The direct effect of x-rays on the blood-forming organs has been widely studied and the results have been repeatedly reviewed (1–4). The histologic changes produced by irradiation were first investigated by Heineke, in 1905 (5). It has been generally agreed that the lymphocytes and polymorphonuclear leukocytes are sensitive to x-rays and that the precursors of all the circulating blood cells are more radiosensitive than the fully developed cells. In the irradiated bone marrow the myeloid cells were found to be greatly damaged and decreased in number, while the erythroblasts might be affected in the same way but to a lesser degree. The latter were found by Siciliano and Banci Buonamici (6) to regenerate more rapidly than the former.

Shouse, Warren and Whipple (7) showed that extensive irradiation caused aplasia of all marrow cells except the connective-tissue and fat cells, endothelium of blood vessels, phagocytes, and occasional normoblasts.

Latta and Ehlers (4) demonstrated that the blood sinuses of the irradiated marrow were more noticeable and nearly empty; the giant cells were decreased in number and stained atypically, while the erythroblastic cells were widely scattered and not found in groups. In the irradiated lymph nodes and spleen there were generally destruction and reduction of the lymphoid tissue, the germinal centers being left as remnants. Phagocytes with granular inclusions were present in the remaining lymphoid tissue.

Akaiwa and Takeshima (8) described the early degenerative changes of the germinal centers in irradiated lymph nodes as consisting of nuclear disintegration of the lymphoid cells followed by phagocytosis of the damaged cells. Kolodny (9) showed that the irradiated spleen was anemic and composed mainly of splenic pulp, while lymphoid cells in the pulp and malpighian bodies were scarce.

In the circulating blood, lymphopenia and polymorphonuclear leukopenia have been constant findings, but there has been diverse opinion as to the fate of the red blood cells. Piney and Mayneord (10) found no change in their number, while Aubertin and Beaujard (11) and Wright and Bulman (12) reported experimental anemia following irradiation. Leukopenia and anemia were reported in x-ray workers and patients exposed to x-radiation (13).

There are few reports concerning changes in the unirradiated parts of the hemopoietic system of an animal which has received radiation in some other part of the body. Aubertin and Beaujard (11) described the unirradiated marrow in rabbits as hyperplastic and red in appearance. Siciliano and Banci Buonamici (6) found morphological alteration and modified distribution of the cellular elements. The percentage of immature red cells was increased,
while the white blood cells were decreased; the red blood cells predominated over the white. Akaiwa and Takeshima (8) described the same changes in the unirradiated lymph nodes as in the irradiated ones, but of less extent.

Some additional data on the histologic changes in the irradiated and unirradiated bone marrow, lymph nodes, and spleen will be presented here.

**Material and Method**

Normal male albino rats, six to nine months old and weighing 350–450 gm., were used throughout the experiment. Only the posterior surface of the right hind limb was exposed to irradiation, the factors being 100 kv., 4 ma., 4 mm. aluminum filter, and a skin-target distance of 30 cm. The effective wavelength was 0.315 Å. Daily doses of 125 r in ten minutes were delivered to the skin. Six animals received a total of 1000 r in eight days, four 2500 Y in twenty days, and four 5000 r in forty days. On the day following the completion of irradiation the animal was killed with ether, and the spleen, sub-maxillary lymph nodes, and bone marrow of both femurs were removed for study either by means of Zenker formalin fixation and methyl green pyronin stain or "Susa" fixation and azocarmine GX stain.

**Observations**

The histologic changes of the blood-forming organs were quite constant in all animals, though varying in degree.

**Changes in the Irradiated Bone Marrow:** The irradiated bone marrow showed three prominent changes in structure: (a) increase of fat cells, (b) hypoplasia of erythrocytes in active proliferation, (c) hypoplasia of leukocytes.

(a) The number of fat cells increases in direct proportion to the amount of x-rays administered (Figs. 1, 5, 9). In Fig. 1, which represents the bone marrow following the administration of 1000 r, the fat cells can be seen between the cell cords and sinusoids as oval or spherical bodies. Their number is increased as compared to normal (see Ma, 14: Figs. 2, 4, 9 and 10), but is less than in animals receiving 2500 r and 5000 r. In the more intensely irradiated bone marrow, the fat cells are greatly increased in number and are polygonal in shape (Fig. 5).

(b) The erythroblasts and erythrocytes of irradiated bone marrow are reduced as compared with the bone marrow of normal rats. In marrow that received 1000 r (Fig. 1) there are cell cords and sinusoids between the fat cells. The cell cords are composed of great numbers of erythroblasts and a smaller number of young and adult leukocytes, while the sinusoids consist mainly of early and adult erythrocytes. In the cell cords active transformation of nucleated into non-nucleated red blood cells can be seen. In the sinusoids, the early erythrocytes, which stain deeply, are numerous, grouped together in large masses. The relative number of the early erythrocytes in the irradiated bone marrow is greater than normal. The distinction between the cell cords and the sinusoids, which is clear in normal bone marrow, becomes obscure following irradiation. This is explained by the fact that the cell cords are constantly in the process of transformation into sinusoids, so
FIGS. 1-4. IRRADIATED AND UNIRRADIATED BONE MARROW OF ANIMAL RECEIVING 1000 r

Figs. 1 and 2 show the irradiated marrow (X 235 and 450); Figs. 3 and 4 the marrow of the opposite, unirradiated side of the same animal (X 235 and 800). A. Amitotic myeloid cells. P. Phagocytic cells.

All preparations illustrated were stained with methyl green pyronin after Zenker formalin fixation except Fig. 23, in which staining was with azo-carmine GX after "Susa" fixation.

that the elements of the two are in direct continuation. When the immature cells in the cell cords become mature, sinusoids are formed.

With heavier irradiation, the tissues between the fat cells in the bone marrow consist almost entirely of non-nucleated red blood cells and have the appearance of sinusoids. The cell cords disappear entirely. This is shown
Figs. 5–8. Irradiated and Unirradiated Bone Marrow of Animal Receiving 2500 r

Figs. 5 and 6 show the irradiated marrow (× 235 and 450); Figs. 7 and 8 the marrow of the opposite, unirradiated side of the same animal (× 235 and 450). P. Phagocytic cells.

in Fig. 6, a highly magnified portion of Fig. 5, representing tissues that received 2500 r. The same phenomenon is seen even more clearly in bone marrow receiving 5000 r (Fig. 10). In Fig. 23 the sinusoids are seen anastomosing and communicating with the veins. Fig. 24 illustrates more marked changes of the same kind after irradiation with 5000 r; it also shows that when the fat cells are more numerous, the sinusoids are smaller, as demonstrated by a comparison with the corresponding structures in Fig. 23.
The number of the undifferentiated reticular cells and phagocytes in the bone marrow increases with the amount of radiation (Figs. 2, 6, and 10).

(c) Hypoplasia of the leukocytes is indicated by the reduced number of cell divisions and the smaller proportion of leukocytes in irradiated marrow. In bone marrow receiving 1000 r, cell division (either mitotic or amitotic) occurs in the cell cords; it is rare or entirely absent following irradiation with 2500 or 5000 r. The number of the leukocytes in proportion to the red blood
cells is less in the irradiated than in the normal marrow. Furthermore, the leukocytes decrease when the quantity of radiation is increased; with doses of 5000 r, they are few in number or entirely absent.

Changes in the Unirradiated Bone Marrow: The changes in the bone marrow of the unirradiated femur in animals in which the irradiated side received 1000, 2500 or 5000 r (Figs. 3, 7 and 11) were all of about the same degree and did not appear to vary with the quantity of radiation. The changes were (a) increase of fat cells, (b) hyperplasia of erythrocytes, and (c) hypoplasia of leukocytes.

Figs. 13-15. Submaxillary Lymph Nodes from Animals Receiving, over the Right Hind Leg, 1000 r (Fig. 13), 2500 r (Fig. 14), 5000 r (Fig. 15). × 25
(a) The unirradiated bone marrow contained more fat cells than normal but less than the irradiated bone marrow. The distribution of the fat cells showed no marked variations in the different animals. A decrease of fat cells with increasing doses of radiation was the only possible difference.

(b) Hyperplasia of erythrocytes was observed in all the animals (Figs. 3, 4, 7, 8, 11, 12). The erythroblasts are massed in dark areas, and between them are a large number of early erythrocytes. With the oil immersion lens, many of the erythroblasts in the dark areas show amitotic figures in different stages (Fig. 4). In some of the more primitive erythroblasts, like the megaloblasts,
blasts, mitotic divisions may also be present. Cell divisions in erythroblasts are found more frequently in the marrow of the unirradiated side than normal bone.

Some of the sinusoids of the bone marrow of the unirradiated femur contain chiefly erythrocytes and early erythrocytes with few normoblasts; others contain fewer erythrocytes and early erythrocytes but a large number of normoblasts. The sinusoids are smaller in size and less in number than in normal marrow. In comparison with the irradiated bone marrow, the unirradiated shows a reversed phenomenon, i.e. many large cell masses and a few small sinusoids.

(c) In the unirradiated bone marrow of all animals in which the opposite side received radiation, both young and adult leukocytes are fewer than in normal control rats. In some heavily irradiated animals cells of the leukocytic group appear to be less numerous than in lightly irradiated ones.

**Changes in the Submaxillary Lymph Nodes:** The prominent change in the submaxillary nodes is a reduction of the cortical thickness and a corresponding expansion of the medullary area. The cortex may be reduced to a very thin layer or may be entirely absent, with the medulla occupying the whole organ. The reduction of the cortex is due to a decrease of the nodules, while the medulla is expanded by extension of the sinusoids. Figs. 13, 14, and 15 represent the changes in the lymph node after different doses of radiation applied to the right leg. Fig. 13 shows small cortical nodules and large, expanded medullary sinusoids (right side of the figure) following a dose of 1000 r. In the more heavily irradiated animals these changes are more pronounced. In the animals receiving 5000 r the nodules are almost entirely absent and the sinusoids of the medulla extend throughout the entire organ (Fig. 15).

In addition to the changes in the general structure of the lymph node there are also changes in the cell types. The normal lymph node contains lymphocytes among which a few plasma cells may be found. In the lymph nodes of the experimental animals the plasma cells become more numerous, apparently increasing in proportion to the dosage of radiation. In the lymph nodes of animals receiving 5000 r most or even all of the cells may be plasma cells.

In the sinusoids, undifferentiated reticular cells can be demonstrated in large numbers with azocarmine GX stain after “Susa” fixation.

**Changes in the Structure of the Spleen:** The bulk of the white pulp decreases in correspondence with the amount of radiation to which the animal is exposed (Figs. 16, 17 and 18). The splenic nodules or follicles seem to be more resistant than those of the lymph node. In animals receiving 5000 r, the nodules in the spleen may still be of considerable size, although they are entirely absent from the lymph node. The lymphoid sheath in animals irradiated with 5000 r is still thick and the lymphocytes are arranged in concentric layers surrounding the artery (Fig. 21). Between the layers, the connective-tissue septa and their accompanying undifferentiated reticular cells show constrictions.

The bulk of the red pulp increases in correspondence with the amount of radiation delivered to the animal (Figs. 16, 17 and 18). Most of the sinusoids in the red pulp are formed at the time of the formation of the erythrocytes
Fig. 19. Spleen of animal irradiated with 5000 r, showing erythroblasts of different stages in the form of islands scattered throughout the pulp. × 295.

Fig. 20. Spleen of an animal irradiated with 5000 r, showing erythroblastic islands in connection with vein. × 370
Fig. 21. Spleen of a rat receiving 5000 r over the right hind limb, showing the lymphocytes in concentric layers with connective-tissue septa surrounding an artery.

Fig. 22. Unirradiated bone marrow of a rat which received 5000 r over the right hind limb showing free communication of many sinusoids with a vein. × 125

Figs. 23 and 24. Irradiated bone marrow (2500 r, Fig. 23; 5000 r, Fig. 24), demonstrating the passing of the erythrocytes from the sinusoids into the veins. V. Vein. LV. Large vein. × 125

which originate in the spleen. The formation of sinusoids from degenerated arteries, as described by one of us (Ma, 15), may also occur but is rare. Islands of erythroblasts of different stages can be found scattered throughout the red pulp (Fig. 19). Some of the erythroblastic islands containing large
numbers of early erythrocytes are directly connected with the veins (Fig. 20). The majority of the cells between the erythroblastic islands are of the phagocytic type. Many cells, including fixed and free undifferentiated reticular cells, contain yellowish or brownish droplets and remnants of decomposed erythrocytes. The phagocytes become more and more numerous as the animal is more heavily irradiated.

With methylene green-pyronin stain after Zenker-formalin fixation, the phagocytes can be seen undergoing gradual transformation into megaloblasts. The transformation of phagocytes into erythroblasts is apparent from the fact that some of the latter contain yellowish-brown pigment which exactly resembles that found in the former.

**DISCUSSION**

In the present experiment, changes in the irradiated bone marrow are considered as direct effects and those in the bone marrow of the unirradiated side, lymph nodes, and spleen as indirect effects.

**Direct Effects:** In the irradiated bone marrow the erythroblasts and erythrocytes are less numerous than normal. The decrease in these cells cannot be regarded as due to degeneration occurring in the erythropoietic system, since the erythroblasts and early erythrocytes which can still be found in heavily irradiated bone marrow show no evidence of degenerative change. Their diminution in number may be due to several factors: (a) rapid drainage, (b) lack of compensation, and (c) intensive formation of fat cells.

(a) The rapid transformation of nucleated into non-nucleated erythrocytes leads to a gradual transition between the cell cords and the sinusoids of the marrow. The erythrocytes, as soon as they are formed, become free and pass out of the bone marrow through the veins. This phenomenon has been demonstrated in the normal bone marrow (14) but it is not as prominent as in irradiated bone marrow. In the latter, the transformation and subsequent drainage of cells are rapid, leaving only a small number of erythrocytes behind.

(b) Normally the undifferentiated reticular cells compensate for the loss of erythroblasts by differentiating into the latter type. While this process goes on rapidly in the irradiated bone marrow, as evidenced by the increase of the undifferentiated reticular cells, it is insufficient for complete compensation.

(c) It has been shown that the fat cells increase in direct proportion to the amount of radiation delivered to the animal. It has been found, also, that in the undifferentiated reticular cells, which may or may not contain pigment of decomposed blood corpuscles, there are small fat vacuoles or large droplets or globules of fat, indicating that the fat cells are derived from undifferentiated reticular cells. Roentgen radiation seems to have a special influence in bringing about transformation of the latter into fat cells. As the number of fat cells increases with irradiation, they may eventually occupy a great part of the bone marrow. Since a great number of reticular cells are differentiated into fat cells, the formation of the necessary erythroblasts suffers and their number becomes reduced.

As to the myeloid series in the irradiated bone marrow, it has been shown
that the myeloid cells become less and less active with the increase of radiation because the number of cells in mitosis is reduced. This decrease of mitoses in myeloid cells leads to a state of hypoplasia, or even aplasia of mature leukocytes when irradiation is intense. As mentioned above, the myeloid cells are more radiosensitive than the circulating blood cells and the erythroblasts in the bone marrow. Clinical experience in the treatment of patients suffering from myeloid leukemia shows the extreme sensitiveness of this type of cell as reported by Piney and Riach (18) and others. In the irradiated bone marrow, the erythroblastic cells show first hyperactivity and then failure, while the myeloid cells show decrease of activity with hypoplasia.

The present work has shown that the end-stage of hyperplasia of hematogenic cells may be succeeded by a stage of failure, in which the marrow becomes hypoplastic or even aplastic. With aplasia, fat cells predominate, as in the bone marrow of patients suffering from idiopathic aplastic anemia (19). In such patients the bone marrow may also show hyperplasia (20) or aplasia of the blood cells. In the irradiated bone marrow, the cells of the myeloid series are more involved than those of the erythroblastic series because of the difference in radiosensitivity.

**Indirect Effects:** In the bone marrow of the unirradiated side, the small number and size of the sinusoids may be attributed to the rapid formation of erythrocytes on the one hand and the active differentiation of the erythroblasts on the other. More undifferentiated reticular cells are differentiated into erythroblasts than fat cells. These erythroblasts are non-mobile cells which may divide mitotically or amitotically to form cell cords; the increase of mitosis and amitosis indicates a more active formation of erythrocytes. In Fig. 22 showing the unirradiated bone marrow after a dose of 5000 r to the opposite femur, it is seen that a vein receives a number of sinusoids, which communicate freely with one another. Since, when the erythrocytes are formed and become free, they can drain into the vein immediately, there are not many remaining between the cell cords to fill the sinusoids.

In the submaxillary lymph node there are three prominent changes: (a) reduction in number and size of the lymph nodules in the cortex, (b) increase in number and size of the sinusoids in the medulla, (c) increase of plasma cells. As one of us (16) has pointed out, nodules and cords may normally be transformed into sinusoids and vice versa. When lymphocytes accumulate, a cord or a nodule is formed; when they are discharged, a sinusoid is the result. After irradiation lymphocytes are produced to form cords and nodules and are discharged into the sinusoids, which become larger in size and greater in number. In the more heavily irradiated animals the lymph node may become entirely free from nodules and consist of a large number of wide sinusoids with some medullary cords (Fig. 15). The failure of the lymphocytes to accumulate and to form nodules and cords indicates excessive activity of the lymph node, since in the most heavily irradiated animals the lymph node (Fig. 15) consists almost entirely of plasma cells. These cells have been considered as rapidly formed cells which undergo transformation into lymphocytes when they are discharged into the circulatory system.

The white pulp of the spleen is affected in the same way as the nodules of
the lymph node but less markedly. As mentioned previously, the undifferentiated reticular cells in the lymphoid sheath show constriction, but these constricted cells, as has been shown by one of us (15), continue to give rise to lymphocytes.

The red pulp of the spleen is also active in erythropoiesis, as shown by the presence of sinusoids containing erythrocytes and of islands of erythroblasts of different stages. The connection of the erythroblastic islands containing early erythrocytes with the vein shows that, when the nucleated erythrocytes are transformed into non-nucleated elements, these islands or cords change into sinusoids draining into the vein, as occurs also in the unirradiated bone marrow.

As mentioned above, the phagocytes between the erythroblastic islands in the pulp of the spleen have the potentiality of transformation into erythrocytes. This process adds further evidence of increased erythropoiesis in the spleen of the locally irradiated animal.

To summarize the indirect effects, all the changes found in the lymph node, spleen, and unirradiated bone marrow point to hyperactivity of the hemopoietic tissue.

The lymphocyte is one of the most radiosensitive cells in the circulating blood. Since complete circulation of the blood requires from fifteen to twenty-three seconds in man at rest and much less during exercise (21, 22), it is probable that most of the blood cells pass periodically through the irradiated areas during the exposure of the animal to x-rays. When an extremity is exposed for ten minutes daily the accumulated effect will cause some damage of the circulating cells. As a result of this damage these delicate cells soon die, whereupon new cells must be produced to restore the normal status of the circulatory system. Consequently hyperplastic changes for the purpose of compensation are seen in the blood-forming organs. This may be particularly true in the lymphoid tissue of the lymph nodes and the spleen, as there is very little evidence for the presence of lymphoid tissue and lymphocytopoiesis in the normal bone marrow of the rat (14).

Clinical as well as experimental evidence has shown that, when an individual is lightly irradiated, only leukopenia follows; with heavier irradiation anemia occurs. This indicates a greater radiosensitivity of the white blood cells. In our experiment the unirradiated bone marrow showed evidence of hyperplastic changes of the erythrocytic cell series while the leukocytic cell series became hypoplastic, suggesting that failure succeeds compensation. More evidence for compensation of the red blood cells may be seen in the spleens of those animals where erythropoiesis can be found in the red pulp. This compensatory picture in the unirradiated bone marrow and spleen is quite similar to that in patients with secondary anemia due to chronic hemorrhage (23). In such patients MacCallum found hyperplasia with compactly crowded formative cells and their products in the bone marrow and spleen. If our explanation of the findings is correct, it may be concluded that the peripheral blood is damaged by the local irradiation, the amount of damage depending upon the radiosensitivity of the various forms of blood cells, and that the changes in the unirradiated hematopoietic organs depend in turn upon the degree of the damage of the various blood cells.
As stated above, Akaiwa and Takeshima (8) found the same kind of changes in the unirradiated lymph nodes of an animal as in the irradiated nodes, but in less degree. In the irradiated lymph node, the cells in the lymph follicles first became enlarged; disintegration of the nuclei of these cells then occurred and finally they were taken up by phagocytes. Owing to this disintegration of lymphocytes there was a marked decrease in the number of these cells in the lymph follicles. The changes of the lymph tissue in the lymph node and spleen of our experiment are of a different nature and are considered to be a result of hyperactivity of this kind of tissue. The reduction in size and number of lymph nodules, the increase of the size of the sinusoids, and the increase of plasma cells all point to a compensatory hyperplasia.

**SUMMARY AND CONCLUSION**

The posterior surfaces of the right hind limbs of rats were irradiated with x-rays in doses of 1000, 2500, or 5000 r. The bone marrow of both femurs, the submaxillary lymph nodes, and the spleens of the animals were studied microscopically. The changes in these organs vary in direct proportion with the amount of radiation delivered. In the irradiated bone marrow the changes are increase of fat cells, hyperplasia of erythrocytes, and hypoplasia of leukocytes. The hyperplasia of erythrocytes is shown by the lack of distinction between the small cell cords which contain erythroblasts and young and adult erythrocytes, and the large sinusoids, which always contain mature erythrocytes. With increase of irradiation, the fat cells increase in number and the sinusoids decrease in size. The hypoplasia of the leukocytes is shown by the lowering of the number of mitotic cell divisions in this group. When heavily irradiated, the bone marrow eventually becomes aplastic in appearance.

The changes in the bone marrow of the non-irradiated side are also increase of fat cells, hyperplasia of erythrocytes, and hypoplasia of leukocytes. The hyperplasia of the erythrocytes is shown by large cell cords containing many erythroblasts and even megaloblasts with mitotic divisions, and by the small sinusoids. Hypoplasia of leukocytes is shown by the marked decrease in number or even absence of cells of this type. The changes in the lymph nodes are reduction in number and size of the lymph nodules in the cortex, increase in number and size of the sinusoids in the medulla, and increase of plasma cells. In the white pulp of the spleen, the lymph follicles or cords are reduced but less so than in the lymph nodes, and there is evidence of differentiation of undifferentiated reticular cells into lymphocytes. With the reduction of the white pulp of the spleen, there is an increase of the red pulp, which consists of sinusoids filled by erythrocytes, and of islands of erythroblasts of different stages. Phagocytes increase with increase in amount of radiation, and evidence for the transformation of phagocytes into erythroblasts is also seen. The changes in the lymph nodes, the spleen, and the unirradiated marrow are considered as compensating the effect of the local irradiation on the circulating blood.

In conclusion, it has been demonstrated that when an animal is irradiated locally, hypoplastic or aplastic changes are found in the irradiated bone marrow and compensatory hyperplastic changes in the unirradiated hemopoietic system.
EFFECTS OF ROENTGEN RADIATION ON BLOOD-FORMING ORGANS

Literature