EFFECTS OF X-RAY ON LYMPHOID AND HEMOPOIETIC TISSUES OF ALBINO MICE

By G. Brecher, M.D., K. M. Endicott, M.D., H. Gump, A.B., and H. P. Brawner

H. EINEKE, investigating effects of x-rays on internal organs, found the lymphoid tissues peculiarly susceptible to irradiation. This observation suggested the possibility of studying the function of the lymphoid apparatus by observing animals deprived of their lymphoid tissue by means of selective injury by x-ray. For this purpose it is necessary to suppress lymphopoiesis without interfering with other bodily functions of the experimental animals used. Permanent destruction of the lymphoid tissues cannot be accomplished without deleterious effects upon other organs, as shown by Hughes and Job. However, with a single whole body irradiation of 400 r, Henshaw produced in albino mice marked temporary lymphopenia accompanied by only slight neutropenia and anemia. The dosage used by Henshaw thus appeared to produce the highly selective damage of lymphoid tissues necessary for functional studies of this system. The present study was undertaken to furnish further details of the selectivity of radiation injury produced with this dosage by measuring the initial damage to all blood-forming tissues and the reticulo-endothelial system, the extent and duration of the suppression of their activity, and the rate of their recovery.

MATERIAL AND METHODS

Inbred female mice of the CFW strain, 2 months old, were given single whole body radiation of 400 r as measured in air with the Victoreen ionization chamber. (186 KV, 10 Millamps., at 50 cm. with 0.15 mm. copper and 0.55 mm. aluminum filters, for 6.24 minutes.) All animals survived the period of the experiment without obvious illness. The animals were examined in groups of six on the 1st, 2nd, 4th, 7th, 10th, 14th, 21st and 28th day after irradiation. On three mice of each group of six, peripheral white blood cell counts and differential counts were done, as well as hemoglobin determination with the Evelyn photoelectric colorimeter, standardized by Van Slyke's method. On the remaining three mice of each group, the peripheral red blood cells and reticulocytes were counted and the hematocrit values determined using van Allen's method. All six mice were then killed with chloroform. The animals were weighed and autopsies were performed. The superficial cervical lymph nodes, thymus, liver and spleen were weighed and fixed in Helly's fluid. Kidneys, heart and lungs were also fixed in Helly's fluid, but not weighed. The gastrointestinal and genital tracts were inspected for gross lesions. The brain and cord were not examined. Paraffin sections were prepared and stained routinely with hemalum azure eosin and with hematoxylin-eosin. In addition, the Rio-Hortega-Foot silver reticulum stain, the Berlin blue reaction for iron, and the periodic acid-leukofuchsin stain were used extensively on sections of the lymph nodes, spleen and thymus. Frozen sections of lymph nodes for the demonstration of fat were prepared in a few instances.

The bone marrow of one femur was used for the preparation of marrow smears. Smears were routinely stained with Giemsa and frequently also with the peroxidase stain. The other femur and several thoracic vertebrae were fixed in formalin and decalcified paraffin sections of them stained by van Gieson's method. Photomicrographs of the marrow of the epiphysis, metaphysis and shaft of the femur and of one vertebra were then prepared. Each photomicrograph was superimposed upon a cross ruled transparent grill.

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The larger cells of the early granulocyte series and the small normoblasts tend to accumulate at opposite ends of the smear. For this reason, the beginning, middle and end portions of each smear were counted, going from the middle to one edge of the slide in each of these areas. Using this method, total cell counts can be traced to failure to take counts from at least one exception. Subgroups of the myeloid series are reported as percentages of the total myeloid cells, as shifts to the left or right are meaningful with reference to the myeloid series only and not with reference to the available marrow space.

The classification of the granulocyte series in the bone marrow of small rodents presents certain difficulties, which have been discussed in an earlier paper by Endicott and Ott. In the mouse as in the rat, maturation of the nuclear chromatin and of the cytoplasm of the granulocytes may proceed independently. "Young" nuclei of round or oval shape with finely divided chromatin are frequently present in cells with "old" neutrophilic cytoplasm, while other cells show "young" basophilic cytoplasm and "old" ring form nuclei with coarsely distributed chromatin. This independent maturation of nucleus and cytoplasm makes any attempt to arrange the granulocytic series into definite promyelocyte, myelocyte and metamyelocyte stages highly artificial and subject to extreme variation from differences of interpretation. However, the very young and the definitely mature granulocytes are easily identifiable. For this reason the following classification was adopted:

a. Young forms: Relatively large cells with blue cytoplasm, with or without azurophilic granules, with round or early ring form nuclei, and finely divided nuclear chromatin.
b. Old forms: Cells with pale blue or neutrophilic cytoplasm, with ring form nuclei showing early indentation or fully developed segmentation and coarsely distributed chromatin.
c. Intermediate forms: All other cells of the granulocyte series, i.e., cells showing partial maturation of cytoplasm and nuclear chromatin in various combinations.

The above classification cannot be correlated exactly with the standard division of granulocytes into promyelocyte, myelocyte and metamyelocyte stages. It is felt that this disadvantage is outweighed by the ease with which differential counts on the same specimen can be reproduced by different observers. Any discrepancies can usually be traced to failure to take counts from at least 3 representative areas. The larger cells of the early granulocyte series and the small normoblasts tend to accumulate at opposite ends of the smear. For this reason, the beginning, middle and end portions of each smear were counted, going from the middle to one edge of the slide in each of these areas. Using this method, total cell counts usually comprise 800 to 1500 cells.

The average weights of the animals, the organ weights, peripheral blood counts and myelograms of the first series of mice killed in groups of six at the above stated intervals after irradiation are recorded in tables 1-4. Duplicate experiments were performed on a second series of animals of the same strain, sex and age and on a third series of mice of the same strain and sex, but 5 to 6 months old. The peripheral blood counts and myelograms for the latter two groups showed no significant deviation from the data of the first series recorded in our tables. Histologic findings were identical in all experiments. In a further small series of animals sections of lymph nodes, thymus, spleen, and bone marrow were examined 1, 2, 3, 7 and 18 hours after irradiation, but no blood counts or myelograms were taken.

Four groups of six control mice of the same strain and sex were examined at various intervals in the course of the experiment. The age of the animals was 2 months in 3 of these groups, and 5 months in the 4th group. In contrast to the narrow range of WBC counts in irradiated animals, individual WBC counts in the 14 control animals ranged from 3800 to 25,000. However, leukocyte counts of control animals in any one group of six tended to be on either the high or the low side, and fell within a relatively narrower range.
TABLE 1.—Body and Organ Weights (in Grams) of Mice Given Whole Body X-radiation of 400 r. (Means and Standard Deviations)

<table>
<thead>
<tr>
<th>Days after x-ray</th>
<th>Mouse</th>
<th>Liver</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Submaxillary lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.2 ±1.1</td>
<td>1.71 ±0.09</td>
<td>0.018 ±0.004</td>
<td>0.011 ±0.010</td>
<td>0.011 ±0.003</td>
</tr>
<tr>
<td>2</td>
<td>15.6 ±1.4</td>
<td>1.00 ±0.17</td>
<td>0.011 ±0.004</td>
<td>0.017 ±0.011</td>
<td>0.006 ±0.006</td>
</tr>
<tr>
<td>4</td>
<td>17.4 ±4.6</td>
<td>0.95 ±0.13</td>
<td>0.014 ±0.004</td>
<td>0.008 ±0.006</td>
<td>0.010 ±0.003</td>
</tr>
<tr>
<td>7</td>
<td>17.7 ±3.2</td>
<td>1.08 ±0.21</td>
<td>0.021 ±0.006</td>
<td>0.014 ±0.006</td>
<td>0.019 ±0.017</td>
</tr>
<tr>
<td>10</td>
<td>20.6 ±3.2</td>
<td>1.06 ±0.07</td>
<td>0.031 ±0.012</td>
<td>0.019 ±0.009</td>
<td>0.023 ±0.007</td>
</tr>
<tr>
<td>14</td>
<td>17.2 ±1.9</td>
<td>1.09 ±0.14</td>
<td>0.110 ±0.064</td>
<td>0.026 ±0.009</td>
<td>0.039 ±0.036</td>
</tr>
<tr>
<td>21</td>
<td>15.1 ±1.8</td>
<td>1.02 ±0.12</td>
<td>0.083 ±0.014</td>
<td>0.013 ±0.009</td>
<td>0.015 ±0.008</td>
</tr>
<tr>
<td>28</td>
<td>15.5 ±1.6</td>
<td>0.92 ±0.10</td>
<td>0.103 ±0.019</td>
<td>0.034 ±0.016</td>
<td>0.023 ±0.017</td>
</tr>
<tr>
<td>Control</td>
<td>16.0 ±1.0</td>
<td>0.93 ±0.14</td>
<td>0.093 ±0.019</td>
<td>0.011 ±0.008</td>
<td>0.003 ±0.006</td>
</tr>
</tbody>
</table>

For estimates of the degree of leukopenia in irradiated animals, it is desirable to know the lower limit of the normal leukocyte count rather than the average count. For this reason the means and the standard deviations of the leukocyte count of each of the four groups of control mice were computed. The group
showing the lowest average leukocyte count is recorded as control group in our tables. The standard deviation of this group indicates that 1/2 of any group of animals with low normal counts may be expected to have lymphocyte counts of 500 or more. Myelograms of all four control groups did not differ significantly.

**Table 4.—Peripheral Blood Counts (WBC per cu. mm.) in Mice Given Whole Body X-radiation of 400 r. (Means and Standard Deviations)**

<table>
<thead>
<tr>
<th>Days after x-ray</th>
<th>Total WBC</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Segmented</td>
<td>Juvenile</td>
</tr>
<tr>
<td>1</td>
<td>1100 ± 700</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>2</td>
<td>1100 ± 700</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>3</td>
<td>800 ± 300</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>4</td>
<td>1100 ± 300</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>7</td>
<td>1100 ± 300</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>10</td>
<td>1100 ± 900</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>14</td>
<td>900 ± 500</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>18</td>
<td>800 ± 800</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
<tr>
<td>Control</td>
<td>8500 ± 1300</td>
<td>2200 ± 100</td>
<td>50 ± 50</td>
<td>5 ± 50</td>
</tr>
</tbody>
</table>

**Organ Weights**

The weight of the lymph nodes, spleen and thymus was considerably below normal 1, 2 and 4 days after irradiation. The weight of the thymus and the lymph nodes was again within normal limits 7 days after irradiation. The weight of the spleen was considerably increased over previous low values at 10 days, but did not reach normal until the 14th day after irradiation. The weights of all organs examined 21 and 28 days after irradiation did not differ significantly from those of the controls. The size of the lymphoid tissues observed at autopsies corresponded to the variations in weight recorded in table 1.

**Histologic Findings**

*Lymph nodes (controls).* Forty superficial cervical lymph nodes from 12 control mice, 2 months old, were studied as a basis for comparison with those of x-rayed mice. The capsule of the nodes is thin. As a rule there are no trabeculae. Medullary cords form the central core of the nodes and project in narrow bands toward the surface, dividing the cortical lymphoid tissue into ovoid masses of varying size. Follicles with more or less well developed secondary centers are present in greatly varying numbers. They are always located near the surface of the node, either embedded in a mass of cortical lymphoid tissue, from which they are demarcated by a rim of closely packed lymphocytes, or flanked by medullary cords.

The term cortical lymphoid tissue, as used here, is identical with the "substance corticale" of Jolly and the "Rindenknoten" of Hellman. In a loose network of reticulum cells and fibrils it contains free cells, mostly small lymphocytes, whose nuclei are frequently angular, indented or lobated, occasionally to an extent simulating a mitosis. Mitotic figures are rare in the cortical lymphoid tissue.

The follicles consist of medium sized and large lymphocytes, a few of which are seen in mitosis, and of a small number of large phagocytic cells. These contain
"Tangible bodies" of Fleming, frequently clearly identifiable as disintegrating lymphocytes. A zone of closely packed, small lymphocytes surrounds the paler center. The term secondary center is used by us for this paler central zone, while the term secondary nodules is reserved for the entire follicle consisting of the paler center and the peripheral rim of closely packed lymphocytes. Reticulum is very scanty in the secondary centers.

The medullary cords consist of a central vessel surrounded by a concentric collar of large cells. These cells have a broad, slightly irregular rim of deeply basophilic cytoplasm and often somewhat eccentric nuclei of the lymphocytic type. They have been described as polyblasts and as "plasmoidocytes." Occasionally cells in mitosis are present. Plasma cells in varying numbers can usually be found in the medullary cords. The intermediary and medullary sinuses are sharply outlined by the marginal reticulum fibrils limiting the medullary cords.

The peripheral and intermediary sinuses are usually empty. A few medullary sinuses near the hilum frequently contain large numbers of polygonal or round cells, with an ample amount of oxyphilic cytoplasm. These probably represent desquamated lining cells of the sinuses. Mast cells in varying numbers are often present in the intermediary and medullary sinusoids. No appreciable amount of connective tissue is present in the hilum of the nodes.

**Lymph nodes of irradiated animals.** Sections taken 2 and 3 hours after irradiation showed widespread disintegration of lymphocytes. Large amounts of nuclear debris were present in macrophages in the secondary centers and occasionally in the cortical lymphoid tissue. At 7 hours cellular debris was also present in the medullary cords. At 18 hours hardly any lymphocytes remained in the secondary centers, which contained only swollen, poorly staining cells and large macrophages filled with cellular debris. The peripheral rim of closely packed lymphocytes, normally surrounding the secondary centers, was still well preserved in some of the follicles (fig. 1a). Other follicles had lost this collar of small lymphocytes and the former secondary centers, containing debris-laden macrophages, bordered directly on the peripheral sinuses (fig. 1b). Small accumulation of nuclear debris were still present in medullary cords and in the cortical lymphoid tissues. Presumably this debris was to a large extent ingested in macrophages, but the phagocytosing cells did not stand out clearly except in the secondary centers. A few granulocytes were occasionally present in the sinuses.

After 24 hours nuclear debris was seen only in macrophages in the remnants of the secondary centers. All follicles had now lost their peripheral rim of small lymphocytes. The medullary cords were partly collapsed and their cells often showed pyknotic nuclei. The medullary sinuses were correspondingly widened and the reticulum condensed (fig. 1c). Granulocytes were occasionally present in some of the sinuses.

The macrophages seen in the secondary centers at 7, 18 and 24 hours contained, in addition to nuclear debris, numerous small globules of oxyphilic cytoplasmic material, which stains vividly with periodic acid-leukofuchsin stain. These globules were slightly acid-fast and slightly sudanophilic. Similar macrophages were sometimes also seen in the cortical lymphoid tissue and medullary cords. A
few secondary centers contained large lipophages which stained brilliantly with oil red O in frozen sections.

At 48 hours no cellular debris remained and the secondary centers had disappeared. Many of the medullary cords were similar to those seen at 2.4 hours. Others had regained their normal size (fig. 1d), largely due to accumulation of plasma cells. A few granulocytes were occasionally found in the medullary cords. The outstanding feature of the nodes at 48 hours, apart from their reduced size, was the absence of secondary nodules and of any mitotic activity.

At 4 days the nodes showed band-shaped and nodular subcapsular accumulations of medium sized lymphocytes with a few mitotic figures and macrophages. The phagocytic cells contained tingible bodies identical with those seen in secondary
centers of control animals. Such cells were scattered through both band-shaped and nodular areas of proliferation of lymphocytic cells. The size and appearance of medullary cords at 4 days was within normal limits. Mitotic figures were occasionally present.

At 7 and 10 days the marginal areas of proliferating lymphoid tissue had grown in size and number. Most of these areas had the shape of follicles. They usually lacked differentiation into lighter centers and peripheral rims of closely packed cells, characteristic of secondary nodules, and frequently bordered directly on peripheral sinuses (Fig. 2b). They were often of ovoid shape with the long axis parallel to the surface of the node. Mitoses and phagocytic cells were numerous in these areas. Medullary cords still contained numerous plasma cells, as well as the large lymphocytic cells with a relatively broad rim of basophilic cytoplasm, which are characteristic of normal medullary cords. They differed from normal cords by the presence of accumulations of small lymphocytes. Such accumulations of lymphocytes were commonly seen in the medullary cords from the 7th day on (Fig. 2a).

At 14 days fully developed secondary nodules were present as well as ovoid follicles without differentiation into center and periphery. Lymphopoietic activity, judged by the number and size of follicles and by the mitotic activity in follicles and medullary cords appeared to be comparable to, or in excess of, that of control nodes. In some of the nodes medullary cords contained an occasional center of myelopoiesis.

At 3 weeks there was extensive myelopoiesis in the medullary cords (Fig. 2c). The peripheral areas of lymphocytic proliferation did not differ from those seen at 2 weeks.

At 4 weeks myelopoiesis in medullary cords continued. In some areas many of the granulocytes were eosinophilic. Almost all follicles were now round and had the typical appearance of secondary nodules with a pale center and a peripheral rim of closely packed lymphocytes. The number of follicles was generally somewhat increased, but differences were often slight, so that only the extensive medullary myelopoiesis distinguished these nodes with certainty from those of control animals.

Thymus. At 2, 3, 7 and 18 hours there was evidence of extensive destruction of lymphocytic cells in the form of large amounts of nuclear debris in the cortex and to a lesser extent in the medulla. The thickness of the cortex was diminished and after 24 hours only a narrow and incomplete rim of cortical tissue remained. The dense accumulations of nuclear debris made it difficult to ascertain the amount of active phagocytosis. At 24 hours cortical areas showed occasional large macrophages against a background of swollen, poorly staining cells and edema fluid. This appearance was similar in all respects to that of secondary centers in lymph nodes at the same time. Granulocytes in small numbers were present in a few cortical areas and occasionally in the pericapsular tissue.

At 48 hours the usual subdivision into cortex and medulla was no longer recognizable. The vessels and reticulum fibrils of the subcapsular zone stood out unusually well and were somewhat condensed, indicating the greater shrinkage of
the cortex as compared with the medulla. Lymphocytes were still present in reduced numbers in the central portion of the thymus. This occasionally produced a
somewhat more cellular and darker appearance of the medulla as compared with the cortical areas (fig. 3a).

At 4 days a narrow rim of large cells resembling lymphoblasts was visible beneath the capsule (fig. 3b).
At 7 days a well developed cortical zone was present, but the medulla still contained fewer lymphocytes than normal. From the 14th day on, the appearance and relative size of both cortex and medulla were within normal limits.

**Spleen.** At 2, 3, 7 and 18 hours nuclear debris was present in large amounts in the Malpighian follicles and to lesser extent in the red pulp. Debris-laden macrophages were clearly seen in some of the follicles (fig. 3c). The small, but fairly numerous centers of erythropoiesis normally present in the mouse spleen, were decreased in size and numbers. Large amounts of brownish, iron-containing pigment appeared in the red pulp. The megakaryocytes were reduced in number and frequently showed pyknotic nuclei. At 24 hours the spleen was greatly reduced in weight. Nuclear debris was seen only in rare macrophages in the follicles which were somewhat reduced in size. Nuclear debris had completely disappeared at 48 hours.

Erythropoietic centers were no longer recognizable. Iron-containing pigment was diffusely distributed through the red pulp, partly phagocytosed in the cells of the condensed reticulum, which often contained numerous red blood cells and few lymphocytes. In Giemsa stained sections the follicles stood out sharply against the uniformly pink-staining red pulp. In sections stained for iron the red pulp took a rather intense blue color, while the follicles took the color of the counter stain only (fig. 3d). Megakaryocytes were markedly reduced in number. The appearance of the spleen remained unchanged at 4 and 7 days, except for an occasional mitosis in the follicles. A marked increase in the size of the spleen occurred between the 7th and 10th day, partly due to increase in the size of the follicles, partly due to formation of subcapsular areas of hemopoiesis (fig. 4). These circumscribed foci of hemopoiesis were frequently visible grossly as light-red or whitish subcapsular
nodules, projecting slightly above the surrounding surface of the spleen. In sections these foci were seen to consist chiefly of round cells with a small rim of cytoplasm with large nuclei and well defined nucleoli (fig. 5). Occasional mitoses were seen. In smears, the majority of these cells were identified as pronormoblasts, among which were a small number of myeloblasts. By the 14th day the spleen had enlarged further and its weight, as well as the size of the Malpighian follicles, was within normal limits. Hemopoiesis was in progress throughout the red pulp. Numerous normoblasts and occasional granulocytes were now intermingled with stem cells. The amount of pigment, already reduced at 10 days, now rarely exceeded that of controls. Megakaryocytes were again present in normal numbers. At 3 and 4 weeks the spleen showed essentially the same appearance as at 2 weeks. Myelopoiesis was somewhat more extensive, but erythropoiesis continued to dominate the picture.

The accumulation of hemosiderin, noted in the spleen after irradiation, was not found in any other organ examined. Hemosiderin in the spleen was most abundant from the 1st to the 7th day. The period of greatest accumulation of hemosiderin thus coincided with the time of greatest reduction in the size of the spleen. Some of the apparent increase in the amount of pigment may therefore have been due to collapse of the splenic stroma with resulting condensation of pigment.

From the histologic appearance alone it was impossible to decide whether this hemosiderosis developed within 24 hours and remained constant over a period of a week, or whether hemosiderin continued to accumulate during the first week. The hemosiderin present at 24 hours was so abundant that any further increase could hardly have changed the histologic picture.

Bone marrow. The diminution and subsequent increase of the total cellular bone marrow are recorded in table 2. Sections showed an increase in the size of blood filled vascular spaces concomitant with the loss of cellular marrow following irra-
radiation. A very small amount of cellular debris and slight phagocytosis were noted from 1 to 24 hours after irradiation. Our paraffin embedded sections were generally unsuitable for the study of finer cellular details. The bone marrow changes, as studied by means of quantitative myelograms, are reported with the hematologic findings.

**Summary of the histologic findings.** Within 24 hours after irradiation, there was extensive destruction of the cells of the secondary centers and medullary cords of the lymph nodes, almost complete destruction of the thymic cortex, diminution of the lymphocyte content of the thymic medulla and marked decrease in size of Malpighian follicles. The weight of the lymphoid tissues was markedly decreased. Regeneration of lymphocytes was first noted at 4 days after irradiation. The lymphoid tissues regained normal weight at 7-14 days. As judged from the histologic appearance, lymphopoiesis was frequently in excess of normal from 14 days on through the rest of the period of observation.

The spleen showed marked hemosiderosis from the first to the 7th day. Due to the marked collapse of splenic stroma during this time, part of this hemosiderosis was probably more apparent than real. No hemosiderosis was found in other organs examined.

Extramedullary hemopoiesis was marked in the spleen from the 10th day on and comprised both the myeloid and erythroid series. Extramedullary myelopoiesis was also prominent in the lymph nodes at 3 and 4 weeks.

**Hematologic Findings**

*Cellularity of the bone marrow* decreased during the first 4 days after irradiation. The first evidence of recovery was noted at 7 days. The total cellularity was still below normal at 2 weeks. Over 90 per cent of the available marrow space was occupied by active marrow at 3 and 4 weeks. The different cell types showed marked differences in rate of diminution and regeneration.

The first regeneration of nucleated red cells of the marrow was noted at 7 days. Average indices reached normal at 10 days, but fell again by 14 days and remained below normal throughout the period of observation.

The statistically significant fall in nucleated red cells at 3 weeks and the renewed increase at 4 weeks is in essential agreement with the data of Langendorff and Papperitz, who believe that regeneration of hematopoietic tissues after x-ray injury proceeds in waves. The possibility of excessive myelopoiesis suppressing erythropoiesis appears to fit the data well, especially as the slight decrease in myelopoiesis at 4 weeks is accompanied by a corresponding increase in nucleated red cells.

*The reticulocyte* count in the peripheral blood dropped from its average normal value of 2.0 per cent to 0.2 per cent within 24 hours after irradiation. It remained at this low level at 2 and 4 days. After 7 days up to 0.8 per cent were found. On the 10th day reticulocyte counts varied from 2.0 per cent to 22.0 per cent. From the 14th day on reticulocyte levels ranged from 2.0 per cent to 5.0 per cent.

The rapid reticulocyte drop within 24 hours after irradiation would indicate a rather short lived reticulocyte stage of the red cell in the mouse.
Total red cell counts, hematocrit and hemoglobin values dropped only slightly during the first 4 days after irradiation. They were markedly reduced between the 7th and 14th day. The lowest average values were observed in one group of six animals examined and killed on the 10th day (RBC 6,600,000 ± 800,000; Hb 14.0 ± 2.0; hematocrit 33 ± 9). At 3 weeks only slight residual anemia was present. At 4 weeks total red cell counts were frequently within normal limits (Control group: RBC 9,600,000 ± 1,300,000; Hb 18.4 ± 1.8; hematocrit 51.0 ± 0.5). In most groups examined the 2-week-values were below the one-week-levels but occasionally the reverse was the case.

The myelopoietic marrow diminished more gradually than the red cell precursors. The lowest level was reached at 4 days. Regeneration proceeded quickly and the myelopoietic indices were above normal from the 14th day on. The period of observation is too short to determine whether the slight but significant drop in myelopoiesis at 4 weeks indicates a return to normal values or not.

The peripheral granulocyte counts reflected the marrow changes accurately. As in the marrow the lowest granulocyte level, amounting in some animals to agranulocytosis, was reached at 4 days. There was slight recovery by the 7th day. A marked left shift was present both in the marrow and in the peripheral blood at 10 days.

Marrow lymphocytes, normally representing almost 20 per cent of the active marrow cells, dropped to a low value within 24 hours. Lymphocytes in the marrow remained scarce until the end of the observation period, except for an abrupt, temporary rise around the 10th day. The cells recorded as lymphocytes in our 10-day-data included a varying number of large cells with dark blue cytoplasm and with somewhat finer distribution of nuclear chromatin than that of mature lymphocytes. Some of these cells showed great similarity to myeloblasts, but lacked nucleoli. These cells are probably identical with the lymphoidocyte of Pappenheim. We feel that the morphology of these cells is not sufficiently distinctive to permit them to be differentiated from lymphocytes when they are present in small numbers only. We have therefore recorded them with the lymphocytes.

As to the function of these cells, however, there is reason to believe that they are stem-cells of the granulocytic series. Cells of this type did not appear in the peripheral blood, a fact which supports the contention that they are stem cells. Their appearance in the bone marrow coincided with a marked left shift in the granulocytic series and preceded a rapid increase of myelopoiesis. This suggests that these lymphocyte-like cells are stem-cells of the myeloid series.

DISCUSSION

There is general agreement in the literature, as reviewed by Dunlap,11 that lymphoid, myelopoietic and erythropoietic tissues show increasing resistance to x-radiation in the order named. In contrast, the almost complete disappearance of normoblasts from the bone marrow within 24 hours of irradiation in our experiments points to a considerable sensitivity of red cell precursors to radiation. The bone marrow did not show the striking phagocytosis of cellular debris found in the lymphoid tissues. However, in view of the vascularity of the bone marrow and
the probable intravascular development of erythroblastic cells, cellular debris might be expected to be flushed out rapidly. For this reason, the small amount of cellular debris and phagocytosis in the bone marrow does not exclude the possibility of widespread disintegration of red cell precursors, such as would account for the drop in normoblasts and the rapid accumulation of hemosiderin in the spleen. Evidence of direct damage to, and disintegration of, erythroblasts in the bone marrow following x-ray injury has recently been described by Bloom and Bloom. These authors used x-ray dosages similar to ours, but different technics for the study of the bone marrow. They concluded that the erythroblasts in mice, rabbits and rats are more susceptible to radiation damage than are myelopoietic cells. The erythroblast of the chicken appeared at least as sensitive to radiation as did the small lymphocytes which are commonly found in nodules in the chicken marrow.

Peripheral blood counts showed an almost immediate drop in lymphocytes, and a more slowly developing granulocytopenia. This is in keeping with the longer life span of granulocytes and the slower diminution of myeloid cells in the bone marrow. Granulocyte levels rose again with myeloid regeneration. Lymphopenia was, however, more prolonged than granulocytopenia. At 2 weeks the lymphocyte count was still only 50 per cent of the lower limit of normal, although the lymphoid tissues had regained their normal weight and lymphopoiesis appeared to be slightly in excess of normal. The lymphopenia in the bone marrow was even more marked and prolonged. This lag between resumption of active lymphopoiesis in the tissues and recovery of normal lymphocyte levels in the peripheral blood has been observed in other species. The significance of this lag period is obscure. It is conceivable that the slow return of the peripheral lymphocyte count to normal levels is due to the fact that lymphopoiesis exceeds normal only by a slight margin during regeneration. After replacing the daily loss of lymphocytes from the peripheral blood, only a small excess would remain and a considerable time would be required to restore the peripheral lymphocyte count from its low level following irradiation. On the other hand, the lag in recovery of peripheral lymphocyte counts may be due to disturbances of the mechanism regulating the release of lymphocytes from the tissues and their distribution throughout the body. Most likely several factors are in operation following irradiation and the rate of recovery of the peripheral lymphocyte count should not be expected to reflect necessarily the activity of, or the amount of original damage to, the lymphoid tissues.

No evidence of diminution or proliferation of the reticuloendothelial cells was found. The only function of the cells of the RES that can be studied morphologically, their ability to phagocytose, appeared to be intact throughout the period of observation. In the lymph nodes phagocytosis of cellular debris was extensive during the first 24 hours, due to extensive destruction of lymphocytes, and was again marked, in conjunction with active regeneration of lymphocytes, from the 4th day on. In the spleen large amounts of hemosiderin were present in phagocytic cells from the 1st to the 7th day after irradiation. The ability of the RES to phagocytose appeared also intact in experiments (unpublished) in which carbon particles were injected intravenously in mice of the same strain, age and sex, at varying intervals after irradiation with 400 r. In these experiments active phagocytosis of
carbon particles by the reticuloendothelial cells of the liver, spleen and bone marrow was comparable to, or in excess of, that observed in nonirradiated mice.

Our basic problem, to estimate the duration of suppression of lymphoid and hematopoietic activity, can receive only a qualified answer. Estimates based on the histologic appearance of the lymphoid tissue suggest that the limit of marked suppression of lymphopoiesis was about 14 days. Peripheral lymphocyte levels were still below normal at that time and this would indicate a longer time interval between irradiation and recovery. Total granulocyte counts reached normal levels at 14 days. Again estimates of the recovery time may vary somewhat, as the number of fully segmented granulocytes reached normal levels somewhat later than the total granulocyte count. The practical problem is simplified by the fact that damage to the myeloid tissues, though probably of slighter degree, is still quite severe during the first 10 days. This would certainly leave too narrow a time margin for any experiments designed to demonstrate the relative function of granulocytes and lymphocytes based upon a supposed selective suppression of one cell type. The lack of any morphologic evidence of damage to the reticuloendothelial system suggests that functional studies concerned with the relative functions of the lymphoid tissues and the RES may be undertaken during the first 2 weeks following irradiation under the experimental conditions used. Such experiments must be interpreted cautiously because of the concomitant damage to the bone marrow and because of the possibility that the RES may have been altered in functional capacities other than its ability to phagocytose.

SUMMARY

Quantitative myelograms, peripheral blood counts and the histologic appearance of lymphoid tissues were studied in albino mice of the CFW strain during a period of 4 weeks following single whole body radiation of medium dosage.

Nucleated red blood cells disappeared almost completely from the marrow within 2-4 hours after irradiation. Regeneration of the erythroid series commenced around the 7th day. Only mild to moderate anemia developed, presumably due to the longevity of red blood cells.

Suppression of mitotic activity and diminution of the myeloid marrow was marked during the first week after irradiation. Granulocytopenia in the peripheral blood was severe for a very short period only. In contrast, early damage to the lymphoid tissue was more pronounced and lymphopenia was of longer duration than granulocytopenia in spite of early regeneration of the lymphoid tissues. Differences in the relative amount of damage to lymphoid and myeloid tissues and in their rates of regeneration were not of sufficient magnitude to indicate a truly selective damage to the lymphoid tissues under the conditions of our experiments.

No morphologic evidence of damage to the reticuloendothelial system was found. The possible use of irradiated animals for functional studies of the lymphoid apparatus is discussed.

REFERENCES

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EFFECTS OF X-RAY ON LYMPHOID AND HEMOPOIETIC TISSUES OF ALBINO MICE

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