

THE COMPARATIVE EFFECTS OF NEUTRONS AND X-RAYS ON THE WHOLE BODY*

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In a previous paper (1) the marked biological effects of neutrons on mammalian tissue were demonstrated, as evidenced by the production of a lymphopenia in white rats after whole body irradiation. As is true after x-rays, the lymphocytes were more sensitive to neutrons than were the other blood cells. In a further study (2), it was found that neutrons were effective *in vitro* on Sarcoma 180, a transplantable neoplasm of white mice. At that time also, the whole bodies of mice were exposed to various doses of x-rays and neutrons, and the lethal effect of neutrons was demonstrated. The preliminary observations indicated that the mechanism of death after irradiation with neutrons was similar to that after x-radiation.

It is important that we understand the effects of whole body irradiation with neutrons for two reasons. First, throughout the world, there are at present numerous laboratories using this new form of radiation in studies in the field of nuclear physics. The workers in these laboratories are exposed in a greater or less degree to neutrons, concerning whose biological effects we know little. The early and late damaging effects suffered by the early workers with x-rays force us to consider the possibility of similar serious injury from exposure to neutrons. Secondly, the biological action of neutrons on mammalian normal and neoplastic tissue, suggests their trial in the treatment of

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neoplastic disease in animals and in man. If we are to use this form of radiation therapeutically, it is imperative that we understand its effects on the normal tissue which surrounds the neoplasm.

Recently we have studied the comparative effects of filtered 200 kv. x-rays¹ and neutrons on a large number of normal mice, in association with studies on neoplastic tissue. The experimental set-up, methods of measurement of dosage and the quantitative results will be reported in another paper. We wish to report here the clinical, bacteriological and pathological changes which take place after exposure to lethally equivalent amounts of these two forms of radiation.²

Experimental Procedure and Methods

In these experiments normal male and female Swiss mice, 6 to 8 weeks of age, weighing approximately 20 gm. were used. Mice of this strain are particularly vigorous and are naturally immune to mouse typhoid, and seemed ideal for these studies. They were kept at room temperature, several animals in a cage, and on a diet of dog chow and water, with oats and lettuce added three times a week. The neutron and x-ray studies were carried on simultaneously. After irradiation the animals were observed at frequent intervals for any change in their condition and weights were taken daily. Previous work (1, 2) had demonstrated the occurrence of leucopenia after neutron irradiation. Frequent bleedings for blood studies give rise to an anemia in mice, so in these experiments blood counts were not performed. All animals dying after irradiation were carefully examined postmortem, including bacteriological study of every animal. In the case of those animals autopsied immediately after death, or freshly killed, the organs were preserved in 10 per cent formalin and 95 per cent alcohol for microscopic study. Using the wood chamber

¹ The x-rays were produced by a 200 kv. constant potential apparatus and (in addition to the tube wall which is equivalent to 0.2 mm. of Cu) were filtered with 0.5 mm. of Cu and 1 mm. of Al. In groups of ten the animals were irradiated in a flat cardboard box at a distance of 55 cm. At this distance the output was 35 r per minute. Each exposure was quantitated by means of a thimble ionization chamber from a Victoreen condenser r-meter. For neutron exposure, the mice were placed in pairs in two holes of a wood chamber, placed near the source of neutrons. Each exposure was quantitated with the same thimble ionization chamber placed within the wood cylinder. Neutrons were produced in the cyclotron (3) by means of bombarding beryllium with 5 million volt deuterons at 10 micro-amperes. Gamma rays are also produced in this reaction, but the ionization produced in the tissues is chiefly due to neutrons. The exposure times for neutrons and x-rays did not vary by more than a factor of two.

² By "lethally equivalent" we mean doses of the two forms of radiation which will cause the deaths of groups of animals in about the same number of days after irradiation.

for neutron exposure, it was found that per unit of ionization measured within the wood chamber, equivalent lethal effects were obtained with one-fourth the necessary x-ray dose. For example, 1000 r of x-rays was approximately equivalent to 250 "r"³ of neutrons (death always occurring after a few days at these doses.) The work here reported is not concerned, however, with the quantitative relation between neutrons and x-rays in terms of biological effects but only with the qualitative effects in biologically equivalent doses. As a basis for the pathological study, we have killed animals irradiated with 1000 r of x-rays, and approxi-

TABLE I*
Results of Irradiating Mice with 200 Kv. Filtered X-Rays

Number of mice	Average weight	Dose	Length of life after irradiation	Average number of days	Remarks
	<i>gm.</i>	<i>roentgens</i>	<i>days</i>		
6	19.7	400			Survived
10	19.5	450			1 died on 11th day 2 developed diarrhea
15	19.7	500			5 died 12th to 18th day 1 developed diarrhea
11	19.5	550			5 died 12th to 49th day 3 developed diarrhea
14	19.7	600			10 died 9th to 23rd day 8 developed diarrhea
14	19.8	704	9-14	10.3	All died 6 developed diarrhea
12	19.9	800	6-11	9	All died 6 developed diarrhea
14	19.9	900	3-10	7	All died 7 developed diarrhea
10	20	1000	3-8	4.7	All died 8 developed diarrhea

* See footnote Table II.

mately 250 r of neutrons, 1, 2, 3, 4 and 5 days after irradiation and preserved their organs for microscopic study. Also animals irradiated with smaller doses, which died or were killed at various periods after irradiation, were similarly studied.

Clinical Findings

In Tables I and II are shown the results of irradiating a large number of mice with x-rays and neutrons. All animals receiving 700 r or more

³ We have arbitrarily defined an equivalent roentgen or "r" of neutrons as that amount of ionization produced in the thimble chamber of the r-meter which would be produced by the incidence of a roentgen of x-rays.

of x-rays and 212 equivalent r or more of neutrons died. At doses as low as 450 r x-rays and 124 r neutrons some of the animals died, an increasing percentage of the mice dying as the doses were increased. The first clinical signs of the effects of irradiation were ruffling of the fur and arching of the backs, associated with loss of weight. There was always a latent period of 2, 3 or 4 days before these signs

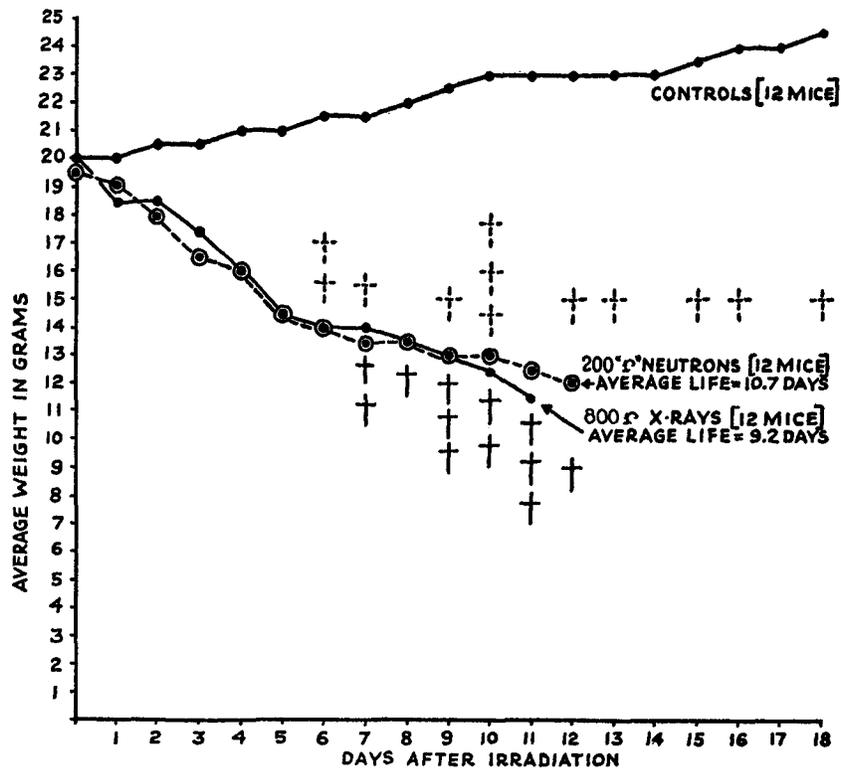
TABLE II*
Results of Irradiating Mice with Neutrons

Number of mice	Average weight	Neutron dose	Average dose	Length of life after irradiation	Average number of days	Remarks
	gm.	roentgens	roentgens	days		
15	19.5	124-140	133			5 died 10th to 28th day 2 developed diarrhea
15	19.8	142-153	147			6 died 8th to 18th day 3 developed diarrhea
15	19.7	153-164	159			7 died 4th to 13th day 7 developed diarrhea
15	19.7	164-171	168			11 died 8th to 42nd day 10 developed diarrhea
15	20.3	172-187	179			10 died 3rd to 43rd day 10 developed diarrhea
15	19.6	189-199	194			10 died 3rd to 46th day 9 developed diarrhea
15	19.6	199-212	207			13 died 3rd to 15th day 12 developed diarrhea
15	19.9	213-233	223	3-19	6.9	All died 13 developed diarrhea
15	19.7	234-265	245	3-11	5.4	All died All developed diarrhea
14	20.2	265-368	298	3-5	3.5	All died 11 developed diarrhea

* Animals living more than 60 days after irradiation are considered survivors. Nearly all of these were alive and clinically normal several months later.

appeared, the latent period tending to be shorter after the larger doses of neutrons and x-rays. Roughly within the range studied the length of life after irradiation was inversely proportional to size of dose given. It is also noted in the tables that as the doses were increased, there was an increasing tendency toward diarrhea which sometimes was bloody. At the higher doses, most of the animals developed diar-

rhea. All of the animals were weighed daily for at least a month. At the lower doses, there was a temporary loss of weight, with return to the normal rate of gain when the animals survived. In Text-fig. 1 are seen the weight curves of three groups of animals, namely, control group, a group receiving on the average 200 r of neutrons and another receiving 800 r of x-rays. All of the irradiated animals in the two



TEXT-FIG. 1. Weight curves of irradiated and control animals.

groups progressively lost weight, became emaciated and, as indicated by the crosses, died during the 18 days after irradiation. In both groups crusting lesions occasionally developed around the eyes. Death was usually preceded by a short period of coma and occasionally terminal generalized convulsions. The animals which received sublethal doses of radiation eventually recovered and except for a tempo-

rare sterility in the males and a permanent sterility in the females, seemed to be normal.

Postmortem Findings

Gross.—All of the animals which died after irradiation with neutrons or x-rays were carefully examined postmortem. Although the colony of animals was inspected at least hourly night and day, it was not always possible to examine each animal immediately after death. However, this was done in many instances; and, as noted previously, some of the animals were killed at various periods after irradiation and their organs examined immediately.

After both forms of radiation in biologically equivalent doses, the gross pathological findings were quite similar.

The lungs usually were normal except for congestion in some cases; the livers were also normal except that after larger doses some of them were fatty. Occasionally, in animals surviving longer periods, multiple yellowish areas were found in the livers, which proved to be focal necroses. The spleens were uniformly decreased in size, after the larger doses being only one-tenth normal size. The lymph nodes (axillary, submaxillary, inguinal, retroperitoneal) were decreased in size. The small intestine from the pylorus to the cecum usually was dilated and had many injected blood vessels. Their contents were often bloody. The large intestines were involved in this reaction to a less degree. Occasionally there were

⁴ Along with other studies, numerous breeding experiments have been carried out on animals which have survived irradiation with neutrons. Out of 48 female mice which survived doses of from 65 to 210 r, and which were placed with normal males 2 to 3 months after irradiation, only one (140 r) became pregnant. She gave birth to a litter of six, all of which died 24 hours after birth. The young were normal in the gross. Another group of irradiated females was placed with normal males, 10 to 18 days after irradiation. There have been only two litters of one and two young each, all dying within 48 hours after birth. Preliminary observations indicate that sublethally irradiated males sire normal young, 2 or 3 months after irradiation. Twelve male mice were mated with normal females within 3 weeks after irradiation of the males. Seven of these have sired nine litters averaging 2.55 animals per litter. This strain of mice normally have 8 to 10 per litter. Thus these experiments indicate that the ovaries are quite sensitive to neutron irradiation and that particularly in male animals the germ cells (spermatozoa) are affected, resulting in small litters, possibly the result of translocations. These studies have been continued by Snell and Aebersold (9), who have investigated in detail the mechanism of the sterility induced in males.

hemorrhages⁵ into the subcutaneous tissues and into the walls of the stomach. The other organs were normal in the gross.

Bacteriological.—In the animals freshly killed or autopsied immediately after death, complete bacteriological studies were carried out. This included culturing a platinum loop of the heart's blood in blood broth and on a blood agar plate, placing small portions of the spleen and liver in blood broth and mincing similar portions of these organs on a blood agar plate followed by streaking. In addition, one or more of similar specimens were cultured anaerobically in meat media. Then, in the case of all animals which were not killed or found freshly dead, but which died after irradiation, complete postmortem examinations were performed and a portion of each liver cultured in a tube of blood broth.

The bacteriological studies consisted of noting the appearance of the colonies on blood agar plates, staining by the method of Gram, and studying the fermentation reactions on dextrose, lactose, sucrose and dulcitol, and in some cases on numerous other sugars.

The purpose of these bacteriological studies was twofold. First, we wished to be certain that no epidemic disease was present among the irradiated animals. Secondly, we wished to get information concerning the mechanism of death after irradiation with both x-rays and neutrons. As noted above, the gross appearance of the viscera of the irradiated animals did not lead us to believe that infection was the primary cause of death, in spite of the well known leucopenia which occurs after irradiation. It was unusual to find multiple abscesses in the liver, spleen or other organs, and when these were found they were in animals which had received relatively small doses and died a long time after exposure.

The organisms found by culture were of the colon-*aerogenes* group, being Gram-negative bacilli, which fermented glucose and lactose with the formation of gas. There was one non-lactose fermenter. It did not agglutinate with *aertrycke* or *enteritidis* antiserum and hence did not belong to the mouse typhoid group. Also, since the sugar reactions were observed only for 48 hours, it may have been a slow lactose fermenter. In other words, the organisms found were those which are normal inhabitants of the gastro-intestinal tract.

In Table III are shown the bacteriological results on x-rayed animals which were cultured immediately after death or after being killed. It is to be noted that when mice receive 1000 r of x-rays and are killed,

⁵ These are presumably due to the marked decrease in the blood platelets following irradiation (10).

TABLE III
Irradiation of Mice with 200 Kv. X-Rays

No.	Dose roentgens	Killed time after irradiation days	Autopsy, time after death	Cultures*			Pathology*
				Heart	Liver	Spleen	
Group 1 401	1000	1	At once	BB-neg. Meat } neg. BB	BAP } neg. BB	BAP } neg. BB	No sections
402	1000	1	"	Meat } neg. BB	BAP } neg. Meat } neg. BB	BAP } neg. BB	"
407	1000	1	"	BAP } neg. Meat } neg. BB	BAP } neg. Meat } neg. BB	BAP } neg. BB	"
408	1000	1	"	BB-neg.	Lung BB-neg.	Peritoneum BB-neg.	Swollen cells in crypts with slight necrosis Same as 408
409	1000	1	"	BB-neg.	BB-neg.	Peritoneum BB-neg.	
403	1000	2	"	BB-neg. Meat } neg. BB	BAP } neg. BB	BAP-B. subtilis BB-neg.	No sections
404	1000	2	"	Meat } neg. BB	BAP } neg. Meat } neg. BB	BB-neg. BAP } neg. BB	"
410	1000	2	"	BAP } neg. Meat } neg. BB	BAP } neg. Meat } neg. BB	BAP } neg. BB	"

411	1000	2	"	"	BB-neg.		Peritoneum BB-neg.	Edema of villi, necrosis of cells of crypts; slight exudate in submucosa Same as 411 No sections
412	1000	2	"	"	Meat } neg. BB }	BAP } neg. Meat } BB }	BAP } neg. BB }	" "
405	1000	3	"	"				
406	1000	3	"	"	Meat-+ BB-neg.	Meat-+ BB-+ Lung BB-+ Lung BB-neg. BAP-neg. Meat-+ BB-+	BB-+ Peritoneum BB-+ Peritoneum BB-neg. BAP-neg.	"
419	1000	3	"	"	BB-neg.		BB-Gram-pos. coc- cus	Same as 411 but more extensive with sloughing Slight regeneration in crypts No sections
420	1000	3	"	"	BB-neg.			
421	1000	3	"	"	BAP } neg. Meat } BB }	BAP } neg. Meat } BB }	BAP } neg. BB }	"
422	1000	3	"	"	BAP } neg. Meat } BB }	BAP } neg. Meat } BB }	BAP } neg. BB }	"
413	1000	4	"	"	BB-+	Lung BB-+	Peritoneum BB-neg.	Same as 419 but more extensive with mucosal ulceration
414	1000	4	"	"	BB-neg.		Peritoneum BB-neg.	Same as 419 but more extensive

BAP = blood agar plate.
 Meat = meat medium (anaerobic).
 BB = blood broth.
 EMB = eosin-methylene blue plate.
 + = growth of *B. coli* or colon-aerogenes organisms.
 Neg. = no growth.
 Col. = colonies.
 * See footnotes to Table IV.

TABLE III—Concluded

No.	Dose roentgens	Killed, time after irradiation days	Autopsy, time after death	Cultures*			Pathology*
				Heart	Liver	Spleen	
Group 1 415	1000	4	At once	BAP } neg. Meat } BB }	BAP-neg. Meat-Gram-pos. coccus BB-neg. BAP } Meat } staphylococ- BB }	BAP } neg. BB } BAP-staphylococcus BB-neg.	No sections
416	1000	4	"	BAP } Meat } neg. BB }	BAP } Meat } staphylococ- BB }	BAP-staphylococcus BB-neg.	"
417	1000	5 1/2	"	BAP } Meat } neg. BB }	BAP } Meat } staphylococ- BB }	BAP-staphylococcus BB-neg.	Intestinal ulcers
418	550	11	"	BAP } Meat } neg. BB }	BAP } Meat } staphylococ- BB }	BAP-staphylococcus BB-neg.	Mucosa intact; focal ne- croses in liver
Group 2 281	1000	Died, time after irradiation days 3 1/6	20 min.	BB-neg.	BB-+ BAP-neg.	Peritoneum BB-neg.	No sections
299	700	4 13/24	At once	BB-+ BAP-14 col.	BB-+ BAP-neg.	BB-neg. BAP-1 col.	"
294	1000	4 7/8	"	BAP-10 col.	BB-+ BAP-many col. BAP-many col.	BAP-50 col.	"
280	1000	5 1/24	"	BB-+ BB-+ BAP-50-100 col.	BB-+ BAP-many col. BAP-many col.	BB-+ BB-+ BAP-50 col.	"
301	900	6 7/8	25 min.	BB-+ BB-+ BAP-50-100 col.	BB-+ BAP-many col. BAP-many col.	BB-+ BB-+ BAP-50 col.	"
255	800	7 11/12	30 "	BB-+ BB-+ BAP-50-100 col.	BB-+ BAP-many col. BAP-many col.	BB-+ BB-+ BAP-50 col.	"
320	800	8 2/3	15 "	BB-+ BB-+ BAP-50-100 col.	BB-+ BAP-many col. BAP-many col.	BB-+ BB-+ BAP-50 col.	" Postmortem changes

275	800	9 1/12	At once	BB- +	BAP-neg.	BB- +	No sections
303	900	9 19/24	" "		BB- +	BB- +	"
254	800	9 5/6	" "	BB- +	BB- +	BB- +	Normal
319	800	10 1/2	20 min.	BAP-many col.	BAP-very many col.	BAP-many col.	Mucosa intact. Bacterial masses in blood vessels of all viscera
257	700	11 1/24	At once	BAP-many col.	BB- +	BB- +	No sections
240	715	11 3/24	" "	BAP-many col.	BB- +	BB-neg.	Intestine normal. Focal necroses in liver
234	500	11 1/8	" "		BB- +	Spleen	Ulcers in large intestine; focal necroses in liver
277	800	11 13/24	20 min.	BB- +	BAP-5 col.	BAP-4 col.	No sections
286	600	11 5/8	30 min.	BAP-1 col. EMB-neg.	BB- +	BB- +	Mucosa intact; bacterial clumps in submucosa. Bacterial masses in liver
260	600	12 5/6	At once	BAP-neg.	BAP-60 col.	BAP-many col.	Mucosa intact
242	715	14 1/12	10 min.	BAP-30 col.			"
Group 3		Killed, time after irradiation					
256	700	2 mos.	At once	Meat } neg. B }	B-neg.		Normal
285	600	54 days	" "	Meat } neg. B }	B-neg.		"
202	550	5 mos.	" "		BB-neg.		No sections
223	550	5 "	" "		BB-neg.		Normal
236	500	5 "	" "		BB-neg.		"

1, 2, 3 or 4 days later, bacteriologically all of the viscera usually are sterile. In those animals dying during the 1st week, the cultures are usually positive. This indicates that at these high doses, bacterial invasion is more or less of a terminal event. As the doses decrease and the mice live longer after irradiation, cultures of the heart and liver usually show an increasing degree of bacterial invasion. The small group of animals receiving sublethal doses, and killed several months later, showed negative cultures.

Table IV similarly depicts the results on numerous mice which died or were killed at various periods after exposure to various doses of neutrons. Here the situation is quite similar to that after x-rays, namely, after large doses when the animals die or are killed during the first few days, cultures are usually negative. Then as the doses are progressively decreased, and life is lengthened, first the visceral and then the heart's blood cultures become positive.

Microscopic.—In order to follow the progress of and compare the anatomical changes in the tissues of the mice irradiated with neutrons and x-rays, the following groups of animals were examined histologically.

1. Mice Irradiated with Lethally Equivalent Doses of X-Ray and Neutrons and Sacrificed at Varying Intervals from 1 to 5 Days Thereafter.—

Two groups of mice were irradiated, one with neutrons and the other with x-rays, with doses which had previously been found to cause death of the mice in about 5 days (neutron 230 to 290 r, x-ray 1000 r). Two animals in each series were sacrificed daily during the 5 days following irradiation. The organs were cultured and sections of viscera were taken for histological examination.

The microscopic findings revealed essentially similar changes in the two groups of animals with the changes developing in the same temporal sequence in both. The organs most extensively affected were the intestines, spleen and bone marrow.

Spleen.—The spleens from all of the animals were markedly shrunken due to an almost complete loss of lymphoid cells. The Malpighian corpuscles were tiny and only the collapsed reticulum of the white pulp indicated their sites (Fig. 1). The lymphocytes throughout the framework of the red pulp were likewise missing and here also the reticular framework was all that remained. In some instances

this was collapsed and the sinuses obliterated, while in other instances the pulp was filled with masses of adult red blood cells throughout which strands of delicate reticulum marked out the sinus structure. In none was there evidence of fibrous tissue increase and the capsules and trabeculae were not thickened. There were no histologically detectable differences between the spleens taken at the different time intervals.

Intestines.—The changes observed in the intestines were most marked in the small intestine. At 24 hours only a minimal change was noted with cells in the crypts presenting a somewhat hyperchromatic and swollen appearance with swollen vesicular nuclei. Occasional cells here appeared necrotic and disintegrated. The villi were intact and lined by tall columnar well stained epithelium. 48 hours after irradiation the cell changes in the crypts of the glands were more marked and the villi now appeared edematous. The epithelial cells covering the villi were swollen, had a cuboidal shape with rounded bulging edges and a pale granular vacuolated cytoplasm and large watery nuclei. Throughout the edematous stroma occasional polymorphonuclear leucocytes were present. In the large intestine the secretory activity of the mucous cells was noticeably increased so that most of the cells contained vacuoles in their cytoplasm. At 72 hours the mucosal changes were more striking. The villi appeared short and widened by the extreme edema. The epithelial cells appeared more swollen and their cytoplasm was markedly vacuolated. In some zones the surfaces of the villi were denuded. In the crypts of the glands many cells were in a state of mitotic division and numerous cells with large vesicular nuclei and prominent nucleoli lined the lumina of the glands, apparently newly regenerated epithelial cells. The stroma of the mucosa had a scant but diffuse infiltration of polymorphonuclear leucocytes. At 4 days the same process was more extensive, with the villus structure destroyed and large surfaces denuded of epithelium (Fig. 3). One x-rayed mouse in this series showed masses of bacteria superimposed on the surfaces of these denuded zones. At 5 days the x-rayed mice showed marked evidence of regenerative activity with most of the surface again epithelialized, although in one animal large ulcerated zones remained and these were covered by masses of bacteria. The ulcers destroyed the entire mucosa in the zone although there was no cellular exudate at the margins of these zones. The intestines of neutron animals in the same period showed the mucosa intact although edema of the interstitial tissue was still present. In all sections of the intestine of these two series the lymphoid follicles were absent.

Bone Marrow.—The bone marrow from the femur 24 hours after irradiation showed the cellular components as numerous and distributed in the same proportions as in the normal control. At 48 hours and in all the subsequent specimens up to 5 days after irradiation the marrow was entirely devoid of young forms and the marrow spaces were filled with adult red blood cells (Fig. 2). There were no adult white cell elements in this blood nor in the peripheral blood present in the various organs examined histologically.

Other Viscera.—The sections of liver, pancreas, stomach, lungs, brain, supra-

TABLE IV
Irradiation of Mice with Neutrons

No.	Dose <i>roentgens</i>	Killed, time after irradiation <i>days</i>	Autopsy, time after death	Cultures*			Pathology†
				Heart	Liver	Peritoneum	
Group 1							
181	292	1	At once	BAP } neg. BB }	BAP-3 col. BB- +		Intestines normal
518	265	1	"	EMB } neg. BB }	EMB } neg. Meat } BB }		Swollen cells in crypts of small intestine
164	288	2	"	BAP } neg. BB }	BAP-10 col. Meat-neg. BB- +		Edema of villi, necrosis in cells of crypts. Scant exudate in submucosa
166	230	2	"	BAP } neg. BB }	BAP } neg. BB }		Same as 164
42	368	3	"	BAP } neg. Meat } BB }		BAP } neg. Meat } BB }	Same as 164 but more ex- tensive with sloughing of epithelium
196	292	3	"	BAP } neg. BB }			Same as 164
165	245	3	"	BAP-neg. BB- +	BB- + BAP-neg. BB- +		Same as 164, plus begin- ning regeneration in crypts
141	272	4 5/12	"	BAP } neg. BB }	BAP-1 col. BB-neg.		Same as 165
150	250	4 7/24	"	EMB } neg. BB }	EMB } neg. Meat } BB }		No sections

123	240	4 11/12	"	BAP-many col. BB- +	BAP-very many col. BB- +	Mucosa intact with swollen cells and numerous mitoses. Edema of submucosa
Group 2 144	340	2 2/3	15 min.	BAP } neg. EMB } BB }	BAP-1 col.	No sections
127	250	3	30 "	EMB-neg. BB- +	BB- + EMB- + BB- +	"
24	350	3 1/6	10 "	BAP } neg. BB }	BAP-25 col. BB- +	Same as 165
142	292	3 1/4	At once	BAP } neg. BB }	BAP-many col. BB- +	No sections
110	189	3 1/4	15 min.	BB-neg.	BB- +	"
113	319	3 7/24	30 "		BB- +	Edema and necrosis in crypts. Postmortem change

* As controls seven normal Swiss mice were killed. Liver and heart's blood cultures taken immediately after death on four of these were negative. The remaining three were cultured 1, 2 and 3 hours postmortem, respectively. All of these were also negative. In the tables it is noted that some of the cultures were taken at varying periods up to 30 minutes after death. Undoubtedly postmortem bacterial invasion of the viscera and blood stream is more rapid in irradiated than in control animals, hence some of these cultures may not give a true picture of the conditions at death.

† Since the changes in the bone marrows and spleens of this group of animals were more or less uniform as described in the text, only changes in the intestines and other organs are mentioned here. All of the viscera of animals surviving irradiation were normal.

TABLE IV—Concluded

No.	Dose <i>roentgens</i>	Died, time after irradiation <i>days</i>	Autopsy, time after death	Cultures*			Pathology†
				Heart	Liver	Peritoneum	
Group 2							
32	290	3 5/8	30 min.	Meat- + BB-neg. EMB } neg. BB }		BAP } BB } neg.	Same as 165
534	213	3 19/24	15 "	BB- + BB- + EMB-neg. BB- +	EMB-many col. BB- + BB- +	BAP-neg. BB- +	No sections
520	241	4	5 "	BB- +	BB- +		No small intestine sections
530	219	4 7/8	10 "	EMB } neg. BB }	EMB-neg. BB- +		No sections
524	209	5	30 "	EMB-35 col. BB- +	EMB-many col.		"
134	275	5 1/12	At once	BAP-1 col. BB- +	BAP-200 col.		Ulcers of intestinal mucosa
133	232	5 1/6	15 min.	BAP-25 col.	BAP-100 col. BB- +	(Non-lactose fer- menter but neg. agglutination with <i>aertrycke</i> and <i>enteri-</i> <i>tidis</i> antiserum	No sections
100	214	5 13/24	At once	BAP } neg. BB }	BAP-4 col. BB- +		"
103	227	5 7/12	While dying	BAP } neg. BB }	BAP-1 col. BB- +	(Spleen) BAP-neg. BB- +	Slight edema of mucosa
511	194	6 1/24	30 min.	BB- +	BB- +		No sections
192	208	7	10 "	BAP-50 col. BB- +	BAP-many col.		Ulcers of intestinal mucosa

195	166	7 7/8	30 "	BAP-15 col.	BAP-many col.	Normal intestinal mucosa				
104	191	9	At once	BAP-26 col.	BAP-many col.	Ulcers of large intestine				
189	203	9 1/4	10 min.	EMB-many col.	EMB-very many col.	No sections				
522	130	10	5 "	EMB-36 col.	EMB-100 col.	Ulcers of large and small intestines				
117	234	10 1/24	10 "	Meat- + BB- +	BAP-100 col.	Intestinal ulcers. Focal necrosis in liver				
503	241	11 19/24	10 "	EMB } neg. BB } +	EMB-neg. BB- +	Intestinal mucosa intact				
170	155	12 7/8	20 "	BAP-50 col.	BAP-many col.	Ulcers large intestine.				
51	174	13 11/12	30 "	BB- +		Hepatitis with necroses of liver cells				
175	199	14 3/4	30 "	EMB } neg. BB }	EMB-neg. B- +	Intestinal mucosa intact; slight edema				
Group 3										
3	217	Killed, time after irradiation								
149	196	nos.								
132	185	6	At once		B-neg.	Viscera normal				
105	177	5	"		B-neg.	Viscera normal, except for large peritoneal abscess				
4	176	2	"	Meat } neg. B }	B-neg.	Viscera normal				
44	153	5	"		B-neg.	"				
528	139	6	"		B-neg.	"				
		4	"		B-neg.	Intestines normal				
						Viscera normal				

renals, testes and ovaries examined in this series showed no significant deviation from the usual histological appearances.

2. Animals Dying from 3 to 14 Days after Varying Doses of Neutrons or X-Rays.—

A second group of irradiated animals which received either neutrons in doses varying from 130 to 350 r or x-rays in doses ranging from 500 to 1000 r and dying at intervals of from 3 to 14 days after irradiation were also studied anatomically. The changes found in these two groups were again similar and will be described together.

Spleen.—The spleens in all of the animals were atrophic with destruction of the lymphoid tissue as was described in the group of sacrificed animals.

Bone Marrow.—The bone marrow also showed the same marked aplastic state that was found in the animals sacrificed during the first 5 days after irradiation. A few animals with small doses of irradiation (neutron or x-ray) and surviving 10 or 11 days, showed a few islands of hematopoietic activity in an otherwise aplastic marrow.

Intestines.—In animals dying during the first 5 days after irradiation the intestinal changes were essentially similar to those described for the sacrificed group of animals. In the longer periods after irradiation the changes were somewhat variable. In most instances the mucosa of both the small and large intestines was intact and well preserved except for numerous large ulcers. These zones showed complete destruction of the mucosa with enormous masses of bacteria superimposed on the necrotic material and extending throughout it. In many instances bacterial masses were present in the blood vessels and lymphatics immediately beneath these ulcerated zones. The striking feature of all these ulcerations was the complete lack of any significant inflammatory exudate at the borders of the lesions. This was, of course, in keeping with the almost complete lack of leucocytes in the marrow or peripheral blood stream. As in the first series, the lymphoid follicles were entirely absent in all of the sections and a collapsed reticular network indicated the sites of the larger lymphoid patches. The sections of intestines of a few animals in both series showed the mucosa intact and well preserved. These occurred in animals living 9 to 14 days after irradiation.

Other Viscera.—In animals living 9 to 14 days after irradiation, the livers occasionally showed evidence of change in the form of focal necrosis without exudate at the margins of the zones. In some of these foci bacterial masses could be found. In instances where the bacterial masses were prominent, necropsies had not been done until 15 to 30 minutes after the death of the animal. The possibility that these represented postmortem growth, if not invasion, must be considered since these findings were not present in animals examined immediately after death.

The lungs, hearts, kidneys, suprarenals, testes and brain sections studied showed no significant alteration

3. *Animals Surviving Irradiation.*—

Several animals that received either x-irradiation in doses varying from 500 to 700 r or neutron irradiation in doses varying from 139 to 185 r survived the exposures and lived from 2 to 6 months. These animals were all sacrificed and histological examination of the viscera revealed normal structures throughout.

DISCUSSION

These clinical, bacteriological and pathological studies on a large number of mice irradiated over the whole body with neutrons and x-rays, indicate that the effects are similar after both forms of irradiation. There is usually a latent period of 2 or more days before the animals have clinical evidence of illness, such as ruffling of the fur, arching of the back, loss of weight and diarrhea. The pathological studies show that the mucosa of the small intestine, the lymphoid tissue, the bone marrow and the spleen, are the most radiosensitive. After sublethal amounts of radiation these various tissues have recuperative power, as evidenced by the normal viscera in animals surviving several months. With the doses used, the other viscera, for example the kidney, liver, nervous tissue, lung, heart and muscle, seem morphologically to be radioresistant. Functionally, the ovaries and testes seem sensitive to neutrons, as evidenced by the temporary sterility in the males, and the permanent sterility in the females, after sublethal doses. It is well known that these latter tissues are sensitive to x-rays and Snell (4) has shown that after male mice receive testicular irradiation in relatively small amounts, the litters sired by these mice are small in number. He has interpreted these findings as being due to translocations taking place. It is quite probable that neutrons act similarly.

Chrom (5) has irradiated mice with moderate doses of x-rays and finds that a week later a bacteremia is constant. When the livers and spleens were shielded with lead, the bacteremia does not occur. He gives good experimental evidence that the reticulo-endothelial system is damaged by radiation and hence cannot combat the presence of bacteria which gain entrance into the circulation through a damaged intestinal mucosa. He concludes that the "cause of the well known 'Roentgen death' in ordinary experimental animals is to be looked for

in a combination of several concurrent destructive agencies, including anemia, intoxication and enterogenous bacteremia. But in most cases it will be impossible to say which of these agencies has been the more decisive of the fatal outcome." Warren and Whipple (6-8) in their studies on Roentgen intoxication in dogs, using large doses, find that death is causally related to damage to the mucosa of the small intestine and that generalized infection is not an important factor. Our studies, both bacteriological and pathological, lead us to conclude that after irradiation with large doses of x-rays (and neutrons also) leading to death within a few days, infection is not a necessary finding. Death is associated with marked destructive changes in the various viscera mentioned above, giving rise to a toxemia from tissue breakdown products.

However, as the doses are decreased and the animals live longer, bacteremia is a usual finding and infection probably a more important factor in the cause of death. This is supported by the anatomical finding of numerous ulcers in an otherwise normal intestinal mucosa and focal necroses in the liver in some of the animals surviving 9 to 14 days after irradiation. Furthermore, the animals in this group uniformly had positive postmortem blood cultures. Presumably the organisms (usually *B. coli*) gain entrance to the circulation through the damaged mucosa of the intestine. These results harmonize those of Chrom (5) and Warren and Whipple (6-8) who used small and large doses of x-rays, respectively.

CONCLUSIONS

Irradiation of the whole bodies of mice with neutron rays in sufficient quantities, leads to a clinical, bacteriological and anatomical picture similar to that following Roentgen irradiation. The mucosa of the small intestine and the lymphoid and hemapoietic tissues are the most radiosensitive. The mechanism of death after both forms of radiation seems to be a combination of tissue destruction and enterogenous infection, the former predominating in the acute deaths after large doses. Aside from any possible delayed effects from exposure to small doses over a long period of time, concerning which we have no information, these changes after relatively large doses make it imperative that workers in laboratories generating neutrons protect them-

selves from exposure by screening. For the same amount of ionization measured by a small bakelite-walled thimble chamber, neutrons are more biologically destructive than x-rays. The average daily dose to those working with neutrons should not exceed one-fourth of the tolerance dose accepted for x-rays. Whether daily doses of this magnitude, over a long period of time, will result in damage is not known. Also, if neutrons are tried therapeutically normal tissue must be protected from undue irradiation.

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EXPLANATION OF PLATES

PLATE 23

FIG. 1. Sections of spleen. Hematoxylin and eosin. All $\times 40$. (a) Control. Large Malpighian corpuscles and lymphocytes in pulp. (b) $4\frac{1}{2}$ days after whole body irradiation with 272 r of neutrons. Total absence of Malpighian corpuscles and lymphocytes in pulp with collapse of stroma. (c) 4 days after whole body irradiation with 1000 r of x-rays. Essentially the same as (b).

FIG. 2. Section of bone marrow from femur. Hematoxylin and eosin. $\times 185$. (a) Control. (b) $4\frac{1}{2}$ days after whole body irradiation with 272 r of neutrons. Aplastic marrow with only adult red blood cells filling spaces. (c) 4 days after whole body irradiation with 1000 r of x-ray. Same as (b).

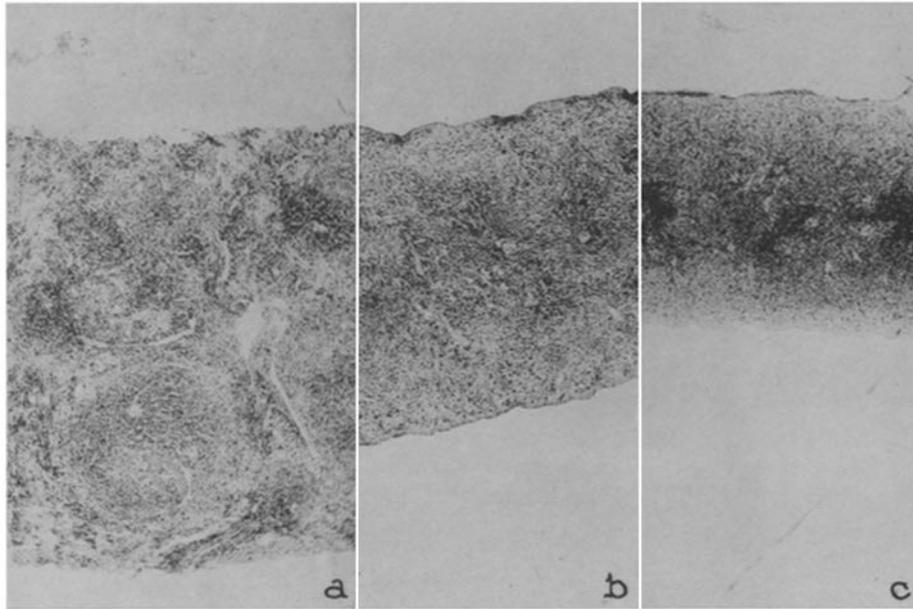


FIG. 1

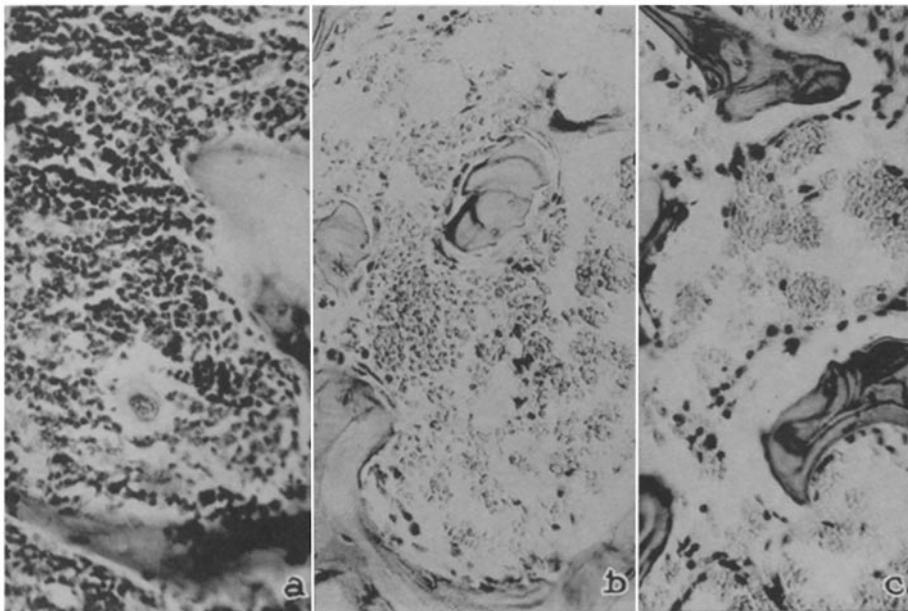


FIG. 2

(Lawrence and Tennant: Effects of neutrons and x-rays on body)

PLATE 24

FIG. 3. Section of small intestine. Hematoxylin and eosin. $\times 125$. (a) Control. (b) $4\frac{1}{2}$ days after whole body irradiation with 272 r of neutrons. Marked distortion of mucosa, sloughing of surface, edema of stroma. (c) 4 days after whole body irradiation with 1000 r of x-rays. Change essentially the same as in (b).

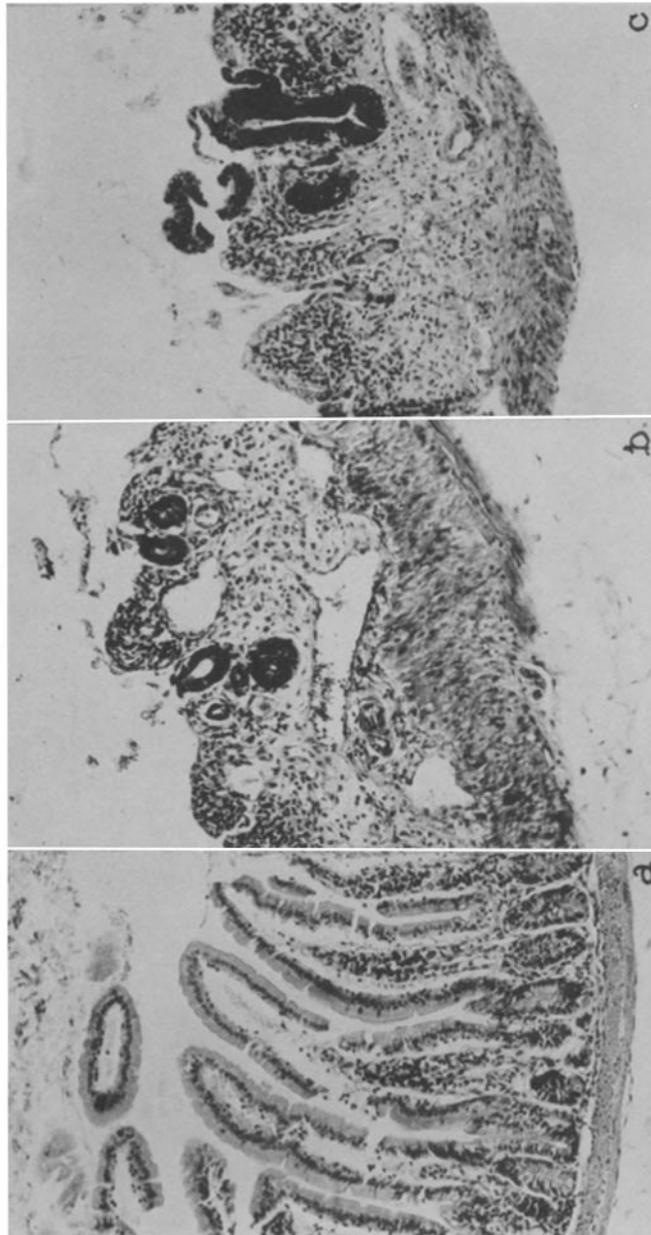


FIG. 3

(Lawrence and Tennant: Effects of neutrons and x-rays on body)