# Effect of Thymectomy on Hematopoietic Organs of the Opossum "Embryo."* (30007)

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It is now well established that the thymus plays an important role in insuring the development and maintenance of lymphoid structures, and associated immunological functions (1). In embryonic life small and medium lymphocytes§ are first found in the thymus and only later in other lymphoid tissues, and thereafter in the circulation(2,3,4). The cat is a possible exception. In 1909 Maximow stated that lymphocytes were first found in lymph nodes of the 13 mm cat embryo(5). In 1927, he said that formation of lymphocytes was first demonstrable in the thymus of the 22 to 30 mm cat embryo(6). In some species, the lymphoid tissues of animals thymectomized at birth and examined after the *first* month of life show a decrease in number of small and medium lymphocytes. This is associated with defects in some types of immune response, particularly homograft rejection(7,8,9,10). The hypothesis has been suggested that the thymus influences lymphoid development either by migration of thymic lymphocytes to other areas or by a thymic humoral factor or both(11). Strong evidence for a humoral mechanism has recently been obtained(12).

At birth, the mouse already has small and medium lymphocytes in the circulation and in the spleen. Although mice thymectomized at birth eventually show severe lymphoid hypoplasia, there are no detailed studies of their lymphoid tissues during the first few weeks after thymectomy. It is therefore not known whether thymectomy at birth influences the origin and initial proliferation of extrathymic lymphocytes or influences the number of these cells which have previously developed by virtue of other mechanisms(13, 14,15). Solution of these problems can be met by studying the effect of thymectomy *prior* to the origin of extrathymic lymphatic tissue. This presents a major technical problem since, in the eutherian mammalian species studied, small and medium lymphocytes occur outside the thymus before birth.

In the opossum, *Didelphys virginiana*(2), large lymphocytes are found in very small numbers first in the thymus on the first day of life and in large numbers on the second day of life. Medium lymphocytes are found in the thymus on the second or third days and small lymphocytes on the 5th or 6th days. Medium lymphocytes are first found in the cervical nodes on the 3rd and 4th days and in the mediastinal nodes on the 5th day. Small lymphocytes appear in lymph nodes one or two days after medium lymphocytes. Medium lymphocytes are first found in the circulation on about the 7th day and small lymphocytes on about the 11th day. Medium lymphocytes are first found in the spleen in periarterial regions during the seventeenth through 22nd days and a few small lymphocytes appear a few days thereafter. Large lymphocytes first appear in lymph nodes and spleen about the 30th day. A study of the effect of thymectomy on the origin and development of extrathymic lymphocytes and lymphatic tissue is presented here.

*Materials and methods.* Most of the opossum pouch young were born in the laboratory in Denver. The exact age of these "embryos" was therefore known. Other animals were born prior to arrival at the laboratory in Denver. The age of the latter was estimated by comparing their weight and the histology of their blood forming tissues to that of a series of opossums of known age(2).

Thymectomies were performed in pouch "embryos" while still attached to the nipple,

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§ Small, medium and large lymphocytes are defined according to the criteria of Bloom and Fawcett, Textbook of Histology, 8th Ed., W. B. Saunders Co., Philadelphia & London, 1962.

the mother being anaesthetized with nembutal. An "embryo" was delivered through a hole in a piece of clean bloth covering the maternal pouch, care being taken not to detach the "embryo" from the nipple. The "embryo" was placed on its back over a piece of foam rubber and its limbs strapped with tape to the material covering the pouch. The operations were performed under a dissecting microscope by a technique to be described elsewhere. Surgical mortality was due to bleeding, very slight amounts of blood loss being incompatible with life. Mortality from operations performed under 5 days of age was 92%, between 5 and 10 days, 70%, and between 10 and 25 days, 60%. About ⅔ of the opossums in the pouch of each mother were thymectomized, the others being left untreated. The animals were sacrificed at various times at 28 or 45 days of age, blood was collected for cell counts, tissues fixed in Zenker-formol, cut at 4 $\mu$ in nitrocellulose and stained with haematoxylin-eosin-azure (16). Every fourth section of the upper mediastinum and of the pancreatic lymph nodes in all animals subjected to operative procedures was examined.

*Results.* Between 7 and 10 days after birth, the lymph nodes of the normal opossum do not show demarcation into cortex and medulla: they consist of a stroma of mesenchymal (reticular) cells and sinusoids. Small and medium lymphocytes and myelocytes are scattered among the mesenchymal cells and numerous basophil erythroblasts occur in the sinusoids(2). Cortical and medullary regions become distinct between 10 and 12 days, small lymphocytes being then densely packed in the cortex which does not contain erythrocyte or granulocyte precursors. Myelocytes become relatively rare in the medullary cords by 20 days; basophil and more mature erythroblasts still fill the sinusoids becoming rare only after the thirtieth day and disappearing from the lymph nodes after 50 days. Plasma cell precursors may be found as early as 45 days. The lymph nodes of animals subjected to incomplete thymectomy showed all the features characterizing the lymph nodes of normal, unoperated pouch-mate controls (Fig. 1).

The lymph nodes of animals subjected to complete thymectomy were clearly deficient in small lymphocytes and showed an abnormal persistence of immature (basophil) erythroblasts and, in most cases, of myelocytes (Fig. 2). All of the nodes examined in 5 animals thymectomized at 10 to 12 days and sacrificed at 24 to 28 days consisted of a framework of reticular tissue, without demarcation into cortex and medulla, with an excessive number of basophil erythroblasts in the sinusoids and many myelocytes in the mesenchymal cords. Large lymphocytes were present and small and medium lymphocytes were grossly reduced in number. One animal thymectomized at 24 days and sacrificed at 44 days had lymph nodes showing cortical and medullary regions, focal accumulation of small and medium lymphocytes in the cortex, numerous large lymphocytes and basophil erythroblasts scattered in the medulla, persisting myelocytes and some plasma cell precursors. The findings on these animals are summarized in Table I. One animal, not included in the table, was thymectomized at 6 days. It was the youngest animal to survive complete thymectomy. It was sacrificed at 21 days because it appeared wasted. It weighed one gram, half the weight of a partially thymectomized control and had very minute lymph nodes consisting only of strands of mesenchymal cells.

At about 10 to 20 days after birth the mesenchymal framework of the spleen of the normal opossum contains mainly the following cell types: erythroblasts in varying stages of maturation (basophil, polychromatophil and eosinophil) and more or less evenly distributed throughout the splenic substance; granulocyte precursors, mostly eosinophil myelocytes, more numerous along arterioles and arterial capillaries and adjacent to the capsule; and scattered, evenly distributed, megakaryocytes(2). By day 20 the spleen is solidly and diffusely filled with these myeloid cells; from day 23 onwards their concentration decreases. The ratio of immature (basophil) to mature (polychromatophil and eosinophil) erythroblasts is less than 1 (Fig. 3). At this time the white pulp is clearly evident around the arterioles, small, medium and occasionally large lymphocytes being found among the mesenchymal cells surrounding

TABLE I. Effect of Thymectomy on

| Treatment | Age at operation (days) | Age of sacrifice (days) | No. in group | Body wt (g)* | Spleen wt (mg)* | Spleen body ratio* | Lymph nodes: Distinction between cortex and medulla | Lymph nodes: Small lymphocytes | Lymph nodes: my per* 1000 nuclei in medulla |
|---|---|---|---|---|---|---|---|---|---|
| Thymectomy | 10–12 | 28 | 5 | 3.6 | 16.0 | 4.3 ± .9 | Absent in 5 animals | Grossly decreased in 4 animals; moderately decreased in 1 animal | 140 |
| Partial thymectomy | 10–12 | 28 | 4 | 3.8 | 7.1 | 1.9 ± .2 | Present | Densely packed in cortex | 20 ± 4 |
| Untreated | | 28 | 7 | 4.6 | 15.2 | 3.3 ± .3 | " | *Idem.* | 10 ± 2 |
| Thymectomy | 24 | 45 | 1 | 9.0 | 54.1 | 6.0 | " | Moderately decreased | 30 |
| Untreated | | 45 | 2 | 9.7 | 19.3 | 3.0 | " | Densely packed in cortex | 10 |

* Avg value.
± Standard error.

these vessels (Fig. 3). By 45 days, granulocyte formation is restricted to occasional foci and the white pulp has increased considerably in size. In animals subjected to partial thymectomy and sacrificed between 28 and 30 days of age, the spleen showed essentially the same features as the spleens of normal, unoperated, pouch-mate controls. In only one case was there a slightly increased number of eosinophil granulocytes.

The spleens of totally thymectomized animals were in most cases enlarged (Table I). They showed a strikingly abnormal persistence and proliferation of immature, basophil, erythroblasts. These cells saturated the spleen in dense sheets extending to the edge of the endothelium of blood vessels (Fig. 4). The ratio of basophil erythroblasts to polychromatophil and eosinophil erythroblasts was significantly higher than that found in partially thymectomized or normal pouch-mate controls (Table I). Mitoses and karyorrhexis were greatly increased in basophil erythroblasts. It was impossible to distinguish clearly some of these basophil erythroblasts from intermediate or even true megaloblasts. An abnormal persistence of eosinophil myelocytes was evident especially around the arterioles and arteriolar capillaries and in the vessel walls. Another striking feature of thymectomized animals sacrificed at 28 days was the absence of the white pulp. No small or medium lymphocytes were found around the arterioles and arterial capillaries; instead, the perivascular area was occupied either by basophil erythroblasts (Fig. 4) or in some cases by eosinophil myelocytes. One animal thymectomized at 24 days and sacrificed at 45 days lacked white pulp; sheets of basophil erythroblasts and increased numbers of eosinophil myelocytes were found (Table I). The spleen of the wasted animal, thymectomized at 6 days and sacrificed at 21 days, was quite different from the spleens of the other thymectomized opossums; it was extremely small, weighing about 1 mg, very immature, similar to that of a 5-day-old opossum, with poorly developed sinuses and practically no myeloid cells and no lymphocytes.

Differential counts on blood smears are given in Table I. The blood of thymectomized opossums sacrificed at 28 days lacked small lymphocytes and contained more nucleated red cells than the blood of controls. The one animal thymectomized at 24 days and studied in detail also had an abnormal persistence of nucleated red cells (basophil erythroblasts) and only one small lympho-

Weight, Hematopoietic Organs and Blood.

| | Spleen | | Peripheral blood | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Differential nucleated cell counts* (%) | | | | | | | | |
| | | | | Erythroblasts | | | Myelocytes + granulocytes | | | Mononuclears | | |
| b.e.*/ p.e. + e.e. in medulla | White pulp | b.e. per 1000 nuclei | Total nucleated cell count | b.e. | p.e. | e.e. | n. | e. | b. | l.m. | s.m. | Smudge |
| 3.9 ± .4 | Absent | 320 ± 43 | 18100 | 9.2 | 13.6 | 10.1 | .7 | 1.1 | .1 | .4 | .0 | 64.9 |
| .3 ± .2 | Present | 100 ± 34 | 15750 | 6.3 | 2.6 | 2.6 | 5.8 | 6.8 | 1.1 | 21.6 | 29.5 | 23.7 |
| .5 ± .1 | " | 50 ± 20 | 14670 | 8.8 | 7.5 | 4.0 | 3.2 | 8.0 | 1.6 | 11.2 | 11.0 | 44.7 |
| 2.1 | Absent | 100 | 8000 | 22.0 | 4.5 | 5.0 | 3.0 | 1.6 | .0 | 2.2 | .1 | 61.6 |
| .3 | Present | 20 | 15650 | .8 | 6.1 | 4.4 | 6.9 | 13.7 | .8 | 5.7 | 48.8 | 12.7 |

Abbreviations: b.e., basophil erythroblasts; p.e., polychromatophil erythroblasts; e.e., eosinophil erythroblasts; n., neutrophil; e., eosinophil; b., basophil; l.m., large mononuclear cells; s.m., small mononuclear cells; my., eosinophilic myelocyte.

cyte among 1000 nucleated cells in marked contrast to the findings in its 2 pouch-mate controls (Table I). Smudged cells were more common in thymectomized animals than in controls.

Although the liver, bone marrow and submucosa of the small intestine of thymectomized opossums showed a greater persistence of immature erythroblasts than in controls, the difference between the 2 groups was not as striking as that noted in lymph nodes and spleen.

*Discussion.* In thymectomized opossums the number of small and medium lymphocytes in lymph nodes was grossly reduced and these cells failed to appear in the spleen. This situation was not observed in the 9 untreated and 4 partially thymectomized controls in this experiment, nor in the much larger number of normal opossums previously studied(2). Thus, the *origin* of lymphatic tissue in the spleen appears to be dependent upon the presence of a thymus. These experiments, however, do not exclude the possibility that thymectomy simply caused a delay in the *origin* of splenic lymphatic tissue since all of the animals in this series were sacrificed between 28 and 45 days of age.

These findings are not relevant to the origin of lymphatic tissue in lymph nodes since these nodes were present in the youngest animals that survived thymectomy. Medium and small lymphocytes were grossly reduced in number in the nodes of animals thymectomized 2 weeks prior to sacrifice in comparison to those of controls of the same age even though, at the time of thymectomy, such nodes already contained lymphocytes. This implies that the thymus plays a role in the continued formation and/or proliferation of lymphocytes. Our experiments, however, do not determine whether such cells emigrate from the thymus or whether they arise locally as a result of a thymic humoral mechanism.

A striking and unanticipated finding in the spleen and lymph nodes of thymectomized opossums was an abnormal persistence and even increase in myeloid cells, particularly erythroblasts. This indicates that the thymus plays a role not only in the origin and maintenance of lymphatic tissue but also in suppression of myeloid tissue. The thymus might thus influence the origin of lymphatic tissue either by a direct (cellular or humoral) mechanism or indirectly by first suppress-

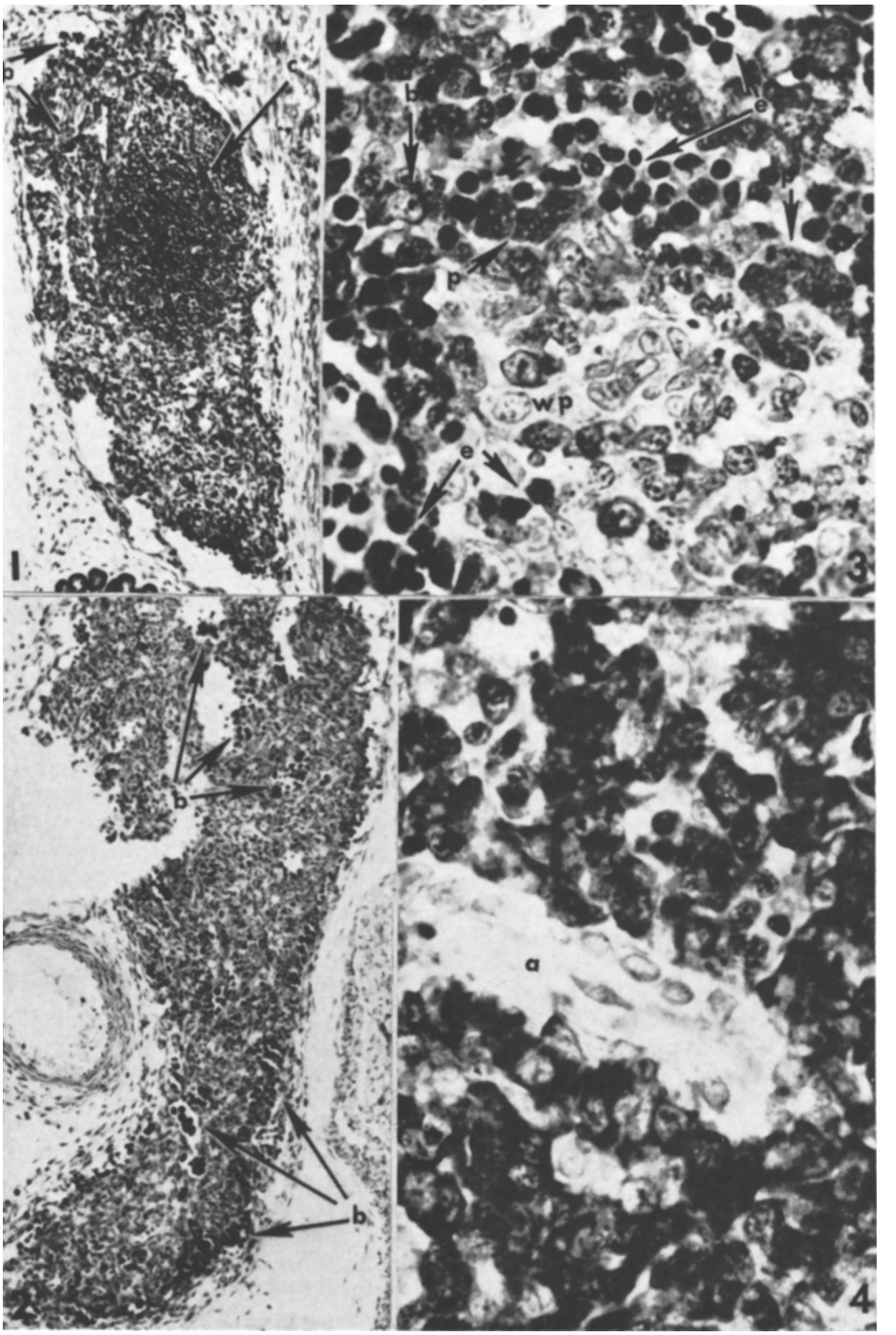


FIG. 1. Pancreatic lymph node of an opossum partially thymectomized at 12 days and sacrificed at 28 days. Cortex (c), densely packed with lymphocytes, is distinct from medulla. There are only a few basophil erythroblasts (b) in the medullary sinuses. Magnification too low to show the rare myelocytes. (×122).

FIG. 2. Pancreatic lymph node of an opossum totally thymectomized at 12 days and sacrificed at 28 days. In contrast to Fig. 1 there is no clearly demarcated cortical tissue. There is a deficiency of small and medium lymphocytes and there are numerous basophil erythroblasts (b). Magnification too low to show the numerous myelocytes clearly. (×122).

FIG. 3. Spleen of an opossum partially thymectomized at 12 days and sacrificed at 28 days. Note basophil (b), polychromatophil (p) and eosinophil erythroblasts (e) in red pulp. Polychromatophil and eosinophil erythroblasts grossly predominate over basophil erythroblasts. White pulp (wp) is clearly present and contains small, medium and large lymphocytes. (×600).

FIG. 4. Spleen of an opossum totally thymectomized at 12 days and sacrificed at 28 days. Note dense, diffuse accumulation of erythroblasts, almost exclusively basophil in type, and lack of white pulp about arteriole (a). (×600).

ing myeloid tissue.

The erythroblastic tissue in the spleen and to a lesser extent in the lymph nodes was not only increased in amount but was abnormal in other respects. There was an increased ratio of immature to mature erythroblasts, a large number of mitoses and of dying cells and the erythroblasts were megaloblastoid in appearance. This suggests a defective type of maturation of erythroblasts and an ineffective erythropoiesis resembling that seen in megaloblastic diseases, di Guglielmo's syndrome and Thalassemia major(17).

The changes described after thymectomy cannot be ascribed to nonspecific factors resulting from surgical trauma since partial thymectomy was not associated with alterations in hematopoietic tissues. Furthermore, the weight gain of thymectomized and partially thymectomized animals was comparable, with the single exception of the animal surviving thymectomy at 6 days of age. It is unlikely, therefore, that the effects observed were the result of vitamin or other nutritional deficiencies.

*Summary.* Opossums were thymectomized when lymphatic tissue had developed in some lymph nodes but not in the spleen. They were sacrificed 2 weeks later. Lymph nodes were grossly deficient in small and medium lymphocytes and lymphatic tissue did not appear in the spleen. Myeloid tissue not only persisted but was found greatly increased in amount at a time when it would have normally decreased. Maturation and proliferation of this tissue was abnormal, being characterized by maturation arrest and ineffective erythropoiesis. These findings indicate that the thymus influences the origin of lymphatic tissue, at least in the spleen, maintenance of lymphatic tissue in lymph nodes, and suppression of myeloid tissue in lymph nodes and spleen.

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