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SOME IN VIVO EFFECTS OF LOCALIZED NUCLEAR DISINTEGRATION PRODUCTS ON A TRANSPLANTABLE MOUSE SARCOMA

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Introduction.—The destruction of living tissues by x-ray and fast neutron radiation is well known. The effects are directly traceable to the action of energetic electrons resulting from the absorption of the x-rays in the one case, and in the other, from recoil nuclei, such as those of hydrogen, carbon and oxygen which have been projected by neutral impact. However, in both cases the destructive action occurs throughout the irradiated tissue, and no satisfactory method has been found for localizing the damage, in the case of cancer therapy, to the tumor zone. Thus skin damage usually sets an upper limit to the dose which can be delivered through the skin to underlying tissue.

Since the passage of slow neutrons through body tissues is not accompanied by the production of energetic recoil protons, there should be little or no resulting damage to the tissue. However, if these slow neutrons be introduced into a zone which has been perfused with certain chemical elements such as boron or lithium, or their compounds, nuclear capture reactions will occur which release very energetic particles, and result in the local destruction of tissue. That is, for boron:

$$_5B^{10} + {}_0n^1 \rightarrow {}_3Li^7 + {}_2He^4 + 2.2 \text{ MEV (million electron volts)}$$

or for lithium:

$$_3Li^6 + {}_0n^1 \rightarrow {}_2He^4 + {}_1H^3 + 4.6 \text{ MEV.}$$

The energies released by these reactions are approximately 2.2 MEV and 4.6 MEV, respectively. The cross-section[4] for the boron process is 57.5×10^{-24} cm.^{2}, as compared with 60×10^{-24} cm.^{2} for the lithium process. Thus while the energy release for lithium is larger, approximately five times the atomic concentration of lithium is necessary to obtain the same total energy release as with boron. Both reactions are competitive with capture

of the slow neutrons by hydrogen, with emission of high energy gamma rays,[5] ${}_1H^1 + {}_0n^1 \rightarrow {}_1H^2 + \gamma$ (2.2 MEV), but the capture probability for this process is only 0.3×10^{-24} cm.^{2}, so only very small atomic concentrations of boron or lithium are required to capture an appreciable fraction of the neutrons.

The foregoing considerations suggest an investigation of the applicability of neutron-boron or neutron-lithium techniques to the localized treatment of tumors. The physical principles have been discussed at length by Kruger (1) in connection with his experimental findings that mammary carcinoma, lymphoma and an undifferentiated sarcoma showed decreased "takes" subsequent to immersion in boric acid solution and *in vitro* irradiation by slow neutrons.

In an effort to test the effectiveness of the neutron-boron process *in vivo*, experiments were designed to attempt to localize boron or lithium atoms within or around the tumor mass of Mouse Sarcoma 180 during the period of irradiation with slow neutrons. This tumor type was considered suitable for such work because of its known radio-sensitivity and its standardizable characteristics.

Experiments.—The implantation technique together with the growth characteristics of Mouse Sarcoma 180 have been fully described by Sugiura (2), *et al.* Suffice it to say that when inoculated subcutaneously in the axillary region with a 2-mm. cube of freshly excised tumor tissue, male mice of 20–22 grams will ordinarily die during the third or fourth week following implantation. The implanted fragment undergoes rapid growth; metastases are not formed. Death presumably is due to impoverishment of the animal by the growing tissue mass, together with a toxemia syndrome resulting from the by-products of necrosis.

The aim was to inject the tumor with various boron or lithium preparations, followed by neutron irradiation of the whole animal with a dose somewhat below that which would ordinarily kill the animal due to general irradiation effects. It was hoped that by taking advantage of the neutron-boron reaction at the site of the tumor, one could develop a local ionization sufficiently intense to destroy the malignant tissue.

The mouse to be irradiated was placed in a small, perforated aluminum shell. Each shell was placed in a paraffin well capped with a paraffin block. For dimensions of these structures see figure 4. The blocks, piled in stacks of three, were placed within the radiation zone of the cyclotron target, in most experiments about forty centimeters from the target.

The neutrons were produced in the cyclotron at Columbia University by bombarding a beryllium target with protons of approximately 7 MEV energy. The neutrons emitted by this reaction have a spectrum rich in neutrons of 0.5 to 2 MEV and comparatively few have energies above 3 MEV.

Many of the neutrons from the cyclotron were slowed down by impacts with the hydrogen nuclei in the paraffin blocks. The thickness of the paraffin walls was a compromise between that necessary to obtain the maximum slow neutron radiation density in the region of the organism to be irradiated and the minimum ionization due to fast neutrons, taking into account the low intensity of the primary neutron beam. One inch of brass (the chamber wall) reduced the gamma radiation from the target, so that the background ionization due to gamma rays in the region of the tumor was less than 20% of the total ionization under these conditions. The increased ionization in a region containing boron is illustrated in figures 1 and 2. Sufficient space was not available for additional lead, but under more favorable conditions, its proper use could reduce the gamma ionization to a still lower value.

Dosages were adjusted from empirical biological observations, aided by existing information on the reaction of mice to known dosages of x-rays. This was necessary, first, because no accurate measure of the energy release of the slow neutron-boron reaction could be made at the tumor site; second, because the biological effectiveness of this energy is not quantitatively understood.

Before undertaking the boron experiments *per se* it was necessary to determine the lethal limit of irradiation in terms of time for the whole animal. Data given by Lawrence, Aebersold and Lawrence (3) indicate that whole-animal x-ray irradiation of 500 roentgens reduces the average life of mice following irradiation to 17 days; 600 r to 12 days; 700 r to 10.5 days; 800 r to 7 days; 1000 r to 5 days. Because of the scatter around each of these mean periods it was arbitrarily assumed that any of our irradiated animals surviving a period of 35 days had escaped the lethal effects of irradiation, and had not been subjected to a total ionization of more than the biological *equivalent* for the mouse of 400 r of x-rays. The validity of this assumption is borne out by Sugiura's (2) findings that 81% of mice receiving 400 r survive indefinitely.

Experiment 1 of table 1 indicates that 18 hours of irradiation in the paraffin wells was 100% lethal. In experiment 2 the irradiation period was reduced to ten hours, and it was found that 66.6% of the animals survived, indicating an approach downward toward the non-killing threshold. From a study of the Lawrence, Aebersold and Lawrence data and that of Sugiura, it was assumed that during somewhat under a ten hour period our animals were being subjected to an effective biological ionization equivalent of between 300 r and 500 r of x-radiation, which according to Sugiura's data is on the borderline between lethal and sub-lethal. The neutron dosage (without the paraffin filter) as read on a standard Victoreen r-meter was approximately 30 "n" per hour.

TABLE 1

TABLE PRESENTING DATA ON MICE SUBJECTED TO VARIOUS TYPES OF TUMOR INJECTIONS TOGETHER WITH SLOW-NEUTRON IRRADIATION

EXPER. NO.	MATERIAL INJECTED	QUANTITY	RADIATION PERIOD	NO. OF ANIMALS		NO. OF ANIMALS ALIVE AT END OF 35 DAYS	% OF TOTAL LIVING AND SHOWING TUMOR CURE OR REGRESSION	% OF TOTAL DYING FROM TUMOR, GENERAL RADIATION EFFECTS OR NATURAL CAUSES	% CURE OR REGRESSION DUE TO BORON-NEUTRON PROCESS
1	No tumor	...	6 hrs. on each of 3 successive days. Total—18 hrs.	Irradiated	18	none*	..	100.0	..
				Non-irradiated	24	21	..	12.5	..
2	No tumor	...	5 hrs. on each of 2 successive days. Total—10 hrs.	Irradiated	18	12	..	33.3	..
				Non-irradiated	24	22	..	8.4	..
3	Particulate boron in sesame oil	0.1 cc.	5 and 6 hrs. on each of two successive days. Total—11 hrs.	Irradiated	12	2	16.6	83.4	8.3
				Non-irradiated	12	1	8.3	91.7	..
3a	None	...	Same as in No. 3	Irradiated	12	1	8.3	91.7	..
				Non-irradiated	12	2	16.6	83.4	..
4	Lithium metaborate in sesame oil	0.1 cc. 50 mg.	6 hrs.	Irradiated	24	13	54.1	45.9	16.0
				Non-irradiated	21	8	38.1	61.9	..
5	Same as in No. 4	0.05 cc. 0.05 cc. Tot. 50 mg.	3 hrs. on each of two successive days. Total—6 hrs.	Irradiated	18	12	66.6	33.4	22.2
				Non-irradiated	18	8	44.4	55.6	..
6	Boric acid in sesame oil	0.05 cc. 0.05 cc. Tot. 50 mg.	4.5 and 3.6 hrs. on two successive days. Tot. 8.2 hrs.	Irradiated	24	12	50.0	50.0	45.0
				Non-irradiated	20	1	5.0	95.0	..
7	Same as in No. 6	0.1 cc.	7 hrs.	Irradiated	18	10	55.5	44.5	15.5
				Non-irradiated	20	8	40.0	60.0	..

* All animals in this experiment were dead within the first 25 days, presumably from general irradiation effects.

Since the appropriate time-dosage period was ascertained grossly to be between six and ten hours, it was necessary to develop a technique for retaining a high concentration of injected material at the tumor site for long periods. Preliminary experiments designed to test the permeability and diffusion characteristics of various forms of boron and lithium indicated: (1) that a saturated aqueous solution of boric acid injected in or around the tumor would not sustain itself in sufficiently high concentration for more than ten or fifteen minutes, as ascertained by making qualitative analyses for boron content of the tissue by the use of the quinalizarine tests of Feigl (4); (2) that finely pulverized metallic boron particles (in the order of 0.5–2.0 μ in dimension) when injected in oil suspension would localize largely at the oil-tissue interface and would not diffuse through the cell membranes or far into the intercellular spaces of the compact tumor tissue. Since the range of the disintegration products of the neutron capture processes was limited to less than fifty microns, the ionizing effect during radiation would be too local for a general tumor-killing effect. (3) That when powdered boric acid suspended in oil in liquid-paste form (one gram doubly pulverized boric acid suspended in two cubic centimeters of sesame oil) and injected into or around the tumor, a large excess of the boric acid could be localized and remain harmless to the tissue, slowly being taken into aqueous solution by the body fluids bathing the tumor and its environs. Thus a relatively high concentration of aqueous boric acid could be sustained at the tumor site for as long as several hours, draining out of the oil suspension into the soluble water phase. (4) That when lithium meta-borate (which is much less water-soluble than boric acid, and which in aqueous solution hydrolyzes into lithium hydroxide and boric acid) is likewise suspended in oil and injected, it goes into body solution even more slowly than the boric acid oil suspension, and therefore was considered suitable for injection preceding very long radiation periods. The toxicity of these materials will be discussed subsequently.

The exact method of injection is a point which warrants description. The needle was inserted under the skin about an inch posterior to the tumor, then passed subcutaneously into the tumor site and through the center of the tumor mass, and extended into the connective tissue anterior to the tumor. The needle was then slowly withdrawn as the plunger was applied so as to deposit material anterior to, within and posterior to the tumor. Before withdrawing the needle from the skin more material was deposited on either side of the tumor. The purpose, of course, was to bring the actively proliferating tumor tissue into close contact with the boron or lithium-bearing oil.

Having found the time-dosage threshold, it was necessary to establish the independent effects on the tumor of each of several variables. The first was radiation itself without injection. Twelve animals with growing, week-old

tumors were irradiated for eleven hours. Only 8.3% of these survived the 35-day period, the others dying presumably because of the sarcoma growth. In the non-irradiated control to this experiment 16.6% of the animals survived. Since the difference between 8.3 and 16.6, in view of the size of the sample, is hardly significant, it can be assumed that the whole body irradiation did not affect a significant regression of the tumors. This is rather what would be expected in view of the fact that from Sugiura's data it requires an equivalent of 1000 r to effect a 50% *in vivo* regression in shielded animals.

The second variable was the toxicity of the injected materials on both the whole animal and the tissue at the site of the injection. Preliminary experiments indicated that amounts of both boric acid and lithium metaborate could be injected subcutaneously into healthy mice in considerable excess to the 25–50 mg. doses injected for the experiments, without any observable ill effects. However, in experiments 4, 5, 6 and 7 it will be seen that the non-irradiated tumor-bearing mice receiving control injections equal to those in the irradiated specimens showed tumor regression or ab-

DESCRIPTION OF PLATE

Figures 1 and 2. Increased ionization produced in regions containing boron when bombarded with neutrons. Fig. 1: Without boron. Eastman Alpha Particle Spectroscopic plate, emulsion No. 129,975. Film exposed to cyclotron irradiation within aluminum shells and paraffin-well in position identical to that occupied by mice in the experiments described in the text. Three tracks due to protons projected by neutrons are clearly visible in the field, together with general photographic grain reduction due to protons projected at various angles to the plane of the film, together with projected nuclei such as carbon, and to gamma-ray background. This photo illustrates essentially the amount of ionizing energy released in an equivalent volume of hydrogen-rich tissue during an equivalent period under conditions described in the text when no boron is present. Magnification: × 1000. Fig. 2: With boron. Same type of film and same exposure time as in figure 1, except emulsion before exposure was dipped into a 2% aqueous solution of boric acid and allowed to dry. Shows numerous alpha particle tracks resulting from slow neutron-boron capture. Many tracks do not lie in plane of the photograph. Illustrates essentially the amount of ionizing energy released in an equivalent volume of tissue in the area of the tumor in which an equivalent concentration of boron following injection with boron salts was maintained. Cf. figure 1. It is the ionization differential illustrated in these two photographs which presents the basic rationale of the experiments described in the text.

Figure 3. Cyclotron with paraffin bricks *in situ*. In each brick are two wells of dimensions indicated in figure 4. Loosely placed in each well is a perforated aluminum shell for housing the living mouse during the radiation. The bricks, in stacks of three, were placed in an arc around the beryllium target in a position designed, so far as possible, to equalize the dosage for each animal. Thus twenty-four mice could be irradiated at one time.

Figure 4. Close view of paraffin brick and wells used in all the experiments. A mouse enclosed in the aluminum shell could be retained in relatively comfortable confinement for more than six hours. The wells were closed from above with paraffin slabs loosely fitted for ventilation.

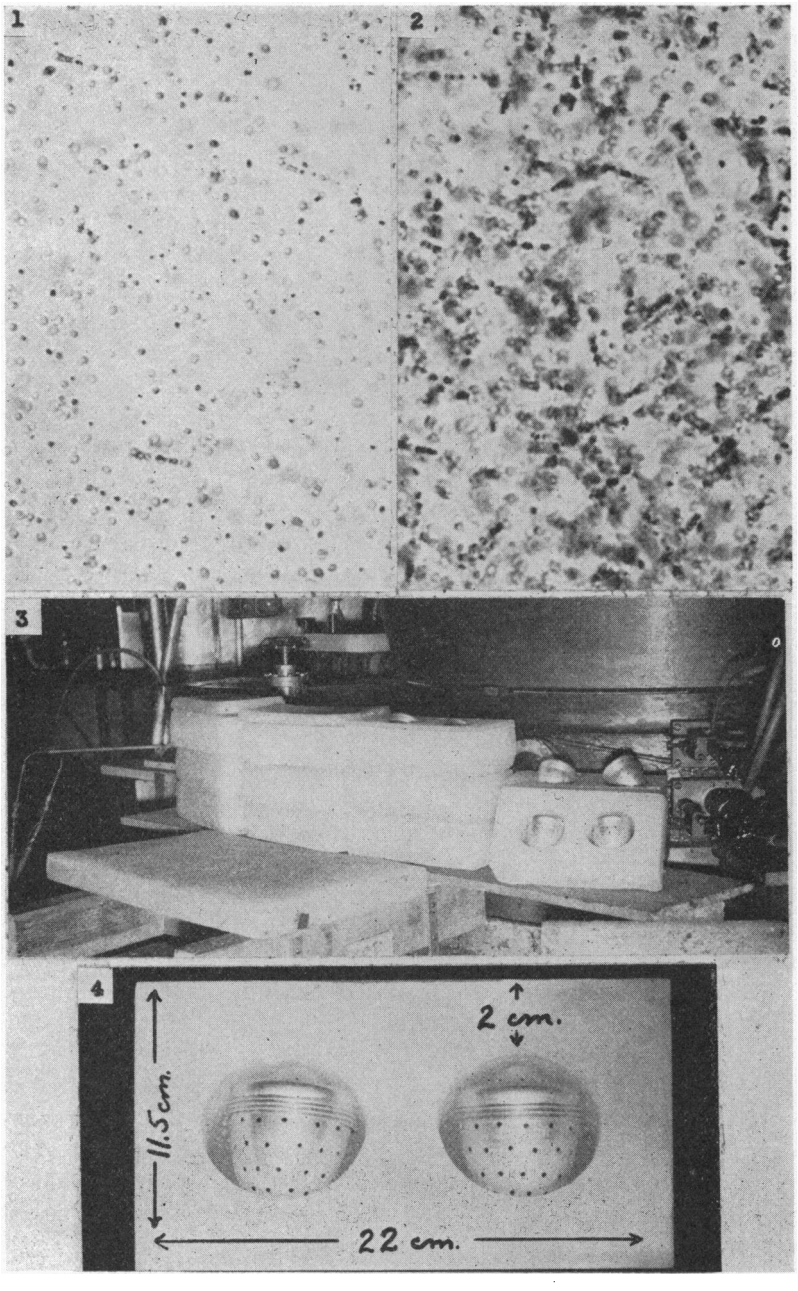

PLATE 1

See opposite page for description of plate.

sorption considerably higher than is normally found in non-injected and non-irradiated tumor-bearing mice. In the four experiments cited the percentages of regressions following injection alone were respectively 38.1, 44.4, 5.0 and 40.0, as compared with the 16% regression in the untreated mice.

This is taken to mean that one or both of the following factors are operative in causing this regression: (1) mechanical injury of the inserted needle to the growing tumor tissue, to the blood channels feeding it or to the connective ground tissue; (2) toxic or destructive effects of the injected materials on the growing tumor tissue, on the blood channels feeding it or on the connective ground tissue. One is inclined to believe that a combination of these two factors is responsible for the increased regression incidence following mere injection of the materials under question.

It is obvious that only percentages of regression significantly above that caused by these two factors can be considered as due to the slow neutron-boron process in the experiments which combine the irradiation and the localization of the boron or lithium compounds within or around the tumor. A study of the data of experiments 4, 5, 6 and 7 of the table clearly indicates that the regression in mice receiving both irradiation and the boron or lithium injections is considerably higher than in those receiving the injections alone, the percentage differences in these four cases being respectively 16.6, 22.2, 45.0 and 15.5. The differences or boron-neutron cures are not large, but since in all four experiments they are consistent, their significance seems conclusive.

Discussion.—The actual variation in the magnitude of these regression differences seems to be subject to any of several explanations: (1) Although the animals, in relation to the source of the irradiation, were placed in such a position so as nearly as possible to equalize the dosage of all the animals being irradiated at one time, complete uniformity was impossible to achieve, due to the architecture of the cyclotron. (2) Fluctuations in the output of the cyclotron during the excessively long radiation periods. (3) Variations in the effectiveness of the injections: the amount of injury, and diffusion factors of the injected materials. (4) Variation in the normal frequency of regression.

The ionization at the site of the injection was not known. As cited above from Sugiura's experiments, a radiation equivalent to 1000 r of x-rays is necessary to cause a 50% regression *in vivo*. With 750 roentgens of x-rays Sugiura reports a 20% regression *in vivo*. Considering the magnitude of our regressions as between 15.5 and 45.0 per cent, and postulating the actual destruction of the malignant cells, it is necessary to assume that an ionization approximately equivalent to 750–1000 roentgens of x-rays was being achieved within or at the tumor, whereas the whole body was being subjected to less than the equivalent of 400 r. We assume that this energy

difference was the result of the ionization caused by the disintegration products of the slow neutron-boron process.

On the other hand, due to the peculiar selective permeability properties of the living cell membrane, we find it difficult to believe, following the injection of boric acid or lithium meta-borate into and around the tumor mass, that as the material slowly goes into aqueous solution a high concentration is maintained uniformly both within and out of the malignant cells. Even if material were injected and retained at the site of the tumor, it was early doubted whether the boric acid or lithium hydroxide would diffuse uniformly throughout the tissue mass in concentrations necessary to be effective in capturing a large proportion of the slow neutrons. Indeed, the question of whether the ions would easily permeate the membranes of tumor cells has not yet been established, and there is little evidence to indicate that they would concentrate in sufficiently large quantities to be effective. It is more likely that they diffuse into and through the intercellular spaces. But in the case of a compact tumor such as Mouse Sarcoma 180 the amount of intercellular diffusion is limited.

For this reason, and because of the relatively high incidence of ulceration around the tumor following the injection-radiation treatment, at the present time the curative regression is interpreted as due to one or a combination of the following factors: (1) alpha particle or proton destruction of some or all of the malignant cells, (2) impairment of the vascular system feeding and draining the tumor, (3) radiation effect on the connective tissue base of the tumor.[6]

One of the interesting aspects of this work is that, unlike x-ray therapy where shielding of tissues not under treatment must be carefully applied, we were able to subject the whole body to the same extrinsic energy as the tumor; but that because of the neutron-boron process we were able to set up a high ionization differential between the tumor and the rest of the body.

The authors consider that for any possible future employment of the slow neutron-boron process in tumor therapy, some device other than simple hypodermic injection should be developed for localizing either boron or lithium or related materials in malignant tissue. This is particularly essential in the case of involved metastasizing and deeply situated growths. Experiments involving the intravenous injection of large particle colloidal dyes to which the lithium atom is attached have indicated considerable localization of lithium in spontaneous mouse tumors. This work is being continued.

It is to be mentioned also that concurrently with the work described in this communication, experiments were undertaken in which boron in one form or another was injected into the mouse testis, followed by slow neutron irradiation. A clearly observable effect on the germinal cells of the seminiferous tubules much more extensive than either the radiation effect alone

or the effect of the chemicals also was observed. These results will be published elsewhere.

Summary.—Transplantable mouse sarcomas were injected with various forms of slow-neutron-capturing materials. When the whole animal whose tumor was so injected was irradiated with slow neutrons, a significant increase in tumor regression was observed. This increase is attributed to the localized ionization resulting from the nuclear disintegration products of the capture process.

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[4] The cross-section is a measure of the probability of interaction of the neutron with the nucleus, i.e., if there is a flux of one neutron per centimeter squared per second and one atom per cubic centimeter, the cross-section represents the probability of this neutron being captured by the atom.

[5] Other elements in tissue also capture some slow neutrons but the resulting contribution to *local* ionization is not extensive. It should be noted that the small absorption within the tissue of the 2.2 MEV γ-radiation resulting from slow neutron capture by hydrogen largely offsets the efficiency of this capture process, at least as regards local ionization.

[6] Sugiura (5) and others have demonstrated that the incidence of tumor-takes is significantly lower in areas which have been previously subjected to irradiation, thus indicating the importance of the physiological condition of the tissue base to the proper growth of introduced cancerous tissue.