10	76.5	24	3,280	70
8	10	84.3	24	3,620	80
9	10	87.4	24	3,750	50
10	10	92.5	24	3,970	70
11	10	97.5	24	4,180	70
12	10	99.8	24	4,280	60
13	10	113.0	24	4,850	40
14	10	114.0	24	4,890	40
15	10	115.0	24	4,930	40
16	15	120.0	24	5,150	60
17	10	126.0	24	5,400	10
18	10	131.0	24	5,620	30
19	10	143.0	24	6,130	0
20	10	151.0	24	6,480	0
21	10	160.0	24	6,860	0
22	10	164.0	24	7,040	0
23	10	216.0	24	9,270	0

At the end of this period of time, the tumor fragments were removed from the P^{32} solution to a Petri dish containing a sheet of semi-moist filter paper and were immediately implanted into mice by the usual trocar method, each animal receiving a single graft.

The results obtained from these experiments are presented in Table I.

The data in Table I show clearly that the growth capacity of mouse sarcoma 180 was not altered when tumor fragments were immersed for 24 hours at 4-5° C. in a solution of P^{32} of about 50 μc. per cc., the takes and growths being the same as in untreated controls. The inhibiting action, however, sharply increased with the increase in concentration of P^{32}. Thus immersion in a solution containing 75 μc. of P^{32} per cc. resulted in about 25 per cent inhibition and slight delayed growth. Marked inhibition and retardation of growth were caused by immersion in P^{32} solutions of 100 and 125 μc./cc. (about 50 and 75 per cent inhibition respectively). The viability of the tumor was completely destroyed by immersing in P^{32} solution of 150 μc./cc.

It may be of interest to mention that under the stated conditions of irradiation, the beta rays emitted from the radioactive phosphorus have not induced any stimulating influence upon mouse sarcoma 180.

Histological examinations of a number of the tumor tissues were made after exposure to P^{32}. The general structure of nonirradiated tissue showed a very cellular, small spindle and polyhedral cell sarcoma, in areas degenerated and necrotic. The sections of irradiated tissues showed no definite changes, the tumor cells appearing to be viable. Yet the growth energy of the transplants which had been immersed in P^{32} solution of 143 μc./cc. or more for 24 hours at 4-5° C., was completely destroyed.

Comparison with Effects of X-Rays

The lethal effect produced by beta rays of P^{32} on a transplantable mammalian tumor was compared with that produced by 200 kv. roentgen rays. With the method used by Marinelli (37), it is possible to calculate the beta-ray tissue dose received by the tumor fragments in the radioactive phosphorus solution in terms of "equivalent roentgens."

The beta particles produced by the disintegration of P^{32} have an average energy of 700 kv. and can penetrate between 2 and 4 mm. of animal tissue. According to Marinelli's calculation, if the beta ray energy of 1 μc. of P^{32} is released in a gram of tissue during a 24 hour period, 42.9 equivalent roentgens will be delivered to that tissue. By using the conversion figure, the *maximum* equivalent roentgen values for given microcuries were obtained as shown in the fifth column of Table I. These are maximum values based on the energy absorbed by the solution itself. The tumor fragments received lesser doses for two reasons: (a) Each was not necessarily surrounded by enough solution of P^{32} to receive a full complement of the beta radiation. (b) The P^{32} concentration, within the tumor fragment, was zero at the beginning of the immersion period, and therefore initially the tumor cells in the fragment could receive only beta rays originating in the solution outside of the fragment. As P^{32} diffused into the tissue, the cells received additional beta radiation from points in the fragment itself. The contribution to the total radiation received by the tumor cells from this P^{32} increased gradually with time. It

reached a maximum when no further diffusion of P^{32} took place. This point, however, may not have been reached during the period of immersion (24 hours). We know, however, that after this period the concentration of P^{32} in the tissue is about 60 per cent of that in the surrounding solution.

As may be seen from the data in Fig. 1, the transplantability of mouse sarcoma 180 was not altered appreciably by irradiation of tumor fragments with 2,500 equivalent roentgens (about 100 per cent takes). An exposure of 3,500 equivalent roentgens gave about 20 per cent inhibition. Marked inhibition was caused by an exposure of 4,500 and 5,500 equivalent roentgens (about 50 and 80 per cent inhibition, respectively). The viability of the tumor was completely destroyed by an exposure of 6,500 equivalent roentgens.

In the present experiment the tumor fragments were immersed in P^{32} solution at the bottom of the weigh-

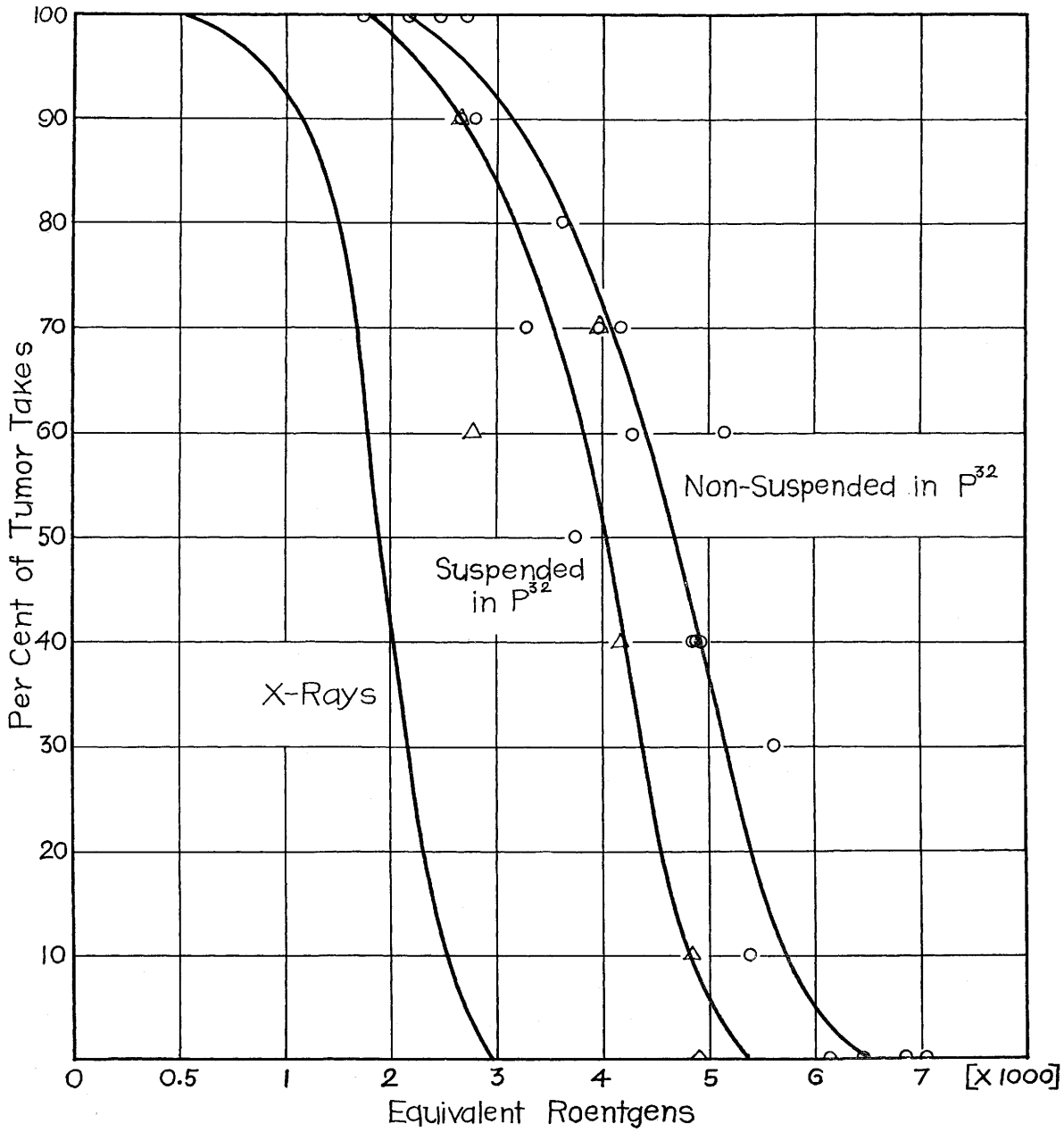

FIG. 1.—Survival curves of mouse sarcoma 180 exposed to beta rays from radioactive phosphorus and to x-rays. "Nonsuspended" fragments rested on bottom of bottle.

ing bottle. Initially some tumor fragments fell side by side, thus possibly preventing full effect of beta rays emitted from P^{32}. Therefore, in order to give tumor tissues maximum radiation effect the tumor fragments were placed in a wide mesh gauze bag and suspended in the radioactive solution. Parallel experiments were run with tumor fragments immersed in P^{32} solution in the usual manner. After standing for 24 hours at 4-5° C. the suspended and nonsuspended tumor fragments (those lying on the bottom of the bottle) were implanted into mice. The results are presented in Table II.

The results of these comparative experiments show that tumor fragments suspended in P^{32} solution gave definitely fewer takes than those not suspended in P^{32} solution; *i.e.,* there was about 30 per cent increase in tumor destruction. Of course, in the case of the suspended fragments, irradiation is from all sides, whereas those on the bottom of the bottle received little radiation on their lower sides.

TABLE II: RESULTS OF TRANSPLANTING TUMOR FRAGMENTS SUSPENDED AND NONSUSPENDED IN P^{32} SOLUTION FOR 24 HOURS

Experiment number	Number of tumor transplants	P^{32} (μc./cc.)	Equivalent roentgens	Condition of tissue exposure	Growth of transplants (per cent)
1	10	61.7	2,650	Suspended	90
	10	61.7	2,650	Nonsuspended	90
2	10	63.0	2,790	Suspended	60
	10	65.0	2,790	Nonsuspended	90
3	10	92.5	3,970	Suspended	70
	10	92.5	3,970	Nonsuspended	70
4	10	97.5	4,180	Suspended	40
	10	97.5	4,180	Nonsuspended	70
5	10	113.0	4,850	Suspended	10
	10	113.0	4,850	Nonsuspended	40
6	10	114.0	4,890	Suspended	0
	10	114.0	4,890	Nonsuspended	40

ABSORPTION OF P^{32} BY TUMOR TISSUE

In the course of the investigation a study was made on the extent of absorption of P^{32} by the tumor tissue. Twenty small pieces of tumor tissue, each weighing about 6 mgm., were placed in 2.0 cc. portions of P^{32} solution of 86 μc./cc. The weighing bottles containing the tumor fragments were kept in a refrigerator at 4-5° C. for 6, 24, and 48 hours. At the end of these time intervals, the tumor fragments were removed from the bottles, dipped into a Locke-Ringer solution for a second, blotted on filter paper, and then weighed and ashed in an electric furnace at 500° C. Afterwards the ashes were measured for beta-ray activity, using standard electroscopic methods. Tissue ash so measured was found after 6 hours soaking to yield 46 μc./gm., after 24 hours, 53 μc./gm., and after 48 hours, 60 μc./gm. It is to be noted that after the first 6 hours of soaking in phosphate solution, the tissue exhibits a P^{32} concentration which is about 53 per cent of the P^{32} concentration of the soaking solution; and that after 24 hours the P^{32} concentration within the tissue has risen to 62 per cent. It is evident that tumor fragments so treated and implanted into mice carry with them a considerable quantity of radioactive phosphorus, which continues to emit beta radiation after the tumor fragments have been inoculated into mice.

DISCUSSION

The primary purpose of this study was to compare the biological effectiveness of P^{32} radiation energy and that of x-rays, and to determine if the doses of radiation needed to achieve the effects under study are of the same order of magnitude in each case. A more quantitatively precise comparison would require a closer evaluation of the role played by the experimental conditions; that is, diffusion rate of phosphate ions into the soaking tissue, etc. It is very probable that when a tumor fragment has been soaked in radioactive phosphate solution it is subjected not to uniform irradiation, as in the case with x-rays, but rather the strength of the dose grades decreasingly from periphery to center of the tissue mass.

In Fig. 1 is also included the survival curve of mouse sarcoma 180 exposed *in vitro* to filtered 200 kv. roentgen rays (43). It shows that when mouse sarcoma 180 is irradiated *in vitro* the dose of filtered roentgen rays necessary to kill all the fragments of tumor is between 2,800 and 3,000 r (measured in air). On inspection, this would seem to indicate that in inhibiting the growth of tumor tissues, the energy released by P^{32} was found to be half as effective as x-rays under the condition of this experiment. However, this conclusion cannot be accepted as categorical because it is shown that tumor fragments immersed in the radioactive solution absorb a concentration of the isotope only about one-half that of the concentration in the surrounding fluid. Therefore, it could be argued that the total radiation effect on the tumor fragment was being produced by one-half the concentration of isotope used for the equivalent roentgen values upon which the curves in Fig. 1 are based; and that instead of the isotope energy being twice as biologically effective as x-rays, it is approximately the same.

SUMMARY AND CONCLUSIONS

1. An investigation has been made of the effect of immersing fragments of mouse sarcoma 180 in radioactive phosphorus solution prior to transplantation.

2. The growth capacity of mouse sarcoma 180 was unaffected when tumor fragments were immersed for

24 hours at 4-5° C. in P^{32} solution having an activity of 50 μc./cc. Immersion in P^{32} solution of 75 μc./cc. resulted in about 25 per cent inhibition. Marked inhibition and retardation of growth were caused by exposure to P^{32} solution of 100 and 125 μc./cc. (about 50 and 75 per cent inhibition, respectively). The viability of the mouse sarcoma 180 was completely destroyed by immersion in P^{32} solution of 150 μc./cc.

3. The lethal effect produced by beta rays of P^{32} on the tumor was compared with that produced by roentgen rays at 200 kv. since it is possible to calculate the beta-ray emission of the radioactive phosphorus in terms of equivalent roentgens. Under the stated conditions of the experiment it was found that the transplantability of mouse sarcoma 180 was not altered appreciably by irradiation of tumor fragments with 2,500 equivalent roentgens. An exposure of 3,500 equivalent roentgens gave about 20 per cent inhibition. Marked inhibition was caused by an exposure of 4,500 and 5,500 equivalent roentgens (about 50 and 80 per cent inhibition, respectively). The viability of the tumor was completely destroyed by an exposure of 6,500 equivalent roentgens.

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