The property of preparations of homologous bone marrow and spleen of promoting hematopoietic regeneration and preventing death in laboratory animals, when injected after otherwise lethal whole-body x-radiation, is well established (1, 7, 8). It has been shown, further, by Congdon and Lorenz (8), that a single intravenous injection of rat bone marrow suspension into x-radiated mice significantly increases the survival at 21 days. Histological evidence of hematopoietic tissue regeneration was observed by these workers, and the occurrence of late deaths (e.g., 3–4 weeks) was noted. These results were confirmed recently in this laboratory (2), with the additional finding that cortisone, given in conjunction with rat marrow, appears to enhance its protective effect. As discussed in these latter papers (2, 3), the question arises as to the mode of action of the injected rat bone marrow: Is it the source of a "humoral" substance which stimulates regeneration of the host's marrow; or do the injected rat cells, as such, repopulate the recipient's marrow cavity?

The observations of Wachstein (9) and of Gomori (5) that leukocytes in rat blood smears give an intensely positive histochemical reaction for alkaline phosphatase, whereas similar smears from mice are negative for this enzyme, suggested to us a means of resolving this question by permitting the identification of rat marrow cells after their injection into mice. This report is a preliminary account of our experimental results, with the use of this technic.

METHODS

Histochemical determinations of alkaline phosphatase were first made on marrow and blood smears of normal mice and rats. Thin smears1 of bone marrow (diluted with 0.9 per cent aqueous saline) and tail blood were air-dried and fixed in absolute ethanol for 30 minutes. The slides were then treated according to the histochemical procedure of Gomori for alkaline phosphatase, as given by Cowdry (4). The time of contact with the glycerophosphate2 substrate was 90 minutes. Counterstaining was done with alcoholic eosin. Control marrow smears were treated in the same fashion, except that glycerophosphate was omitted from the substrate solution.

Groups of 30 LAF2 mice (males, 14–16 weeks of age) were given whole-body exposure to x-rays (250 kvP) at an LD100 dose level (810 r). Approximately 1 hour later the mice received a single intravenous injection of a suspension of fresh rat bone marrow equivalent to 70 mg. wet weight marrow/mouse, as described previously (2). Another group of irradiated mice was given injections of approximately 1 mg/mouse of homologous mouse bone marrow, and a third group was irradiated but received no injection. A fourth group, nonirradiated, was given injections of rat bone marrow. Mice from each group were serially sacrificed, and blood and bone marrow smears were treated for alkaline phosphatase, as described above.

RESULTS

Normal animals.—It was found (Figs. 1, 2) that the mature neutrophilic leukocytes of rat marrow showed a heavy black or dark brown precipitate, indicative of alkaline phosphatase activity; the immature myeloid cells, megakaryocytes, and nucleated erythrocytes were various lighter shades of brown; while the mature erythrocytes and lymphocytes were negative. In mouse marrow (Figs. 3, 4), all the cellular elements were negative for

1 It was essential that smears be of unicellular thickness, since heavy or uneven smears occasionally gave false positive results, as also observed by Wachstein (9).
2 Eastman Kodak Co., 52 per cent Na-alpha-glycerophosphate.
phosphatase, except for an occasional site where hematopoietic cells were fortuitously clumped together with bone spicules or with fragments of connective tissue stroma. Since it is known that these osseous and stromal elements are high in alkaline phosphatase activity, it was felt that such foci could be excluded from consideration with respect to phosphatase activity in the hematopoietic cells.

The histochemical findings on the blood smears were similar to those obtained from marrow and, in agreement with those of Wachstein (9) and of Gomori (5), the mature neutrophilic leukocytes of the rat gave a strongly positive phosphatase reaction. The mouse neutrophils were negative, as were all the other peripheral cellular elements in both species. Thus, the apparent “all-or-none” difference in alkaline phosphatase activity between the rat and mouse made possible an approach to the problem of the identity and fate of rat bone marrow injected into irradiated mice.

**Irradiated animals.**—The experimental results obtained are summarized in Table 1 and Figures 5–8. Each value represents the mean from two or more mice. The “total marrow cellularity” in a given animal was estimated from routine sections of one femur; it represents the fraction of the marrow cavity occupied by hematopoietic elements. The normal value in these mice is approximately 90 per cent. The number of “phosphatase-positive cells” was obtained by counts of individual cells in marrow smears from the other femur. Total marrow cellularity in both injected groups of irradiated mice followed the characteristic time sequence of degeneration and regeneration described previously by Lorenz et al. (8) in marrow-treated x-irradiated mice.

However, the occurrence of cells positive for alkaline phosphatase was strikingly different in the two groups. Whereas, in the mice injected with homologous (i.e., mouse) marrow, no positive phosphatase cells were detected at any time during the 28-day period studied, in the group receiving heterologous (i.e., rat) marrow, phosphatase-positive cells were already present in the marrow at the first sampling time after injection (2 hr.) and persisted despite the precipitous decrease in total marrow cellularity during the first 4 days. Concomitant with beginning regeneration, evident at 7 days, there was a marked increase in the percentage of phosphatase-positive cells. During the 2d, 3d, and 4th weeks after irradiation, phosphatase-positive cells comprised virtually the entire population of the marrow and were morphologically indistinguishable from those seen in normal rats.

Some mice from each group, sacrificed during this period, were grossly and microscopically free of infection, while others showed lung and liver abscesses of different degrees of severity. Although these infections varied with the amount of marrow regeneration in each individual case, they did not influence the “all-or-none” phosphatase difference between the two treated groups. Most of the animals of the homologous group survived throughout this period and showed complete marrow regeneration. However, in the heterologous group there was a continuing rate of mortality, as previously described (2), and the marrow of sacrificed animals showed evidences of decreasing cellularity with heavy infiltration by eosinophils. These changes are reflected in the low total cellularity figures in the heterologous group at 21 and 28 days.

The blood smears from the two treated groups showed the same marked difference with respect to phosphatase activity as was found in the marrow smears. No phosphatase-positive cells ever appeared in the homologous group, whereas, in the heterologous group, the concentration of positive cells paralleled that in the marrow.

**Splenic imprints from both groups on the 11th day after irradiation, and from a normal mouse**

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Material injected</th>
<th>Time after injection (days)</th>
<th>Total cellularity of marrow (per cent)</th>
<th>Cells positive for phosphatase (per 100 marrow cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat bone marrow</td>
<td>(2 hr.)</td>
<td>75</td>
<td>7</td>
</tr>
<tr>
<td>Mouse bone marrow</td>
<td>1</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>15</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>60</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>45</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>55</td>
<td>94</td>
</tr>
<tr>
<td>Marrow from normal mouse</td>
<td>1</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Marrow from normal rat</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>75</td>
<td>0</td>
</tr>
</tbody>
</table>
| X-ray dose 810 r (whole-body exposure). Bone marrow suspension injected approximately 1 hour after irradiation.

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1It is recognized that mouse bone marrow does actually contain some alkaline phosphatase, as demonstrated chemically (6). However, the conditions of the histochemical technic used here are such as to produce an apparent qualitative difference.
and rat, showed cellular alkaline phosphatase distribution in every way similar to that noted in the blood and bone marrow.

In the irradiated, noninjected mice, all of which died within 11 days, no phosphatase-positive cells were found in the blood or in the bone marrow at any time.

Following the injection of rat marrow into the group of normal, nonirradiated mice, progressively fewer phosphatase-positive cells were found in blood and marrow smears at 2 hours, 1 day, and 3 days after injection. Seven days following the injection, and thereafter, no phosphatase-positive cells could be detected.

**DISCUSSION**

These experimental findings indicate that intravenously injected rat bone marrow cells are transported to the bone marrow cavity of the recipient mice within a few hours and lodge there in viable form for at least several days. Further, the results imply that these cells are able to divide and, eventually, to repopulate the irradiated mouse marrow cavity with rat cells. It would seem, therefore, that the apparent regeneration of bone marrow in these mice is not a phenomenon of autochthonous regeneration but, rather, is an overgrowth of transplanted rat marrow cells which persist and function hematopoietically in the recipient mice. As an alternative possibility, it could be postulated that the injected rat cells elicit an increase in alkaline phosphatase in the regenerating mouse marrow cells to the extent that it can be demonstrated histochemically by the technic used. Although this explanation appears rather unlikely, studies designed to resolve this point are under way.

As suggested previously (2), it is possible that the late deaths occurring in the heterologous group during the 2d—4th weeks are a result of a delayed immunological response of the host, resulting in partial destruction of the heterologous marrow. This aspect of the problem has not been solved as yet. In any event, it is of considerable interest that, in all the animals from the heterologous group examined during this later period, the marrow that did remain was phosphatase-positive, and there was no evidence of the return of phosphatase-negative (i.e., mouse) cells.

Data beyond those at 4 weeks are, at present, extremely limited because of the continuing deaths in the heterologous group. However, a few mice from previous experiments have survived beyond the 2d month, and, in those examined, phosphatase-positive cells were found in blood smears up to 16 months after irradiation and injection of rat marrow.

**SUMMARY**

Leukocytes in rat blood and bone marrow smears stained black or brown when treated histochemically for alkaline phosphatase.

Mouse leukocytes in similar smears were uniformly phosphatase-negative.

Following intravenous injection of rat marrow cells into x-radiated (810 r) mice, phosphatase-positive cells were present in marrow smears within 2 hours.

With beginning marrow "regeneration" at 7 days, the phosphatase-positive cells rapidly increased in numbers.

By 14—28 days, when "regeneration" was nearly complete, essentially all the cells were phosphatase-positive, and the smears were indistinguishable from rat marrow.

Smears from mice injected with mouse bone marrow remained uniformly phosphatase-negative throughout all stages of regeneration.

These findings indicate that injected rat marrow cells survive, divide, and eventually repopulate the marrow cavity of irradiated mice. Prolonged survival of these mice, in some cases beyond 30 days, appears to be correlated with the continued growth of phosphatase-positive marrow. This suggests that transplanted rat marrow is actually functioning hematopoietically in the heterologous host.

**ADDENDUM (Added in Proof)**

Since this paper was originally submitted for publication, additional mice have survived beyond 30 days after x-radia-
tion and treatment with rat bone marrow. A number of these
have shown, on examination, a transition from phosphatase-
positive to phosphatase-negative leukocytes in blood and bone
marrow smears, suggesting that the heterologous bone marrow
has been replaced by cells of mouse origin.

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