THE DISTRIBUTION OF LARGE DIVIDING LYMPH NODE CELLS IN SYNGENEIC RECIPIENT RATS AFTER INTRAVENOUS INJECTION*

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(Received for publication 28 July 1969)

It has been shown by Gowans and Knight (1) that when small lymphocytes obtained from rat thoracic duct lymph are injected intravenously into other rats, the cells rapidly "home" to lymphoid tissue, that is, to the white pulp of the spleen, lymph nodes in all locations, and lymphoid tissue of the gut, including Peyer's patches. There is considerable evidence that this process is physiological, and reflects the continuous recirculation of small lymphocytes from lymphoid tissue to blood via the major lymphatic ducts and then back again to lymphoid tissue. The mechanism for the "homing" has not been thoroughly elucidated, but it appears to depend upon certain sugar-containing receptors on the lymphocyte surface (2, 3); in any case, there is no reason to believe that it depends upon immunologically specific recognition mechanisms.

In contrast to the behavior of small lymphocytes, Gowans and Knight (1) found that large lymphocytes from thoracic duct lymph localize principally in the lymphoid tissue of the gut, with a few being found in the spleen, and virtually none in peripheral lymph nodes. They suggested that this selective localization might be due to the fact that most large lymphocytes in the thoracic duct are derived from intestinal lymphoid tissue, where they may have been "sensitized" to antigens commonly found in the gut. If this explanation were correct, it might be expected that large lymphocytes obtained from other lymph nodes would distribute themselves differently within lymphoid tissues after intravenous injection. In the course of studies concerned with the fate of rapidly

* Supported by United States Public Health Service Grant No. AI-AM-07651 and from the Fonds National Suisse de la Recherche Scientifique Médicale.

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dividing cells appearing in lymph nodes shortly after immunization, we made observations relevant to this problem. Suspensions of rat lymph node cells obtained from various sites were incubated in vitro with ^{3}H-thymidine to label the dividing cells, injected intravenously into syngeneic recipients, and their fate and distribution followed by radioautography or counts of radioactivity. It was found that these large dividing cells "homed" to lymphoid tissue in all locations, in a fashion similar to small lymphocytes. Further, it was confirmed that rapidly dividing cells obtained from the thoracic duct localize preferentially in the gut mucosa and gut lymphoid tissue (Peyer's patches and mesenteric lymph nodes), and in addition, it was found that cells obtained from mesenteric lymph nodes also localize preferentially in gut-associated lymphoid tissue. In striking contrast, cells obtained from peripheral nodes were found to localize preferentially in peripheral nodes. It was further shown that cells from donors immunized with a particular antigen showed some selective localization in lymph nodes containing that antigen, which may provide the explanation for the different migration pattern of lymphocytes obtained from various regions.

Materials and Methods

Inbred rats of Lewis or W-F strains were obtained from Microbiological Associates, Inc., Bethesda, Md. Noninbred, germfree rats were obtained from Charles River Breeding Laboratories, North Wilmington, Mass.

Donor Rats.—Most donor animals weighed between 150 and 200 g. Both immunized and nonimmunized donors were used. Immunization was carried out by injecting 2×10^9 killed *Bacillus pertussis* (Eli Lilly and Co., Indianapolis, Ind.) in 0.1 ml of saline in each footpad, either alone or mixed with 50 μg of 2,4-dinitrophenyl (DNP)-bovine gamma globulin (DNP-BGG), 50 groups/mole, prepared according to Benacerraf and Levine (4). Immunized rats were killed 4 days after injection.

Cell Transfers.—Donor rats were killed with ether and their lymph nodes quickly excised. The lymph nodes were separated into groups according to the following definitions.

"Peripheral" lymph nodes: popliteal, superficial and deep axillary, lumbo-aortic nodes, and superficial and deep cervical nodes.

Mesenteric lymph nodes: the lymph nodes present in the mesentery (or against the caecal wall) at the ileocaecal junction, including a thin, elongated, soft lymph node, present in the mesentery along the last 3 cm of the ileum. These nodes were fairly constant in size and location; usually four nodes were found.

In experiments involving transfers between an immunized donor and a recipient injected with the same antigen, only the lymph nodes directly draining the site of antigen injection, i.e., the popliteal and axillary nodes, were used as donor cells.

Excised lymph nodes were freed of fat, then minced in medium 199 (Grand Island Biological Co., Grand Island, N. Y.) at room temperature into very small fragments with fine scissors. Node fragments were pressed through wire mesh No. 100 with the plunger of a syringe, with frequent washings with medium 199. The cells were centrifuged at 700 *g* for 10 min at 25°C, and washed twice in 199. The cells were then resuspended in a siliconized Erlenmeyer flask in 10–50 ml of medium 199 containing 15% rat serum and 2 μc/ml of H_3-thymidine (5 c/mM, New England Nuclear Corp., Boston, Mass.) or 10 μc/ml of ^{3}H-uridine (8 c/mM

Schwarz Bio Research Inc., Orangeburg, N. Y.). After incubation for 60–120 min at 37° in a shaking water bath, the cells were centrifuged and washed twice with medium 199; when clumps were present at the end of the incubation, they were removed by filtration through wire mesh. A small portion of the cell pellet obtained after the last centrifugation was resuspended in one or two drops of rabbit serum to prepare cell smears, and the bulk of the pellet was resuspended in an amount of medium 199 calculated to give a cell concentration between 200 and 500 million cells/ml. Cell viability of this suspension, as judged by the trypan blue exclusion method, was usually higher than 90%.

When thoracic duct cells were used for transfer, lymph of the first or second day after cannulation was collected in an Erlenmeyer flask containing a small amount of heparin and streptomycin. The cells in a 24 hr collection were separated, washed, and incubated in the manner described for lymph node cells. All injections of cells were given intravenously.

In most experiments, 200–400 million cells were injected into a recipient rat weighing 60–100 g. Recipient rats were always of the same strain as the donor, except in experiments involving germfree rats as recipients. In some experiments, the recipients were splenectomized, usually a few hours before transfer.

Study of Recipient Rats.—Rats were killed with ether at various times after transfer (generally 18–24 hr). For histologic sections and radioautographs, blocks of tissue were fixed in 10% buffered formalin; smears for radioautographs were prepared from suspensions of the tissue which were teased in rabbit serum. In experiments in which counts of radioactivity were performed, lymph nodes were separated into peripheral and mesenteric groups according to the definitions outlined above, and stored until used at −20°C. In these experiments, Peyer's patches were also removed and pooled. All the Peyer's patches grossly visible from the ileocaecal junction and up were taken; their number varied between 16 and 21. In all instances, the lymph nodes as well as the Peyer's patches were removed by the same person, so that the identification of these groups of lymphoid structures were made on the basis of fairly constant criteria.

Radioautographs were prepared from tissue sections or methanol-fixed smears with Kodak NTB 3 liquid emulsion, and were developed in Kodak 19B developer after a period of exposure at 4°C, varying between 3 to 7 days for smears and 1 to 10 wk for sections. Smears were stained with methyl green–pyronin, Wright or Giemsa stains, sections with hematoxylin-eosin or methyl green–pyronin.

For electronmicroscopic studies, cell pellets were fixed in 3% glutaraldehyde in phosphate buffer 0.1 M pH 7.4, followed by 2% osmic acid (in Millonig's phosphate buffer). The pellets were embedded in Epon and processed for electronradioautography (5).

For counts of radioactivity, pooled groups of nodes or fragments of tissues were processed, usually after a period of storage at −20°C overnight or for 2 days. Tissues were weighed, then homogenized in 0.5 M perchloric acid with the help of a motor-driven Potter type homogenizer. When radioactivity was to be counted in a single, very small lymph node (popliteal, for instance), "carrier" lymph nodes of a normal animal were added in order to insure that the amount of acid insoluble material was roughly equivalent in all specimens. The homogenized tissues were kept in ice for 10 min, then the acid insoluble precipitates were washed three times in 0.25 M perchloric acid, twice in ethanol–ether 3:1, and once in ether. The final pellets were dissolved either in 0.5 ml formic acid and subsequently mixed in 4 ml ethanol and 15 ml scintillation medium (Omnifluor, New England Nuclear Corp., Boston, Mass.), or in 0.7 ml of NCS (Nuclear-Chicago Corp., Des Plaines, Ill.) mixed with 15 ml of scintillation medium. Radioactivity was counted in a Packard Tri-Carb scintillation counter. The degree of quenching was determined by an external standard, and correction for uneven quenching among the various samples coming from the same animal was made, when necessary, by using an internal standard.

RESULTS

Distribution in Radioautographs of Intravenously Injected Cells from Peripheral Lymph Nodes of Pertussis Immunized Donors Labeled In Vitro with ^{3}H-Thymidine

These experiments were designed to follow the fate of large dividing cells obtained from peripheral draining lymph nodes 4 days after injection of *B. pertussis*, which was chosen as an antigen because in the rat it produces an especially vigorous immune response.

Nature of the Cells Labeled In Vitro with ^{3}H-Thymidine.—In order to determine the nature and the location within the nodes of the cells dividing after immunization with *B. pertussis*, 3 control rats and 12 rats which had been injected 1–4 days previously in the foot pads with *B. pertussis* were given a single intravenous injection of ^{3}H-thymidine and killed two hr later. Radioautographs of sections of draining lymph nodes showed a marked increase in labeled cells on the 3rd and 4th days. The labeled cells were scattered throughout the cortex, and in large numbers in the medulla (Figs. 1 and 2). These cells were classified as large lymphocytes, large pyroninophilic cells, or intermediate forms. In addition, increased numbers of germinal centers were also found; virtually all of the cells within them were labeled, always rather lightly so. Since germinal centers were sometimes found in control nodes, it was clear that not all of them had resulted from the *B. pertussis* injection.

The extent and nature of the in vitro labeling of the cell suspensions used for transfer are shown in Table I. About 3–3.5% of the cells were labeled. The labeled cells were almost equally divided between large lymphocytes and large pyroninophilic cells (Fig. 3). In many instances the distinction between large lymphocytes and large pyroninophilic cells was arbitrary, as intermediate forms were frequently seen. Labeled small lymphocytes were not found. It is apparent that some of the labeled cells must be germinal center cells, unless they are unusually fragile cells which are destroyed in the preparation of the cell suspensions. However, it was estimated from the historadioautographs that fewer than 25% of the labeled cells in the suspensions could have represented germinal center cells.

In order to determine more precisely the nature of the labeled cells used for injection, radioautographs of ultrathin sections of cell pellets were studied by electron microscopy. Of 60 labeled cells observed, 2/3 were large cells containing large amounts of ribosomes, either isolated or arranged in polysomes, and a few short fragments of endoplasmic reticulum, without any regular arrangement or concentric organization around the nuclei (Figs. 4 and 5); most of these correspond to cells classified as large pyroninophilic cells in smears. The other 1/3 was about equally divided between large cells without endoplasmic reticulum membranes (Fig. 6), classified as large lymphocytes, or cells with

rather well developed endoplasmic reticulum structures showing concentric organization around the nucleus, classified as plasmoblasts (Fig. 7), which in

TABLE I

Types of Labeled and Unlabeled Cells in Donor Cell Suspensions After In Vitro Incubation with ^3H-Thymidine*

Source of cells‡	Small lymphocytes	Large lymphocytes	Large pyroninophilic cells
Peripheral nodes			
Percentage of total cells in smear	81 –88	12.4–16	1.7
Percentage of labeled cells in each cell type	0	10 –17	70 –77
Percentage of cell type among labeled cells	0	58 –60	40 –42
Mesenteric nodes			
Percentage of total cells in smear	91.3–93.3	5.3– 7.2	0.7– 1.4
Percentage of labeled cells in each cell type	0	7.5– 9.4	50 –81
Percentage of cell type among labeled cells	0	30 –61.5	38.5–70
Thoracic duct lymph			
Percentage of total cells in smear	83	16.5	0.5
Percentage of labeled cells in each cell type	0	11	90
Percentage of cell types among labeled cells	0	80	20

* Based on counts of 3000 cells from each of 2 donors. Radioautographs were exposed for 7 or 14 days.

‡ Peripheral lymph nodes obtained from rats 4 days after footpad injections of *B. pertussis*. Mesenteric nodes and thoracic duct cells obtained from nonimmunized donors.

Classification of cells: (*a*) small lymphocytes: cells less than 8 μ in diameter, with scanty cytoplasm and dense nucleus. (*b*) large lymphocyte: cells 8–13 μ with a less dense nucleus and slightly more cytoplasm than the small lymphocyte. The cytoplasm is slightly too moderately pyroninophilic. (*c*) large pyroninophilic cell: cells usually larger than 15 μ with vesicular nuclei and prominent nucleoli with abundant moderate to intense pyroninophilic cytoplasm. Distinction between these cell types, especially the last two categories was often arbitrary, because of transitional forms and variation in size in different smears.

smears might be classified either as large pyroninophilic cells or large lymphocytes.

Fate of the Labeled Cells Injected into the Recipient.—At least two recipients were killed at 30 min, 2, 4, 24, and 36 hr after injection, and the distribution of labeled cells in various tissues was studied in radioautographs. At 30 min, a few labeled cells were found in peripheral lymph nodes, in the spleen (both white

and red pulp), and occasionally in the lung or liver. Within lymph nodes, labeled cells were occasionally encountered traversing postcapillary venules. At all later intervals, large numbers of labeled cells were found in the white pulp of the spleen and in peripheral lymph nodes, where they were scattered throughout the cortex and in the paracortical areas (Fig. 8); they were virtually never present in follicles or germinal centers. The labeled cells in lymphoid tissue included both large lymphocytes and large pyroninophilic cells, as identified in smears of recipient nodes at 4 and 20 hr; it was estimated that anywhere from 20 to 50% were large pyroninophilic cells, and the rest large lymphocytes. In order to determine if the labeled cells recirculate, as small lymphocytes are known to do, thoracic duct cannulation was performed in two recipient rats. A few labeled large lymphocytes and large pyroninophilic cells were found in lymph collected during the first 24 hr, indicating recirculation. Although not relevent to this report, it is of interest to note that at later intervals (48–96 hr) most of the labeled cells in the tissues of the recipients had the typical appearance of small lymphocytes, although some in the spleen had the appearance of plasma cells. Virtually no labeled cells were found in the thymus. In some animals moderate numbers were found in hepatic sinusoids; only rare cells were found in other organs. A striking observation was that although labeled cells were numerous in peripheral nodes (Fig. 8), only a few cells were found in the lymphoid tissues associated with the gut, i.e., mesenteric lymph nodes, Peyer's patches, or intestinal mucosa (Figs. 9 and 10; see cell distribution at 20 hr, Table II).

In order to see if the difference in behavior of cells from peripheral nodes was in some way peculiar to *B. pertussis* immunized donors, the distribution of cells from peripheral lymph nodes of nonimmunized animals was studied. It was found that these cells, like the cells from *B. pertussis* donors, accumulated predominantly in peripheral nodes.

Distribution in Radioautographs of Intravenously Injected Cells from Mesenteric Nodes or thoracic Duct Lymph Labeled In Vitro with ^3H Thymidine.—As noted above, the finding of relatively few labeled cells in the mesenteric lymph nodes and intestine contrasted strikingly with the observations of Gowans and Knight (1), concerning the distribution of labeled cells after injection of thoracic duct lymphocytes which had been incubated in vitro with ^3H-thymidine. Accordingly, it was decided to study the fate of cells derived from mesenteric nodes or from the thoracic duct. Nonimmunized donors were used. The percentage of labeled cells (Table I) was found to be smaller with mesenteric node cell suspensions than with suspensions of peripheral nodes from *B. pertussis* stimulated animals (1–1.5% vs. 3–3.5%), probably reflecting the less intense antigenic stimulation of the mesenteric nodes. Electron microscopic radioautographs of labeled cells of mesenteric nodes showed results comparable to those obtained with cells from peripheral nodes.

Thoracic duct lymphocytes from nonimmunized donors obtained within 24 hr

of cannulation were also labeled in vitro with ^3H thymidine under the same conditions (Table I). These cells were not studied by electron microscopy.

The distribution of labeled cells, as observed in radioautographs in recipients killed at 20 hr after injection, is shown in Table II. Cells from mesenteric nodes showed a striking tendency to accumulate to a greater extent in mesenteric

TABLE II

*Distribution of Labeled Cells in Various Lymphoid Tissues After Injection of Cells of Different Origins**

Source of donor cells	Recipient tissue	Number of labeled cells/number of fields	Average number of cells per field
Peripheral nodes‡	Peripheral node (popliteal)	84/9	9.3
	Mesenteric node	23/26	0.88
	Peyer's patches	21/25	0.84
	Gut mucosa	2/10	0.2
	Spleen	63/6	10.5
Mesenteric nodes	Peripheral node (popliteal)	10/20	0.5
	Mesenteric node	66/10	6.6
	Peyer's patches	61/6	10.1
	Gut mucosa	39/15	2.6
	Spleen	79/12	6.6
Thoracic duct lymph	Peripheral node	8/36	0.22
	Mesenteric node	40/50	0.80
	Peyer's patches	20/30	0.66
	Gut mucosa	84/58	1.45
	Spleen	50/30	1.62

* Cells labeled in vitro with ^3H-thymidine. Recipients killed 20 hr after injection. Distribution of cells in one recipient in each group estimated in radioautographs by counting labeled cells per field at 400 × magnification.

‡ From donors injected with *B. pertussis* 4 days previously.

Distribution of labeled cells was studied in 3 other recipients of either peripheral or mesenteric node cells, and in one other recipient of thoracic duct cells and the results were essentially the same.

nodes, Peyer's patches, and in the intestinal mucosa than in peripheral nodes (Table II and Fig. 11). A similar pattern was observed with labeled thoracic duct cells; however, these cells accumulated in the highest concentration in the gut mucosa, rather than in Peyer's patches or mesenteric nodes, in confirmation of the finding of Gowans and Knight (1). It should be noted that cells from peripheral nodes, mesenteric nodes, or thoracic duct lymph displayed an approximately equal tendency to accumulate in the white pulp of the spleen.

Studies of Distribution of ^3H Thymidine Labeled Cells Based on Counts of Radioactivity.—In order to obtain more quantitative data concerning the distri-

bution of labeled cells, experiments were carried out in which total radioactivity in various groups of nodes was determined. For this purpose, all of the peripheral nodes in a recipient were pooled, as were all of the mesenteric nodes or Peyer's patches (see Materials and Methods). The results are shown in Table III. It is clear that the distribution of radioactivity exhibits the same pattern as the labeled cells seen in radioautographs, although the differences are less marked.

In order to see if the spleen played any role in the differences in distribution, six recipients were splenectomized several hours prior to transfer (four injected with peripheral node cells and two with mesenteric node cells); the relative

TABLE III

Average Distribution of Radioactivity in Recipients of Lymphoid Cells Labeled In Vitro with ^{3}H-Thymidine*

Source of donor cells	Peripheral nodes			Mesenteric nodes			Thoracic duct lymph		
Recipient nodes	Peripheral nodes	Mesenteric nodes	Peyer's patches	Peripheral nodes	Mesenteric nodes	Peyer's patches	Peripheral nodes	Mesenteric nodes	Peyer's patches
Percentage of radioactivity in each region	58.5	22.5	19	19	36	45	13	33	54
Range of total radioactivity recovered in each group of recipients	6,000–16,000 cpm			1,500–8,000 cpm			35,000–85,000 cpm		
Number of recipients studied	8 (4 splenectomized)			4 (2 splenectomized)			2		

* Recipients killed 20 hr after transfer.

amounts of radioactivity in the groups of nodes in these animals did not differ from that found in normal recipients.

To explore the possibility of preferential territorial migration within the group of peripheral nodes, three recipients were injected with cervical node cells from nonimmunized animals. Cervical nodes were used because they are hyperplastic in normal animals. The distribution of radioactivity did not differ from that in recipients of pooled peripheral node cells from *B. pertussis* immunized donors, and no preferential accumulation was found in cervical nodes.

Distribution of Lymphoid Cells Labeled In Vitro with ^{3}H-Uridine after Intravenous Injection.—The results given so far were obtained with cells which incorporate ^{3}H-thymidine in vitro, i.e., dividing cells. In order to see if similar migration patterns would be observed when the entire population of cells from a given region is labeled, experiments were performed in which the cells were incubated in vitro with ^{3}H-uridine. Radioautographs of donor smears showed that

over 90% of the cells were labeled. The distribution of radioactivity in peripheral nodes, mesenteric nodes, and Peyer's patches in recipients killed at 20 hr is shown in Table IV. It can be seen that there was little, if any, difference in the migration patterns of cells from peripheral nodes, mesenteric nodes, or thoracic duct lymph. Since in these experiments the bulk of the radioactivity was present in small lymphocytes, these results provide no evidence that selective territorial migration is a property of small lymphocytes. However, the possibility that a small percentage of these cells exhibit such behavior cannot, of course, be excluded by the present observations.

Preferential Migration of Lymph Node Cells to Antigen.—As noted above, one explanation for the selective migration of cells from mesenteric nodes or thoracic

TABLE IV

Average Distribution of Radioactivity in Recipients of Lymphoid Cells Labeled In Vitro with ^3H-Uridine*

Source of donor cells	Peripheral nodes			Mesenteric nodes			Thoracic duct lymph		
Recipient nodes	Peripheral nodes	Mesenteric nodes	Peyer's patches	Peripheral nodes	Mesenteric nodes	Peyer's patches	Peripheral nodes	Mesenteric nodes	Peyer's patches
Percentage of radioactivity recovered in each region	53	32	15	44	39	17	47.5	35	17.5
Range of radioactivity recovered in each group of recipients	25,000–66,000 cpm			21,000–87,000 cpm			2800–3000 cpm‡		
Number of recipients	7			3			2		

* Recipients killed 20 hr after transfer.

‡ Incubation performed in medium 199 which contains some ^1H uridine.

duct lymph towards gut-associated lymphoid tissue or gut mucosa is that these sites contain the antigens which stimulated the proliferation of large lymphocytes and large pyroninophilic cells, and that these cells can "recognize" antigen and be either attracted to or arrested by it. In one attempt to test this possibility, germfree rats were used as recipients of peripheral node cells (two recipients), mesenteric node cells (two recipients), or thoracic duct cells (one recipient) labeled with ^3H-thymidine. Radioactivity counts in different lymphoid structures showed results essentialy similar to those observed with normal recipients. However, as discussed below, these results do not rule out attraction or arrest by antigen as the explanation for preferential migration.

Therefore, in order to explore the possibility of preferential migration towards antigen in a more controlled fashion, experiments were performed in which the recipients of intravenously injected labeled cells had been injected in the foot pads on one side with an antigen against which the donor animal had been im-

munized. The number of labeled cells or radioactivity in the draining nodes was compared with that found in the contralateral nodes. As a control, other recipient rats received the same unilateral antigen injection, but were injected with the cells of nonimmunized donors or of donors immunized with an unrelated antigen.

The donors were animals which had been immunized with either *B. pertussis* or DNP-BGG plus *B. pertussis*[1], or were nonimmunized. In the case of the immunized donors, only the draining nodes (popliteal and axillary) were used.

TABLE V

Radioactivity in B. Pertussis-Containing and Contralateral Lymph Nodes in Recipients of Labeled Lymphnode Cells‡ from B. Pertussis or Nonimmunized Donors*

	Pertussis donors			Nonimmunized donors	
	Ratio of total radioactivity in pertussis/ uninjected side§	Ratio of cpm/ mg of tissue in pertussis/ uninjected side		Ratio of total radioactivity in pertussis/ uninjected side	Ratio of cpm/ mg of tissue in pertussis/ uninjected side
Rat 1			Rat 4		
axillary	5.4	3.5	axillary	2.0	1.3
popliteal	13.7	4.0	popliteal	3.3	1.5
Rat 2			Rat 5		
axillary	5.6	3.2	axillary	2.0	1.3
popliteal	10.1	4.0	popliteal	2.7	1.7
Rat 3			Rat 6		
axillary plus popliteal	6.7	3.2	axillary	2.1	1.1
			popliteal	4.3	1.7
Mean	8.3	3.6	Mean	2.7	1.4

* Recipients injected in left front and rear footpads 1 hr before transfer with 2×10^9 *B. pertussis* in 0.1 ml. Recipients killed 20 hr after transfer.

‡ Cell suspension labeled in vitro with ^{3}H-thymidine.

§ Counts on uninjected side ranged from 200 to 800 cpm.

With nonimmunized donors, cervical nodes were also included. The recipients of cells from *B. pertussis* were injected in front and hind footpads on one side with *B. pertussis* 1 hr before transfer. The recipients of cells from DNP-BGG donors were similarly injected with DNP-BGG. As controls, recipients of nonimmunized donors were injected on one side with *B. pertussis*, and recipients of cells from *B. pertussis*-immunized donors were injected on one side with DNP-BGG.

[1] *B. pertussis* was used in the DNP-BGG immunized animals for its adjuvant effect, which results in enhanced cell proliferation and immune response against DNP-BGG.

The results are illustrated in Figs. 12 and 13 and in Tables V and VI, where they are expressed as the ratio of counts in the lymph node containing the antigen and the contralateral node. It was found that lymph nodes containing *B. pertussis* showed an increased accumulation of labeled cells, even when nonimmunized donors were used (Table V). This nonspecific effect was much weaker or absent with DNP-BGG containing nodes (Table VI). The effect correlated fairly well with increase in size of the node (which averaged about double the normal size in the nodes draining *B. pertussis*). Therefore, the results are also expressed as the ratio of counts per minute/per milligram. of lymph node

TABLE VI

Radioactivity in DNP-BGG-Containing and Contralateral Lymph Nodes in Recipients of Labeled Lymph Node Cells from Donors Immunized with DNP-BGG and B. Pertussis or B. Pertussis Alone*

Rat No.	Donors immunized with DNP-BGG and *B. pertussis*		Rat No.	Donors immunized with *B. pertussis*	
	Ratio of total radioactivity in DNP-BGG/ uninjected side‡	Ratio cpm/mg of tissue in DNP-BGG/ uninjected side		Ratio of total radioactivity in DNP-BGG/ uninjected side	Ratio cpm/mg of tissue in DNP-BGG/ uninjected side
1	3.0	2.2	6	1.2	1.0
2	4.3	3.2	7	1.3	0.7
3	3.8	1.7	8	1.7	1.0
4	4.6	2.9	9	1.1	0.8
Mean	3.9	2.5	Mean	1.3	0.9

* Donors immunized in 4 footpads with 50 μg DNP-BGG plus *B, pertussis* 2×10^9. Recipients injected in left front and rear footpads with DNP-BGG 1 hr before transfer.

‡ Counts on uninjected side ranged from 200 to 1000 cpm. Only popliteal nodes were used.

tissue, and it can be seen that in these terms, the nonspecific effect is only moderate with *B. pertussis* and absent with DNP-BGG.

More significantly, as can be seen in Tables V and VI, there is also a definite migration of cells toward specific antigen, i.e., the increase in radioactivity in the antigen injected side is about three times higher when donor cells of the same specificity were used than when nonspecific donor cells were used.

The preferential accumulation of cells in nodes containing specific antigen was confirmed using radioautographs, with similar experimental design, using three recipients in each group. When *B. pertussis* immunized donors were used and the recipients injected with DNP-BGG on one side, the number of labeled cells appeared equal on each side. When donors immunized with DNP-BGG plus *B. pertussis* were used, the DNP-BGG-containing nodes had 2.2–4.6 more labeled cells per field (average of 20 comparable fields in each recipient) than the contralateral nodes.

DISCUSSION

In the present study it was found that large dividing cells obtained from lymph nodes of rats exhibited the property of "homing" to lymphoid tissue after intravenous injection in syngeneic recipients. The cells, which were labeled in vitro with ^{3}H-thymidine, consisted prinicipally of large lymphocytes and large pyroninophilic cells.[2] By electronmicroscopy the majority of labeled cells showed only scanty endoplasmic reticulum. Within a few hours after injection, numerous labeled cells were found in lymph nodes, where they were sometimes seen crossing postcapillary venules, and in the white pulp of the spleen; only a few cells were found in other organs except for the liver, where a moderate number of cells were often found. It was also shown that at least some labeled cells recirculated in the recipient, since a few were found in the thoracic duct lymph in the first 24 hr.

These findings show that large dividing cells appearing in lymph nodes during the immune response possess the capability of "homing"to lymphoid tissue and recirculating, similar to the fashion of small lymphocytes. It seems very likely that this behavior occurs not only under the artificial conditions of the present experiment, but also under physiological conditions. Thus, Hall et al. (7) have shown that similar types of cells were found in the efferent lymph coming from antigenically stimulated lymph nodes. Evidence has also been obtained (8, 9) that similar cells may enter the blood from the spleen shortly after intravenous immunization.

In addition to finding that large dividing lymph node cells "homed" to lymphoid tissue in all locations to some extent, it was discovered that they showed different patterns of distribution, depending upon the source of the donor cells; mesenteric lymph node cells or thoracic duct cells were found to accumulate preferentially in lymphoid tissue within or adjacent to the gut, whereas cells from peripheral nodes localized preferentially in peripheral lymph nodes. Cells from any of these sources accumulated to an equal extent in the white pulp of the spleen.

It was found that small lymphocytes did not exhibit a similar tendency to accumulate selectively in lymphoid tissue in certain regions; thus, lymph node cells or thoracic duct cells which had been labeled in vitro with ^{3}H-uridine distributed themselves fairly randomly among lymphoid tissue, regardless of their

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[2] The possibility that some of the labeled large pyroninophilic cells traced in the present experiments were germinal center cells warrants consideration, especially in view of the findings of Wakefield and Thorbecke, (6) who showed that cell suspensions from splenic white pulp containing large germinal centers home to lymphoid tissues of the spleen, lymph nodes, and Peyer's patches, where many of them appeared to turn into small lymphocytes. However, it is clear that most of the cells employed for transfer in the present studies were not of germinal center origin.

source. However, the possibility that a few of the small lymphocytes among those injected did show selective localization cannot be excluded.

Another salient finding in the present study was that large dividing lymph node cells from donors immunized with an antigen (DNP-BGG or *B. pertussis*) showed a greater tendency to accumulate in a recipient lymph node containing that antigen than in the contralateral node.

Two general explanations can be proposed for the preferential localization of cells from different groups of nodes in various regions in the recipient; one, that the lymph nodes in which preferential localization occurs contain antigens which were responsible for the proliferation of donor cells and that circulating large lymphoid cells are capable of "recognizing" the antigen, and two, that there are two populations of cells with different homing mechanisms. On the basis of the findings in the present study, it cannot be decided which (if either) of these interpretations is correct. If the first explanation is valid, it is necessary to postulate further that different naturally occurring antigens are usually present in the different groups of nodes studied. In support of this interpretation is the finding that preferential accumulation of large dividing lymph node cells from immunized donors does in fact occur in recipient nodes containing the specific antigen. However, certain observations are difficult to reconcile with this explanation. The finding that mesenteric lymph node cells accumulate preferentially in the gut-associated lymphoid tissue in germfree animals indicates that at least bacterial antigens are not responsible; however, it is clear that nonbacterial antigens also normally provide a stimulus for gut-associated lymphoid tissue, since mesenteric nodes are often hyperplastic in germfree animals. More difficult to explain is the finding that when peripheral nodes from *B. pertussis*-immunized rats are used as the source of donor cells, relatively few cells localize in mesenteric nodes, even in nonimmunized recipients. Since many of the donor cells were stimulated by *B. pertussis* antigens which are presumably not present in the recipients, one would expect a random homing pattern. However, it is conceivable that the peripheral nodes of donors and recipients contain in common certain naturally occurring antigens which provide a stimulus for proliferation in the donor nodes when the adjuvant action of *B. pertussis* is added. Against this explanation is the observation that cells from cervical lymph nodes, which like mesenteric nodes are hyperplastic in nonimmunized animals, failed to accumulate preferentially in cervical nodes, as compared with other peripheral nodes.

Although there is no direct evidence for the second hypothesis, namely that there are two populations of cells with different homing patterns, this possibility cannot be excluded. In this connection it is known that the factors affecting the homing of small lymphocytes to the white pulp of the spleen are different from those affecting homing to lymph nodes, (2, 3, 10, 11), presumably because different receptor mechanisms are involved. If the second hypothesis is correct, it

might provide an explanation for the marked preponderance of IgA-producing cells in gut-associated lymphoid tissue, i.e., it might be that cells with the potential to produce IgA contain receptors which cause them to home preferentially to the gut.

Aside from the question of the factors causing selective localization in certain territories, what can be said concerning the mechanisms accounting for the preferential accumulation of large dividing cells in lymph nodes containing specific antigens? In a general way this must depend upon an antigen recognition mechanism, which either causes the cells to be attracted to, or, as seems more likely, arrested by antigen. The antigen recognition mechansim could depend upon the presence of antibody on the cells, which could either be produced by the cells or passively coat the cells. Support for the interpretation that active antibody production is involved is provided by the fact that lymphoid cells with ultrastructural features similar to those of the cells used in the present experiments have been shown to be capable of producing antibody and of fixing specific antigen in vitro (12–15). The possibility that the results could be attributed to cytophilic antibody coating the cells would appear to be excluded, since the donor cells were taken so early after immunization and since the types of donor cells used are not known to bind cytophilic antibody. Another possibility is that the cells which accumulate preferentially at the site of antigen injection are cells responsible for the mediation of delayed sensitivity reactions. Since the nature of the interaction between such cells and antigen is unknown, nothing further can be said about this mechanism.

Whatever the mechanisms involved in the localization of the large dividing cells in lymphoid tissue, it seems likely that their homing and recirculation could be of the significance in the chain of events occurring in the immune response. The localization of large dividing cells in other lymph nodes could lead to the propagation of the immune response, as postulated by Hall et al. (7). Furthermore, preferential localization in lymphoid tissue containing antigen would be expected to occur. This in turn might lead to further proliferation and differentiation of antibody-producing cells into plasma cells. The finding (alluded to in the results) that after a few days most of the labeled cells in lymph nodes were small lymphocytes, suggests that in the absence of antigen most of the large dividing cells turn into small lymphocytes. However, in lymph nodes containing antigen many of the labeled cells seen after 4 days are plasma cells.[3] Similarly, the finding of Gowans and Knight (1), that large lymphocytes which migrate to the gut are transformed into plasma cells, might be explained by the presence of antigen.

In the present study, it was shown only that the type of preferential localization of large dividing lymph node cells which is dependent upon antigen can oc-

[3] Griscelli, C., D. Guy-Grand, P. Vassalli, and R. T. McCluskey. Unpublished observations.

cur in lymph nodes; whether similar results would be obtained in other tissues is not known, but it seems reasonable to assume that this would occur.

SUMMARY

The distribution of large dividing lymph node or thoracic duct lymph cells, labeled in vitro with ^{3}H-thymidine, was studied in syngeneic recipient rats after intravenous injection. In most experiments the donor rats had been immunized with *Bacillus pertussis* 4 days earlier, but in some instances cells from nonimmunized donors were used. In smears, the labeled donor cells had the appearance of large lymphocytes or large pyroninophilic cells. By electronmicroscopy, the majority of labeled donor cells were seen to have only scanty endoplasmic reticulum.

It was found that the labeled cells rapidly "homed" to lymphoid tissue and recirculated in the recipient, in a fashion resembling that of small lymphocytes. However, the distribution of labeled cells was found to depend upon the source of the donor cells. Cells from mesenteric lymph nodes or thoracic duct lymph showed a marked preferential accumulation in lymphoid tissue within or adjacent to the intestine, whereas cells from peripheral nodes accumulated preferentially in peripheral lymph nodes. Cells from any of these sources showed an equal tendancy to accumlate in the white pulp of the spleen.

Suspensions of small lymphocytes, labeled in vitro with ^{3}H-uridine, did not display a similar tendency to localize preferentially in lymphoid tissue in certain regions.

It was also found that large dividing lymph node cells from donors immunized with an antigen (2,4-dinitrophenyl-bovine gamma globulin (DNP-BGG) or *B. pertussis*) showed a greater tendency to accumulate in a recipient lymph node containing that antigen than in the contralateral node.

It was not determined whether the selective accumulation of large dividing lymphoid cells from different sources in lymphoid tissue of different regions in recipients was due to an antigen recognition mechansim or was the result of two different populations of cells with different "homing" mechanisms.

We would like to thank Mr. N. Fournier for his help in the electron microscopic part of the study and Miss B. Leo, Mr. R. Fernandez, and Miss M. C. Clottu for their technical help.

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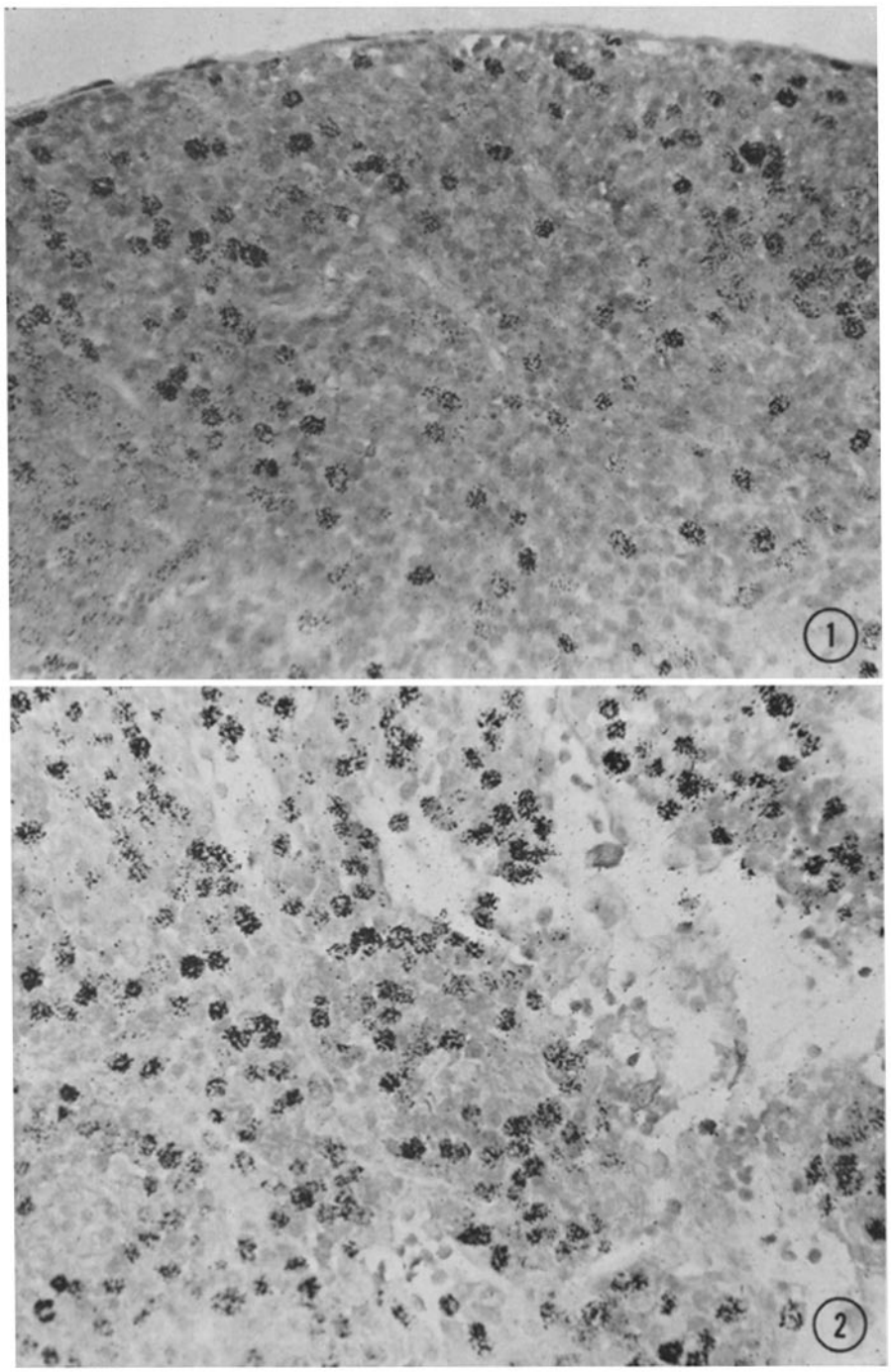

FIG. 1. Radioautograph of peripheral lymph node from a rat immunized 4 days earlier with *B. pertussis* and killed 2 hr after an intravenous injection of ^{3}H thymidine. Many isolated labeled cells are seen in the cortex. Methyl green–pyronin stain. × 375.

FIG. 2. Medullary portion of the same lymph node shown in Fig. 1. A very large number of labeled cells are seen. Methyl green–pyronin stain. × 375.

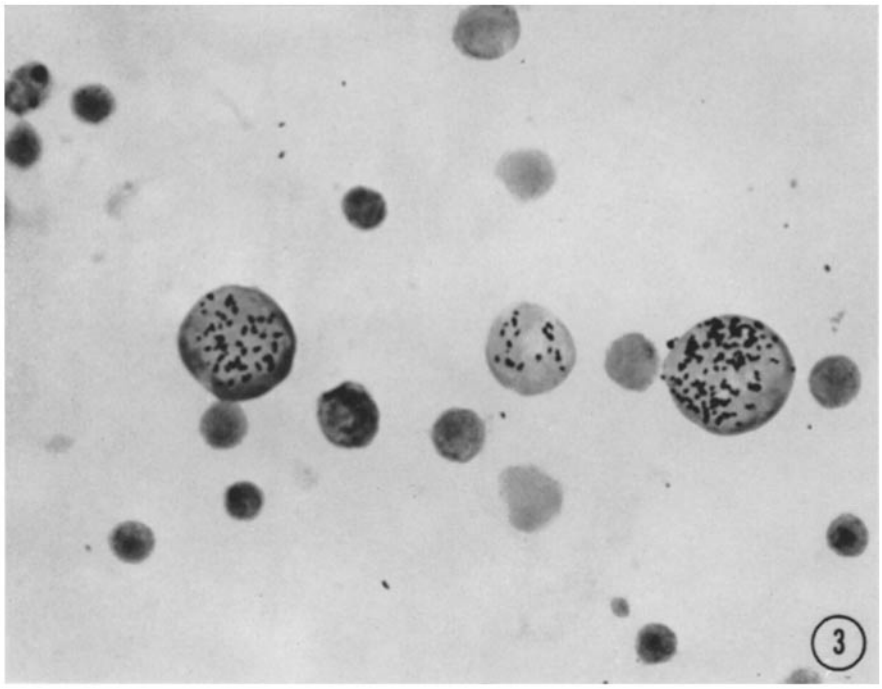

FIG. 3. Radioautograph of smear of cell suspension of peripheral node from *B. pertussis*-immunized donor labeled in vitro with ^{3}H-thymidine. Two labeled large pyroninophilic cells and a labeled large lymphocyte are shown. × 1000.

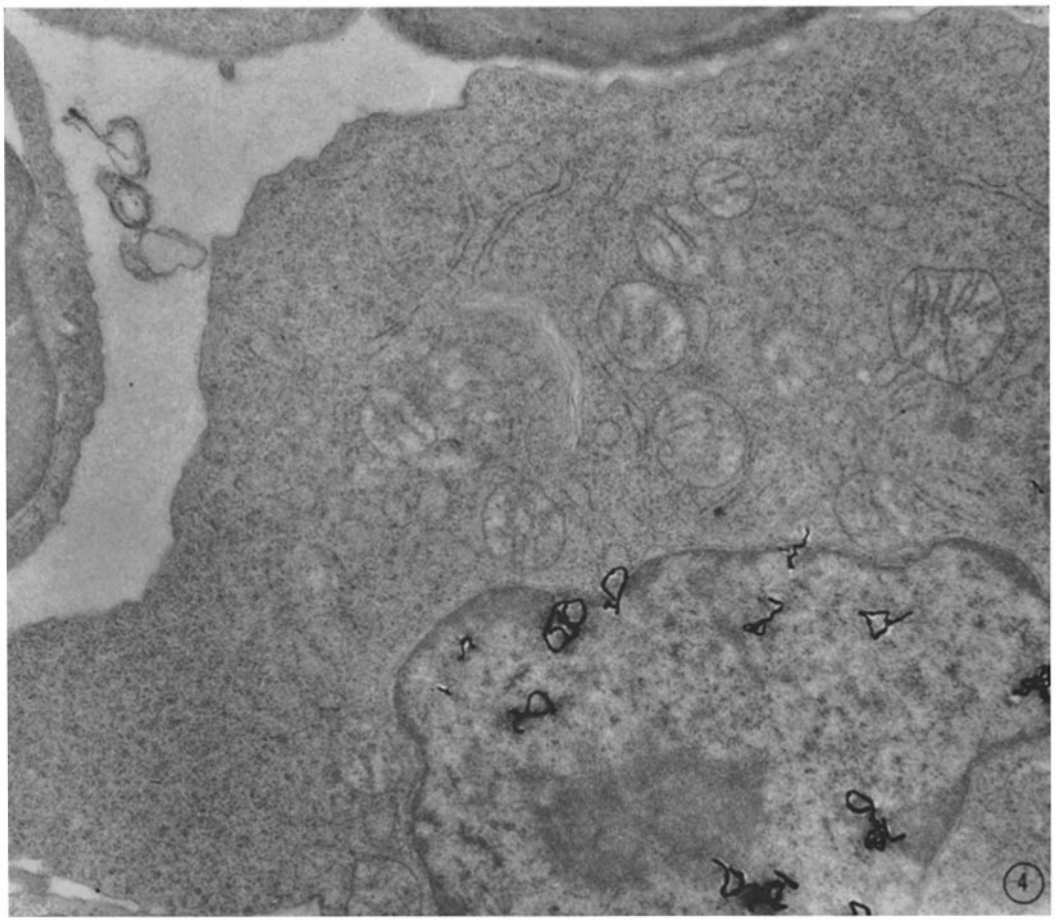

FIG. 4. Electronmicro-radioautograph of donor cell from peripheral node labeled in vitro with ^{3}H-thymidine. This cell would be classified in smears as a large pyroninophilic cell. Isolated structures of endoplasmic reticulum are seen, but ribosomes are not distinctly arranged in clusters. × 28,000.

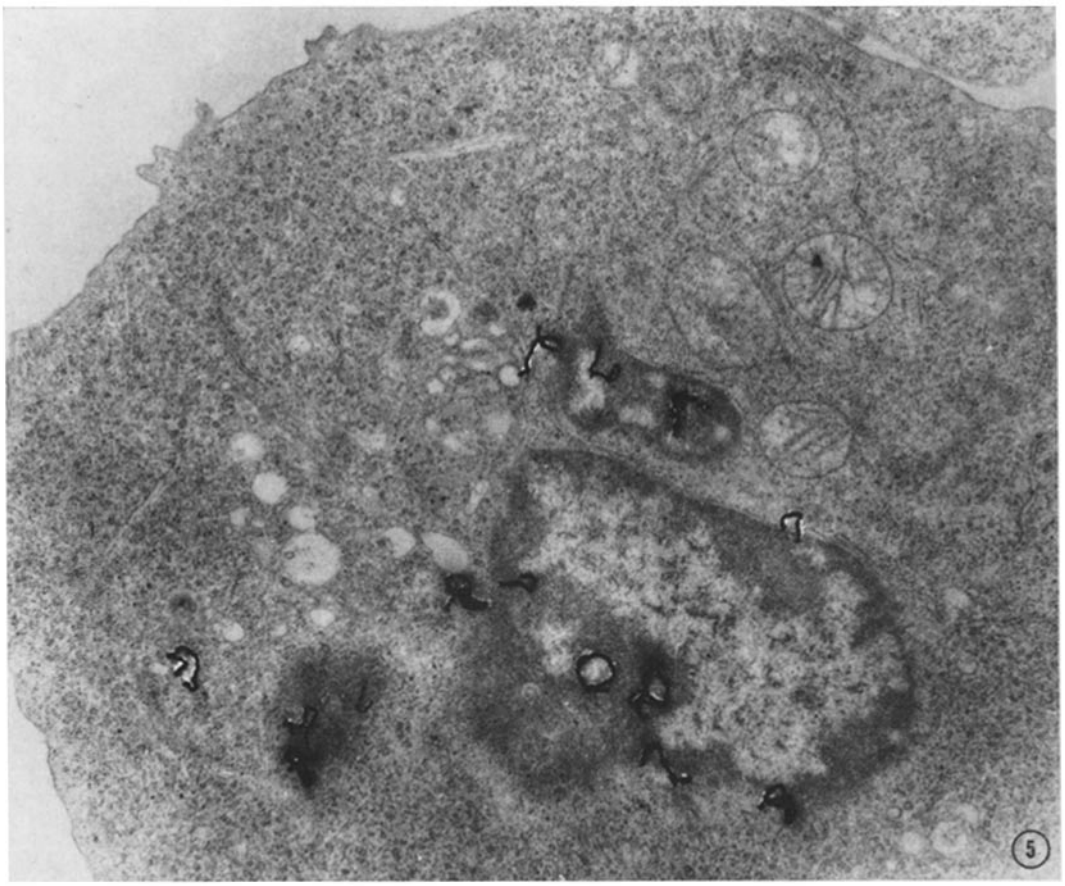

FIG. 5. Electronmicroradioautograph of donor cell from peripheral node labeled in vitro with ^{3}H-thymidine. This cell, which would also be classified as a large pyroninophilic cell, shows the ribosomes arranged in clusters. × 27,000.

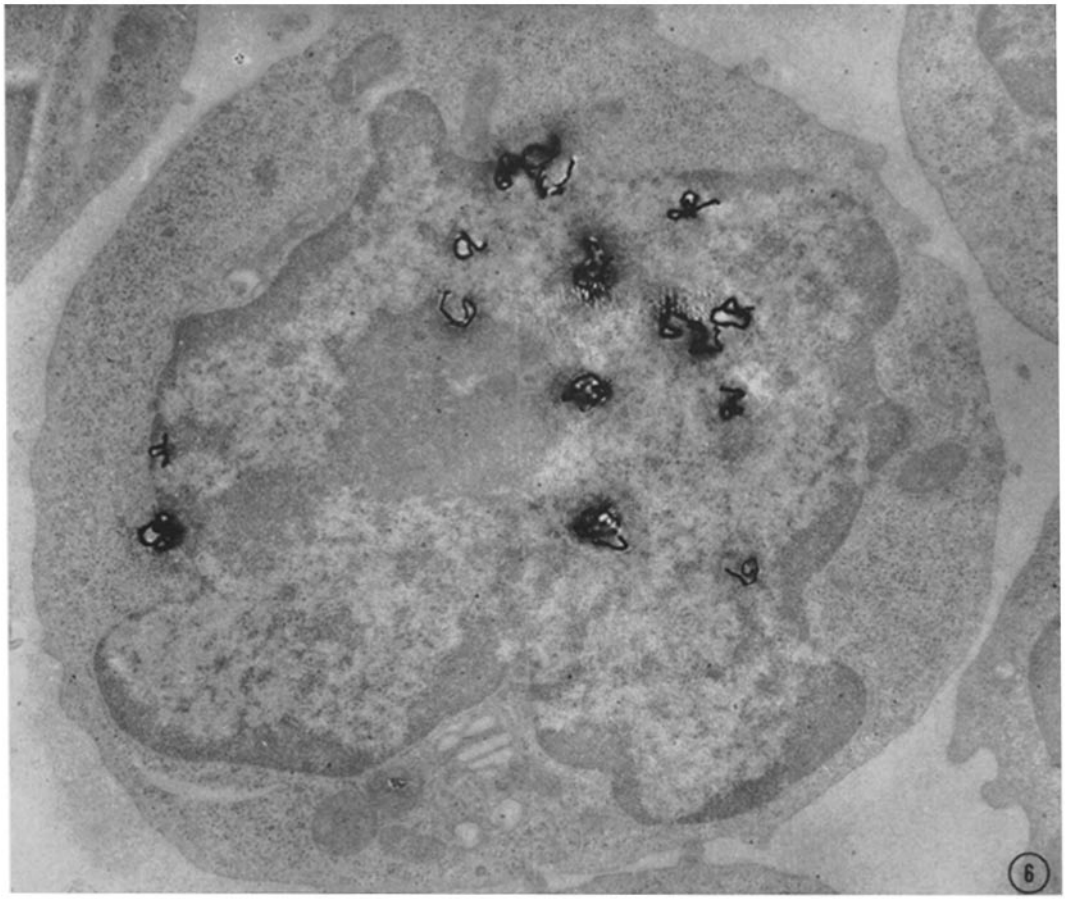

FIG. 6. Electronmicroradioautograph of donor cell from peripheral node labeled in vitro with ^{3}H thymidine. This cell would be classified as a large lymphocyte. The cytoplasm is relatively scanty and contains no endoplasmic reticulum. × 21,000.

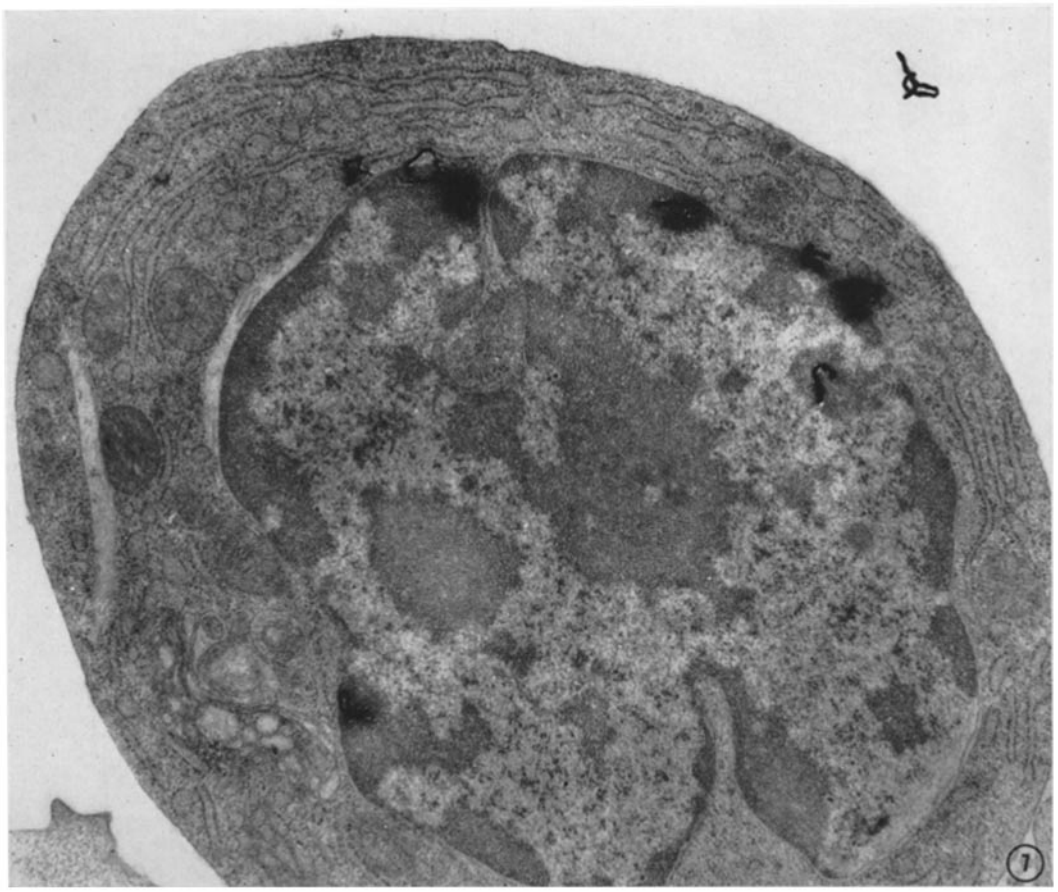

FIG. 7. Electronmicroradioautograph of donor cell from peripheral node labeled in vitro with ^{3}H-thymidine. This cell has a rather well developed endoplasmic reticulum arranged in a concentric pattern. The cell can be considered an immature plasma cell and might be classified in smears as either a large pyroninophilic cell or as a large lymphocyte. × 22,000.

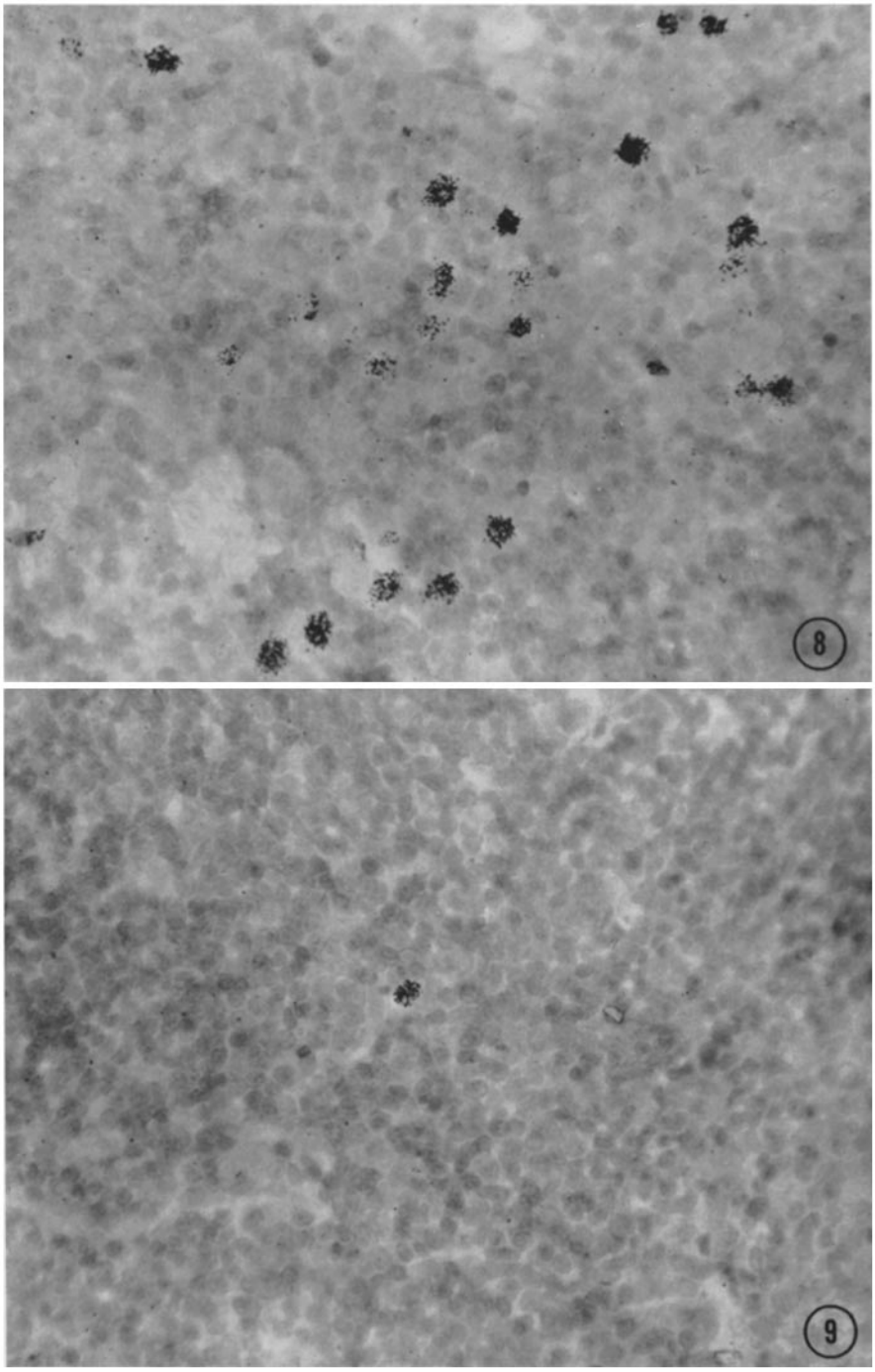

FIG. 8. Peripheral lymph node from recipient of peripheral node donor cells 20 hr after injection. Numerous labeled cells are seen. Methyl green–pyronin. × 400.

FIG. 9. Mesenteric lymph node from recipient of peripheral node donor cells. Only one labeled cell is seen. Methyl green–pyronin. × 400.

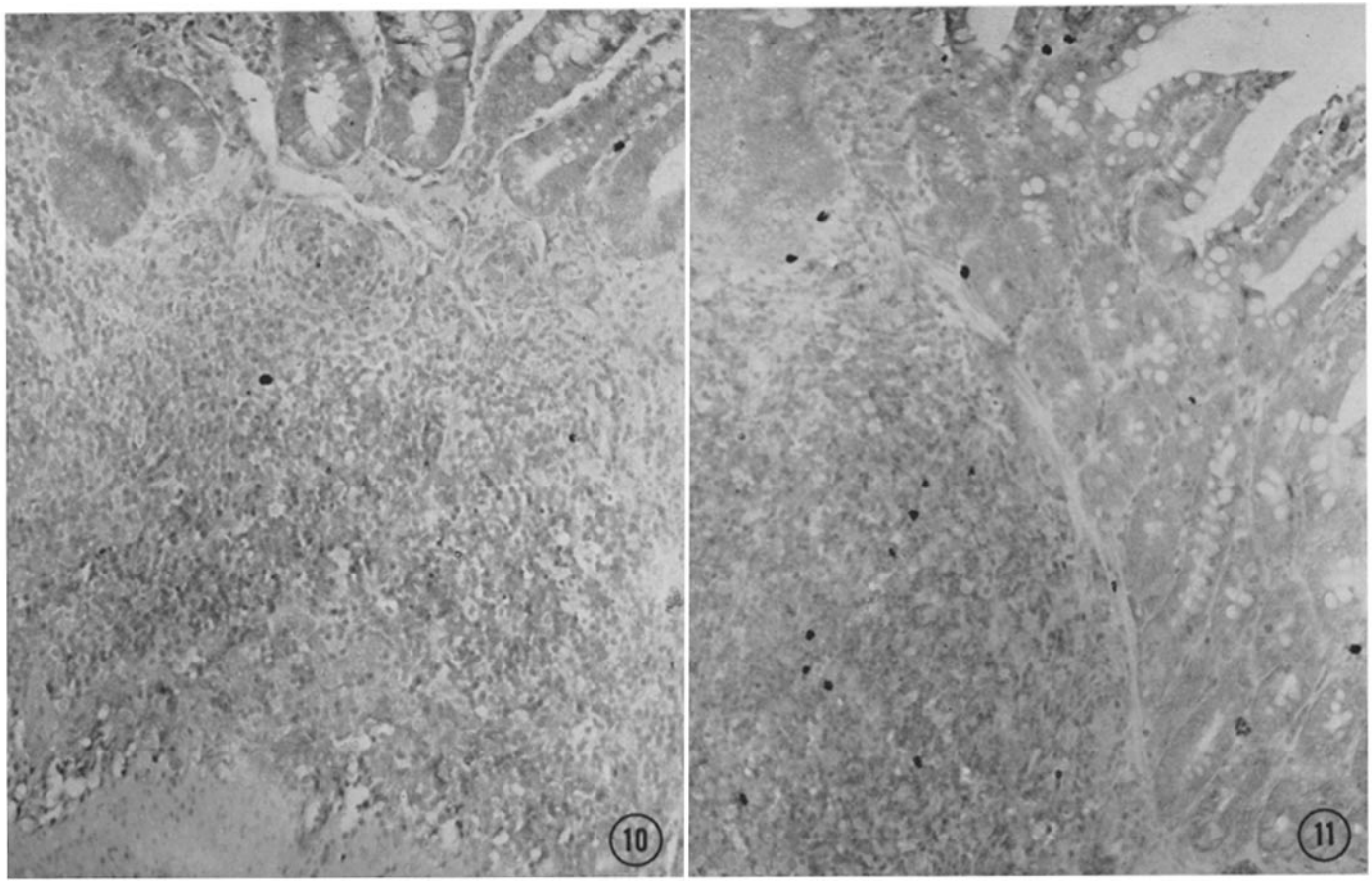

FIG. 10. Peyer's patch and intestinal mucosa from recipient of peripheral node cells. One labeled cell is present in the mucosa and one in the lymphoid tissue. Methyl green-pyronin. × 125.

FIG. 11. Peyer's patch and intestinal mucosa from recipient of mesenteric node cells. Many labeled cells are seen in the lymphoid tissue and mucosa. × 125.

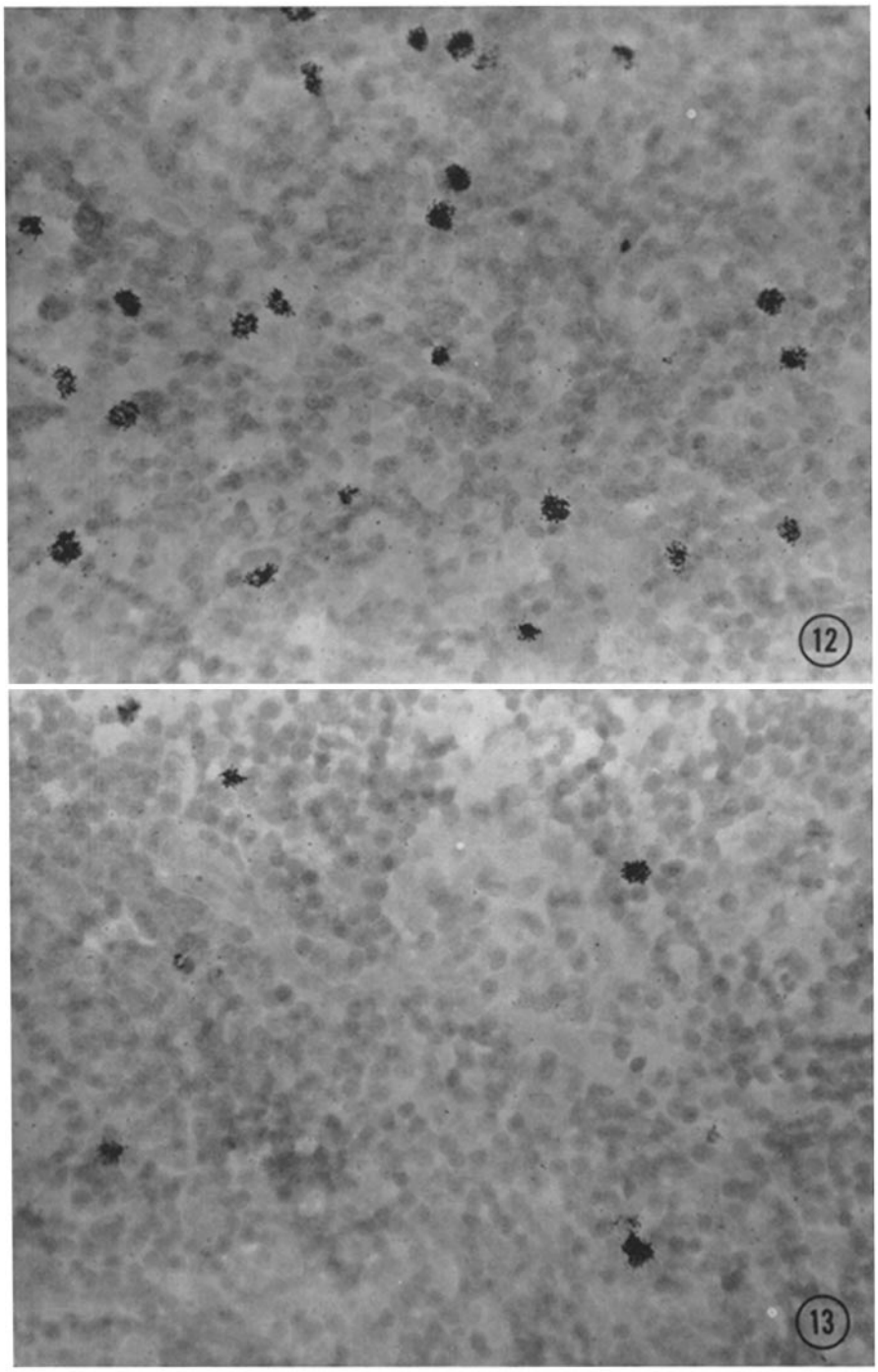

FIG. 12. Lymph node draining an injection of *B. pertussis* in recipient of cells from *B. pertussis*-immunized donor. Numerous labeled cells are seen. Methyl green–pyronin. × 400.

FIG. 13. Contralateral lymph node from animal shown in Fig. 12. This node, which does not contain antigen, shows considerably fewer cells. Methyl green–pyronin. × 400.