ROLE OF THE ADULT THYMUS IN IMMUNE REACTIONS

I. Observations on Lymphoid Organs, Circulating Lymphocytes and Serum Protein Fractions of Thymectomized or Splenectomized Adult Mice

Henry A. Azar, M.D.; Gerda Naujoks, M.D. and Julia Williams, B.S.

From the Department of Pathology, Columbia University College of Physicians and Surgeons, and the Francis Delafield Hospital, New York, N. Y.

Recent observations have suggested that the thymus of newborn animals, and by inference that of infants, was intimately linked with the development of certain immunologic functions. Thymectomy in adult animals has been associated with either no appreciable [1] or moderate impairment [2] of antibody formation. In contrast to these observations, removal of the bursa of Fabricius,[3] a lymphoid organ of the posterior gut, in newly hatched chickens and thymectomy in newly born rabbits [4] resulted in severe immunologic deficiency in the mature animal. More recent reports have indicated that thymectomy in newborn mice [5] and newborn rats [6–8] was associated with marked impairment of the immune response to standard antigens and skin homografts, as well as severe depletion in the lymphocyte population of the blood and lymphoid tissues. It has been postulated that clones carrying primary immunologic potentialities were developed in the thymus and that the thymus might represent the major primordium of the antibody-forming system.[9] The role of the thymus in the development of immunologic competence may not be limited strictly to the neonatal period since skin homotransplantation across weak histocompatibility barriers was successfully achieved in mice thymectomized at 30 days of age.[10]

The experiment here reported was undertaken to study changes in lymphoid organs, circulating lymphocytes and serum electrophoretic protein changes of thymectomized or splenectomized young adult mice in the nonimmunized state or after repeated subcutaneous administration of human gamma globulin (serum Cohn fraction II) in Freund's adjuvant. The findings indicate that the thymus of adult mice does not play an appreciable role in the formation of plasma cells or its precursors and in gamma globulin synthesis. The thymus of adult mice appears to be, however, an important source of circulating lymphocytes or of a factor stimulating lymphocytosis.

This study was supported by research grant A-5418 of the National Institutes of Health, United States Public Health Service.

Accepted for publication, January 25, 1963.

Material and Methods

A total of 150 three-month old C3H/HeJ mice of both sexes was used (obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.). The animals were fed Rockland pellets and given water *ad libitum*. They were divided into groups of sham-operated controls and those with thymectomy or splenectomy. Approximately half of the mice were given 3 subcutaneous injections of human serum Cohn fraction II (provided by the American Red Cross, Washington, D.C.) in Freund's adjuvant, and the remainder was left untreated.

The operations were performed under a combination of pentobarbital and ether anesthesia. Thymectomy was done through a midline vertical incision in the upper third of the sternum. The thymic lobes were removed with the help of two fine-curved forceps, one of which served as a retractor. Splenectomy was performed through a left upper quadrant abdominal incision. Mock operations consisted of either a laparotomy or an incision in the upper portion of the sternum. Surgical wounds were closed with metallic clips. Over-all mortality was about 16 per cent.

Immunization was begun 2 weeks after the date of operation. Immunized animals received 3 subcutaneous injections, each 0.5 ml., administered at weekly intervals into the flanks. Each dose consisted of 25 mg. lyophilized human serum Cohn fraction II in a mixture of equal parts of saline and Difco complete Freund's adjuvant. All animals were sacrificed under ether anesthesia 8 weeks following operation. The same determinations were carried out in all animals.

Immediately before the animals were sacrificed, blood was obtained from the tail for the following determinations: (a) leukocyte count, using the usual counting technique with a hemocytometer; (b) hematocrit, using 75 mm. by 1.5 mm. heparinized capillary tubes sealed at one end and spun in an International Hematocrit Centrifuge at 11,500 r.p.m. for 2 minutes; (c) blood smears stained with Wright's solution for differential leukocyte count. In addition, immediately after sacrifice of the animals, heart blood was obtained in nonheparinized capillary tubes. The serum was separated by centrifugation and used for the following: (a) total serum protein determination with a Hitashi PRP-B hand protein refractometer; (b) fractionation of serum proteins by paper electrophoresis using barbital (veronal) buffer at pH 8.6. Separation was carried out at room temperature, using a constant current of 2.5 ma. per cell for 16 hours. The strips were stained with bromphenol blue dissolved in methanol. The quantitative analysis of the various protein fractions was accomplished with a Spinco Analytrol Densitometer.

At necropsy, the total body weight of each animal was recorded on a Harvard trip balance (sensitivity 0.1 gm.). The thymus and the spleen, when *in situ*, as well as the largest left axillary lymph node, were weighed after formalin fixation on an analytic balance (sensitivity 0.1 mg.). The left axillary node was chosen arbitrarily because of its convenient location and was considered a satisfactory sample of lymph nodal tissue. All tissues were weighed under the same conditions of fixation, care being taken to remove excessive formalin by blotting.

Formalin-fixed blocks of the thymus, spleen, and left axillary lymph node were sectioned at 5 μ and stained with hematoxylin and eosin. Brachet's modification of the Unna-Pappenheim methyl green-pyronine procedure for ribonucleic acid [11] was used where necessary.

Results

Response of Lymphoid Organs to Ablation of Thymus or Spleen and to Immunization

The mean value and range in the total body weight and weight of the thymus, spleen and largest left axillary lymph node in the various animal groups are shown in Table I. The total body weight as well as the weight

TABLE I

EFFECTS OF THYMECTOMY OR SPLENECTOMY IN YOUNG ADULT NONIMMUNIZED AND IMMUNIZED MICE

Group	No. of animals M	No. of animals F	Total wt. (gm.) Mean & range	Thymus (mg.) Mean & range	Spleen (mg.) Mean & range	Lymph node* (mg.) Mean & range
Control	15	10	24.1 (17–28)	36.6 (18–40)	97.2 (60–149)	5.4 (3–11)
Thymectomy †	16	10	24.9 (22–27.5)		110.1 (76–146)	4.9 (2–10)
Splenectomy †	10	10	23.5 (21–25.5)	32.7 (23–38)		6.7 (3–12)
Control, immunized ‡	8	10	24.1 (20.5–27)	31.4 (27–35)	146.2 (100–213)	12.6 (5–25)
Thymectomy, immunized ‡	10	6	24.5 (20.5–27)		148.7 (106–205)	11.6 (6–25)
Splenectomy, immunized ‡	10	10	24.1 (21–26.5)	33.0 (27–42)		11.1 (5–17)

* Only the largest left axillary lymph node was examined.

† Animals were sacrificed 8 weeks after operation.

‡ Animals were sacrificed 4 weeks after a third subcutaneous injection of human serum Cohn fraction II in Freund's adjuvant.

of the thymus remained fairly constant in all groups. There was a negligible decrease in the mean weight of the thymus in all but nonimmunized sham-operated mice. The mean weight of the spleen in immunized animals was approximately 50 per cent above that in control nonimmunized mice. Thymectomy did not seem to affect the weight of the spleen appreciably. The weight of the largest left axillary lymph nodes was not significantly affected by either thymectomy or splenectomy; however, the mean weight in all immunized animals was about twice that seen in all nonimmunized groups.

Plasma Cell Reaction and Pyroninophilia of Lymphoid Cells in Lymphoid Organs and at the Site of Immunization

Table II shows the degree of pyronine affinity in the cytoplasm of lymphoid cells, including plasma cells, in the thymus, spleen, largest left

TABLE II

PYRONINE AFFINITY OF LYMPHOID CELLS IN LYMPHATIC ORGANS AND AT SITE OF INJECTION OF HUMAN SERUM COHN FRACTION II IN FREUND'S ADJUVANT *

Group	Pyronine affinity of lymphoid cells			
	Thymus	Spleen	Lymph node	Granuloma
Control	±	+	+	
Thymectomy		+	+	
Splenectomy	±		+	
Control, immunized	±	++	++	++
Thymectomy, immunized		++	++	++
Splenectomy, immunized	±		++	++

* Pyronine affinity indicates the cytoplasmic affinity of all lymphoid cells, including plasma cells, as evaluated by means of the methyl-green-pyronine staining procedure: ±, minimal; +, moderate; ++, marked.

axillary lymph node, and in granulomas at the site of immunization in the flanks. Plasma cell reaction and pyroninophilia of lymphoid cells were negligible in the thymus in both nonimmunized and immunized control and splenectomized mice (Fig. 1). A slight degree of plasma cell reaction and pyroninophilia was observed in the spleen and lymph nodes in nonimmunized animals and a moderate degree in immunized groups (Figs. 2 and 3). Plasma cell reaction and pyroninophilia occurred with equal intensity in the Freund's adjuvant granulomas in all 3 immunized groups.

Changes in Circulating Leukocytes and Hematocrit Values

The mean and range of the total number of circulating leukocytes per cu.mm., the number of the various types of leukocytes and the hematocrit values are shown in Table III. Certain distinct trends were noted in spite of the wide range observed in both total leukocyte and differential counts.

TABLE III

TOTAL LEUKOCYTE COUNT, DIFFERENTIAL COUNT AND HEMATOCRIT IN NONIMMUNIZED AND IMMUNIZED MICE

Group	Leukocyte count (cu. mm.) Mean & range	Differential count (cu. mm.) Mean & range				Hematocrit (%) Mean & range
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	
Control	11,396 (7,875–17,125)	3,050 (903–5,985)	8,025 (4,800–10,965)	286 (0–1,197)	35 (0–516)	43.3 (38–50)
Thymectomy	8,631 (4,200–11,750)	3,085 (1,496–5,175)	5,330 (1,890–8,225)	207 (0–560)	9 (0–54)	43.3 (37–50)
Splenectomy	15,643 (10,000–22,500)	4,700 (995–9,930)	10,315 (4,088–17,124)	408 (0–1,452)	220 (0–1,452)	40.4 (34–45)
Control, immunized	13,056 (8,000–17,800)	4,760 (2,520–7,480)	7,770 (4,480–10,295)	526 (0–1,908)	0	41.3 (38–52)
Thymectomy, immunized	9,606 (3,450–16,000)	3,900 (1,001–7,400)	5,400 (1,725–9,120)	306 (0–954)	0	42.2 (38–47)
Splenectomy, immunized	15,333 (8,200–19,000)	5,750 (2,730–7,200)	9,200 (7,770–14,060)	322 (0–680)	61 (0–380)	37.5 (20–44)

TABLE IV

TOTAL SERUM PROTEIN CONCENTRATION AND PERCENTAGE OF SERUM ELECTROPHORETIC PROTEIN FRACTIONS IN NONIMMUNIZED AND IMMUNIZED MICE

Group	Total serum protein (gm. %) Mean & range	Serum protein fractions (%) Mean & range			
		Albumin	Alpha globulin	Beta globulin	Gamma globulin
Control	5.6 (5.3–6.2)	65.0 (58.7–72.4)	7.9 (4.9–10.6)	18.3 (13.6–24.8)	8.8 (5.4–11.2)
Thymectomy	5.1 (4.4–5.9)	68.1 (54.8–80.0)	6.3 (3.3–10.7)	17.8 (8.3–27.6)	7.8 (4.9–12.1)
Splenectomy	5.3 (4.7–5.8)	72.7 (56.5–79.5)	6.4 (3.3–10.5)	14.2 (9.6–21.7)	6.7 (4.1–12.7)
Control, immunized	6.2 (5.8–6.5)	61.9 (44.3–69.1)	8.4 (5.7–16.9)	16.6 (14.7–24.3)	13.1 (7.7–18.1)
Thymectomy, immunized	5.7 (5.2–6.1)	58.4 (55.1–65.0)	10.5 (7.9–13.9)	18.7 (14.7–24.4)	12.4 (9.5–14.6)
Splenectomy, immunized	5.6 (5.2–6.1)	61.0 (50.0–71.0)	8.6 (4.8–12.5)	19.0 (14.9–26.6)	11.3 (5.7–14.3)

The mean number of total leukocytes in nonimmunized thymectomized mice was approximately one third lower than that in the control group. This low level of circulating leukocytes was entirely accounted for by a selectively low number of lymphocytes. No other significant differences were noted in other types of leukocytes. Similar low values of circulating lymphocytes were observed in immunized thymectomized animals. Splenectomized animals tended to have a higher mean number of leukocytes with an increase in the number of all types of cells. No attempt was made in this experiment to differentiate between small, medium and large lymphocytes.

Hematocrit values appeared to be essentially similar in all but the splenectomized groups, which showed a significantly lower percentage of packed red cells. The lowest hematocrit values were noted in splenectomized immunized animals.

Changes in Serum Protein Fractions

The total serum protein level and serum protein electrophoretic fractions in the various animal groups are shown in Table IV. Both of the groups with thymectomy and splenectomy tended to show slightly lower total serum protein levels as well as lower gamma globulin concentrations than control groups. However, thymectomized and splenectomized mice as well as sham-operated controls showed an equally marked rise in serum gamma globulin concentration following immunization.

Discussion

The findings here described lead to the impression that the thymus of adult C_3H mice does not play an essential role in the differentiation or proliferation of immunologically competent cells of the plasma cell and pre-plasma cell varieties. A corollary of the above impression is that the adult thymus does not seem to participate in gamma globulin synthesis. It is clear from this and other studies [12–14] that the thymus, unlike the spleen and lymph nodes, fails to produce pyroninophilic lymphoid cells in response to parenteral administration of antigens. Ablation of the adult thymus, unlike thymectomy in the immediate postnatal period, does not seem to alter the immunologic response of the host appreciably. It is interesting to note also that ablation of the spleen, a site of active plasma cell and pre-plasma cell proliferation, did not likewise affect appreciably the serum gamma globulin concentration in both immunized and nonimmunized animals in this experiment. This finding suggests that there is a considerable reservoir of immunologically competent cells outside the spleen. For example, the mean weight of the left axillary lymph nodes in immunized animals was about twice that of nonimmunized mice, whereas

the spleen showed an increase in weight of only 50 per cent above that of nonimmunized mice.

The concept of a widely scattered reservoir of immunologically competent cells in the adult, as opposed to the finding that thymectomy in the newborn was followed by a marked impairment of immunologic functions, was further borne out in another report.[15] When adult mice underwent thymectomy together with splenectomy and were then subjected to repeated intraperitoneal injections of horse serum, their ability to form gamma globulin was markedly reduced but still maintained. A similar impairment was not observed after either thymectomy or splenectomy alone.

The mode of participation of the thymus in immune reactions is still obscure. In the newborn, it has been postulated that thymic lymphocytes are seeded in the spleen and other lymphoid tissues where they develop into immunologically competent cells.[5,9] Thymic lymphocytes do not seem to mature into plasma cells or their precursors within the thymus itself. Marshall and White[16] have postulated that the normal rat and guinea pig thymus possesses a barrier against the entry of antigens. They have shown that direct injection of antigens into the thymus of guinea pigs induces germinal center, plasma cell and antibody formation. These findings are, however, not unique to the thymus since introduction of antigen in any tissue is followed by essentially the same immunologic responses. On the other hand, O'Gara and Ards[17] demonstrated that thymic grafts taken from neonatal C57B1 mice grew equally well in the spleens of young adult thymectomized and intact animals of the same strain. The transplanted thymocytes could be distinguished morphologically from the lymphocytes of the spleen by their greater hyperchromasia. These observations favored the theory that there might be several breeds of lymphocytes, one of which was of thymic origin.

The thymus of rodents is considered to be an important source of circulating lymphocytes although more direct observations are needed to prove this point.[18,19] In another experiment,[20] it was shown that irradiation of the thymic region in adult C3H mice with 3,000 r tissue dose resulted in a selective drop in the number of circulating lymphocytes. The lymphopenia persisted for about 1 week, after which the number of circulating lymphocytes returned to pre-irradiation levels. Lymphopenia was associated with destruction of the thymic cortex which is rich in lymphocytes, whereas restitution of the normal level of circulating lymphocytes was accompanied by repopulation of the thymic cortex. These observations, however, neither negated nor confirmed the existence of Metcalf's lymphocytosis-stimulating factor.[21]

Lymphocytes are believed to play an important, if not exclusive role

in cell-borne immunologic reactions such as the homograft rejection and the tuberculin reactions.[22-24] According to Burnet's clonal selection theory,[25] lymphocytes play a central role as the carrier of genetic information determining antibody specificity. It is a common observation that lymphocytes participate in a great variety of inflammatory reactions in which they are frequently seen with plasma cells and other leukocytes. It is possible that lymphocytes carry to the site of tissue injury the genetic apparatus responsible for the identification of an offending antigen and for the mediation of immunologic specificity. Whereas plasma cells and their precursors are the sites of "crude" gamma globulin synthesis,[26,27] the further elaboration of specific antibodies may depend on the genetic information or "memory" conveyed by lymphocytes. It is interesting to note that although thymectomy in the neonatal period results in depletion of circulating lymphocytes and marked immunologic deficiency, the serum gamma globulin concentration and plasma cell reaction in these animals are not appreciably different from those of sham-operated animals.[6,8,28,29]

Summary

Young adult C3H mice, thymectomized or splenectomized at 3 months of age, were observed in the nonimmunized state and after repeated subcutaneous administration of human gamma globulin in Freund's adjuvant.

Thymectomy at the age of 3 months resulted in a decrease of approximately one third in the total number of circulating lymphocytes. Other leukocytes and hematocrit values were not affected. Mice thymectomized or splenectomized at 3 months showed slightly lower levels of serum gamma globulin than control animals. However, thymectomized or splenectomized mice responded to antigen administration with a rise of serum gamma globulin concentration comparable to that observed in control immunized animals. Thymectomy did not affect significantly the intensity of plasma cell reaction in the spleen, lymph nodes and Freund's adjuvant granulomas in immunized animals.

The thymus in young adult mice appears to be an important source of circulating lymphocytes or of a factor stimulating lymphocytosis. The thymus does not, however, seem to play any appreciable role in the formation of plasma cells or in gamma globulin synthesis.

References

1. MacLean, L. D.; Zak, S. J.; Varco, R. L., and Good, R. A. The role of the thymus and spleen in antibody production: an experimental study of the immune response in thymectomized rabbits. *Transplant. Bull.*, 1957, 4, 21–22.

2. FICHTELIUS, K. E.; LAURELL, G., and PHILIPSSON, L. The influence of thymectomy on antibody formation. *Acta path. et microbiol. scandinav.*, 1961, **51**, 81–86.

3. GLICK, B.; CHANG, T. S., and JAAP, R. G. The bursa of Fabricius and antibody production. *Poultry Sc.*, 1956, **35**, 224–225.

4. ARCHER, O., and PIERCE, J. C. Role of thymus in development of the immune response. (Abstract) *Fed. Proc.*, 1961, **20**, 26.

5. MILLER, J. F. A. P. Effect of neonatal thymectomy on the immunological responsiveness of the mouse. *Proc. Roy. Soc. London, s.B*, 1962, **156**, 415–428.

6. JANKOVIC, B. D.; WAKSMAN, B. H., and ARNASON, B. G. Role of the thymus in immune reactions in rats. I. The immunologic response to bovine serum albumin (antibody formation, Arthus reactivity, and delayed hypersensitivity) in rats thymectomized or splenectomized at various times after birth. *J. Exper. Med.*, 1962, **116**, 159–176.

7. ARNASON, B. G.; JANKOVIC, B. D.; WAKSMAN, B. H., and WENNERSTEN, C. Role of the thymus in immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocyte. *J. Exper. Med.*, 1962, **116**, 177–186.

8. WAKSMAN, B. H.; ARNASON, B. G., and JANKOVIC, B. D. Role of the thymus in immune reactions in rats. III. Changes in the lymphoid organs of thymectomized rats. *J. Exper. Med.*, 1962, **116**, 187–206.

9. BURNET, F. M. The immunological significance of the thymus: an extension of the clonal selection theory of immunity. *Australasian Ann. Med.*, 1962, **11**, 79–91.

10. GOOD, R. A.; DALMASSO, A. P.; MARTINEZ, C.; ARCHER, O. K.; PIERCE, J. C., and PAPERMASTER, B. W. The role of the thymus in development of immunologic capacity in rabbits and mice. *J. Exper.Med.*, 1962, **116**, 773–796.

11. PEARSE, A. G. E. Histochemistry, Theoretical and Applied. Little, Brown & Co., Boston, 1960, ed. 2, 998 pp.

12. BJØRNEBOE, M.; GORMSEN, H., and LUNDQUIST, F. Further experimental studies on the role of plasma cells as antibody producers. *J. Immunol.*, 1947, **55**, 121–129.

13. FAGRAEUS, A. Antibody production in relation to the development of plasma cells; *in vivo* and *in vitro* experiments. *Acta. med. scandinav.*, 1948, **130**, Suppl. 204, 1–122.

14. AZAR, H. A. Pyronin affinity of the cytoplasm of lymphoid cells and thymocytes. An experimental study in rabbits undergoing immunization. *Arch. Path.*, 1960, **70**, 29–34.

15. AZAR, H. A., and WILLIAMS, J. Role of the adult thymus in immune reactions. II. Effect of combined thymectomy and splenectomy on serum gamma globulin and precipitin antibodies in adult mice immunized with horse serum. (To be published)

16. MARSHALL, A. H., and WHITE, R. G. The immunological reactivity of the thymus. *Brit. J. Exper. Path.*, 1961, **42**, 379–385.

17. O'GARA, R. W., and ARDS, J. Intrasplenic transplantation of neonatal thymus. *J. Nat. Cancer Inst.*, 1961, **27**, 277–297.

18. KINDRED, J. E. Quantitative studies on lymphoid tissues. *Ann. New York Acad. Sc.*, 1955, **59**, 746–754.

19. LEBLOND, C. P., and SAINTE-MARIE, G. Models for Lymphocyte and Plasmocyte Formation. In: Haemopoiesis; Cell Production and Its Regulation. Ciba Foundation Symposium. WOLSTENHOLME, G. E. W., and O'CONNOR, M. (eds.). Little, Brown & Co., Boston, 1960, p. 152.

20. GHOSSEIN, N. A.; AZAR, H. A., and WILLIAMS, J. Local irradiation of the thymus. Histologic changes with observations on circulating lymphocytes and serum protein fractions in adult mice. *Am. J. Path.*, 1963. (To be published)

21. METCALF, D. The thymic lymphocytosis-stimulating factor. *Ann. New York Acad. Sc.*, 1958, **73**, 113–119.

22. MEDAWAR, P. B. The behaviour and fate of skin autografts and skin homografts in rabbits. (A report to the War Wounds Committee of the Medical Research Council.) *J. Anat.*, 1944, **78**, 176–199.

23. TOOLAN, H. W., and KIDD, J. G. Association of lymphoid elements with cancer cells undergoing distinctive necrobiosis in resistant and immune hosts. (Abstract) *Fed. Proc.*, 1949, **8**, 373.

24. CHASE, M. W. The cellular transfer of cutaneous hypersensitivity to tuberculin. *Proc. Soc. Exper. Biol. & Med.*, 1945, **59**, 134–135.

25. BURNET, F. M. The Clonal Selection Theory of Acquired Immunity. Vanderbilt University Press, Nashville, Tenn., 1959, 209 pp.

26. COONS, A. H.; LEDUC, A. H., and CONNOLLY, J. M. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exper. Med.*, 1955, **102**, 49–60.

27. ORTEGA, L. G., and MELLORS, R. C. Cellular sites of formation of gamma globulin. *J. Exper. Med.*, 1957, **106**, 627–640.

28. PARROTT, D. M. V., and EAST, J. Studies on fatal wasting syndrome in mice thymectomized at birth. (In press)

29. AZAR, H. A.; SNYDER, R. W., and WILLIAMS, J. Dissociation between serum gamma globulin and precipitin antibody in rats thymectomized at birth. (Abstract) *Fed. Proc.*, 1963, **22**, 600.

[*Illustrations follow*]

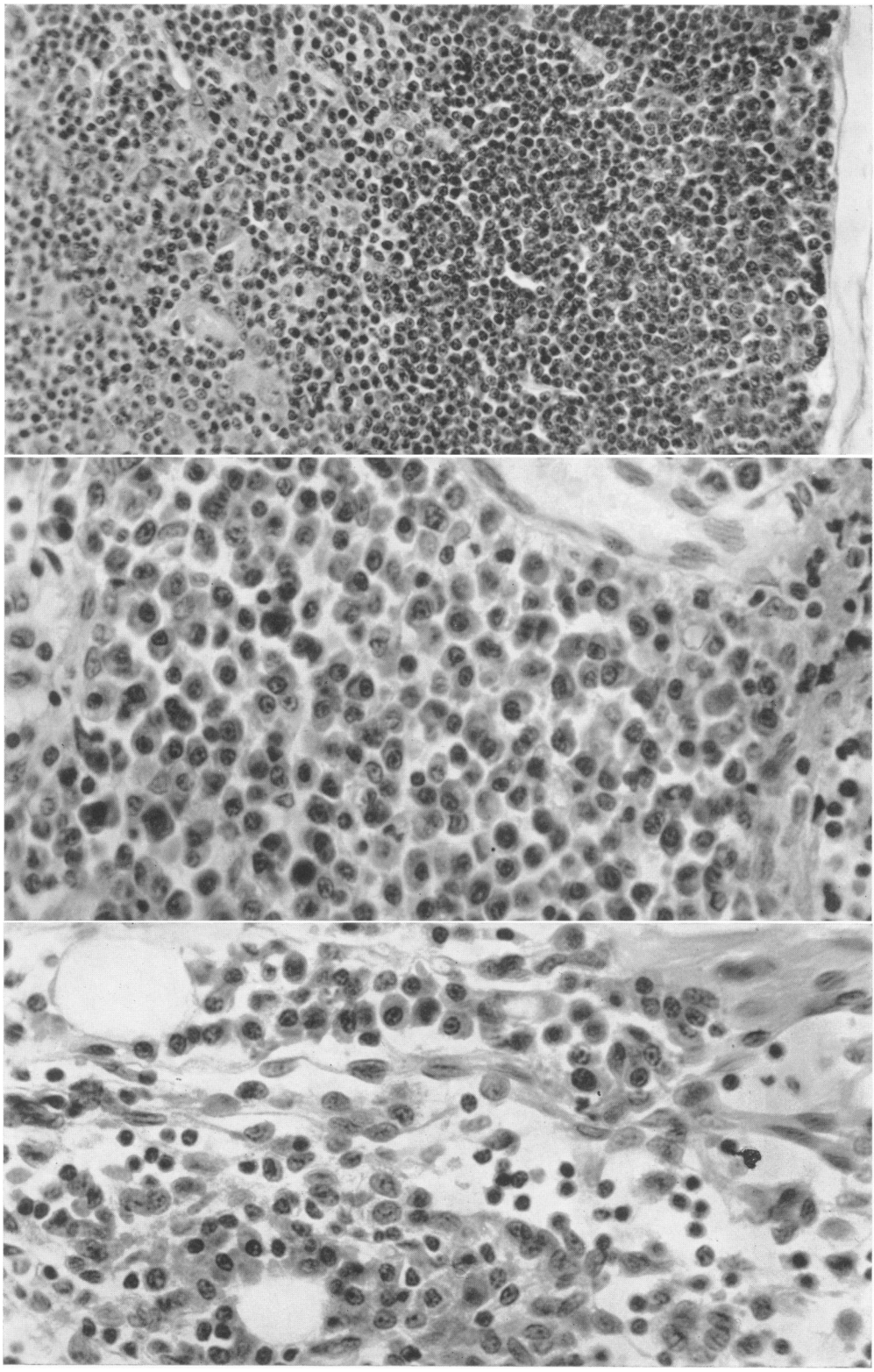
LEGENDS FOR FIGURES

Photomicrographs are of sections stained with hematoxylin and eosin.

FIG 1. Thymic cortex and medulla in an intact young adult C_3H mouse after repeated subcutaneous injections with human gamma globulin in Freund's adjuvant. The thymic lymphocytes exhibit relative uniformity, and plasma cells and pre-plasma cells are absent from both cortex and medulla. × 425.

FIG. 2. Red pulp in the spleen of a mouse thymectomized at 3 months of age and then immunized with human gamma globulin in Freund's adjuvant. A preponderance of typical plasma cells and pre-plasma cells is manifest. × 730.

FIG. 3. Medullary cords in an axillary lymph node of a mouse thymectomized at 3 months and then immunized with human gamma globulin in Freund's adjuvant. Plasma cells and pre-plasma cells are evident. The plasma cell reaction in the lymph nodes and spleen of thymectomized animals appears to be comparable to that of control or splenectomized mice. × 730.



1

2

3