THE LYMPHOID TISSUES AND IMMUNE RESPONSES OF NEONATALLY THYMECTOMIZED MICE BEARING THYMUS TISSUE IN MILLIPORE DIFFUSION CHAMBERS*

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Plates 8 to 15

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It is now well established that mice thymectomized at birth have a marked diminution of lymphocytes in their blood and tissues and severe defects in immune responses (1). The reactivity of such mice can be restored to normal either by injecting adult lymphoid cells or by grafting thymus tissue. These results have led to the notion that an important role of the thymus is to establish a functioning immune mechanism. Two alternative, but not necessarily mutually exclusive, hypotheses have been proposed to explain the mechanism by which the thymus influences the maturation of immunological faculty. The thymus would act either by providing a highly specialized environment for the differentiation of lymphoid cells which would colonize the peripheral lymphoid tissues where they could function as immunologically competent cells or by elaborating a specific humoral factor which would enable lymphoid cells preexisting in the peripheral lymphoid organs to participate in immune reactions. There is no direct unequivocal evidence to support either theory although indirect suggestive evidence can be cited in support of both.

In support of the cell colonization theory, it has been claimed that cell production in the thymus exceeds the requirement for normal growth and that the rate of cell death is not higher than 15 per cent of cell production. This has been taken to imply that some cells must leave the thymus (2–4). Experiments in which thymus cells were labeled *in situ* with tritiated thymidine showed that suprisingly small numbers of labeled cells seeded out of the organ (5).

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Auerbach (6, 7), using tissue culture techniques, found that when 12-day-old embryonic thymus was separated into its epithelial and mesenchymal components by trypsin, the epithelial component gave rise to lymphocytes which reached peak differentiation at about 12 to 14 days and thereafter gradually changed to non-lymphoid cell types. A suspension of 14-day-old embryonic spleen cells did not give rise to any lymphocytes or form follicles *in vitro* under identical conditions. If, however, 12-day-old embryonic thymus cells were added to the suspension of spleen cells, lymphoid follicles were formed. The results suggested that "spleen differentiation is not autonomous but depends upon acquisition of cells of extrinsic origin" (7).

Fichtelius injected P^{32}-labeled thymus cells into the circulation and traced them to the spleen (8). He suggested that the spleen may be an important destination for thymus lymphocytes. Ford and Micklem (9), utilizing a chromosome marker technique, found that marrow cells injected into an irradiated adult host proliferated in both thymus and lymph nodes whereas injected thymus and lymph node cells proliferated only in the lymph nodes. Whereas myeloid as well as lymphoid elements were seen in the lymph nodes, only the normal thymus elements, *i.e.* lymphocytes, were found in the host thymus. This was taken as evidence that the numerous marrow derived cells of the thymus were differentiating into lymphocytes. Since reformation of lymphoid elements occurred sooner in the thymus than in the lymph nodes it was suggested that "the thymus continues to seed the other lymphoid tissues."

Studies of subcutaneous thymus grafts have revealed an initial stage of degeneration of lymphoid elements in the graft followed by a stage of lymphoid regeneration (10). By means of a cytological technique, it was shown that the great majority of cells dividing in the graft 2 or more weeks after grafting were derived from the host, not from the thymus donor (11, 12). This was also the case in hosts that had been thymectomized at birth (13). Furthermore, examination of the peripheral lymphoid organs of such mice showed them to be populated almost completely with cells having the cytogenetic characteristics of the host (13–15). The cells populating these lymphoid tissues could have been derived either from host cells which differentiated in the thymus graft, or from lymphoid precursor cells which differentiated in the lymphoid tissues as a result of a humoral factor elaborated by the thymus graft.

The evidence for a humoral thymus factor is only suggestive. Implantation of thymus tissue, depleted of lymphocytes by irradiation and therefore consisting mainly of the radio-resistant epithelial-reticular complex, has increased mitosis in neighboring lymph nodes (16). Injections of thymus extracts into various species of rodents have produced a temporary lymphocytosis (17–19). Metcalf (19) isolated a heat-labile, filterable, non-dialyzable lymphocytosis-stimulating factor (LSF) from the thymus. Injection of this extract into newborn mice produced a temporary increase in lymphocyte levels in the peripheral blood which reached its peak at 3 to 7 days. Other workers have found it difficult to confirm this work (20). Whether LSF acts directly on peripheral lymphoid tissues or whether it stimulates the thymus to release cells is not known. Repeated injections of saline extracts of neonatal thymus tissue failed however to equip neonatally thymectomized mice with normal lymphocyte populations and immune mechanisms (15).

The present experiments were designed to test the possibility that a humoral

factor from the thymus may play a part in the development of the immune system. Preliminary reports of this work have been published elsewhere (13, 21).

Materials and Methods

Mice.—Mice of the highly inbred strain, CBA, and F_1 hybrids between CBA and T6 were used in the experiments. After weaning, at 4 weeks of age, they were fed ordinary laboratory diet and given water *ad libitum*. All mice, both experimental and control, were weighed 2 to 3 times weekly on a standard animal scale.

Thymectomy.—Thymectomy or sham operation was performed on mice less than 24 hours old by a technique similar to that used in this laboratory for adult mice (22). The immediate operative mortality was less than 5 per cent but the mortality from neglect or cannibalism was considerably higher in some litters. After the operation, the mice were returned to their mothers or to a foster mother of the same strain and left undisturbed until required for further operative procedures.

Diffusion Chambers.—Millipore[1] filter membranes with a rated pore size of 0.3 μ were cut into squares of average size 0.7 cm^2. The chambers were constructed by approximating two squares together and sealing the edges on three sides by dipping them into a thin film of acetone. The assembled chambers were sterilized in a dry oven at 60°C for 24 hours and stored in sterile Petri dishes. Each chamber was filled with either one lobe of 1-day-old thymus or one to three lobes of 14-day-old embryonic thymus. The thymuses were dissected out aseptically from mice of the CBA strain and placed in sterile Ringer phosphate solution. A dissecting microscope was used to remove the thymic lobes from embryonic mice. After filling the chambers, the opening was sealed by approximating the two sides and dipping the edges into a film of acetone. The filled chambers were implanted, within 5 to 10 minutes after filling, into the peritoneal cavities of 7-day-old mice. Empty chambers were implanted into controls. The operation was performed under light ether anesthesia and the abdominal incision was closed with 1 or 2 black silk sutures.

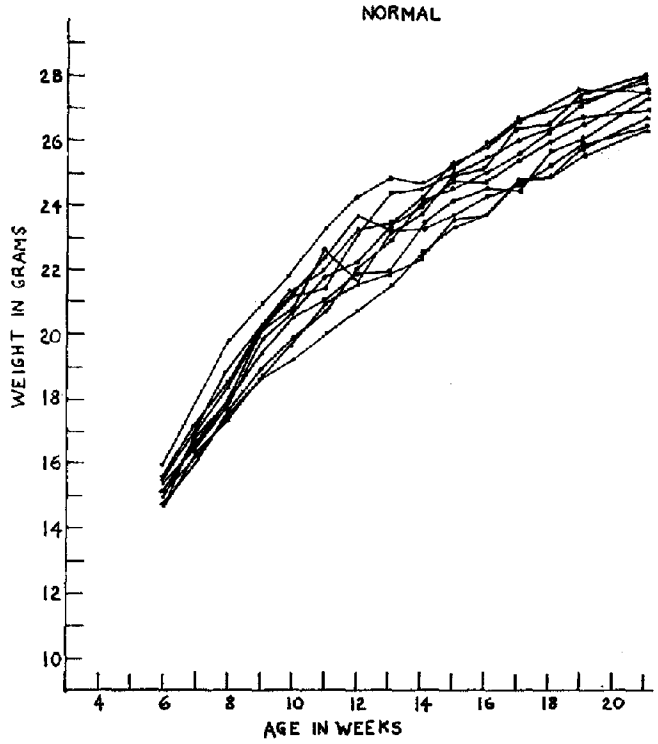
Hematology.—Absolute and differential white cell counts were performed on tail vein blood of 6- to 12-week-old experimental and control mice. Blood smears for differential counts were stained with new rapid Giemsa (George T. Gurr, Ltd., London).

Immunization.—Sheep erythrocytes (Wellcome Reasearch Laboratories, Beckenham, England) from whole sheep blood in Alsever's solution were washed three times in 0.9 per cent sodium chloride and made up to a 20 per cent suspension for injection. Mice were injected intraperitoneally with 0.2 ml of this suspension when they were about 10 weeks old.

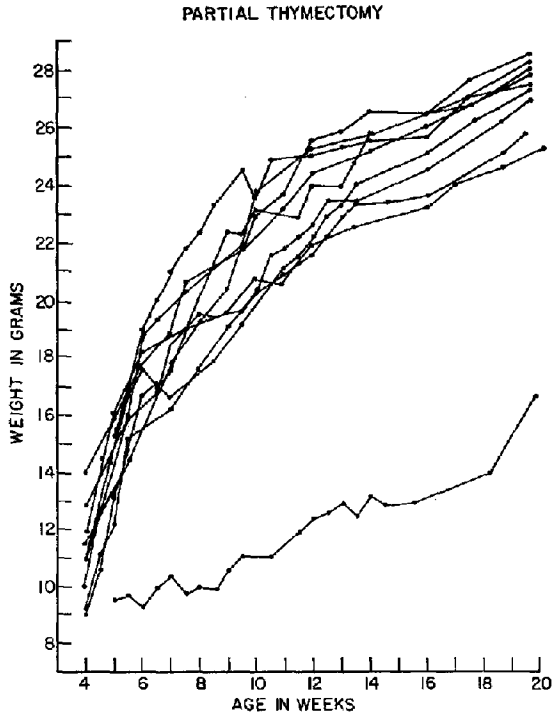
Antibody Titrations.—Blood was obtained from the retroorbital sinus of mice 10 days after injection of sheep erythrocytes. It was allowed to stand in the refrigerator for 1 hour before the serum was separated by centrifugation. Hemagglutinin titers were performed in plastic agglutination trays. Serial twofold dilutions of serum were made in 0.9 per cent sodium chloride beginning at a dilution of 1 in 2. Sheep erythrocytes were washed three times in 0.9 per cent sodium chloride and resuspended so as to make a 2 per cent suspension. One volume of sheep erythrocytes was added to one volume of diluted serum and the suspension was mixed and left at room temperature for 2 hours. The cells were examined microscopically for agglutination and the end-point was the last dilution of serum which showed this agglutination. It was expressed as $\log_2$ of the reciprocal of the dilution.

Skin Grafting.—Full thickness skin grafts were performed by the method of Billingham and Medawar (23). The mice were first skin grafted at 5 weeks of age and the graft was first examined at 7 days. Its survival was estimated from the gross appearance. Second set skin grafts were performed on some of the mice 3 to 4 weeks after the first graft.

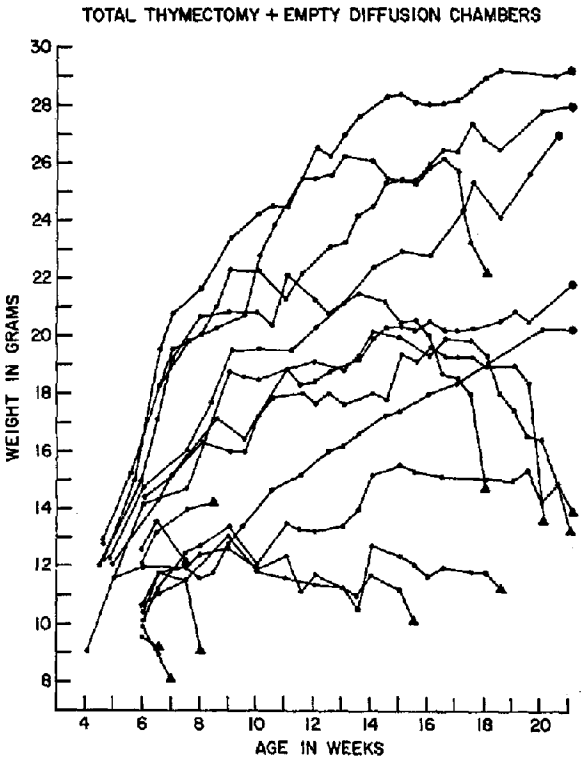
[1] Millipore Filter Corp., Bedford, Massachusetts.



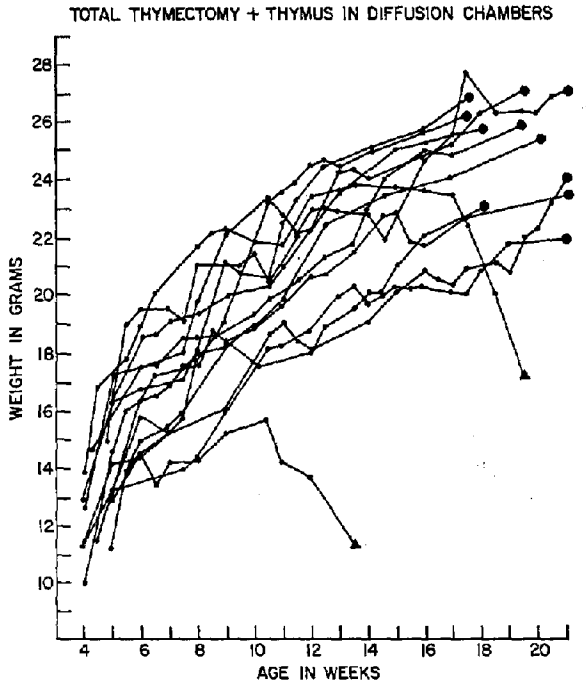
TEXT-FIG. 1. Body weight curves of normal, untreated, (CBAXT6)F_1 mice.



TEXT-FIG. 2. Body weight curves of (CBAXT6)F_1 mice partially thymectomized at birth.



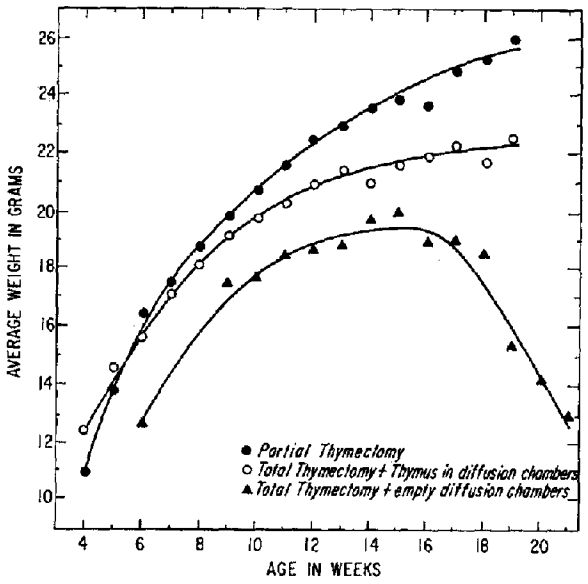
TEXT-FIG. 3. Body weight curves of (CBAXT6)F_1 mice totally thymectomized at birth and implanted intraperitoneally at 7 days with empty Millipore diffusion chambers. ▲, mice dying before 21 weeks; ●, mice surviving beyond 21 weeks.



TEXT-FIG. 4. Body weight curves of (CBAXT6)F_1 mice totally thymectomized at birth and implanted intraperitoneally at 7 days with CBA thymus tissue enclosed in Millipore diffusion chambers. ▲, mice dying before 21 weeks; ●, mice surviving beyond 21 weeks.

Histology.—Mice used for histological studies were killed at about 10 weeks of age and their lymphoid system was examined macroscopically and microscopically. The absence of mediastinal thymic tissue in all thymectomized mice was verified both in the gross and by microscopic sectioning. The thymus area, spleen, lymph nodes, Peyer's patches, liver, and diffusion chamber were fixed in Bouin's fluid. They were cut and stained as routine with hematoxylin and eosin and the tissue in the diffusion chamber was also stained with the periodic acid–Schiff reagent.

Cytology.—Mice used for cytological purposes were killed by cervical dislocation 60 to 90



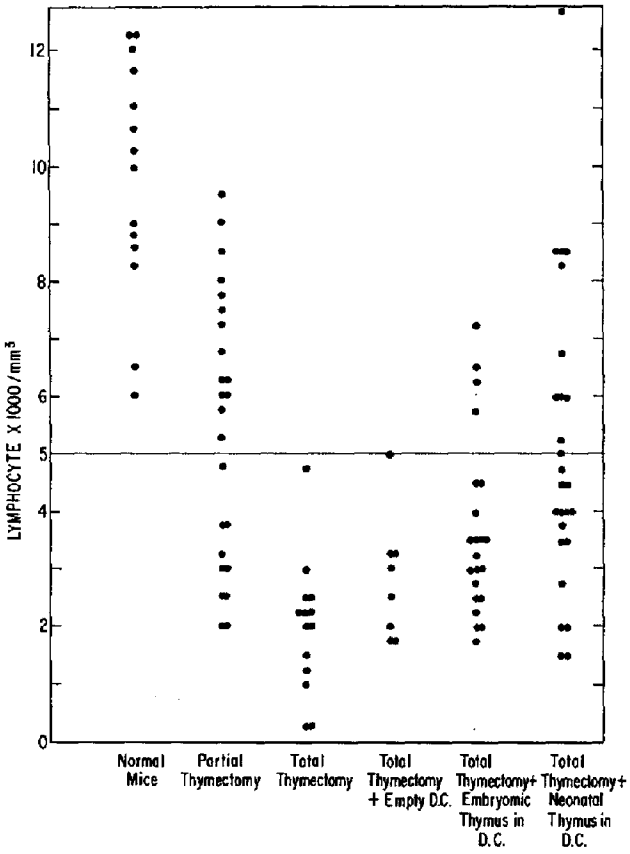
TEXT-FIG. 5. Mean values for body weights of partially thymectomized (CBAXT6)F_1 mice, totally thymectomized (CBAXT6)F_1 mice with CBA thymus in diffusion chambers, and totally thymectomized (CBAXT6)F_1 mice with empty diffusion chambers.

minutes after receiving an intraperitoneal injection of 0.2 ml of 0.02 per cent colcemid (Ciba Pharmaceutical Products, Inc., Summit, New Jersey) per 10 gm body weight. Cell suspensions were prepared from lymph nodes and, when possible, from the tissue in the chamber, incubated in 0.9 per cent sodium citrate for 20 minutes at 37°C, fixed in chilled 3:1 alcohol acetic acid mixture for 1 to 2 hours and resuspended in 60 per cent acetic acid for examination. The cells were spread by an air-drying technique (24).

RESULTS

Weight Curves.—Text-figs. 1 to 5 illustrate body weight curves of representative normal mice, partially thymectomized mice, totally thymectomized mice, and totally thymectomized mice bearing thymus tissue enclosed in Millipore diffusion chambers. It can be seen that 9 to 10 partially thymectomized mice (Text-fig. 2) gained weight steadily and at the same rate as normal controls (Text-fig. 1). Mice subjected to complete thymectomy (Text-fig. 3) began to

loose weight at periods ranging from 6 to 14 weeks. This loss of weight was accompanied by ruffling of the fur, hunching, lethargy (Fig. 1), diarrhea, and eventually death. Five of the mice died at about 8 weeks of age and a further 7 died by 21 weeks; the remaining 5 mice survived beyond 21 weeks. The mice



TEXT-FIG. 6. Absolute numbers of lymphocytes in the peripheral blood of the various groups of (CBAXT6)F_1 mice aged between 6 and 12 weeks. D.C., diffusion chamber.

subjected to total thymectomy at birth and implantation of thymus tissue in diffusion chambers gained weight steadily at a slightly lower rate than normal or partially thymectomized mice. Only 2 died of wasting disease, the remaining 11 surviving beyond 21 weeks (Text-fig. 4) and appearing in good health (Fig. 1). Weight curves of all operated mice are summarized in Text-fig. 5.

Lymphocyte Counts.—The absolute lymphocyte counts in the peripheral blood of 6- to 12-week-old control and experimental mice are shown in Text-fig. 6. All normal mice had counts above 5000 per mm^3. Ten of 24 partially thymectomized

mice had counts below 5000. All mice subjected to total thymectomy at birth had counts below 5000. Implantation of empty diffusion chambers in these mice had no effect on their blood lymphocyte levels. Most totally thymectomized mice bearing embryonic thymus in diffusion chambers had counts below 5000, but 11 of 26 mice bearing neonatal thymus in chambers had counts above 5000.

Hemagglutinin Production.—The immune response of the various groups of mice to a challenge of sheep erythrocytes is shown in Table I. Unchallenged normal mice did not produce any detectable titers of 7S hemagglutinins. Three of 10 challenged mice, which had been subjected to total thymectomy at birth and implantation of empty chambers, had no detectable levels of hemag-

TABLE I

Hemagglutinin Production by (CBAXT6)F_1 Mice Thymectomized at Birth and Bearing Thymus Tissue in Millipore Diffusion Chambers

Treatment at birth	Diffusion chamber	Challenge	No. of mice in group	No. of mice showing following $\log_2$ titer of hemagglutinins								
				0	1	2	3	4	5	6	7	8
None	None	None	10	10								
None	None	Sheep erythrocytes	10							1	2	7
Total thymectomy	Empty	Sheep erythrocytes	10	3			1	2	2	1		1
Total thymectomy	Neonatal thymus	Sheep erythrocytes	10						1		2	7

glutinins and a further 5 had titers below normal levels. Implantation of neonatal thymus in diffusion chambers enabled 9 of 10 challenged thymectomized mice to produce normal hemagglutinin titers.

Skin Homograft Immunity.—Previous work had shown that mice thymectomized at birth failed to reject skin homografts not only from donors which differed from the host at the strong histocompatibility locus, H-2, but also from rat donors (14). In the present experiments, it was of interest to determine whether thymus tissue enclosed in Millipore diffusion chambers could enable neonatally thymectomized mice to reject allogeneic skin grafts when the immunogenetic differences between donor and host were only slight. Neonatally thymectomized (CBAXT6)F_1 (agouti, H-2k) mice were challenged with Ak (albino, H-2k) skin homografts. These strains differ at histocompatibility loci other than H-2 and thus have only weak immunogenetic differences. The results are presented in Tables II and III. Most mice subjected to partial thymectomy at birth could reject first set skin homografts as intact, non-thymectomized, mice. In contrast, none of the 14 totally thymectomized mice with

or without empty diffusion chambers could reject Ak skin within 25 days and only one rejected the skin by 50 days. The majority of totally thymectomized mice (27 out of 37) bearing either neonatal or embryonic thymus in chambers rejected the skin grafts within 25 days and a further 4 rejected them by 50 days

TABLE II

Survival of First Set Ak Skin Homografts on (CBAXT6)F_1 Mice Thymectomized at Birth and Bearing Thymus Tissue in Millipore Diffusion Chambers

Treatment	No. of mice in group	No. of mice with skin grafts surviving for		
		< 25 days	25 to 50 days	> 50 days
None	11	11	0	0
Partial thymectomy at birth	17	15	2	0
Total thymectomy at birth	6	0	0	6
Total thymectomy at birth + empty diffusion chambers	8	0	1	7
Total thymectomy at birth + neonatal CBA thymus in diffusion chambers	23	18	3	2
Total thymectomy at birth + 14-day embryonic CBA thymus in diffusion chambers	14	9	1	4

TABLE III

Survival of Second Set Ak Skin Homografts on (CBAXT6)F_1 Mice Thymectomized at Birth and Bearing Thymus Tissue in Millipore Diffusion Chambers

Treatment	No. of mice in group	No. of mice with skin grafts surviving for:		
		< 10 days	10 to 25 days	> 25 days
First set skin graft only	10	10	0	0
Partial thymectomy at birth, first set skin graft	9	6	3	0
Total thymectomy at birth + empty diffusion chambers, first set skin graft	4	0	0	4
Total thymectomy at birth + 14-day embryonic CBA thymus in diffusion chambers, first set skin graft	8	6	2	0

(Table II). All the grafts in this group were infiltrated with lymphocytes. The response of some of the mice to a second set graft of the same skin is shown in Table III. Six of 9 partially thymectomized mice showed evidence of immunity to second set skin grafts but none of the totally thymectomized mice bearing empty chambers rejected the second skin graft. Six of 8 thymectomized mice

with embryonic thymus in the chambers showed evidence of a second set response.

Histological and Cytological Findings.—

Host tissues: The microscopic appearance of lymph nodes, spleen, Peyer's patches, and liver of the mice in the various groups is shown in Figs. 2 to 20.

The lymph nodes of neonatally thymectomized mice uniformly showed absence of the normal follicular structure, absence of germinal centers, and conspicuous deficiency of small lymphocytes (Fig. 3). Spaces normally occupied by lymphoid follicles showed reticuloendothelial cells, histiocytes, macrophages, and only few scattered lymphocytes most of which were immature (Fig. 4). A few mature plasma cells were seen in the medullary cords.

TABLE IV

Extent of Population of Lymphoid Tissues of Neonatally Thymectomized Mice Implanted with Thymus Tissue in Millipore Diffusion Chambers

Treatment	Tissue examined	No. of mice examined	No. of mice showing following appearance of lymphocytic fields:		
			Normal	Moderately depleted*	Markedly depleted‡
Thymectomy at birth; embryonic thymus in diffusion chambers	Lymph nodes§	10	2	5	3
	Spleen	11	3	7	1
	Peyer's patch	7	3	3	1
Thymectomy at birth; neonatal thymus in diffusion chambers	Lymph nodes§	13	5	6	2
	Spleen	7	0	5	2
	Peyer's patch	5	2	2	1

* Lymphocytic fields were classed as moderately depleted when they showed about half of the average normal number of small lymphocytes per high power field (× 500).

‡ Lymphocytic fields were classed as markedly depleted when they showed about a quarter of the average normal number of small lymphocytes per high power field (× 500).

§ One axillary and one inguinal lymph node were examined for each mouse.

After implantation of thymus tissue in diffusion chambers, the lymph nodes from 16 of 23 mice examined still showed some deficiency of small lymphocytes in the cortex (Table IV). Lymph nodes from the other 7 mice showed in the cortex a normal follicular structure with islands rich in small lymphocytes and, often, numerous germinal centers (Figs. 5 to 8). The medullary cords were usually rich in plasma cells. All the lymph nodes examined cytologically in implanted mice had dividing cells bearing the T6 marker chromosome. These cells could not, therefore, have been direct descendants of cells from the thymus tissue enclosed in the chambers.

The spleens of mice subjected to neonatal thymectomy alone showed ill-defined, inactive follicles, no germinal centers, and a marked deficiency of small lymphocytes (Fig. 10). Evidence of lymphocyte deficiency was still present in some thymectomized mice implanted with chambers containing thymus tissue (Table IV, Fig. 11). In others, the normal follicular structure and lymphocyte complement was present (Figs. 12 to 13).

TABLE V

Body Weights, Lymphocyte Levels, and Immune Responses of Individual Thymectomized Mice Bearing Neonatal Thymus in Millipore Diffusion Chambers

No. of mouse	Body weight at week:					Average blood lympho-cyte	Population of lymph nodes	Hemagglutinin titer (logs)	Survival of Ak skin graft	Tissue in diffusion chamber
	4	6	8	10	12					
	gm	*gm*	*gm*	*gm*	*gm*	*counts/mm³*			*days*	
1	16	20	22	22	24	6000	Normal	8	13	Viable
2	16	22	23	24	25	6000	Normal	7	15	Viable
3	16	21	22	21	24	6000	Normal	5	15	Viable
4	10	14	14	18	20	8500	Normal	Not done	13	Viable
5	14	16	18	18	19	3500	Normal	7	19	Viable
6	12	15	20	20	22	8500	Moderately depleted	Not done	13	Viable
7	10	16	18	20	22	13,500	Moderately depleted	Not done	14	Viable
8	12	14	16	18	20	4000	Moderately depleted	8	15	Viable
9	16	20	21	21	24	3750	Moderately depleted	8	15	Viable
10	16	18	19	19	21	4750	Moderately depleted	8	15	Viable
11	14	16	18	17	18	5250	Moderately depleted	8	29	Viable
12	8	15	15	19	20	4000	Markedly depleted	0	>211	Necrotic
13	14	16	17	16	18	3500	Markedly depleted	4	> 42	Necrotic

The Peyer's patches in neonatally thymectomized mice were usually atrophic (Fig. 15) and at times even absent. After implantation of chambers containing thymus, they showed varying degrees of restoration (Table IV). Some still showed a deficiency of small lymphocytes (Figs. 16 to 17) while others had normal lymphoid architecture.

The variation shown by individual mice in the extent to which their tissues were populated with lymphocytes can be appreciated from Table IV.

The liver of neonatally thymectomized mice usually showed a general in-

crease in the size and number of the Kupffer cells (Fig. 18). In about 50 per cent of mice dying from wasting disease, acute necrotic patches with no specific location in the lobules were seen (Fig. 19). In thymectomized mice bearing thymus tissue in chambers, hypertrophy of the Kupffer cells was still present but no necrotic areas were found (Fig. 20).

Tissue in diffusion chambers: The diffusion chambers were usually encapsulated in a loose fibrous matrix and showed no evidence of perforation or separation of the membranes. Tissue could be recovered from most of the thymus-filled chambers. It appeared as a whitish mass either spread out as a thin sheet on one of the walls or adhering to the wall as a compact mass 2 to 3 mm in length. It was composed of epithelial-reticular cells (Figs. 21 to 24) lying either in clumps (Fig. 23) or scattered singly in a loose bed of fibrous tissue (Fig. 24). There were no recognizable lymphoid elements. In some cases, the epithelial cells tended to form acinus-like structures (Fig. 21) containing a colloidal material which gave a positive reaction with the periodic acid–Schiff reagent. At no time was there any direct histological evidence of cells traversing the chamber walls. In some mice, which failed to reject skin grafts and behaved as thymectomized controls, the tissue in the chamber was entirely necrotic (Fig. 25).

Correlation between Increase in Body Weight, Lymphocyte Production, and Immune Response.—In order to see whether a correlation can be made between the various parameters studied in mice thymectomized at birth and bearing neonatal thymus in diffusion chambers, the results obtained from individual mice in a group of 13 are shown in Table V. There was not in every case a good correlation between the rise in body weight, extent of repopulation of lymphoid tissues, and immune responses. For instance, animal 9 had a satisfactory increase in body weight, normal hemagglutinin titers and rejection of foreign skin, but low lymphocyte counts and moderately depleted lymphoid tissues. The two mice listed at the bottom of Table V, which had necrotic remnants in the chamber, behaved as neonatally thymectomized controls.

DISCUSSION

Previous studies have shown that the deficiencies of neonatally thymectomized mice can be corrected either by grafts of neonatal thymus tissue or by an injection of cells prepared from lymph nodes and spleen of adult donors of the same inbred strain (14, 15). It was tempting to conclude that the thymus implant was producing lymphocytes which colonized the deficient lymphoid tissues of the host thus producing a lymphoid chimaera. Cytological studies showed, however, that 80 to 100 per cent of the cells dividing in both the thymus implant and in spleen and lymph nodes were of host origin (13). Furthermore, discriminant spleen assays revealed that host cells were responsible for immunological reactivity (25). It was thus concluded that the role of the thymus was not just to provide directly the cells which populated the lymphoid tissues but

to exert some influence on the host's own lymphoid system. The present experiments were performed in order to determine whether this influence was humoral in nature. Thymus tissue was enclosed in cell-tight Millipore diffusion chambers and these were implanted into neonatally thymectomized mice. These mice gained weight satisfactorily, did not develop wasting disease, could produce serum antibodies, and reject allogeneic skin homografts. The extent to which their tissues became populated with lymphocytes was, however, variable: some mice had perfectly normal lymphoid structures but most still showed a diminution of lymphocytes in spite of having produced normal immune responses to sheep erythrocytes and skin homografts.

The fact that a small amount of tissue, as was enclosed in the chambers, can influence so many widely scattered tissues, suggests the existence of a humoral mechanism. The restorative effects of the chambers could, however, be attributed either to an adjuvant effect of the Millipore material (26), or to passage of cells through the chamber walls. Control thymectomized mice bearing empty chambers did not have the capacity to produce immune responses. It is unlikely, therefore, that the Millipore material itself played any role in restoring to normal the reactivity of the mice in the experimental group. Furthermore, those mice with necrotic thymus remnants in the chambers behaved as thymectomized controls. Cytological and histological examinations showed that only host cells were dividing in the lymphoid tissues and that no lymphocytes were present in the chamber itself. If cells had been able to gain entry into the chambers, one might have expected, by analogy with what takes place in subcutaneous thymus grafts, to find repopulation of the epithelial-reticular thymus complex with lymphocytes. Further, there was never any direct histological evidence of cells traversing the chamber walls. The available evidence, therefore, indicates that a humoral factor produced by the tissue enclosed in the chamber was responsible for correcting the immunological inadequacies of its host.

The cellular source of the humoral factor may be the epithelial-reticular cells or the lymphoid cells. Only the epithelial-reticular elements of the enclosed thymus were recovered from the chambers, whether these had contained embryonic epithelial thymus rudiments or neonatal lymphoidal thymuses. The absence of lymphoid cells from chambers containing neonatal thymus suggests that such cells were not able to survive the prolonged periods of time that the chambers were present in the mice. This agrees with the observations of Grégoire (27), who failed to find thymocytes in 120 autografts of thymus enclosed in porous cellulose membranes for periods up to 29 days after implantation into the subcutaneous tissues or peritoneal cavity of rats and guinea pigs thymectomized in adult life. The absence of lymphoid cells from chambers containing embryonic thymus tissue indicates either that lymphoid differentiation failed to take place or that lymphoid cells which had differentiated from

the epithelial cells had died out by the time the examination was made in the present experiments. Further histological studies of embryonic epithelial thymus rudiments enclosed in chambers revealed, however, no lymphoid differentiation during the first 3 weeks after implantation (28). This leaves the thymus epithelial-reticulum as the principal cellular source of the humoral factor.

Clearly there may be one or more thymus factors and the effect may be produced directly or indirectly. Thus, for instance, one could argue that prevention of the wasting syndrome was achieved by a direct effect on growth at a critical phase in development. Wasting diseases, similar to that seen after neonatal thymectomy, do however occur in association with many other conditions resulting in lymphoid dysplasia. This suggests that the common denominator in the pathogenesis of such diseases is the inadequacy of the lymphoid system (29). One might argue further that the rejection of skin homografts, by the thymectomized mice bearing thymus tissue in chambers, was accomplished by a direct local action of the humoral factor at the site of the homograft. Since, however, it has been shown that the phenomenon of homograft rejection in intact animals is a function of immunologically competent lymphocytes (30), it is probable that the same mechanism occurs in the mice of the present experiments. It is likely, therefore, that the humoral thymus factor exerts its effect indirectly through the host's own lymphoid system.

There are two important ways in which a humoral thymus factor (or factors) could influence the lymphoid system. It may stimulate lymphopoiesis in a manner similar to the lymphocytosis stimulating factor postulated by Metcalf (19), and it may endow lymphoid cells with the property of immunological competence (13). The high mitotic activity of thymic lymphoid cells (2, 3), the lymphocytosis observed in animals injected with acellular thymus extracts (17–19) and the fall in circulating lymphocytes that occurs in animals after thymectomy at any age (1, 31, 32) are indirect evidence in favor of a lymphocytosis stimulating effect of the thymus factor.

Rats thymectomized at birth show marked diminution in the population of small lymphocytes and diminished capacity to produce serum antibodies and yet show normal levels of γ-globulin (33). In the present work, many mice thymectomized at birth and bearing thymus tissue in chambers produced normal immune responses and yet had a reduced population of lymphocytes (Tables IV and V). The lack of correlation between the extent of population of the lymphoid tissues, the ability to produce immune responses and to synthesize γ-globulin suggests that the functioning of the immunological apparatus depends not so much on the quantity of lymphoid cells or γ-globulin present, but rather on the capacity of the cells to react specifically under appropriate stimulation. The thymus may thus be responsible for endowing lymphoid cells with immunological competence. This effect must take place very early in life,

since mice thymectomized later than 1 week after birth do not show any significant immunological defects (14).

Recently, strong evidence has been obtained suggesting that primary immune responses in general may be initiated by the interaction of antigens with small lymphocytes (30). These cells would interact with antigens (or antigens which have been "processed" by reticuloendothelial phagocytes, reference 34), become fixed in lymphoid tissues and give rise to a dividing cell line which generates within lymphoid tissues the "effector" cells which either destroy grafts or synthesize antibodies. Since mice thymectomized at birth are in general incapable of producing primary immune responses it may be that their small lymphocytes are incapable of interacting with antigens. This is unlikely to be due to defects in the phagocytic function of the reticuloendothelial system (35). Perhaps the antigen recognition mechanism has failed to differentiate. At a certain stage in ontogenesis, lymphoid cells may have the inherent capacity for synthesizing globulin molecules, even though they may not be able to recognize, and therefore interact with, antigen, and thus would be non-inducible with regards to production of specific antibody. Presumably the thymus factor would allow further differentiation of the cells to a stage at which an antigenic stimulus could then interact with the cell to put into effect some mechanism to allow derepression of the specific combination of genes which provide the cytoplasm with the information required for the synthesis of specific antibody.

The existence of a humoral thymus factor in no way negates the possibility that the thymus is the chief cellular source of lymphocytes, particularly in embryonic life and in germ-free conditions (36). Experiments are in progress to identify the nature of this thymus factor and to determine its mechanism of action at the cellular level.

Since this manuscript was completed, the preliminary report of Levey, Trainin, and Law (37) has appeared. These authors studied the effects of neonatal thymus tissue in Millipore diffusion chambers implanted intraperitoneally into neonatally thymectomized mice. They claimed that "the spleen, lymph nodes, and Peyer's patches were rich in lymphocytes and in primary and secondary lymphoid nodules." They did not give data on immune responses of their mice nor did they report any variation in the extent to which tissues in individual mice were populated with lymphocytes. Their data on peripheral blood counts, when analyzed in detail, are essentially similar to those reported here.

SUMMARY

Neonatally thymectomized mice were implanted intraperitoneally at 7 days of age with Millipore diffusion chambers containing either embryonic or neonatal thymus tissue. Mice which received either empty diffusion chambers

or no further treatment following neonatal thymectomy served as controls. In contrast to these controls, most of the mice implanted with thymus-filled chambers gained weight satisfactorily, did not develop a wasting syndrome, and had the capacity to produce serum antibodies in response to sheep erythrocytes and to reject allogeneic skin grafts. Lymphoid follicles were present in the lymph nodes, spleen, and intestinal tract of the implanted mice but most still showed some diminution in the population of lymphocytes in both blood and tissues. Control thymectomized mice had markedly depleted lymphoid tissues and low peripheral blood lymphocyte levels. The tissue recovered after 1 to 2 months from the diffusion chambers showed only epithelial-reticular cells but no lymphoid cells. It is suggested that a humoral factor produced by the thymus epithelial-reticular complex may be responsible for endowing lymphoid cells with immunological competence.

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EXPLANATION OF PLATES

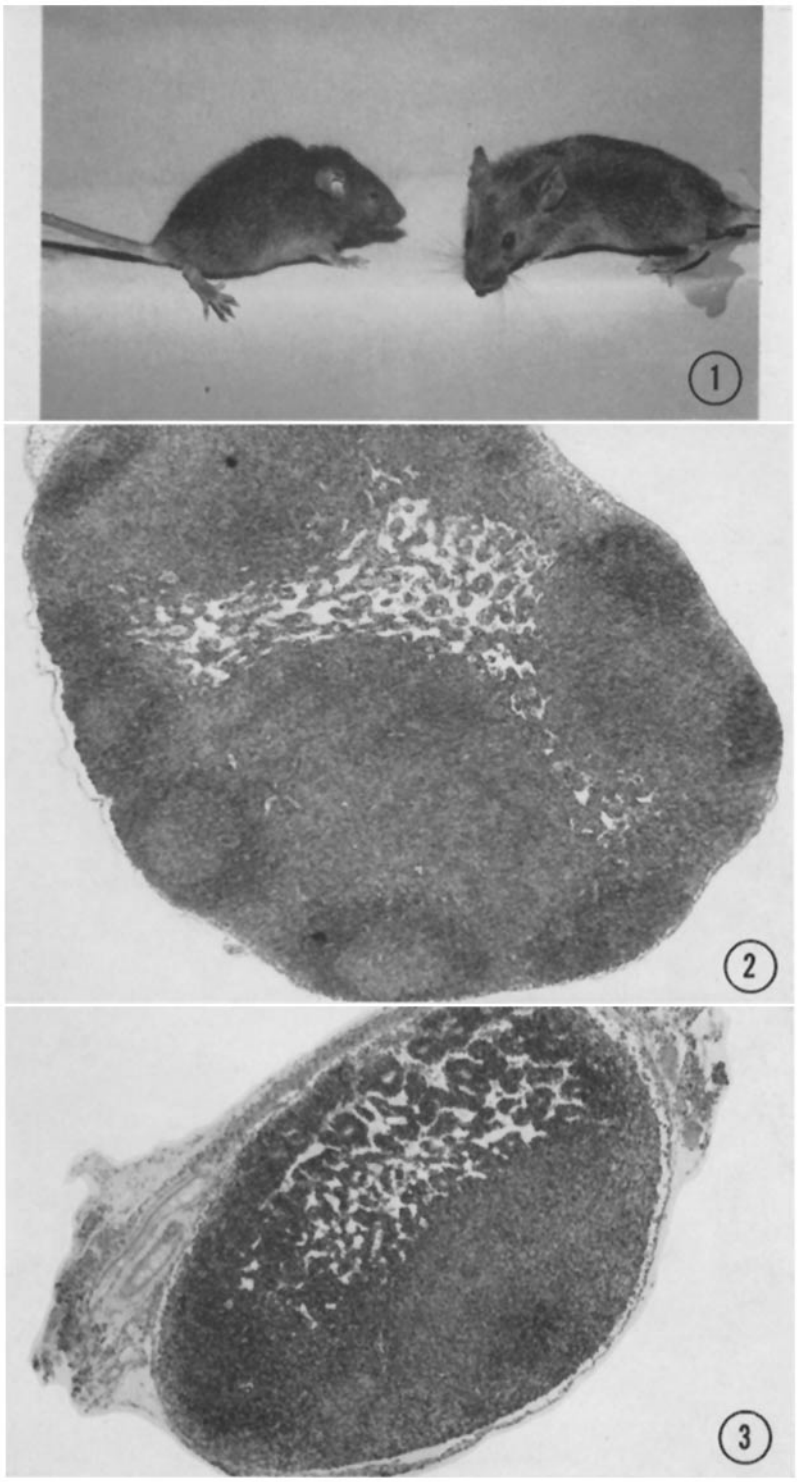
The sections of Figs. 2 to 20, 24, and 25 inclusive were stained with hematoxylin and eosin; those of Figs. 21 to 23 were stained with the periodic acid–Schiff reagent.

PLATE 8

FIG. 1. Wasting disease: both mice have been totally thymectomized at birth and are shown here at 8 weeks of age. The mouse on the left received no further treatment and is showing signs of wasting disease. The mouse on the right was implanted at 7 days of age with neonatal thymus in diffusion chamber and appears healthy.

FIG. 2. Left axillary lymph node of a normal 10-week-old (CBAXT6)F_1 mouse. Note germinal centers and lymphocyte-filled cortex. × 50.

FIG. 3. Left axillary lymph node of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth. Note generalized atrophy, markedly depleted cortex and complete absence of follicles and germinal centers. × 50.



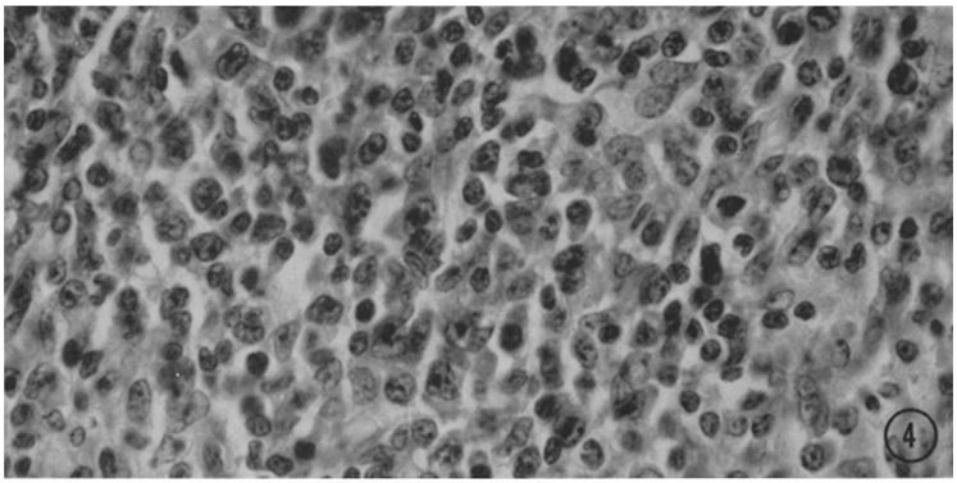
(Osoba and Miller: Lymphoid tissues and immune responses)

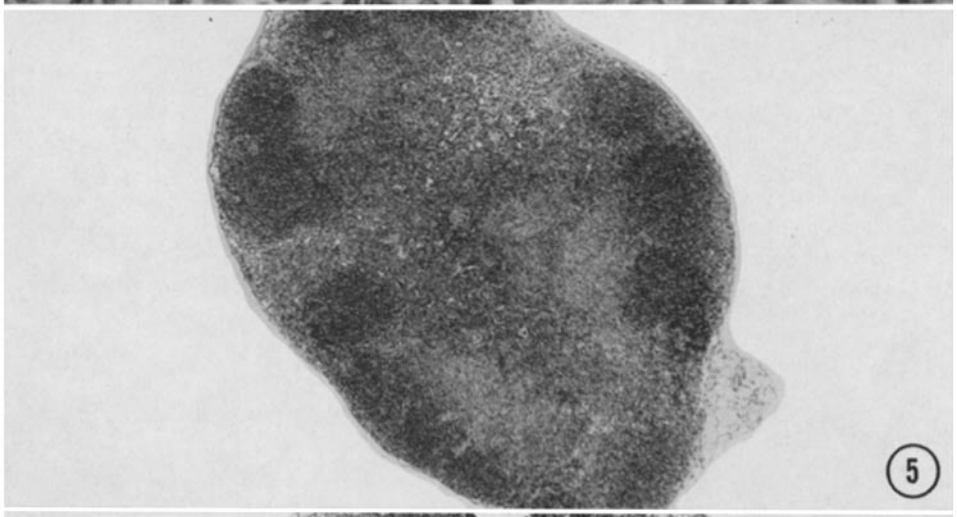
PLATE 9

FIG. 4. Left axillary lymph node of a 10-week-old mouse totally thymectomized at birth. Note predominance of reticuloendothelial cells, histiocytes, macrophages, and some immature lymphoid cells. × 675.

FIG. 5. Left axillary lymph node of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing embryonic thymus. Note islands of lymphocytes in cortex. × 50.

FIG. 6. Left axillary lymph node of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing embryonic thymus. Note numerous germinal centers. × 50.





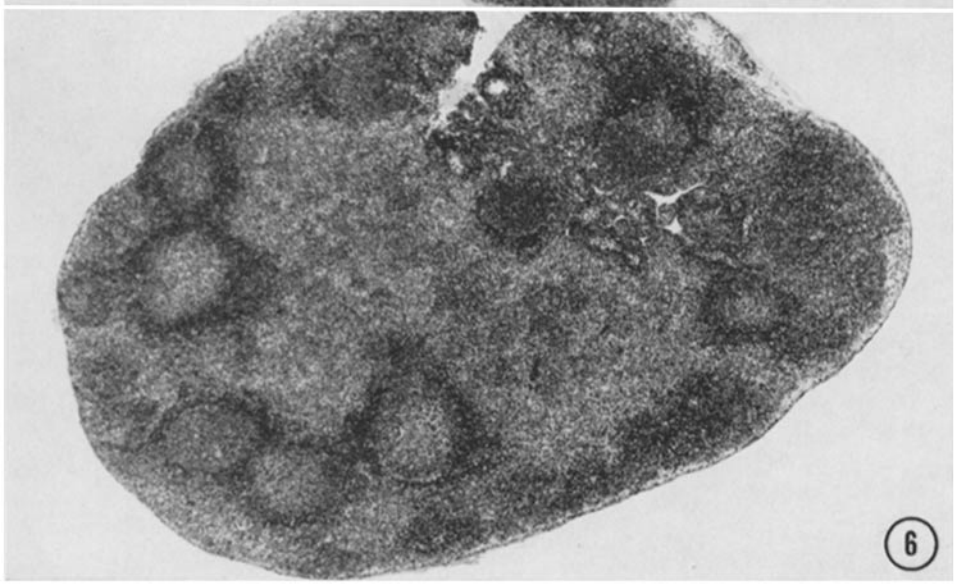


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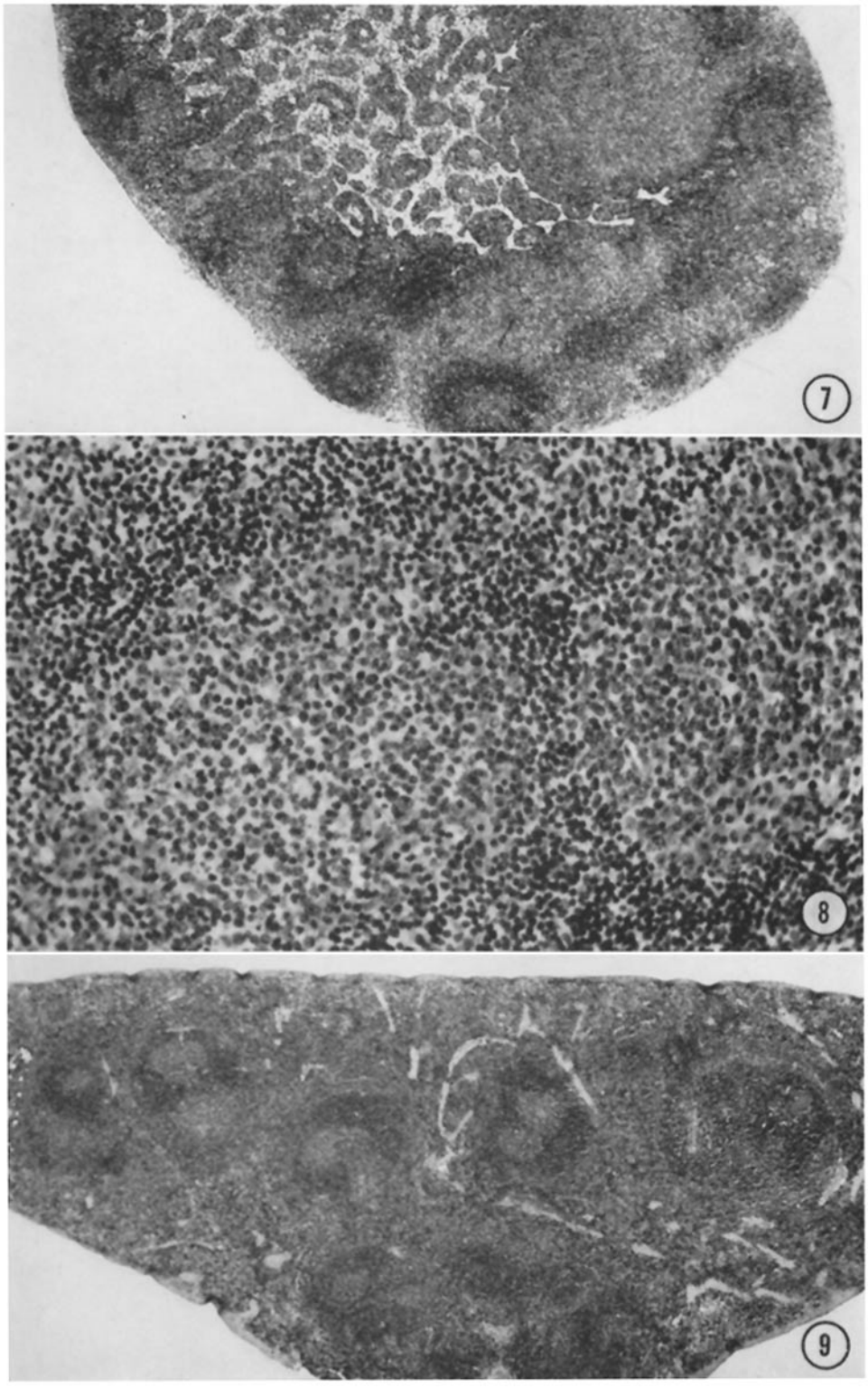
PLATE 10

FIG. 7. Left axillary lymph node of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing neonatal thymus. Note lymphocytic fields and germinal centers in cortex. × 50.

FIG. 8. Left axillary lymph node of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chambers containing embryonic thymus. Note germinal centers and numerous small, medium, and large lymphocytes. × 300.

FIG. 9. Spleen of a 10-week-old normal (CBAXT6)F_1 mouse. Note lymphoid follicles and germinal centers. × 50.



(Osoba and Miller: Lymphoid tissues and immune responses)

PLATE 11

FIG. 10. Spleen of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth. Note poorly developed follicular structure and absence of germinal centers. × 50.

FIG. 11. Spleen of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chambers containing embryonic thymus. Note moderate lymphocyte depletion. × 50.

FIG. 12. Spleen of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing embryonic thymus. Note lymphoid follicles. × 50.

FIG. 13. Spleen of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing neonatal thymus. Note normal follicular architecture. × 50.

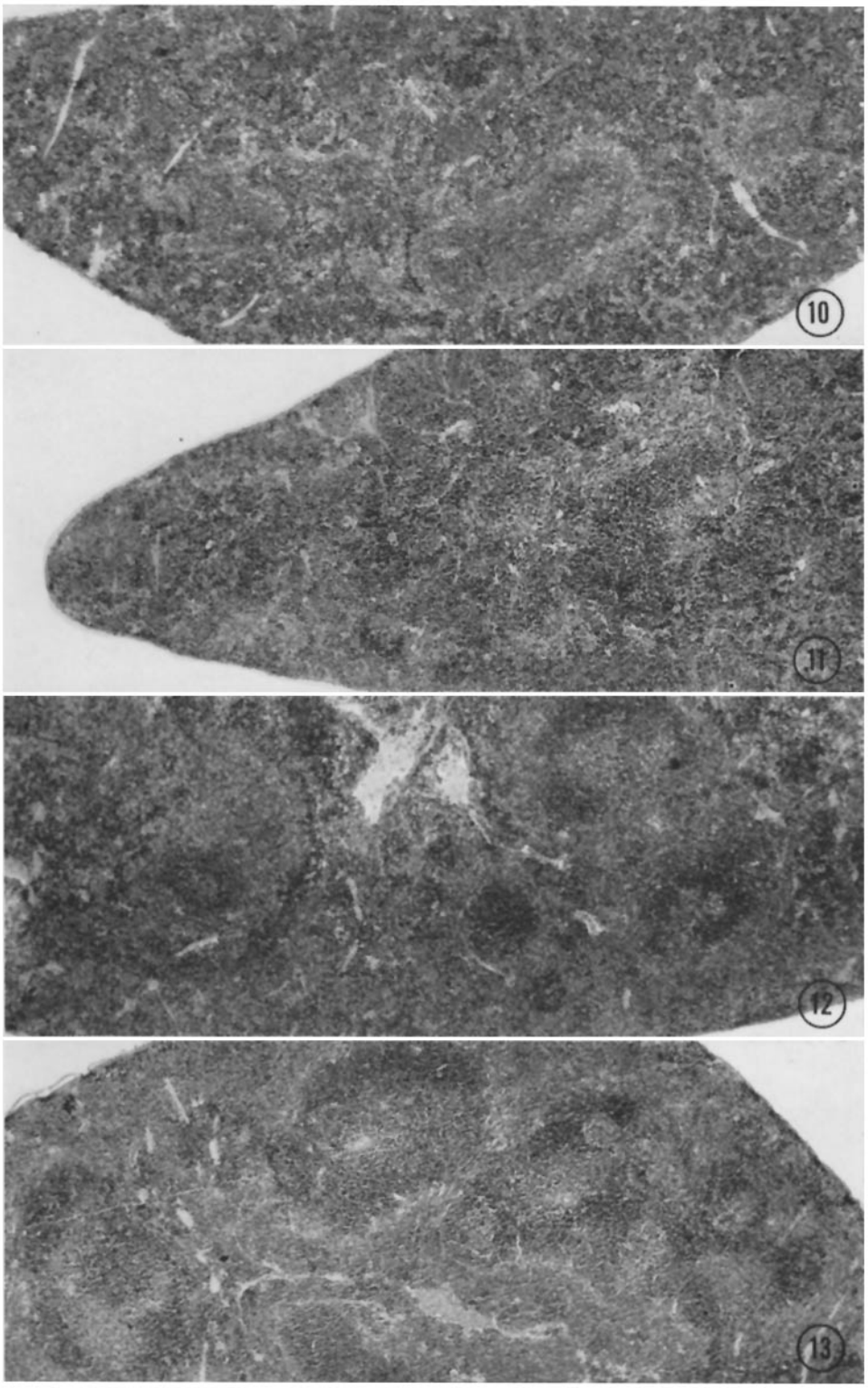


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PLATE 12

FIG. 14. Peyer's patch of a 10-week-old normal (CBAXT6)F_1 mouse. × 160.

FIG. 15. Peyer's patch of a 10-week-old (CBAXT6)F_1 mouse thymectomized completely at birth. Note depletion of lymphocytes and atrophy of patch. × 160.

FIG. 16. Peyer's patch of a 10-week-old (CBAXT6)F_1 mouse thymectomized completely at birth and implanted at 7 days with Millipore diffusion chamber containing embryonic thymus. Note moderate depletion of lymphocytes. × 160.

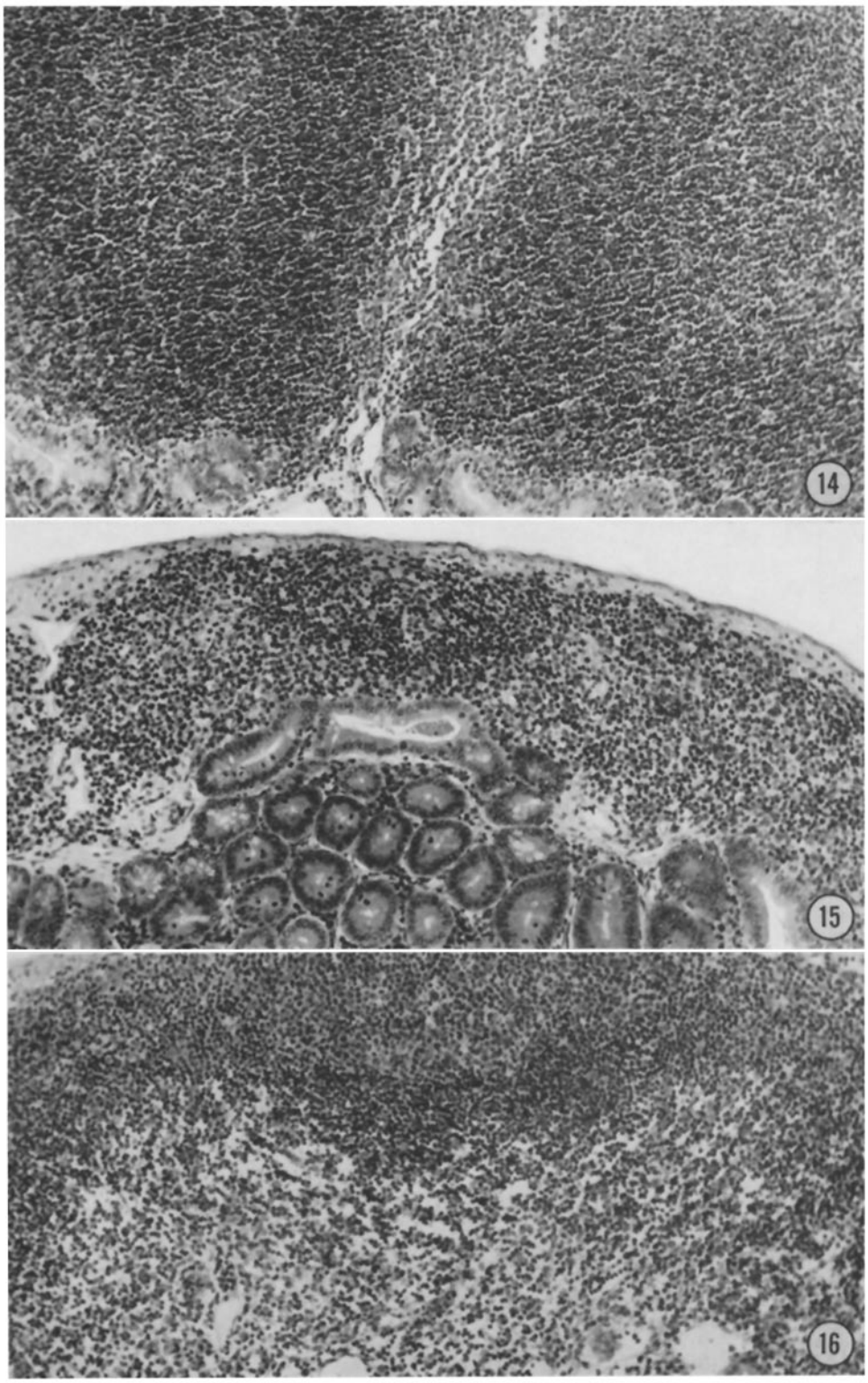

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PLATE 13

FIG. 17. Peyer's patch of a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with Millipore diffusion chamber containing neonatal thymus. There is still some depletion of small lymphocytes. × 160.

FIG. 18. Liver from a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth. Note numerous prominent Kupffer cells. × 160.

FIG. 19. Liver from 9-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and dying of wasting disease. Note large granulomatous lesions composed of polymorphonuclear and mononuclear cells and giant cells. × 160.

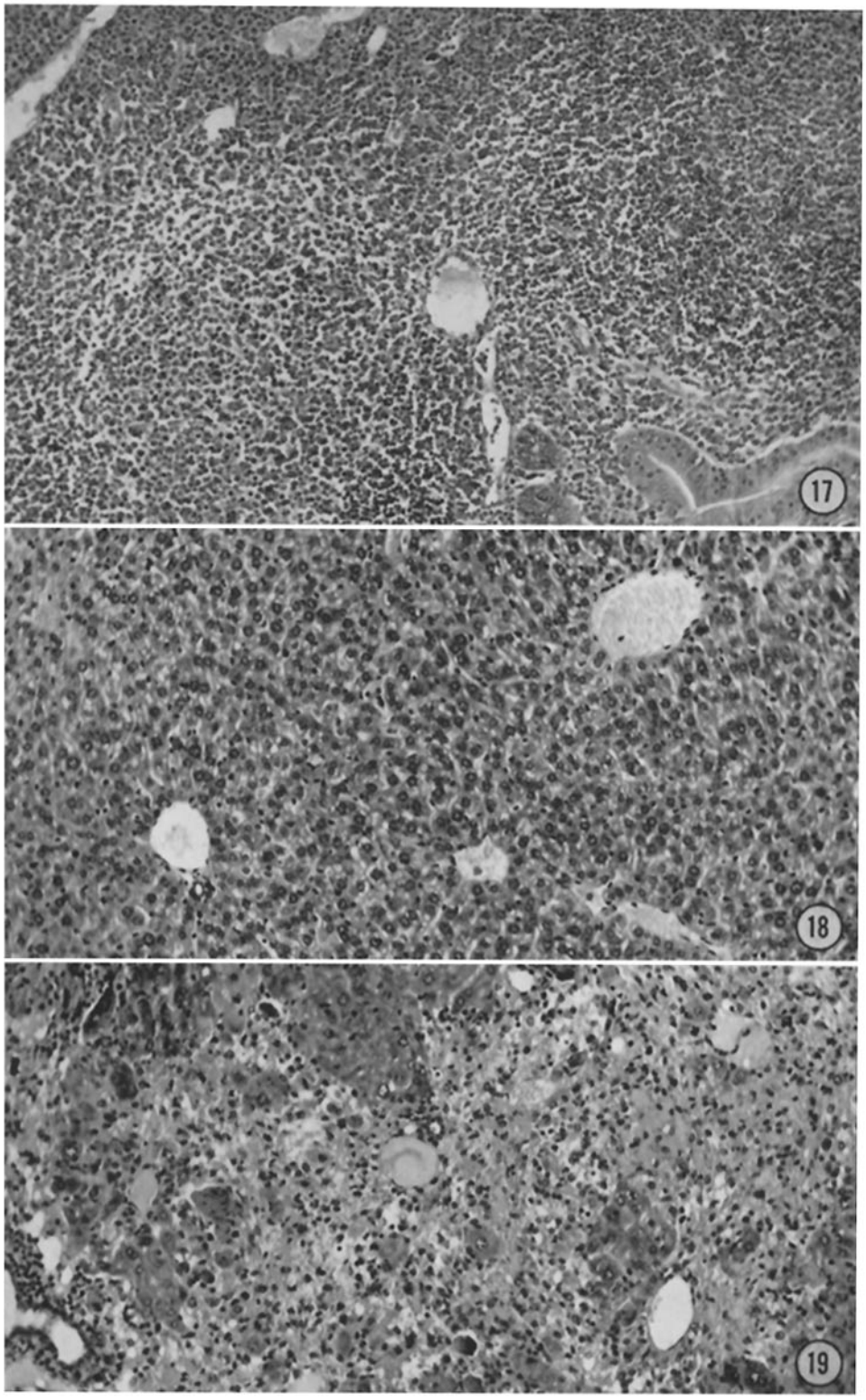

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PLATE 14

FIG. 20. Liver from a 10-week-old (CBAXT6)F_1 mouse totally thymectomized at birth and implanted at 7 days with embryonic thymus in Millipore diffusion chamber. There is still some hypertrophy of the Kupffer cells. × 160.

FIG. 21. Embryonic thymus tissue recovered from Millipore diffusion chamber 46 days after implantation. Note absence of lymphocytes and formation of acini. × 375.

FIG. 22. Higher magnification of embryonic thymus tissue shown in Fig. 21. Note numerous viable epithelial-reticular cells. × 1100.

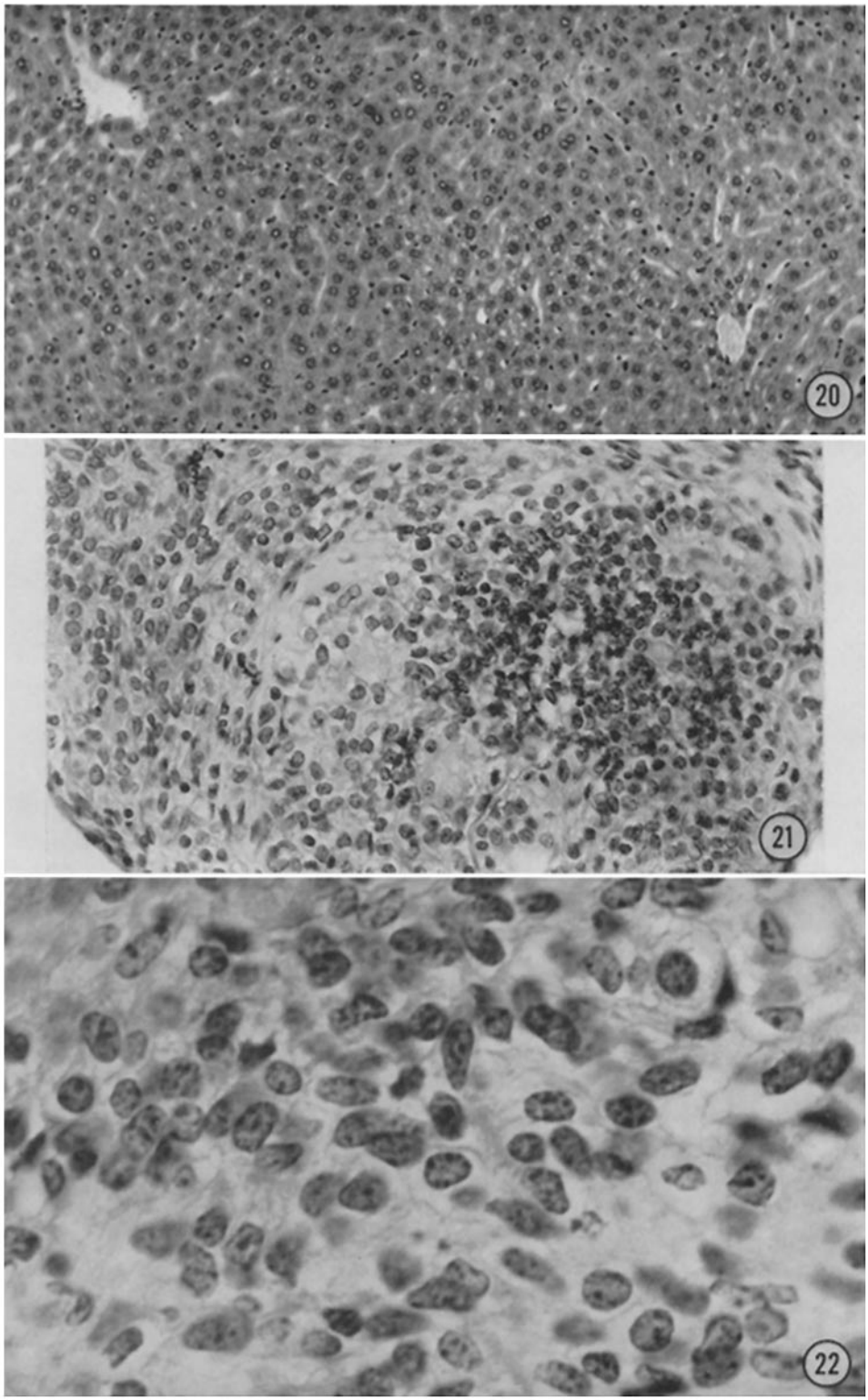

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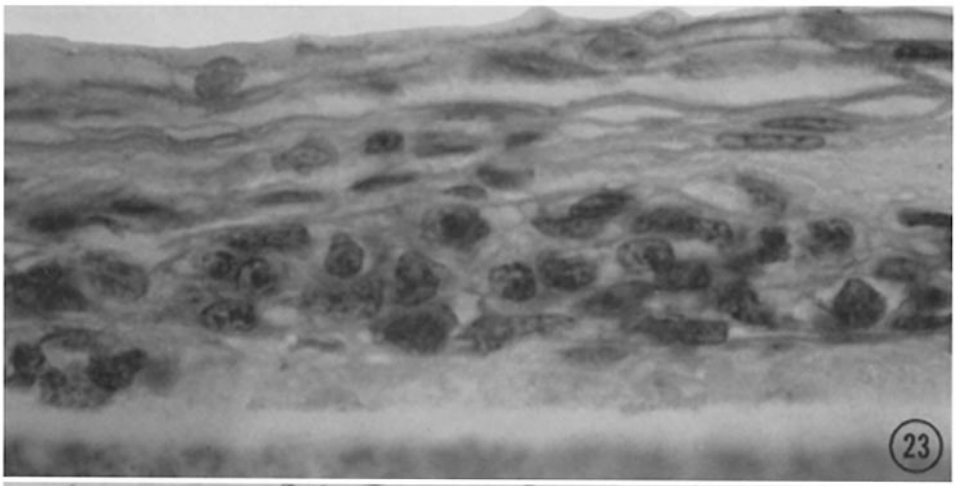
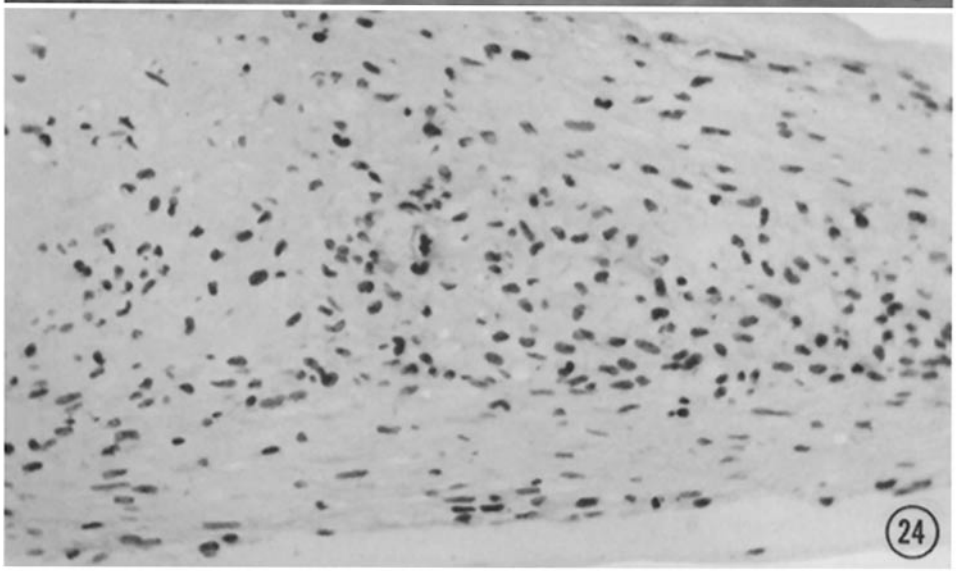
PLATE 15

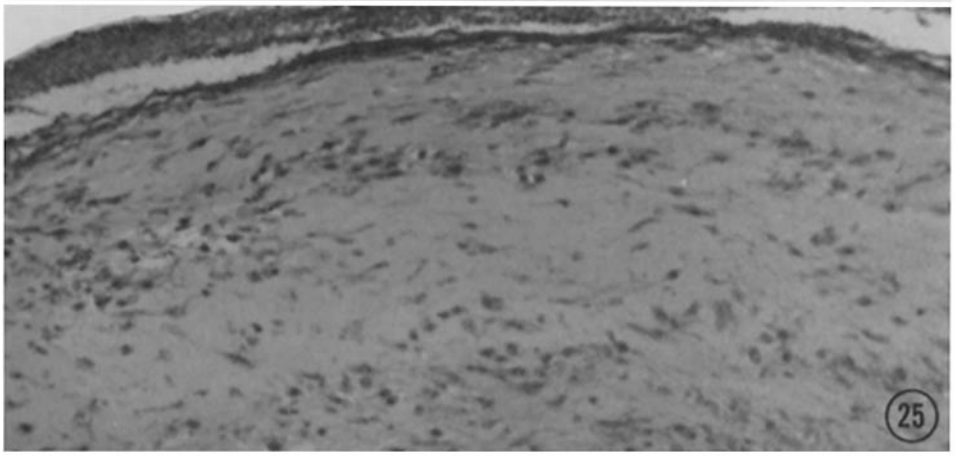
FIG. 23. Clumps of epithelial-reticular cells in tissue recovered from Millipore diffusion chamber 9 weeks after implantation of chamber containing embryonic thymus tissue. × 1100.

FIG. 24. Neonatal thymus enclosed in diffusion chamber for 56 days. Note absence of lymphocytes and presence of epithelial-reticular cells scattered in lose fibrous tissue. × 300.

FIG. 25. Necrotic remnant of neonatal thymus enclosed in Millipore diffusion chamber for 56 days. This mouse behaved as a thymectomized control. × 300.







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