MAGNITUDE AND PATTERN OF THYMIC LYMPHOCYTE MIGRATION IN NEONATAL MICE*

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The thymus in mammals is essential for the development and maintenance of the lymphoid system throughout the body and for full expression of immune responsiveness (1–3). There is increasing evidence that thymic function relies upon properties of the thymic stroma which provide for optimal proliferation and differentiation of hematogenous precursor cells into immunocompetent lymphocytes (4–6). The ultimate fate of thymic lymphocytes, however, still is a matter of controversy. The finding that a considerable fraction of lymphocytes in peripheral lymphoid tissues of mice, like thymic lymphocytes, have the θ-antigen on their surface (7–9), although highly suggestive of, does not offer definite proof for the thymic origin of these elements. The possibility has not been excluded that (a) some lymphocytes might acquire the θ-antigen without actually passing through the thymus or, (b) thymus-derived cells lose the θ-antigen during further differentiation in peripheral organs. Therefore, it should be postulated that studies on thymic cellular migration be based on stable cell markers such as radioactively labeled specific DNA precursors or marker chromosomes. The use of the latter necessitates the creation of artificial chimeras by cell or organ transfer from one animal to another and, although it is well suited for demonstrating peripheralization of lymphocytes from thymic grafts (10, 11), it does not necessarily reflect the physiological situation. In addition, identification of thymus-derived migrants with this method is restricted to cells in mitosis.

Although thymic cell emigration has been shown by regional labeling of the thymus with tritiated thymidine, followed by radioautographic and/or radiochemical analysis of peripheral lymphoid organs (12–16), the magnitude of this process is still disputed. It has been postulated that an overwhelming majority of thymic lymphocytes disintegrate within the thymus (17). Kinetic studies on mice, however, have provided support for an extensive migration of lymphoid cells from the thymus to peripheral lymphoid organs in the neonatal period (18–20). Experiments in the calf utilizing both continuous intra-arterial infusion of the thymus with tritiated thymidine and thymus-specific antigen as markers have indicated that a substantial peripheralization of thymic lymphocytes is maintained beyond the neonatal period (21).

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Additional studies on the magnitude and pattern of migration of thymic lymphocytes are particularly desirable in neonatal animals since it is at this time, or before, that thymectomy is most effective in suppressing immune responsiveness. Newborn mice are especially suited for experiments of this type since the peripheral lymphoid organs are poorly developed at birth and contain relatively few lymphoid cells (22). Within 2 or 3 days of postnatal life, however, there is a marked increase in the number of lymphocytes in these tissues. Kinetic studies suggested that during this same developmental period a large number of cells leave the thymus (19). In the present study a modified technique of local labeling of the thymus was used in neonatal mice and the migration of labeled cells to spleen, Peyer's patches, lymph nodes, and bone marrow was followed with time. The results provide direct evidence for the thymic origin of the majority, or in certain tissues at least a large fraction, of lymphocytes which appear within the peripheral lymphoid organs during the first few days of life. It is also demonstrated that during this period, i.e. when the bacterial flora is developing in the intestinal tract, lymphocytes of thymic origin enter preferentially the gut-associated lymphoreticular tissues and do not migrate in appreciable numbers to the bone marrow.

Materials and Methods

Animals.—Charles River albino mice of both sexes (Charles River Breeding Laboratories, Wilmington, Mass.) 18–30 hr of age and weighing 1.8–2.2 g each (mean thymic weight 6.2 mg), were used for intrathymic injection of thymidine-3H. After the labeling procedure the mice were returned to their dams until sacrificed.

Method of Regional Labelling.—Mice were anesthetized with ether, and with the aid of a stereomicroscope, the thymus was exposed as previously described (23). Tritiated thymidine (thymidine-3H, 1 1.8 Ci/µmole, New England Nuclear Corporation, Boston, Mass., diluted with physiologic saline to contain 0.02 µCi/µl) was injected subcapsularly into the frontal portion of both thymic lobes (0.5 µl/lobe) with specially prepared, calibrated glass microneedles measuring approximately 25 µ outer diameter. Based on body weight, the amount of thymidine-3H used was 0.01 µCi/g. Injection time was 2–3 min. A small amount of Evan's blue was added to the thymidine solution to visualize intrathymic injection. The incision was closed as reported earlier (23). Animals in which leakage of dye at the point of needle insertion and/or in which visible thymic damage was noticed were excluded from the experiment. Newborns, which after the injection procedure, did not exhibit weight gains comparable to a group of nonoperated mice were also discarded.

Histological and Radioautographic Procedure.—Groups of 10 mice were sacrificed at 1 hr, 2 hr, 1, 2, 3, 5, and 7 days after intrathymic injection of thymidine-3H. The thymus, spleen, mesenteric lymph node, Peyer's patches, popliteal lymph node, and bone marrow were fixed in Bouin's solution, sectioned at 5 µ, and processed for radioautography using NTB-2 emulsion (Eastman Kodak Co., Rochester, N. Y.). The sections were exposed for 57 days (4°C) and stained with nuclear fast red.

The distribution of labeled cells in the various tissues was determined as follows:

Correction for Background and Systemic Labeling.—To correct for background and systemic labeling which might have resulted from the passage of thymidine-3H into the blood during injection, grains were counted over proliferating splenic erythroblasts and intestinal crypt

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1 Abbreviation used in this paper: Thymidine-3H, tritiated thymidine.
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An average of only 1 in 500 of these cells was covered by four grains, with the vast majority showing no label throughout the observation period. Based on these counts, cells with four or more grains over the nucleus were considered to have been labeled in the thymus.

Thymus.—Radioautographic studies with thymidine-3H in newborn mice indicated that thymic lymphoid cells migrate from the outer cortical zone to the corticomedullary junction from where most of them apparently disappear (24). The labeling index in the cortex was, therefore, determined by counting cortical lymphocytes near the corticomedullary junction. Since local injection of thymidine-3H did not result in a uniform distribution of labeled cells throughout the thymus, cortical lymphocytes were counted all around the medulla on cross sections taken from at least three different areas, i.e., apical, middle, and caudal parts of each thymic lobe. A separate labeling index was established for the medulla. An average of 1200 lymphocytes were counted per thymus.

Peripheral Lymphoid Tissues.—Labeling indices were determined from counts made along several straight line paths set at equal distance and covering each lymphoid tissue. In Peyer’s patches the lines were directed from the epithelium to the serosal surface; in lymph nodes, from the subcapsular sinus to the medullary sinus; and in the spleen, from the central arteriole to the outer edge of the lymphoid nodule. A minimum of 500 lymphocytes were counted per tissue per animal.

Statistical Evaluation.—The difference of two means was examined by the t test and was considered to be significant with P values equal to or smaller than 0.05.

Histology.—For histological evaluation of changes in peripheral lymphoid organs during the neonatal period, tissues were fixed in glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon. Semithin sections (1 μ) were cut with an ultramicrotome and stained with Giemsa.

RESULTS

Thymus.—Except for mice sacrificed 5 and 7 days after intrathymic injection of thymidine-3H, labeled cells were observed in all sections of thymus although their distribution was dependent upon the position of the microneedle. 1 and 2 hr after injection of the radioactive precursor the highest concentration of labeled cells was in the outer cortical zone, however, some heavily labeled elements were also seen in the inner cortex (Fig. 1 a). Cells labeled initially were by comparison larger than the vast majority of unlabeled lymphocytes in the mid- and inner cortex. By 24 hr postinjection, and especially by 48 hr, labeled lymphocytes of all sizes were distributed throughout the thymic cortex (Fig. 1 b). Labeling indices for cortical lymphocytes near the corticomedullary junction and medullary lymphocytes are shown in Fig. 2 and Table I as a function of time after injection of thymidine-3H. There was a steady increase in the over-all labeling index of lymphocytes in the innermost zone of the cortex reaching a peak of 13 % on day 2. Thereafter, the per cent of labeled lymphocytes decreased rapidly. The labeling index of lymphocytes in the medulla, which was initially higher than that of inner cortical lymphocytes, fell sharply between days 1 and 2, and on day 2 was significantly lower (P < 0.01) than that of cortical lymphocytes. From day 2 to day 7 the labeling index of medullary lymphocytes decreased slowly. At no time interval after labeling did we observe a relative increase in the number of pyknotic nuclei or nuclear fragments in the thymus.

Peripheral Lymphoid Tissues.—Grain counts on proliferating erythroblasts in
the spleen and intestinal crypt cells remained low throughout the experiment, indicating minimal systemic labeling either through leakage of thymidine-3H during injection or through release and reutilization of label after the death of labeled cells. No labeled cells (after correction for background and systemic labeling) were detected in any of the peripheral lymphoid tissues 1 and 2 hr after intrathyamic injection of thymidine-3H (Figs. 3 a, 4 a, and 5 a). Thereafter, consid-

![Image](image.jpg)

**Fig. 1.** Labeled areas of the thymic cortex after intrathyamic injection of thymidine-3H indicating migration of lymphoid cells from the subcapsular zone (C) to the corticomedullary junction (M). (a) 2 hr after labeling. (b) 2 days after labeling; note that most labeled cells have moved toward the corticomedullary junction and, because of repeated divisions, have reduced grain counts as compared to (a). (Radioautographs.) × 400.

cerable numbers of labeled lymphocytes were observed in all peripheral lymphoid organs at all times.

In the strain of mice used in this study, the Peyer's patches at birth had the histological appearance of primitive lymphoid organs containing a large proportion (up to 50%) of reticular, endothelial, and histiocytic cells. Within 1-3 days after birth, however, the number of lymphocytes increased markedly so that by day 4 they constituted 90-95% of the cells present. Labeling indices of Peyer's patch lymphocytes are shown in Fig. 6 and Table I. 2 days after intrathyamic injection of thymidine-3H numerous labeled lymphocytes were found throughout the Peyer's patches (Fig. 3 b) as well as within the epithelium cover-
Fig. 2. Per cent labeled lymphoid cells in the thymic cortex near the corticomedullary junction (TC) and in the thymic medulla (TM) at various time intervals after a subcapsular, intrathymic injection of thymidine-3H in newborn mice.

TABLE I

Labeling Pattern of Lymphocytes in the Thymus and in Peripheral Lymphoid Tissues of Newborn Mice at Various Time Intervals after Intrathymic Injection of Thymidine-3H

| Time interval and corresponding labeling pattern | Thymic cortex (CMJ) | Thymic medulla | Mesenteric lymph node | Peyer's patch | Spleen follicle | Popliteal lymph node |
|-------------------------------------------------|---------------------|----------------|-----------------------|---------------|----------------|---------------------|
| 1 hr                                            |                     |                |                       |               |                |                     |
| Per cent labeled                                | 7.3 ± 4.2*          | 15.3 ± 5.9*    | 0                     | 0             | 0             | 0                   |
| Mean grain count                                | 11.3 ± 2.5          | 12.3 ± 3.2     |                       |               |                |                     |
| 1 day                                           |                     |                |                       |               |                |                     |
| Per cent labeled                                | 11.8 ± 5.6*         | 15.0 ± 7.0*    | 6.5 ± 1.4‡           | 6.6 ± 2.2†    | 2.2 ± 1.1      | 2.7 ± 1.2           |
| Mean grain count                                | 7.6 ± 1.0*          | 10.1 ± 1.9     | 10.0 ± 2.1‡          | 10.1 ± 1.1    | 10.4 ± 2.8     | 11.4 ± 1.5          |
| 2 days                                          |                     |                |                       |               |                |                     |
| Per cent labeled                                | 13.2 ± 4.2*         | 6.6 ± 1.8      | 9.8 ± 2.4‡           | 8.1 ± 2.1‡    | 3.4 ± 1.8      | 5.3 ± 1.3           |
| Mean grain count                                | 6.9 ± 0.7‡          | 7.3 ± 0.9      | 8.2 ± 1.3            | 8.5 ± 1.7     | 8.0 ± 1.8      | 8.4 ± 1.3           |
| 3 days                                          |                     |                |                       |               |                |                     |
| Per cent labeled                                | 9.1 ± 4.3*          | 5.7 ± 1.8      | 5.4 ± 1.3            | 4.4 ± 1.5     | 3.1 ± 1.3      | 4.6 ± 1.7           |
| Mean grain count                                | 6.2 ± 0.5*          | 6.8 ± 1.3      | 7.8 ± 1.1            | 7.9 ± 1.2     | 8.5 ± 1.4      | 7.8 ± 1.3           |
| 5 days                                          |                     |                |                       |               |                |                     |
| Per cent labeled                                | 2.0 ± 0.6           | 4.8 ± 1.8      | 3.8 ± 1.2            | 2.6 ± 0.6     | 2.0 ± 0.6      | 2.5 ± 0.9           |
| Mean grain count                                | 5.1 ± 0.4*          | 6.3 ± 0.8      | 7.2 ± 1.1            | 7.5 ± 1.0     | 6.0 ± 1.0      | 7.5 ± 1.4           |
| 7 days                                          |                     |                |                       |               |                |                     |
| Per cent labeled                                | 1.1 ± 0.5           | 2.4 ± 1.9      | 2.5 ± 1.3            | 2.1 ± 0.7     | 1.8 ± 0.5      | 2.1 ± 1.3           |
| Mean grain count                                | 5.3 ± 0.6‡          | 6.3 ± 1.7      | 7.6 ± 1.5            | 8.3 ± 1.5     | 7.4 ± 1.0      | 7.5 ± 1.4           |
| 2 days                                          |                     |                |                       |               |                |                     |
| Per cent thymus-derived cells (corrected)‡      | 100                 | —              | 74                    | 61            | 26            | 40                  |

Values are expressed as mean per tissue ± standard deviations in groups of 10 mice per time interval.

CMJ, thymic cortex near corticomedullary junction.

* Significantly different from corresponding values for lymph nodes, Peyer's patches, and spleen follicles (P < 0.05).

‡ Significantly different from corresponding values for spleen follicles and popliteal lymph nodes (P < 0.05).

§ These are minimum values based on the assumptions that the thymic cortex is the main source of migrants and that unlabeled thymic lymphocytes behaved like labeled elements (see text).
ing these gut-associated lymphoid structures. The labeling index reached a peak of
8% on day 2, decreased quite sharply between days 2 and 3, and then declined
slowly between days 3 and 7. Thus, during the first 3 days postinjection, the
labeling index curve for Peyer's patch lymphocytes paralleled that for thymic
cortical cells. The per cent labeled lymphocytes within the epithelium overlying
the Peyer's patches corresponded to that found in the subepithelial lymphoid
tissue. Labeled mitotic figures were frequently seen, particularly 1 and 2 days
after injection of thymidine-\(^{3}H\). In only rare instances was a labeled migrant
found in the intestinal wall outside the Peyer's patches.

Changes in the lymphoid cell content of the mesenteric lymph nodes were
essentially the same as those described for Peyer's patches, i.e., a marked in-
crease in the proportion of lymphocytes was observed within 2-3 days after
birth. On microscope examination, it was not possible to subdivide the cortex
into mid- (paracortical) and superficial cortical regions, however, a rudimentary
medulla was distinguishable. Within 2 days after intrathymic injection, numer-
ous labeled lymphocytes appeared throughout the entire lymph node cortex.
(Fig. 4 b) although the concentration at this time was higher near the venules. Rarely was a labeled migrant found in the medulla. As shown in Fig. 6 and Table I, the labeling pattern was similar to that seen in Peyer's patches. On day 2 the mean labeling index of lymph node lymphocytes was higher than that of Peyer's patch lymphocytes, however, this difference was not statistically significant. As in Peyer's patches, labeled mitotic figures were frequent.

At birth, the popliteal lymph node was morphologically similar to the mesenteric lymph node. The accumulation of lymphoid cells after birth, however, occurred more slowly. As shown in Fig. 6 and Table I, the percent of labeled lymphocytes in the popliteal lymph nodes at 1 and 2 days postinjection was significantly less ($P < 0.05$) than the percent of labeled lymphocytes in the mesenteric lymph node or Peyer's patches. The labeling index reached a peak on day 2 and then decreased gradually over the next 5 days. By the 3rd day postinjection, the labeling index of lymphocytes in the popliteal lymph node was not significantly different from that in the mesenteric lymph node and Peyer's patches.
The spleen at birth was composed principally of myelopoietic and erythropoietic tissue, however, small, loose lymphocytic follicles surrounding central arterioles were seen throughout the organ (Fig. 5 a). These lymphoid nodules expanded slowly after birth. The magnitude and pattern of lymphocyte labeling in the splenic lymphoid tissue was similar to that in the popliteal lymph node (Figs. 6 and 5 b, Table I). Labeled migrants were seen throughout lymphoid nodules, however, at early intervals the concentration was greater near the central arterioles. An occasional isolated, heavily labeled migrant was seen in areas of myelopoiesis or erythropoiesis.

One parathymic lymph node was sectioned along with the thymus in a mouse sacrificed 3 days postinjection. The labeling index and intensity in this lymph node were comparable to that of the corresponding popliteal lymph node.

Heavily labeled lymphocytes (> 12 grains) persisted in the peripheral lymphoid tissues for the duration of the experiment. On the 7th day postinjection these cells constituted 11–16% of all labeled lymphocytes in the mesenteric nodules.
lymph node, Peyer's patches, spleen, and popliteal lymph node whereas no heavily labeled cells were found in the thymic cortex. In the thymic medulla 5% of the labeled lymphocytes were covered with more than 12 grains. The mean grain counts of labeled lymphocytes in the various lymphoid tissues vs. time after intrathymic injection of thymidine-3H are shown in a semilogarithmic plot in Fig. 7 and in Table I. In all tissues the mean grain count deviated from a simple exponential decrease. In the peripheral lymphoid tissues and thymic medulla very little change in mean labeling intensity occurred after day 3 while the mean grain count in the thymic cortex continued to decrease until day 5 where it began to approach the established correction level of four grains. Mean grain counts of labeled lymphocytes in the peripheral lymphoid tissues were at all time intervals significantly higher \((P < 0.05)\) than those of thymic cortical lymphocytes, and by the second day after injection were higher than the mean grain counts of medullary lymphocytes although the difference was not statistically significant in all instances. There was no significant difference in the mean grain counts of the various peripheral lymphoid tissues. With the exception of a few questionable cells, no labeled lymphocytes were found in the bone marrow cords.

**DISCUSSION**

Peripheral lymphocytes may appropriately be termed "thymus-derived" if it can be shown with the aid of stable cell markers that they, or their progenitor cells, have originated from the thymus. Proof for this migration under physiological conditions in intact animals relies heavily on local, intrathymic labeling with radioactive DNA precursor substances. Some of the possible hazards and
limitations of this procedure have been discussed previously (15). In our experiments, using a glass microneedle of 25 μ outer diameter and slow infusion of thymidine-³H into the subcapsular zone of the thymic cortex, minimal mechanical disruption and no detectable leakage was observed in animals used for evaluation. No evidence for increased intrathymic cell death, i.e. pyknotic nuclei, was found during the course of the experiment, provided that only mice which exhibited normal weight gains were included. Possible effects of stress such as cellular disintegration within the thymus and/or increased lymphocyte migration from the thymus, if they occurred, appeared to be negligible since both size and lymphocyte content of the thymus as well as of peripheral lymphoid organs of experimental animals were indistinguishable from those of controls.

In view of possible radiotoxic effects, the dose of thymidine-³H used for local injection was kept low. The initial mean grain count per labeled lymphoid cell in the thymus after an exposure time of 57 days was approximately 12. Although the mean labeling intensity of labeled lymphoid cells in the immediate area of injection was higher by a factor of 2, this value compares favorably with

Fig. 7. Mean grain counts per labeled lymphoid cell in the thymic cortex, thymic medulla, lymph nodes, Peyer's patches, and spleen follicles at various time intervals after intrathymic injection of thymidine-³H in newborn mice (for symbols see legend to Figs. 2 and 6).
mean grain number over initially labeled elements in the thymic cortex found after systemic labeling with 1 $\mu$Ci of thymidine-3H (specific activity 1.9 Ci/mmole) per gram body weight and 51 days radioautographic exposure time (19). This latter dose is five times smaller than the dose known to cause an increase in the relative number of pyknotic labeled lymphocytes in the circulating blood of young adult rats (25). It should also be noted that the doses of thymidine-3H, in terms of radioactivity per unit weight of thymus, used by other authors (12, 14, 15) for intrathymic injection were from 16 to 80 times higher than in the present experiment. In order to prevent reutilization of label, Weissman (14), in his study on thymus cell migration in newborn rats, in addition to an intrathymic injection of 10 $\mu$Ci of thymidine-3H, gave the animals an immediate intravenous injection 1 mg of cold thymidine supplemented by subcutaneous doses of up to 10 mg daily until sacrifice. These doses of cold nucleoside are apt to be toxic (26). It remains to be examined if and to what extent earlier estimates on the magnitude of thymic lymphocyte emission and increased intrathymic cell death after injection were influenced by the factors mentioned above.

The highest values reported for the proportion of radioactively labeled, thymus-derived cells in peripheral lymphoid organs of newborn animals relate to the mesenteric lymph nodes of rats and vary between 9 and 19% (14). The present results provide direct evidence for more extensive migration in newborn mice of cells from the thymus to the same peripheral tissue. On day 2 after intrathymic injection of thymidine-3H the mean labeling index of lymphocytes in the mesenteric lymph node and gut-associated lymphoid tissue (Peyer's patches) was 61–74% of that in the thymus. Assuming that nonlabeled lymphocytes migrated in the same manner as labeled lymphocytes, it can be concluded that the majority of lymphocytes present within these peripheral lymphoid tissues were of thymic origin. The origin of lymphocytes already present at these sites before labeling cannot be determined by the present data, however, there is evidence that thymic cell migration in mice begins before this time (18, 24, 27). Consequently, there is a definite possibility that virtually all lymphocytes present in the peripheral lymphoid tissue during this early stage of development are thymus-derived. The reason why thymic lymphocyte peripheralization has previously been underestimated may in part relate to the fact that most other investigations of this type have been performed at a time when there are large numbers of lymphocytes already present in the peripheral tissues. In addition, the postulate to avoid the toxic effects of locally injected radioactive DNA precursor necessitates the use of rather small doses of thymidine-3H and, therefore, requires careful radioautographic analysis which includes weakly labeled cells. This is exemplified in Fig. 1 where the difference can be clearly seen between the labeling intensity of initially labeled cells in the outer cortical zone 2 hr postinjection and the labeling intensity of cortical lymphocytes near the corticomedullary junction 2 days later. Grain counts in peripheral lymphoid
organisms should be compared with those in the innermost areas of the thymic cortex since it is from this zone that cells appear to leave the organ, and they should not be restricted to "heavily labeled" lymphocytes. It is also important to consider that the relative proportion of labeled cells in the periphery depends upon the time lapse between intrathymic injection of thymidine-\(^{3}H\) and sampling.

The region of origin within the thymus of the labeled migrants is not completely clear from the present results. Labeling indices in the peripheral lymphoid organs, particularly those of the gut-associated tissues, paralleled those of cortical thymic lymphocytes suggesting the cortex as the major source of migrants. This interpretation is in accord with earlier suggestive evidence indicating that in fetal and newborn mice lymphocytes migrate from the outer cortex to the corticomedullary junction and then emigrate (24). It should also be considered that in the thymus of newborn mice, the cortex contains approximately eight times more lymphocytes than the medulla. This does not exclude the possibility that lymphocytes initially labeled in the medulla also contribute to the emigrating population.

The magnitude of migration of labeled thymic cells to the spleen and popliteal lymph node was significantly less \((P < 0.05)\) as compared to the mesenteric lymph node and Peyer's patches. This was paralleled by histological evidence of a less rapid expansion of the lymphoid cell population in the spleen and popliteal lymph node. This difference seems to depend largely on antigenic stimulation from the intestinal flora which develops during the first days of life. Miller (1969) injected 18-day old mouse fetuses with thymic lymphocytes obtained from newborns and labeled in vitro, and found the concentration of labeled cells in the spleen, Peyer's patches, and mesenteric lymph nodes to be essentially the same. In a similar experiment performed in 6-day old mice, the highest concentration of labeled lymphocytes was found in mesenteric lymph nodes. Collectively these data suggest that at birth thymic lymphocyte migration changes rapidly from a random process to one favoring migration to lymphoid tissues regional to sites of intense antigenic stimulation, i.e., under physiological conditions the gut. It may be recalled in this context that in young guinea pigs significantly more thymic lymphocytes were shown to migrate to the lymph nodes regional to the site of sensitization with dinitrochlorobenzene than to the contralateral ones (28). The mechanisms responsible for antigen-dependent, preferential entry of thymic lymphocytes into peripheral lymphoid organs remained to be clarified. Newborn animals ingest, with colostrum, gamma globulins which pass the intestinal epithelium and also enter into the Peyer's patch lymphoid tissue at least for a short time period after birth. Sordat et al. (29) found high concentrations of immunoglobulin in the wall of postcapillary venules of human tonsils and proposed that the localization of antigen-antibody complexes in the wall of these specialized vessels may be one of the factors controlling lymphocyte recirculation. Further studies are necessary to examine this hypothesis.
The preferential and extensive migration of thymic lymphocytes to Peyer's patches in newborn mice (20) leads one to revise the hypothesis that these structures are primary lymphoid organs independent from the thymus. Indeed, the migrational pattern of thymic lymphocytes to these tissues was the same as to the mesenteric lymph node, a recognized "secondary" lymphoid organ. Using methods of alloantigenic markers, Raff and Owen (27) came to a similar conclusion. Therefore, Peyer's patches are, in part at least, thymus dependent. It should also be emphasized that in the present study thymus-derived lymphocytes were found throughout the peripheral lymphoid organs and were not confined to so-called "thymus-dependent areas" (30). Similar observations have been made by other authors in young adult guinea pigs (28) and calves (16). It can thus be concluded that at least during early periods of life, the development of the entire lymph node cortex and splenic nodule is dependent upon migration of cells from the thymus. If the follicular cortex of lymph nodes contains lymphocytes which are bone marrow derived as has been proposed (31), these cells must come into prominence only at a later time. Since the cellular deficit in discrete areas of lymphoid organs in rats thymectomized at birth (3) can be corrected by an intravenous injection of thoracic duct lymphocytes, the lymphocyte deficiency of such animals appears to be related primarily to a decrease in circulating small lymphocytes (32). The latter population is most probably comprised of thymus-derived cells having immunological memory (33).

The absence in newborn mice of a noticeable thymic cell migration to the bone marrow is in accord with earlier findings in neonatal rats (14) and calves (16). In this context it has been reported that bone marrow-derived cells which repopulate the thymus in whole body irradiated rats have the ability to return to the bone marrow in considerable numbers only for a limited period of time (34).

In the present study the mean grain count per labeled lymphocyte as a function of time deviated from a simple exponential decrease in all tissues examined. By 2 days postinjection, however, the mean grain counts of labeled lymphocytes in all peripheral lymphoid tissues were higher than the mean grain counts of labeled lymphocytes in the thymus. At 7 days postinjection heavily labeled lymphocytes (>12 grains) constituted 11–16% of the labeled population in peripheral lymphoid tissues while they were much less frequent in the thymic medulla and totally absent from the thymic cortex. These results indicate that a fraction of thymus-derived cells, upon settling in the periphery, remained in, or reentered, a nonproliferative phase for at least 7 days. Many thymus-derived lymphocytes underwent division in the periphery as demonstrated by numerous labeled mitotic figures. The present findings also suggest at least one way in which thymus-derived lymphocytes might be lost. Numerous labeled cells were found within the epithelium covering Peyer's patches just adjacent to the lumen of intestine and there is no reason not to believe that many of these cells eventually disintegrate within the gut.

It may be of particular interest to compare the present results with studies
on newborn mice using the theta (θ) alloantigen as a marker of thymus-derived cells. Based on this method, Raff and Owen (27) reported that mesenteric lymph nodes of 1- and 2-day old CBA and BALB/c mice contained between 14 and 23% θ-bearing cells. This is less than the relative number of thymus-derived cells found in the present study at this site during the same period of time. More experimentation is necessary, therefore, to test the possibility that at least a fraction of thymus-derived cells may no longer show detectable θ-antigen as they reach the peripheral organs and contact antigen. Before this problem is solved, θ-negative lymphocytes should not automatically be referred to as “bone marrow derived.” Further studies are also needed to examine if and to what extent in the neonatal period of mice a central lymphoid organ other than the thymus contributes to the peripheral population of immunologically competent or active lymphocytes. In young adult rodents the bone marrow produces and emits lymphocytes (35), however, to our knowledge no information is available with regard to the magnitude of bone marrow lymphocyte peripheralization in intact newborns. It has been reported that in certain instances large doses of antigen to day-old mice results in the formation of large numbers of antibody-forming cells (36). This bears on the important question of whether or not thymus-derived cells alone can initiate immunoglobulin synthesis without the cooperation of a separate population of lymphocytes of bone marrow origin.

**SUMMARY**

Neonatal mice were given a subcapsular, intrathymic injection of thymidine-\(^{3}H\) using a modified microneedle technique, and the migration of labeled cells to spleen, lymph nodes, Peyer’s patches, and bone marrow was followed radioautographically with time. Assuming that nonlabeled lymphocytes migrated in the same manner as labeled lymphocytes, it can be concluded that the majority of lymphocytes present within mesenteric lymph nodes (74%) and Peyer’s patches (61%), and a large proportion of those located in popliteal lymph nodes (40%) and the spleen (26%), were of thymic origin. Evidence is presented indicating that these are minimum values. The difference in the magnitude of thymic cell migration to gut-associated lymphoid tissue on the one hand and to the spleen and popliteal lymph node on the other hand was tentatively attributed to antigenic stimulation from the intestinal flora which develops during the first days of life. Thymus-derived lymphocytes were scattered throughout the lymph node cortex and splenic follicles. No noticeable thymic cell migration to the bone marrow was found. Labeling indices in the peripheral lymphoid organs paralleled those of cortical thymic lymphocytes suggesting the thymic cortex as the major source of migrants.

By 2 days postinjection, the mean grain counts of labeled lymphocytes in all peripheral lymphoid tissues were higher than the mean grain counts of labeled lymphocytes in the thymus. At 7 days postinjection heavily labeled cells con-
stituted 11–16% of the labeled population in peripheral tissues while they were absent from the thymic cortex. These results indicate that a fraction of thymus-derived cells, upon settling in the periphery, remained in, or reentered, a non-proliferative phase for at least 7 days. Conversely, many thymus-derived lymphocytes underwent division in the periphery and/or penetrated the intestinal epithelium.

Since the relative number of thymus-derived cells found in the mesenteric lymph nodes of 1- and 2-day old mice was considerably higher than the percentage of cells at this site having the theta (θ) alloantigen, as reported by other authors, the possibility exists that θ-antigen on thymus-derived lymphocytes may, at least in a fraction of these cells, no longer be detectable as they reach the peripheral organs.

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