RESTRICTED

REC D-2085

CH-386A
Health and Biology
General

DECLASSIFIED

METALLURGICAL LABORATORY
University of Chicago

THE HEMATOLOGICAL EFFECTS OF IONIZING RADIATIONS

by

L. O. Jacobson, Edna K. Marks and Egon Lorenz

October 1947

Medical Division

This document contains information affecting the national defense of the United States within the meaning of the Espionage Act, U. S. C. 50; 31 and 32. Its transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law.

Report Received: April 9, 1948
Figures Received: May 3, 1948
Issued: May 19, 1948

Contract No. W-7401-eng-37

RESTRICTED

DECLASSIFIED

12111

Table of Contents

	Page
Abstract	
1. Introduction	5
2. The Comparative Effects of Acute Whole Body Exposure to X-rays and Fast Neutrons.	6
2.1 Acute Exposure to Penetrating X-rays.	7
a. Abortive rise in Leucocytes and Reticulocytes after Acute Exposure.....	23
b. Special Studies on the Effect of Penetrating Radiations on Coagulation of Blood.....	23
c. The Effect of Irradiation on Induced Anemia. ...	25
2.2 Acute Exposure to Fast Neutrons.	36
a. Dosimetry	36
b. Probe-Object Distance and Rate of Exposure	36
c. Effects on Hematological Constituents	37
3. Hemtological Effects of Chronic Whole Body Exposure of Mammals to Externally Originating Radiation.	47
3.1 Rabbits, Mice, and Guinea Pigs.	48
3.2 Recurrent Anemia after Chronic Exposure to Gamma Radiation.	64
3.3 Exposure of Humans to Single and Divided Doses of Externally Originating Radiation.	65
3.4 Morphological Changes in the Cells of the Peripheral Blood Following Acute and Chronic Irradiation.....	66
4. The Comparative Effects of Parenterally and Orally Administered Radioactive Elements (Acute and Chronic Studies).....	80
4.1 Plutonium	80
4.2 Radium	82
4.3 Yttrium (Y^{91})	84
4.4 Sodium (Na^{24})	85
4.5 Barium-lanthanum ($Ba-La^{140}$)	85
4.6 Strontium (Sr^{89})	86

2411

RESTRICTED

Table of Contents (Continued)

	Page
5. So-called Stimulating Effects of Radiation on Hemopoiesis.	90
6. Studies on the Peripheral Blood of Plutonium Project Personnel Exposed or Potentially Exposed to Radiations.	91
7. Literature Cited.	93

THE HEMATOLOGICAL EFFECTS OF IONIZING RADIATIONS

by

L. O. Jacobson, Edna K. Marks, and Egon Lorenz

1. Introduction

The objectives of the Health Division of the Plutonium Project were essentially (1) to study the fundamental and comparative biological action of external radiations and internally administered radioactive materials, (2) to apply the findings toward the protection of individuals who worked in the Project, and (3) to use the findings generally for the protection of the public which was potentially in danger of exposure to these physical hazards.

The hematologic studies conducted on the Plutonium Project were divided into four main categories, namely, (1) the effects of acute^{*} whole body exposure to externally administered penetrating radiations, (2) the effects of chronic^{**} whole body exposure to externally administered penetrating radiations, (3) acute toxicity of parenterally and enterally administered radioactive elements, and (4) chronic toxicity of parenterally and enterally administered radioactive isotopes.

In order properly to correlate studies on the peripheral blood effects with those occurring in the hemopoietic tissue, the animals in the same or parallel experiments were sacrificed at appropriate intervals for histological study. All experiments included an adequate number of controls, and the frequency of sampling depended upon the chronic nature of the experiment.

*Single exposure
**Repeated exposures

2. The Comparative Effects of Acute Whole Body Exposure to X-rays and Fast Neutrons.

In considering the irradiation of the whole body with penetrating radiations of external origin, those of significance in so far as direct effects on the blood and blood forming tissue are concerned are confined to X-rays, gamma rays, fast neutrons, and slow neutrons. The beta and alpha rays that originate from radioactive substances can largely be neglected because penetration in tissue is only a few millimeters in the former and a fraction of a millimeter in the latter. The studies on animals by Raper and Barnes,⁽¹⁾ Raper, Zirkle and Barnes,⁽²⁾ Raper, Henshaw, and Snider,⁽³⁾ and others have demonstrated that although beta rays may penetrate through the skin, the hematologic effects, if any occur, are minimal and are secondary to other more serious pathological changes. No experiments similar to those of Raper and Zirkle have been performed using alpha rays. Since alpha rays have such shallow penetrability, e.g., those emitted by plutonium will only penetrate approximately forty micra into the skin, no direct effects and only minimal indirect effects on hemopoietic tissue appear likely.

Studies on the acute exposure of animals to slow neutron flux have been carried out by Zirkle and Raper^(4a). This slow neutron source, however, was contaminated with fast neutrons and thus the effect on the hemopoietic system, as reported by Raper, Henshaw and Snider⁽³⁾, is difficult to interpret. As has been pointed out by Zirkle,^(4a,b) it is not the slow neutron itself that produces

biological effects but rather the gamma rays and fast atomic nuclei emitted upon nuclear capture of the slow neutron in certain elements within the body. It is likely, therefore, that the exposure of experimental animals or man to a pure slow neutron flux would produce biological effects comparable to those induced by gamma rays plus fast neutrons. Henshaw, Snider, et al⁽⁵⁾ conducted studies in which relatively heavy doses of gamma rays (i.e., 500 and 700 r) were given to mice in one acute exposure within a period of minutes. Hematological studies were not adequate however, to make a comparison with X-ray effects. Since, however, gamma rays differ from X-rays only in wave length, the biological action of gamma rays can, for all practical purposes, be expected to be qualitatively the same as that of penetrating X-rays.

2.1 Acute Exposure to Penetrating X-rays: The whole body exposure of rabbits, rats, mice, chicks, humans, goats, guinea pigs, and dogs to X-radiation given in one dose has revealed that in terms of sensitivity of the blood and blood-forming tissue these animals may be listed as above in appropriate order of increasing sensitivity. (6 to 16)

The X-rays administered in these experiments were generated in a 200 kvp machine operating at 15 ma. A 0.5 mm copper filter and a 1 mm aluminum filter were used. The half-value layer in copper of the filtered beam was 0.98 mm. The exposures were measured with a Victoreen condenser r-meter equipped with a 100 r chamber. Measurements were made in air within a treatment box at the position occupied

by the center of the animal's body. Victoreen chambers of 250 r full scale reading were used as monitors. For rats, the dose-rate at 34 cm average 12 r per minute; for rabbits, 12-16 r at 87.5 cm; for mice, 15 r at 79 cm; guinea pigs, 12 r at 86 cm; dogs, 6 r at 107 cm; goats, 3.3 r at 132 cm. As the dose was measured at the center of the body, the average dose the animal received would vary according to its size. For mice, the average body dose would be the same as the air dose; for guinea pigs and rats, it would be slightly smaller; for the larger animals it would not only be considerably smaller, but also the actual dose received by the various tissues would differ to some extent. To obtain a more uniformly distributed dosage, the larger animals were given one-half of the total dose to one side and the other half to the other side.

The most sensitive indicator of radiation effect in any of these species which can be recognized by a study of the peripheral blood is a reduction in the number of total lymphocytes per cu mm. In general, this effect is not significant in these species with doses below 25 r. The general pattern of acute radiation response of the leucocytes in the peripheral blood of the rabbit is shown in Figures 1, 2, and 3. Lymphocyte values are reduced below control values after 25 r within 24 to 48 hours. With doses of 100 r and above a reduction occurs after 3 hours and a maximum depression is reached by 24 to 48 hours. Recovery proceeds slowly, requiring more than 60 days with doses of 300 r and above. After irradiation, the monocytes of the peripheral blood follow a pattern of response similar to the lymphocytes but characteristically return to normal values or show an absolute increase

Figure 1. Acute radiation response of the leucocytes of the peripheral blood of the rabbits.

8211

FIG. 1

Figure 2. Acute radiation response of the lymphocytes of rabbits.

10x111

Figure 3. Acute radiation response of the heterophils of rabbits.

12/2/11

FIG. 3

between the 4th and 6th day after exposure to 100 r and above. An increase in the neutral red granules of the lymphocytes is observed after doses of 600 r. An increase in heterophil motility after 24 hours is observed following doses of 200 r and above.

A significant initial rise in absolute heterophil values in the peripheral blood of the rabbit follows a dose of 400 r and above. After doses of 500 and 800 r two initial peaks at about 12 and 24 hours are observed. No significant reduction in heterophil values occurs with doses of 300 r and below. After 400 to 800 r, a significant reduction in heterophils is apparent after 96 hours with recovery to normal limits by 23 days or less.

The platelet values of the peripheral blood as indicators of acute X-ray damage of the hemopoietic system are comparable to the granular series except that reduction in their number occurs later. In the rabbit, platelets are markedly reduced after doses of 500 or 800 r, reaching a minimum on the 9th day after exposure. Recovery follows usually within one or two weeks. Gross and microscopic bleeding into the skin and other tissues is quite apparent in rabbits after doses of from 500 to 800 r. This is due in part to the platelet reduction and capillary abnormality, but the hyperheparinemia described by Allen et al (14, 17, 18) probably plays a vital role in the process.

No anemia of significance has been observed in groups of rabbits on the basis of mean averages with doses below 500 r given in an acute dose to the whole body. Occasionally an animal developed a slight anemia after exposure to 300 r. A progressively larger fraction of

experimental animals developed anemia after 500, 600, 700 and 800 r. An acute dose of 800 r produced a significant normochromic anemia which reached a maximum at 14 days with recovery by 23 days. Figure 4 illustrates the anemia which develops after 800 and 500 r. Many of the animals that succumbed within the first three weeks after receiving doses of 600 to 800 r died with severe terminal anemia of a relatively abrupt onset. Figure 5 illustrates the terminal erythrocyte count in a group of unselected nonsurvivors after a whole body exposure of 800 r.

Reticulocytes are not reduced significantly in the peripheral blood of rabbits with doses below 100 r. With doses above this level the degree of depression becomes significantly greater with increasing doses. After the exposure of rabbits to 800 r, the reticulocytes fall from a normal value of 3 to 5 per cent to less than 0.1 per cent by 72 hours, and as is shown in Figure 6, recovery proceeds slowly during the first 14 days and reaches normal values by 23 days.

The description of the effects of X-ray upon the cellular constituents of the peripheral blood of the rabbit typifies the pattern of response seen in rats, mice, guinea pigs, dogs, man, goats, and chicken with certain exceptions. A dose of whole body exposure that kills 50 per cent of a group of rabbits within 30 days* produces an anemia which is usually normochromic. In other species, particularly the rat and human, a definite macrocytic anemia may develop. (7, 10) The LD 50 of a group of rabbits is approximately 800 r; the LD 50 for rats is 600 r; for dogs 315 r; mice 540 r; and goats 350 r. These data, of course, hold only for the animals used in this experiment

*Hereafter referred to as LD 50.

Figure 4. Anemia in rabbits after 500 and 800 r total body radiation.

16 4/11

FIG. 4

Figure 5. Representative erythrocyte counts on nonsurviving animals that received 800 r X-radiation.

184111

FIG. 5

Figure 6. Reticulocyte counts in rabbits after 800 r.

20X11

FIG. 6

17410

and there may be considerable difference in the LD 50 dose for different strains or hybrids of the same species. The effect of 800 r (LD 50) on rabbits is thus roughly comparable to the effect of 315 r on dogs. A difference of recovery rates exists between species, however. The extreme example of this difference in recovery rate is seen when one compares the rabbit and the rat; the rat recovers much more rapidly than the rabbit after comparable irradiation damage.

a. Abortive Rise in Leucocytes and Reticulocytes after Acute Exposure. Between the 4th and 11th day after irradiation of the rabbit with 500 r or more, an "abortive rise" in reticulocytes, lymphocytes, and heterophil values of the peripheral blood occurs. This phenomenon as it involves the lymphocytes and heterophils is illustrated in Figures 2 and 3. As to the nature of this phenomenon, only speculation is possible. It may be related to the "waves of regeneration" that are seen histologically in the hemopoietic tissue of irradiated animals and that have been described by Bloom and his associates⁽¹⁵⁾ and by Jacobson and Block.⁽¹⁶⁾ The fact that grossly abnormal forms, especially of the myeloid series, are seen both in the peripheral blood and bone marrow during this period followed by a recurrent wave of degeneration suggests that this "abortive rise" actually represents effects produced at the time of irradiation in the precursors which after a series of divisions no longer survive.

b. Special Studies on the Effect of Penetrating Radiations on Coagulation of Blood. A prolonged bleeding time, increased capillary fragility, thrombocytopenia, and prolonged clotting time have been

described following heavy acute exposure of animals to X-radiation by Shouse, Warren and Whipple⁽¹⁹⁾, whose findings have been confirmed by Jacobson et al.^(13, 14) It is evident from our experience on the Plutonium Project that the clotting time may be increased in the human as a result of acute over-exposure to penetrating radiations. The explanation of the prolonged bleeding time was considered to be related to the thrombocytopenia; neither the increased capillary fragility nor the prolonged clotting time had been adequately explained.

Allen, Jacobson et al.^(14, 17, 18) working with dogs have shown that this increased clotting time is definitely related to an increase in the amount of a circulating heparin-like substance, and is a major cause of death from acute irradiation injury. This anticoagulant increase may be sufficient in certain dogs to render the blood incagu-
lable in the test tube. Also, anticoagulant present in the blood of some of these animals is sufficient to alter the coagulability of the blood of normal animals in the test tube. This hemorrhagic state can be repeatedly reversed for varying periods of time (24 to 72 hours) by administration of purified toluidine blue and certain other of the thionin dyes as well as protamines. This hemorrhagic defect is also produced in experimental animals by radioactive elements given in relatively large acute doses.^(20, 21) The explanation for the hyper-heparinemia is not entirely clear. The prolonged clotting time as a result of an increased amount of circulating heparin is not specifically a radiation effect. It has been produced in rabbits by Jacobson, Marks, et al.⁽²²⁾ by the administration of a nitrogen mustard (methyl bis (B chloroethyl) amine) given intracardially in one single dose of 5.0 mg per kilogram of body weight. It has also been observed by Smith, Jacobson, et al.⁽²³⁾ in human subjects who have been given this

nitrogen mustard for the therapy of Hodgkin's disease. It seems likely therefore that hyperheparinemia and thus a prolonged clotting time may result from any severe acute damage to the hemopoietic system. The fact that toluidine blue and protamine have been reported by Allen, Bogardus, Jacobson, et al⁽²⁴⁾ to be effective in temporarily controlling the hemorrhagic manifestations of some cases of acute leukemia, idiopathic thrombocytopenia and the like, even though no increase in circulating heparin can be unequivocally demonstrated, opens a new avenue of approach to research in this field.

The work of Prosser et al^(13, 25), Jacobson et al⁽²⁶⁾ and Brues et al⁽²⁷⁾, indicates that the death of animals after a single exposure to ionizing radiations such as X-rays and fast neutrons is more frequently due to a cumulative effect of derangement in several organ systems than to the failure of one single system such as the hemopoietic system. Thus, for example, death may be due to initial shock within the first 24 hours, to overwhelming infection with failure of immune mechanisms including severe lymphopenia and neutropenia, or intractable hemorrhage due to hyperheparinemia and thrombocytopenia.

It is of interest to note that whereas single or repeated exposures in the LD 50 range may produce a prolonged clotting time due to the increase in circulating heparin or heparin-like substance, chronic irradiation, though sufficient to produce panhematopenia and death, produced no apparent change in the blood clotting time of animals. (41).

c. The Effect of Irradiation on Induced Anemia: One of the problems facing those charged with the responsibility of the protection of personnel from radiation hazards on the Plutonium Project was whether

individuals with a pre-existing anemia would be more adversely affected by externally originating total body irradiation than individuals with normal values. In an attempt to study this problem, a regenerative anemia was produced in rabbits by the subcutaneous administration of phenylhydrazine hydrochloride^(29a) or by repeated phlebotomies^(29b). The hematological effects of 800 r total body X-radiation on such anemic animals were compared with those in normal (non-anemic) animals given the same dose. A group of normal untreated and nonirradiated animals was studied simultaneously.

The characteristic initial rise and subsequent reduction in heterophils and immediate precipitous fall in the lymphocyte values followed the administration of 800 r in the group in which a regenerative anemia had been produced as well as in the irradiated normal animals. The time of recovery of these elements to normal limits in the two groups was likewise comparable. An "abortive rise" in the leucocyte values of both of these groups was noted between the 5th and 11th day after irradiation.

The group of animals which received X-radiation only developed maximum anemia at 14 days, with recovery by 25 days. The animals in which a regenerative anemia was produced by phenylhydrazine or by bleeding and which were irradiated at or near the point of maximum anemia developed no further anemia, and the blood values returned to normal limits by 25 days. The phenylhydrazine administration or bleeding was discontinued in these animals 2 days prior to irradiation.

The erythrocyte and hemoglobin values of the group in which an anemia was induced and which were allowed to recover spontaneously, returned to normal within 14 days (Figs. 7 and 7a).

The reticulocyte values of animals that received phenylhydrazine or were bled had reached approximately 27 per cent when X-radiation was given. This mean value was reduced to a minimum of circa 2.0 per cent between 5 and 6 days after irradiation, and it returned to normal limits or higher within 24 hours. The mean reticulocyte value of normal control animals that were given 800 r fell maximally within 2 days, reached a minimum of less than 0.1 per cent, and recovered slowly but completely by 23 days (Fig. 7b).

The failure of 800 r total body X-radiation to produce further anemia in animals with a previously induced regenerative anemia or to materially interfere with recovery is dependent in part on the number of surviving erythroblasts in the erythropoietic tissue.

Histological studies made on animals prepared in a comparable manner and sacrificed at varying appropriate intervals indicate that erythropoietic tissue was completely destroyed by 72 hours in normal rabbits that were given 800 r, and recovery proceeded slowly. On the other hand, although massive destruction of erythropoietic tissue occurred after exposure to 800 r in animals in which a regenerative anemia had been produced, the destruction was incomplete and apparently delayed (Fig. 8). These observations appear to indicate that either (1) there may be a delayed response of previously stimulated erythropoietic tissue after exposure to X-radiation or (2) actively dividing cells of the erythropoietic series are actually less sensitive to irradiation.

Figure 7. Red count values of animals with an induced anemia prior to 800 r radiation.

FIG. 7

Figure 7a. Hemoglobin values of animals with an induced anemia
prior to 800 r total body radiation.

29 x 311

FIG. 7c

308,111

Figure 7b. Reticulocyte values of animals with an induced anemia
prior to 800 r radiation.

31211

FIG. 7b

Figure 8. The hematologic effects of 800 r on normal and hyperplastic bone marrow of the rabbit: 1A, B, C-range of normal control marrow; 2A, B, C-bone marrow after phenylhydrazine-induced hyperplasia 3, 4 and 6 days, respectively, after phenylhydrazine withdrawal; 3A, B, C-bone marrow of phenylhydrazine-induced hyperplasia at 1, 3, and 5 days, respectively, after 800 r, and 3, 4, and 6 days, respectively, after phenylhydrazine withdrawal; 4A, B, C-bone marrow of normal rabbits exposed to 800 r at 1, 3, and 5 days after exposure. (Mag. 16x.)

992/111

FIGURE 8

2.2 Acute Exposure to Fast Neutrons: Although survival data are available on several species of animals exposed to fast neutrons (4b,30), hematological data in our laboratories are confined to rabbits (26). An exhaustive study was undertaken to obtain quantitative information on the relative biological effectiveness of fast neutrons as compared to X-rays. It must be pointed out that the fast neutron unit used here is an arbitrary unit based on the reading given by the Victoreen ionization chamber when exposed to neutrons.

a. Dosimetry: Fast neutron doses were measured with Victoreen condenser r-meters, equipped with 100 r chambers which were placed within lead shields in cardboard containers between two rabbit boxes or between two tiers of mouse boxes.

The Victoreen thimble chambers used for the measurement of ionization by fast neutrons had been calibrated in the fast neutron field of the University of Chicago cyclotron against an instrument of this type first used for this purpose at the Radiation Laboratory of the University of California. Ionization by fast neutrons sufficient to cause a discharge of one division on the r scale of the California instrument has been considered one n, the n being a unit of fast neutron dosimetry. Measurement of the ionization by fast neutrons emanating from the Chicago cyclotron should be comparable with determinations made on those emanating from the University of California cyclotron if the same reaction is used.

b. Probe-Object Distance and Rate of Exposure: Animals exposed in one set of experiments were approximately 55 cm from the tip of the

probe, and the fast neutrons were administered at a rate of 1 to 6 n per minute. In another group of experiments the animals were approximately 64 cm from the source, and the rate averaged 0.6 times that in shield A.

c. Effects on Hematological Constituents: The neutron dose required for an LD 50 in 30 days is circa 120 n. In general, the pattern of effect observed in the peripheral blood of rabbits exposed to fast neutrons from the cyclotron is qualitatively similar to that of X-ray exposure. This is illustrated in Figures 9 and 10.

An initial increase in heterophils followed exposure to fast neutrons in doses of 9 to 128 n units. An initial rise occurred within the first 24 hours. A reduction that reached a minimum between 3 to 5 days followed the initial rise after doses ranging from 26 to 128 n units. No significant reduction in heterophils occurred after an exposure to 9 n units. Recovery to normal limits occurred in all groups exposed to a total dose below 128 n units. The group exposed to 128 n expired prior to the 16th day after exposure without apparent recovery.

The number of lymphocytes in the peripheral blood was reduced after the administration of doses of fast neutrons ranging from 9 to 128 n units. This reduction was apparent as early as 3 hours after exposure with a maximum reduction at 2 to 3 days. Although the lymphocyte values of animals exposed to 9 n units were again within the normal control range by 5 days, the lymphocytes of those animals exposed to 26 n units and above had not returned to normal by 37 days when blood counts were discontinued.

Figure 9. Eterophils of rabbits after exposure to fast neutrons.

372 m

FIG. 9

Figure 10. Lymphocytes of rabbits after exposure to fast neutrons.

FIG. 10

A severe normochromic anemia occurred in animals exposed to doses of fast neutron units in the LD 50 range or above (circa 120 n). The maximum anemia in those animals that recovered occurred at about 14 days, with recovery by 23 days. Reticulocytes were reduced to 0.1 per cent or less by 3 days in animals exposed to doses of 128 n units or above of fast neutrons (Fig. 11).

After doses of 70 to 85 and 100 n units of fast neutrons, a maximum reduction of approximately 75 per cent in the platelet values of the peripheral blood occurred between the 5th and 10th day with return to normal values by 15 days (Fig. 12).

An "abortive rise" in heterophils and lymphocytes occurred after fast neutron exposure (Figs. 9 and 10) and is comparable to that which occurred after the exposure of rabbits to X-rays.

The morphological changes in the cellular constituents of the peripheral blood after fast neutron exposure are identical with those which occur in the peripheral blood of rabbits after "comparable" doses of X-rays.

Sacher⁽³¹⁾ has made a statistical analysis of the hematological data obtained by Jacobson et al⁽²⁶⁾ from rabbits exposed to X-rays and fast neutrons. He has derived a ratio of relative effectiveness of these two radiations. Using the data on the effect of fast neutron exposure and X-rays on the number of circulating heterophils and lymphocytes, he estimated the X/n ratio to be of the order of 6.3. These estimates are in essential agreement with the X/n ratio derived from survival data on rabbits (using the same type of instruments) by Hagen and Zirkle⁽³⁰⁾. It should be pointed out again that the n unit is

Figure 11. Reticulocytes of rabbits after exposure to fast neutrons.

FIG. II

Figure 12. Platelets of rabbits after exposure to fast neutrons.

44211

FIG. 12

arbitrary and that the I/n ratio does not mean that neutrons are 6.3 times more effective than X-rays in producing the same biological effect for the same amount of energy absorbed per gram of tissue. Since an exposure of 1 n results in the same energy absorption per gram as an exposure of 2.5 r, the relative biological effectiveness can be obtained by dividing the I/n ratio by 2.5.

3. The Hematological Effects of Chronic Whole Body Exposure of Mammals to Externally Originating Radiations.

Prior to the war no precise experimental information was available on the biological effects of chronic daily whole body exposure to penetrating radiations arising externally to the body in the range of the tolerance or "permissible" dose. The penetrating radiations which were of significance on the Plutonium Project were gamma rays, X-rays and neutrons. In studying these radiations, the animal exposures were given daily for periods of weeks to years. The daily exposure was given in a relatively short period (minutes) in one group of experiments, whereas other daily exposures were given continuously over an 8 or 24 hour period.

In the former category (brief daily exposures), the general biological effects of pile radiations (fast neutrons, slow neutrons and gamma rays) were studied by Zirkle et al ^(4b,32), Raper et al ⁽³³⁾, and Henshaw et al ^(5,34,35). These studies were limited largely to mice. Hematological studies were not extensive enough in these latter experiments to be of interest in this particular presentation.

Extensive studies of the peripheral blood were carried out by Lorenz et al ^(28,43) in a group of experiments involving the chronic exposure of animals (mice, guinea pigs, and rabbits) to gamma rays.

These experiments are of particular interest because of the fact that although most experimental irradiation of animals has involved relatively short daily exposures, these experiments largely duplicated the type of daily laboratory exposure that scientists and their assistants sustain in radiation laboratories.

3.1 Rabbits, Mice, and Guinea Pigs: The three species studied were rabbits (crosses of Dutch and American Blue), Mice (LAF₁ genetically homogeneous hybrids and strain A), and guinea pigs (hybrid and inbred). The animals were placed in cages that were arranged in a spherical manner about a centrally located radium source. Groups of the three species of animals were exposed to doses of 0.11, 1.1, 2.2, 4.4, or 8.8 r for 8 hours per day, others 24 hours per day, 7 days weekly. Although many observations of a biological nature were made on these animals while thus exposed over periods extending beyond three years, only the significant hematological effects will be discussed in this paper.

The degree of effect on the hemopoietic organs as reflected in the peripheral blood varied with the species: guinea pigs were most sensitive, mice intermediate, and rabbits relatively resistant. An equivocal reduction in the total leucocyte count in guinea pigs on the 0.1 r exposure level and a definite reduction in leucocytes (chiefly lymphocyte reduction) on the 1.1 r exposure level occurred within the first year of exposure. On the other hand, no comparable effects were observed in mice or rabbits exposed on these dose levels. With daily doses of 2.2, 4.4 and 8.8 r, significant hematological effects were

eventually apparent in all three species. Figures 13 to 19 illustrate the hematological effects produced by chronic exposure to gamma radiation. As has been universally true in our experiments in which animals were given single whole body exposures to penetrating radiations, a reduction in the lymphocytes in the peripheral blood was the earliest abnormality noted after chronic radiation exposure. In no instances were absolute increases in lymphocytes, eosinophils, or monocytes apparent. However, a relative increase in lymphocytes was common in animals that developed a leucopenia in the course of the exposure. Even though a marked lymphopenia or, in fact, an insidious reduction in other hematological values occurred, histological studies in general failed to show sufficient unequivocal evidence of change in the hemopoietic organs to account for the abnormalities encountered in the peripheral blood.

The acute onset of a severe anemia in guinea pigs on the 2.2, 4.4 and 8.8 r exposure was a common cause of death. This observation has been made in animals given whole body exposure to single doses of X-rays⁽⁶⁾ and fast neutrons⁽²⁶⁾ in the LD 50 range, as well as in animals given relatively large doses of parenterally administered radioactive elements such as plutonium^(21,36), radium⁽³⁷⁾, strontium⁽²⁰⁾ and phosphorus⁽³⁸⁾. This cannot always be accounted for by hemorrhage or by interruption of erythropoiesis. Schwartz *et al*⁽³⁹⁾ have some evidence to indicate that an increased hemolysis may play a role.

A macrocytic anemia appeared in certain of the animals exposed chronically to gamma rays. As is pointed out elsewhere in this report,

Figure 13. Chronic radiation response of the leucocytes of male guinea pigs.

FIG 13

11/20/52

Figure 14. Chronic radiation response of the lymphocytes of female guinea pigs.

11/2/5

FIG. 14

5-28-111

Figure 15. Chronic radiation response of the erythrocytes of female guinea pigs.

FIG. 15

5/22/54

Figure 16. Chronic radiation response of the leucocytes of rabbits.

524 III

FIG. 16

807

Figure 17. Chronic radiation response of the lymphocytes of rabbits.

57411

FIG. 17

Figure 18. Chronic radiation response of the leucocytes of female mice.

11/24/11

FIG. 18

60r

Figure 19. Chronic radiation response of the lymphocytes of female mice.

61211

FIG. 19

628111

macrocytic anemias have appeared in animals after exposure to single doses of penetrating radiation and after the parenteral administration of certain radioactive materials. Such anemias occur in humans after subacute whole body X-irradiation^(40,41) and after the administration of radioactive phosphorus⁽³⁸⁾. The etiology of the macrocytosis is obscure at the present time. No evidence of associated megaloblastic changes in the bone marrow has been found either by histological study of erythropoietic tissue or by studying dry smears of bone marrow. That it is not due to direct radiation of the gastrointestinal tract is brought out by the fact that radioactive elements that localize in blood-forming tissue and not in the gastrointestinal tract may also produce a marked macrocytosis^(20,36,37,38). Jacobson *et al*⁽⁴²⁾ have attempted unsuccessfully to influence the macrocytic anemia by the parenteral administration of folic acid in rats having strontium⁸⁹ intoxication.

3.2 Recurrent Anemia after Chronic Exposure to Gamma Radiation:

The observation of Lorenz, Jacobson, *et al*^(43,49) on guinea pigs exposed to 8.8 r given in 8 hours per day is of interest. If the irradiation of these guinea pigs was stopped after an anemia as low as 2.6 to 2.8 million erythrocytes per cu mm had developed (total accumulated doses ranging from 800 r to 1400 r), one of three things happened: (1) about fifty per cent of the animals developed further anemia and died within a few weeks; (2) approximately 20 per cent recovered to essentially normal limits and remained normal; and (3) the remaining 30 per cent

after recovering to an approximately normal blood count for weeks eventually had a recurrence of anemia and death ensued. This is an extremely interesting observation that deserves further intensive study.

3.3 Exposure of Humans to Single and Divided Doses of Externally Originating Radiation (Whole Body X-radiation): Few studies have been made on human subjects in which whole body exposure to single or divided doses of X-radiation was used. In those studies that have been reported, the subjects were often so ill as a result of the primary disease when they were treated with X-rays that interpretations of the hematological data were difficult, if not impossible.

Low-Bear and Stone⁽⁴¹⁾ undertook treatment of a number of patients with chronic degenerative disease of the joints with whole body exposure to X-rays. The total doses administered varied from 100 to 320 r given daily in divided doses of 5 to 20 r on the skin. The daily treatment required only a relatively few minutes of exposure. Jacobson and Dick⁽⁴⁰⁾ undertook comparable studies. These studies indicated a sensitivity of the hemopoietic system comparable to guinea pigs according to Lorenz et al⁽²⁸⁾, and comparable to dogs according to Prosser and Jacobson et al⁽⁴⁴⁾. With total accumulated doses of 150 r (estimated average dose for the whole body) leucopenia and lymphopenia occurred, the severity of which, however, varied widely from individual to individual. A striking effect was the definite macrocytic anemia that developed in the human subjects in about 200 days in practically all cases exposed to total doses of this magnitude. Spontaneous recovery of the hematological constituents of the peripheral blood of these individuals was universal but the time required for recovery varied widely.

3.4 Morphological Changes in the Cells of the Peripheral Blood

Following Acute and Chronic Irradiation: Morphological changes in the cellular constituents of the peripheral blood of rabbits given whole body X-radiation paralleled the histopathological changes seen in the hemopoietic system (15, 16). After 800 r and to a lesser extent with doses below this level, nucleated erythrocytes appear in the peripheral blood of the rabbit in increased numbers. The maximum number is not seen in the early period following irradiation but between the 10th and 25th days. This represents the period of maximum anemia in the peripheral blood and the maximum regeneration in the erythropoietic tissue. The degree of polychromatophilia, poikilocytosis, and anisocytosis depends largely on the type and chronicity of the anemia that was obtained in individual animals. Occasionally pyknotic changes are seen in the nucleated red cells (Fig. 20); Howell-Jolly bodies are rarely found. Occasionally a few stippled red cells are seen during the stage of anemia. Examination of the reticulocytes stained with brilliant cresyl blue fails to show any change in the morphological character of the reticulum (Fig. 21).

Degenerative morphological changes in the platelets are found in the peripheral blood of animals following LD 50 or higher doses of total body X-radiation. Giant platelets are frequently found concomitantly with the reduction in platelet values. Except for the increased size and distinctive granular degeneration, no further morphological alterations are discovered in the platelets of the peripheral blood. Only on very rare occasions are megakaryocytes found in the peripheral blood after total body X-radiation in the LD 50 range (Fig. 22).

Figure 20. Nucleated red cell from the blood of dog 72 hours after the administration of plutonium.

Figure 21. Reticulocyte from the blood of a goat 12 days after the injection of strontium⁸⁹.

Figure 22. Megakaryocyte from the blood of a rabbit 96 hours after 800 r total body radiation.

Figure 23. Lymphocyte from the blood of a dog 15 days after the administration of plutonium.

FIGURE 22

FIGURE 20

FIGURE 23

FIGURE 21

11/1/69

The morphological changes in the lymphocytes of the peripheral blood increase with increasing amounts of radiation. Clumping of nuclear chromatin may be seen early. Histological studies indicate that this change that occurs with relatively small doses (circa 50 r) may actually be reversible in some lymphocytes⁽¹⁵⁾. As it becomes more pronounced, pale bluish opaque areas are encountered. In addition, lymphocytes are seen with rounded chromatin masses seemingly free within the nuclear border or free within the cells, with an apparent disappearance of the nuclear membrane (Fig. 23). At the time of maximum lymphocyte reduction in the peripheral blood after irradiation, many lymphocytes are basophilic and the nucleoli are often prominent. These latter cells are indistinguishable from "blast" forms (Fig. 24). Occasionally mitotic "blast" cells appear after LD 50 or higher doses (Fig. 25). Other lymphocytes appear to have a split nucleus (Fig. 26) with considerable fragmentation. With LD 50 doses of acute total body irradiation, these destructive changes are more pronounced. Remnants of nuclear fragments, without nuclear outline and which cannot be distinguished as belonging to either the myeloid or lymphoid series (Fig. 27) appear. Many lymphocytes are bi-lobed, while others are difficult to distinguish from monocytes. During the peak of lymphocyte destruction, phagocytic monocytes with engulfed nuclear debris are found in the peripheral blood smears (Fig. 28).

During the phase of rapid reduction of heterophil values in rabbits, granulocytes which appear to be degenerating are seen in the peripheral blood. These cells show nuclear deformity and disintegration

Figure 24. Blast form from the blood of a dog 14 days after the administration of plutonium.

Figure 25. Mitotic blast cell from the blood of a rabbit 24 hours after 800 r total body X-irradiation.

Figure 26. Lymphocyte from the blood of a rabbit 96 hours after the administration of 800 r.

Figure 27. Cell from the blood of rabbit 25 days after 800 r.

FIGURE 26

FIGURE 24

FIGURE 27

FIGURE 25

7003/11

to such an extent that it is impossible to determine at which stage of maturity the cells were damaged (Figs. 29 and 30). The nuclear membrane is destroyed, and in most instances bluish non-specific granules of uneven contour fill the entire cell, covering the nucleus and cytoplasm to such an extent that it is impossible to identify the cell other than to say it is a damaged leucocyte (Fig. 31). Other bizarre forms are also seen that are difficult to describe. The cytoplasm of the heterophil may undergo changes following irradiation. Occasionally it contains vacuoles and has a mottled appearance. In some instances the granules of the heterophils appear to be damaged, as cells are found in which some granules cling to the nuclear membrane while the remainder of the cytoplasm remains clear (Fig. 32). Another type of cell seen in rabbits is one that resembles a basket cell, but differs from it in having one or two distinct chromatin masses that are deep blue against a network of destroyed perichromatin and cytoplasmic disintegration (Fig. 33).

A typical eosinophile characterized by irregularity in the granules both with regard to size and shape (Fig. 34) are occasionally seen in the blood smears of dogs following LD 50 or higher doses of total body X-radiation. Giant polymorphonuclear leucocytes are frequently seen which appear swollen (Figs. 35 and 36) and contain multilobed nuclei that are spread over the cytoplasm like globules having an extremely fine filament branching into 8 or more lobes (Fig. 37).

Figure 28. Phagocytic monocyte from the blood of a rabbit 11 days after 800 r.

Figure 29. Heterophil from the blood of a rabbit 96 hours after 800 r.

Figure 30. Heterophil from the blood of a rabbit 48 hours after 800 r.

Figure 31. Cell from the blood of man following daily total body exposure to 125 r for a period of 30 days.

FIGURE 30

FIGURE 28

FIGURE 31

FIGURE 29

936

Figure 32. Heterophil from the blood of a dog 9 days after the administration of strontium⁸⁹.

Figure 33. Cell from the blood of a rabbit 96 hours after 800 r.

Figure 34. Eosinophil from the blood of dog 95 days after the administration of plutonium.

Figure 35. Giant heterophil from the blood of a rabbit 48 hours after 800 r.

FIGURE 34

FIGURE 32

FIGURE 35

FIGURE 33

25411

Changes similar to those described above are found in patients after total body radiation with X-rays totaling 300 r in air administered in divided doses over periods of approximately 20 days. The most consistent morphological abnormality in the peripheral blood of these patients is the appearance of the basophilic degenerating cell described previously (Fig. 31). It appears as early as 3 days after the first treatment, and may be present as long as 2½ months after treatment ceases. Giant multilobed polymorphonuclear cells are frequently seen, and degenerating neutrophils and lymphocytes are found that show the characteristic massing of nuclear chromatin and general disintegration. Occasionally such cells contain round and red shaped bodies within the cytoplasm. Although they have the appearance of lymphocytes, it is possible that they are atypical and abnormal monocytes containing phagocytized material.

Radioactive isotopes, such as plutonium, strontium and phosphorus, give essentially the same results. As early as 4 days after treatment, the peripheral blood smears of patients to whom these elements have been administered may contain monocytes with nuclear deformities that show disintegration, lymphocytes with split or bi-lobed nuclei, and other morphological alterations previously described. These morphological changes are produced likewise with the administration of nitrogen mustards (38,45,46) (Figs. 38 and 39).

Figure 36. Giant eosinophil from the blood of a dog 4 days after the administration of plutonium.

Figure 37. Giant multi-lobed neutrophil from the blood of a rabbit 72 hours after 800 r.

Figure 38. Giant eosinophil from the blood of man 18 days after the administration of mustard gas.

Figure 39. Giant platelets 15 days after the injection of mustard gas in a human.

FIGURE 38

FIGURE 36

FIGURE 39

FIGURE 37

282111

4. The Comparative Effects of Parenterally and Orally Administered
Radioactive Elements (Acute and Chronic Studies)

The general biological effects of the various radio-elements involved in fission studies have been described in detail by Brues et al (47a,b,c.), Bloom et al (15), Prosser et al (25,48), and Jacobson et al (20,21,36,37). As was recently pointed out by Jacobson and Marks (49), the magnitude and nature of the effect of radioactive isotopes upon the constituents of the peripheral blood depend in a large measure on the dose administered, the type of emission-alpha, beta, gamma-, the energy of the radiation, the half-life of the isotope, the rate of elimination, and the distribution or localization in the body. With all the radio-elements that we have studied thus far, whether they were localized to bone or more generally distributed in the body, a reduction in the lymphocyte values of the peripheral blood was the most sensitive indicator of acute or subacute effects. Lymphocyte reduction may occur in the absence of histological evidence of radiation damage to lymphatic tissue. The dose of radioactive elements, such as strontium⁸⁹ and radium, required to produce bone sarcomas, lymphomas, and the like (50,51) is practically identical with that required to produce perceptible mean reduction of the leucocytes of the peripheral blood.

4.1 Plutonium: Plutonium which has a half-life of 2.4×10^4 years is an alpha emitter which localizes largely in bone and to a lesser extent in the liver when given intramuscularly or intravenously. Hematological studies of the peripheral blood after the administration

of plutonium as the citrate in the plus 6 state or as the nitrate in the plus 4 or 6 states have been made on mice, rats, and rabbits⁽²¹⁾. Intravenous and intracardiac doses of 0.001 to 0.119 $\mu\text{c/g}$ have been given to mice; intracardiac doses of 0.0006 to 0.006 $\mu\text{c/g}$ have been given to rabbits; and intravenous and intramuscular doses of 0.003 to 0.125 $\mu\text{c/g}$ have been given to the rat. There appeared to be no significant species difference in the sensitivity of the hemopoietic system of the mouse, rat, and rabbit to plutonium on the basis of the above experiments, nor was there any apparent strain difference within a species, or sex difference within a strain. No significant hematological alteration in the peripheral blood occurred after intracardiac, intravenous or intramuscular injection of 0.0003 or 0.0006 $\mu\text{c/g}$ of plutonium in the mouse, rat or rabbit during approximately 300 days of observation. A dose in the range of 0.003 $\mu\text{c/g}$, however, produced a minimum to moderate depression of the leucocytes and platelets in the three species within the first 100 days. A dose of 0.0063 $\mu\text{c/g}$ produced a moderate to severe and sustained leucopenia in all three species and caused an anemia in mice and rats. This latter dose caused early death in rats and mice (13 days). Doses above 0.0063 $\mu\text{c/g}$ produced correspondingly more severe and sustained anemia, leucopenia, reticulocyte reduction, and platelet deficiency in mice and rats.

A macrocytic anemia, as evidenced by an increased erythrocyte diameter and increased mean corpuscular hemoglobin, became apparent in rats after the parenteral administration of doses of plutonium between 0.0063 and 0.063 $\mu\text{c/g}$. This effect was less significant in

mice and rabbits after comparable doses. The acute and subacute cellular changes seen in the peripheral blood following plutonium administration were paralleled by concomitant histological changes in the hemopoietic tissue.

The fact that effects upon the hemopoietic system may be reflected in the peripheral blood of plutonium-injected animals, while comparable doses of injected radium produce no such blood change and yet produce bone tumors earlier and in greater number, is probably related to the difference in the site of deposition in the skeleton of the two elements. In general, the peripheral blood fails to reflect the development of bone sarcoma in radium-injected animals. The development of an increase in mean corpuscular hemoglobin and erythrocyte cell size independent of reticulocyte increase or decrease seems to be characteristic of plutonium when given parenterally within certain dose limits. It is likely that other internally deposited elements that affect the hemopoietic system will do likewise if chronically administered within certain dose limits. The greatest degree of macrocytosis thus far encountered in the experimental animal has been in the rat^(20,42) and goat⁽¹¹⁾ after strontium⁸⁹ administration.

4.2 Radium: The biological effects of radium which has a half-life of circa 1500 years are due almost exclusively to alpha radiation. This element localizes in bone as does plutonium and radiostrontium. Hematological studies⁽³⁷⁾ of the peripheral blood of rats, mice, and rabbits after the intraperitoneal, intravenous, or intracardiac injection of radium chloride indicate the following:

1. There appeared to be no significant species difference in the sensitivity of the hemopoietic systems of the rat and mouse to radium chloride, but rabbits were slightly more resistant than these species. CFI* female mice appear to be somewhat more resistant than A back cross male mice.** This may represent either a sex or strain difference.

2. Doses of 0.01 μg or lower caused no detectable hematological alterations in the peripheral blood of rats, mice, or rabbits. Doses in the range of 0.02 to 0.03 μg resulted in anemias and leucocyte reductions in rats and ABC male mice.

Doses as high as 0.1 μg , however, failed to produce an anemia in the rabbit, but did cause a reduction in leucocytes. A dose of 0.94 μg in the rat resulted in reticulocyte and platelet reduction.

3. Radium intoxication produced morphological changes in the nucleated cells of the peripheral blood comparable to those that occur after exposure to external radiations and parenterally administered plutonium.

4. A macrocytic anemia developed in rats, mice, and rabbits following the parenteral administration of radium chloride in doses between 0.1 and 0.2 μg .

*CFI, raised by Carverth Farms. - Homozygous recessive for aa bb cc

** A back cross - Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Heterogeneous Stock.

4.3 Yttrium (Y^{91}): Radioactive yttrium (Y^{91}) was administered by gavage to a group of rats in single doses of 5, 10, and 20 $\mu\text{c/g}$ ⁽⁵²⁾ in an attempt to determine the effects of beta radiation on the gastrointestinal tract. This element, which is a beta emitter and which has a half-life of 57 days, was given as the chloride. Absorption from the gastrointestinal tract was negligible. In another group, daily gavages of from 0.3 to 2.0 $\mu\text{c/g}$ were continued up to 178 days. A single dose of 20 $\mu\text{c/g}$ produced a marked initial elevation in erythrocyte and hemoglobin values, followed by a severe anemia that reached its maximum at 20 days. A 75 per cent reduction in lymphocytes occurred within 5 days but the lymphocyte count reached a normal value by 35 days. The heterophil values after this dose were extremely erratic. A dose of 10 $\mu\text{c/g}$ produced a temporary lymphopenia and only an erratic heterophilia. No hematological effect was observed after single doses of less than 10 $\mu\text{c/g}$.

The daily gavage of yttrium ⁹¹ in amounts from 0.3 to 2.0 $\mu\text{c/g}$ produced no anemia. A sustained heterophilia was present in all groups receiving daily gavage. In one experiment the daily gavage of 0.3 and 1.0 $\mu\text{c/g}$ produced a moderate lymphopenia which reached its maximum at 60 days, but gradual recovery to normal values occurred by 90 days even though yttrium administration continued through 178 days. In another experiment, daily ingestion of 0.3, 1.0 and 2.0 $\mu\text{c/g}$ produced a lymphopenia that reached its maximum at 90 days. Upon discontinuation of yttrium ⁹¹ by gavage at 90 days, a prompt return of the lymphocyte values to normal levels occurred. It is suggested that the lymphopenia described above results from the local effects of beta ray irradiation on the lymphoid tissue of the gastrointestinal tract.

No significant morphological change in the nucleated cells or erythrocytes occurred after single or repeated doses of ingested yttrium⁹¹.

4.4 Sodium (Na^{24}): Groups of A back cross and CFI male mice were injected intraperitoneally with sodium²⁴ as the chloride in doses of 12, 25, 25, 48, 75 or 95 $\mu\text{c/g}$ (55). This element, which has a half-life of 15 hours, is a pure gamma emitter. When thus administered it is distributed almost uniformly throughout the body. Leucocyte counts and differential blood smears were made on all animals, before the injection of the radioactive sodium and at intervals thereafter through 10 days in one experiment and through 15 days in another. A moderate to severe leucopenia followed the injection of all the above doses except the 12 $\mu\text{c/g}$. Death of the animals in the groups that received 48, 75 and 95 $\mu\text{c/g}$ occurred before the eighth day. The neutrophils per cu mm rose slightly by 3 days in the group that received 95 $\mu\text{c/g}$. No further sampling was possible in this group because of the death of all the animals. No reduction of neutrophils followed the injection of 25 $\mu\text{c/g}$ in one experiment, while 25, 48, 74 $\mu\text{c/g}$ produced a marked neutropenia in another experiment. Lymphopenia occurred in all-groups of animals that received 25, 25, 48, 74 and 95 $\mu\text{c/g}$ and remained thus depressed until death of the animals or until sampling was discontinued.

4.5 Barium-Lanthanum (Ba-La^{140}): These two radio-isotopes are considered together because upon the disintegration to barium (Ba^{140}) short-lived lanthanum daughter (La^{140}) is formed. Ba^{140} has a half-life of 12.5 days and La^{140} has a half-life of 40 hours. Barium emits only

a beta ray whereas lanthanum emits a beta and a gamma ray. The radiation effect of these two isotopes in the mouse may be entirely attributed to the beta rays which are essentially completely absorbed in the body, whereas the gamma ray with its long range and consequent diffuse dissipation of energy results in a considerable escape of energy from the body⁽⁵⁴⁾. (Ba-La)¹⁴⁰ administered intraperitoneally to mice in a dose of 0.2 $\mu\text{c/g}$ produced only a questionably significant decrease in the leucocytes, lymphocytes, and heterophils per cu mm during the 29 days of observation⁽⁵⁵⁾. A dose of 1.9 $\mu\text{c/g}$ produced in these constituents a significant and rapid reduction that was sustained for approximately 60 days. Rapid reduction in these values and death within 15 days followed the administration of 17 $\mu\text{c/g}$. No significant change occurred in erythrocyte or hemoglobin values with these doses during the 60 days of observation.

4.6 Strontium (Sr^{89}): This element, which emits beta rays only, has a half-life of 55 days and like radium and plutonium localizes in bone. Hematological studies of the peripheral blood of rabbits, rats, and mice after the intraperitoneal or stomach tube administration of Sr^{89} in doses from 0.015 to 14.5 $\mu\text{c/g}$ for varying periods up to 108 weeks indicated that a reduction in the heterophil values was of a comparable sensitivity to that of lymphocyte reduction as an indicator of acute and subacute effects⁽²⁰⁾. Moderate but significant reduction in heterophil values followed the intraperitoneal injection of 0.068 $\mu\text{c/g}$ in A back cross male mice; no effect was apparent after the administration of 0.015 $\mu\text{c/g}$ by the same route in A back cross males and

Carworth CF females. Progressively more significant and sustained reductions in heterophil and lymphocyte values followed higher doses. No anemia occurred in mice given doses of 0.034 μg or lower, but a moderate anemia was apparent with a dose of approximately 2.0 μg . The minimal dose exerting a mean detectable reduction in heterophil values in rats was between 0.22 and 0.25 μg . Doses above this produced a progressively more severe heterophil depression and lymphopenia.

Stomach tube administration of the isotope was studied in rats for comparison with doses given by the intraperitoneal route. The intraperitoneal dose required to produce a comparable effect on the cellular constituents of the peripheral blood is approximately twice that required by stomach tube. An intraperitoneal dose of 1.0 μg produced a significant reduction of heterophil values in the rabbit. Following an intraperitoneal dose of 3.0 μg a significant reduction in hemoglobin and erythrocyte values occurred with a rapid recovery. A sustained heterophil and lymphocyte reduction of significance was also apparent after this dose. Although drastic depletion of hematopoiesis in the bone marrow follows Sr^{89} intoxication, only moderate anemia developed in mice with doses up to 2.0 μg , due to the rapid development of extramedullary erythropoiesis in the spleen. Jacobsen, Simmons, et al⁽⁵⁶⁾ have shown that this dose produced a severe anemia in splenectomized animals (Fig. 40).

Studies of the effect of Sr^{89} on dogs and goats were also done^(11,57). The minimum effective dose required to produce hematological effects in these animals has not been determined. The lowest dose

Figure 40. Erythrocyte values of splenectomized rats following 22 μg /g strontium⁸⁹.

FIG. 40

FIG. 40

given to dogs was 0.10 $\mu\text{c/g}$, intramuscularly. Four days after this dose, the leucocytes were reduced approximately 60 per cent. The dogs showed no clinical symptoms, however, and all survived. The approximate LD 50 range for dogs and goats is 1.0 $\mu\text{c/g}$. The most spectacular myelocytosis yet encountered in animals made anemic by exposure to external penetrating radiations or paravertebral administration of radioelements was seen in rats⁽²⁰⁾ and goats⁽¹¹⁾ given Sr^{90} .

5. So Called Stimulating Effects of Radiation on Hemopoiesis

The idea that small doses of radiation "stimulate" cellular activity and cellular multiplication is not correct. In many of the experiments described above, the hematological effects of radiations applied acutely or chronically in relatively large to small doses were carefully studied with this in mind. In no instance was primary stimulation detected. On the other hand, compensatory increases were not infrequently encountered after initial depressions of the various cellular constituents of the peripheral blood. This is in agreement with the work of Murphy⁽⁵⁸⁾, who has often been misquoted. After acute total body exposure to penetrating X-rays and fast neutrons in the LD 50 range, a marked initial increase in heterophils occurs in rabbits within the first 24 hours without a preceding depression. A marked reduction occurs subsequently. This heterophil increase has been interpreted by Isaacs⁽⁵⁹⁾ to represent a hastened maturation of the myeloid precursors and may represent a "stimulation". Low-Bear and Stone⁽⁴¹⁾ reported a marked myelocytosis in patients with chronic degenerative arthritis after total body radiation with divided doses of penetrating X-rays totaling up to 300 r. This phenomenon did not

occur in patients similarly treated by Jacobson and Dick⁽⁴⁰⁾ but the underlying disease was not the same. It seems likely that the monocytosis was due to an altered reaction to radiation related to the underlying disease and not a primary stimulation of monocyte production.

6. Studies on the Peripheral Blood of Plutonium Project Personnel Exposed or Potentially Exposed to Radiations

As reported by Jacobson and Marks^(49,60), an extensive study of the hematological constituents of the peripheral blood of Plutonium Project personnel was undertaken at the inception of the program and still continues. This was done in an attempt to discover evidence of incipient injury to personnel from radiation exposure. We were aware of the fact that significant biological effects which could be detected by clinical laboratory examination invariably meant that serious damage to health had already occurred. Prophylactic measures designed to prevent or minimize the danger of irreversible damage from these physical agents were therefore of paramount importance.

All employees accepted for employment and placed in a particular job or position were classified in terms of individual occupation hazard in one of two groups: (1) the control group, and (2) the work hazard group. This division was not made in terms of the usual understanding of occupational hazards but rather in terms of the radiation hazards peculiar to the Plutonium Project.

The control group consisted of all employees working in areas in which exposure to radiations was negligible or absent. The Work Hazard Group consisted of all individuals in the laboratory who worked in areas where radiation or radio-active materials were being used and

who might, therefore, be exposed to them. The frequency of the hemtological examinations which were conducted in these two groups varied from daily studies to studies conducted every three months.

Relatively few instances were encountered in which changes in the hemtological constituents of the peripheral blood of personnel might be attributed to contact with external radiation or the deposition of radioactive materials within the body. The great variation in the steady state of the various hemtological constituents of the peripheral blood from one individual to another, the inaccuracy of modern methods for measuring these constituents, and the individual physiological variation from hour to hour, day to day, and month to month made it almost impossible to interpret minor fluctuations possibly resulting from acute or chronic radiation exposure. Therefore, more emphasis on basic biological research on the mechanism of action of ionizing radiations is required before one can logically devise clinically applicable tests to determine and evaluate incipient damage from these agents. The concept that no amount of ionizing radiation can be tolerated by the human obviously cannot be accepted if work is to continue in this field, but a firm biological basis for a permissible dose should be established.

7. Literature Cited

1. Raper, J. and Barnes, K. K.: Effects of External Irradiation with Beta Rays on the Peripheral Blood of Rabbits. *PER* Vol. 22B.
2. Raper, J., Zirkle, R. and Barnes, K. K.: Comparative Lethal Effects of External Beta Irradiation. *PER* Vol. 22B.
3. Raper, J., Henshaw, P. S., and Snider, R. S.: Effects of Total Surface Beta Irradiation. *Radiology* 49: 315, 1947.
4. (a) Zirkle, R.: Components of the Acute Lethal Action of Slow Neutrons. *Radiology* 49: 269, 1947.
(b) Zirkle, R., Raper, J., Riley, E. F., Stapleton, G. E.: Additivity of Fast Neutrons and Gamma Rays in their Acute Lethal Action on Mice. *PER* Vol. 22B.
5. Henshaw, P. S., Snider, R. S., Riley, E. F., Stapleton, G. E., and Zirkle, R.: Comparative Late Effects of Single Doses of Fission Neutrons and of Gamma Rays. *PER* Vol. 22B.
6. Jacobson, L. O., Marks, E. K., Simmons, E., Hagen, C. and Zirkle, R.: Effects of X-rays on Rabbits. Part II. The Hematological Effects of Total Body X-irradiation in the Rabbit. *PER* Vol. 22B.
7. Stearns, P., Simmons, E. and Jacobson, L. O.: The Effects of Total Body X-irradiation on the Peripheral Blood and Blood Forming Tissues of the Rat. *PER* Vol. 22B.
8. Sacher, G.: Lethal Action of Single Doses of X-rays on Mice. Unpublished Data.
9. Jacobson, L. O.: Effect of X-rays in the Hematology of the Chick. *PER* Vol. 22B.
10. Nickson, J. J., Cantrell, S. T., and Jacobson, L. O.: Blood Changes in Humans Following Total Body Irradiation. *PER* Vol. 20B.
11. Swift, M., Prosser, C. L., and Mica, E.: Effects of Sr^{89} and X-radiation on Goats. *PER* Vol. 22B.
12. Hagen, C.: Acute Lethal Action of X-rays on Guinea Pigs, Chickens and Amphibians. *PER* Vol. 22B.
13. Prosser, C. L., Painter, E., Swift, M., and Jacobson, L. O.: Clinical Physiology of Dogs Exposed to Single Total Body Doses of X-rays. *PER* Vol. 22B.

14. Allen, J. G., Snadersen, M. and Jacobson, L. O.: Hyperheparinemia as cause of Post-Irradiation Hemorrhage. *PER Vol. 22B*.
15. Bloom, W.: Histological Changes Following Radiation Exposure. *Radiology 49: 344, 1947*
16. Jacobson, L. O., and Block, M.: Unpublished Data.
17. Allen, J. G., and Jacobson, L. O.: Hyperheparinemia: Cause of the Hemorrhagic Syndrome Associated with Total Body Exposure to Ionization Radiation. *Science 105: 388, 1947*.
18. Allen, J. G., and Jacobson, L. O.: Irradiation Hemorrhage. *Proc. Inst. Med. of Chicago. 16: 376, 1947*.
19. Shouse, S., Warren, S. L., and Whipple, G.: II. Aplasia of Marrow and Fatal Intoxication in Dogs Produced by Roentgen Radiation of All Bones. *Jr. Exp. Med. 52: 421, 1951*.
20. Simmons, E., and Jacobson, L. O.: Radiotoxicity of Injected Strontium⁸⁹ for Rats, Mice and Rabbits. Part IV. The Hematological Effects of Enterally and Parenterally Administered Strontium⁸⁹ in Mammals. *PER Vol. 22B*.
21. Jacobson, L. O. and Simmons, E.: Acute and Chronic Toxicity of Plutonium for Mice, Rats, and Rabbits. Part IV. Hematological Effects. *PER Vol. 22B*.
22. Jacobson, L. O., Marks, E. K., Gaston, E.: Effect of Nitrogen Mustards on the Whole Blood Coagulation Time of the Rabbit. *Quarterly Report, Biology Division, Nov. 1947*.
23. Smith, T. R., Jacobson, L. O., Spurr, C., Allen, J. G. and Block, M.: Coagulation Defect Produced by Nitrogen Mustard. *In Press*.
24. Allen, J. G., Bogardus, G., Jacobson, L. O., and Spurr, C. L.: Some Observations on Bleeding Tendency in Thrombocytopenic Purpura. *Annals of Internal Medicine 27: 382, 1947*.
25. Prosser, C. L., and Jacobson, L. O.: The Clinical Sequence of Physiological Effects of Ionizing Radiation in Animals. *Radiology 49: 299, 1947*.
26. Jacobson, L. O., and Marks, E. K.: Comparative Action of Cyclotron Fast Neutrons and X-rays. Part II. Hematological Effects Produced in the Rabbit by Fast Neutrons. *PER Vol. 22B*.
27. Brues, A., and Lisco, H.: Tumor Induction and Other Late Effects of Single Doses of X-ray in Rats and Mice. *Unpublished Data*.
28. Lorenz, E., Heston, W., Jacobson, L.O., Eschenbrenner, A. B., Shinkin, M. and Derringer, M.: Biologic Effects of Long Continued Whole Body Irradiation by Gamma Rays in Mice, Guinea Pigs, and Rabbits. *PER Vol. 22B*.

29. (a) Jacobson, L. O., Marks, E. E., and Simmons, E.: Effect of Total Body X-irradiation on a Pre-existing Induced Anemia in Rabbits. Part I. Response to Animals with Phenylhydrazine. *PER Vol. 22B*.
(b) Jacobson, L. O., Marks, E. E., and Gaston, E. O.: Effect of Total Body X-irradiation on a Pre-existing Induced Anemia in Rabbits. Part II. Response to Animals with an Anemia Induced by Bleeding. *PER Vol. 22B*.
30. Hagen, C., and Zirkle, R.: Comparative Action of Cyclotron Fast Neutrons and X-rays. Part I. Lethal Action on Mice and Rabbits. *PER Vol. 22B*.
31. Sacher, G., and Pearlman, H.: The Comparative Action of Cyclotron Fast Neutrons and X-rays. Part III. Statistical Analysis of Blood Data. *PER Vol. 22B*.
32. Zirkle, R. E., and Raper, J.: Use of the Clinton Pile for Radiobiologic Experiments to Fast Neutrons and Slow Neutrons. *PER Vol. 22B*.
33. Raper, J., and Zirkle, R.: Preparations of Artificial Gamma Ray Sources for Radiobiology. *PER Vol. 22B*.
34. Henshaw, P. S., Snider, R. S., Riley, E. F., Stapleton, G. E., and Zirkle, R.: Effects of Periodic Doses of Slow Neutrons on Mice. *PER Vol. 22B*.
35. Henshaw, P. S., Riley, E. F., and Stapleton, G. E.: Biologic Effects of Pile Radiations. *Radiology* 49: 349, 1947.
36. Jacobson, L. O., and Edwards, R.: Hematological Effects of Parenterally Administered Plutonium in the Dog. *PER Vol. 22B*.
37. Jacobson, L. O., and Simmons, E.: Radiotoxicity of Injected Radium. Part II. Hematological Effects. *PER Vol. 22B*.
38. Jacobson, L. O., Spurr, C. L., Smith, T. R., and Dick, G.: Radioactive Phosphorus (P^{32}) and Alkylamines (Nitrogen Mustards) in the Treatment of Neoplastic and Allied Diseases of the Hemopoietic System. *Med. Clin. of N. Amer.* Jan. 1947.
39. Schwartz, S., Entz, E., Porter, L., Jacobson, L. O., and Watson, C. J.: Studies on the Hemolytic Effects of Radiation. *PER Vol. 22B*.

40. Jacobson, L. O., and Dick, G. F.: Studies in Total Body X-Irradiation of the Human. Unpublished Data.
41. Low-Beer, A., and Stone, R. S.: Studies on Total Body X-Irradiation. PFR Vol. 21.
42. Jacobson, L. O., Stearns, P., and Simmons, E.: The Effect of Folic Acid (*Leuconococcus casei* factor) on the Response of the Peripheral Blood and Blood-Forming Tissue of the Rat to Parenterally Administered Sr^{89} PFR Vol. 22B.
43. Lorenz, E., Jacobson, L. O., and Eschenbrenner, A. B.: Studies on the Recovery from Anemia Induced by Chronic Gamma Ray Exposure of Guinea Pigs. To be published.
44. Prosser, C. L., Moore, M., Painter, E., Jacobson, L. O. et al: Clinical Physiology of Dogs Exposed to Daily Total Doses of X-rays. PFR Vol. 22B.
45. Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T. R., Lushbaugh, C., and Dick, G. F.: Studies on the Effect of Methyl bis (B chloroethyl) Amine Hydrochloride (Nitrogen Mustard Therapy) on Neoplastic Diseases and Allied Disorders of the Hemopoietic System. Jr. A.M.A. 132: 263, 1946.
46. Spurr, C. L., Jacobson, L. O., Smith, T. R., and Barron, E.S.G.: Clinical Application of Methyl bis (B Chloroethyl) Amine Hydrochloride to the Treatment of Lymphomas and Allied Dyscrasias (Abstract): Cancer Research 7: 51, 1947.
47. (a) Brues, A. M., Lisco, H., and Finkle, M.: Acute Radio-toxicity of Injected Sr^{89} for Rats, Mice and Rabbits. PFR Vol. 22B.
48. (b) Brues, A. M., Lisco, H., and Finkle, M.: Acute Toxicity of Plutonium for Mice, Rats, and Rabbits. PFR Vol. 22B.
- (c) Lisco, H., Brues, A. M., and Finkle, M.: Late Effects of Insoluble Ingested Y^{91} and Other Fission Products. PFR Vol. 22B.
48. Swift, M., Prosser, C. L., Painter, E., Edwards, R., and Jacobson, L. O.: Clinical Physiology of Dogs Injected with Sr^{89} . PFR Vol. 22B.
49. Jacobson, L. O., and Marks, E. E.: The Hematological Effects of Ionizing Radiations in the Tolerance Range. Radiology 49: 286, 1947.
50. Brues, A. M., Lisco, H., and Finkle, M. P.: Carcinogenic Action of Some Substances Which May Be a Problem in Certain Future Industries. (Abstract) Cancer Research 7: 48, 1947.

51. Lisco, H., Finkle, M. P., and Brues, A. M.: Carcinogenic Properties of Radioactive Fission Products and of Plutonium Radiology 49: 361, 1947
52. Jacobson, L. O., and Simmons, E.: Radiotoxicity of Enterally Administered Y^{91} . Part VIII. Hematological Effects. PFR Vol. 22B.
53. Finkle, M., Snyder, R., Jacobson, L. O., and Simmons, E.: Acute Radiotoxicity of Injected Sodium (Na^{24}). Part II. Effects on the Leucocytes of the Peripheral Blood PFR Vol. 22B.
54. Finkle, R., Snyder, R., and Tumkins, P.: Acute Radiotoxicity of $(Ba-La)^{140}$ in Rats and Mice. Part I. Preparation and Administration on the Emitters. PFR Vol. 22B.
55. Jacobson, L. O.: Acute Radiotoxicity of $(Ba-La)^{140}$ in Rats and Mice. Part V. The Effects of $(Ba-La)^{140}$ on the Hematological Constituents of the Peripheral Blood of Rats and Mice. PFR Vol. 22B.
56. Jacobson, L. O., Simmons, E., and Block, M.: Radiotoxicity of Injected Sr^{89} in Splenectomized Mice. PFR Vol. 22B.
57. Swift, M. M., and Prosser, C. L.: The Excretion, Retention, Distribution and Clinical Effects of Strontium⁸⁹ in the Dog. Part I. Report of Experimental Work. PFR Vol 22B.
58. Murphy, J. B.: The Lymphocyte in Resistance to Tissue Grafting. Monograph 21, Rockefeller Institute for Medical Research, 1926.
59. Isaacs, R.: The Relation of Cell Types in Leukemia to Sensitivity to Radiation. Vol. Hemat. 52: 414, 1934.
60. Jacobson, L. O., and Marks, E. K.: Clinical Laboratory Examinations of Plutonium Project Personnel. PFR Vol 21.

END