MIGRATION OF LYMPHOCYTES AND THYMOCYTES IN THE RAT*

I. The Route of Migration from Blood to Spleen and Lymph Nodes

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Interest in the thymus has centered on its role in development of the lymphoid system and immunological responsiveness of rodents and fowl. Animals thymectomized at birth or soon after hatching often show incomplete development of lymphoid tissue, lymphopenia, and impaired immunological performance (1, 2). Even in the adult, thymectomy can cause a progressive decrease of small lymphocytes in lymph nodes, blood, and thoracic duct lymph (3–5), and gradual weakening of the animal's immunological capability (6–8). The effects are dramatic in adult mice exposed, after thymectomy, to a large dose of whole-body X-irradiation. Thymectomized and irradiated mice never regenerate a normal lymphocyte population, and the animals become immunological cripples (9, 10).

The unresponsiveness in mice thymectomized at birth can be corrected by injections of spleen and lymph node cells (11, 12), or thoracic duct lymphocytes (13) from normal, adult donors. The ability of lymphocytes (presumably free of phagocytic cells) to correct the unresponsiveness is important for two reasons. First, it suggests that the immunological deficiency in thymectomized mice is related solely to a lack of circulating lymphocytes. Second, it virtually excludes the possibility that the unresponsiveness is the result of a defect in cellular "processing" of antigen or an unfavorable environment for proliferation of immunologically compentent cells. The experiments of Miller and his colleagues (13) support this contention. They demonstrated that the peripheral pool of circulating lymphocytes in mice thymectomized at birth is deficient in both small lymphocytes and its content of potential antibody-forming cells.

Results of the present experiments suggest that the cellular deficit in rats thymectomized at birth also is limited to cells in the peripheral lymphocyte pool. The lymphocyte deficiency in such animals is similar to that induced in adult rats by chronic drainage of lymph and cells from a thoracic duct fistula.

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Chronic lymph drainage causes a specific deficiency of small lymphocytes (14–16). The lymphocyte deficiency in neonatally thymectomized rats, like that in lymphocyte-depleted adult rats, can be corrected by injections of thoracic duct lymphocytes from normal, syngeneic donors.

The results disclose the route by which small lymphocytes enter splenic white pulp from blood, and a relationship between movement of small lymphocytes across postcapillary venules and the structure of endothelium lining these vessels. The experiments also suggest that cells normally resident in thymus can join the peripheral lymphocyte pool. A few radioactively labeled small thymocytes injected intravenously colonize lymphoid tissue. Small thymocytes which emigrate to splenic white pulp and the cortex of lymph nodes and Peyer's patches follow the pathway of circulating small lymphocytes.

Methods

Animals.—The subjects of these experiments were male and female Lewis rats (Microbiological Associates, Walkersville, Md.), DA rats,[1] and members of the F_1 hybrid cross between these inbred strains. Animals were always inoculated with cells from donors belonging to the same strain.

Operative Procedures.—

Thymectomy: Newborn (Lewis × DA)F_1 rats were thymectomized by the method of Janković et al. (17) with the following minor modifications. The sternum was divided in the midline. The thymus was then exposed by applying traction on single sutures encompassing each sternal fragment. After thymectomy, the sutures were used to close the incision. Cannibalism was greatly reduced by anesthetizing the mother with pentobarbital, 2 mg/100 g body weight, before the thymectomized rats were returned to the nest.[2] At the end of each experiment, the mediastinum was inspected and, in some cases, tissue was examined histologically for thymus remnants. Thymectomy was complete in all animals employed in the experiments.

Cannulation of the thoracic duct: The thoracic duct of male rats, 180–250 g body weight, was cannulated in its short intra-abdominal course by the method of Bollman et al. (18). After the operation, the animals were placed in restraining cages (19) where they remained unanesthetized during the period of lymph drainage. The general management of the restrained animals has been described by Gowans and Knight (20). After 6–8 days of lymph drainage, the thoracic duct fistula was "closed" by cutting the cannula short and pushing it under the skin (14).

Preparation of Thoracic Duct Cells and Thymocytes.—Lymph from the thoracic duct of freshly cannulated rats was collected at room temperature, for periods of 8–16 hr into, sterile flasks containing 5 ml of Krebs-Ringer solution, 100 units of heparin and 500 μg of streptomycin.

Thymuses from adult rats were rinsed in 0.85% sodium chloride and freed of adherent lymph nodes by blunt dissection under a low power microscope. Cells were then expressed from the thymus by teasing it in tissue culture medium 199 (21) containing 1 unit of heparin per ml. Approximately 90% of the cells obtained in this way were small thymocytes (cell diameter 8 μ or less). The remaining cells were arbitrarily classified as "large thymocytes."

Suspensions of lymphocytes and thymocytes were filtered through several layers of surgical

[1] Nucleus of colony kindly supplied by Dr. R. E. Billingham.

[2] Suggested by Dr. Kenneth S. Warren.

gauze to remove tissue debris. The cells were centrifuged from the medium for 10 min at 100 *g*, washed once in fresh, heparin-free medium, and resuspended at a concentration of 50×10^6–100×10^6 cells per ml.

Radioactive-Labeling of Cells.—Lymphocytes and thymocytes were radioactively labeled by incubating the cells in vitro for 1 hr at 37°C in tissue culture medium 199 containing 1 unit of heparin and 10 μc per ml of tritiated uridine (^{3}H-5-uridine, 8 c per mmole, Schwartz BioResearch, Inc., Orangeburg, N. Y.). After incubation, the cells were washed twice in nonradioactive, heparin-free, medium containing 60–120 μg of ^{2}H-uridine per ml, and resuspended at a concentration of 10^8 cells per ml for injection.

Many thymocytes and a few lymphocytes lysed during the incubation procedure. Material released from these cells was removed by washing. Only suspensions in which more than 95% of the remaining cells were viable, as judged by their ability to exclude trypan blue, were used in experiments.

TABLE I

Radioactive Labeling of Cells with ^{3}H-5-Uridine*

Cell type	Radioautographs (days' exposure)	Large‡		Small§	
		Per cent labeled	Mean grain count/cell	Per cent labeled	Mean grain count/cell
Thoracic duct lymphocytes	1	83	22	49	4
	4	98	>50	90	17
Thymocytes	1	85	30	35	2
	4	95	>50	81	6

* From male and female Lewis rats 8–24 wk of age.

‡ Cell diameter greater than 8μ.

§ Cell diameter 8μ or less.

Table I shows that lymphocytes and thymocytes (both large and small) became radioactively labeled in cell suspensions prepared in this way. Presumably, ^{3}H-5-uridine was incorporated exclusively into the ribonucleic acid (RNA) of labeled cells (22). Grain counts over labeled cells showed that small lymphocytes were more heavily labeled than small thymocytes whereas large thymocytes were more heavily labeled than large lymphocytes.

In one experiment, thymocytes were radioactively labeled by incubating them in vitro for 1 hr in the presence of 0.1 μc of tritiated thymidine (^{3}H-thymidine, 6 c per mmole, Schwartz BioResearch, Inc.) per milliliter. The labeled cells were then washed once in nonradioactive, heparin-free medium containing 1 μg of ^{2}H-thymidine per ml. This technique labeled 75% large thymocytes and less than 1% small thymocytes in radioautographs exposed for 14 days. The few small thymocytes which became radioactively labeled had a cell diameter of 7–8 μ.

Histology and Radioautography.—Air-dried smears of lymphocytes, thymocytes, and peripheral blood were fixed in methyl alcohol. Tissues were fixed in 80% v/v ethyl alcohol, 10% v/v glacial acetic acid, and 10% v/v chloroform, embedded in paraffin wax and sectioned at 4 μ. Smears and tissue sections were extracted for 20 min at 4°C in two changes of 5% trichloracetic acid then washed for at least 1 hr in cold, running tap water. The smears were prepared as radioautographs with NTB-2 liquid photographic emulsion (Eastman Kodak, Rochester, N. Y.) and stained after development with iron hematoxylin. Tissue sections

stained with periodic acid–Schiff reagent (PAS) were prepared as radioautographs and counterstained, after development, with alum hematoxylin. In addition to the radioautographs, sections of all tissues were stained with either methyl green–pyronin or PAS.

RESULTS

Histological Changes in Lymphoid Tissue of Rats.—Histological changes in lymphoid tissues of rats thymectomized at birth were similar to those induced in adult rats by drainage of lymph and cells from a thoracic duct fistula for 8 days. In both cases, the animals showed a gross deficiency of small lymphocytes in splenic white pulp, lymph nodes, and Peyer's patches; and a great reduction of small lymphocytes in peripheral blood and thoracic duct lymph.

The most obvious change in splenic white pulp was a reduction of small lymphocytes in the periarteriolar sheath. In rats thymectomized at birth, and in lymphocyte-depleted rats, the sheath was reduced to a reticulum lattice containing only a few small lymphocytes and a small number of large, pyroninophilic cells (Figs. 1–3). In neonatally thymectomized animals, there often was increased hematopoiesis in splenic red pulp and an increase in reticular cells in the marginal zone between red pulp and white pulp.

Neonatally thymectomized rats and lymphocyte-depleted rats showed a great reduction of small lymphocytes in diffuse lymphocyte fields deep in the cortex of lymph nodes and Peyer's patches (Figs. 4 and 5). The superficial cortex, containing lymphoid follicles, and a narrow band of tissue at the corticomedullary junction were not obviously depleted of lymphocytes. Medullary cords contained many plasma cells as they do in normal rats.

An impressive change was observed in postcapillary venules in diffuse lymphocyte fields of lymph nodes and Peyer's patches. Venules in this location normally are lined by endothelial cells which have abundant cytoplasm; and the venules contain many small lymphocytes in their lumen and wall (Fig. 6). Postcapillary venules in rats thymectomized at birth, and in lymphocyte-depleted rats, were dilated and empty. Endothelial cells lining the venules had relatively little cytoplasm: and the cytoplasm lacked its normal affinity for pyronin (Fig. 7).

Table II shows the number of small lymphocytes in blood and thoracic duct lymph of normal rats, neonatally thymectomized rats, and rats depleted of lymphocytes by drainage from the thoracic duct. Neonatally thymectomized animals and lymphocyte-depleted rats showed a severe lymphopenia. In addition, the daily output of small lymphocytes from the thoracic duct of such animals was much less than the output from freshly-cannulated, normal rats.

Distribution of ^{3}H-5-Uridine-Labeled Lymphocytes.—The migration of lymphocytes from blood to lymphoid tissue was studied by injecting rats intravenously with 10^{6}, ^{3}H-5-uridine–labeled thoracic duct cells per gram body weight. The distribution of labled lymphocytes was the same in normal, neonatally thymectomized, and lymphocyte-depleted recipients.

In animals sacrificed 10 min after injection, radioactively labeled small lymphocytes were found in the marginal sinus (perifollicular space) and outlying sinuses of the marginal zone between splenic red pulp and white pulp (Fig. 8). Only a few labeled small lymphocytes were found in the periarteriolar sheath of white pulp. However, labeled small lymphocytes increased in the sheath between 10 min and 6 hr, then gradually decreased (Figs. 9–13). Spleen sections examined with the electron microscope showed many small lymphocytes in the luman or the marginal sinus and in gaps between endothelial cells of the sinus wall (Fig. 14).

TABLE II

*Small Lymphocytes in Peripheral Blood and Thoracic Duct Lymph of Rats**

Treatment	No. rats	No. of small lymphocytes—mean (range)	
		Peripheral blood (per mm^3)	Thoracic duct lymph (24 hr output $\times 10^6$)
None	10	8244 (7600–11400)	—
	5	—	940 (760–1080)‡
Thymectomy at birth	8	999 (680–1220)	—
	3	838 (714,860,920)	253 (185,284,290)‡
Chronic lymph drainage§	5	844 (182–1460)	84 (62–108)‖

* Male and female rats 8–14 wk of age.

‡ Output during first 24 hr of lymph drainage.

§ Drainage from thoracic duct fistula for 8 days.

‖ Output during final 24 hr of lymph drainage.

Radioactively labeled large lymphocytes, unlike small lymphocytes, rarely penetrated splenic white pulp. Large lymphocytes entered red pulp from blood but the portal of entry is unknown. Labeled large lymphocytes increased in splenic red pulp between 10 min and 3 hr, then gradually decreased.

Many radioactively labeled small lymphocytes and a few labeled large lymphocytes passed from blood to lymph nodes and Peyer's patches. Migrant cells penetrated the wall of postcapillary venules. Labeled small lymphocytes were confined to the venule lumen and wall 10 min after injection (Figs. 15 and 16). These lymphocytes traversed the wall within 20 min and increased in the cortex during the first 48 hr. Radioactively labeled small lymphocytes accumulated in diffuse lymphocyte fields: they did not penetrate the subcapsular portion of the cortex, lymphoid follicles, or medullary cords (Figs. 17 and 18). Many labeled lymphocytes were released into medullary sinuses. Apparently, lymphocytes enter efferent lymph from the cortex, but the route followed is unknown.

Repopulation of lymph nodes and Peyer's patches was accompanied by changes in endothelium of postcapillary venules. Endothelial cells acquired more cytoplasm, and the cytoplasm regained its affinity for pyronin (Fig 19). By 24 hr, the postcapillary venules appeared normal.

A few radioactively labeled small lymphocytes were found in the thymus medulla of normal and lymphocyte-depleted rats, and in bone marrow of all recipients. Radioactively labeled lymphocytes (both large and small) decreased rapidly in lung capillaries and sinusoids of liver. Labeled large lymphocytes increased in the intestinal wall from the 1st hr on. The large lymphocytes were found in lamina propria (Fig. 20) between epithelial cells, and occasionally in the intestinal lumen.

During the first 24 hr, there was an apparent shift of radioactivity from nucleus to cytoplasm in labeled small lymphocytes in lymph nodes and Peyer's patches, and in labeled large and small lymphocytes in spleen. No obvious shift of radioactivity was observed in labeled lymphocytes in liver or the intestinal wall.

Distribution of ^{3}H-5-Uridine-Labeled Thymocytes.— The distribution of thymocytes in tissues was studied by injecting rats intravenously with 10^{6}, ^{3}H-5-uridine-labeled thymocytes per gram body weight. Labeled thymocytes followed similar pathways in normal recipients, neonatally thymectomized rats, and rats depleted of small lymphocytes by chronic drainage from the thoracic duct.

Both small and large thymocytes filtered through lung capillaries more slowly than labeled lymphocytes and, unlike lymphocytes, they accumulated in large numbers in liver sinusoids. A few small thymocytes passed from blood to splenic white pulp along the route followed by small lymphocytes (Fig. 21). More large thymocytes than large lymphocytes entered splenic red pulp. Radioactively labeled small thymocytes penetrated diffuse lymphocyte fields of lymph nodes and Peyer's patches by traversing the wall of postcapillary venules (Fig. 22). However, only a small number of thymocytes followed this migration pathway when compared with the heavy traffic of small lymphocytes through venules of rats inoculated with labeled thoracic duct cells. In neonatally thymectomized rats and lymphocyte-depleted rats inoculated with thymocytes, no obvious change was observed in "atrophic" endothelial cells lining postcapillary venules.

Radioactively labeled small thymocytes were found in thymus and bone marrow after the 1st hr. There was a progressive increase of large thymocytes in the intestinal wall, where they were found in the same locations as large lymphocytes.

During the first 24 hr after injection, radioactivity became increasingly concentrated in the cytoplasm of labled small thymocytes in all lymphoid organs, and in some large thymocytes in spleen. No obvious change in intracellular radioactivity was observed in labeled thymocytes in liver or intestine.

Distribution of ^{3}H-Thymidine-Labeled Thymocytes.—The migration of large thymocytes from blood to tissue was studied further by injecting normal rats intravenously with 10^6, ^{3}H-thymidine–labeled thymocytes per gram body weight. In recipients of such inocula, radioactively labeled large thymocytes were found in lung, liver, splenic red pulp, and intestinal wall. Labeled large thymocytes rarely penetrated splenic white pulp and none was found in lymph nodes, thymus, or Peyer's patches.

DISCUSSION

Rats thymectomized at birth show a decrease in small lymphocytes which normally circulate from blood to central lymph through lymphoid tissue. This conclusion emerges from a comparative study of the cellular deficit in lymphoid tissue of neonatally thymectomized rats and adult rats depleted of circulating small lymphocytes by chronic drainage from a thoracic duct fistula. In both cases, there is a decrease in small lymphocytes in splenic white pulp, lymph nodes and Peyer's patches, lymphopenia, and selective impairment of the animal's immunological performance (16, 17, 23, 24). The lymphocyte deficiency and unresponsiveness caused by chronic lymph drainage can be corrected by injections of thoracic duct cells, containing both large and small lymphocytes, or by inocula consisting almost exclusively of small lymphocytes (14–16). The ability of thoracic duct lymphocytes to correct the cellular deficit in rats thymectomized at birth implies that the lymphocyte deficiency, and possibly the unresponsiveness, of such animals is related solely to a decrease in circulating small lymphocytes.

Waksman et al. (24) described discrete areas of lymphocyte depletion in tissues of neonatally thymectomized rats. These areas were the periarteriolar sheaths of splenic white pulp and diffuse lymphocyte fields ("primary lymphoid masses") in the cortex of lymph nodes and Peyer's patches. Recently, Parrott et al. (25) demonstrated similar areas of lymphocyte depletion in tissues of neonatally thymectomized mice. They injected mice intravenously with radioactively labeled spleen cells or thymocytes, and showed that labeled cells localized in "thymus-dependent" areas of spleen and lymph nodes. The present experiments corroborate the presence of thymus-dependent areas in lymphoid tissue of rats. In addition, they demonstrate that thymus-dependent areas are occupied by members of the circulating lymphocyte pool. Periarteriolar sheaths of splenic white pulp and diffuse lymphocyte fields of lymph nodes and Peyer's patches are severely depleted of small lymphocytes by neonatal thymectomy or chronic lymph drainage—procedures which greatly reduce the number of small lymphocytes in blood and central lymph. Further, intravenously injected small lymphocytes colonize thymus-dependent areas of lymphoid tissue, but rarely localize elsewhere.

There is no satisfactory explanation for segregation of small lymphocytes in lymphoid tissue. Good and his colleagues (2) have suggested that cells in thy-

mus-dependent areas and follicles are members of different cell populations. They hold that delayed hypersensitivity responses and tissue transplanatation reactions are mediated by cells found in thymus-dependent areas whereas antibody-forming cells develop from precursors in lymphoid follicles. The view that two, anatomically separate, cell systems are involved in development of lymphoid tissue and immunological responsiveness receives some support from studies using chickens (26, 27). The thymus has an important role in formation of splenic lymphocytes and development of cellular immunity in the chicken. Another lymphoid organ, the bursa of Fabricius, controls development of lymphoid follicles and the ability to synthesize circulating antibody. It cannot be concluded that similar events underlie development of lymphoid tissue and immunological responsiveness in mammals. Mammals have no bursa. Nevertheless, extirpation experiments in the rabbit (28), and the study of patients with defects in immunological performance (29) suggest that the vermiform appendix, tonsils, and possibly other, gut-associated, lymphoid organs have a bursal role. The only alternative is that follicular lymphocytes and those in thymus-dependent areas are members of the same cell population at a different stage in development. This notion is based on the premise that cells in thymus-dependent areas derive from precursors in lymphoid follicles. However, studies using ^{3}H-thymidine provide no evidence to support this possibility. In the rat, there is no large-scale migration of cells from splenic follicles into the periarteriolar sheath as the follicular lymphocytes divide and differentiate (30).

Small lymphocytes pass in blood to splenic white pulp through gaps in the wall of the marginal sinus. The route followed is identical in normal rats, neonatally thymectomized rats, and rats depleted of small lymphocytes by chronic drainage from the thoracic duct. Studies using the electron microscope have shown that the marginal sinus forms an incomplete barrier between blood and the periarteriolar sheath (31). Small lymphocytes are found frequently in the lumen, and in gaps between endothelial cells lining the sinus wall. The observations imply that small lymphocytes migrate through the sinus wall but they do not indicate the direction of movement. The idea that small lymphocytes pass from sinus to sheath is based on the present study of serial radioautographs. Here it was shown that intravenously injected small lymphocytes, labeled with ^{3}H-5-uridine, "home" into the marginal sinus and other vascular spaces of the marginal zone. Radioactively labeled small lymphocytes traverse the wall of the marginal sinus and enter the periarteriolar sheath where large numbers accumulate. The marginal sinus receives blood from capillaries within the follicle (32, 33). The possibility cannot be excluded that a few small lymphocytes enter the sheath through the wall of follicular capillaries, although the present experiments provide no evidence that they do.

The fact that many (perhaps all) small lymphocytes enter splenic white pulp via the marginal sinus has important functional implications. In the rat spleen, the marginal zone contains many cells shown to be phagocytic by their ability

to engulf ^{125}I-labeled *Salmonella* antigens (34) and antigenically inert material (33, 34). Passage of small lymphocytes through the marginal zone could provide an opportunity for interaction of small lymphocytes with phagocytic cells. Fishman (35–38) has suggested that such an interaction may occur at an early stage in primary immune responses.

There is little doubt that small lymphocytes also migrate from splenic white pulp to blood but the route followed is unknown. In the rat, chronic lymph drainage depletes the periarteriolar sheath of small lymphocytes (14, 16). Ford and Gowans (39) studied the movement of small lymphocytes through isolated, perfused rat spleens. When spleens were perfused with lymphocyte-deficient blood, approximately 6×10^7 lymphocytes/hr were added to the venous effluent. The flow of lymphocytes from spleen to blood was associated with rapid depletion of small lymphocytes in the periarteriolar sheath. No obvious depletion occurred in spleens perfused with blood containing a normal concentration of small lymphocytes.

Gowans and Knight (20) have shown that rat small lymphocytes normally circulate from blood to central lymph along a route which includes postcapillary venules in the cortex of lymph nodes and Peyer's patches. The present experiments demonstrate that neither the ability of small lymphocytes to colonize lymphoid tissue nor their affinity for endothelium of postcapillary venules are thymus-dependent phenomena. ^{3}H-5-uridine–labeled small lymphocytes injected intravenously cross the wall of postcapillary venules in neontally thymectomized rats as efficiently as they do in normal rats.

The movement of small lymphocytes across postcapillary venules is reflected in the appearance of the endothelium. Postcapillary venules in the rat and other mammalian species, including man, normally are lined by endothelial cells which have abundant cytoplasm (20). In contrast, endothelial cells of post capillary venules in rats thymectomized at birth have little cytoplasm; and the cytoplasm lacks its normal affinity for pyronin. Similar changes are observed in postcapillary venules of rats depleted of lymphocytes by thoracic duct drainage, and in patients with thymic alymphoplasia (unpublished observations). Results of the present experiments suggest that the endothelial changes are related to a lack of circulating small lymphocytes. Thus, endothelial cells lining the venules in neonatally thymectomized rats and lymphocyte-depleted rats revert to normal within 24 hr after the animals are injected intravenously with thoracic duct lymphocytes from normal, syngeneic donors.

Burwell (40) studied vascular changes in lymph nodes draining first-set bone allografts in the rabbit. He found that endothelial cells of postcapillary venules have an increased amount of intensely pyroninophilic cytoplasm. It is tempting to speculate that changes in endothelium were caused by *increased* traffic of small lymphocytes through the vessel wall, and not by antigenic stimulation as Burwell suggested.

Rat small lymphocytes follow a unique pathway through the wall of post-

capillary venules in lymph nodes of normal rats (41). The lymphocytes do not penetrate endothelial junctions as other leukocytes do in venules of inflamed lymph nodes. The lymphocytes pass through cytoplasm of endothelial cells. Perhaps, in lymphocyte-deficient rats, and in antigen-stimulated rabbits, the observed changes in endothelium are related to the operation of a mechanism for transporting small lymphocytes across the vessel wall. Another possibility is that the changes reflect synthesis of membranous material to replace that stripped away by small lymphocytes as they penetrate and traverse endothelial cells.

Intravenously injected thymocytes, like lymphocytes, emigrate from blood to lymphoid tissue. However, only a few radioactively labeled small thymocytes enter splenic white pulp via the marginal sinus when compared with the heavy traffic of small lymphocytes across the sinus wall. Similarly, small thymocytes do not show the same affinity as small lymphocytes for endothelium of postcapillary venules in lymph nodes and Peyer's patches. In fact, radioautographic studies and experiments which measure tissue radioactivity suggest that approximately 10 times as many small lymphocytes as small thymocytes injected intravenously circulate from blood to thoracic duct lymph through lymph nodes in the intestinal lymphatic bed.[3]

It should be emphasized that the fate of intravenously injected thymocytes provides no information about the flow of cells from the intact thymus. The idea that cells emigrate from thymus to lymph nodes in guinea pigs (42, 43), mice (44), and rats (45) is based on the results of studies using ^{3}H-thymidine and chromosome markers. If emigration does occur, the present experiments suggest that the migrant cells are morphologically identical to small lymphocytes and have at least one of their properties—the abilty to localize in thymus-dependent areas of lymphoid tissue.

SUMMARY

The cellular deficit in rats thymectomized at birth is primarily one of circulating small lymphocytes. The lymphocyte deficiency is similar to that induced in adult rats by chronic drainage from a thoracic duct fistula. In both cases, the animals show a reduction of small lymphocytes in peripheral blood, thoracic duct lymph, and in circumscribed areas of lymphoid tissue. The lympocyte deficiency in lymphoid tissue can be corrected by an intravenous injection of thoracic duct lymphocytes. The evidence suggests that the deficiency is corrected by small lymphocytes.

Small lymphocytes pass from blood to lymphoid tissue along a route which includes the marginal sinus in splenic white pulp and postcapillary venules in the cortex of lymph nodes and Peyer's patches. Neither the ability of small

[3] Goldschneider, I., and D. D. McGregor. Migration of lymphocytes and thymocytes in the rat. II. Recirculation of lymphocytes and thymocytes from blood to lymph. To be published.

lymphocytes to colonize lymphoid tissue nor their ability to traverse postcapillary venules are thymus-dependent phenomena. However, movement of small lymphocytes across postcapillary venules appears to modify the structure of endothelium.

Intravenously injected small thymocytes migrate to lymphoid tissue in smaller numbers than small lymphocytes inoculated by the same route. The few thymocytes which localize in lymphoid tissue follow the same pathway as circulating small lymphocytes.

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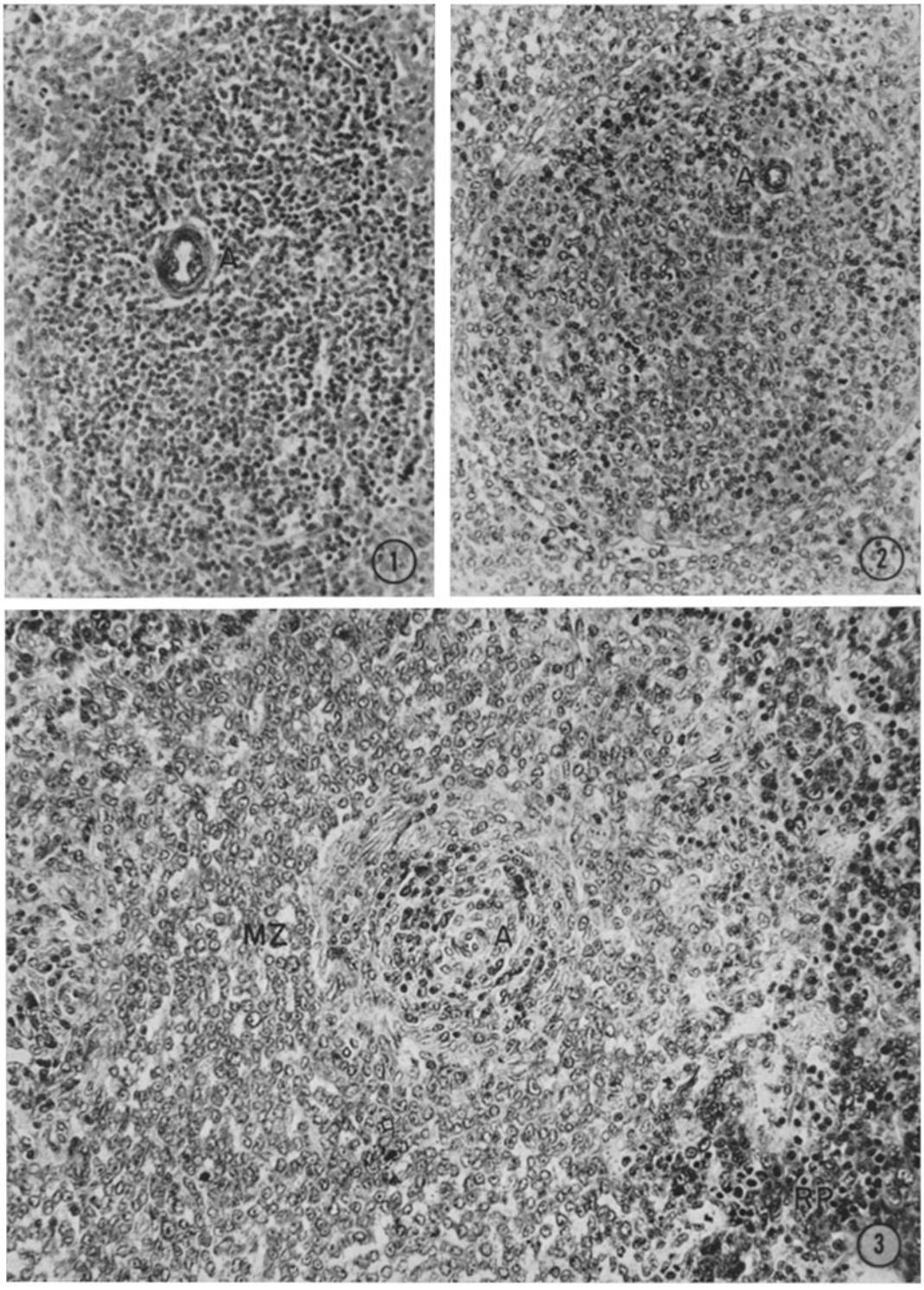
EXPLANATION OF PLATES

PLATE 23

FIG. 1. Spleen from a normal rat. The central arteriole (*A*) in the white pulp is enveloped by a sheath containing many small lymphocytes. Methyl green–pyronin. × 225.

FIG. 2. Spleen from a rat after 8 days of drainage from a thoracic duct fistula. The lymphocyte sheath is severely depleted of small lymphocytes, and several large, pyroninophilic cells are present around the central arteriole (*A*). Methyl green–pyronin. × 215.

FIG. 3. Spleen from a rat thymectomized at birth. The lymphocyte sheath is reduced to a narrow cuff of small lymphocytes around the central arteriole (*A*). The prominent marginal zone (*MZ*), between red pulp and white pulp, contains concentric sinuses and reticular cells. Many mature and immature plasma cells are located in the red pulp (*RP*). Periodic acid–Schiff. × 355.



(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

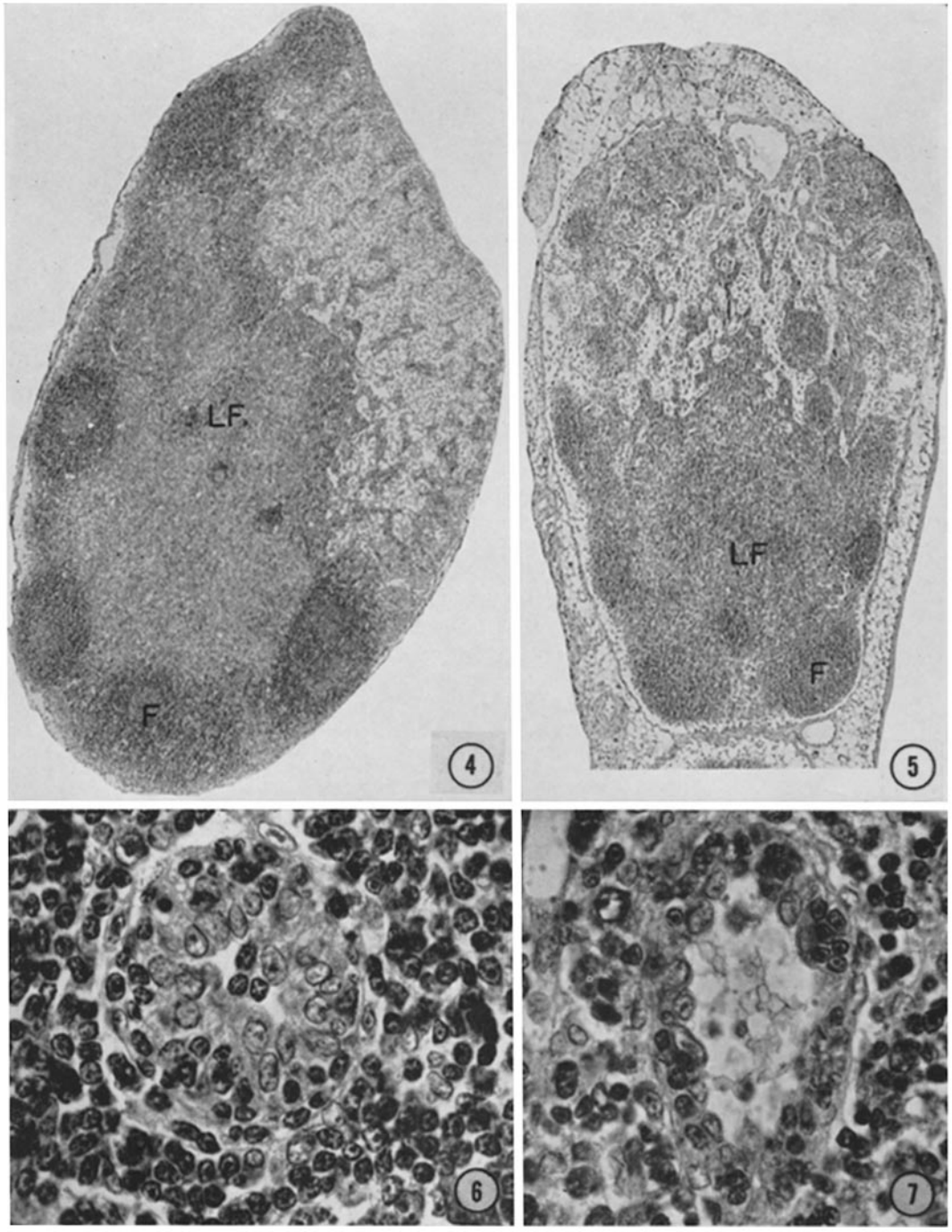
PLATE 24

FIG. 4. Mesenteric lymph node from a rat thymectomized at birth. The diffuse lymphocyte field (*LF*), deep in the cortex, is severely depleted of small lymphocytes. In contrast, the superficial zone, containing lymphoid follicles (*F*), and a narrow band of tissue between cortex and medulla contain a normal number of lymphocytes. Methyl green–pyronin. × 55.

FIG. 5. Mesenteric lymph node after 8 days of drainage from a thoracic duct fistula. There is a reduction of small lymphocytes in the diffuse lymphocyte field (*LF*) but no reduction of lymphocytes in and around lymphoid follicles (*F*). Methyl green–pyronin. × 55.

FIG. 6. Postcapillary venule in mesenteric lymph node from a normal rat. The venule is lined by endothelial cells which have abundant cytoplasm, and there are many small lymphocytes in the lumen and wall. Methyl green–pyronin. × 740.

FIG. 7. Postcapillary venule in mesenteric lymph node from a rat thymectomized at birth. Endothelial cells lining the venule are smaller than normal. There is a reduction of small lymphocytes in the vessel wall, and in the surrounding cortex of the node. Methyl green–pyronin. × 740.



(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

FIGS. 8–13 (Plates 25 and 26). Radioautographs of sections through splenic white pulp of neonatally thymectomized rats and rats depleted of small lymphocytes by drainage from a thoracic duct fistula for 8 days. The animals were injected intravenously with 10^6, ^{3}H-5-uridine–labeled thoracic duct cells per gram body weight. Exposure, 8 days.

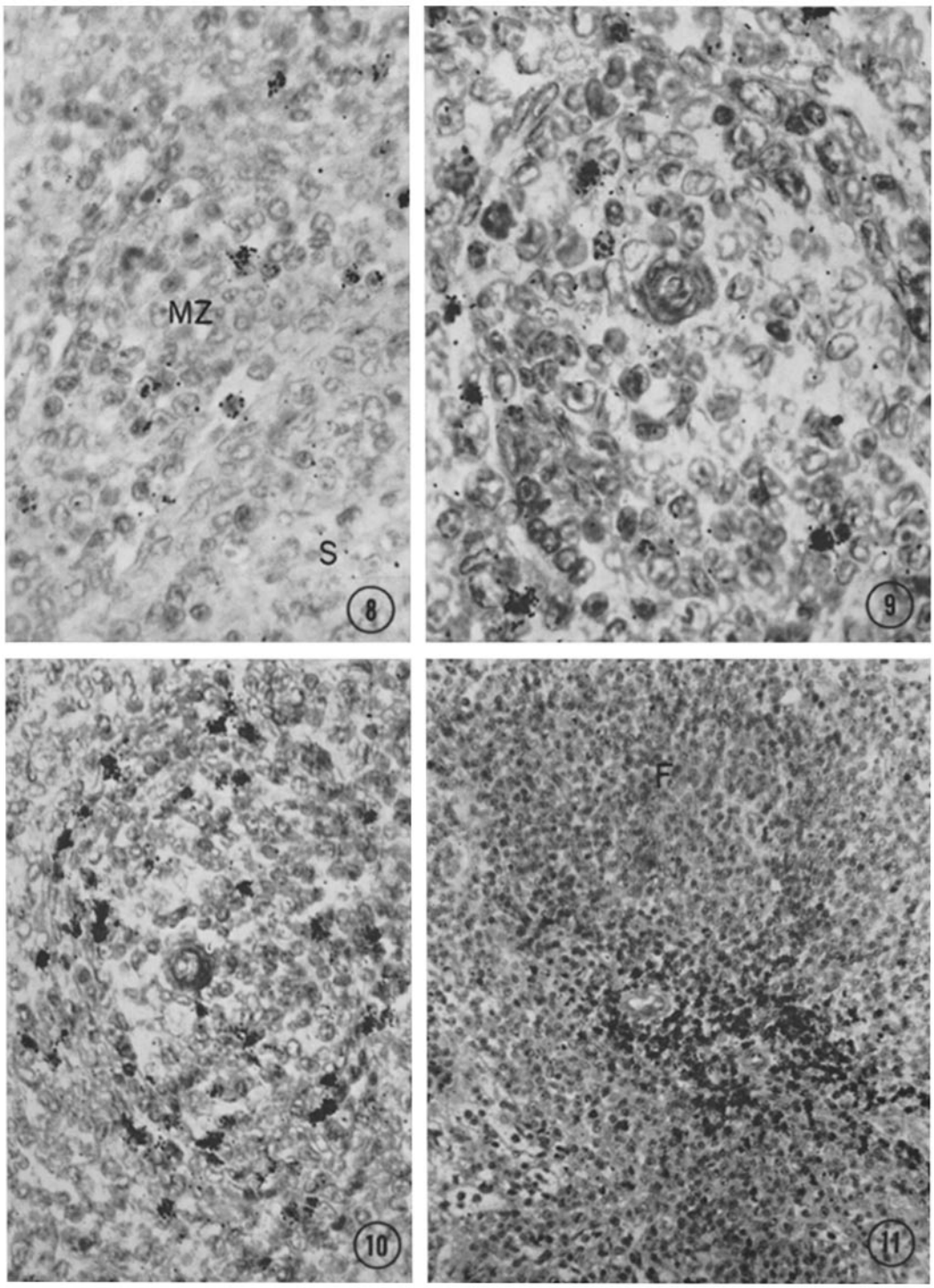
PLATE 25

FIG. 8. 10 min after inoculation, radioactively labeled small lymphocytes are confined to the marginal sinus and other vascular spaces of the marginal zone (*MZ*). No labeled cells have penetrated the periarteriolar sheath (*S*). × 600.

FIG. 9. A few radioactively labeled small lymphocytes have moved from the marginal sinus into the periarteriolar sheath 20 min after inoculation. × 750.

FIG. 10. Many radioactively labeled small lymphocytes are present in the marginal sinus and adjacent region of the periarteriolar sheath 30 min after inoculation. There are only a few labeled lymphocytes deep in the sheath. × 450.

FIG. 11. By 3 hr, radioactively labeled small lymphocytes have congregated around the central arteriole. No labeled lymphocytes have penetrated the lymphoid follicle (*F*). × 225.



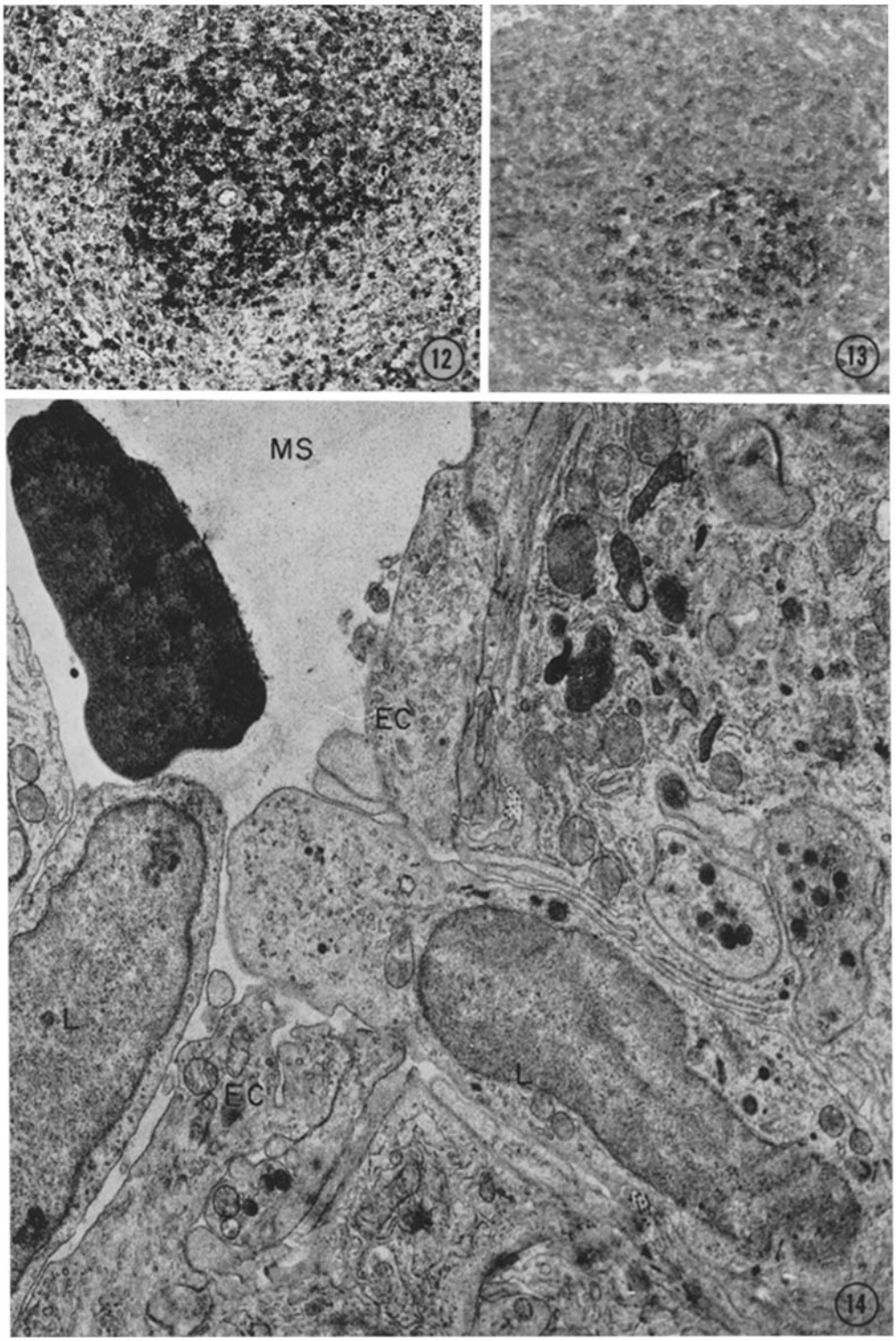
(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

PLATE 26

FIG. 12. 6 hr after inoculation, the periarteriolar sheath is uniformally populated with radioactively labeled small lymphocytes. × 205.

FIG. 13. Radioactively labeled small lymphocytes have decreased in the periarteriolar sheath 18 hr after inoculation. × 205.

FIG. 14. Electron micrograph of a section through the marginal zone of the spleen from a lymphocyte-depleted rat. The animal was inoculated intravenously with 10^6, thoracic duct cells per gram body weight and sacrificed 10 min later. The marginal sinus (*MS*) contains two small lymphocytes (L) and a red blood cell. One of the lymphocytes is traversing a gap between endothelial cells (*EC*) lining the sinus wall. × 10,320.



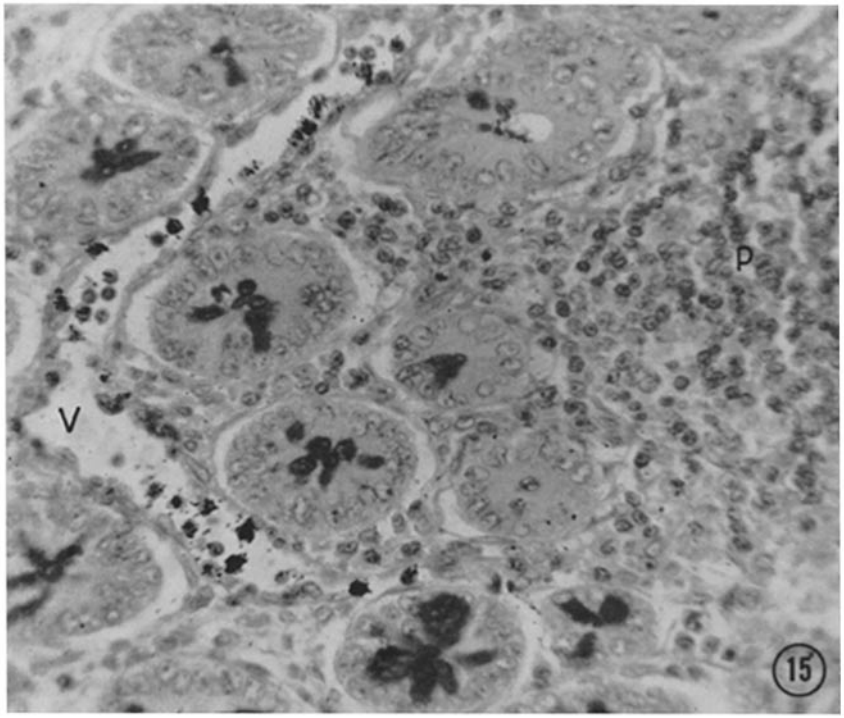
(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

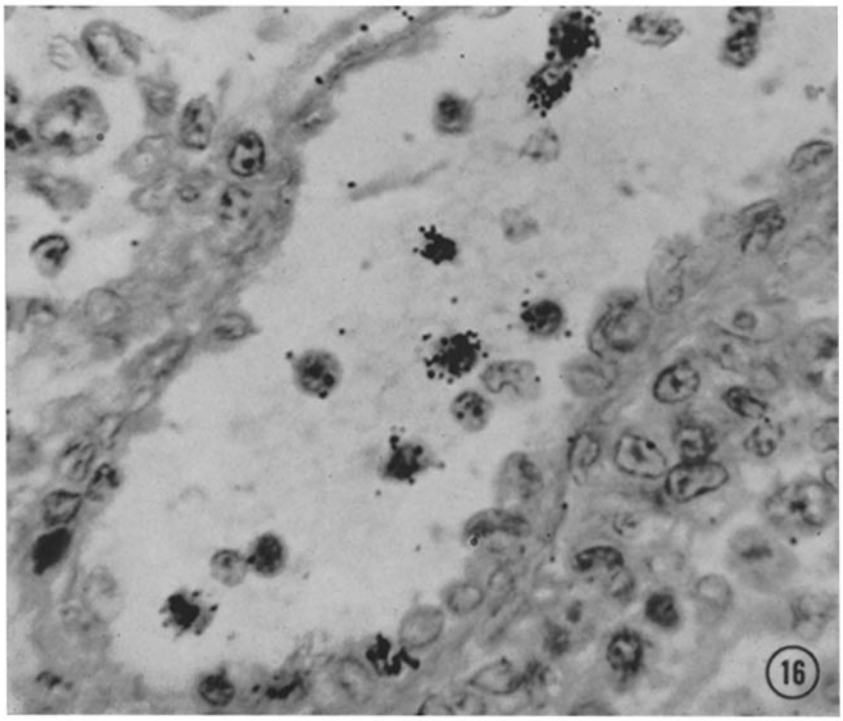
FIGS. 15–20 (Plates 27–29). Radioautographs of tissue sections from neonatally thymectomized rats and rats depleted of small lymphocytes by drainage from the thoracic duct for 8 days. The animals were inoculated intravenously with 10^6, ^{3}H-5-uridine–labeled thoracic duct cells per gram body weight. Exposure, 8 days.

PLATE 27

FIG. 15. Peyer's patch (*P*) in wall of terminal ileum of a lymphocyte-depleted rat 10 min after injection. Radioactively labeled small lymphocytes are confined to the lumen and wall of a dilated postcapillary venule (*V*) × 575.

FIG. 16. Postcapillary venule in mesenteric lymph node from a lymphocyte-depleted rat 10 min after injection. The venule, containing labeled and unlabeled small lymphocytes, is lined by endothelial cells which have relatively little cytoplasm. × 1500.



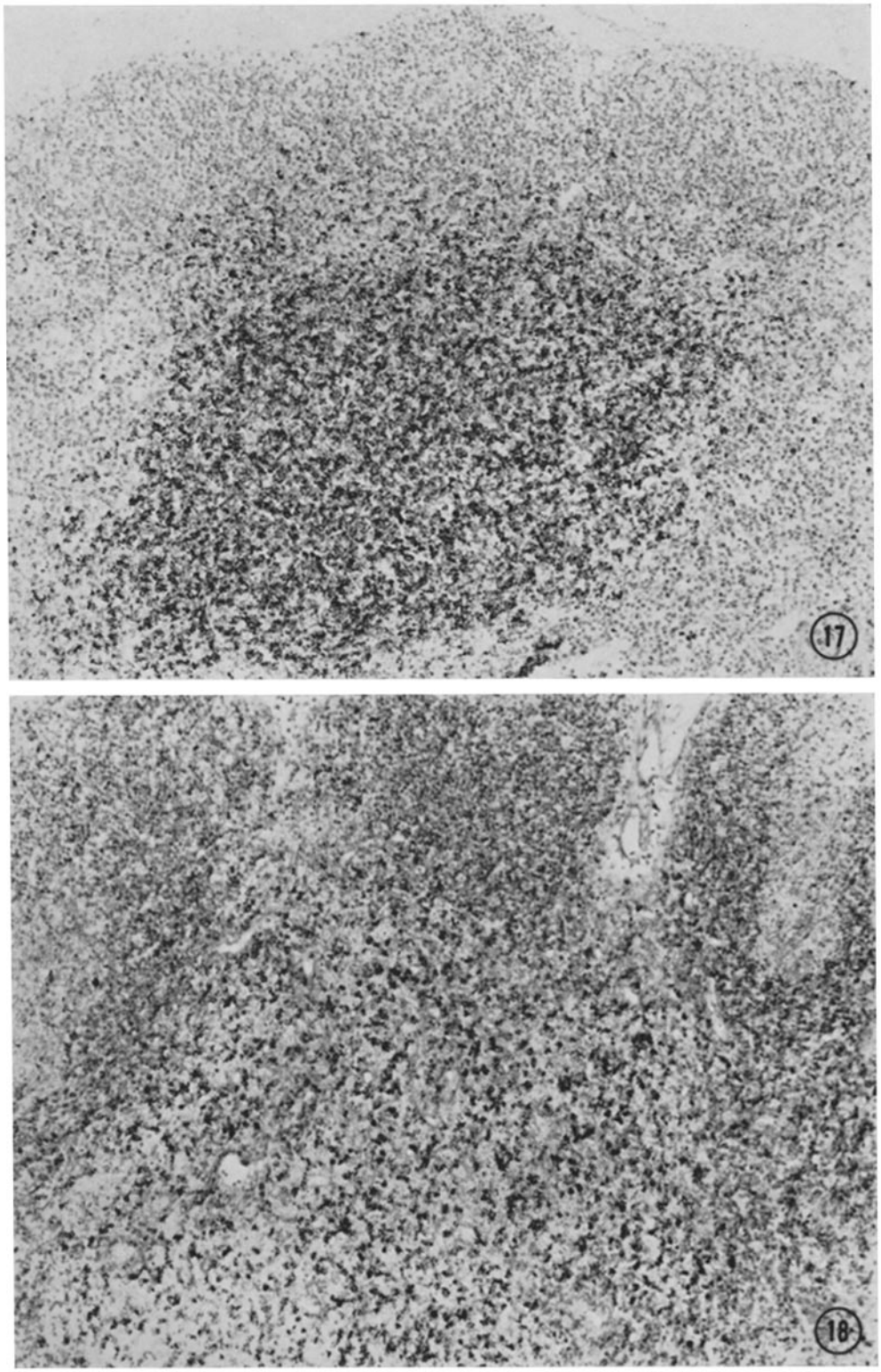


(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

PLATE 28

FIG. 17. Section through cortex of mesenteric lymph node from a lymphocyte-depleted rat 48 hr after injection. Radioactively labeled small lymphocytes have migrated in large numbers into the diffuse lymphocyte field, whereas only a few labeled lymphocytes have penetrated the superficial cortex. × 133.

FIG. 18. Section through cortex of mesenteric lymph node from a rat thymectomized at birth. The animal was sacrificed 48 hr after injection. Radioactively labeled small lymphocytes are confined almost exclusively to the diffuse lymphocyte field. × 133.



(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)

PLATE 29

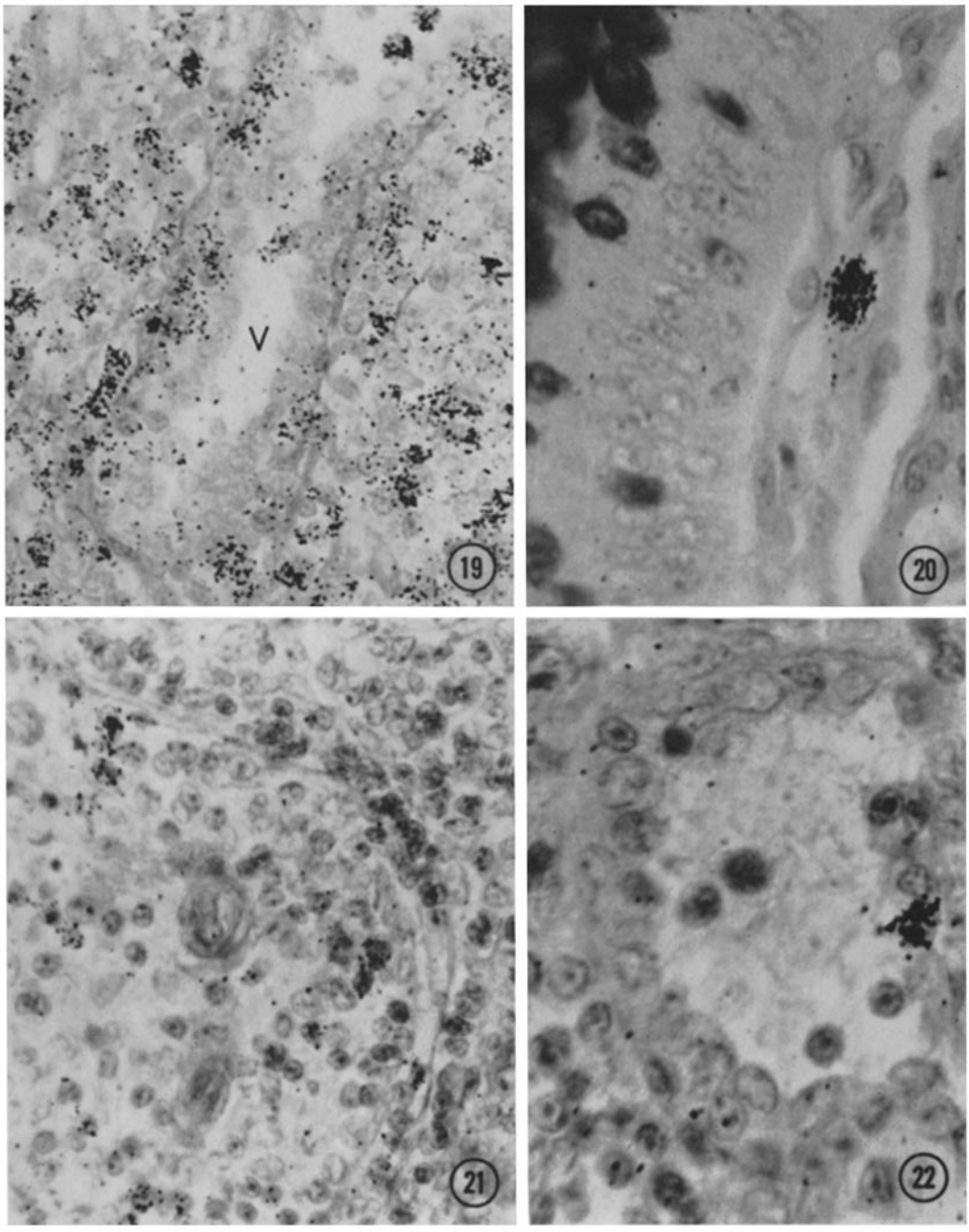
FIG. 19. Section through diffuse lymphocyte field of mesenteric lymph node from a lymphocyte-depleted rat 24 hr after injection. Endothelial cells lining a large postcapillary venule (*V*) have abundant cytoplasm. Many radioactively labeled small lymphocytes are present in the vessel lumen and wall, and in the adjacent cortex of the node. × 620.

FIG. 20. Section through wall of terminal illeum of a neonatally thymectomized rat 24 hr after injection. A radioactively labeled large lymphocyte is located in connective tissue beneath the intestinal epithelium. × 1100.

FIGS. 21 and 22. Sections through lymphoid tissue from neonatally thymectomized rats injected intravenously with 10^6, ^{3}H-5-uridine–labeled thymocytes per gram body weight.

FIG. 21. Splenic white pulp 3 hr after injection. A few radioactively labeled small thymocytes are present in the marginal sinus and periarteriolar sheath (*S*) × 600.

FIG. 22. Postcapillary venule in mesenteric lymph node 30 min after injection. A labeled small thymocyte lies against an "atrophic" endothelial cell lining the vessel wall. × 1775.



(Goldschneider and McGregor: Migration of lymphocytes and thymocytes)