of 19% ethyl alcohol (equivalent to 0.06 cc of 95% ethyl alcohol) caused no change in the tumor, whereas 0.02 cc of 95% ethyl alcohol produced marked reduction in the tumor size. This was confirmed by histological studies (Fig. 1). The histological changes seen in the tumors of animals receiving the concentrated alcohol resemble those obtained with bis(β-chloroethyl)sulfide (*1*), the main feature of which is diffuse cell necrosis throughout the entire tumor. These changes are qualitatively not unlike spontaneous cellular changes seen in large lymphoid tumors; however, quantitatively the difference is quite striking.

Since there is much current interest in the response of lymphoma to various chemical agents, it is an appropriate time to call attention to the fact that the normal lymphocyte can be affected both in the circulating blood and in lymphoid structures by indirect action of chemical substances. Moreover, the data presented show that this same type of response can be produced in malignant lymphoid tissue. At present we are not prepared to say that this effect of concentrated alcohol on the malignant lymphocyte is mediated through the adrenal, as Selye has suggested for the normal lymphocyte in the case of colchicine-treated animals. However, it appears that the nonspecific damage caused by the concentrated alcohol, and not the ethyl alcohol itself, is responsible in some way for the malignant lymphocyte effect.

References

1. Bass, Allan D., and Freeman, Marion L. H. *J. nat. Cancer Inst.*, 1946, **7**, 171.
2. Elmadjian, Fred, and Pincus, Gregory. *Endocrinology*, 1945, **37**, 47.
3. Lu, C. S., and Kirschbaum, Arthur. Reported at 4th International Cancer Research Congress, 1947.
4. Selye, Hans. *J. clin. Endocrinol.*, 1946, **6**, 117.

Some Biological Effects Due to Nuclear Fission[1]

C. A. Tobias, P. P. Weymouth, and L. R. Wasserman

Division of Medical Physics, University of California, Berkeley

G. E. Stapleton

Clinton Laboratories, Monsanto Chemical Company

Since direct interaction of neutrons with the atoms and molecules of the animal body is small, most of the biological effects produced by neutrons are due to secondary ionizing radiations. As is well known, fast neutron effects are due mainly to recoil nuclei from elastic collisions in the tissue (*1*). Thermal neutrons exert their effect primarily through ionizing radiations produced in transmutations of the elements composing the body and, to a lesser extent (~ 1%), through neutron-induced radioactivities. The relative importance of various nuclear reactions in causing biological effects has been elucidated by Zirkle (*17*) and his collaborators.

Localized effects may be artificially produced in the animal body or in selected organs if one deposits in the body or organ some element A, the cross section of which is relatively high for a suitable slow neutron-induced transmutation. If the organism is then bombarded with slow neutrons, the action of the disintegration products and of induced radioactivities resulting from the interaction of the neutrons and atoms of element A will be superimposed on the general effects of the neutrons. Several elements with suitable properties for such use are known. Among them is boron, where the $_5B^{10}$ (n, α)$_3$ Li^7 reaction has a high cross section. The lithium nucleus and the alpha particle emitted in this reaction produce ionizations in the tissue. Kruger (*8*) suspended tumor slices in boric acid solution and bombarded them with slow neutrons. With neutron doses above a certain level these tumors would no longer grow after transplantation. On the other hand, control tumor slices untreated with boric acid were not affected by neutron doses up to a much higher level. Likewise, boric acid without neutrons was without effect. Zahl, *et al.* (*15*) infiltrated sarcomata *in vivo* with lithium and boron compounds. Li^6 splits into an alpha particle and tritium on slow neutron bombardment. These disintegration products caused regression of some of the sarcomata. The same authors also showed that azo-dyes containing lithium can be localized to some extent in tumors.

To our knowledge, fissionable materials have not been used thus far in studying the biological effects of fission fragments and their radioactivity produced *in vivo*.[2] The study of such effects is interesting for at least three reasons. First, the knowledge of the specific effectiveness of heavily ionizing fragments may be of importance in understanding the mechanism of radiation effects. It should be mentioned that alpha particles and protons are known to cause change in animal tissue more effectively per unit energy transferred than electrons, and it is not unreasonable to assume that the heavily ionizing fission fragments might be even more effective than alpha particles. Secondly, the use of fission *in vivo* also has potential application in radiation therapy, particularly if fissionable elements can be incorporated into compounds which may localize at specific sites in the animal body. Steps in this direction have been made for example, by McClintock and Friedman (*10*), who were able to combine uranium with certain antibodies. Finally, the knowledge of *in vivo* fission effects is of interest from the point of view of radiation health protection in atomic energy plants and laboratories.

An over-all view of numerical factors involved in a comparison of the properties of neutron disintegration of elements important to this discussion may be ob-

[1] It is a pleasure to thank J. H. Lawrence for his active participation. We are also grateful to A. Hollaender, P. S. Henshaw, and J. G. Hamilton for discussions and use of their facilities; to R. L. Dobson, H. O. Anger, Constance Tregillus, and Jean Luce for their collaboration; and to the Radiological Unit at Clinton Laboratories for courteous cooperation. This document is based on work performed under contract No. W-7405-eng-48 for the Atomic Energy Project at the Radiation Laboratory, University of California.

[2] Zahl, *et al.* (*15*) mentioned the possibility of the use of uranium.

tained from Table 1. H and N are the elements primarily responsible for slow neutron effects in normal tissues; other tissue constituents—C, O, P, Ca, S, etc.—make only minor contributions. It is assumed in the calculations that the size of the tissue is quite large, so that all the energy in the 2.2 Mev gamma ray liberated in neutron capture by hydrogen is absorbed. This table indicates that usually a rather small concentration of Li, B, or U should give observable localized tissue effects with neutron bombardment. In certain experiments even smaller concentrations may be effective. For example, in small animals such as the mice used in our experiments, the gamma rays produced in neutron capture by hydrogen are only slightly absorbed (~ 3%); consequently, smaller amounts of localized material suffice to give equivalent effects. In man, approximately 30% of the gamma rays would be absorbed. However, if fissionable materials are used, there are certain limiting factors: the alpha radioactivity should not exceed a certain level if one is to avoid long-time radiation effects, and the amount introduced into the tissue should not be in such a form or in sufficient quantities to produce toxic reactions. The figures in Table 1 are only true when thermal neutrons are used. Slow neutrons obtained from conventional sources usually are not strictly thermal. Finally, a correction should be made for absorption of neutrons in the overlying tissue, which cuts down the neutron intensities for the deeper layers by a considerable factor. The table also shows the mean energy loss of the disintegration fragments per micron of tissue. Fission recoils have extremely high energy loss and correspondingly short range, a few cell diameters only.

TABLE 1

COMPARATIVE DISINTEGRATION DATA OF A FEW ELEMENTS BOMBARDED WITH THERMAL NEUTRONS

Isotope	$_1H^1$	$_7N^{14}$	$_3Li^6$	$_5B^{10}$	$_{92}U^{235}$
Isotope abundance (%)	99.98	99.62	7.5	18.4	0.71
Transmutation process	(n, γ)	(n, p)	(n, H^3)	(n, α)	(n, f)
Process cross section $\times 10^{24}$ cm^2	0.31 (*12*)	1.7 (*11*)	860 (*12*)	3,525 (*12*)	420 (*2*)
Energy liberated/disintegration in Mev	2.2	0.6	4.6	2.8	159* (7)
Mean energy loss of disintegration products/μ tissue† (Mev/μ)	0.002 secondary electrons	0.067 (protons)	.16 (alphas)	.15 (alphas)	9 (*11*) fission recoils
Amount of isotope needed to liberate same energy as 1 gm wet tissue (gm)	0.104	0.96	1.06×10^{-4}	7.1×10^{5}	2.6×10^{-4}
Correction factor for biological effectiveness of dense ionization	1	4 (*16*)	7 (*16*)	7 (*16*)	27‡
Isotope needed to produce biological effect equal to neutrons alone in 1 gm wet tissue (gm)	0.112	0.25	16×10^{-6}	11×10^{-6}	7×10^{-6}

* Radioactivity of fission products (— 25 Mev) neglected.
† 10% hydrogen and 3% nitrogen assumed in tissue.
‡ As determined in the present paper.

From consideration of the table given, with corrections made for the laboratory mouse, it was believed that as little as 2.2 μg of U^{235}/gm of mouse tissue would exert a demonstrable effect on the tissue after neutron bombardment. In the experiments, however, much higher concentrations (of the order of 1 mg of U^{235}/gm of tissue) were used in the hope that the ensuing effects would be clearly demonstrated even at a low neutron flux. Such concentrations of uranium were produced by administration of a relatively nontoxic compound, colloidal UO_2 (suspended in 5% glucose in water). When 5 mg of U in this form was injected intravenously into Bagg albino mice, only 20% of the animals died in the first 4 weeks. This form of uranium is much less toxic than uranyl nitrate, for which the LD_{50} is about 150 μg of U. The toxicity of UO_2 colloids was found to depend on the mean particle size. When this was 0.15 μ, most of the uranium was taken up and fixed in the reticuloendothelial system.[3] Table 2 shows typical distribution data, obtained by counting the alpha particles from aliquots of ashed tissue.

The distribution remains essentially the same for at least 10 days. On the whole, the distribution data are quite similar to those given by Jones, *et al.* (*5, 6*) for colloidal chromic phosphate labeled with P^{32}. It is possible that the uranium deposited in the bones and kidneys comes from the fraction of the colloid containing very small particles or UO_2^{++} ions.

UO_2 colloid prepared from enriched U^{235} (95%) was given intravenously to 22 8-week-old Bagg Albino mice.

[3] This was proven by alpha-ray autoradiography (to be published).

The dose was 2 mg of uranium contained in 0.3 ml. Three days later, these mice were bombarded by a sublethal dose of slow neutrons, using the techniques and experimental facilities previously developed by P. S. Henshaw and his collaborators at the Clinton pile. All of the mice in this group EUB (enriched uranium bombarded) died within 3 weeks after bombardment. In order to demonstrate that the presence of uranium fission was important in causing lethal effects, a number of control groups of mice of the same strain were also run. Toxicity or alpha radioactivity of uranium did not cause the observed effects, because all mice in control groups UC (uranium control) and EUC (enriched uranium control) who had received colloid with U^{238} and enriched U, respectively, but no exposure to neutrons, lived. Neutron bombardment alone did not kill any of the mice in the NB (normal bombarded) group. In addition to the above 4 groups of mice, a fifth one, EUX (enriched uranium X-rayed), showed that irradition of mice containing enriched uranium was not lethal unless fission was also produced. In this experiment, X-rays of a biologically equivalent dose were substituted for neutrons.[4] These data are summarized in Table 3.

TABLE 2

TYPICAL DISTRIBUTION OF URANIUM IN THE TISSUES OF BAGG ALBINO MICE, 5 DAYS AFTER INTRAVENOUS INJECTION OF 2 MG OF UO_2 COLLOID

Organ	% of total amount given	Density of distribution (mg/gm tissue)
Liver	58	.45
Spleen	25	1.8
Carcass (incl. fat, muscle, brain, bone marrow)	6.7	
Gut	1.3	
Lungs	1.0	
Kidney	0.6	
Excreted in first 48 hr	0.5	
Tail (site of injection)	3.1	

Total accounted for, 96.2.

The animals in group EUB showed the signs of radiation effects: ruffled fur appeared 2 or 3 days after bombardment; in 3–14 days most of them had arched backs, and some developed diarrhea. This group showed an average weight loss of 0.7 gm daily until death, the mean time of which was 8 days. The control groups, UC, EUC, NB, and EUX, lost less weight during the first few days, regained this loss within a week, and gained weight thereafter. Postmortem examination failed to give positive evidence of the cause of death, but the effects of radiation on the organs which had the most uranium were unmistakable. In all animals, the spleen was small in size at the time of death (spleen weights were roughly $\frac{1}{8}$ of the normal). The spleens were paper thin and gray or black in color. The dose to the spleen, due to fission recoils, was about 15,000 r.e.p.,[5] in addition to about 3,000 r.e.p. from delayed effects of the radioactive fission products. Liver weights were generally below normal. The livers appeared pale, were often mottled, some had round necrotic areas, and others looked hemorrhagic. The liver fission dose was approximately 3,700 r.e.p. The kidneys were pale in most of the animals. In a few, intraperitoneal hemorrhage was present; lymph nodes were small and occasionally hemorrhagic; anemia was apparent in many at the time of death.

TABLE 3

ACUTE LETHAL EFFECTS IN AN EXPERIMENT TO EVALUATE BIOLOGICAL EFFECTIVENESS OF URANIUM FISSION

Bagg Albino mice group	Code	Amount of U in UO_2 suspension injected intravenously (mg)	Type and dose of exposure	No. of mice in group	% dead in 6 weeks*
Enriched uranium bombarded	EUB	2	1.7×10^{12} n/cm²†	22	100‡
Uranium control	UC	2	–	25	0
Enriched uranium control	EUC	2		18	0
Enriched uranium X-rayed	EUX	2	310 r†	12	0
Normal bombarded	NB	–	1.7×10^{12} n/cm²†	20	0
Normal X-rayed	NX	–	310 r†	25	0
Normal control	NC	–	–	20	0

* All groups were transported by air on the 10th day after neutron bombardment from Oak Ridge, Tennessee, to Berkeley, California. No ill effects were noted on the colony subsequently. Of the EUB group, 90% had already died at the time of the transportation.

† Both the X-ray and neutron doses are $\frac{1}{3}$ of the LD_{50}; on the basis of our physiological studies we consider 1.7×10^{12} n/cm² on its gross effects equivalent to 310 r of 150 kv X-ray on our particular mice. The thermal neutrons were contaminated with some γ rays and fast neutrons.

‡ All mice died within 3 weeks.

Tissues for histological studies were taken from a number of animals of the EUB group immediately following death. In these the spleens, livers, and kidneys showed the most marked changes. The germinal centers of the spleens were shrunken with a decrease in the number of lymphocytes; the livers showed severe central fatty degeneration; and in some of the animals, the kidneys showed patchy tubular necrosis, probably due to uranium poisoning.

[4] Neutron dose (D_n) and X-ray dose (D_x) were considered to be biologically equivalent if $\frac{D_n}{\text{Ld neutron}} = \frac{D_x}{\text{Ld X-rays}}$. A separate experiment was carried out, substantiating this for Bagg Albinos (see, also, *9*). The circumstances of X-ray bombardment resembled those described by Ellinger (*3*) : 150 kv, .5 mm copper screen, no back-scattering.

[5] One r.e.p. (roentgen equivalent physical) = 83 ergs/gm.

In addition to those described here, other groups were treated in a way similar to that for the EUB, EUC, NB, and NC groups described above. The uranium used for the first two groups was 85% enriched. A number of mice from each group were sacrificed at various time intervals following treatment to obtain a more complete series of tissue samples for histological study. Blood counts were done routinely on mice from each of these groups. The results obtained in these studies will be presented in detail elsewhere. Briefly, they amplified and confirmed the results reported above. One day after bombardment both NB and EUB groups developed leucopenia. After the third day, the leucocyte count of group NB began to increase, whereas that of group EUB continued to drop. The white blood cell count of EUC mice remained normal for several days and then decreased slightly. Three or four days after bombardment, anemia began to develop in mice of group EUB and continued to increase in severity. Anemia also developed in EUC, but to a less marked degree. No change occurred in the erythrocyte count of NB animals. The spleen size of group EUB animals started to decrease immediately after bombardment and continued to drop until the weight reached $\frac{1}{8}$ of the original; NB spleens decreased in weight and recovered after 3–5 days; EUC spleens slowly decreased in size because of the alpha activity of the uranium. On the first day after bombardment microscopic examination of the liver showed central hydropic degeneration with swollen pale cells in the lobules. In certain areas there was frank necrosis with loss of cell outline and destruction of nuclei and scattered nuclear debris. In animals examined two days later these findings were much less evident, the livers having more normal histological appearance. In one case many mitotic figures were found scattered throughout the sections. Still later, the livers appeared normal. These findings correspond to the histological picture one obtains in the liver after a massive dose of whole body X-rays (*14*). In the EUC group, about half of the liver sections examined exhibited the same sort of central degeneration of hepatic cells as was found in the EUB group; in the rest of the animals the livers appeared normal. Animals sacrificed in subsequent days in this group all had normal livers. The livers in the NC and NB groups were all entirely normal histologically.

A rough comparison can be made of the dose necessary to kill mice by fission and by beta particles from P^{32} in chromic phosphate. Acute lethal effects have been observed by Jones, *et al.* (*5*) when 100,000 r.e.p. was given by *in vivo* chromic phosphate to the mouse liver. The ratio of the phosphorus liver dose to the fission liver dose is 27; this value cannot be taken as final, but suggests that fission recoils are more effective in producing lethal effects than beta rays.

References

1. Aebersold, P. C., and Lawrence, J. H. *Ann. Rev. Physiol.*, 1942, **4**, 25.
2. Booth, E. T., Dunning, J. R., and Shack, F. G. *Phys. Rev.*, 1939, **55**, 876.
3. Ellinger, F. *Radiology*, 1945, **44**, 125.
4. Henshaw, P. A., Riley, E. F., and Stapleton, G. E. *Radiology*, in press.
5. Jones, H. B., Dougherty, E. C., and Lawrence, J. H. (To be published.)
6. Jones, H. B., Wrobel, D. J., and Lyons, W. R. *J. clin. Invest.*, 1944, **23**, 873.
7. Kanner, M. H., and Barshall, H. H. *Phys. Rev.*, 1940, **51**, 372.
8. Kruger, P. G. *Proc. nat. Acad. Sci.*, 1940, **26**, 181.
9. Lawrence, J. H., and Tennant, R. *J. exp. Med.*, 1937, **66**, 667.
10. McClintock, L. A., and Friedman, M. M. *Amer. J. Roentgenol. rad. Therap.*, 1945, **54**, 704.
11. Norris, L. D., Snell, A. H., Meiners, E. P., and Slotin, L. Manufacture of carbon 14 in the chain reacting pile, 1946, MDDC 36.
12. Segrè, E. Isotope Chart MDDC 184.
13. Segrè, E., and Wiegand, D. *Phys. Rev.*, 1946, **70**, 808.
14. Warren, S., and Friedman, N. B. *Arch. Path.* (Art. IV), 1942, **34**, 749.
15. Zahl, P. A., and Cooper, F. S. *Radiology*, 1941, **37**, 673–682; *Science*, 1941, **93**, 64; Zahl, P. A., Cooper, F. S., and Dunning, J. R. *Proc. nat. Acad. Sci.*, 1940, **26**, 589.
16. Zirkle, R. E. *Occ. Publ. AAAS*, 1936, **4**, 220.
17. Zirkle, R. E. Chicago Meeting, North American Radiological Society, December 1946.

Protection Against Bacterial Endotoxins by Penicillin and Its Impurities[1]

C. Phillip Miller, Walter D. Hawk, and Alden K. Boor

Department of Medicine and *University Health Service, University of Chicago*

Previous reports have described protection of mice against the endotoxins of meningococcus (*1*) and gonococcus (*6*) by the subcutaneous administration of large doses of penicillin. Further investigation demonstrated that such protection is best provided by repeated intraperitoneal injection of penicillin during the 24-hr period before endotoxin is given (*2*). Recent work has shown that mice can be protected by this same means against the endotoxins of a number of other gram-negative bacteria—*S. paratyphi* A, *S. paratyphi* B, *Shigella paradysenteriae, S. enteritidis, S. aertrycke,* and *A. aerogenes.* Negative results were obtained only with the endotoxin of *Shigella shigae.* It has also been found that certain impure penicillin preparations provide a much greater degree of protection than does crystalline penicillin.

Endotoxin is prepared by the method previously described (*1*) and is injected intraperitoneally in 1-cc volume. Protection is measured by comparing the LD_{50} of endotoxin (as computed by the Reed-Muench formula) for untreated and for penicillin-treated mice. A typical determination is presented in the experiment shown in Table 1.

It will be seen that the LD_{50} of endotoxin for mice treated with crystalline penicillin was 3 times that for

[1] This investigation is supported by a grant from the Antibiotics Study Section, National Institute of Health, U. S. Public Health Service. A preliminary report of the work was presented at the Conference on Antibiotic Research held at Washington, D. C., February 1, 1947, under the auspices of the Antibiotics Study Section, National Institute of Health.