Effect of Neonatal Thymectomy on Hemopoietic Tissue in Mice

By Peretz Resnitzky, Dov Zipori, and Nathan Trainin

The influence of neonatal thymectomy on the behavior of hemopoietic stem cells of bone marrow and spleen was investigated by studying the colony-forming capacity (CFC) of these cells. Bone marrow cells (2 × 10^4) from neonatally thymectomized C3H mice, injected into lethally irradiated (850 R) recipients, showed a reduced CFC in relation to that of intact control donors. This decrease, which was more evident in female than in male mice, started early in life after thymectomy, and persisted throughout all the ages investigated. In most of the experiments a concomitant decrease in the total number of nucleated bone marrow cells was observed following thymectomy. When spleen cells (10^5) from neonatally thymectomized mice were tested under similar experimental conditions, they manifested a CFC equivalent to that of intact controls. Neonatal thymectomy increased the number of spontaneous colonies appearing in the spleen of mice after a sublethal (650 R) total-body irradiation. In contrast, when neonatal splenectomy was tested, a moderate increase in the CFC of bone marrow and in the total number of bone marrow cells was observed. The present results suggest that the thymus plays a role in the proliferative pattern of hemopoietic stem (progenitor) cells located in the bone marrow, whereas it does not influence the behavior of more mature (precursor) hemopoietic cells populating the spleen.

Although extensive information is available on the immunological function of the thymus,1 very little is known about the possible influence of the thymus on hemopoiesis, either experimentally or clinically. There are well-documented data on the migration of cells in irradiated animals from bone marrow to thymus,2,3 or to thymus grafts in intact or thymectomized mice,4 and from shielded bone marrow to the thymus in parabiotic rats.5 Although migration of cells with hemopoietic properties from the thymus has not been definitely demonstrated, there are some reports on changes in hemopoietic tissues following thymectomy in different species. In neonatally thymectomized mice, extramedullary hemopoiesis in the spleen was observed by Miller and Howard,6 but the authors postulate that this phenomenon may have been the consequence of some environmental influence, such as an infectious agent. It has been shown that in adult mice...
thymectomy combined with sublethal irradiation led to a more extensive erythropoiesis and myelopoiesis in the regenerative spleens than that in animals subjected to irradiation alone. In adult opossums subjected to prenatal thymectomy, an increase of myeloid tissue in the lymph nodes and spleen was noted at an age when a decrease of this tissue normally occurs; these thymectomized animals showed, in addition, an increased ratio of immature to mature erythroblasts in their bone marrow. Moreover, in vitro studies on interaction between adult bone marrow and embryonic thymus, both of mouse origin, have indicated that lymphoid cells appear in the marrow channels, and that under such conditions there is a stimulatory effect on granulopoiesis. An increase was observed in the number of blasts and of young erythroblasts in the bone marrow of rats thymectomized within 48 hr of birth. There was, however, no change in the bone marrow when thymectomy was performed in adult rats. Furthermore, it has been suggested that thymic cells exert a stimulating effect on erythropoiesis when injected simultaneously with bone marrow cells into heavily irradiated mice. Absence of the thymus (congenital agenesis) leads, in infants, to the development of a syndrome that is considered a clinical counterpart of the immunological picture obtained in neonatally thymectomized animals, manifested by the absence of delayed hypersensitivity and of homograft immunity, together with a striking predisposition of infections. Although in these cases there is a pronounced fall in the number of peripheral blood lymphocytes, and a depletion of paracortical lymphocytes in the lymph nodes, abnormalities of the hemopoietic tissue were not reported, and the possibility cannot be excluded that these patients did not survive long enough to develop a manifest alteration in hemopoiesis. In elderly people, another type of syndrome has been described, consisting of an association between a tumor of the thymus (thymoma) and a special type of aplastic anemia, namely, pure red cell aplasia. In addition, an immune deficiency state was also described. This clinical syndrome does not seem to be purely coincidental, though there is as yet no reasonable explanation for this association. However, in several cases the presence of an inhibitor of erythropoietin or of a marrow-suppressing factor was demonstrated in the blood of these anemic patients.

In the present communication we describe experiments performed to determine whether any functional changes occurred in the hemopoietic organs of neonatally thymectomized animals. For this purpose, the Till and McCulloch technique seemed appropriate, since variation in the colony-forming capacity (CFC) of hemopoietic tissue should reflect changes in the stem cell population. A possible influence of neonatal thymectomy on the behavior of the primitive hemopoietic cells of the bone marrow and spleen was investigated by this method. In a previous communication we found that the cloning capacity of bone marrow cells from neonatally thymectomized mice is lower than that of normal animals. The present work confirms this finding, provides additional information with reference to the age and sex of the experimental animals, and investigates the colony-forming capacity of the spleen cells of these mice. The possible influence of neonatal thymectomy on spontaneous spleen colony formation after sublethal irradiation was also studied.
MATERIALS AND METHODS

Mice

Inbred C3H/eb mice, originally obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were bred at the Weizmann Institute of Science by sibling mating. They were used as donors or recipients of bone marrow and spleen cells. Intact and thymectomized male and female mice, aged between 7 and 240 days, were used as donors. Normal and thymectomized mice of both sexes, aged between 80 and 100 days, were used as recipients. Throughout the experiments the animals were housed in metal cages, in air-conditioned rooms kept at 21–25°C, and fed Purina Laboratory Chow pellets and tap water ad libitum.

Thymectomy

Whole litters of mice were thymectomized at 3 days of age, under ether anesthesia, by an adaptation of Miller’s technique.22 The animals were used as donors of bone marrow and/or spleen; their mediastinum was examined grossly and microscopically, and any mouse found to contain a thymic remnant was discarded. Comparable litters of mice were left intact as controls. Thymectomized mice were also used in experiments in which spontaneous colony formation was studied.

Splenectomy

The mice were splenectomized within 24 hr after birth, under ether anesthesia, by laparotomy and separation of the spleen from the blood vessels with fine scissors. After removal of the spleen, the incision was closed with one stitch.

X-irradiation

The mice were exposed to a single dose of 650 or 850 R total-body irradiation from a General Electric Maximar III 250 X-ray machine, 215 kV, 15 ma, 40 rpm, using filters of 0.5 mm Cu and 1 mm Al, at a target distance of 50 cm, in a field of 20 × 20 cm.

Preparation of Bone Marrow Cell Suspensions

Bone marrow was obtained from the tibiae and femora of each individual mouse. Pooling of the material was omitted, in order to test separately the cells of one mouse at a time, and also to prevent any possible interaction of cells from different donors. A 27-gauge needle, mounted on a 1.0-ml syringe containing Tyrode’s solution, was inserted into one end of the bone, and the marrow was then discharged by forcing the solution through the marrow cavity. Nucleated cells were counted in a hemocytometer. The final suspension was adjusted by dilution with Tyrode’s solution to a concentration of 2 × 10^4 cells per 0.5 ml. The entire procedure was carried out at ice temperature.

Preparation of Spleen Cell Suspensions

Spleens were removed aseptically, and the cells were dispersed by pressure through a stainless steel mesh into Tyrode’s solution. The cells were further dissociated by means of a 27-gauge needle mounted on a 2.0-ml syringe, and then counted in a hemocytometer. The final cell suspension was adjusted by dilution with Tyrode’s solution to a concentration of 10^5 cells/0.5 ml. As in the case of the preparation of the bone marrow suspensions, the entire procedure was carried out at ice temperature.

Experimental Groups

In most of these experiments, bone marrow or spleen cells were taken from individual thymectomized and intact animals of the same sex, ages 7–240 days, adjusted to the appropriate dilution, and injected immediately into irradiated recipients of the same sex, ages 80–100 days. A solution containing 2 × 10^4 cells for bone marrow, or 10^5 for spleen,
EFFECT OF NEONATAL THYMECTOMY ON HEMOPOIETIC TISSUE

Influence of Neonatal Thymectomy on the Cloning Capacity of Bone Marrow Cells

Bone marrow cells (2 × 10⁴), taken from normal and thymectomized mice at different ages and of both sexes, were injected intravenously into intact heavily irradiated (850 R) recipients. The spleen colonies were counted at day 9. The general pattern of these experiments points to a reduced CFC by the bone marrow cells of neonatally thymectomized mice. This capacity, however, showed variations according to the sex and age of the donor animals. Figures 1 and 2 present the results of 44 experiments, 20 with female and 24 with male mice. Each point of the curves is the average of 2 to 3 individual experiments, each one including from 8 to 12 animals. (The point at 160 days for the females is the average of a single experiment.)

In female mice (Fig. 1), the drop in colony-forming units (CFU) started...
at approximately the age of 21 days. Before this period, when tested at 8 and 11 days, a moderate increase in the cloning capacity of bone marrow from thymectomized mice was noted (Table 1). Following this short period, there was a significant decrease in the CFC of the bone marrow cells from thymectomized animals, which persisted throughout all the ages investigated—that is, up to 225 days. The thymectomized male mice showed a pattern of cloning similar to that of the thymectomized females (Fig. 2), and manifested a transient increase in CFC in the youngest age groups (Table 1). The drop in CFC started at approximately day 20; thereafter, the decrease in CFC

### Table 1.—Colony-forming Capacity of $2 \times 10^4$ Bone Marrow Cells From Young Normal (N) and Thymectomized (Tx) Mice

<table>
<thead>
<tr>
<th>Age of Donor (days)</th>
<th>Sex</th>
<th>Source of Bone Marrow Injected</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>♀</td>
<td>N</td>
<td>2.5 ± 0.38</td>
</tr>
<tr>
<td>8</td>
<td>♀</td>
<td>Tx</td>
<td>4.5 ± 0.42</td>
</tr>
<tr>
<td>11</td>
<td>♀</td>
<td>N</td>
<td>6.5 ± 0.38</td>
</tr>
<tr>
<td>11</td>
<td>♀</td>
<td>Tx</td>
<td>6.5 ± 0.38</td>
</tr>
<tr>
<td>11</td>
<td>♂</td>
<td>N</td>
<td>2.4 ± 0.35</td>
</tr>
<tr>
<td>11</td>
<td>♂</td>
<td>Tx</td>
<td>5.7 ± 0.42</td>
</tr>
<tr>
<td>13</td>
<td>♀</td>
<td>N</td>
<td>3.4 ± 0.35</td>
</tr>
<tr>
<td>13</td>
<td>♀</td>
<td>Tx</td>
<td>3.9 ± 0.30</td>
</tr>
<tr>
<td>18</td>
<td>♀</td>
<td>N</td>
<td>4.0 ± 0.42</td>
</tr>
<tr>
<td>18</td>
<td>♂</td>
<td>Tx</td>
<td>5.1 ± 0.35</td>
</tr>
</tbody>
</table>

Fig. 2.—Colony-forming capacity of $2 \times 10^4$ bone marrow cells from normal (solid line) and neonatally thymectomized (dashed line) male mice at different ages. Each point represents the average number of colonies from 2–3 individual experiments, each including from 8 to 12 animals. (The last point of the curve represents the average of 5 individual experiments.) The standard deviation is indicated by vertical lines.
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Fig. 3.—Relative colony-forming capacity of neonatally thymectomized male (closed circle) and female (open circle) mice in relation to respective normal animals (dashed line). Each point in the two curves represents the ratio of the average number of colonies in neonatally thymectomized mice to the normal animals at the different ages shown in Figs. 1 and 2, for female and male mice, respectively.

Persisted throughout the entire period investigated, though in most of the experiments the difference between normal and thymectomized male mice was less marked than in the females, especially in the older age groups. The cloning capacity of bone marrow from thymectomized female and male mice in relation to that of normal animals is expressed in Fig. 3. The data presented in this figure summarize those presented in Figs. 1 and 2. Each point of this figure was obtained by dividing the average number of CFU from thymectomized mice, at a certain age, by that of normal animals of the same age. As seen in Fig. 3, the drop in CFC in female mice was more evident than that in males. Table 1 gives the results of some of the experiments with mice of the younger age groups (up to 18 days).

Influence of Neonatal Thymectomy on the Cloning Capacity of Spleen Cells

Spleen cell (10⁶) from normal and neonatally thymectomized male and female mice were injected intravenously, at different ages, into intact heavily irradiated (850 R) recipients. The spleen colonies were counted at day 9 after irradiation. Figs. 4 and 5 summarize the results of 38 experiments, 18 with female and 20 with male mice. Each point in the curves represents the average of 2 to 3 individual experiments, each one including 8 to 12 animals (except for the points at 160 and 207 days, each of which represents the average of a single experiment). It can be seen that there is no difference between the CFC of spleen cells from normal and neonatally thymectomized mice, with the exception of the point at days 115–131 for females, where the average number of colonies was higher in thymectomized mice. Furthermore, these figures indicate a significant increase in the CFC in the youngest age groups of both normal and thymectomized mice.
Influence of Neonatal Thymectomy on Number of Bone Marrow Cells

As an extension of the previous experiments, we also investigated the total number of nucleated cells in four large bones (two femora and two tibiae)
**EFFECT OF NEONATAL THYMECTOMY ON HEMOPOIETIC TISSUE**

Table 2.—Total Number of Nucleated Cells in Four Large Bones* of Normal (N) and Neonatally Thymectomized (Tx) Mice At Different Ages

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Sex</th>
<th>N</th>
<th>Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>♀</td>
<td>16.0</td>
<td>10.0</td>
</tr>
<tr>
<td>18</td>
<td>♀</td>
<td>20.2</td>
<td>8.4</td>
</tr>
<tr>
<td>21</td>
<td>♀</td>
<td>27.2</td>
<td>25.2</td>
</tr>
<tr>
<td>28</td>
<td>♂</td>
<td>25.5</td>
<td>24.8</td>
</tr>
<tr>
<td>29</td>
<td>♂</td>
<td>21.6</td>
<td>15.8</td>
</tr>
<tr>
<td>36</td>
<td>♀</td>
<td>26.4</td>
<td>26.0</td>
</tr>
<tr>
<td>40</td>
<td>♂</td>
<td>31.2</td>
<td>30.8</td>
</tr>
<tr>
<td>41</td>
<td>♀</td>
<td>28.8</td>
<td>25.0</td>
</tr>
<tr>
<td>53</td>
<td>♀</td>
<td>24.4</td>
<td>22.4</td>
</tr>
<tr>
<td>54</td>
<td>♂</td>
<td>27.6</td>
<td>20.4</td>
</tr>
<tr>
<td>59</td>
<td>♂</td>
<td>48.2</td>
<td>29.8</td>
</tr>
<tr>
<td>98</td>
<td>♂</td>
<td>25.4</td>
<td>24.6</td>
</tr>
<tr>
<td>122</td>
<td>♀</td>
<td>18.8</td>
<td>10.4</td>
</tr>
<tr>
<td>131</td>
<td>♀</td>
<td>16.0</td>
<td>14.8</td>
</tr>
<tr>
<td>140</td>
<td>♂</td>
<td>28.4</td>
<td>22.2</td>
</tr>
<tr>
<td>160</td>
<td>♀</td>
<td>30.6</td>
<td>20.6</td>
</tr>
<tr>
<td>170</td>
<td>♂</td>
<td>21.6</td>
<td>11.4</td>
</tr>
<tr>
<td>201</td>
<td>♂</td>
<td>31.0</td>
<td>20.0</td>
</tr>
<tr>
<td>240</td>
<td>♀</td>
<td>32.2</td>
<td>15.6</td>
</tr>
</tbody>
</table>

* Two femora and two tibiae.

of the mice used as donors. Table 2 shows the number of bone marrow cells in these four large bones in normal and thymectomized male and female mice, at different ages. In most of the experiments, a decrease in the total number of nucleated cells was observed in the thymectomized donors, as compared with adequate normal controls.

**Influence of Neonatal Splenectomy on the Cloning Capacity of Bone Marrow Cells**

Since the mouse spleen is closely related to the hemopoietic as well as to the lymphopoietic systems, we investigated whether any detectable changes occurred in the total population and CFU of the bone marrow of animals splenectomized instead of thymectomized at newborn age. This experiment was performed in order to learn whether the changes in the bone marrow after neonatal thymectomy were specific to the absence of this organ or were the mere expression of the removal of any sizable compartment of the lympho-hemopoietic system. Bone marrow cells of splenectomized male mice were tested on lethally irradiated male recipients, as in the previous experiments. As can be seen in Table 3, there was a moderate increase in the CFU of the bone marrow cells of neonatally splenectomized male mice, as compared to intact donors. In addition, in most of these experiments the number of nucleated bone marrow cells counted in a sample of four large bones (two femora and two tibiae) was higher in the splenectomized than in the intact donor animals. Furthermore, by multiplying the number of nucleated cells counted in the bone marrow by the values of the CFU obtained by injecting
Table 3.—Total Number of Nucleated Bone Marrow Cells and Colony-forming Units* in Four Large Bones of Normal (N) and Neonatally Splenectomized (Sx) Male Mice

<table>
<thead>
<tr>
<th>Age of Donor (days)</th>
<th>Source of Bone Marrow Injected</th>
<th>Number of Bone Marrow Cells (10⁶) Counted</th>
<th>Number of CFU in 2 × 10⁶ Cells</th>
<th>Total Number of CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>N</td>
<td>29.2</td>
<td>5.0</td>
<td>14,600</td>
</tr>
<tr>
<td>49</td>
<td>Sx</td>
<td>38.2</td>
<td>6.4</td>
<td>24,400</td>
</tr>
<tr>
<td>49</td>
<td>N</td>
<td>22.8</td>
<td>4.4</td>
<td>10,033</td>
</tr>
<tr>
<td>49</td>
<td>Sx</td>
<td>22.0</td>
<td>7.0</td>
<td>15,400</td>
</tr>
<tr>
<td>50</td>
<td>N</td>
<td>29.2</td>
<td>6.4</td>
<td>18,680</td>
</tr>
<tr>
<td>50</td>
<td>Sx</td>
<td>28.5</td>
<td>9.2</td>
<td>26,320</td>
</tr>
<tr>
<td>50</td>
<td>N</td>
<td>33.0</td>
<td>5.8</td>
<td>19,140</td>
</tr>
<tr>
<td>50</td>
<td>Sx</td>
<td>38.2</td>
<td>8.1</td>
<td>30,980</td>
</tr>
<tr>
<td>111</td>
<td>N</td>
<td>26.4</td>
<td>4.8</td>
<td>12,672</td>
</tr>
<tr>
<td>111</td>
<td>Sx</td>
<td>33.4</td>
<td>6.0</td>
<td>20,040</td>
</tr>
</tbody>
</table>

* The total number of CFU was calculated by multiplying the number of nucleated cells in four large bones (two femora and two tibiae) by the number of CFU in 2 × 10⁶ bone marrow cells.

2 × 10⁶ cells, it was possible to calculate the probable total number of CFU in the whole population of the four large bones. Thus, the total number of colony-forming cells in the bone marrow of these four bones was much higher in neonatally splenectomized mice than that found in the respective intact controls (see Table 3).

Table 4.—Number of Endogenous Spleen Colonies in Normal (N) and Neonatally Thymectomized (Tx) Mice Submitted to Sublethal Irradiation (650 R)

<table>
<thead>
<tr>
<th>Age at Time of Irradiation (days)</th>
<th>Sex</th>
<th>Treatment</th>
<th>Number of Colonies in Individual Spleens</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>♀</td>
<td>N</td>
<td>2,2,3,4,4,5,6,7,10,10,14</td>
<td>5.8</td>
</tr>
<tr>
<td>66</td>
<td>♀</td>
<td>Tx</td>
<td>3,4,6,9,11,13,13,21,21,21,21</td>
<td>11.2</td>
</tr>
<tr>
<td>67</td>
<td>♀</td>
<td>N</td>
<td>0,0,2,3,4,5,5,9,14,20,25</td>
<td>7.5</td>
</tr>
<tr>
<td>67</td>
<td>♀</td>
<td>Tx</td>
<td>3,6,7,9,11,14,14,18,19,25</td>
<td>12.6</td>
</tr>
<tr>
<td>72</td>
<td>♀</td>
<td>N</td>
<td>1,1,3,3,3,3,9,9,10,10,11,12</td>
<td>6.0</td>
</tr>
<tr>
<td>72</td>
<td>♀</td>
<td>Tx</td>
<td>1,4,4,5,8,8,9,10,10,14,15,20,25</td>
<td>10.2</td>
</tr>
<tr>
<td>66</td>
<td>♂</td>
<td>N</td>
<td>5,8,8,9,9,10,12,13,15,15,16,18</td>
<td>11.5</td>
</tr>
<tr>
<td>66</td>
<td>♂</td>
<td>Tx</td>
<td>0,2,6,9,10,13,20,21,25,25,25</td>
<td>13.1</td>
</tr>
<tr>
<td>73</td>
<td>♂</td>
<td>N</td>
<td>1,4,5,6,6,7,8,9,10,15,18</td>
<td>8.1</td>
</tr>
<tr>
<td>73</td>
<td>♂</td>
<td>Tx</td>
<td>2,4,5,6,7,7,8,9,9,25</td>
<td>8.2</td>
</tr>
<tr>
<td>73</td>
<td>♂</td>
<td>N</td>
<td>3,3,3,6,7,7,7,9,9,10,12,20</td>
<td>8.0</td>
</tr>
<tr>
<td>73</td>
<td>♂</td>
<td>Tx</td>
<td>0,2,3,7,7,8,10,15,25,25,25,30</td>
<td>11.6</td>
</tr>
</tbody>
</table>
Influence of Neonatal Thymectomy on Spontaneous Colony Formation in the Spleens of Mice Submitted to Sublethal Irradiation

When mice are irradiated with sublethal doses of X rays, hemopoietic colonies are formed spontaneously, and can be observed macroscopically in their spleens 8-10 days after the irradiation. It was interesting to study the effect of neonatal thymectomy on this type of endogenous colony formation. Table 4 summarizes the results of experiments with intact and neonatally thymectomized mice submitted to sublethal total-body irradiation (650 R). Male and female mice, 2-25 months old, were used, since at this age the difference in the number of colonies between intact and thymectomized mice was striking. As seen in Table 4, the number of spontaneous colonies in the spleens of female mice was higher in thymectomized animals than in intact controls. This difference was less pronounced when the same type of experiment was performed in males.

Discussion

The results of the present experiments confirm our initial observation that neonatal thymectomy in mice decreases the colony-forming capacity of bone marrow cells following their injection into lethally irradiated recipients. This effect, however, was not uniform, and age and sex differences were observed. In most cases, there was a period of 2-3 wk after thymectomy that was characterized by a slight rise in the CFC of the bone marrow. After this short period, a pronounced and prolonged decrease in the CFC of bone marrow from thymectomized animals followed. This drop persisted in female mice throughout all the ages investigated (up to 225 days), whereas in the males it was less striking, especially in the older age groups. From bone marrow cell counts we learned, in addition, that there was a marked decrease in the total number of nucleated cells in the marrow of thymectomized mice as compared with intact animals. These changes in the CFC, and in the number of bone marrow cells of neonatally thymectomized mice, suggest that the thymus, in addition to its well-known lymphopoietic and immunologic functions, also plays an important role in the proliferative pattern of the hemopoietic cells. Whether this interaction between thymus and bone marrow tissue is of a cellular or humoral nature, as has been discussed for other aspects of thymic activity, is still a subject for further investigation.

The possibility that these changes in bone marrow proliferation following neonatal thymectomy were merely the result of eradication of a sizable part of the lymphatic tissue was tested by submitting some groups of mice to neonatal splenectomy. In splenectomized animals, however, both the CFC and number of nucleated cells in the bone marrow were even higher than in the intact mice (Table 3). It has been reported that when the cloning capacity of neonatally splenectomized mice was tested by an in vitro method, no differences were observed between splenectomized and intact mice. However, cells responsible for colony formation in vivo and in vitro seem not to be the same ones, a fact that could explain this discrepancy. At any rate, unlike neonatal thymectomy, neonatal splenectomy failed to reduce the CFC of bone marrow cells.
Another explanation for the decreased cloning capacity of the bone marrow population in neonatally thymectomized mice could be an alteration in the biophysical properties, such as an increased fragility, of certain hemopoietic cells, which could diminish the actual number of viable cells injected. Examination of osmotic fragility of bone marrow cells by means of the fragiograph\textsuperscript{34} did not reveal any difference between cells of intact and of thymectomized mice.\textsuperscript{35} Further investigation is required to elucidate the mechanism of the interaction between the thymus and the bone marrow cell population.

Whereas the CFC of bone marrow cells was impaired after neonatal thymectomy, that of the spleen was found to be similar to the CFC of intact controls, in both male and female animals. According to Bennett and Cudkowicz,\textsuperscript{36} the primitive hemopoietic cell pool can be classified into two categories: one of “precursor cells,” which are capable of hemopoietic repopulation and colony formation only, and another of “progenitor cells,” which, in addition, are also capable of self-replication. The latter category refers, therefore, to the hemopoietic stem cells. These authors have found both precursor and progenitor cells in mouse bone marrow, while the spleen of these animals contained chiefly precursor cells. Since in the present experiments we have found that neonatal thymectomy reduced the CFC of the bone marrow, but not of the spleen cells, it may be postulated that progenitor cells present in the bone marrow are under thymus control, whereas more differentiated precursor cells found in the spleen do not depend on thymus function. This hypothesis might also explain the appearance of a higher number of endogenous spleen colonies in thymectomized animals submitted to sublethal irradiation than in intact irradiated controls. Assuming that precursor hemopoietic cells in the spleen of these animals are unaffected by thymectomy, they could possibly compensate for the impaired activity of stem cells in the bone marrow.

The different pattern of bone marrow cloning capacity in male and female mice after thymectomy deserves further comment. As seen in Fig. 3, the impairment in CFC following thymectomy was more pronounced in female mice, suggesting the participation of sex hormones in hemopoiesis. In fact, an increase in spontaneous spleen colony formation in mice treated with testosterone before irradiation has been reported,\textsuperscript{37} and androgens have also been found to stimulate hemopoiesis.\textsuperscript{38} The milder drop in the CFC of bone marrow after thymectomy in male mice may therefore be explained on these grounds.

In conclusion, the results of the present experiments suggest that hemopoiesis is influenced by the thymus, since thymectomy impairs the CFC of bone marrow, as demonstrated by the in vivo cloning method. Cytological studies of the bone marrow and spleen, and histological patterns of spleen colonies of neonatally thymectomized mice, will provide an answer as to whether the changes found in these animals are an expression of maturation arrest of the bone marrow population, as hinted by preliminary results previously published.\textsuperscript{21}
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