ment of ramus with $P\bar{3}$, $M\bar{1}$, $M\bar{2}$ and the posterior portion of $M\bar{3}$, Plate 1, figures 2 and 2*a*.

Locality.—Sespe upper Eocene, Locality 202.

Characters.—The individual represented by the type specimen is smaller than *Peratherium innominatum* and is distinctly smaller than *P. marsupium*. Both of these species are known from the Bridger Eocene of Wyoming. The trigonids of the molars in the Sespe specimen are low and the talonids are relatively short. Length from anterior end of $P\bar{3}$ to posterior end of $M\bar{3}$ is 5 mm.

The two additional specimens are referred to:

Peratherium species

Material.—No. 1942 Calif. Inst. Tech Vert. Pale. Coll., a fragment of ramus with $M\bar{2}$, Plate 1, figures 1 and 1*a*. No. 1944, a fragment of ramus with $M\bar{2}$, $M\bar{3}$ and the posterior portion of $M\bar{4}$, Plate 1, figures 3 and 3*a*.

Locality.—No. 1942 is from Locality 180 and No. 1944 is from Locality 202.

Characters.—These specimens represent smaller animals than that of the species *Peratherium marsupium*, No. 13046 Amer. Mus. Coll. The depth of jaw in Nos. 1942, 1944 is more like that in *P. innominatum*. No well defined cingulum is present at the base of the protoconid-paraconid blade as in the Bridger species. Length of $M\bar{2}$ in No. 1942 is 1.8 mm. Length from anterior end of $M\bar{2}$ to posterior end of $M\bar{4}$ in No. 1944 is 5.5 mm.

[1] Simpson, G. G., *Amer. Mus. Nov.*, 307 (1928).

THE BIOLOGICAL ACTION OF NEUTRON RAYS

BY JOHN H. LAWRENCE AND ERNEST O. LAWRENCE

DEPARTMENT OF INTERNAL MEDICINE, YALE UNIVERSITY SCHOOL OF MEDICINE, DEPARTMENT OF PHYSICS, UNIVERSITY OF CALIFORNIA

Communicated December 17, 1935

Introduction.—Neutron rays have the property of penetrating dense substances such as lead more readily than light substances containing hydrogen. This behavior arises from the circumstances that, being uncharged particles, neutrons pass unimpeded through electron clouds of atoms and are slowed down or absorbed only when they encounter the much more dense atomic nuclei.

The collision of a neutron with the nucleus of an atom is understandable in very simple terms; for both neutron and nucleus behave as tiny, very dense, solid spheres.[1] The neutron has a mass very nearly equal to that of the hydrogen nucleus, the proton, so that in a head-on collision the proton recoils with the full speed of the neutron while the neutron is brought to rest. Glancing impacts likewise give rise to recoil protons of various smaller speeds with the result that neutrons on the average lose half their energy per collision. On the other hand, when a neutron strikes the nucleus of a heavy element, as for example lead, which is more than two hundred times heavier, the neutron rebounds with little loss of energy. Momentum is conserved in the impact and the heavy nucleus recoils with a small amount of energy which is in inverse proportion to its mass. The latter situation is not unlike a billiard ball colliding with a cannon ball.

It is for this reason that neutrons are able to penetrate such great thicknesses of dense substances—for inasmuch as little energy is lost in each impact, the neutrons make many nuclear collisions and hence travel great distances before being brought practically to rest. Likewise, it is clear that they are more readily absorbed in substance containing hydrogen such as biological materials.

The recoil nuclei, resulting from the passage of neutrons through a substance, being heavy charged particles, rapidly lose their acquired kinetic energy by intense ionization along their paths. Recoil protons produce more than one hundred times as much ionization per unit distance of path as is produced by secondary electrons generated in matter by x-rays. In other words, in ionizing power the recoil particles are similar to alpha rays rather than electrons.

This vastly different mode of ionization by neutron rays at once raises interesting questions concerning their biological action. In the case of x-rays there is some evidence[2] that the biological effects produced are proportional to the total amount of ionization, quite independently of the wave-length of the x-rays which influences the distribution of the ionization. It might be inferred from this that neutron rays would manifest themselves biologically in a manner indistinguishable from x-rays, that the mechanism of ionization and the mode of distribution is of no importance. However, Zirkle[3] has shown by a series of careful experiments with alpha rays that biological action in the case of the fern spore (Pteris longifolia) does depend on the distribution or intensity of the ionization in the cell. He finds that along its path over which the density of ionization varies by a factor of two, the alpha particle has a biological effectiveness that varies about as the five halves power of the ion density. Thus, although this empirical law probably does not hold over a wide range including the ionization produced by x-rays, Zirkle's experiments nevertheless point very strongly to the conclusion that neutron rays which generate ionizing particles like alpha rays within the substance, are much more biologically effective per unit of ionization than x-rays. This is a matter which is of much interest from the standpoint of our understanding of the fundamental processes involved, and also is of great practical concern; for it is important to have some information on the biological action of neutrons as a basis for precautionary measures to safeguard the health of those working with this new radiation.

With these considerations in mind some experiments have recently been carried out in the Radiation Laboratory of the University of California. A study of the effects of neutrons on the blood of rats which is the subject of this paper, and an investigation of the growth inhibiting effect of the rays on wheat seedlings, reported in an accompanying paper by Zirkle and Aebersold, corroborated each other in pointing to the conclusion that neutron rays are in fact considerably more effective biologically than x-rays.

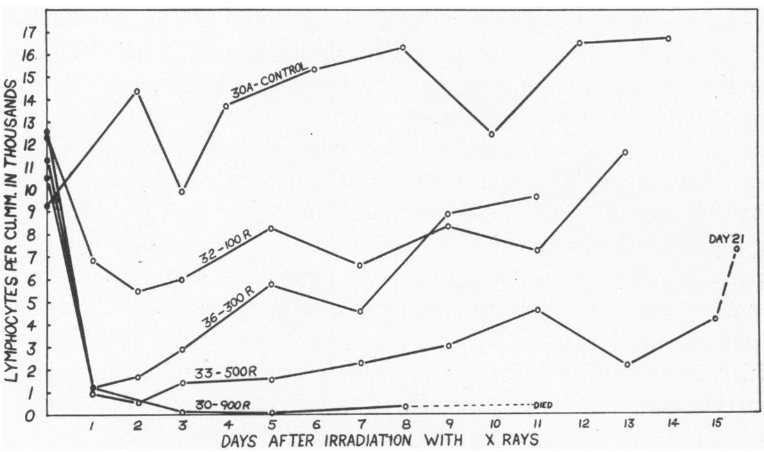

FIGURE 1

Showing the fall in lymphocytes after irradiation with neutrons.

Experimental Procedure.—Various considerations made it apparent that a simple mode of attack which would yield significant information would be to compare as directly as possible the biological effects of neutron rays and x-rays. Changes produced in the blood picture of rats was chosen as a convenient indicator of biological action, partly because the blood picture of the rat is well known and partly because these small animals could be placed near the source of neutrons where the radiation intensity (and therefore biological effect) was great.

Accordingly, the procedure of the experiments was simply to expose rats to measure dosages of neutrons and x-rays and to follow through the course of days the blood counts of the exposed rats along with controls taken from the same litter.

Physical Measurements.—The neutron radiation was produced by bombarding a beryllium target with several microamperes of 3.5 million volt deuterons.[4] Although there is no difficulty in recognizing the fact that the reaction of these high speed deuterons with beryllium nuclei is the most intense source* of neutrons at present available, it is not an easy matter to measure the number and the speed of the neutrons emitted from this or any other source. In the present experiment, however, we were not especially interested in the neutrons themselves, but in the ionization they produced in the tissues of the rats. A measure of this was obtained by placing in the position occupied by the rats during exposure an air ionization chamber which had previously been calibrated in roentgen units. In this way, neutron dosages could be expressed arbitrarily in these x-ray units, and in that sense the neutron effects could be compared directly with the x-ray effects.

From the considerations outlined in the introduction, it is evident that such a procedure does not compare directly the ionization produced by the neutrons and that by the x-rays in the tissues of the rat. It is to be expected that since organic matter is relatively rich in hydrogen, the ionization of the neutrons in the rats would be relatively greater than that by the x-rays. On the other hand, a very probable process of ionization of air by neutrons is one in which nitrogen is disintegrated with the formation of boron and alpha-particles. The alpha-particles are formed with a great deal of energy, and accordingly produce a great many ions in the air. There are possibly still other nuclear processes that give rise to ionization by the neutrons, in both air and the tissues of the rat in addition to that produced by recoil nuclei formed in nuclear collisions. Fortunately, however, the net effect of all ionization processes can be determined by experimental test.

In order to get a direct measure of the ratio of the ionization per roentgen of neutrons and x-rays in the rat, the air ionization chamber was filled with methane, and the change in the ionization in the chamber produced by a neutron beam of constant intensity was noted. It was found that replacing the air with methane increased the ionization by a factor of 3. On the other hand, the x-ray ionization was reduced to $^5/_7$ because of the lower density of the organic gas. The increase in the neutron ionization in methane is ascribable largely to the presence of four hydrogen atoms per molecule. In the tissues of the rat the proportion of hydrogen is something like half as great, so that the ionization factor for the neutrons would be more like 1.5. Taking account also of the factor $^5/_7$ for the x-rays, then, it is estimated that one roentgen of neutron rays produces something like 2.1 times as much ionization in the rat as an equal dosage of x-rays.

In practice it was found convenient to measure the neutron dosages by the radioactivity induced in pieces of sulphur placed adjacent to the rat. When sulphur is exposed to the neutron rays, radio-phosphorus is formed, and since the half-life of this substance is more than two weeks, the amount of radioactivity generated in the course of exposure is accurately proportional to the total exposure. It was, of course, a very easy matter to measure the radioactivity of the pieces of sulphur with an ordinary ionization chamber, and also it was a simple matter to calibrate these radioactive indicators in terms of roentgen units.

For the sake of radiation intensity, the rats were placed in positions as near the beryllium target as possible. Their heads and hind legs were about 4 cm. and 14 cm., respectively, from the target. By comparing the radioactivity produced simultaneously in several pieces of sulphur in various positions, it was possible to determine the intensity of the neutron radia-

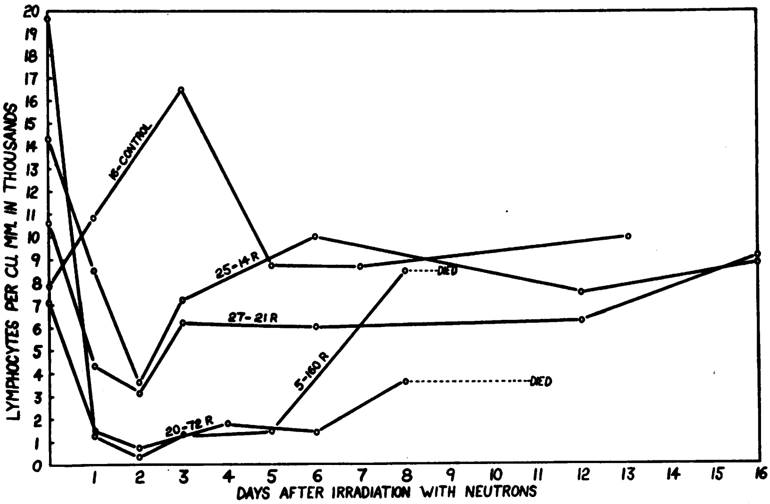

FIGURE 2

Showing the fall in lymphocytes after irradiation with neutrons.

tion at various points. Over the body of the rat, the radiation varied in intensity by more than a factor of 2, and the intensity in the region 1 cm. below the shoulders of the rat was taken as an average value. This arbitrary procedure is doubtless open to much question and precludes really quantitative conclusions.

The emission of neutrons from the beryllium target was such as to produce average exposures to the rat of 0.15 r per microampere per minute. Deuteron currents up to 6 microamperes were available so that exposures of 100 roentgens were obtainable in about an hour and a half.

Inasmuch as the neutrons were comparatively little absorbed in the rats, it was thought desirable in making comparisons with x-rays, to use correspondingly penetrating radiation. Accordingly the rats were exposed to x-rays generated by the Sloan x-ray machine of the University of California

Hospital, operating at 900,000 volts with the radiation filtered through 1 mm. of lead. All animals were exposed for one hour at varying distances.

TABLE 1

		BEFORE IRRADIATION				AFTER IRRADIATION				
RAT NO.	DOSE	W. B. C.	P.	L.	TOTAL LYMPH.	W. B. C.	P.	L.	TOTAL LYMPH.	LYMPH. C. AFTER / LYMPH. C. BEFORE
					X-ray					
$\frac{31+32}{2}$	100 r	14,800	18	80	11,840	8,325	30	69	5,740	48%
$\frac{37+38}{2}$	200	16,250	15	83	13,487	7,975	27	71	5,662	42%
$\frac{35+36}{2}$	300	17,450	17	82	14,309	3,350	54	45	1,507	11%
$\frac{34+33}{2}$	500	12,750	20	77	9,817	2,775	76	23	638	6%
$\frac{30+47}{2}$	900	15,600	18	80	12,480	125	30	70	88	0.7% (Died)
39	Control	11,800	10	88	10,384	11,400	2	95	10,830	
30 A	Control	12,400	25	75	9,300	15,500	33	64	9,930	
43	Control	12,600	16	79	9,954	9,250	13	87	8,047	
					Neutron					
$\frac{25+26}{2}$	14 r	15,400	9	83	12,782	5,900	23	69	4,071	32%
$\frac{27+28}{2}$	21	17,500	21	70	12,250	6,725	44	49	3,295	26%
$\frac{41+42}{2}$	31	22,400	10	82	18,368	5,400	26	69	3,726	20%
44	Control	14,600	21	69	10,074	13,300	22	77	10,241	
18	19	16,350	20	72	11,772	4,750	28	59	2,802	24%
48	20	13,600	5	94	12,784	5,100	29	71	3,621	28%
19	42	15,025	6	81	12,170	6,400	43	52	3,328	27%
20	72	9,000	16	79	7,110	1,450	43	49	710	10% (Died)
5	160	26,250	19	75	19,687	1,500	76	20	300	1.5% (Died)
16	Control	10,950	23	72	7,884	11,750	22	73	8,577	
4*	30	6,350	9	85	5,397	650	44	40	260	5%
1*	30	9,950	21	64	6,368	1,550	68	26	403	6%
	200					400	86	6	24	0.4% (Died)

* Young rats, 4 weeks old.

The same ionization chamber was used for both the neutron and the x-ray dosage measurements.

Biological.—The rats used were albino, some of them of the Wistar strain, $2^1/_2$ to 3 months of age. Due to the small size of the compartment available for neutron exposure, it was not possible to use fully matured rats but since the blood picture of these rats at 80 to 90 days of age is that of the fully grown rat, and since the ages of the rats were the same in the groups exposed to x-rays and neutrons, there was no objection to using them. Males and females were used indiscriminately. All of the animals were kept under the same conditions and on the same standard diet. Blood was obtained from the tail by cutting off a small portion with a razor blade. The studies included counts of the total number of white and red cells, differential counts of the white cells, and observation of the general condition of the animals after exposure. Counts were done daily for several days before irradiation, then daily for the first three days after exposure, and then less frequently until return to normal or the death of the animal. The normal blood picture of the strain of rats used showed on the average from 10,000 to 15,000 white blood cells per cu. mm.. but some animals had lower and others higher values. In the differential counts the lymphocytes predominated, being on the average 75 to 80 per cent with 15 to 20 per cent polymorphonuclear leukocytes. The values for the red cells varied between 7,000,000 and 9,000,000 cells per cu. mm.

Results.—In the leukopenia produced by exposure of rats to x-rays, it is well known that the lymphocytes are affected to a greater degree than the other white cells. This proved to be true also in the leukopenia produced by exposure to neutrons. In table 1 are given the values for the white blood cells with percentages of neutrophilic polymorphonuclear cells and lymphocytes, before and after irradiation, for the animals exposed to x-rays and neutrons, respectively. The lowest value for the lymphocytes after irradiation was taken and this usually was within the first three days. The values for the control animals are also included, the second value being the lowest of the subsequent counts. In the last column is given the per cent of original lymphocytes per cu. mm. for the minimum after irradiation. In figures 1 and 2 the total number of lymphocytes are plotted against days after irradiation of some of the animals. In the case of those animals exposed to less than 100 r units of x-rays, there was no fall in the number of white cells. In those animals receiving up to 500 r units of x-rays there was a fall with a gradual return of the counts to normal, and there were never any signs of illness. The two animals receiving 900 r units of x-rays, developed a progressively increasing leukopenia, and died on the 11th and 16th days after exposure. The animals which were exposed to from 14 to 45 r units of neutrons, always developed a leukopenia, followed by a gradual return to normal without signs of illness. Rats Nos. 20, 5 and 1 received relatively large doses of neutrons. No. 20 receiving 72 r units over a period of 1 hr. 50 min., developed a marked leukopenia. On the fifth day after irradiation, the animal was obviously sick, with rough fur and arched back, was not taking food or water, and had lost weight. The total number of white cells increased (see Fig. 2) and the red cells increased from 9,600,000 to 13,000,000. Crusting lesions developed about the eyes which finally became sealed shut and swelling of the tissues around the mouth occurred. The animal died on the 11th day after exposure (see Fig. 3). At autopsy there were no gross abnormalities other than the sloughing lesions of the eyes including the eyeballs and swelling of the tissues around the mouth. Previous to death the animal lost weight markedly, which with the increase in white and red cells, were interpreted as evidence of dehydration.

Rat No. 5 receiving 160 r units died nine days after exposure with a picture similar to No. 20.

Rat No. 1, a young animal receiving 230 r of neutrons, died three days after exposure, the day previously having been ruffled and hunched with

FIGURE 3

Rat No. 20, before death. This animal received 72 r units of neutrons. Note the lesions of the eyes.

eyes closed. No increase in the white cells occurred before death. Thus, those animals dying after exposure to neutrons, unlike the two animals dying after exposure to 900 r units of x-rays, developed necrotizing lesions about the head. These lesions may be secondary to infection, or due directly to the effect of neutrons on the tissues. Further work is necessary to clarify this point. Incomplete data on the weights of the animals after irradiation indicate that the suppression of gain in weight is relatively greater for neutrons. Rat No. 41, which received 31 r units of neutrons, gave birth to a litter of eight normal offspring, thirty days after irradiation. The young developed normally. In the rats receiving up to 500 r units of x-rays there was no significant change in the number of red cells per cu.

mm. However there was a marked decrease in the number of red cells in those rats exposed to 900 r units. After irradiation with neutrons, there was a tendency toward a decreasing red cell count, except in those animals receiving the higher doses, when there was an increase due probably to dehydration.

In figure 4 are plotted the dosages of x-rays and neutrons against the per cent of original lymphocytes per cu. mm. for the minimum after irradiation. The basic points for these curves are those values obtained when two rats were exposed to the same dose. These curves suggest that as measured by the fall in the total number of lymphocytes, neutrons are roughly ten times as biologically effective as x-rays. This factor is also suggested

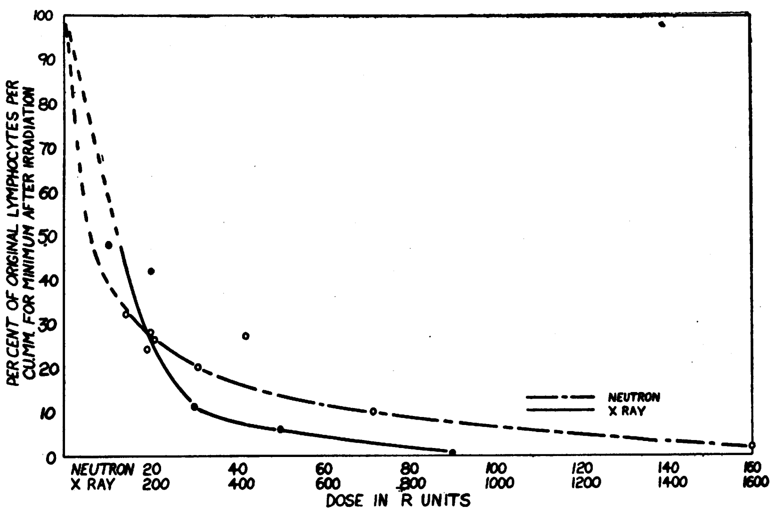

FIGURE 4

Curves showing per cent of original lymphocytes for minimum after irradiation with x-rays and neutrons.

by the dosages necessary to produce death in the two types of irradiation.

Discussion.—These experimental results show that for irradiation in terms of roentgens, neutron rays are roughly ten times as biologically effective as x-rays, in respect to alteration of the blood picture of the rat. It follows, from the considerations brought out above, that per unit of ionization in the rat, neutrons are possibly five times as biologically effective. This means that in this case ion distribution does play a rôle in biological action. However, inasmuch as the ion densities produced by the neutrons and x-rays differ enormously (more than one hundredfold), it is evident that the biological effectiveness of ionizing radiation is a very slowly varying function of the ion distribution. It would seem to follow, therefore, that at least in regard to blood changes in the rat the biological effectiveness of x-rays per roentgen is practically independent of the wave-length of the radiation, since over the useful range of wave-lengths the ion distribution varies appreciably less than by a factor of ten.

From long experience, roentgenologists have established that the maximum dosage of x-rays which a human being can tolerate daily, without apparent harm, is 0.1 roentgen. If it is assumed that neutron rays produce similar biological effects to those produced by x-rays, and this assumption might well be questioned, it follows from the present experiments that the maximum allowable daily dosage of neutrons is 0.01 roentgen. This should constitute a warning inasmuch as many laboratories will soon be using neutron generators of such power that individuals in the vicinity of the apparatus will be exposed to many times this allowable dosage in the course of a few minutes unless adequate protective screening is provided.

We are indebted to many of our associates for assistance in these experiments and we want to expresss our thanks especially to Dr. R. E. Zirkle and P. C. Aebersold for their cordial coöperation and to Miss M. E. McLaughlin who did some of the blood counts. We wish to thank also Dr. R. T. Legge for his kindness in placing at our disposal the laboratory facilities of nearby Cowell Memorial Hospital; and Dr. R. S. Stone for the use of the high voltage x-ray machine in the University Hospital. The rats used in these experiments were kindly furnished us by the Institute of Experimental Biology and the Department of Zoölogy of the University of California. We acknowledge with thanks also gifts of certain essential materials from the Beryllium Products Corporation and from the Climax Molybdenum Company.

We are grateful for the general support of the Research Corporation and the Chemical Foundation and for a grant-in-aid of this investigation from the Josiah Macy, Jr., Foundation.

[1] J. R. Dunning, *Phys. Rev.*, **45**, 586–600 (1934).

[2] This matter is still controversial. The literature concerning the wave-length factor has been reviewed by C. Packard, *Am. Jour. Cancer*, **16**, 1257–1274 (1932); and by G. Failla, *Am. Jour. Roentgenol.*, **29**, 352–362 (1933).

[3] R. E. Zirkle, *Am. Jour. Cancer*, **23**, 558–567 (1935).

[4] A description of the magnetic resonance accelerator that provided the high speed deuterons, and therefore the neutrons, for these experiments has been published (E. O. Lawrence and M. S. Livingston, *Phys. Rev.*, **40**, 19–35 (1932)).

* Gamma rays are also emitted from the beryllium target. However, measurements with the Wilson Cloud Chamber showed that the ionization produced by the gamma rays was only a few per cent of that produced by the neutrons.