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FURTHER STUDIES OF A LYMPHOCYTIC HEMOGRAM AND ITS RELATION TO LYMPHOCYTOPOIESIS

I. VARIATIONS IN MITOCHONDARIAL CONTENT IN BLOOD LYMPHOCYTES IN RELATION TO THE POSTNATAL DEVELOPMENT OF THE LYMPHATIC APPARATUS IN THE RAT*†

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As is well known, the number of nuclear segments of the polymorphonuclear leukocyte is an indication of cell maturity. In the lymphocyte, however, the nuclear configuration shows on significant variations which can serve as a reliable indication of the age of the cell. Therefore, other criteria for estimating lymphocyte maturity should be sought in the cytoplasm, viz., the amount of cytoplasma or cell size, the degree of basophilia and the number of mitochondria, in particular. Among these, the amount of cytoplasm or cell size and the mitochondrial content are easily measurable, quantitatively.

In our laboratory an attempt has been made for the past several years to establish a classification of lymphocytes according to the number of mitochondria supravitally stained with *Janus* green B, which may reflect changes in production and delivery of lymphocytes in lymphnid organs. The previous observations on rabbits by *Osogoe* et al. (1953) and *Awaya* (1956) have demonstrated that there occurs a significant rise in the average number of mitochondria in circulating lymphocytes, coincident with an extensive new formation of secondary nodules of the *Flemming* type in the lymphatic apparatus. This was observed not only in the normal course of postnatal development but also after intravenous injection of foreign protein (ovalbumin). It also has been shown that the secondary nodules of the *Flemming* type first appear as foci of active proliferation of medium-sized lymphocytes.

On the basis of these observations, it is inferred that a rise in mitochondrial content in circulating lymphocytes is linked with an extensive new formation of *Flemming*'s secondary nodules in lymphoid organs, which, in turn, indicates an increased production and delivery of lymphocytes.

In or der to obtain further evidence to support this inference, a series of observations have been made on rats and mice. This report describes the variations in

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mitochondrial content in circulating lymphocytes in relation to the postnatal development of the lymphatic apparatus of the rat.

MATERIAL AND METHODS

Eighty normal healthy rats (a modification of the Wistar strain) of both sexes, evenly distributed among various age groups from 1 week to 12 months (Tables 1–3), were studied.

For staining mitochondria of lymphocytes, virtually the same supravital technique with *Janus* green B and neutral red was used as in the earlier studies by *Osogoe* et al. (1953) and *Awaya* (1956). A mixed solution of *Janus* green B (*Grübler*) and neutral red (*Grübler*) in absolute alcohol was used for preparing slides. Each 10 ml of this solution contained 20 drops of *Janus* green B and 30 drops of neutral red, both saturated in absolute alcohol. A drop of blood from the tail vein was placed upon such a slide, covered with a glass slip and sealed about the edges with vaseline. After the slides thus prepared had been kept in a thermostat at 37°C. for 20 minutes, they were examined at room temperature. Routine blood examinations were made of another drop of blood from the tail vein.

Mitochondria thus stained were counted in 100 lymphocytes from each animal so as to give a total 1000 or 500 lymphocytes in each age group (Table 2). The lymphocytes were classified into six groups according to their mitochondrial content as shown in Table 2, and the average number of mitochondria per lymphocyte was determined. In addition, a mitochondrial index, which indicates the predominating type of lymphocytes, was computed using the same data. For example, using the data presented in the extreme upper row of Table 2, calculation may be made in the following manner:

$$\text{M.I.} = \frac{1\times 1.7+2\times 19.3+3\times 54.4+4\times 19.9+5\times 3.3+6\times 1.4}{100} = 3.08$$

In blood smears stained with Giemsa the lymphocytes were classified according to their cell size into 3 types: small lymphocytes measuring less than 9 μ; medium-sized lymphocytes, 9–13 μ; and large lymphocytes, more than 14 μ in diameter.

From each age group two or three animals were killed by cervical dislocation and bleeding. As representatives of lymphoid organs, the mesenteric lymph nodes, spleen and thymus were examined after fixing in Zenker-formol, embedded in paraffin, cut serially at 5 μ and stained with *Mayer*'s acid hemalum and eosin.

Observations

1. Variations in mitochondrial content in blood lymphocytes

The results of blood examinations in various age groups are tabulated in Table 1 and illustrated in Figure 1. Both the total white cell count and lymphocyte count were at considerable low levels until 4 weeks after birth. Thereafter, however, both counts rose abruptly, reaching a first peak at 4 months, and then fell sharply at 5 months. At 12 months of age, a second increase was observed in both counts.

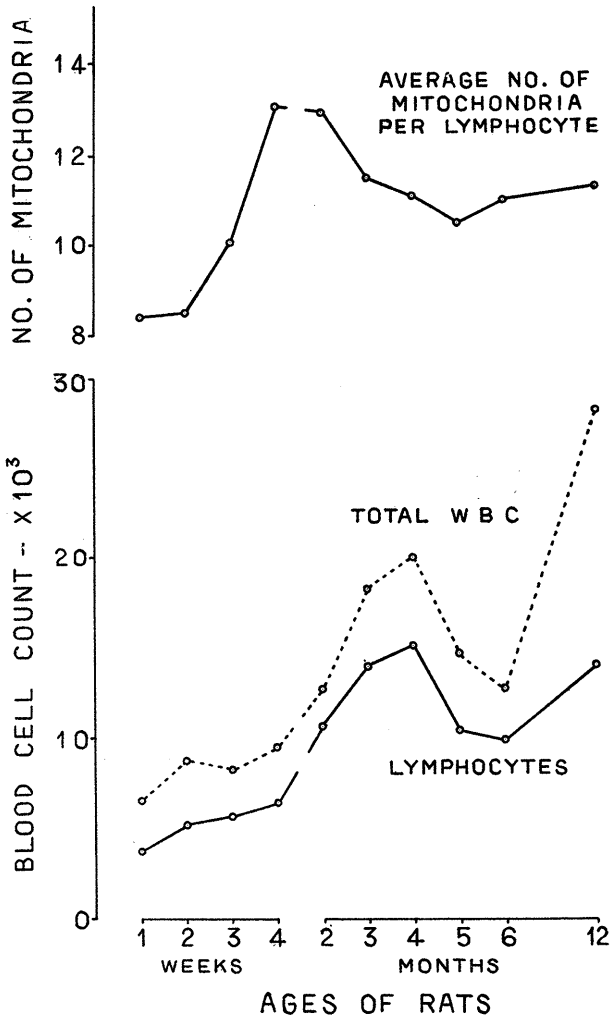

Fig. 1. Variations in blood cell counns and average number of mitochondria per lymphocyte in the peripheral blood of normal rats at different ages.

Table 1. Blood cell counts and average number of mitochondria per lymphocyte in the peripheral blood of normal rats at different ages.

Age in weeks or months	No. of rats examined	Body weight (g)	RBC ($\times 10^4$)	Hb (%)	WBC*	Lymphocyte count*	Total No. of lymphocytes counted	Avg. No. of mitochondria per lymphocyte	Mitochondrial index of lymphocyte	Degree of development of secondary nodules** in mesenteric lymph nodes
1 week	10	9	327		6550	3820	1000	8.44	3.08	−
2 weeks	10	11	494	33	8930	5260	1000	8.56	3.11	+
3 weeks	10	21	548	45	8265	5669	1000	10.15	3.42	+
4 weeks	10	37	659	71	9555	6496	1000	13.16	4.02	++
2 months	10	124	958	112	12980	10775	1000	13.06	4.01	+++
3 months	10	160	1045	133	18368	14026	1000	11.59	3.68	+++
4 months	5	178	917	110	20040	15273	500	11.11	3.61	+++
5 months	5	188	1003	118	14760	10404	500	10.59	3.51	++
6 months	5	200	876	112	12760	9844	500	11.08	3.62	++
12 months	5	286	833	169	28400	14086	500	11.03	3.57	++

* The total white cell count and lymphocyte count are subject to considerable individual variation.

** Immature and mature forms of *Flemming*'s secondary nodules (cf. Table 3).

Table 2. Differential counts of lymphocytes in the peripheral blood of normal rats at varying ages.

Age in weeks or months	No. of lymphocytes counted	Classification of lymphocytes according to mitochondrial content						Classification of lymphocytes according to cell size*		
		I 0	II 1-5	III 6-10	IV 11-15	V 16-20	VI 21-	Small L.	Medium-sized L.	Large L.
1 week	1000	1.7	19.3	54.4	19.9	3.3	1.4	82.1	17.1	0.6
2 weeks	1000	1.2	19.3	53.5	19.7	5.2	1.1	84.9	14.3	0.6
3 weeks	1000	1.4	13.7	44.7	16.6	9.1	4.5	84.8	14.4	0.7
4 weeks	1000	1.0	2.9	23.6	44.2	19.7	8.6	83.8	14.5	1.6
2 months	1000	1.0	2.8	28.7	37.9	20.0	9.6	78.5	19.6	1.8
3 months	1000	2.1	3.5	40.4	36.3	13.3	4.4	83.3	15.0	1.5
4 months	500	2.0	5.6	41.4	34.4	13.4	3.2	84.8	13.9	1.1
5 months	500	1.8	4.6	46.0	37.0	9.4	1.2	84.2	14.1	1.6
6 months	500	1.6	6.6	41.8	33.6	12.4	4.0	84.3	13.2	2.4
12 months	500	4.8	3.8	41.2	34.2	10.8	5.2	74.6	22.9	2.3

* Small lymphocytes measure less than 9μ; medium-sized lymphocytes, 9-13μ; and large lymphocytes, more than 14μ in diameter in blood smears stained with *Giemsa*.

In contrast, the average number of mitochondria per lymphocyte and mitochondrial index varied in an essentially different fashion. Both values began so increase already at 2 weeks and reached the maximal level at 3 weeks, and were maintained at a similar high level until 2 months after birth. Thereafter, both values considerably decreased and did not rise again. Since both values run quite parallel each other, only the variation in average number of mitochondria per lymphocyte was depicted in Figure 1. Statistical analysis disclosed that the rise in the average number of mitochondria per lymphocyte above the initial value is highly significant ($P < 0.01$), and that the lower values observed from 3 to 12 months after birth is also significant as compared with the higher values at 3 weeks and 2 months ($P < 0.05$).

The results of differential counts of blood lymphocytes on the basis of classifications of these cells according to mitochondrial content and cell size are listed in Table 2. It is seen that the classification of lymphocytes according to mitochnodrial content reveals a shift of the predominating type of these cells from type III to type IV at 3 weeks and 2 months after birth, whereas the classification of lymphocytes according to cell size fails to disclose such a shift at any age.

Most of the mitochondria in lymphocytes appear as small distinct granules which are either scattered around the nucleus, or grouped in a portion adjacent to the nuclear identation. In the morphology of mitochondria no change could be seen among different age groups.

2. *Postnatal development of lymphoid tissue*

In the normal course of its postnatal development, the lymphoid tissue undergoes not only quantitative but also qualitative changes, except for the thymic tissue, which shows only quantitative changes.

In order to roughly estimate the quantitative age changes, variations in weights of lymphoid organs in different age classes from 1 week to 12 months were followed. As seen in Table 3, the weights of thymus and mesenteric lymph nodes reach their maximal level at about 4 months and thereafter tend to decrease, whereas the weight of spleen appears to keep on increasing until 12 months of age. This fairly well agrees with the observations of *Andreasen* (1943), who made extensive, quantitative investigation on the thymolymphatic system in normal rats at different ages, using a far greater number of animals for each age class than in the present work. It is interesting to note that at about 4 months both the total white cell count and lymphocyte count show a first peak (Fig. 1).

The most striking qualitative change in the lymphoid tissue is the formation of secondary nodules, the *Flemming*'s types in particular.

The secondary nodules (or cortical nodules) were classified into 3 main types as described previously by *Awaya* (1956): (1) *Flemming*'s *secondary nodules or mature*

secondary nodules are typical secondary nodules with a pale-staining peripheral zone (Fig. 2); (2) *Solid secondary nodules* of *Groll* and *Krampf* (1920–21) denote nodules that are solidly packed with small lymphocytes (Fig. 3); (3) *Pseudo-secondary nodules* of *Ehrich* (1929) are enormously large masses of diffuse lymphatic tissue, often containing a few solid secondary nodules or *Flemming*'s secondary nodules (Fig. 4) and characterized by the presence of the post-capillary veins with high endothelial cells resembling cuboidal epithelium (Fig. 5).

The early stages of *Flemming*'s secondary nodules were termed *immature secondary nodules* as in the previous study (*Awaya*, 1956), because as they become mature they give rise eventually to *Flemming*'s nodules. The immature secondary nodules first appear as small isolated masses of densely packed, rapidly dividing medium-sized lymphocytes in either diffuse or compact lymphatic tissue. Unless they develop in the solid secondary nodules, they are initially "bare" without any demarcating outer zone of small lymphocytes (Fig. 6); in this case they are identical to "bare germinal centers" of *Conway* (1937). However, they often develop in the pre-existing solid secondary nodules. In such instances an outer zone of small lymphocytes completely or partially surround the germinal centers from the beginning (Fig. 7).

The pale-staining centers of the *Flemming*'s secondary nodules or mature secondary nodules vary considerably in their cellular constitution. If they are almost entirely composed of densely packed, medium-sized lymphocytes with many mitotic figures, they are termed "active" (Figs. 8–9). In numerous nodules of adult rats, however, one-half is active while the other is not. Such nodules are called "half-active" (Fig. 10). In some instances, the center of the nodules is quite "inactive", being composed mainly of reticular cells and devoid of proliferating lymphocytes (Fig. 11). In half-active nodules of the lymph nodes (Fig. 10), the inactive zones are at the capsular end of the nodules, as described by *Rölhich* (1930), *Kindred* (1938) and *De Bruyn* (1948).

The results of observations on the postnatal development of various types of secondary nodules outlined above in the mesenteric lymph nodes and spleen are summerized in Table 3.

In the mesenteric lymph nodes, no nodullar masses are discernible at one week (Fig. 12); it is from the second week on that solid secondary nodules and pseudo-secondary nodules make their appearance. Thereafter neither nodules show any remarkable qualitative changes. The *Flemming*'s secondary nodules, on the other hand, undergo not only quantitative but also qualitative changes; they begin to occur as immature secondary nodules from the second week on and, rapidly growing in size and number, reach their full maturity at about 2 months of age (Figs. 13–14). In later stages, the *Flemming*'s nodules gradually undergo involutionary changes.

It should be emphasized here that formation and maturation of *Flemming*'s

Table 3. Body weight, weights of lymphoid organs, and the degree of development of secondary nodules in the mesenteric lymph nodes and spleen of normal rats at different ages. The activity of myelopoiesis in the splenic pulp is also roughly estimated.

Animal No.	Age in weeks or months	Body weight (g) and sex	Weights of organs (g)			Mesenteric lymph nodes				Spleen		
			Thymus	Mesenteric lymph nodes	Spleen	Solid secondary nodules	Pseudo-secondary nodules	Immature secondary nodules	Mature secondary nodules	Immature secondary nodules	Mature secondary nodules	Myelopoiesis in red pulp
55	1W	12.3♂	0.02	0.01	0.04	−	±	−	−	−	−	+++
97		10.7♀	0.02	0.01	0.03	−	−	−	−	−	−	+++
34	2W	11.3♀	0.02	0.01	0.02	+	+	+	−	−	−	+++
99		22.5♂	0.02	0.01	0.04	++	+	+	−	−	−	+++
57	3W	26.3♀	0.05	0.05	0.12	++	+	+	−	−	−	+++
13		27.2♂	0.09	0.05	0.10	++	+	+	−	−	−	+++
10		20.3♂	0.07	0.03	0.09	++	+	+	−	−	−	+++
8	4W	35.0♂	0.08	0.05	0.12	+	+	++	−	+	−	++
4		32.0♀	0.16	0.08	0.09	+	+	++	−	+	−	++
94		53.0♂	0.19	0.10	0.34	+	+	+	−	−	−	++
118	2M	101 ♀	0.27	0.38	0.32	++	+	−	+++	+	−	+
101		83 ♂	0.16	0.30	0.30	+	+	−	+++	++	−	++
102		83 ♂	0.27	0.30	0.33	+	+	−	+++	+	++	+
49	3M	127 ♀	0.17	0.38	0.37	+	+	−	+++*	++	−	±
105		155 ♀	0.40	0.62	0.40	+	+	−	+++**	+	++	+
106		127 ♀	0.27	0.39	0.31	+	+	+	++	+	++	±
80	4M	188 ♀	0.35	0.55	0.65	+	+	−	++*	−	++*	+
108		180 ♀	0.31	0.41	0.76	+	+	+	+++*	−	+++*	±
109		200 ♀	0.25	0·47	0.65	+	+	−	+++**	−	+++*	±
65	5M	149 ♀	0.14	0.18	0.24	++	+	−	++**	−	++**	+
110		235 ♂	0.27	0.33	0.85	++	+	+	++***	−	++**	++
89	6M	180 ♀	0.23	0.31	0.44	++	+	−	++***	+	++**	++
88		195 ♀	0.17	0.24	0.66	+	+	−	++***	+	++**	++
86	12M	250 ♂	0.25	0.30	0.85	++	+	−	++**	+	++**	±
84		240 ♀	0.22	0.24	0.95	+	+	+	++**	−	++**	++

* The predominating secondary nodules are half-active forms with a few active secondary nodules.

** The predominating secondary nodules are half-active forms with a few inactive secondary nodules.

*** The predominating secondary nodules are inactive forms.

secondary nodules in the mesenteric lymph nodes take place almost coincident with a significant rise in the average number of mitochondria in blood lymphocytes. Strinctly speaking, a rise in mitochondrial content in blood lymphocytes somewhat precedes the period at which the *Flemming*'s nodules have reached their full maturity. No other changes in the mesenteric lymph nodes can be correlated with the observed alteration in the lymphocytic blood picture. Also noteworthy is the fact that after the differentiation of *Flemming*'s nodules has been accomplished, the mitochondrial number in blood lymphocytes markedly drops and does not rise again.

The involutionary changes of secondary nodules in the mesenteric nodes are characterized by a progressive increase in percentage of half-active and inactive forms, but the *Flemming*'s nodules as a whole show a tendency towards decrease both in size and number; such changes become prominent from the 4th or 5th month on.

The differentiation of secondary nodules in the spleen is somewhat delayed but proceeds in a similar fashion as in the mesenteric lymph nodes. Up to 3 weeks after birth, the so-called "periarterial lymphatic sheaths" contain no nodular masses (Fig. 15) and active myelopoiesis, notably erythropoiesis, is taking place in the red pulp (Fig. 16). Immature *Flemming*'s nodules begin to appear at 4 weeks in the periarterial lymphatic sheaths and their full development is attained at 4 months (Table 3; Figs. 17, 18 and 21). In later stages, half-active and inactive forms of *Flemming*'s nodules show a relative increase, but the *Flemming*'s nodules as a whole progressively decrease both in size and number. The morphology of the active, half-active and inactive secondary nodules are essentially the same as in the mesenteric lymph nodes (Figs. 17-20). In half-active nodules in the periarterial lymphatic sheaths of the spleen, the inactive zones are situated opposite the central artery (Fig. 19).

At the height of development of *Flemming*'s nodules, the activity of myelopoiesis in the red pulp is diminished to a large extent. With the advent of regressive changes of *Flemming*'s nodules, its activity appears to become intensified again (Table 3).

Finally, some mention should be made of the abundant occurrence of plasma cells in the mesenteric lymph nodes. These cells begin to appear at 4 weeks in the medullary cord and become very numerous from the second month on. Detailed observations on the postnatal development of plasma cells in lymph nodes will be made elsewhere.

Discussion

The present observations have demonstrated that the mode of lymphocytopoiesis in the mesenteric lymph nodes and spleen undergoes a marked qualitative

change, which is characterized by an extensive new formation of *Flemming*'s secondary nodules in an early period of the postnatal development of these organs, and that this is accompanied by a significant transient rise in average number of mitochondria in blood lymphocytes. No other changes in lymphoid organs can be correlated with the alteration in the lymphocytic blood picture.

The results obtained confirm the earlier observations on rabbits by *Osogoe* et al. (1953) and *Awaya* (1956) in our laboratory that there occurs a marked rise in mitochondrial content in blood lymphocytes, almost coincident with an extensive new formation of *Flemming*'s secondary nodules in the mesenteric lymph nodes and spleen, not only in the normal course of the postnatal development but also after intravenous injection of ovalbumin.

It has been repeatedly demonstrated that the *Flemming*'s secondary nodules first appear as foci of active proliferation of medium-sized lymphocytes and only secondarily they become "reaction centers" (*Conway*, 1937; *De Bruyn*, 1948; and *Awaya*, 1958). Unpublished observations of *Osogoe* and *Ito* on young rats revealed that, when mitoses are arrested at metaphase by the injection of colchicine, metaphases occur in the *Flemming*'s nodules often in much greater numbers than in other parts of the lymphatic tissue. It is therefore indisputable that an extensive new formation of *Flemming*'s nodules in lymphoid organs results in a marked increase in production and delivery of lymphocytes.

On the basis of a number of statistical observations, it is now well established, on the other hand, that younger lymphocytes contain a greater number of mitochondria in their cytoplasm than the mature and older forms (*Wiseman*, 1931; *Miller* and *Taylor*, 1948; *Fukase*, 1949; *Osogoe* et al. (1953); *Ackerman* and *Bellios*, 1955; and *Awaya*, (1956). This is strongly supported by the observations of *Fukase* (1949) on rabbits that the average number of mitochondria per lymphocyte is highest in the lymph nodes, intermediate in the efferent lymph and lowest in the peripheral blood.

From the above considerations it may be stated that a remarkable rise in mitochondrial content in circulating lymphocytes takes place as a consequence of extensive new formation of *Flemming*'s seondary nodules, which, in turn, indicates an increased production and delivery of lymphocytes from the lymphoid organs. This is supported by the fact that after the *Flemming*'s nodules have reach their full maturity, the mitochondrial number shows a marked fall and does not rise again.

It should be emphasized here that the elevation of average number of mitochondria in blood lymphocytes observed in the present research is primarily linked with an increased production of lymphocytes in lymphoid organs. Theoretically, a similar rise in mitochondrial content in lymphocytes, primarily linked with an increase in mobilization and not in production of lymphocytes in lymphoid organs, may take place under special circumstances. Our collaborator *Ito* is now making an extensive study of the latter possibility. A detailed account will be

published later.

It is also worthy of notice that there seem to be certain regional difference as regards the development of *Flemming*'s secondary nodules. In guinea-pigs, *Gyllensten* (1950) observed that *Flemming*'s secondary nodules in different lymphoid organs reach their full maturity in the following order: *Peyer*'s patches and cervical lymph nodes, lymph nodes of extremities, tracheal and mesenteric lymph nodes, white splenic pulp. Although no detailed study was made on this point in the present work, it is certain that development of *Flemming*'s nodules in the mesenteric lymph nodes and white splenic pulp is quite representative, because these organs constitute a large part (approximately 40 %) of the total lymphatic tissue in the rat lymphoid organs (*Monden*, 1955; *Osogoe* et al., 1957). In view of the fact that development of *Flemming*'s nodules in the splenic white pulp is somewhat delayed as compared with that in the mesenteric lymph nodes (cf. Table 3), it seems probable that differentiation of these nodules in different lymphoid organs in the rat occurs in the same sequence as in the guinea-pigs.

Summary

1. In the normal course of the postnatal development of lymphoid organs in the rat, a remarkable transient elevation of the average number of mitochondria was observed in circulating lymphocytes, during the period between 2 weeks and 2 months after birth.

2. This period coincides with the period during which the processes of new formation and differentiation of *Flemming*'s secondary nodules proceed in the mesenteric lymph nodes and splenic white pulp, the former in particular. After maturation of *Flemming*'s nodules in these organs has been accomplished, the mitochonrial number in circulating lymphocytes considerably fell and did not rise again.

3. These findings agree fairly well with the previous observations by *Osogoe* et al. (1953) and *Awaya* (1956) on rabbits and provide further evidence to indicate that a marked rise in mitochondrial number in blood lymphocytes takes place as a consequence of extensive new formation of *Flemming*'s secondary nodules which, in turn, indicates an increased production and delivery of lymphocytes from the lymphoid organs.

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PLATE I

Explanation of Figures

Fig. 2. Two mature secondary nodes of the *Flemming* type in the cortex of the mesenteric lymph node. Rat No. 4, 4 weeks after birth. × 100.

Eig. 3. Solid secondary nodules just developing in the cortex of the mesenteric lymph node. Rat No. 34, 2 weeks after birth. × 100.

Fig. 4. A large pseudo-secondary nodule (PSN) in the cortex of the mesenteric lymph node having four *Flemming*'s secondary nodules toward their surface. Rat No. 4, 4 weeks after birth. × 40.

Fig. 5. A post-capillary vein with extraordinarily large, oval endothelial cells (in the center of the figure) in the cortex of the mesenteric lymph node. Rat No. 55, 7 days after birth. × 400. It is interesting to note that the appearnce of the post-capillary veins in the cortex somewhat precedes the development of the pseudo-secondary nodules.


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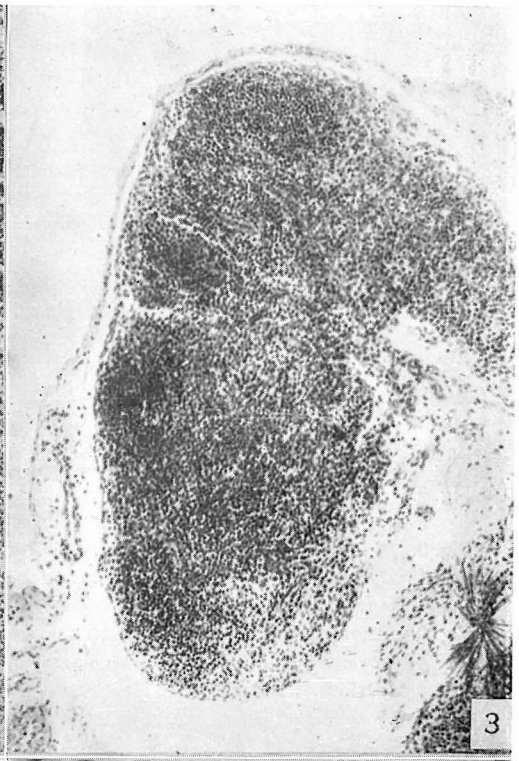
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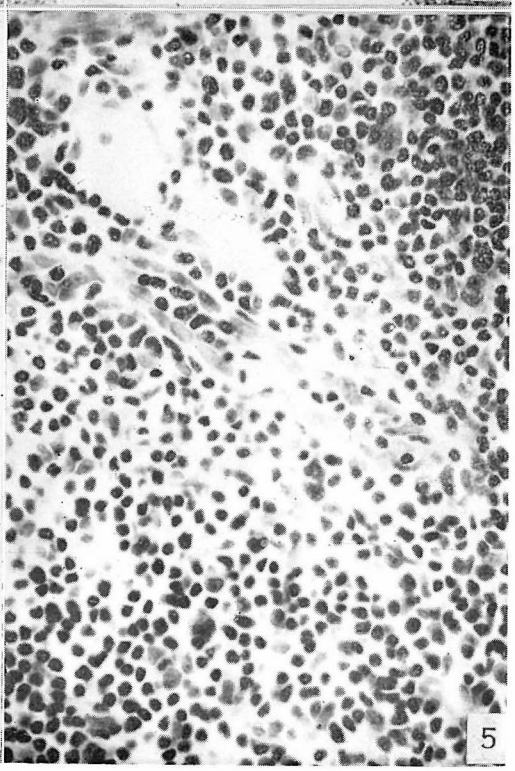
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PLATE II

Explanation of Figures

Fig. 6. Two germinal centers just developing in the diffuse lymphatic tissue in the cortex of the mesenteric lymph node ("bare germinal centers" of *Conway*). Rat No. 99, 2 weeks after birth. ×2000.

Fig. 7. A germinal center just developing in a pre-existing solid secondary nodule in the cortex of the mesenteric lymph node. At No.8, 4 weeks after birth. ×200.

Fig. 8. A mature active secondary nodule of the *Flemming* type in the cortex of the mesenteric lymph node. Rat No. 4, 4 weeks after birth. The long axis of its pale center is paralell to the capsular surface. ×200.

Fig. 9. Another mature active secondary nodule of the *Flemming* type in the cortex of the same node as seen in Fig. 8. The long axis of its pale center is perpendicular to the capsular surface. ×200.

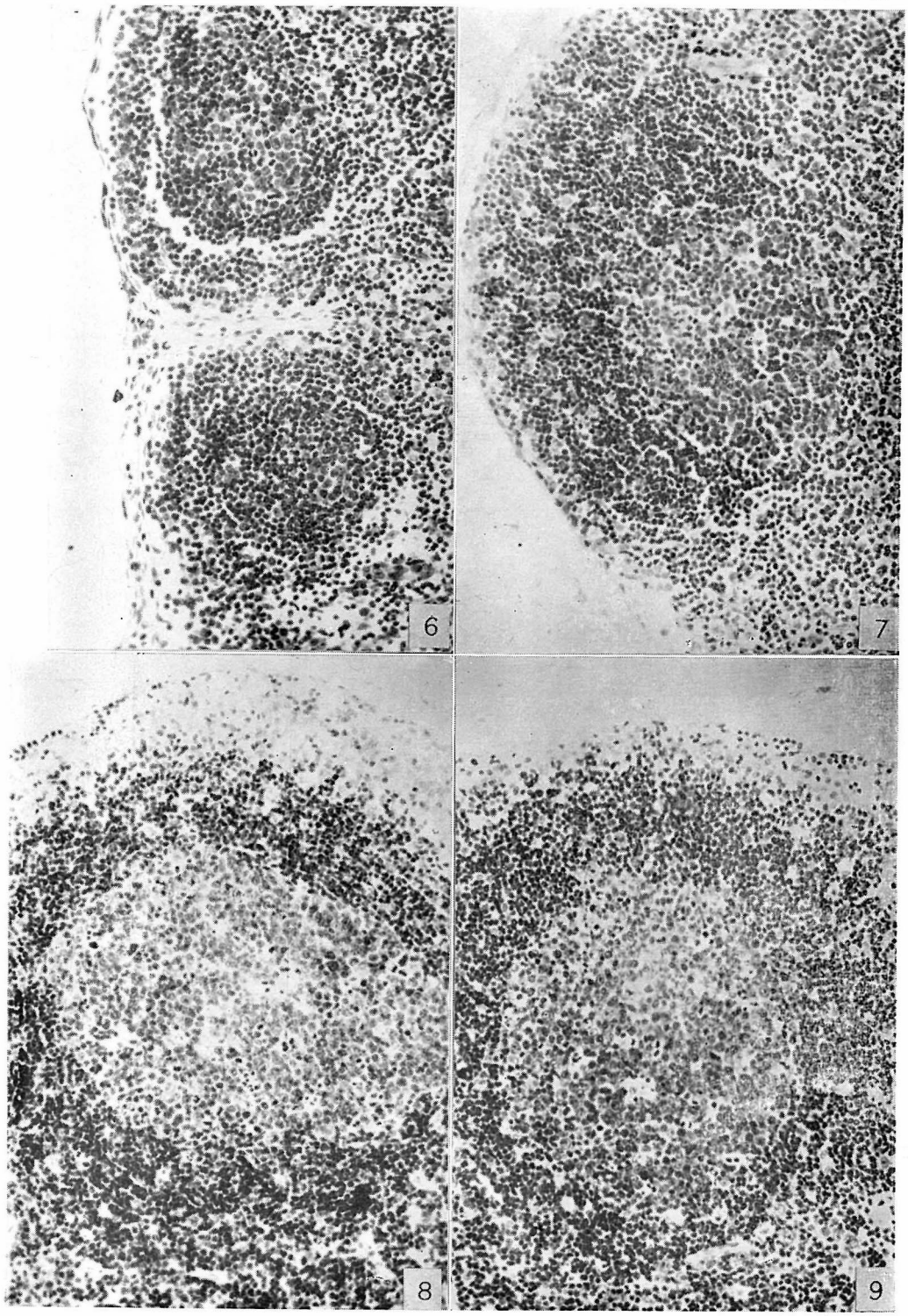
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PLATE III

Explanation of Figures

Fig. 10. A half-active secondary nodule of the *Flemming* type in the cortex of the mesenteric lymph node. Rat No. 108, 4 months after birth. The inactive, lighter zone is at the capsular end of the nodule. ×200.

Fig. 11. An inactive secondary nodule of the *Flemming* type in the cortex of the mesenteric lymph node. Rat No. 89, 5 months after birth. ×200.

Fig. 12. Mesenteric lymph node composed of diffuse lymhatic tissue without nodular masses. No. 55, 7 days after birth. ×100.

Fig. 13. Mesenteric lymph node having numerous fully developed *Flemming*'s secondary nodules. Rat No. 118, 2 months after birth.

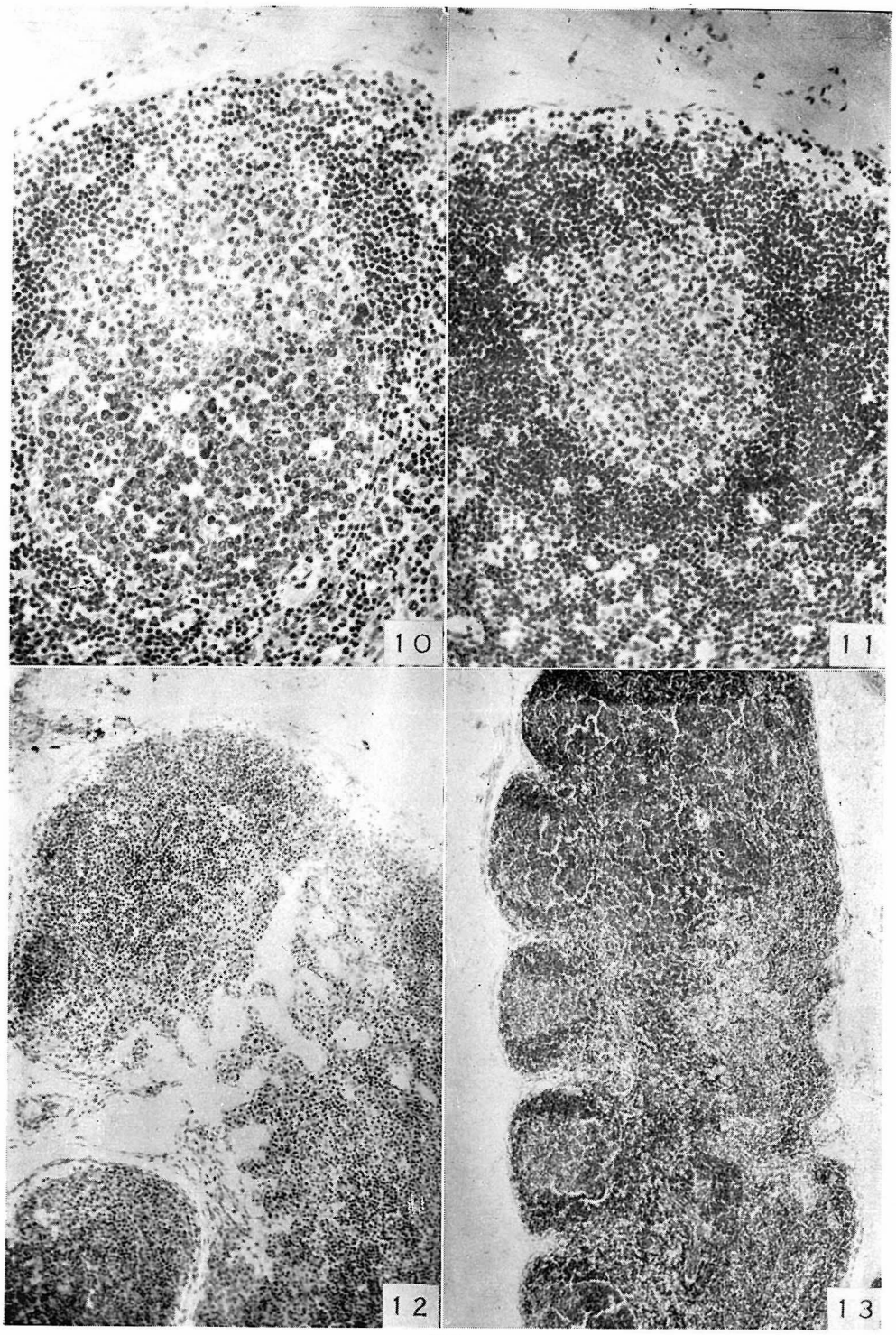
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PLATE IV

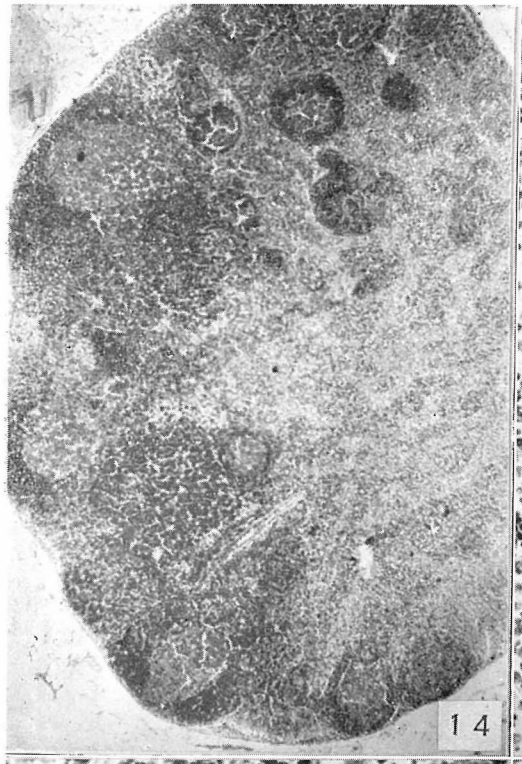
Explanation of Figures

Fig. 14. Mesenteric lymph node having numerous *Flemming*'s secondary nodules in different stages of maturity. Rat No. 118, 2 months after birth. ×40.

Fig. 15. Lymphatic tissue surrounding a central artery (periarterial lymphatic sheath) in an early stage of its development. Rat No 99, 2 weeks after birth. ×200.

Fig. 16. Erythropoietic foci in the splenic red pulp. Rat No. 57, 3 weeks after birth.

Fig. 17. A germinal center just developing in the periarterial lymphatic sheath of the spleen (an immature secondary nodule of the *Flemming* type). Rat No. 8. 4 weeks after birth. ×200.



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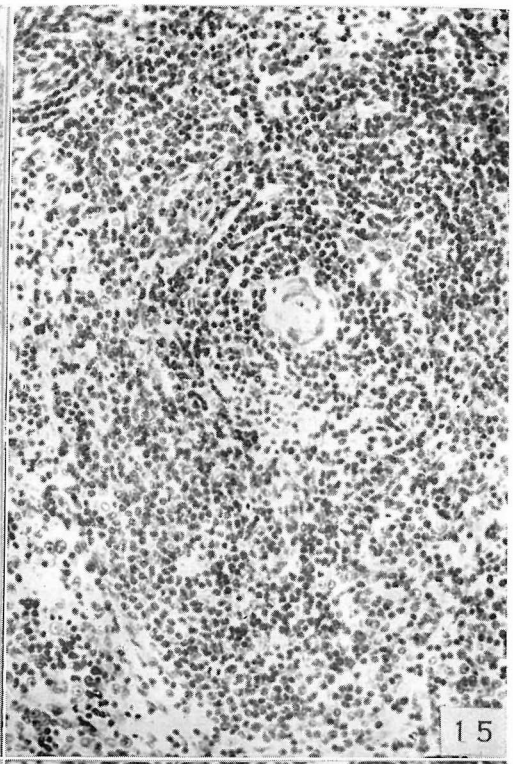

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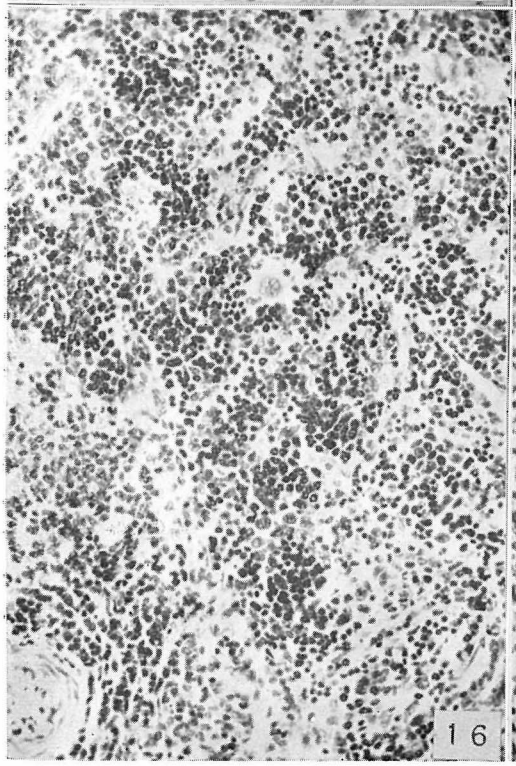

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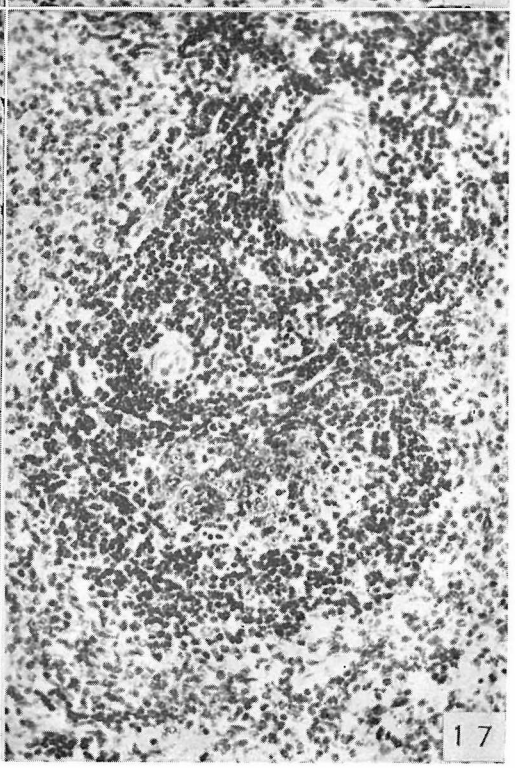

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PLATE V

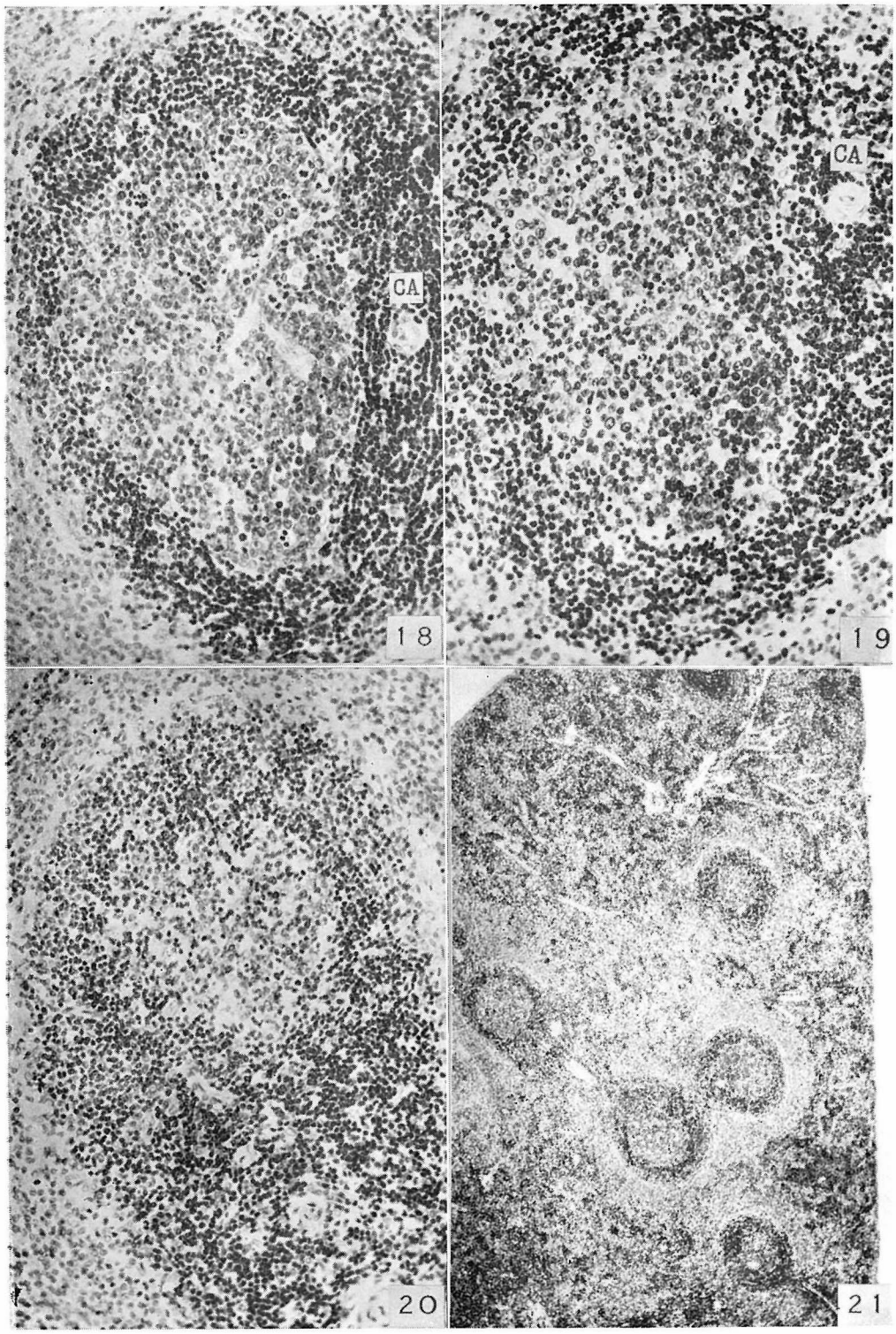
Explanation of Figuees

Fig. 18. A mature active secondary nodule of the *Flemming*'s type in the splenic white pulp. Notice its relation to the central artery (CA). Rat. No. 105, 3 months after birth. ×200.

Fig. 19. A half-active secondary nodule of the *Flemming* type in the splenic white pulp. Rat No. 108, 4 months after birth. Notice that the inactive zone of the pale center is situated opposite the central artery (CA). ×200.

Fig. 20. An inactive secondary nodules of the *Flemming* type in the splenic white pulp. Rat No. 86,12 months after birth. ×200.

Fig. 21. Splenic white pulp containing five full developed secondary nodules of the *Flemming*'s type. Notice the well developed, pale-staining outer zones surrounding the *Flemming*'s nodules. Numerous dark specks in the red pulp represent erythropoietic foci. Rat. No. 105, 3 months after brith. ×40.


CA
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CA
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