# SOME BIOLOGICAL EFFECTS OF NUCLEAR DISINTEGRATION PRODUCTS ON NEOPLASTIC TISSUE*

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Experiments of the type described below were initiated in the autumn of 1938 by Professors B. V. Hall, M. Goldhaber and the author at the Physics Department of the University of Illinois. Since the neutron intensity from the small cyclotron there was insufficient to give any conclusive result, the experiments have been continued in the Crocker Radiation Laboratory where intense neutron sources are available.

In the past, neoplastic tissue has been irradiated with x-ray, $\gamma$-rays and fast neutrons. In the case of x-ray and $\gamma$-ray irradiation the destructive ionization in the tissue is produced by Compton electrons, photoelectrons or positive-negative pair electrons which are ejected or created by the x-rays and $\gamma$-rays. The physical processes involved here are well known, and the resulting ionization per unit distance along the path of the electron is small as compared to heavy particle ionization.[2]

The process of neutron irradiation is quite different from the above, since it involves a collision process between two heavy particles instead of between a photon and an electron. Here the recoil proton obtains energy (varying from zero to the neutron energy) from the neutron and dissipates the energy by producing along its path an ionization which is much more intense than that produced by electrons.[2]

In the experiments discussed below, the ionizing bodies are the disintegration products produced when boron is bombarded with slow neutrons. In nuclear terms the reaction is represented by ${}_5B^{10} + n_s^1 \rightarrow {}_3Li^7 + {}_2He^4$. This reaction is one of the most favorable ones known for use in biological experiments of the type here discussed because the capture cross-section for slow neutrons ($n_s^1$) by boron is about 100 times larger than the collision cross-section for fast neutrons and hydrogen. Thus one would expect this nuclear disintegration process to be more efficient in biology than fast neutron irradiation. Moreover, while the incident slow neutrons have a very small energy (a fraction of an electron volt up to a few electron volts), the disintegration products of the boron slow neutron reaction (${}_3Li^7$ and ${}_2He^4$) have approximately 0.8 m. e. v. and 1.4 m. e. v. energy.[3] These rather large nuclear energies are dissipated in very short distances (approximately 4 and 7 microns) in tissue and so cause an even more intense ionization along their paths than the recoil proton in the fast neutron irradiation process. Thus, from the knowledge of nuclear physics alone, it is clear that the boron slow neutron reaction should cause cell destruc-

tion more efficiently than other types of irradiation, if the disintegration can be produced in the environment of neoplastic cells.

This method has the further potential advantage of localizing the lethal ionization in the region where the boron disintegration takes place and thus removes (in the case of its application to *in vivo* work) the danger of

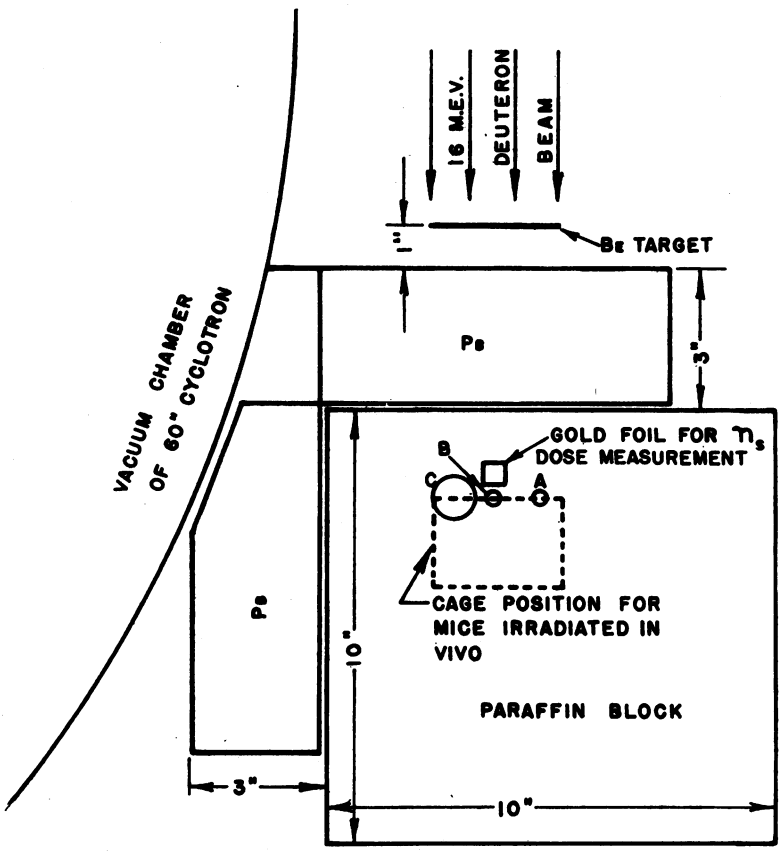


FIGURE 1

Schematic arrangement of beam, target and irradiation positions in the paraffin block.

skin burns and similar disturbing factors which are prevalent in x-ray, $\gamma$-ray and fast neutron therapy. This follows from the fact that slow neutrons have so little energy that any ionization caused by a recoil proton from them is negligible. Also it should be remarked that no element having an appreciable concentration in tissue, has a cross-section for slow neutron capture comparable to boron, and thus no ill effects due to slow

neutron irradiation can occur elsewhere in the body. Consequently one can irradiate with large slow neutron doses, provided care is taken to keep the background dose of fast neutrons and $\gamma$-rays below the lethal amount.

Figure 1 shows, schematically, the experimental arrangement for producing the slow neutrons used for the irradiations and the relative positions of the irradiated samples in the paraffin block. Fast neutrons are produced, by bombarding Be with 16 m. e. v. deuterons in the 60-inch cyclotron,[4] according to the reaction ${}_4Be^9 + {}_1D^2 \rightarrow {}_5B^{10} + {}_0n^1$. These fast neutrons are slowed down by many collisions with hydrogen nuclei in the paraffin block and are thus available at positions *A*, *B* and *C* for irradiating samples placed at *A*, *B* or *C*. The Pb blocks shown in figure 1 were placed between the target, cyclotron and the irradiated samples to reduce the $\gamma$-ray background from the Be target. A thin sheet of gold (Au detector in figure 1) was positioned just in front of hole *B* in the paraffin block and the slow neutron induced radioactivity in the gold used as a measurement of the slow neutron dose for the various irradiations. Measurements of the gold activity were made in the conventional manner using an ionization chamber, amplifier and a scale of four counter. The fast neutron-$\gamma$-ray background was measured with a victoreen dosemeter.

The procedure for preparing small pieces of mammary carcinoma, lymphoma and an undifferentiated sarcoma for irradiation and implantation is as follows. A tumor, about ten days old, is taken from the animal and chopped into small pieces suitable for implantation with a trocar. These are placed in a soft glass test tube about $1/4$ inch in diameter and immersed in a solution made by adding 2 gm. $H_3BO_3$ to 100 cc. of buffer solution. Three such samples are made up. One is kept in the laboratory as a control and is not irradiated. This hereafter will be designated as the boron control. A second, the boron irradiated, is placed in position *A* in the paraffin block. The third is placed inside of a one-inch diameter glass tube and the intervening space filled with $B_6C$. This is placed in position *C* and is designated as a $B + B$ shield. A fourth sample has been prepared for some experiments (mammary carcinoma *A*, *B*, *E* and lymphoma *C*) by omitting the $H_3BO_3$ from the immersing solution. This is placed in hole *B* and is called the buffer control. All tumors used were known by previous experimentation to give essentially 100 per cent takes for normal implants.

During the course of an irradiation the boron irradiated sample (*A*) receives $\gamma$-rays ($\gamma$) and fast neutrons ($n_f$) as background radiation and slow neutrons ($n_s$). Sample *C* receives mostly background $n_f + \gamma$ radiation, the $B_6C$ absorbing a large part of the slow neutrons except for heavy doses. Thus the resultant differential growth between samples *A* and *C* represents, in a rough way, the effect of the slow neutrons. Sample *B* receives $n_f + \gamma + n_s$ and is simply a control to test for any possible effect

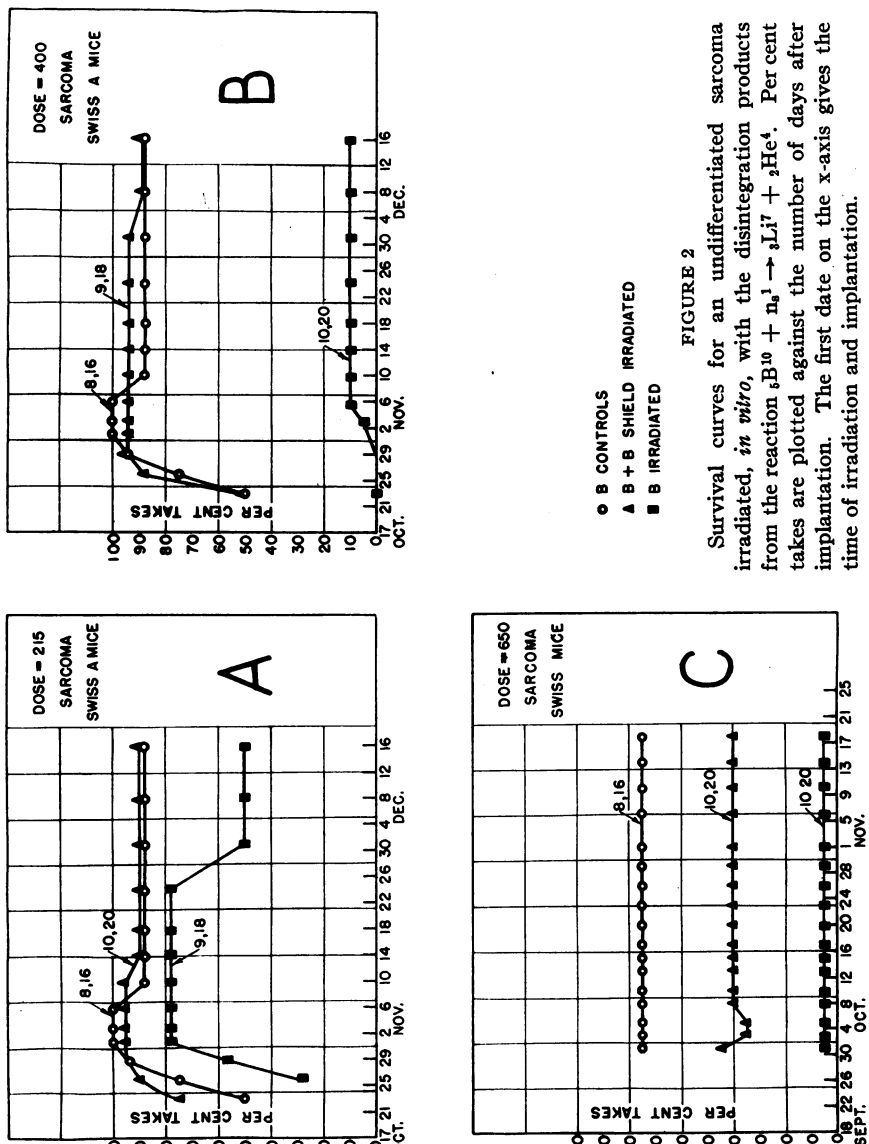


FIGURE 2

Survival curves for an undifferentiated sarcoma irradiated, *in vivo*, with the disintegration products from the reaction ${}_5B^{10} + n_0^1 \rightarrow {}_3Li^7 + {}_2He^4$. Per cent takes are plotted against the number of days after implantation. The first date on the x-axis gives the time of irradiation and implantation.

of irradiation in buffer solution. None was observed as would be expected since none of the elements in the buffer solution have a slow neutron capture cross-section comparable with boron.

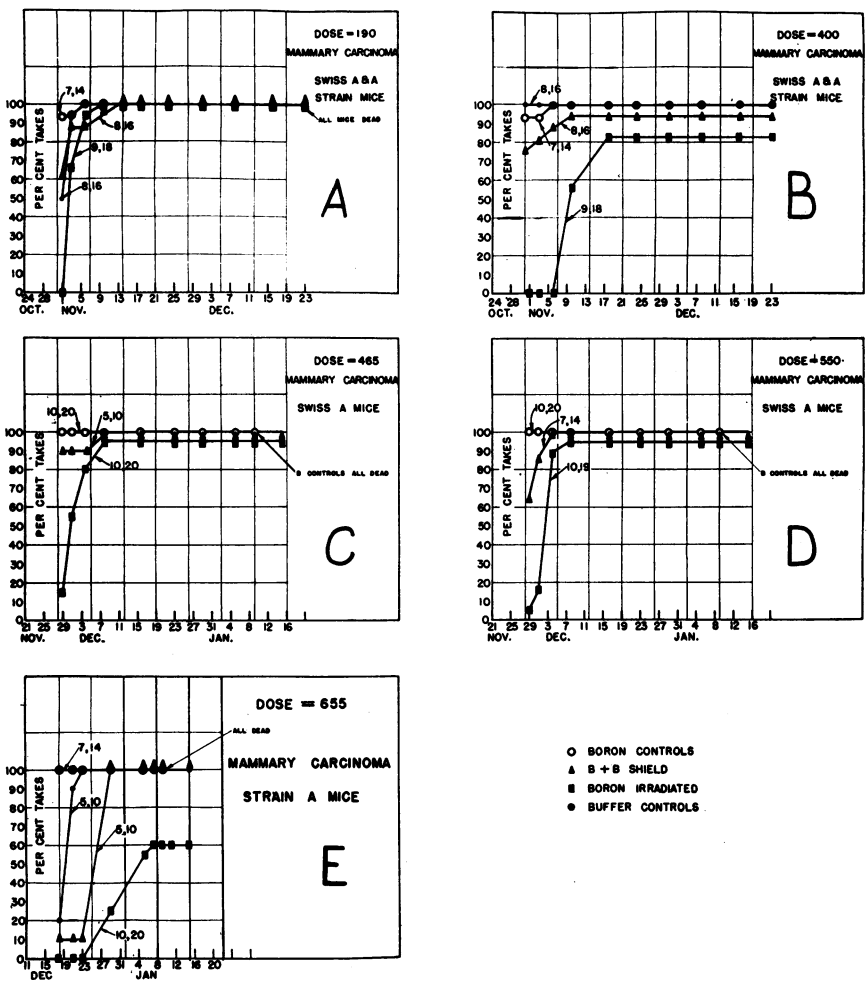


FIGURE 3

Survival curves for mammary carcinoma irradiated, *in vitro*, with the disintegration products from the reaction ${}_5B^{10} + n_0^1 \rightarrow {}_3Li^7 + {}_2He^4$. Per cent takes are plotted against the number of days after implantation.

After irradiation, two tumor particles are implanted in each mouse (one on each side) used. Thereafter for about eight weeks, the number of takes, for each experiment, was checked at least once a week by counting the number of tumors observable in each mouse and by measuring the

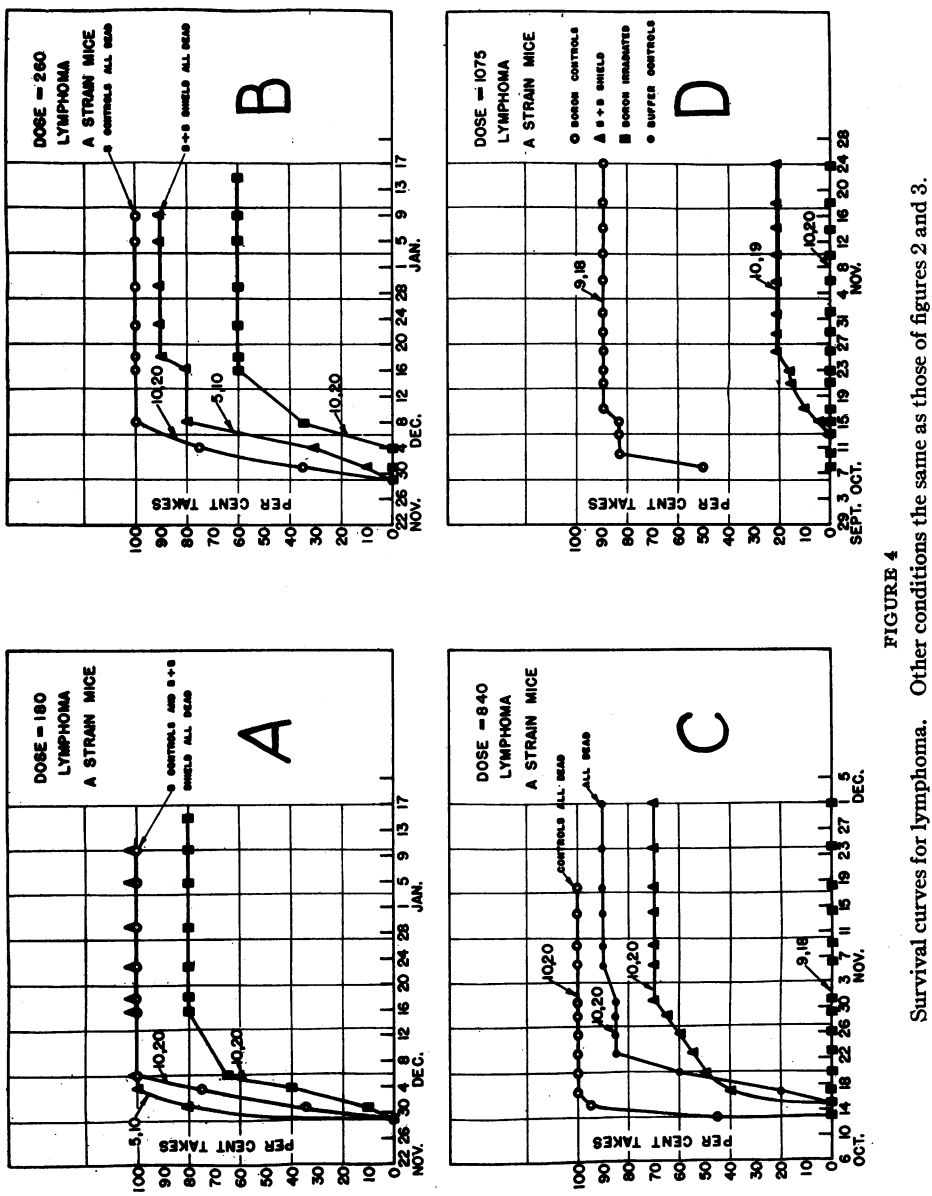


FIGURE 4

Survival curves for lymphoma. Other conditions the same as those of figures 2 and 3.

size of the tumors. The results of these measurements are shown graphically in figures 2, 3 and 4.

Figure 2 shows the effect of the boron disintegration products on an undifferentiated sarcoma which occurred spontaneously in a swiss mouse four years ago, and since then has been observed and studied by Professor B. V. Hall of the University of Illinois. The figure has three sets of curves (*A*, *B* and *C*), one for each of three doses given the tumor particles prepared and irradiated as described above. The dose, given in the upper

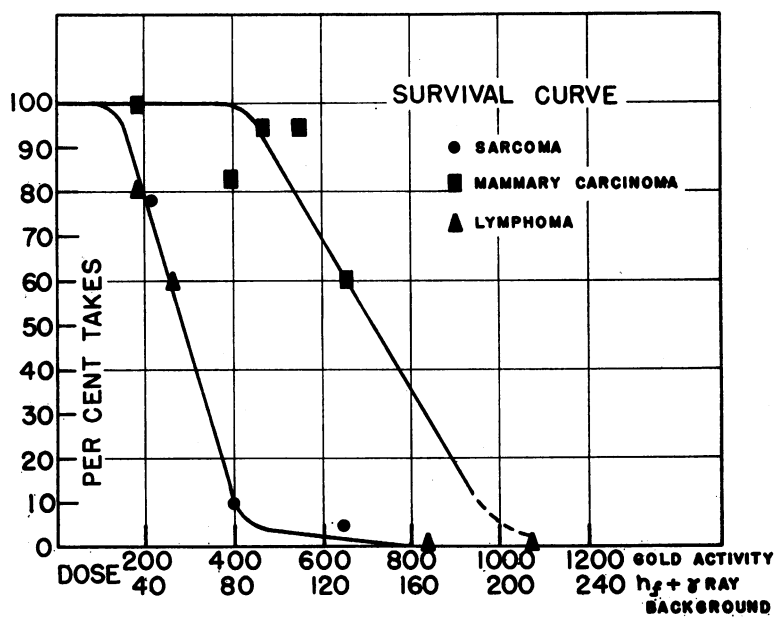


FIGURE 5

Comparison survival curves for sarcoma, mammary carcinoma and lymphoma. Here the maximum per cent number of takes are plotted against the slow neutron dose as measured by gold activity and for reference on another scale the associated $n_f + \gamma$ background dose.

right-hand corner of parts *A*, *B* and *C* of the figure, is the result of the measurement of the radioactivity induced in the gold foil (see Fig. 1) by slow neutrons during the tumor irradiation. For this reason it is a relative dose measurement and, while comparable for all of the experiments described here, has no direct comparison with other dose measurements (i.e., fast neutron doses as measured by a victoreen dosemeter) or even other gold activity dose measurements made under different experimental conditions. Associated with each curve are two numbers, the first one of which gives the number of mice used, the second the number of tumors

implanted (i.e., curve in figure 2*A* for boron irradiated samples has the numbers 9, 18: this means 9 mice and 18 implants).

In a qualitative way the boron shield acts as would be expected. For doses 215, 400 and 650 (Fig. 2*A*, *B*, *C*) the per cent takes are 95, 94 and 40 per cent, respectively. This indicates that for the first two doses enough slow neutrons were absorbed by the $B_6C$ shield so that the transmitted neutrons had little effect on the tumors. However, at dose 650 enough slow neutrons were transmitted to cause 60 per cent deaths. For the

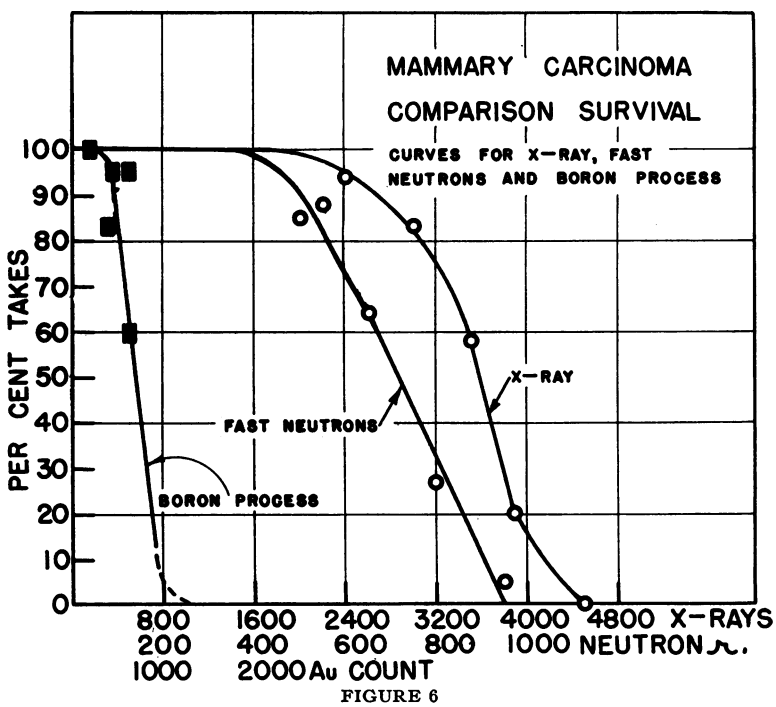


FIGURE 6

Comparison survival curves for mammary carcinoma irradiated with x-ray, fast neutrons and boron disintegration products.

above doses the $n_f + \gamma$ background was approximately 45, 80 and 130 "n." No data concerning the effect of fast neutrons on this sarcoma are available.

Figure 3 shows the effect of the boron disintegration products on mammary carcinoma.[5] The notation here is the same as that for figure 2. In three (*A*, *B* and *E*) of the five experiments performed on this tumor a group of animals were inoculated with implants irradiated in buffer solution as described above. All three groups show 100 per cent takes as was expected from theoretical considerations. The only other similar experi-

ment performed was in part *C* of the lymphoma experiments where 90 per cent takes were observed.

Figure 4 shows the effect of the boron disintegration products on lymphoma.[6] The notation is the same as for figure 2. Here the effect of the boron shield is nicely portrayed. For the doses 180, 260, 840, 1075, the per cent takes for the boron shield irradiated implants are 100, 90, 70 and 21 per cent. The $n_f + \gamma$ background corresponding to the above doses is 35, 50, 170 and 215 "n."

The data in figures 2, 3 and 4 are given in tabular form in table 1.

TABLE 1

SUMMARY OF DATA ON THE *in vitro* IRRADIATION OF SARCOMA, MAMMARY CARCINOMA AND LYMPHOMA WITH DISINTEGRATION PRODUCTS FROM THE REACTION $_5B^{10} + n_0^1 \rightarrow {}_3Li^7 + {}_2He^4$

| DOSE FROM GOLD ACTIVITY | APPROXIMATE BACKGROUND $n_f + \gamma$ | PER CENT TAKES BORON IRRADIATED | PER CENT TAKES B + B SHIELD | PER CENT TAKES BORON CONTROLS | PER CENT TAKES BUFFER CONTROLS |
|---|---|---|---|---|---|
| | | *Sarcoma* | | | |
| 215 | 45 | 78 | 95 | 100 | |
| 400 | 80 | 10 | 94 | 100 | |
| 650 | 130 | 5 | 40 | 75 | |
| | | *Mammary Carcinoma* | | | |
| 190 | 40 | 100 | 100 | 100 | 100 |
| 400 | 80 | 83 | 94 | 100 | 100 |
| 465 | 90 | 95 | 100 | 100 | |
| 550 | 110 | 95 | 100 | 100 | |
| 655 | 130 | 60 | 100 | 100 | 100 |
| | | *Lymphoma* | | | |
| 180 | 35 | 80 | 100 | 100 | |
| 260 | 50 | 60 | 90 | 100 | |
| 840 | 170 | 0 | 70 | 100 | 90 |
| 1075 | 215 | 0 | 21 | 89 | |

In figure 5 there is plotted the maximum per cent number of takes (*B* irradiated sample) taken from the curves in figures 2, 3 and 4, vs. the dose for the three tumors used. Here it appears that the sarcoma and lymphoma have about the same sensitivity to the radiation used and that a dose of 450 (gold activity) will kill both kinds of tumors *in vitro*. This corresponds to a $n_f + \gamma$ background of about 90 "n." The mammary carcinoma is more resistant to radiation and needs a dose of about 1000 (gold count) with a background of 200 "n" for 100 per cent lethal effects.

Figure 6 shows a comparison between the effects of x-rays, fast neutrons and boron disintegration products on mammary carcinoma. The x-ray and fast neutron data are taken from curves published by J. H. Lawrence, P. C. Aebersold and E. O. Lawrence.[2]

The fast neutron curve shows that below approximately 500 "n," no failure of takes occurs. Since, for the boron process, the $n_f$ background accompanying the lethal dose (gold activity 1000) of boron disintegration products is only 200 "n," that background cannot be responsible for the lethal effects observed, and one must conclude that the boron disintegration products are responsible for the death of the tumor cells. Another

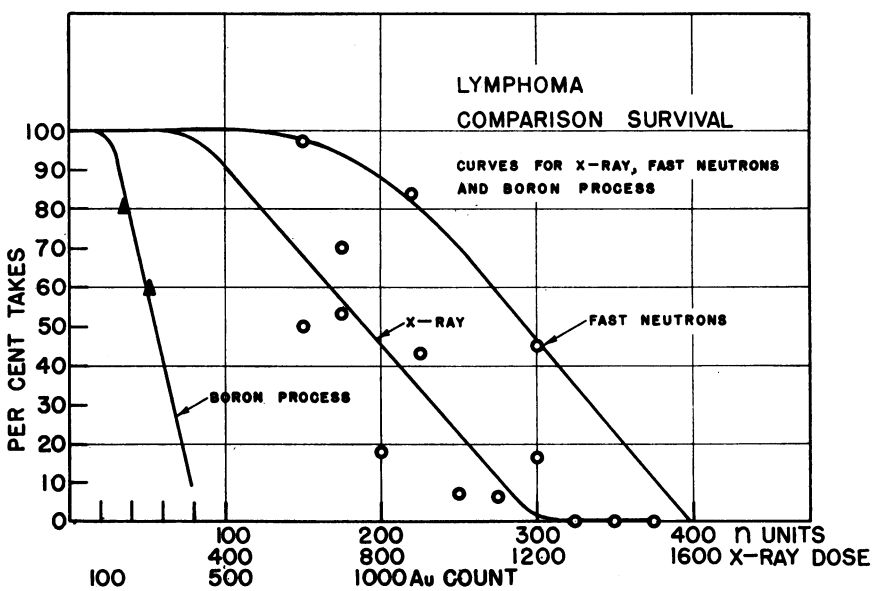


FIGURE 7

Comparison survival curves for lymphoma irradiated with x-rays, fast neutrons and boron disintegration products.

interesting comparison is to note that the dose of fast neutrons for 100 per cent lethal effects is approximately 950 "n," which is about five times the $n_f$ background in the boron process for the same effect. It must be emphasized, however, that the factor five depends on the amount of boron which can be gotten into the tumor and that the factor is meaningless except for the fact that it shows that a sufficient amount of boron can be dispersed throughout the tissue, to accomplish the desired lethal effect.

Figure 7 draws a similar comparison for lymphoma. Here the fast neutron data are taken from preliminary experiments being conducted[7] in the Crocker Radiation Laboratory at the present time and the x-ray

data from exploratory and unconfirmed results. The fast neutron sublethal dose is approximately 175 "n" whereas the $n_f + \gamma$ background accompanying 100 per cent lethal effect in the boron process is about 90 "n" so that again the lethal effects observed here must be due to the boron disintegration products. The fast neutron dose for 100 per cent lethal effect is approximately 400 which is about four times the $n_f + \gamma$ background in the boron process for the same effect.

As shown by the data in table 1, the average per cent number of takes for the boron controls in the three experiments on sarcoma is 92 per cent; for the five experiments on mammary carcinoma it is 100 per cent; and for the four experiments on lymphoma it is 97 per cent. This shows that the boric acid solution when not irradiated has no effect on the growth of these neoplastic tissues.

In considering the data from these experiments, it must be remembered that the number of mice used (see figures 2, 3 and 4) was small so that the shape of the survival curves in figure 5 is known only approximately. It would be of interest to repeat these experiments with a large number of mice to establish the curves more accurately.

In figure 2*A* and *B* there is evidence for some natural regression of the undifferentiated sarcoma. In those cases where regression occurred the tumors grew to good size (1 cc. to 2 cc. volume approximately), became neucrotic and then sloughed off. Eventually some healed completely so that it is unsafe to use this tumor for *in vivo* work.

The results of these *in vitro* experiments also indicate that neoplastic cells can be destroyed *in vivo*, if sufficient boron, in some suitable form, can be applied to the tumor *in vivo*.

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[6] J. W. Lawrence and W. U. Gardner, *Amer. Jour. Cancer*, **33**, No. 1, 112 (May, 1938).

[7] The experiments are being conducted by J. H. Lawrence, P. C. Aebersold and D. Axelrod.

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# *A DECOMPOSITION OF COMPACT CONTINUA AND RELATED THEOREMS ON FIXED SETS UNDER CONTINUOUS TRANSFORMATIONS*[1]

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*1. Definitions and Theorems on F-sets.*—We suppose throughout that $M$ is a compact metric continuum.

DEFINITIONS:[2] A point $p \epsilon M$ is *conjugate* to $q \epsilon M$ provided no point separates $p$ and $q$ in $M$. If $p$ is a non-cut point, $Mp$ is defined to be the set of all points conjugate to $p$. $p \epsilon M$ is an *end-point* of $M$ provided there exists an arbitrarily small neighborhood of $p$ having as its boundary a single point. A set is said to be an *F-set* provided it is (1) an end-point of $M$, (2) a cut point of $M$ or (3) a non-degenerate $Mp$.

THEOREM 1.1: *Any set $Mp$ may be written as a monotone product $Mp = \pi_1^\infty C_i$, where each $C_i$ is a continuum, the closure of the complement of which consists of a finite number of continua, each intersecting $C_i$ in a single point.*

This theorem is proved by a direct construction, making use of the lemma to the effect that there exists a countable basis for the cut points of $M$, i.e., a countable set of points $[p_i]$ such that if any two points of $M$ are not conjugate, some point of $[p_i]$ separates them in $M$. This fundamental theorem implies

THEOREM 1.2: *An F-set is a continuum: the product of an F-set and a continuum is a continuum or vacuous.*

THEOREM 1.3: *$M$ is the sum of its F-sets.*

This theorem is proved by showing that if $p \epsilon M$ is not an end-point and has no conjugate point, it is a cut point. This gives an independent proof of the known result when $M$ is locally connected. For non-locally connected continua the result is new. From this theorem we obtain

THEOREM 1.4: *Each non-cut point of $M$ belongs to one and only one F-set.*

THEOREM 1.5: *In order that two points $p$ and $q$ belong to the same F-set it is necessary and sufficient that $p$ and $q$ be conjugate.*