Mice or rats are protected from the acute lethal effects of X irradiation of the whole body when they are injected with normal bone marrow (1, 2). Histological studies show that the hematopoietic tissues recover in injected animals only (3–5). The question arose as to whether the regenerated cells are derived from the irradiated recipients or from the injected bone marrow. The finding that homologous and heterologous bone-marrow cells also protect (4) suggests that the identity of the repopulating cells could be established if the heterologous or homologous cells could be distinguished from those of the irradiated hosts.

While the present investigation, based on the identification of homologous cells, was in progress, several other studies were reported in the literature based on the identification of homologous or heterologous cells. The presence of foreign cells in the tissues of the irradiated mouse was detected by the staining properties of the cells, their immunological properties, or by marker chromosomes (6–12). The injected cells produced erythrocytes and repopulated the marrow and red pulp of the spleen (6, 7, 10, 11) and under some conditions repopulated the thymus and lymph nodes (8, 9, 12).

In the experiments to be reported here the presence of homologous bone-marrow cells in the tissues of the irradiated, treated mice was determined by the capacity of these tissues to initiate immunity when injected into non-irradiated mice of the same strain as the irradiated ones. When immunity developed in these test mice, a nonvascularized homograft, which had been previously placed in each mouse, disintegrated. A nonvascularized homograft does not initiate immunity (13) but if a homograft, which becomes vascularized, is placed at some other site or if an injection of homologous cells is made, the host becomes immune and the graft disintegrates. The homografts in the test mice were obtained from mice of
the same strain as the donors of the protecting bone marrow. Thus, when homologous cells were present in the tissues of the irradiated, treated mice, the test mice developed immunity to the homologous tissue and the nonvascularized homografts disintegrated. During the first 4 days after treatment a few homologous cells were present in the bone marrow. In 12 to 14 days many more homologous cells had appeared. At 30 days and also at 100 days the lymph nodes, spleen, and thymus as well as the marrow contained many homologous cells.

A comparison of the antigenicity, in test mice, of known amounts of tissue from irradiated recipient mice was made with known amounts of tissue from normal mice of the donor strain. This comparison allowed us to make a rough estimate of the amount of homologous tissue in the tissues of the treated mice. The antigenicity was measured by: 1) the number of test mice in which the grafts disintegrated as compared with the number in which they did not disintegrate; 2) the length of the interval observed after the injection of the tissues before the disintegration of the nonvascularized homografts.

These comparisons were based on two assumptions. First, it was assumed that cells in normal marrow had approximately the same antigenicity as cells in marrow of the treated mice, though the proportions of various cell types may have been different. Second, it was assumed that homologous cells in the irradiated mice had not changed significantly in their antigenicity, though it has been shown that a foreign environment may change cells immunologically (14, 15).

**Materials and Methods**

Four strains of mice or F₁ hybrids were used: C3H, C, \((C \times A)F₁\), and \((L \times A)F₁\). These strains were of the following substrains, respectively: C3H/HeN, BALB/cAnN, \((BALB/cAnN \times A/LN)F₁\), and \((C57L/HeN \times A/HeN)F₁\) (16). The bone-marrow donors were C3H mice. The irradiated recipients were \((L \times A)F₁\), \((C \times A)F₁\), or C mice.

Mice, 3 to 4 months of age, were irradiated with 900 r of X rays to the whole body. The mice received 93 r per minute from 2 oppositely placed tubes whose foci were 54 cm. from the center of the mice. The irradiation conditions were: 186 kvp and 20 ma., 0.25 mm. Cu and 1.06 mm. Al filters.

Within 4 hours after irradiation, the marrow obtained from 1 femur of a C3H mouse and suspended in 0.5 ml. of Tyrode's solution was injected into the tail vein of each irradiated mouse. The marrow donors ranged in age from 2 to 4 months.

At various intervals after treatment, the mice were killed and suspensions of cells from hematopoietic tissues and blood were tested for the presence of homologous cells. The suspending medium for the cells being tested was a tissue-culture fluid containing horse serum, chicken embryo extract, and a balanced salt solution (17). For each bone-marrow suspension, the ends of a femur were cut off and the marrow washed out with 0.5 ml. of the tissue-culture fluid. Thymus glands, lymph nodes, or
spleen were macerated in 0.5 ml. of the culture fluid and the pieces of
tissue were removed with fine forceps. The suspensions contained almost
entirely separate cells except for the spleen cell suspensions in which the
erthrocytes were agglutinated by the horse serum.

Various known fractions of the total number of cells obtained from the
lymph nodes or other tissues of the treated mice were tested. The original
suspension, which contained all the cells from any one tissue, was diluted
until 0.5 ml. of the culture fluid contained the desired fraction. In a few
experiments, the suspension of cells from normal C3H mice was diluted
until it contained the same number of cells in 0.5 ml. of the culture fluid
as the suspension of cells from the treated mice.

A hemocytometer was used to count the number of cells in some of
these suspensions; both erythrocytes and leukocytes were counted.

Cell suspensions to be tested for the presence of homologous cells were
 injected subcutaneously into test mice, which ranged in age from 3 to 10
months. Each of these mice carried a nonvascularized homograft of
harderian-gland tissue transplanted 1 week to several months previously
(13). Harderian-gland tissue, obtained from C3H mice less than 3 days
old, was used because it contains large pigment cells whose disintegration
can be detected easily. Each graft was placed under the skin on one side
of the test mouse, and a glass coverslip, to serve as a window, was placed
under an opening in the skin on the other side. Every 2 or 3 days the skin
was pulled up along the mid-dorsal line so that the graft could be observed
through the window (13). The time of disintegration of the graft was
recorded. If the graft had not disintegrated by 30 days, the result of the
test was considered negative since disintegration rarely took place after 30
days.

Autopsies were performed on many of the irradiated, treated mice at
the time when tissues were taken for the harderian-gland test. Samples
of the tissues tested were prepared for histological study. Sections were
stained with hematoxylin and eosin.

Results

Tests for Presence and Quantity of Homologous Tissue in Marrow of
Irradiated Mice Injected with Homologous Marrow

Tests up to 4 days after irradiation and treatment showed that each
suspension containing the marrow from 1 femur of a treated (L × A)F₁
mouse was, on the average, as antigenic as each suspension containing
1/2048 to 1/512 of the marrow from 1 femur of a normal C3H mouse
(text-fig. 1).

Twelve days after treatment the marrow tissue was more antigenic
than up to 4 days after treatment, and it still maintained this increased
antigenicity at 180 days. The marrow from each femur was as antigenic
as 1/256 to all the marrow from any 1 femur from a normal C3H mouse.
These amounts of normal C3H marrow, ranging from 1/256 to all the
femoral marrow, however, caused approximately the same response.
Therefore, in order to obtain a closer estimate of the amount of homologous
NO. TESTS IN WHICH GRAFTS DID NOT DISINTEGRATE

TOTAL NO. TESTS

TEXT-Figure 1.—Tests to determine how much donor homologous tissue was present in the femoral bone marrow of irradiated recipient mice. A.—Tests on marrow from normal mice of the donor strain (C3H). B.—Tests on marrow from irradiated recipient mice (L × A)F₁. Lines are drawn between the median points. Tests on the entire marrow contents of femurs of C3H mice were made on 1 femur from each mouse. The 23 tests on fractions of the femoral-marrow content were made on 9 femurs from 7 mice. The marrow from each femur of each mouse was tested 0 to 14 days after treatment, but the marrow from only 1 femur was tested at 30 to 180 days.

tissue present in the treated animals, it was necessary to compare the antigenicity of fractions of the marrow content of femurs from treated mice with fractions from normal mice.

At 30 and 60 days after treatment, fractions of the marrow content of the femurs from treated mice were approximately one fourth to equally as antigenic as similar fractions from normal C3H mice (table 1). Further, it was estimated that one eighth to all of the marrow cells from the treated mice were homologous since the suspension prepared from 1 of the femurs of a treated mouse contained approximately 1 ½ times the average number of cells contained in suspensions prepared from the femoral marrow of normal C3H mice. The suspension of marrow from the treated mouse contained 38,000 ± 3,800 cells in 0.5 ml. of suspending fluid at a dilution of 1:1024. Ten suspensions of marrow from the femurs of normal C3H mice, similarly diluted, contained on the average 25,500 ± 3,600 cells with a range from 20,000 to 40,000 cells.

The findings at 100 days indicated that many homologous cells were still present in the marrow of the treated mice.

Tests for Presence and Quantity of Homologous Cells in Blood and in Hematopoietic Tissues of Irradiated Recipient Mice

In all except 1 of the tests on 0.05 ml. of blood taken from mice during the first 14 days after treatment, the nonvascularized harderian-gland
### Table 1.—Results of harderian-gland test for the presence of homologous (C3H) cells in the hematopoietic tissues from mice that had been irradiated and injected with homologous (C3H) marrow

<table>
<thead>
<tr>
<th>No. mice tested</th>
<th>Strain of mice tested</th>
<th>Time after irradiation (days)</th>
<th>Tissue tested</th>
<th>Fractions of tissue tested</th>
<th>E-L-N</th>
<th>E-L-N</th>
<th>E-L-N</th>
<th>E-L-N</th>
<th>E-L-N</th>
<th>E-L-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>C3H</td>
<td>No irradi.</td>
<td>Marrow</td>
<td>1/128</td>
<td>3-1-1</td>
<td>2-1-1</td>
<td>0-0-4</td>
<td>0-0-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>30</td>
<td>Marrow</td>
<td>1/256</td>
<td>2-3-1</td>
<td>1-1-4</td>
<td>1-1-5</td>
<td>0-0-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thymus</td>
<td>1/512</td>
<td>1-0-2</td>
<td>0-1-2</td>
<td>0-0-2</td>
<td>0-1-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph nodes</td>
<td>1/1024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
<td>1/2048</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/4096</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(C × A)F₁</td>
<td>60</td>
<td>Marrow</td>
<td>E-L-N 0-0-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thymus</td>
<td>E-L-N 3-0-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph nodes</td>
<td>E-L-N 1-0-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
<td>E-L-N 3-0-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C3H</td>
<td>No irradi.</td>
<td>Marrow</td>
<td>E-L-N 0-2-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thymus</td>
<td>E-L-N 1-0-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph nodes</td>
<td>E-L-N 3-0-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
<td>E-L-N 1-0-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tests were made on the marrow from each of the femurs of these mice.

†Early disintegration took place in 6 to 17 days, and late disintegration, 18 to 30 days after injection of the tissue being tested.

‡Both of these bone-marrow suspensions contained about 72,000 cells per 0.5 ml.

§Fractions used were 1/384 rather than 1/512.

¶Both of these thymus cell suspensions contained about 94,000 cells per 0.5 ml.
homografts in the test mice did not disintegrate and the test was considered negative. In 30 days or more after treatment, all the tests, except 1 at 30 days, were positive (table 2). Tests on hematopoietic tissues up to 14 days after treatment could not have been positive because of the blood content of the tissues, except for tests on spleen. The suspensions prepared from spleen contained more than the number of erythrocytes found in 0.05 ml of blood, whereas the suspensions prepared from the other tissues contained only a small fraction of this amount. By 30 to 180 days after treatment, when the blood gave a positive test, the suspensions of thymus cells prepared from the entire organ may have contained enough blood cells to affect the test, but the fractions of the suspensions prepared from the various hematopoietic tissues did not contain enough blood to affect it. Thymus suspensions prepared from the whole organ contained less than approximately 16,000,000 erythrocytes. The fractions of suspensions prepared from the various hematopoietic tissues contained less than approximately 900,000 erythrocytes. Twelve tests were negative on blood from C3H mice diluted until 0.5 ml of the suspending fluid contained approximately 1,700,000 to 7,000,000 erythrocytes. Only 3 out of 9 tests were positive when a volume of blood containing approximately 28,000,000 to 56,000,000 erythrocytes was injected.

Tests on marrow, spleen, thymus, and lymph nodes (table 2) indicated that the spleen and lymph nodes, like the marrow, contained a few homologous cells up to 4 days after irradiation and that, like the marrow, these tissues and thymus showed an increased antigenicity in 12 to 14 days after treatment. Furthermore, except when very small fractions were used, all these tissues, like the marrow, were antigenic in every test made 12 to 180 days after treatment.

At 60 days after treatment, a suspension prepared from 9 pooled lymph

**Table 2.--Number of irradiated recipient mice whose hematopoietic tissues caused disintegration of the harderian gland (+ test)**

<table>
<thead>
<tr>
<th>Tissue tested*</th>
<th>Number of + harderian-gland tests</th>
<th>Total number of tests at indicated time after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kind</td>
<td>Amount</td>
<td>0-4 days</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1 femur 1/128 or less</td>
<td>13/14</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>1/3 to all 1/128 or less</td>
<td>1/2</td>
</tr>
<tr>
<td>Thymus</td>
<td>3/4 to whole 1/128 or less</td>
<td>0/2</td>
</tr>
<tr>
<td>Spleen</td>
<td>3/4 to whole 1/1024 or less</td>
<td>2/3</td>
</tr>
<tr>
<td>Blood</td>
<td>1/20 ml.</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*The irradiated recipient mice were (L X A)F1, (C X A)F1, and C. The donor bone marrow came from C3H mice.
nodes contained approximately 15,000 ± 850 cells per 0.5 ml. of suspending fluid at a dilution of 1:1024. This suspension was approximately as antigenic as suspensions, at the same dilution, of femoral marrow cells from normal C3H mice. The average number of cells present in suspensions, each of which was prepared from the marrow of 1 femur of a C3H mouse, was 25,000 ± 3,600 cells at this dilution. Thus, unless the lymph-node cells were more antigenic than the marrow cells, approximately one fourth to all of the lymph-node cells were homologous.

The results of tests on thymus suspensions 60 days after treatment indicated that many homologous cells were present. Tests at 100 days on suspensions containing equal numbers of cells from either a normal C3H mouse or from a treated mouse indicated that one fourth to all the cells from the thymus of the treated mouse were homologous. A sample of the thymus of the treated mouse studied histologically showed atrophy, though cortical tissue was present. The lymph nodes also showed atrophy, whereas the white pulp of the spleen appeared normal.

Pathologic Findings in Hematopoietic Tissues of Irradiated Recipient Mice

In mice killed and autopsied at 60 or 100 days after treatment, the lymph nodes were reduced one fourth to one half the size of that seen in normal mice. The thymus was about half and the spleen about twice the size of that seen in normal mice.

The bone marrow attained normal cellularity within 12 days after irradiation and remained this way, or nearly so, at later intervals. The time of repopulation of the marrow coincided with the time of increase in number of homologous cells as indicated by the results of the harderiangland tests.

After irradiation, the red pulp of the spleen showed hyperplasia of the blood-forming cells from the 12th to the 60th day. At later intervals, the blood-forming cells in the red pulp were present in about normal amounts. The white pulp was atrophic in all the pieces of spleen taken 30 days or less after treatment. This atrophy resembled that seen in the delayed homograft bone-marrow reaction reported by Congdon and Urso (18). By 60 days, the white pulp was atrophic in 1 spleen and slightly atrophic in another. By 90 to 180 days it was normal.

During the first 30 days after irradiation and injection with homologous bone marrow, the lymph nodes showed atrophy, granulopoiesis, and reactive changes just as those described by Congdon and Urso (18). In the present experiments, in 60 to 180 days after treatment, no granulopoiesis was observed and, although the lymph nodes were still atrophic in some mice including 2 whose lymph nodes gave a positive test, others contained lymphocytes and showed lymphopoiesis in varying degrees. A representative lymph node, from the animal that was killed and autopsied at 180 days after irradiation, appeared normal in architecture.

From these histological findings on the lymph nodes, it seems possible that the positive tests, obtained at 30 days and earlier, were due to
homologous granulocytes rather than to lymphocytes. Although no granulopoiesis was found in lymph nodes 60 days or more after treatment, granulocytes may have been present in the pooled suspensions. Therefore, one cannot exclude the possibility that, even at 60 and 100 days after treatment, the positive tests resulted from granulocytes rather than from lymphocytes.

It is known that a temporary regeneration of the thymus occurs in irradiated mice that have been given homologous bone marrow (18), but a secondary atrophy usually follows during the 4th week. In this atrophy the normal architecture of the gland is sometimes preserved. In the mice studied here, regeneration was observed in 12 to 15 days. In 95 to 108 days the thymus showed either atrophy with cortex present or normal architecture. Since granulopoiesis was observed in 1 thymus at 12 days after treatment, it must be considered that the positive tests for thymus may have been due to the presence of granulocytes.

Discussion

Our observations that homologous cells appeared in the marrow of the irradiated, treated mice on the day of injection and that these homologous cells increased markedly in 12 to 14 days agree with the findings of Nowell et al. (7). The results reported in the present paper indicate that at 30 and 60 days after treatment the marrow of the treated mice contained approximately one eighth to all homologous cells. Other studies in which heterologous or homologous spleen or bone marrow was used for protection indicate that all the erythrocytes and granulocytes are derived from the injected cells (8–11). Lindsley et al. (6), however, found that under the conditions of their experiments various proportions of the numbers of erythrocytes were of the host type.

The lymph nodes and thymus glands have been described here as containing many homologous cells. The lymph nodes at 60 days and a thymus at 100 days were estimated to have between one fourth to all homologous cells. Although the homologous cells found before 60 days may have been granulocytes, it does not seem likely that those present in 60 to 100 days were granulocytes. Mitchison (12) recorded that donor antibody-producing cells were active in the irradiated, treated recipients. Also Ford (8, 9) found that even at 100 days after injection of spleen all the cells undergoing mitosis in the thymus and lymph nodes were of the donor type. Ford did not mention the presence of granulopoiesis in the thymus or lymph nodes but it is unlikely that all cells undergoing mitosis at 100 days after treatment could have been granulocytes. Although these observations of Mitchison and Ford indicate that spleen can produce lymphocytes, yet Makinodan (10) and Gengozian and Makinodan (19) concluded that neither isologous nor heterologous marrow injected into irradiated mice contributed to the antibody-forming tissues of the host—suggesting that the marrow does not form lymphocytes. Although the present observations suggest that marrow does form lymphocytes, more work is needed to prove whether it does or does not.
If lymphatic tissue of the irradiated host regenerated, it might result in passive transmission of immunity by the suspensions obtained from the spleen, lymph nodes, or thymus. However, a large volume of tissue is needed to obtain passive transmission (20, 21) and, therefore, only the undiluted suspensions tested at 12 or 30 days could be effective. The harderian-gland test has been found (unpublished work) to be no more sensitive to passive transmission than are other tests.

Summary and Conclusions

Mice were irradiated with 900 r to the whole body, and then each was given an intravenous injection of a suspension of marrow cells from 1 femur of a mouse of another strain (C3H). Tests were made for homologous (C3H) cells in the marrow, blood, spleen, lymph nodes, and thymus of the irradiated recipients. A sufficient number of homologous bone-marrow cells initiated immunity to homologous tissue when injected into test mice. Immunity in the test mice was detected by the disintegration of a nonvascularized C3H homograft that had previously been placed in each test mouse.

During the first 4 days after irradiation and the injection of homologous C3H marrow, each femur contained a small number of homologous cells. Twelve to 14 days after irradiation, the marrow had regained its normal cellularity and the antigenicity had increased. In 30 to 60 days each femoral marrow from the treated mice contained C3H tissue equivalent to from one fourth to the total marrow content of a femur from a normal C3H mouse. These results, and those of others, demonstrate that the marrow of mice irradiated and given marrow or spleen is repopulated by the donor cells.

The spleen and lymph nodes, like the marrow, gave some positive tests after treatment up to 4 days. These tissues and the thymus gave consistently positive tests by 12 days. Blood, in the amount used, did not give consistently positive tests until after 30 days. The thymus glands and lymph nodes in 60 to 100 days contained many homologous cells. At 100 days the thymus of 1 animal contained approximately one fourth to all homologous cells. Although it has been indicated by the work of others that injected spleen repopulates the lymph nodes and thymus of irradiated hosts, this is not yet proved for marrow injections. The findings reported here suggest that the thymus and lymph nodes are repopulated by lymphocytes derived from the donor; however, the evidence is not conclusive because of the possibility that the positive tests were due to homologous granulocytes rather than lymphocytes.

References


