STRUCTURAL AND CELLULAR CHANGES IN THE LYMPH NODES OF YOUNG MICE

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(Received for publication, March 27, 1968)

INTRODUCTION

As study material in immunological studies, mouse lymph nodes are more popular than nodes of other species, but, unfortunately, there is only a small amount of anatomical data on the lymph nodes of this animal. According to recent studies, it is suggested that under different physiological conditions, the lymph nodes contain not only reticular cells and lymphocytes, but plasma cells, mast cells and others[5,12,21,22].

On the other hand, studies in postnatal development of mouse lymph nodes have been reported by ARGYRIS, MASSHOFF & GROSS and TANABASHI. Recently, contrary to the theory that lymphocytes disseminate from the fetal or neonatal thymus to other lymphatic tissues, PARROTT et al. pointed out the existence of thymus-dependent areas in the spleen and the lymph nodes of neonatally thymectomized mice, and suggested that in addition to thymus-dependent areas, there exists another system primarily responsible for production of the plasma cell series.

In this paper, the writers will try to clarify a specific structure in young mice lymph nodes, which is closely related to thymus-dependent areas. Various cell changes in the lymph nodes were also investigated and will offer data in immunological studies.

MATERIALS AND METHODS

One hundred and ten NIH strain mice, neonatal to 10 weeks old, were used in the study (tab. 1). The mice were bred in the Department of Veterinary Anatomy, Hokkaido University. All animals were given a stock diet (MNF type: Oriental Yeast Co.) and water ad libitum in their usual environment.

Submandibular, mesenteric and subiliac lymph nodes were obtained from these animals. The nodes were fixed in CARNOY's fluid, embedded in paraffin, and sectioned at 4 to 6 μ

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TABLE 1 *Average body weight of mice*

AGE	CASES EXAMINED			BODY WEIGHT
	Male	Female	Total	$\bar{x} \pm s$
weeks				g
0*	4	6	10	1.52±0.19
1	5	5	10	3.85±0.69
2	5	5	10	6.92±1.61
3	5	5	10	8.79±2.43
4	5	5	10	12.85±2.18
5	5	5	10	20.75±1.99
6	5	5	10	22.89±5.25
7	5	5	10	24.58±3.28
8	5	5	10	27.15±4.62
9	0	10	10	25.70±3.44
10	5	5	10	29.15±3.43

*: Newborn

through the hilus. Nodes were stained with hematoxylin-eosin (H & E), TAFT's pyronine-methyl green, MCMANUS's PAS reaction, toluidine blue stain of OHNO et al. and SCHMORL's reaction.

In the mature nodes, cells were counted in the medullary cord. The appearance of large pyroninophilic cells and plasma cells is shown as their percentage of 500 cells counted in the cord. The mean number of mast cells and PAS positive cells was determined by counting 20 fields (one field = 10,000 square μ) in the medulla of each node. In small immature nodes without cortex-medulla differentiation, counts were made in the entire parenchyma and sinuses. In these small nodes, there were less than 20 fields (200,000 square μ), so the number of cells counted was multiplied by the appropriate number corresponding to a field of 200,000 square μ. For example, if a field was 100,000 square μ, the number of cells was multiplied by two.

OBSERVATIONS

I General structural changes in mouse lymph nodes

A Age differences of the nodes

(1) The capsules of the lymph nodes of neonatal mice were loose and thick (figs. 9 & 10). But, after the first week of life, as a result of maturation, the capsule became thinner. Through the stage from newborn to 10 weeks of age, the capsule was connected directly with the parenchyma of some areas thus obliterating the marginal sinus (figs. 5 & 9).

(2) The marginal sinuses in neonatal mice sometimes assume the form of lymph vessels (fig. 10); this kind of structure disappeared after the first week of life.

(3) The medullary sinuses are surrounded by the medullary cords. In neonatal lymph

nodes, only a few were found to have this structure (fig. 5). In all of the specimens, medullary sinuses appeared after the second week of life (tab. 2).

(4) According to the arrangement of the lymphatic tissue, lymph node parenchyma is generally divided into cortex and medulla. In neonatal mice, it is difficult to divide parenchyma into cortex and medulla. By the end of the first week of life, the medullary sinuses are differentiated and the cortex and medulla are clearly distinguishable (tab. 2).

TABLE 2 *Changes of the lymph node parenchyma*

AGE	CASES EXAMENED	SUBMAND. L.N.		MESENT. L.N.		SUBILIAC L.N.	
		MS	C/M	MS	C/M	MS	C/M
weeks							
0	10	7	1	1	0	2	0
1	10	10	10	9	9	8	8
2	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10
8	10	10	10	10	10	10	10
9	10	10	10	10	10	10	10
10	10	10	10	10	10	10	10

MS: Cases with medullary sinus
C/M: Cases with cortex-medulla differentiation

TABLE 3 *Relation between age and the appearance of the "light zone"*

AGE	CASES EXAMINED	SUBMAND. L.N.	MESENT. L.N.	SUBILIAC L.N.
weeks				
0	10	0	0	0
1	10	8	9	3
2	10	7	8	4
3	10	1	3	6
4	10	0	0	0
5	10	0	0	0
6	10	0	0	0
7	10	0	0	0
8	10	0	0	0
9	10	0	0	0
10	10	0	0	0

(5) Before the appearance of the mature secondary nodules, a specific area of lymphocyte depletion was found at the outer area of the cortex (figs. 4B, 6 & 13). In H & E stain or pyronine-methyl green stained sections, because of the scarce population of lymphocytes in this area, the area exhibited a lighter staining characteristic in optic microscopic observation. This structure was designated "outer light zone" by TANABASHI in rat lymph nodes. The "light zone" appears between the first and third week of life in mouse lymph nodes, but could not be found in newborn mice (tab. 3).

TABLE 4 *Relation between age and the appearance of secondary nodules*

AGE	CASES EXAMINED	SUBMAND. L.N.		MENSENT. L.N.		SUBILIAC L.N.	
		SN	MS	SN	MS	SN	MS
weeks							
0	10	0	0	0	0	0	0
1	10	0	0	0	0	0	0
2	10	10	0	10	0	7	0
3	10	4	6	8	2	10	0
4	10	0	10	1	9	3	7
5	10	0	10	0	10	1	9
6	10	0	10	1	9	0	10
7	10	0	10	0	10	0	10
8	10	0	10	0	10	0	10
9	10	0	10	0	10	0	10
10	10	0	10	0	10	0	10

SN: Solitary nodules only
MS: Mature nodules only, or with solitary nodules

TABLE 5 *Relation between age and the appearance of post-capillary venules*

AGE	CASES EXAMINED	SUBMAND. L.N.	MESENT. L.N.	SUBILIAC L.N.
weeks				
0	10	0	0	0
1	10	6	6	7
2	10	10	10	10
3	10	10	10	10
4	10	10	10	10
5	10	10	10	10
6	10	10	10	10
7	10	10	10	10
8	10	10	10	10
9	10	10	10	10
10	10	10	10	10

(6) Prior to the first week of life, no secondary nodules were found in mouse lymph nodes. Solitary nodules without germinal centers appeared at the second week of life (fig. 7). The mature secondary nodules with germinal centers were found from the third week (fig. 8). After the third week, secondary nodules appeared in all specimens (tab. 4).

(7) Post-capillary venules (fig. 12) are found in the middle and deep parts of the cortex, juxta-medullary area of the cortex and medulla. Appearance of the venules is shown in table 5; they did not appear in neonatal mouse lymph nodes.

(8) Extramedullary myelopoiesis (figs. 14~16), primarily granulocytopoiesis (fig. 14), and megakaryocytes (fig. 16) were found in the nodes, especially during the first to the third week of life (tab. 6).

TABLE 6 *Appearances of extramedullary myelopoiesis in lymph nodes*

AGE	CASES EXAMINED	SUBMAND. L.N.			MESENT. L.N.			SUBILIAC L.N.		
		E	G	M	E	G	M	E	G	M
weeks										
0	10	0	0	0	0	0	0	0	0	0
1	10	0	9	1	1	3	0	0	6	0
2	10	2	8	5	7	5	4	3	7	5
3	10	5	6	4	5	3	1	6	7	5
4	10	4	5	3	3	2	3	5	5	2
5	10	2	6	3	2	3	0	4	6	5
6	10	1	6	1	0	2	3	2	5	4
7	10	0	4	0	1	3	1	0	2	1
8	10	0	0	0	0	0	1	0	0	1
9	10	0	1	1	0	0	0	0	1	1
10	10	0	0	2	0	0	0	0	0	2

E : Erythrocytopoiesis
G : Granulocytopoiesis
M : Megakaryocytes
Cells were counted in 20 fields (200,000 square μ) in the medulla of each node. Each cytopoiesis contains at least one example of mitosis in the group of cells.

TABLE 7 *Typing of the mouse lymph nodes*

	TYPE					
	Embryonic	Immature			Mature	
Cortex-medulla differentiation	−	+	+	+	+	
Appearance of "light zone"	−	−	+	+	−	
Secondary nodules	−	−	−	SN	SN, MS	

SN : Solitary nodules only
MS : Mature nodules only or with solitary nodules

B Typing of the mouse lymph nodes

As shown in table 7, accroding to cortex-medulla differentiation, appearance of the "light zone", and appearance of the secondary nodules, the mouse lymph nodes were classified as embryonic, immature and mature types. Appearances of the three types of the lymph nodes are shown in table 8.

TABLE 8 *Changing of the types of lymph nodes*

AGE	CASES EXAMINED	SUBMAND. L.N.			MESENT. L.N.			SUBILIAC L.N.		
		E	I	M	E	I	M	E	I	M
weeks										
0	10	9	1	0	10	0	0	10	0	0
1	10	0	10	0	1	9	0	2	8	0
2	10	0	7	3	0	8	2	0	5	5
3	10	0	1	9	0	3	7	0	6	4
4	10	0	0	10	0	0	10	0	0	10
5	10	0	0	10	0	0	10	0	0	10
6	10	0	0	10	0	0	10	0	0	10
7	10	0	0	10	0	0	10	0	0	10
8	10	0	0	10	0	0	10	0	0	10
9	10	0	0	10	0	0	10	0	0	10
10	10	0	0	10	0	0	10	0	0	10

E : Embryonic type
I : Immature type
M : Mature type

(1) Embryonic type (figs. 9 & 10): Without the cortex-medulla differentiation, appearance of the "light zone" and secondary nodules, the embryonic type lymph nodes were most predominant in newborn mice.

(2) Immature type (figs. 5, 6 & 7): The lymph nodes of this classification were found from neonatal to the third week of life in the NIH strain mouse. This type exhibited a differentiated cortex-medulla; the "light zone" was found in most nodes of this type except in some of the younger immature type lymph nodes. In the most advanced group of this type, solitary nodules were found.

(3) Mature type (fig. 8): This type appeared at the second week of life, and was characterized by the differentiated cortex-medulla, disappearance of the "light zone", and more numerous secondary nodules.

The results show that the mouse lymph nodes matured on the fourth week after birth (tab. 8).

II Regional and maturation differences in cell counts of mouse lymph nodes

A Types of the cells counted

Following the studies of SUGIMURA et al.[22] on regional differences in the appearance of various cells in mouse lymph nodes, the writers decided to count four types of cells. The cell counts of the submandibular, mesenteric and subiliac lymph nodes were made on newborn to 10-week-old mice. The four types of the cells counted are described below:

(1) Large pyroninophilic cells (figs. 18 & 19), measuring from 10 to 15 μ: The cytoplasm and large nucleoli stained pinkish-red with pyronine-methyl green and metachromatically with toluidine blue.

(2) Plasma cells (figs. 17~19), measuring from 7 to 12 μ in diameter: The nucleus has an eccentric position; adjacent to the nucleus, a pale area occupies the middle of the cell. The staining property of the cytoplasm of plasma cells is the same as that of large pyroninophilic cells.

(3) Mast cells (figs. 20 & 21), measuring from 10 to 20 μ in diameter: The cytoplasm of these cells is filled with granules. The granules stain metachromatically with toluidine blue and pyronine-methyl green, but appear to be PAS negative.

(4) PAS positive cell type IV (SUGIMURA et al.[22]; fig. 22), ordinarily from 10 to 20 μ: Their cytoplasmic granules are positive by PAS and SCHMORL's reactions.

B The result of cell counts

(1) As shown in table 9 and figure 1, large pyroninophilic cells were found in materials from all ages used in the study, and increased in number with maturation. After the fourth week, regional differences in the cell became clear; the cells were dominant in the mesenteric lymph nodes.

TABLE 9 *Cell counts of large pyroninophilic cells in the medullary cord*

AGE	CASES EXAMINED	SUBMAND L.N. $\bar{x} \pm s$	MESSENT. L.N. $\bar{x} \pm s$	SUBILIAC L.N. $\bar{x} \pm s$
weeks		%	%	%
0	10	0.08±0.10	0.14±0.19	0.08±0.12
1	10	0.34±0.28	0.34±0.27	0.18±0.20
2	10	0.74±0.51	0.60±0.53	0.67±0.90
3	10	0.66±0.58	0.82±0.45	0.60±0.42
4	10	0.74±0.37	1.38±0.59	0.76±0.40
5	10	0.90±0.48	2.26±0.82	1.28±0.95
6	10	2.06±0.92	3.14±0.83	1.70±0.40
7	10	1.76±0.43	2.44±0.94	1.54±0.68
8	10	1.60±0.42	2.72±0.63	1.48±0.39
9	10	1.92±0.40	3.42±1.05	1.72±0.67
10	10	1.94±0.75	3.22±0.75	1.68±0.49

(2) Plasma cells could not be found in the lymph nodes of newborn mice. At the end of the first week, a few plasma cells began to appear in the lymph nodes. After the

FIGURE 1 *Regional and age differences in the appearance of large pyroninophilic cells*

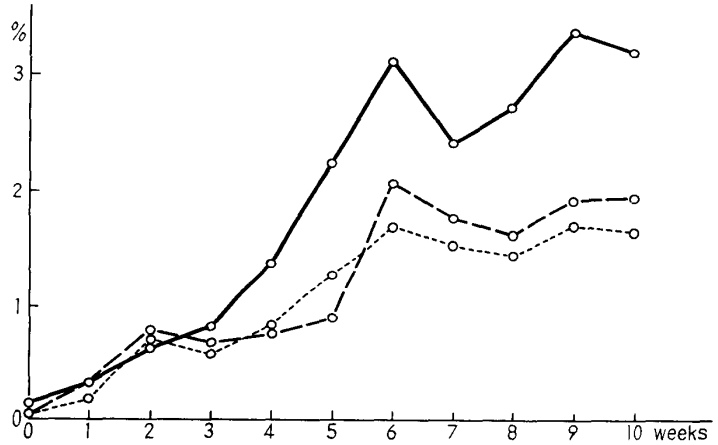


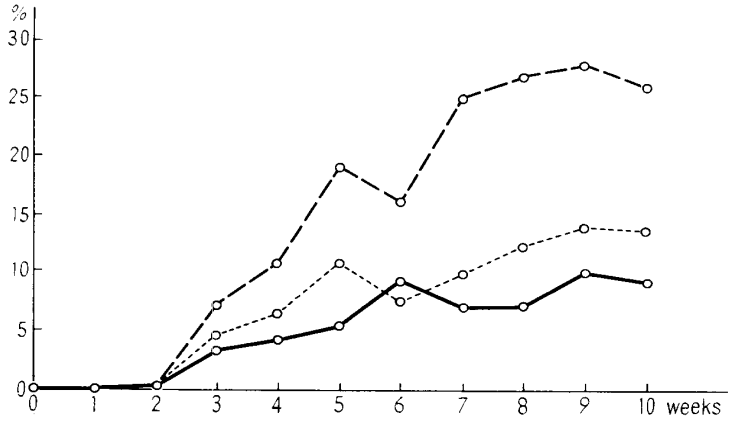
TABLE 10 *Cell counts of plasma cells in the medullary cord*

AGE	CASES EXAMINED	SUBMAND. L.N. $\bar{x} \pm s$	MESENT. L.N. $\bar{x} \pm s$	SUBILIAC L.N. $\bar{x} \pm s$
weeks		%	%	%
0	10	0	0	0
1	10	0	0.08 ± 0.19	0
2	10	0.38 ± 0.43	0.12 ± 0.25	0.10 ± 0.17
3	10	7.20 ± 4.60	3.56 ± 1.23	4.80 ± 1.52
4	10	11.10 ± 3.94	4.26 ± 1.79	6.04 ± 4.50
5	10	19.42 ± 5.97	5.48 ± 2.70	10.92 ± 3.23
6	10	16.08 ± 7.95	8.92 ± 7.56	7.82 ± 3.02
7	10	25.50 ± 8.48	7.30 ± 3.27	9.06 ± 3.21
8	10	27.54 ± 6.06	7.38 ± 1.90	12.36 ± 2.92
9	10	28.44 ± 4.49	10.04 ± 3.93	13.92 ± 3.72
10	10	26.50 ± 5.27	9.62 ± 2.72	13.80 ± 1.46

end of the third week, they increased rapidly (tab. 10 & fig. 2). At the same time, regional differences became clear; the cells were dominant in the submandibular lymph nodes and scarce in the mesenteric lymph nodes.

(3) The appearance of mast cells is shown in table 11 and figure 3. These cells were found in the lymph nodes of neonatal mice. Although their appearance varied greatly, still, they increased in number with maturation. The mast cells found were dominant in the

FIGURE 2 *Regional and age differences in the appearance of plasma cells*



Submandibular lymph node : — — — —
Mesenteric lymph node : ————
Subiliac lymph node : ....

TABLE 11 *Cell counts of mast cells in medulla*

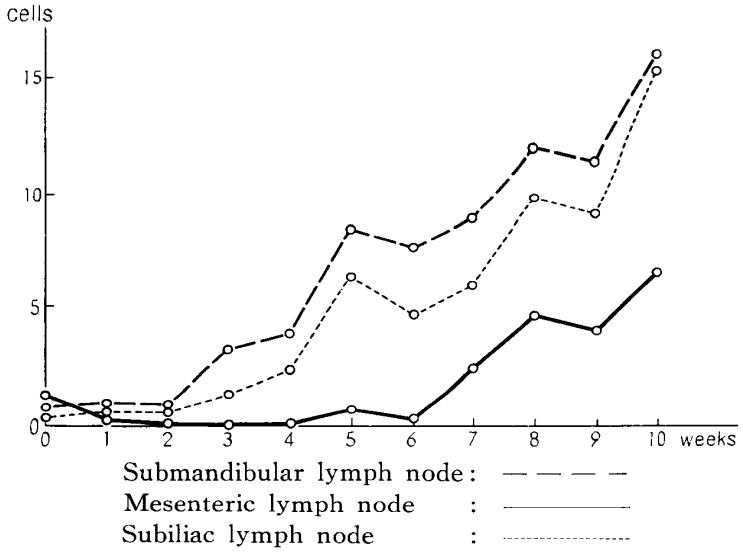
AGE	CASES EXAMINED	SUBMAND. L.N. $\bar{x} \pm s$	MESENT. L.N. $\bar{x} \pm s$	SUBILIAC L.N. $\bar{x} \pm s$
weeks				
0	10	0.70 ± 0.74*	1.10 ± 1.46*	0.30 ± 0.48*
1	10	0.80 ± 0.79*	0.20 ± 0.42*	0.40 ± 0.70*
2	10	0.80 ± 0.79	0	0.60 ± 1.07
3	10	3.20 ± 2.86	0	1.30 ± 1.34
4	10	3.80 ± 3.36	0	2.80 ± 2.44
5	10	7.30 ± 10.56	0.50 ± 3.72	6.30 ± 3.83
6	10	6.50 ± 8.13	0.30 ± 0.48	4.70 ± 5.42
7	10	7.60 ± 3.98	2.70 ± 4.79	6.10 ± 6.05
8	10	12.00 ± 12.51	4.70 ± 5.17	9.90 ± 7.33
9	10	11.50 ± 6.77	4.00 ± 5.21	7.60 ± 6.26
10	10	15.90 ± 7.54	6.60 ± 12.06	15.10 ± 7.82

*: Reference data; cell counts did not attain 200,000 square μ.

submandibular lymph nodes and scarce in the mesenteric lymph nodes.

(4) PAS positive cells were very rare in young mice; very few cells were found in two subiliac lymph nodes of nine-week-old mice.

FIGURE 3 *Regional and age differences of mast cells in medulla*



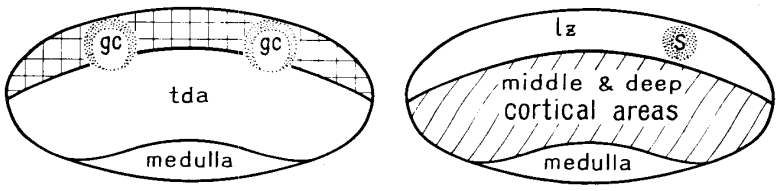
DISCUSSIONS

There are many studies[2,11,13,18] on the development of lymph nodes. These reports show that the development of lymph nodes is completed before birth. Their postnatal development seems to involve the appearance of secondary nodules only. However, in the rat[24] and the mouse[3,15,23], the lymph nodes show some immature structures after birth. In this study, the writers found that the immature characters of the mouse lymph nodes are shown by their capsule-parenchyma connection, tube-like marginal sinus, indistinguishable parenchyma (can not be separated into cortex and medulla), a great deal of extramedullary myelopoiesis, and the appearance of a most conspicuous characteristic; the "light zone". To consider these specific characteristics of mouse lymph nodes, the writers classified mouse lympe nodes into embryonic, immature and mature types. The writers found that the mouse lymph node matures during the fourth week of life.

It will be seen that the most dramatic feature of the postnatal develoment of the mouse lymph node is the appearance of the "light zone". The structure has been reported in newborn rat lymph nodes by TANABASHI; he suggested that the "outer light zone" is the germinal layer of young rat lymph nodes prior to the appearance of the secondary nodules. In the mouse, the writers found that the "light zone" appeared consistently at the same time as the post-capillary venules, and it is considered that there is some special relationship between them. Post-capillary venules are not found in the outer area of the cortex; this finding was also reported by GOWANS & KNIGHT. In their study, they also established

the passing of small lymphocytes through post-capillary venules and the subsequent reappearance of these small lymphocytes in the lymph nodes. According to these findings, recirculated lymphocytes aggregate in the portion where post-capillary venules are located. Therefore, outer areas of the cortex would be depleted of lymphocytes and in this study the area was designated the "light zone". However, in fact, this zone does not have real lymphocyte depletion; actually the multiplication of small lymphocytes in the middle and deep cortical areas is faster than in the "light zone", where no post-capillary venules are located. The source of these recirculating lymphocytes, according to present knowledge[17,19,26], is considered to be thymus. Using labeled thymus and spleen cells for injection into neonatally thymectomized mice, PARROTT et al. found that these cells preferentially localize in the middle and deep cortical areas of lymph nodes. They named this specific area the "thymus-dependent area", and suggested that other than the "thymus-dependent area" exists another system primarily responsible for production of plasma cell series. To compare this study with the report of PARROTT et al., the cortex of mouse lymph nodes seems to be composed of two different parts; i.e., the "light zone" and the "thymus-dependent area" (figs. 4 A & 4 B). Furthermore, ACKERMAN shows that in the fetal cat, the lymphocyte

FIGURE 4 *Relationship of "light zone" and "thymus-dependent area" in the mouse lymph node*



A Scheme of "thymus-dependent area" of PARROTT et al. (tda)
gc: Germinal center of secondary nodules
B Scheme of "light zone" (lz) s: Solitary nodules

formation arises in both lymph node and thymus anlagen, and suggested that lymphocytes derived from the thymus may provide a second population of lymphocytes for other lymphocytic tissue. From these viewpoints, the "light zone" may be considered as the original lymphoid tissue of the lymph node, which provides the node with a primitive population of lymphocytes.

SUGIMURA et al.[22] have clarified the regional differences according to the appearance of large lymphocytes, plasma cells, mast cells, PAS positive cells and adipose tissue in the mature mouse lymph nodes. They classified the mouse lymph nodes into five groups based on their findings. In this study, following

their study, the writers examined the submandibular node (many plasma cell groups), the subiliac node (many mast cell groups) and the mesenteric node (a few plasma cell and mast cell groups) to trace quantitative changes of the cells.

The large pyroninophilic cells, considered to be the same as the pyroninophilic large lymphocyte of SUGIMURA et al.[22], actively produce small lymphocytes. This study produced the same results concerning regional differences as the study of SUGIMURA et al.[22]; the pyroninophilic cells are predominant in the mesenteric lymph nodes after the fourth week of life. Large pyroninophilic cells increase in numer somewhat after antigenic stimulation; they react in a similar manner to thymus stimulating hormone (METCALF). In the writers' work, material from mice six weeks of age contained large numbers of these cells; it is interesting that this time is just after the thymus attains its maximal weight (ITO & HOSHINO).

The fact that plasma cells produce antibodies has been clarified by many investigators. The cells are found in the medullary cords and juxta-medullary area of the cortex of the lymph nodes under normal physiological conditions. The appearance of plasma cells in lymph nodes has been studied. THORBECKE & KEUNING reported that in the rabbit, plasma cells can not be found in the newborn; after 1 to 2 weeks they begin to appear in the intestinal wall and the spleen. AWAYA et al. reported that in the rat plasma cells are found in large quantities during the second week of life. In the mouse, MASSHOFF & GROSS reported that great multiplication of plasma cells was found after the third week of life; however, ARGYRIS found that plasma cells appeared in popliteal nodes of 6-week-old mice. In this study, no plasma cells were seen in the lymph nodes of newborn mice. At the end of the first week, very few cells began to appear in the mesenteric lymph nodes, after the end of the third week they increased rapidly in all the nodes. In lymph nodes, regional differences in the number of plasma cells has been reported by FUJII, JORDAN & MORTON, KELSALL & CRABB and SUGIMURA et al.[22] With the exception of FUJII, all the investigators have found that the cells are dominant in the submandibular nodes. In this study, plasma cells clearly show regional differences, after the third week of life, they were found predominantly in the submandibular nodes and rarely in the mesenteric nodes; the finding suggests that the drainage area of the submandibular nodes receives more antigenic stimulation than the mesenteric nodes which have a barrier of plasma cells in the lamina propria of the intestinal tract.

After a review of recent contributions to the knowledge of the mast cell and its fluctuation with age, SIMPSON concluded that there is no common pattern of mast cell changes with age, and the mast cell population of any tissue is controlled largely by local factors, either physiological or pathological. The results of this study seem to support SIMPSON's viewpoint; the mast cell population in the study

shows a great deal of variety. Mesenteric lymph nodes show a scattered population of mast cells, this corresponds to SUGIMURA's report[22]; but, between 2 to 4 weeks after birth, there are no mast cells found in mesenteric nodes. This finding seems to agree with the finding in the intestinal tract of man; LINDHOLM reported a momentary decrease of mast cells in babies. In the present work, regional differences of mast cells were similar to the results of SUGIMURA et al.[22], but the number of mast cells in the submandibular and subiliac lymph nodes was about the same.

Very few PAS positive cells were found in two mice, however, they have been found in the cat[21] and the mouse[6,22]. SUGIMURA et al.[22] reported that PAS positive cells can not be found in submandibular and subiliac lymph nodes; however, the writers found the cells in the subiliac node of one mouse.

This study seems to suggest that the "light zone" is a primary structure of mouse lymph nodes; on the other hand, it elucidates the importance of the selection of the location of nodes in studies of lymph nodes.

SUMMARY

In this study, the writers concentrated their attention upon histological changes of structures and cells of mouse lymph nodes. According to the difference in structural maturity, the nodes were classified as to embryonic, immature and mature types. The "light zone" was found in immature type lymph nodes. The structure suggests the possibility of independent primitive lymphoid tissue of the lymph node in contrast to the "thymus-dependent area".

The appearance and regional differences of large pyroninophilic cells, plasma cells, mast cells and PAS positive cells were also studied: Large pyroninophilic cells and mast cells were found in the lymph nodes of the neonatal mice and increased in number with age. Plasma cells began to appear in lymph nodes at the end of the first week and increased in number rapidly after the end of the third week. Regional differences of these cells were clear: Large pyroninophilic cells were dominant in the mesenteric lymph nodes. Plasma cells and mast cells were predominantly found in the submandibular lymph nodes. PAS positive cells were very rare in lymph nodes of young mice.

ACKNOWLEDGEMENT

The writers wish to offer cordial thanks to Dr. C. B. SWENSON, Associate Professor of the Department of Veterinary Anatomy, Purdue University, for his kind assistance.

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PLATES

EXPLANATION OF PLATES

PLATE I

Fig. 5 Submandibular node, newborn; youngest immature type lymph node Parenchyma can be divided into cortex and medulla. No "light zone" appeared; part of parenchyma connected with capsule (arrow). H-E × 90

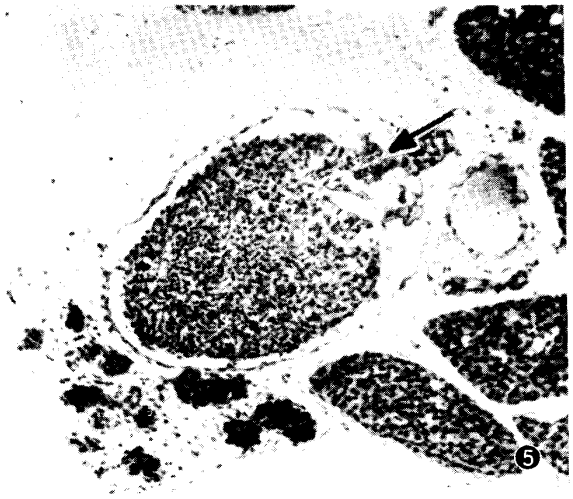
Fig. 6 Mesenteric node, 1 week; immature type lymph node Appearance of "light zone" (within arrows) H-E × 40

Fig. 7 Mesenteric node, 2 weeks; immature type lymph node The node is larger than in figure 6. In the cortex, many solitary nodules are arranged at the light zone area; the "light zone" is not so clear. H-E × 40

Fig. 8 Submandibular node, 3 weeks; Mature type lymph node Three lymph nodules with germinal centers are found in the node. H-E × 40

Fig. 9 Mesenteric node, newborn; embryonic type lymph node Capsule is thick but shows looseness; all the mass of parenchyma seems to connect with the loose capsule (right side). PAS reaction × 140

Fig. 10 Subiliac node, newborn; embryonic type lymph node Marginal sinus appears as lymph vessel, capsule also is thick. Parenchyma can not be separated into cortex and medulla. Pyronine-methyl green × 110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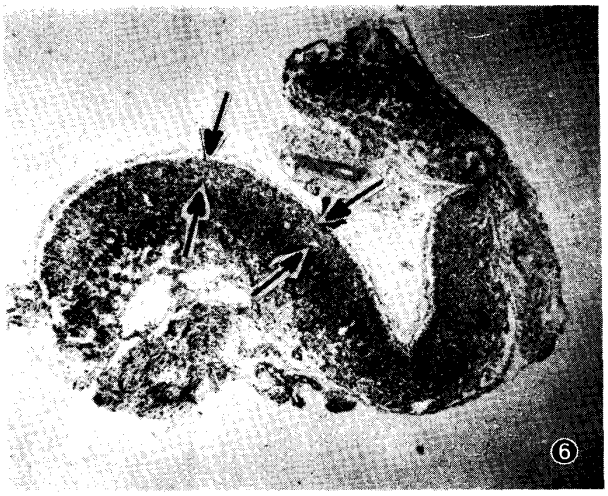
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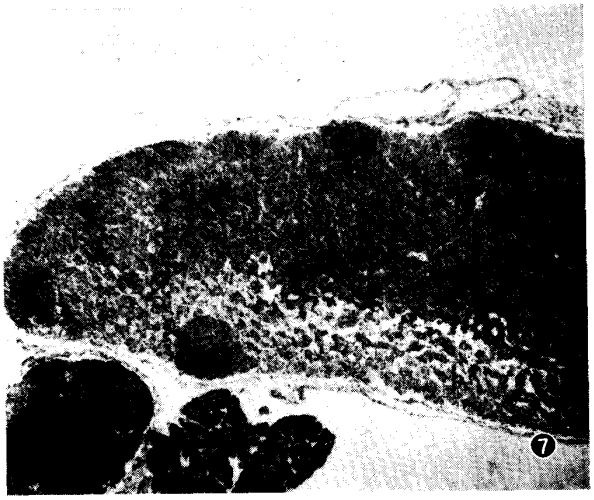
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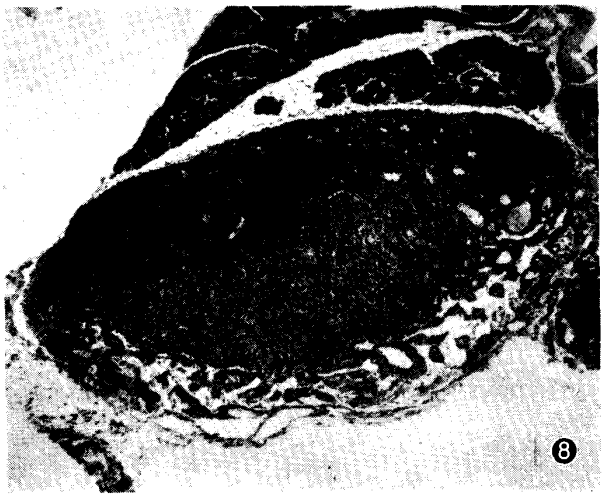
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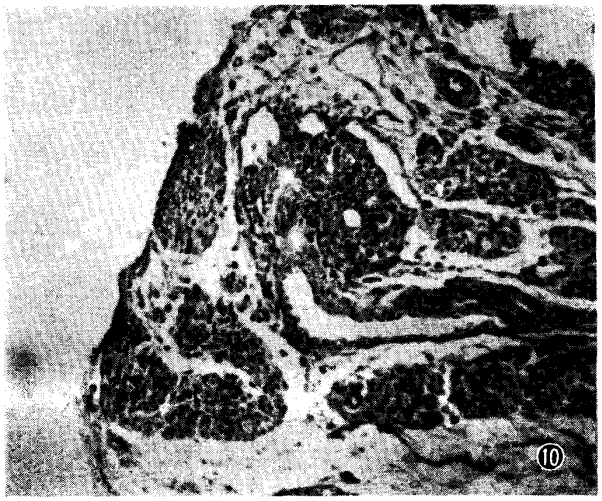
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PLATE II

Fig. 11 Enlargement of figure 10; No plasma cell can be found in the parenchyma. Blood vessels are numerous but no post-capillary venule. Pyronine-methyl green × 450

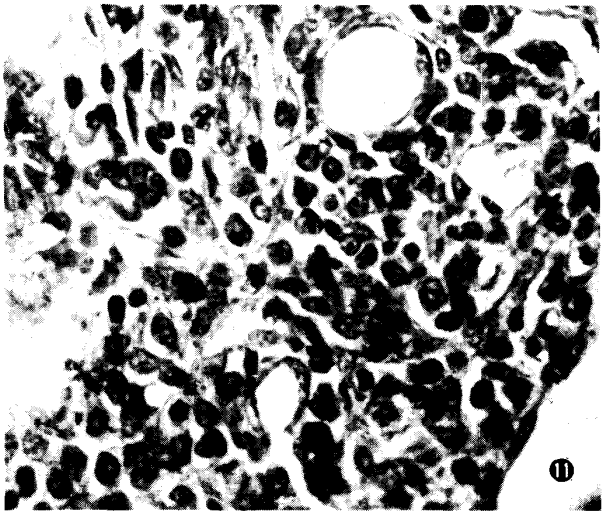
Fig. 12 Submandibular node, 1 week; medulla Many post-capillary venules are found with large endothelial cells; some migrating lymphocytes are found (arrows). Toluidine blue × 450

Fig. 13 Enlargement of figure 6; "light zone" (outer part of cortex) with a few small lymphocytes Some post-capillary venules are found in the middle and deep parts of cortex. H-E × 140

Fig. 14 Subiliac node, 3 weeks; Granulocytopoiesis in medullary cord Arrows show mitosis. H-E × 750

Fig. 15 Mesenteric node, 1 week; Extramedullary myelopoiesis in medullary cord A group of normoblasts with mitosis (arrow) is found. H-E × 750

Fig. 16 Subiliac node, 4 weeks; A large megakaryocyte appears in medulla. H-E × 750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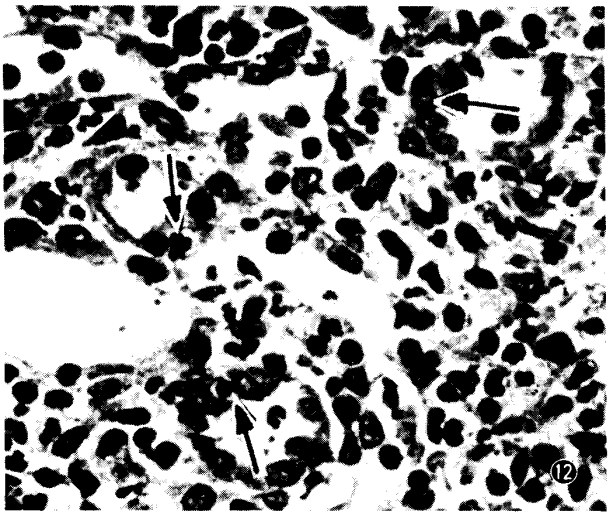
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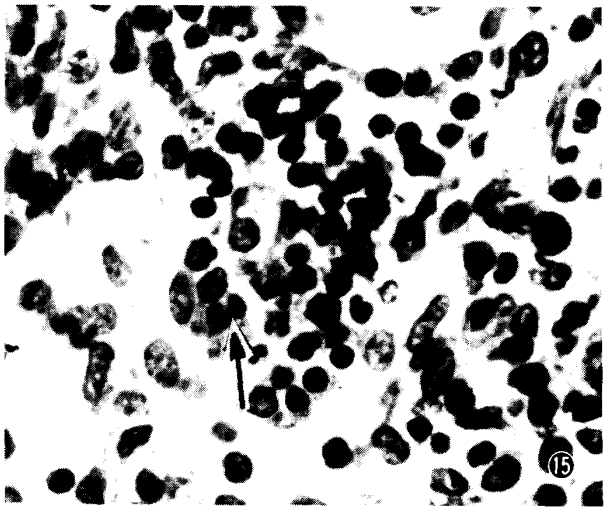
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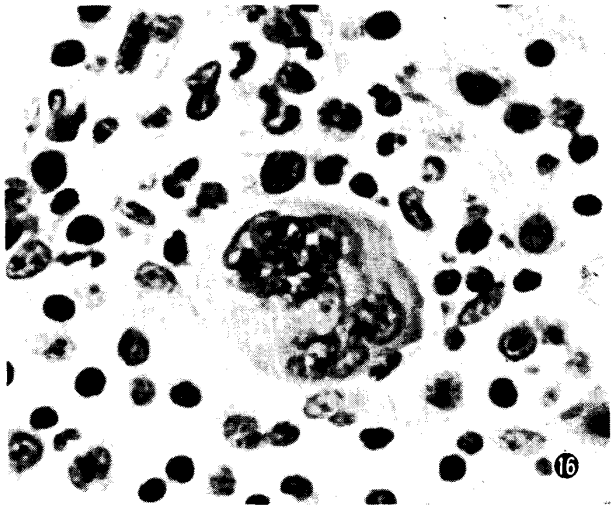
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PLATE III

Fig. 17 Mesenteric node, 1 week; The first appearance of plasma cells (arrows) in medulla Pyronine-methyl green × 750

Fig. 18 Submandibular node, 9 weeks; medullary cord of lymph node Plasma cells are fairly numerous, some large pyroninophilic cells also present. Pyronine-methyl green × 750

Fig. 19 Mesenteric node, 5 weeks; Many large pyroninophilic cells are clearly illustrated with some plasma cells. H-E × 750

Fig. 20 Submandibular node, 5 weeks; two mast cells stained metachromatically appear at upper part of the figure. Pyronine-methyl green × 750

Fig. 21 Submandibular node, 9 weeks; many metachromatically stained mast cells are distributed in medulla of the lymph node. Toluidine blue × 200

Fig. 22 Subiliac node, 9 weeks; the cell (arrow) has positive SCHMORL's reaction SCHMORL's reaction × 750