THYMUS CELL MIGRATION*

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The origin and fate of thymic lymphocytes are still matters of controversy. The thymus is ontogenetically the first lymphoid organ (1), and it has been shown that the origin of lymphocytes in the embryonic thymus is an autonomous process (2). However, the development of lymphoid tissue elsewhere appears to be a thymus-dependent process (3–5). Two ways in which the thymus might influence the development of peripheral lymphoid tissue have been postulated: (*a*) The thymus generates lymphocytes which then migrate to the peripheral tissue; (*b*) the thymus produces a diffusible factor which induces the development of lymphocytes in situ in peripheral lymphoid tissue.

The purpose of this work was to examine the possibility that thymus cells migrate. This problem was investigated by labeling thymus cells in situ with tritiated adenosine or thymidine, and then by following the distribution of labeled cells, as a function of time, in the lymphoid and other tissues of the same animals.

Similar studies employing localized labeling of the thymus have been reported recently (6, 7), but the possible uptake of undegraded label by nonthymic tissues was not rigorously ruled out (8). The present experiments demonstrate that thymus cells labeled in situ do migrate to peripheral lymphoid tissue sites.

Materials and Methods

Animals.—All the animals were members of a noninbred strain of albino rat. Thymic infusions were carried out on both adult (160–250 g) and newborn animals.

Method of Infusion.—Text-fig. 1 illustrates the method of infusion in adult rats. Rats were anesthetized with ether, and then fitted with an endotracheal tube connected to a continuous cycle positive pressure respirator. In this way respiration could be maintained with the thorax opened during thymus infusion. The manubrium and half the length of the body of

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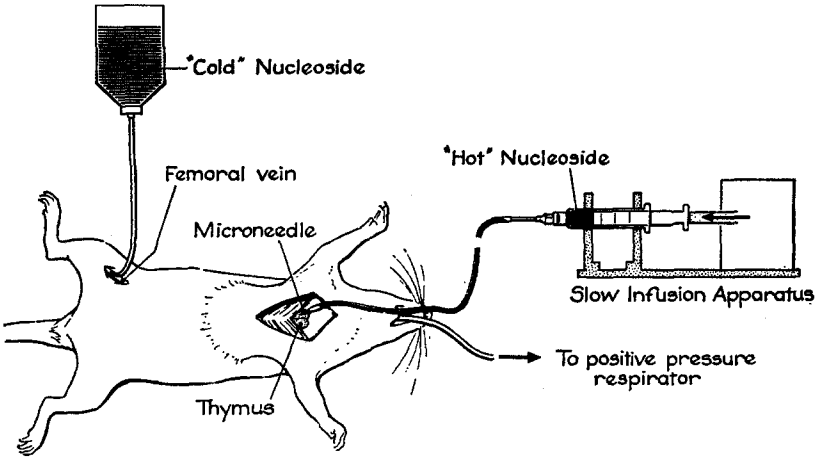
the sternum were split, taking care to avoid damaging the internal thoracic (parasternal) vessels. The thymus was freed of overlying membranes and then covered with sterile gauze moistened with warm saline. At this point, the femoral vein was cannulated with polyethylene tubing and infused continuously with saline at 2 ml/hr by means of gravity feed.

The thymus was infused through a specially prepared glass microneedle connected to a slow infusion pump which was calibrated to deliver 0.03 ml/hr. The infusion apparatus was filled with a single tritiated nucleoside (obtained from the Radiochemical Centre, Amersham, England), either thymidine-^{3}H, 4.17 mc/mg, thymidine-^{3}H, 62.5 mc/mg, or adenosine-^{3}H, 109 mc/mg diluted to 1 mc/ml in saline containing Pontamine sky blue dye. The microneedle was then inserted into a single lobe of the thymus, stabilized, and sealed at its point of entry into the thymus with a drop of rat plasma which was allowed to clot. After the period of thymic infusion, any animal in which dye was not limited exclusively to the thymus was discarded.

The thymus was usually infused for 1.5–2 hr, by which time a total dose of 45–60 μc had



TEXT-FIG. 1: Experimental method, see text.

been delivered. Following infusion, the microneedle was removed, 0.05 ml of nucleoside-^{1}H (1 mg/ml), penicillin (1000 μ/ml), and streptomycin (0.2 mg/ml) in physiological saline was dripped over the infusion site; then the sternum, overlying muscle, and skin were closed by interrupted suture. The endotracheal tube was removed when the rat resumed spontaneous respirations, and the rat was transferred to a restraining cage. The intravenous cannula was then attached to a gravity-feed drip of the corresponding nucleoside-^{1}H (1 mg/ml) in heparinized (2 units/ml) physiological saline, and continuous infusion was carried out at a rate of 2 ml/hr until sacrifice.

Newborn hosts anesthetized by hypothermia received 10 μc tritiated nucleoside intrathymically over a time course of 1–3 min and then an immediate intravenous injection of 1 mg cold nucleoside via the anterior facial vein, supplemented by subcutaneous doses of up to 10 mg daily until sacrifice.

Thymidine-^{3}H, a specific DNA precursor, was infused to trace the fate of cells undergoing DNA synthesis within the thymus, whereas adenosine-^{3}H, a precursor of both DNA and RNA synthesis, was infused to trace both non-DNA and DNA-synthesizing cells. In

practice, it was found that intrathymic infusion of adenosine-^{3}H labeled virtually every cell in the region around the microneedle.

Histological and Radioautographic Procedures.—Infusion-sacrifice intervals varied from a few hours to many days and are set out in Table I. In all studies, both touch imprints and histological sections of tissues were exposed for 2 and 4 wk with an Ilford K-5 photographic emulsion overlay and then stained through the emulsion with either Giemsa or methyl-green pyronin, respectively.

Tissues were fixed in formol alcohol and smears and imprints in absolute methanol. Sections were cut at 5 μ. After exposure, slides exhibiting background activity of greater than 2 gr per 150 μ^2 area were discarded. The following tissues were examined: (*a*) thymus, spleen,

TABLE I

Experimental Guide: Thymus Infusion Experiments

Host	Infusion-sacrifice interval	Thymidine-^{3}H	Adenosine-^{3}H
Adult	3 hr	2	0
	5 hr	1	0
	24 hr	1 (1)*	1 (1)*
	3 days	1 (1)*	1 (1)*
	5 days	1	1
	7 days	1	0
	10 days	1	0
	26 days	1	0
Newborn	2 hr	1	0
	4 hr	1	1
	8 hr	1	0
	1 day	3	1
	3 days	3	1
	5 days	3	0
	7 days	3	0
	10 days	3	0
	20 days	3	0

* Adrenalectomized controls.

Peyer's patches, peripheral lymph nodes[1], bone marrow, free peritoneal cells, and blood; (*b*) kidney, liver, and lung; (*c*) segments of small and large intestine.

Group *a* comprised the tissues to which lymphoid cells might be expected to migrate. Group *b* tissues were chosen in view of the claim that no thymic infusion is physiological. Lymphocytes which have been labeled in vitro may become trapped in the liver, the lung, and to a lesser extent the kidney, after their rapid injection into the blood (personal observation).

Intestinal epithelium was examined radioautographically to check the possibility of leakage of labeled nucleoside from the thymus during infusion, or that of its release into the blood and reutilization after the death of labeled cells. Greater than background radioactivity

[1] Peripheral nodes chosen were axillary, brachial, inguinal, thoracic, superficial and deep cervical, mesenteric, and para-aortic.

was detected in radioautographs of intestinal epithelium after the intravenous infusion into adult rats of as little as 4 μc tritiated thymidine in 1 hr. In newborn animals, both intestinal mucosa and the hematopoietic cells of the liver and spleen are sensitive indicators for leakage and/or reutilization of labeled nucleosides (Figs. 1 and 2).

The possibility of stress-induced cell migration from the thymus was tested by repeating the experiments in hosts that were adrenalectomized by abdominal approach 24 hr prior to thymic infusion and maintained with a single intramuscular injection of 3 mg of deoxycorticosterone trimethylacetate, 5% glucose added to the "cold" nucleoside intravenous infusion, and physiological saline in the drinking tube.

Additional controls were carried out to check the possibility that extrathymic cells entered the thymus along the tract created by the infusion microneedle, became labeled, and then migrated to the periphery. Inbred hooded male rats were used both as donors and recipients in these experiments. Bone marrow cells were obtained from femoral and tibial medullary cavities, washed in Krebs-Ringer solution, resuspended in tissue culture medium 199 (TC 199) at a concentration of 1×10^7 cells/ml and incubated at 37°C for 1 hr with either adenosine-^{3}H (10 μc/ml) or thymidine-^{3}H (1 μc/ml), washed in Krebs-Ringer solution, and resuspended in physiological saline containing 10^{-5} M ^{1}H-nucleoside. 200–400 million cells were slowly injected intravenously into adult hosts during thymic infusion. The thymic infusion and intravenous infusion consisted of 10^{-4} M nucleoside-^{1}H in physiological saline. Hosts were sacrificed 19 and 72 hr after infusion. Thoracic duct lymphocytes were obtained by the method of Bollman et al. (9) during the 8 hr interval beginning 6 hr after cannulation of appropriate donors. They were washed and incubated in TC 199 containing 10 μc/ml adenosine-^{3}H for 1 hr at 37°C. The cells were washed again, resuspended in 10^{-4} M adenosine-^{1}H, and injected intravenously at doses of 2.6×10^8 to 1×10^9 cells to appropriate thymus-infused hosts. Control smears of bone marrow and thoracic duct lymph cells were examined radioautographically to determine the proportion of labeled cells. Three animals received adenosine-^{3}H-labeled thoracic duct lymphocytes at the time of thymic infusion and were killed at 5, 24, and 48 hr, respectively. A fourth rat received labeled cells 24 hr after thymic infusion and was killed 24 hr later.

The analysis of the distribution of labeled cells in the tissues of the recipients was carried out as described below, after selection of slides with minimal background.

Thymus.—Analysis of thymic sections was carried out using a standard eyepiece grid for cell counting. Multiple sample counts were made to determine the ratio of cells per grid in the cortex and the medulla. Then the thymic pieces were outlined at × 25 with a camera lucida apparatus. A random series of straight line paths were drawn on these tracings, and the paths followed on cell-counting runs. At × 950, the number of labeled cells per grid were recorded, the number and site (cortex or medulla) of grids summed up, and the ratio of labeled to unlabeled cells calculated. Although sampling runs were randomly distributed, the entry site of the microneedle dictates the distribution of labeled cells within the thymus, and therefore there is a nonrandom distribution of labeled cells on thymic sections.

Spleen.—For counting purposes, the spleen was divided into red pulp and white pulp, without attempting to distinguish periarteriolar white pulp from germinal follicles, except for qualitative analysis. Quantitation of the ratio of labeled cells per unlabeled cells was accomplished by tracing a × 100 camera lucida projection of each periarteriolar white pulp-germinal follicle complex, counting the number of labeled cells in each of these complexes at × 950 and determining its histological location (periarteriolar white pulp vs. germinal follicle) at both × 100 and × 950. The camera lucida tracing was cut out and weighed; the total number of cells was calculated by comparing the tracing weight with a standard. The standard was established by cutting out and weighing representations of the eyepiece grid at × 100 from the same paper, knowing the number of white pulp cells per grid area. The red pulp was analyzed in the same manner as the thymus.

Lymph Nodes.—Lymph nodes were arbitrarily divided into three portions: the medulla, the germinal-follicles, and the diffuse cortex. The medulla was characterized by cords of pyronine-positive cells, large thin-walled venules, and low cell density. The germinal follicles were characterized as any cortical lymphoid mass which showed definite structural organization, with or without pale pyroninophilic central areas. Diffuse cortex was characterized as the densely packed lymphoid mass between germinal follicles, bounded by medulla and afferent lymphatics, and containing specific vessels with high-walled endothelial cells (postcapillary venules).

Camera lucida tracings of whole lymph nodes were divided into these three portions, cut, and weighed. Labeled cells were found by systematic × 950 examination of the entire node, histologically localized and recorded. Standard grid cell counts of the various node portions were recorded and labeling indices calculated as before.

Nonlymphoid tissues were examined and counted in the same manner as the thymus.

Although background was limited to 2 gr per cell, only cells with 6 gr or more were recorded as labeled cells. All data presented are from 4 wk exposure periods.

RESULTS

The results provide both qualitative and quantitative evidence of thymus cell migration. General comments are derived from all animals in Table I, but only a selection of these have been analyzed quantitatively.

In all experiments in which neonatal and adult hosts were killed immediately after infusion with either adenosine-^{3}H or thymidine-^{3}H, label was found *only in thymus cells.*

In the thymus, labeling varied somewhat with the position of the microneedle. Usually, cortical lymphocytes made up the greatest portion of the labeled cells. However, labeled cells were also found in the medulla and the connective tissue septa. 3 days after infusion, labeled cells were nearly equally distributed between cortex and medulla. By 10 days, only a few labeled cells could be found, predominantly in the medulla. By 26 days, no labeled cells were apparent, either in the thymus or in other lymphoid tissues.

As shown in Figs 3–7, cells heavily labeled during intrathymic infusion of adenosine-^{3}H were found in the thymus, splenic white pulp, and peripheral nodes. Intestinal epithelial cells were not labeled; nor were labeled cells found in liver, kidney, or lung. A quantitative estimate in an adult rat sacrificed 24 hr after infusion is shown in Table II (host 1).

In addition to the organ specificity of their distribution, the recently migrated thymus lymphocytes exhibited considerable specificity with respect to their migration sites within these organs. Every labeled cell found in the lymph nodes was in the diffuse cortex. A few cells were seen in the walls of postcapillary venules. No heavily labeled cells were found in germinal follicles, afferent lymphatics, or the medulla. No labeled plasma cells, reticular cells, or phagocytic cells were observed. In the spleen, labeled cells were limited to the periarteriolar white pulp; no heavily labeled cells were present either in the germinal follicles or the splenic red pulp.

There are two major differences between adenosine-infused and thymidine-

infused adult hosts. The first concerns the character of the thymic labeling. Adenosine-infused hosts demonstrate rapid and universal labeling of all cells in the region of the microneedle, irrespective of morphology or size (Fig. 3 and Table IV, lines 1 and 2). However, thymidine-infused hosts are initially labeled

TABLE II

Labeled Cell Distribution in Adult Hosts after Intrathymic Nucleoside-H^3 Infusion

A Nucleoside-^{3}H	B Interval	C Organ	D No. of labeled cells (>6 gr)	E Total cell No.	F Labeled cells	G* Corrected per cent thymus-derived cells
					%	
(1) Adenosine	24	Thymus, whole	5468	46,920	11.7	100
		Thymus, infused lobe	5072	19,090	26.6	—
		Thymus, noninfused lobe	396	27,830	1.4	—
		Splenic white pulp	26	7,750	0.34	2.9
		Splenic red pulp	0	8,300	0	0
		Mesenteric node, whole	59	98,512	0.06	—
		Mesenteric node, diffuse cortex	59	18,520	0.32	2.7
		Cervical node, whole	48	95,030	0.051	—
		Cervical node, diffuse cortex	48	25,468	0.19	1.6
		Intestinal mucosa	0	>8,000	0	0
		Bone marrow	1	41,800	0.002	0.017
		Peyer's patches	0	4,860	0	0
(2) Thymidine	24	Thymus	3551	54,120	6.56	100
		Splenic white pulp	1	63,848	0.0016	0.024
		Mesenteric node, diffuse cortex	10	126,896	0.0079	0.12
(3) Thymidine	72	Thymus	2608	26,046	10.01	100
		Splenic white pulp	32	49,466	0.065	0.65
		Mesenteric node, diffuse cortex	65	92,311	0.07	0.7

* Column G = column F test organ $\times \frac{100}{\text{column F thymus}}$.

primarily in the large and medium lymphoid cells. As time passes, label is no longer found exclusively in these cells, and appears increasingly in cells of the small lymphocyte class (Table IV, lines 3–5).

The second major difference between adenosine- and thymidine-infused hosts can be seen in the tempo of cell migration from the thymus, and it is probably

related to the first difference explained above. By 24 hr after intrathymic thymidine-^{3}H infusion, very few labeled cells were found in extrathymic sites (Table II, host 2). However, by 72 hr, many labeled cells were evident in the extrathymic sites described after adenosine-^{3}H infusion (Table II, host 3) which represents a delayed migration pulse of thymidine-labeled cells.

Cell size analysis of thymus cell migrants is shown in Table V. Cell imprints were divided into size categories by the following criteria: large, $>11\ \mu$ diameter; medium, 7–11 μ diameter; small, $<7\ \mu$ diameter. In adults labeled with thymidine-^{3}H, either 24 or 72 hr prior to sacrifice, only small and medium lymphoid cell migrants were found, although a considerable proportion of the labeled cells in the thymus were large. 24 hr after infusion of adenosine-^{3}H into adults, all thymus cell migrants were either small or medium. In all three cases, the cell size distribution of labeled cells in the thymus was significantly different from labeled cells in lymph nodes and spleen.

Adult Controls.—Labeling patterns and the phenomenon of thymic cell migration were unaltered in adrenalectomized hosts.

Experiments were carried out to test the possible entry of the extrathymic cells along the thymic infusion tract by intravenous injection of in vitro–labeled thoracic duct lymphocytes or bone marrow cells. Although massive numbers of labeled cells could be identified in spleen, liver, lung, or nodes of these hosts, no labeled cells were found along the infusion tract into the thymus, and only rarely in other sites in the thymus.

Newborn Hosts.—On microscopic examination, very few lymphocytes are seen in the very small lymph nodes or in the spleen of newborn rats. After birth there is an expansion of spleen and lymph node size and lymphocyte content. It is clear from Figs. 8–11 and Table III that thymus-derived labeled lymphocytes comprised a very large proportion of the peripheral lymphoid tissues in newborns. Again, labeled cells were distributed specifically to nodes, splenic white pulp, and infused thymus, whereas kidney, lung, bone marrow, neonatal liver, and intestinal mucosa showed no labeled cells. The architecture of neonatal rat nodes is poorly defined, and it was impossible to assign a precise anatomical location to the labeled cells within them.

The data of Tables II and III seem to indicate a further distinction between adults and neonates. In neonates, thymidine-labeled thymus cell migrants were found to migrate at 24 hr, without the lag observed in adults (Table II, host 2). This could be explained either by early intrathymic thymidine-labeling of small lymphocytes (by direct uptake or rapid differentiation from large cells) or migration of intrathymic labeled large and medium cell types. Tables IV and V demonstrate the labeling pattern of the thymuses of these hosts as well as the size distribution of the migrants. It is clear that while virtually all of the thymus cell migrants in adult hosts were either small or medium lymphocytes, thymus cell migrants in neonatal hosts were distributed throughout all size categories, although the proportion of large cells was significantly smaller in

the migrant pool than thymus cells in situ. Furthermore, the time course–cell size distribution of thymidine-^{3}H–labeled cells in the neonate was strikingly similar to that in adult hosts, i.e., a selective uptake of thymidine-^{3}H by larger cells immediately after infusion with a transition from large to small labeled cells as time passed.

The absence of label in intestinal epithelial cells in animals infused 3 days prior to sacrifice cannot be due to a rapid transit time from crypt to intestinal lumen. As independent studies have demonstrated, pulse-labeled neonatal rat gut crypt cells have a crypt to lumen transit time of greater than 3 days (10).

TABLE III

Labeled Cell Distribution in Neonatal Hosts after Intrathymic Nucleoside-^{3}H Infusion

A Nucleoside-^{3}H	B Interval	C Organ	D Number of labeled cells (>6 gr)	E Total cell No.	F Labeled cells	G Corrected per cent thymus-derived cells
	hr				%	
Adenosine	72	Thymus	1741	8,000	21.8	100
		Splenic white pulp	33	1,620	2.0	9
		Splenic red pulp	0	4,800	0	0
		Mesenteric node	103	2,427	4.2	19
		Other nodes	346	10,371	3.3	15
		Intestinal mucosa	0	>5,000	0	0
		Bone marrow	0	>5,000	0	0
		Hepatic hemopoietic cells	0	>5,000	0	0
Thymidine	24	Thymus	482	7,322	6.58	100
		Splenic white pulp	50	4,263	1.17	18
		Mesenteric node	48	6,180	0.78	12

DISCUSSION

The appreciable numbers of highly labeled cells found in specific lymphoid sites in adult and neonatal rats infused intrathymically with nucleoside-^{3}H in the absence of radioautographically detectable passage of label in the form of nucleosides confirms the hypothesis that cells regularly migrate from thymus to nonthymic lymphoid tissues.

Recirculation of thoracic duct lymphocytes from blood to lymph via lymphoid tissues has been demonstrated (11, 12). This recirculation excludes the thymus. Yet, the specific localization of thymus cell migrants to lymph nodal diffuse cortex and splenic periarteriolar white pulp is reminiscent of the similar localization of recirculating, long-lived thoracic duct small lymphocytes (11, 12). Thoracic duct small lymphocytes are immunologically competent, requiring

antigenic stimulation to set in action the course of events leading to antigraft or anti-sheep red blood cell immunological reactions (13). One may then ask: "Are the thymus cell migrants destined to become part of the long-lived, recirculating, immunologically competent cell population?" (14–17). One may alternatively postulate that recently migrated cells undergo a vigorous selection procedure, eliminating the greater portion of their numbers, a fate predicted for thymus cells in situ by Burnet (18) and Matsuyama et al. (19).

Tritiated adenosine, a precursor of both DNA and RNA, is rapidly taken up by thymus cells of all sizes. Migration of such cells occurs in large numbers within 24 hr after infusion, exclusively to the periarteriolar white pulp of the

TABLE IV

Cell Size Distribution of Labeled Cells Following Pulse-Chase Intrathymic Infusion

Host	Interval	Nucleoside	Large	Medium	Small	Large and medium	Total cells counted
	hr		%	%	%	%	
Adult	24	Adenosine	6.8	21.0	72.2	27.8	396
Adult*	Random	None	7.3	20.4	72.3	27.7	1100
"	5	Thymidine	74.8	21.8	3.4	96.6	500
"	24	"	27.5	36	36.5	63.5	200
"	72	"	4.3	18	77.7	22.3	300
Newborn	1.5	Thymidine	48	51	1	99	100
"	20	"	34	41	25	75	100
"	48	"	16	43	41	59	100
"	72	"	2	16	82	18	100

* This represents the cell size distribution of a typical adult thymus not subjected to intrathymic infusion.

spleen and the diffuse cortex of the lymph nodes. Although all cell categories are labeled in the thymus, only small and medium migrants are found in adults. In addition, although thymidine-^{3}H labels only large and medium cells initially, thymus cell migrants in adults are exclusively small and medium, only appearing in significant numbers as the labeled cell distribution in the thymus shifts from large to small. Therefore it is suggested that the normal sequence of events is as follows: Large thymic lymphocytes give rise to small thymic lymphocytes, either by division or differentiation. Small (and perhaps medium) cells then migrate to splenic periarteriolar white pulp and lymph node diffuse cortex. If this is an accurate description of events, the thymectomy-induced deficit of lymphocytes in these specific sites might be attributed to a deficit of migrating thymocytes rather than a deficit of some thymic humoral factors (5).

In newborns, however, there is one qualitative difference; although intrathymic labeling patterns are essentially the same as those in the adult, migration of all cell categories seems to occur. Although large cells seem to migrate in neonatal hosts, the proportion of labeled migrant cells which are

TABLE V

Comparison of Cell Size Distribution of Thymic Cells and Migrants in the Same Hosts

Host	Interval	Nucleoside	Organ*	Per cent labeled cells				Total cells counted	χ^2‡	*P* (two-tailed test)
				Large	Medium	Small	L & M			
	hr									
Adult	24	Adenosine	Thymus	6.8	21.0	72.2	27.8	396	6.5	<0.025
			Migrants	0	25.6	74.4	25.6	90		
			SWP	0	27.6	72.4	27.6	29		
			MNDC	0	24.6	75.4	24.6	61		
Adult	5	Thymidine	No migrants							
Adult	24	Thymidine	Thymus	27.5	36	36.5	63.5	200		
			SWP	0	0	100	0	1		
			MNDC	0	70	30	70	10		
Adult	72	Thymidine	Thymus	4.3	18	77.7	22.3	300	4.3	<0.05
			Migrants	0	20.6	79.4	20.6	97		
			SWP	0	21.9	78.1	21.9	32		
			MNDC	0	20	80	20	65		
Newborn	20	Thymidine	Thymus	34	41	25	75	100	10.5	<0.005
			Migrants	14.3	52	33.7	66.3	98		
			SWP	18	50	32	68	50		
			MNDC	10.4	54.2	35.4	64.6	48		

* MNDC, mesenteric node, diffuse cortex; SWP, splenic white pulp.

‡ χ^2, distribution testing:

	Large	Not large
Thymus Cells		
Migrants		

large is significantly lower than the proportion of thymic cells which are large. This may be evidence in favor of at least two large lymphoid cell classes in the neonatal thymus, both capable of DNA synthesis, but with one type primarily migrating to peripheral lymphoid sites and the other type giving rise to small thymic lymphocytes in situ. It is unlikely that these large labeled cells in periph-

eral sites are the result of antigen-induced transformation from small cell migrants.

How significant is the thymus cell migration demonstrated in this study? The question must be considered in the two test situations, the newborn and the adult host. In the adult host, infusion occurs at a point in time when the lymph nodes and spleen have a preexisting resident lymphoid population. Any thymus cell migrants are diluted by the pool they enter. Nevertheless, Table II, column G, shows that thymus cell migrants make up 1.6–2.9% of the diffuse cortex of nodes or periarteriolar white pulp of the spleen in an adult host 24 hr after adenosine-^{3}H infusion. If migration continues at this rate, one could expect a complete renewal of these "pools" in 30–60 days. This figure is only approximate, as the sampling error in the thymus could be quite large. Also, many 2–6 gr cells were seen specifically in these same sites but were excluded to avoid counting "background" cells.

In the newborn, one-fourth to one-half of the cells in peripheral lymph nodes are macrophages, reticular cells, and endothelial cells. At least 9–19% of the total cell number in these nodes were thymus-derived. If one takes into account the lymphoid cells present in these nodes prior to infusion and the false low counts obtained by excluding cells labeled with 2–6 gr, it is evident that one cannot rule out the hypothesis that virtually all lymphoid cells in these sites were either thymus-derived, or recent descendants of thymus-derived cells.

Previous studies employing direct thymus infusion with nucleoside-^{3}H were carried out in the absence of a concomitant whole body nucleoside-^{1}H infusion. Such experiments demonstrated labeling in cells of the intestinal mucosa (6) and bone marrow (7) (myeloid series) as well as in the lymphoid tissues. However, the present experiments, in which an excess of cold nucleoside was infused systemically to prevent reutilization, demonstrate labeling only in specific lymphoid sites and not in bone marrow or intestinal mucosa. These results cast doubt on hypotheses that thymic cells may act as multipotential stem cells or trephocytes (20).

Metcalf (19, 21) has put forward evidence against the idea of thymus cell migration. He used two lines of evidence to support his hypothesis: The first is based on a comparison of mitotic and pyknotic counts in the thymus. However, if the duration of morphological "pyknosis" is longer than the duration of "mitosis," then one would ascribe a spuriously high death rate to the cell populations studied. Metcalf's second series of experiments demonstrated that the nonthymic lymphoid tissue content and peripheral blood lymphocyte count remained unaltered in the presence of as many as 48 thymus grafts, all undergoing normal thymic lymphopoietic patterns. However, there are known functional differences between thymus grafts and thymus in situ (22, 23). Although thymus grafts may not function in the same way as a thymus in situ, one must still account for the finding that such grafts do not seem to affect

lymphocyte numbers in the grafted hosts. It has already been established that total thymic graft mass is not subject to host control mechanisms, whereas total spleen graft mass is under such control (21). If further feedback mechanisms exist which rigidly control various extrathymic lymphoid pool sizes (such as loss of cells to intestinal lumina, etc.), results consistent with the present work and Metcalf's experiments would ensue. However, the present experiments do not tell us what proportion of cells labeled in the thymus *do* migrate. They only allow us to detect such migration, to relate it to the existing extrathymic lymphoid mass, and to make some predictions about the relevance of this migration to maintenance of extrathymic lymphoid pool sizes. Thus, we can neither confirm nor deny the hypothesis that most thymic lymphoid cells die in the thymus.

There are certain criticisms of the present work that should be considered. The operative procedure itself is certainly far from physiologic, and one can demonstrate a local damaging effect of the microneedle and a postsurgical wave of pyknosis throughout the thymus cortex. However, the reverse-labeling experiments appear to rule out the infusion tract as a source of cellular influx into the thymus, and the experiments in adrenalectomized animals, again demonstrating cell migration, were not accompanied by such increases in thymic pyknotic counts. It should be emphasized that this experimental method can only trace the migration of labeled cells for a few days and cannot reveal their eventual fate.

There was a cortico-medullary reversal of the distribution of labeled cells within the thymus as time passed. Since it was not possible to limit the initial labeling either to cortex or medulla alone, it was not established whether this "reversal" signified a cortex-to-medulla pathway of thymocyte migration or a differential rate of proliferation and/or migration between cortical and medullary thymocytes.

SUMMARY

The preceding studies have established the following points: Intrathymic labeling of thymic lymphocytes provides an adequate marker system to detect the migration of thymus cells to peripheral lymphoid sites. In the newborn, this comprises a major portion of the total lymphocyte population in lymph nodes and spleen. In the adult, this migration is limited to specific portions of lymph nodes and spleen, i.e., those portions which serve the recirculating pool of small lymphocytes. Kinetic studies of labeling within the adult thymus indicate that large cells give rise to medium and small cells, which then migrate to the specific sites noted above. In the newborn, the kinetic pattern is similar to that of adults, with the single distinction that large cells also migrate, accelerating the tempo of migration in these hosts. The long-term fate and function of thymus cell migrants has not yet been determined.

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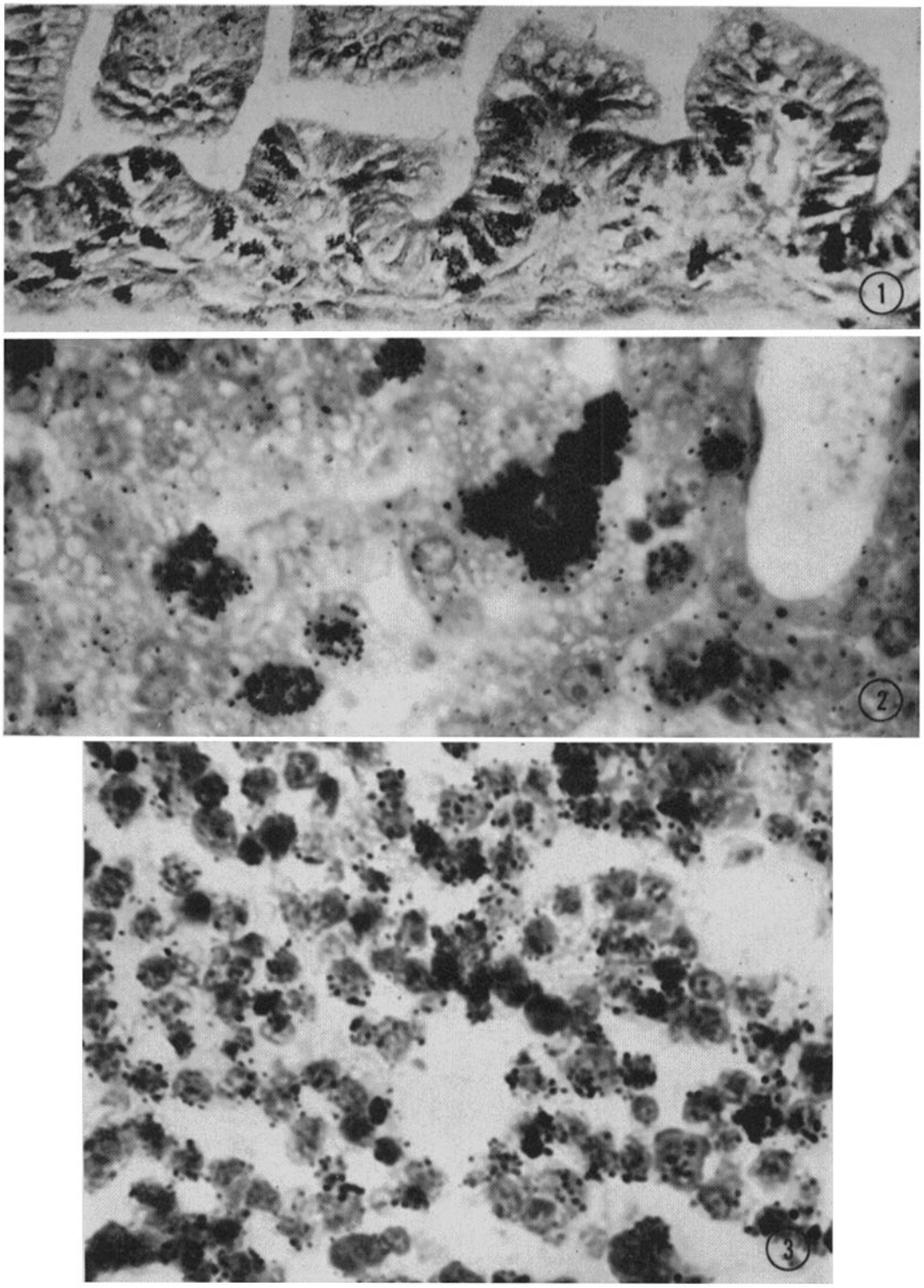
EXPLANATION OF PLATES

PLATE 28

FIG. 1. Heavily labeled gut epithelium in newborn rat 24 hr after intravenous injection of 5 μc thymidine-^{3}H. × 100.

FIG. 2. Heavily labeled hepatic hemopoietic cells from the same host. × 950.

FIG. 3. Thymus of an adult rat infused intrathymically with 60 μc adenosine-^{3}H and sacrificed 24 hr later. Note that all lymphoid cell types are labeled. This is a portion of the thymus which was heavily labeled. Over 90% of the thymus was unlabled. Compare this to the restricted labeling pattern in Fig. 4 in which the thymus was labeled with a DNA precursor. × 950.



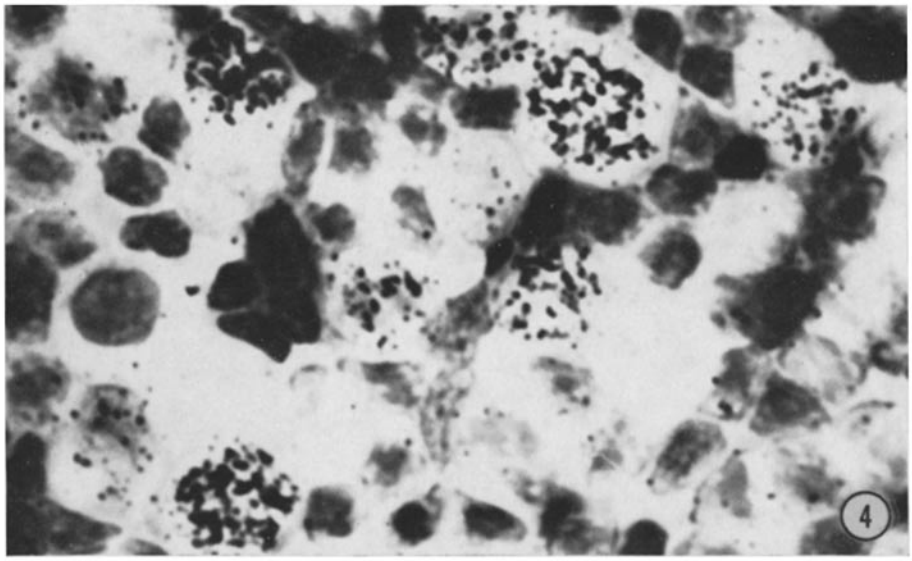
(Weissman: Thymus cell migration)

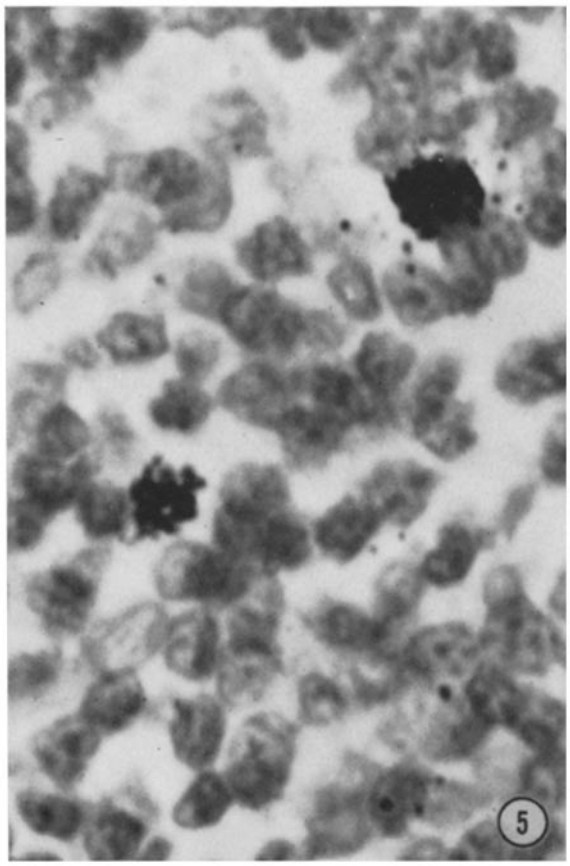
PLATE 29

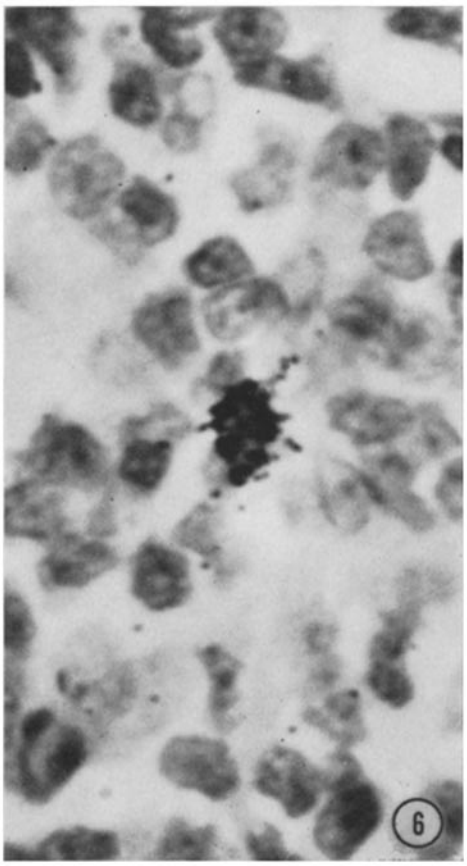
FIG. 4. Thymidine-^{3}H–labeled thymus; note the many heavily labeled cells, predominantly of the large type. × 950.

FIG. 5. Deep cervical node of an adult rat infused intrathymically with 60 μc adenosine-^{3}H and sacrificed 24 hr later. Two heavily labeled cells. × 950.

FIG. 6. Periarteriolar white pulp in spleen of the same host. A heavily labeled cell surrounded by unlabeled cells. × 950.





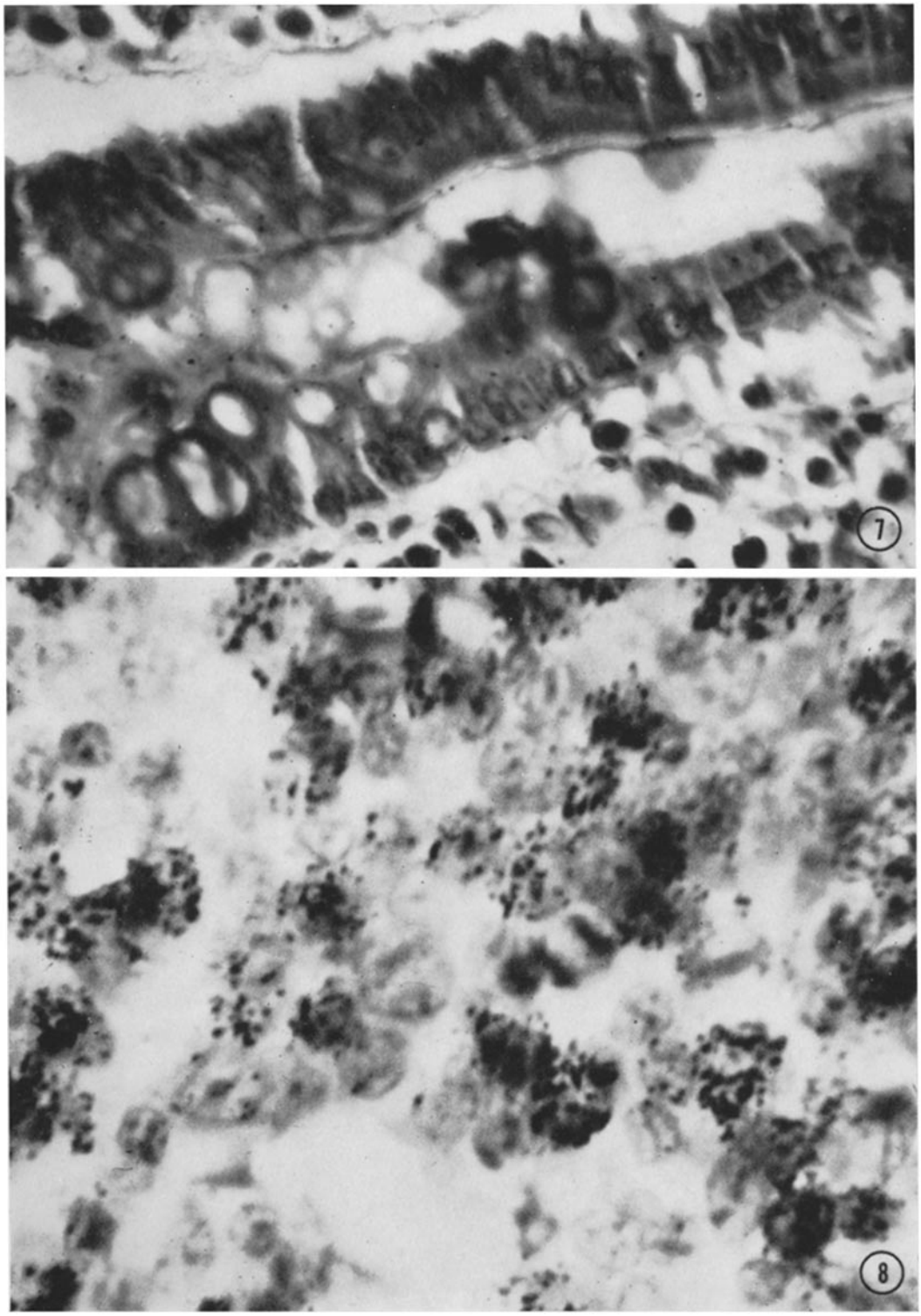


(Weissman: Thymus cell migration)

PLATE 30

FIG. 7. Small intestine of same host as Figs. 3, 5, and 6. Again, multiple sections of small and large intestine revealed no labeled cells. × 950.

FIG. 8. Thymus of a newborn rat infused intrathymically (as described) with thymidine-^{3}H and sacrificed 8 hr later. Many heavily labeled cells in this section. Other regions were relatively lightly labeled. × 950.



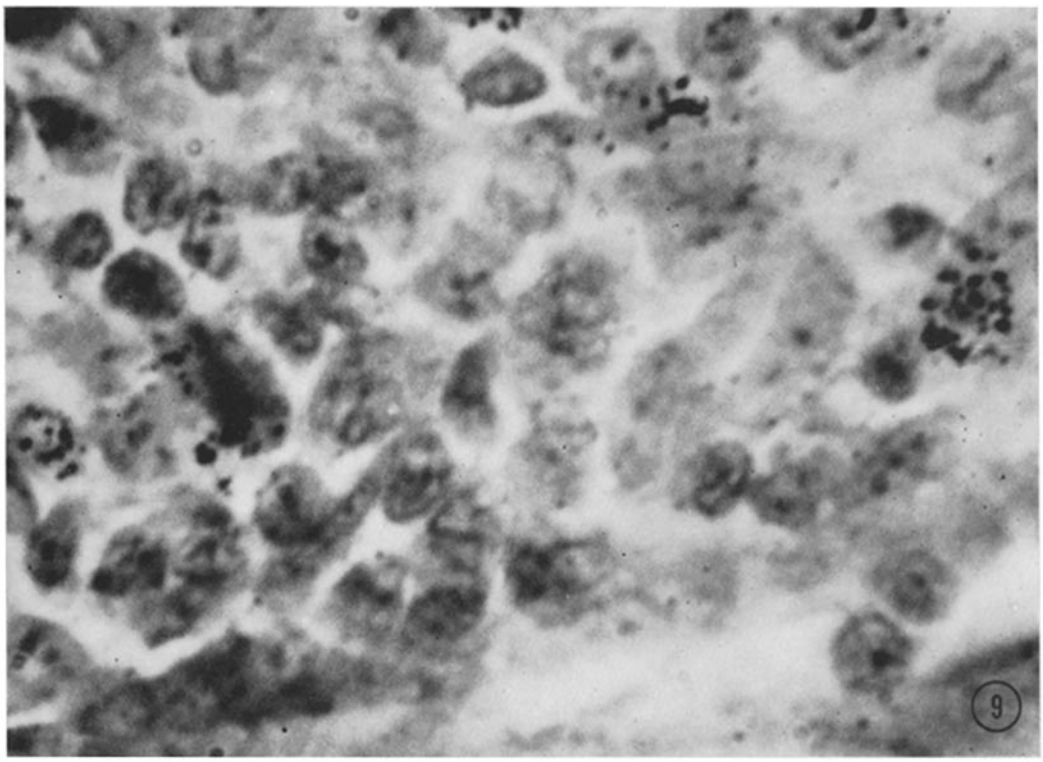
(Weissman: Thymus cell migration)

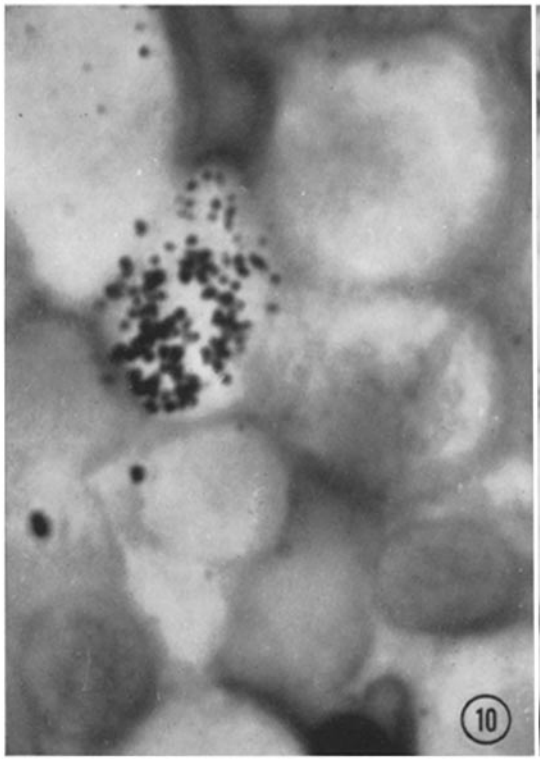
PLATE 31

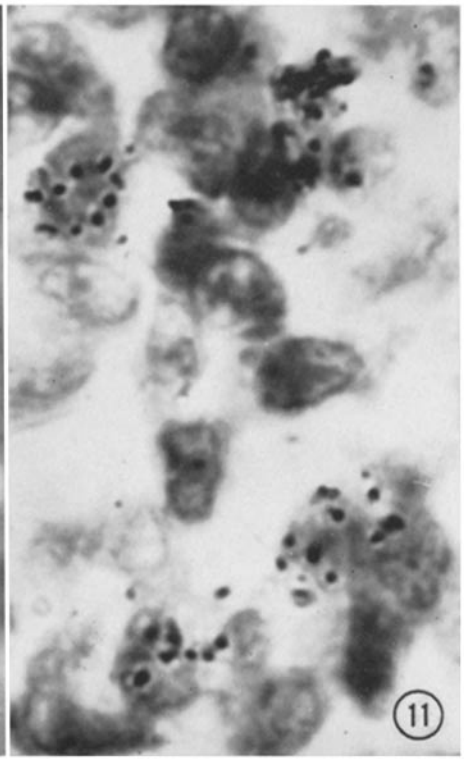
FIG. 9. Inguinal node of same host as Fig. 8. Approximately 10% of the cells were labeled. × 950.

FIG. 10. Spleen imprint of same host. A single, highly labeled cell. × 950.

FIG. 11. Newborn rat, 72 hr after infusion with adenosine-^{3}H. Lymph node; many lightly labeled cells. × 950.







(Weissman: Thymus cell migration)