THE MODE OF THE REACTION OF THE LYMPHATIC SYSTEM TO OVALBUMIN, WITH REMARKS ON THE RÔLE OF SECONDARY NODULES IN LYMPHOCYTOPOIESIS

KAZUHIKO AWAYA

Department of Anatomy, Yamaguchi Medical School, Ube

(Director: Prof. Dr. Bunsuke Osogoe)

(Received January 10, 1956)

I. INTRODUCTION

Among reactions of the lymphatic tissue to various kinds of stimuli the most striking one is new formation of secondary nodules. Hellman (1938, 1943) and his coworkers (A. and H. Sjövall, 1930; Rudebeck, 1932; and Glimstedt, 1936) are of opinion that secondary nodules are produced as a reaction of lymphatic tissue to bacteria or bacterial toxins and do not represent "germinal centers" in the sense originally proposed by Flemming (1885). For this reason Hellman calls them "reaction centers". On the basis of her observation of the reaction of lymphatic tissue to B. monocytogenes infection in rabbits and guinea-pigs, Conway (1937) claims, on the other hand, that the secondary nodule is primarily a focus of proliferation of lymphocytes and only secondarily does it become "reaction center".

In a previous report from our laboratory by Osogoe et al. (1953), it has been demonstrated that in the postnatal differentiation processes of the lymphatic tissue, secondary nodules first appear as lymphocytopoietic centers without demarcating peripheral mantles of small lymphocytes, and that such early nodules rapidly increase in number coincident with a remarkable rise in the mitochondrial content in circulating lymphocytes. Since a rise in the mitochondrial content in circulating lymphocytes signifies an increased delivery of younger lymphocytes which contain in their cytoplasm a greater number of mitochondria than the mature and older forms, the above-mentioned findings suggest that the newly formed, early secondary nodules are important sites of production and delivery of younger lymphocytes responsible for the alteration in the lymphocytic blood picture.

The purpose of the present investigation is to determine whether or not

secondary nodules are newly formed as lymphocytopoietic loci in the lymphatic tissue of adult rabbits and whether new formation of secondary nodules, if really took place, is accompanied by a simultaneous rise in the number of mitochondria in circulating lymphocytes, when the animals had been injected with some agents such as heterogeneous proteins which are known to produce hyperplasia of lymphatic tissue (Wiseman, 1931a; Wiseman et al., 1936; and Horii, 1938). Our collaborator, Fukase (1949), has previously shown that ovalbumin may produce, when injected intravenously into adult rabbits, a marked increase in the average number of mitochondria in circulating lymphocytes within several days. Accordingly, in the present experiments ovalbumin was used as stimulating agent, and the relationships between the reactions of circulating lymphocytes and those of lymphatic tissue to this agent were investigated.

This work was carried out in the laboratory of Professor Dr. Bunsuke Osogoe and constitutes a part of his extensive study of the lymphocyte and the lymphoid tissue. I wish to express my thanks to him for constant guidance in the course of the present research.

II. Material and Methods

A total of 28 adult rabbits of both sexes weighing about 2 kg were used. All showed normal peripheral blood counts and smears. They were divided into two groups: one group received 100 mg of ovalbumin (E. Merck) in a single intravenous injection and another 50 mg of ovalbumin daily also in intravenous injection. Ovalbumin was dissolved in physiological saline as a 1 % solution and pasteurized two or three times at 60°C. for 30 minutes before injection.

Blood examinations were made at intervals of one, three, 5, 7, 14 and 21 days after the initial injection. The mitochondrial content in the blood lymphocytes was examined by means of supravital staining with Janus green B and neutral red, in the same manner as in the previous study by Osogoe et al. (1953). Mitochondria thus stained were counted in 100 or 200 lymphocytes from each animal so as to give a total of more than 400 lymphocytes at each time interval stated above, and the average number of mitochondria per lymphocyte was determined. In blood smears stained with Giemsa the lymphocytes were classified according to their cell size into 3 types: small lymphocytes measuring less than 9μ; medium-sized lymphocytes, 9–14μ; and large lymphocytes, more than 14μ in diameter. The proportions of three types of lymphocytes were determined by counting at least 200 lymphocytes from each smear.

After the initial injection of ovalbumin, each two animals were sacrificed serially at intervals of one, three, 5, 7, 14 and 21 days and the mesenterial

mass of lymph nodes (pancreas Aselli), popliteal lymph nodes, Peyer's patches of small intestine, vermiform appendix, spleen, liver, bone marrow and other organs were examined in tissue sections. The tissues were fixed in Zenker-formol, embedded in paraffin and cut serially in 6μ thickness. The sections were stained with haematoxylin (Mayer's acid haemalum) and eosin.

In the present research, special attention was directed to the changes in number of secondary nodules of differrent types appearing in the mesenterial lymph nodes as well as in the spleen following the ovalbumin administration. Since, however, both organs are too voluminous to enumerate secondary nodules contained in the whole organs on serial sections, the average number of nodules occurring per unit area of cross-sectional cuts of the organs were determined as follows: Both organs were cut into from 5 to 7 pieces of approximately equal size before fixation, from each of which about 300 cross-sectional cuts 6μ thick were made serially after embedding in paraffin. Here, special care was taken to cut the paraffin blocks as perpendicularly to the organ surface as possible in order to reproduce normal proportions between tissue constituents, viz., between the cortex and medulla of lymph nodes and between the red and white pulp of spleen. Since the diameter of secondary nodules was not greater than 450μ, several sections were chosen at random at intervals of 450μ from each series, and classification as well as enumeration of secondary nodules occurring in the whole sections were made. After measuring the area of the sections, the numbers of secondary nodules of different types occurring per 1 cm^2 of sections were determined. The values thus obtained from different portions of the organs were then averaged. In this way the average number of secondary nodules occurring per unit area of sections was computed, decimal fracitons being ignored, and used for comparison. A preliminary examination

TABLE I

Numbers of immature secondary nodules occurring in $1cm^2$ of sections from different portions of the mesenterial mass of lymph nodes. Five days after single injection of 100mg of ovalbumin.

Rabbit No., body weight and sex	Ke 22, 2.0 kg, ♂	Ke 23, 2.0 kg, ♂
Portions of mesenterial mass of nodes	a b c d e f g	a′ b′ c′ d′ e′ f′ g′
Numbers of immature secondary nodules occurring in $1cm^2$ of sections	25 44 35 45 42 41 33	36 48 47 30 43 43 47

disclosed that secondary nodules, notably the immature ones which will be described later, are relatively evenly distributed among different portions of the lymph nodes (Table 1) and spleen.

The secondary nodules were classified into 3 main types: (1) *Flemming's secondary nodules or mature secondary nodules* are typical text-book secondary nodules composed of pale-staining centers and surrounding dark-staining peripheral mantles: (2) *Solid secondary nodules* of Groll and Krampf (1920–21) denote nodules consisting nearly exclusively of small lymphocytes without pale-staining center: (3) *Pseudo-secondary nodules* of Ehrich (1929) are large, round, fairly well circumscribed masses of diffuse lymphatic tissue, frequently measuring 3 mm or more. Surrounding these, toward theire surface, there are usually several solid secondary nodules. Sometimes, they also contain a few Flemming's secondary nodules.

The early stages ef Flemming's secondary nodules were termed *immature secondary nodules* in the present research, because in later stages they give rise to typical Flemming's nodules having pale-staining centers surrounded by dark-staining mantles. These nodules first appear as small isolated masses of densely packed, rapidly dividing, medium-sized lymphocytes in the diffuse lymphatic tissue without demarcating peripheral zones of small lymphocytes. They are identical with the "bare germinal center" of Conway (1937). They were further subdivided according to their developmental stages into three types. Detailed description of each type will be made in the next section.

Supplementary to the present experiments, the normal processes of post-natal development of the rabbit lymphatic apparatus from birth up to old age was re-investigated by the above-mentioned method, based on the same material of the previous study by Osogoe et al. (1953). It must be mentioned here that examination of the rabbits of age varying from 4 to 8 months, which correspond to the animals used in the present experiments, furnishes normal control data.

III. Results

A. Reactions in the blood lymphocytes.

The lymphocytic picture of peripheral blood was markedly altered by the ovalbumin administration. After the single injection of 100 mg of ovalbumin, the average number of mitochondria per lymphocyte considerably increased on the next day, attaining its maximum at 3 days, and abruptly decreased at 5 days. Thereafter it declined slowly (Fig. 1). The rise in the mitochondrial content in lymphocytes at 3 days over the pre-injection level was statistically significant ($P<0.01$). Either in the lymphocyte count or in the total white cell count no significant changes were observed.

When 50mg of ovalbumin was injected daily, the average number of mitochondria per lymphocyte also began to increase from the next day, reaching

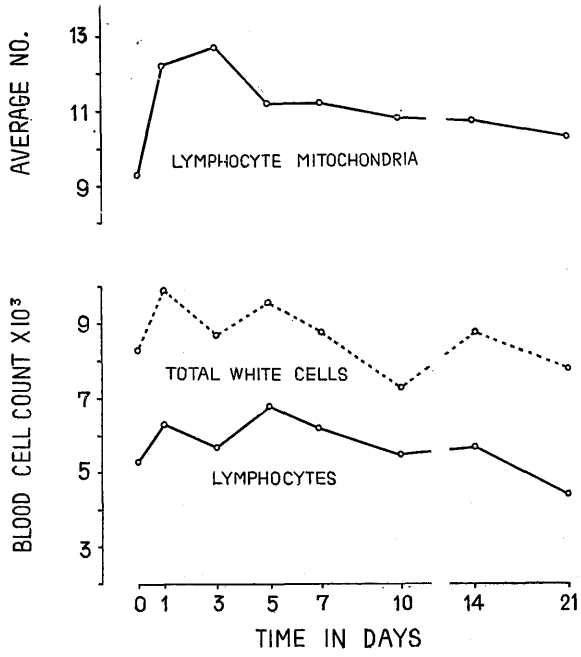


Fig. 1. Changes in the blood cell counts and in the average number of mitochondria per lymphocyte in peripheral blood after a single injection of 100mg of ovalbumin.

its maximum on the 7th day after the initial injection, and then gradually decreased, but a relatively high value was maintained until the 21st day (Fig. 2). The rise in the mitochondrial content in lymphocytes on the 7th day over the pre-injection level was also statistically significant ($P<0.01$). The number of blood lymphocytes was increased by nearly 80 per cent over normal. The total white cell count almost paralelled the lymphocyte count.

The proportions of small, medium-sized and large lymphocytes in blood smears were altered to a considerable extent by the injection of ovalbumin. Following a single injection, a relative increase of medium-sized lymphocytes over normal was prominent during the period from the next day till the 7th day. When injected daily, an almost three-fold increase in the percentage of medium-sized lymphocytes, from 13.3% before injection to 33.9% on the 5th day after the initial injection, was observed (Table 2). In such instances, a marked proliferation of medium-sized lymphocytes was observed in the lymphatic tissue of chief lymphoid organs.

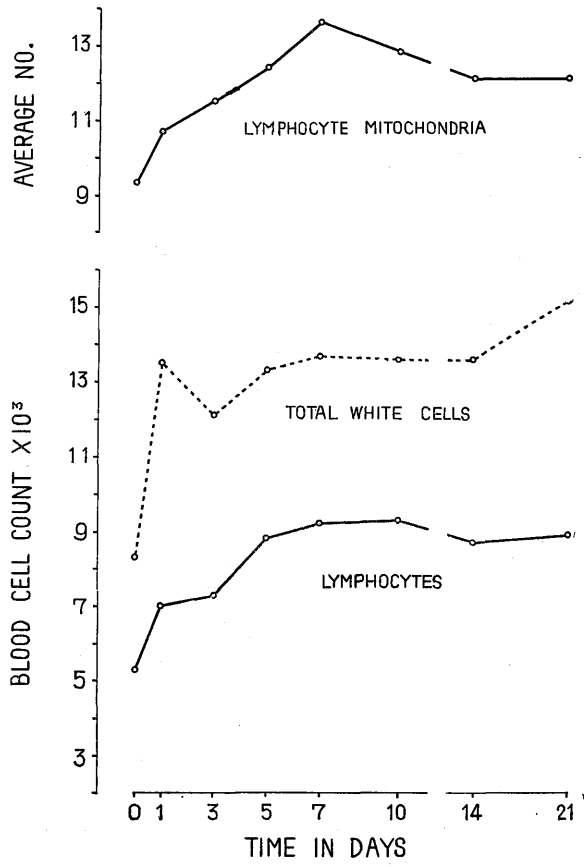


Fig. 2. Changes in the blood cell counts and in the average number of mitochondria per lymphocyte in peripheral blood in the course of daily injection of 50 mg of ovalbumin.

TABLE II

Proportions of small, medium-sized and large lymphocytes in blood smears at various intervals after administering ovalbumin.

Groups	Time after the initial injection	Lympho-cyte counts	Differential counts (%) of lymphocytes		
			Small L.	Medium-sized L.	Large L.
Normal (average 28 rabbits)	Before injection	5300	84.3	13.3	2.4
Single injection of 100 mg of ovalbumin (average 4–8 rabbits)	1 day	6300	70.8	26.1	3.1
	3 days	5700	71.2	26.9	1.9
	5 days	6800	73.6	26.0	0.4
	7 days	6200	70.1	28.2	1.7
	10 days	5500	78.9	19.9	1.2
	14 days	5700	83.3	16.3	0.4
	21 days*	4400	82.1	17.7	0.2
Daily injection of 50 mg of ovalbumin (average 5–8 rabbits)	1 day	7000	76.4	22.0	1.6
	3 days	7300	80.8	18.6	0.6
	5 days	8800	64.7	33.9	1.4
	7 days	9200	69.0	29.2	1.8
	10 days	9300	81.6	17.9	0.5
	14 days	8700	80.5	17.7	1.8
	21 days**	8900	84.9	12.7	2.4

* average 2 rabbits
** average 3 rabbits

Number of mitochondria in circulating lymphocytes varys considerably. Among small lymphocytes there are cells entirely devoid of mitochondria stainable with Janus green B, on the one extreme, and those containing more than 30 mitochondria, on the other. Larger forms of lymphocytes also shows a similar variation in regard to the mitochondrial content, but, generally speaking, the larger lymphocytes contain a greater number of mitochondria than the smaller forms. It can be stated, therefore, that an increase in the percentage of medium-sized forms contributes to a rise in the average number of mitochondria in circulating lymphocytes.

B. Reactions in the mesenterial mass of lymph nodes.

Among lymph nodes the mesenterial mass of nodes showed the most striking changes following the ovalbumin administration. In this mass there was a marked hyperplasia of lymphatic tissue with new formation of immature secondary nodules and with relative increase of active mature secondary nodules of Flemming's type. Neither solid secondary nodules nor pseudo-secondary

nodules were significantly increased in number. Since it was shown by Karasawa (1954) in this laboratory that in adult rabbits the mesenterial mass of nodes is the most important source of blood lymphocytes, the changes in this mass may be regarded as responsible for the alterations in the lymphocytic picture of peripheral blood described in the foregoing section. In describing these changes I wish to begin with the new formation of immature secondary nodules.

1. *Immature secondary nodules.*

The term "immature secondary nodules" as used here denotes early developmental stages of Flemming's secondary nodules without peripheral, dark-staining mantle of small lymphocytes, and thus are identical with the "bare germinal centers" of Conway (1937). They first appear in the diffuse lymphatic tissue as small isolated masses of densely packed, medium-sized lymphocytes which are very often in mitosis, but become gradually surrounded, as they grow, first by demarcating fibrilar capsules and then by outer zones of densely packed small lymphocytes, eventually giving rise to mature secondary nodules of Flemming's type. Gyllensten (1950) distinguished three developmental stages of Flemmsng's nodules and designated them as +, ++, and +++ stages, the first two representing the immature forms. In the present research the immature forms were divided into three types according to their developmental stages and designated as +, ++, +++; and the mature forms as ++++.

Type + represents nodules which assume the appearance of "bare germinal centers" of Conway, consisting mainly of densely packed, medium-sized lymphocytes with a few large lymphocytes and scattered reticular cells (Fig. 12 on Plate I). Neither demarcating fibrilar capsule nor outer zone of small lymphocytes is discernible around them. The nodules usually include many mitotic figures of medium-sized lymphocytes and a few nuclear debris (Fig. 16 on Plate II). They usually measure less than 100μ but may somtimes be larger than 100μ in diameter.

Type ++ denotes nodules in more advanced stages of development, demarcated partially or completely by a thin fibrilar capsule but without outer zone of small lymphocytes (Fig. 13 on Plate I). In this type of nodules, the cellular density of medium-sized lymphocytes is somewhat diminished by an increase in reticular cells and small lymphocytes, but mitotic figures occur more numerously than in the former type (Fig. 17 on Plate II). The nodules of this type usually measure between 100 and 200μ, occasionally even more than

200μ.

Type + + + represents further developed nodules with a fibrilar capsule surrounded partially or completely by a thin layer of densely packed small lymphocytes (Fig. 14 on Plate I). The cellularity of medium-sized lymphocytes of the nodules is diminished by an increase in reticular cells to a greater extent than in the former type, but they still contain numerous mitotic figures. They usually measure from 200 to 300μ, or sometimes over 300μ.

In general, the immature secondary nodules appear most frequently in

TABLE III

Variations in the average number of secondary nodules of various types occurring in 1cm^2 of sections of the mesenterial lymph nodes of normal rabbits at different ages.

Age in weeks or months	Rabbit No., body weight and sex	Immature secondary nodules				Mature secondary nodules ⫲	Total
		+	++	⫲	Total		
1 week	1Wa, 168g, ♂	0	0	0	0	0	0
	1Wb. 170g, ♂	0	0	0	0	0	0
2 weeks	2Wa, 278g, ♂	0	0	0	0	0	0
	2Wb, 158g, ♂	0	0	0	0	0	0
3 weeks	3Wa, 564g, ♂	32	0	0	32	0	32
	3Wb, 238g, ♂	18	0	0	18	0	18
1 month	1Ma, 450g, ♂	21	0	0	21	0	21
	1Mb, 588g, ♂	22	0	21	43	70	113
	1Mc, 610g, ♂	24	6	6	36	128	164
2 months	2Ma, 925g, ♀	6	0	0	6	18	24
	2Mb, 743g, ♂	11	5	5	21	38	59
3 months	3Ma, 1.5kg, ♀	0	0	3	3	44	47
	3Mb, 1.4kg, ♀	7	0	0	7	24	31
4 months	4Ma, 1.6kg, ♀	3	0	0	3	61	64
	4Mb, 1,6kg, ♀	0	0	0	0	29	29
6 months	6Ma, 2.5kg, ♀	0	0	0	0	168	168
	6Mb, 2.6kg, ♀	0	0	0	0	23	23
8 months	8Ma, 3.1kg, ♂	0	0	0	0	93	93
	8Mb, 3.3kg, ♀	0	0	0	0	42	42
12 months	12M, 3.8kg, ♂	0	0	0	0	79	79
18 months	18M, 3.6kg, ♀	0	0	0	0	14	14

Designations: + indicates secondary nodules in the early developmental stages devoid of both demarcating fibrilar capsule and outer zone of small lymphocytes (bare germinal centers of Conway); ++ those surrounded by a thin fibrilar capsule, but without outer zone of small lymphocytes; ⫲ those with fibrilar capsule surrounded moreover partially or completely by a thin layer of small lymphocytes; ⫲ fully developed secondary nodules with distinct outer zone of small lymphocytes.

deeper zones of the cortex, at the cortico-medullary junctions in particular, but they may also develop in any other parts of the lymphatic tissue. It must again be emphasized here that the most striking feature of the immature secondary nodules is the occurrence of numerous mitotic figures. That most of the cells in mitosis are medium-sized lymphocytes is indisputable from their nuclear size and because many of them are in prophase (Figs. 16–17 on Plate II). It is also worthy of notice that the immature secondary nodules are devoid of demarcating peripheral mantles consisting of densely packed small lymphocytes, because this condition favors mobilization of lymphocytes newly formed in the nodules.

In normal rabbits, the average number of different types of secondary nodules occurring per unit area of sections of the mesenteric lymph nodes varys considerably with age. The data from animals, from one week to 18 months of age, are given in Table 3. It can be seen that Flemming's secondary nodules first appear as type + at 3 weeks after birth and at one month of age types

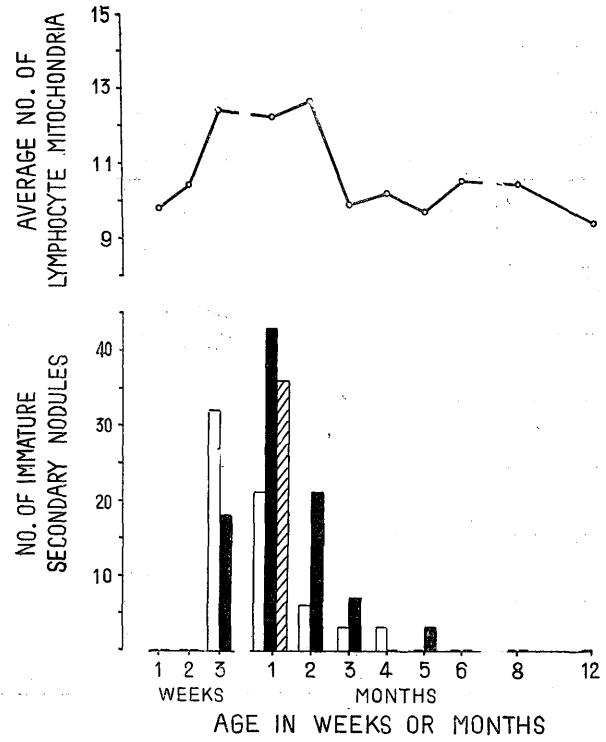


Fig. 3. Variations in the average number of immature secondary nodules occurring per 1 cm^2 of sections of the mesenterial lymph nodes and in the average number of mitochondria per lymphocyte in peripheral blood in normal rabbits at different ages.

++ and +++ also occur, and that the total number of immature forms reaches its maximum at one month, diminishing abruptly thereafter. In animals older than 3 months of age they are seldom found. In this connection the fact should be noticed that the mature secondary nodules begin to appear from one month of age and rapidly increase in number. Since it is known that, in normal rabbits, the average number of mitochondria in circulating lymphocytes is markedly raised after birth and reaches its maximum during the period from three weeks to two months of age (Osogoe et al., 1953), it can be stated that the above-mentioned increase in the number of immature secondary nodules in the mesenterial nodes occurs simultaneously with a remarkable rise in the mitochondrial content in circulating lymphocytes (Fig. 3).

TABLE IV

Changes in the average number of secondary nodules of various types occurring in 1 cm^2 of sections of the mesenterial lymph nodes after administering ovalbumin.

Groups	Time after the initial injetion	Rabbit No., body weight and sex	Immature secondary nodules				Mature secondary nodules |||	Total
			+	++	|||	Total		
Single injection of 100 mg of ovalbumin	12 hours	Ke28, 2.0kg, ♂	0	0	1	1	102	103
		Ke29, 2.1kg, ♀	0	0	0	0	125	125
	1 day	Ke26, 2.2kg, ♀	2	1	2	5	169	174
		Ke27, 2.2kg, ♀	0	0	5	5	175	180
	3 days	Ke24, 2.0kg, ♀	2	1	1	4	63	67
		Ke25, 2.1kg, ♀	3	0	1	4	40	44
	5 days	Ke22, 2.0kg, ♂	8	2	15	25	151	176
		Ke23, 2.0kg, ♂	6	5	25	36	136	172
	7 days	Ke20, 2.0kg, ♂	3	2	2	7	64	71
		Ke21, 1.8kg, ♂	5	0	1	6	47	53
	14 days	Ke18, 2.0kg. ♂	0	0	0	0	48	48
		Ke19, 2.1kg, ♂	3	0	0	3	226	229
	21 days	Ke15, 2.1kg, ♂	0	0	0	0	28	28
		Ke16, 1.8kg, ♀	0	0	0	0	86	86
Daily injection of 50 mg of ovalbumin	3 days	Ke34, 1.9kg, ♂	1	1	3	5	121	126
		Ke35, 2.0kg, ♂	1	0	5	6	182	188
	5 days	Ke32, 2.0kg, ♂	4	4	6	14	163	177
		Ke33, 2.0kg, ♂	2	1	6	9	157	166
	7 days	Ke30, 2.1kg, ♂	6	4	16	26	123	149
		Ke31, 2.1kg, ♂	3	1	12	16	87	103
	14 days	Ke12, 2.4kg, ♀	0	0	0	0	100	100
		Ke13, 2.2kg, ♂	0	0	0	0	108	108
	21 days	Ke11, 2.2kg, ♀	0	0	0	0	108	108
		Ke14, 2.4kg, ♀	0	0	0	0	48	48

The designations, +, ++, ||| and ||||, are the same as used in Table 3.

Following the ovalbumin administration, new formation of immature secondary nodules also occurred substantially in the same way as in the postnatal differentiation processes. As seen in Table 4, a maximal increase in the total number of immature secondary nodules per unit area of sections was observed on the 5th day in the case of single injection and on the 7th day in the case of daily injection, respectively. Thereafter these nodules abruptly decreased in number. When contrasted with the alterations in the lymphocytic picture of peripheral blood described in the foregoing section, it is to be seen that the observed increase in the average number of immature secondary nodules in the mesenterial nodes occurs almost simultaneously with a remarkable rise in the mitochondrial content in circulating lymphocytes (Figs. 4 and 5). Here, the fact must again be stressed that after their maximum increase the immature secondary nodules abruptly decrease in number. This implies that many of them have been transformed into the mature forms.

As has been repeatedly stated, the immature secondary nodules appears as

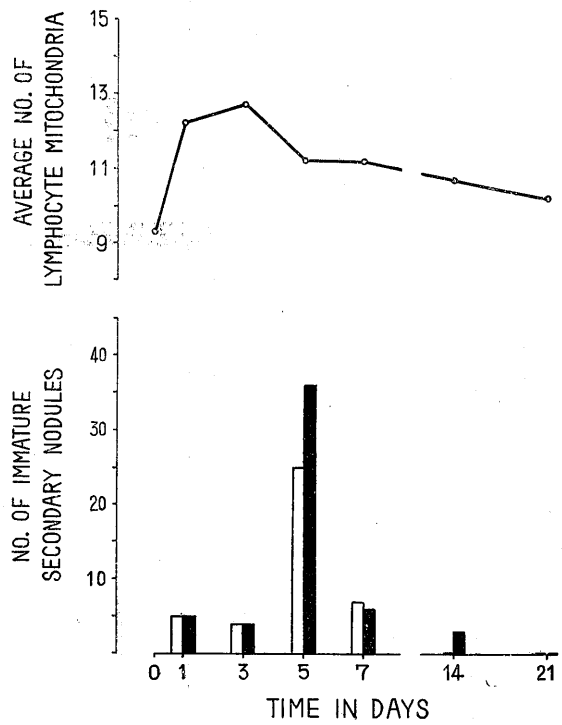


Fig. 4. Changes in the average number of immature secondary nodules occurring per 1 cm^2 of sections of the mesenterial lymph nodes and in the average number of mitochondria per lymphocyte in peripheral blood after a single injection of 100 mg of ovalbumin.

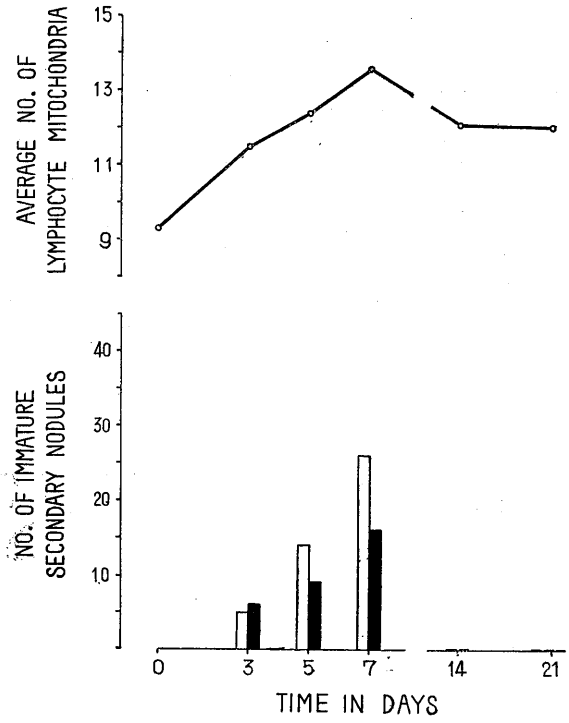


Fig. 5. Changes in the average number of immature secondary nodules occurring per 1 cm^2 of sections of the mesenterial lymph nodes and in the average number of mitochondria per lymphocyte in peripheral blood in the course of daily injection of 50 mg of ovalbumin.

isolated round masses of densely packed, medium-sized lymphocytes which are very often in mitosis. Accordingly, their increase in number signifies an enhanced proliferation of medium-sized lymphocytes in the lymphatic tissue. Since medium-sized and large lymphocytes normally occur numerouly in the diffuse lymphatic tissue, the medullary cords in particular, as well as in the intermediary sinuses, it is of interest to determine whether these lymphocytes also enter into active proliferation or not. Following the ovalbumin administration, a marked increase in number of larger lymphocytes which are frequently in mitosis was obesrved in the medullary cords as well as in the intermediary sinuses, coincident with new formation of immature secondary nodules. It is conceivable therefore that the lymphatic tissue of lymph nodes responds to the stimulation of ovalbumin with an extensive, generalized proliferation of larger lymphocytes, notably the medium-sized forms, which leads to a new formation of immature secondary nodules, on the one hand, and to a diffuse hyperplasia of larger lymphocytes in other parts of lymphatic tissue without formation of secondary nodules, on the other.

2. *Mature secondary nodules.*

"Mature secondary nodules" here termed denote Flemming's secondary nodules having pale-staining centers surrounded by dark-staining mantles. Following ovalbumin administration, the average number of mature secondary nodules occurring per unit area of sections of the mesenterial mass of nodes was subject to a considerable individual variation without consistent tendency towards increase. Qualitatively, however, a remarkable alteration was to be seen in these nodules.

It is well known that the Flemming's centers vary considerably in their cellular constitution. If they are almost entirely composed of densely packed, medium-sized lymphocytes with many mitotic figures, they arse termed "active"; otherwise, they are called either "inactive" or "half-inactive" secondary nodules

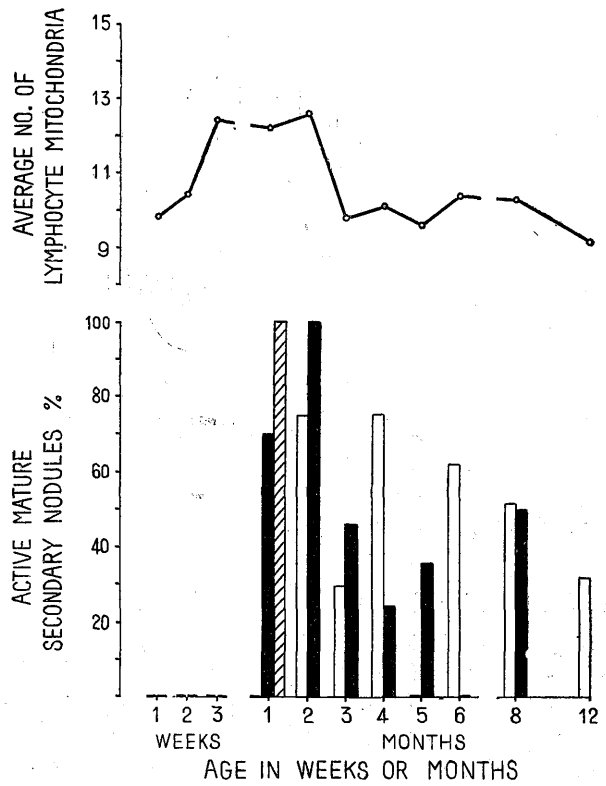


Fig. 6. Variations in the percentage of active mature secondary nodules in the mesenterial lymph nodes and in the average number of mitochondria per lymphocyte in peripheral blood in normal rabbits at different ages.

in this research. The secondary nodules here termed 'inactve' correspond to the "reaction centers" of Hellman constituting chiefly of reticular cells. According to Röhlich (1930), the Flemming's centers in the cortex of cat's lymph nodes usually consist of two portions, the superficial pale-staining and the deep dark-staining; the former being composed mainly of reticular cells and the latter of densely packed medium-sized lymphocytes. Such nodules as observed by Röhlich were also seen in the rabbit lymph nodes and designated here "half-inactive" secondary nodules.

In normal rabbits from one to two months of age, in which the mitochondrial content of circulating lymphocytes is significantly raised, the overwhelming majority of mature secondary nodules are in active state, but with advancing age the active nodules rapidly decrease in percentage and in animals older than 5 months of age they often disappear completely (Fig. 6).

Following the ovalbumin administration the percentage of active mature

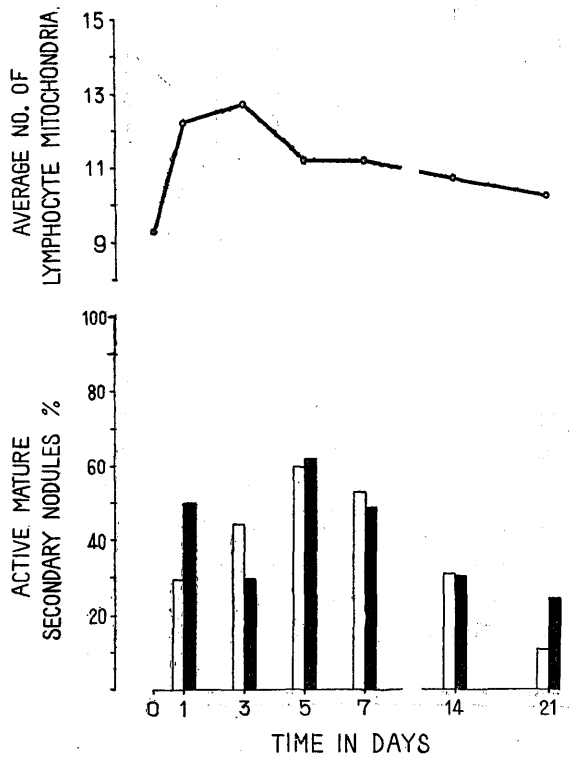


Fig. 7. Changes in the percentage of active mature secondary nodules in the mesenterial lymph nodes and in the average number of mitochodria per lymphocyte in peripheral blood after a single injection of 100 mg of ovalbmin.

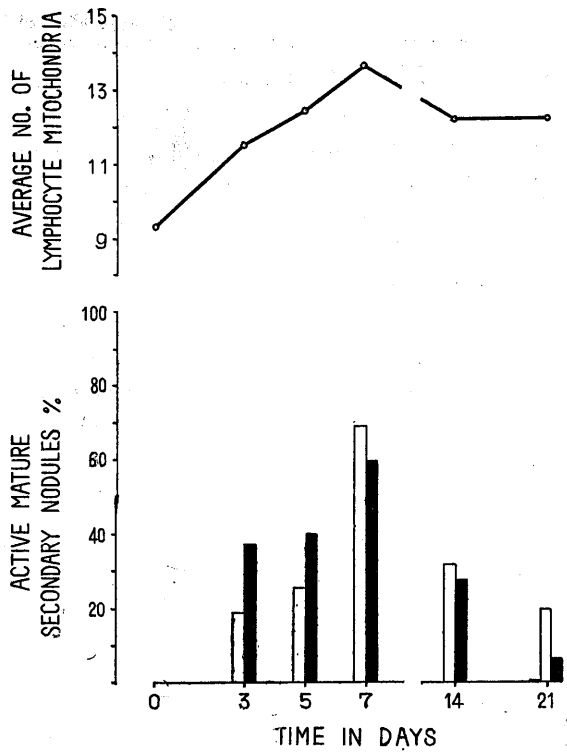


Fig. 8. Changes in the percentage of active mature secondary nodules in the mesenterial lymph nodes and in the average number of mitochondria per lymphocyte in peripheral blood in the course of daily injection of 50 mg of ovalbumin.

secondary nodules also increased, attaining its maximum on the 5th day in the case of single injection and on the 7th day in the case of daily repeated injection, respectively. Thereafter it decreased gradually. When contrasted with the alterations in the lymphocytic picture of peripheral blood already described, it is to be seen that the observed increase in the percentage of active mature secondary nodules occurs almost simultaneously with a remarkable rise in the mitochondrial content in circulating lymphocytes (Figs. 7 and 8). The results may be interpreted as due to transformation of newly formed immature secondary nodules into the mature forms through maturation.

3. *Solid secondary nodules and pseudo-secondary nodules.*

The solid secondary nodules of Groll and Krampf consist almost exclusively of small lymphocytes. According to Ehrich (1929), they are the first lymphatic nodules to develop before birth. The pseudo-secondary nodules of Ehrich are

large round masses of diffuse lymphatic tissue, around which, towards their surface, several solid secondary nodules are usually present. Sometimes, they also contain a few Flemming's nodules. The nodules of this type are said also to develop before birth (Ehrich, 1929).

In normal rabbits, solid secondary nodules and pseudo-secondary nodules are already found at one week after birth, and thereafter the number of each type of nodules rapidly increases, reaching its maximum at three weeks or one month, when both types of nodules begin gradually to decrease with advancing age (table 5). Therefore, they appear to increase almost parallel to an increase of immature secondary nodules. (Compare Tables 3 and 5.)

Following the ovalbumin administration, however, neither solid secondary nodules nor pseudo-secondary nodules were significantly increased in number (Table 6). In this connection the facts must be taken into consideration, first, that the solid secondary nodules as well as the pseudo-secondary nodules develop

TABLE V

Variations in the average number of solid and pseudo-secondary nodules occurring in 1 cm^2 of sections of the mesenterial lymph nodes of normal rabbits at different ages.

Age in weeks or months	Rabbit No., body weight and sex	Solid secondary nodules	Pseudo-secondary nodules
1 week	1Wa, 168g, ♂	10	5
	1Wb, 170g, ♂	10	5
2 weeks	2Wa, 278g, ♂	20	0
	2Wb, 158g, ♂	40	20
3 weeks	3Wa, 564g, ♂	112	16
	3Wb, 238g, ♂	87	16
1 month	1Ma, 450g, ♂	124	30
	1Mb, 588g, ♂	43	15
	1Mc, 610g, ♂	60	6
2 months	2Ma, 925g, ♀	54	6
	2Mb, 743g, ♂	43	5
3 months	3Ma, 1.5kg, ♀	22	5
	3Mb, 1.4kg, ♀	33	13
4 months	4Ma, 1.6kg, ♀	3	0
	4Mb, 1.6kg, ♀	10	4
6 months	6Ma, 2.5kg, ♀	26	5
	6Mb, 2.6kg, ♀	8	7
8 months	8Ma, 3.1kg, ♂	14	5
	8Mb, 3.3kg, ♀	20	6
12 months	12M, 3.8kg, ♂	10	0
18 months	18M, 3.6kg, ♀	3	0

TABLE VI

Changes in the average number of solid and pseudo-secondary nodules occurring in 1 cm^2 of sections of the mesenterial lymph nodes after administering ovalbumin.

Groups	Time after the initial injection	Rabbit No., body weight and sex	Solid secondary nodules	Pseudo-secondary nodules
Single injection of 100mg of ovalbumin	12 hours	Ke28, 2.0kg, ♂ Ke29, 2.1kg, ♀	24 14	8 8
	1 day	Ke26, 2.2kg, ♀ Ke27, 2.2kg, ♀	17 23	11 8
	3 days	Ke24, 2.0kg, ♀ Ke25, 2.1kg, ♀	18 23	7 11
	5 days	Ke22, 2.0kg, ♂ Ke23, 2.0kg, ♂	22 23	13 10
	7 days	Ke20, 2.0kg, ♂ Ke21, 1.8kg, ♂	9 19	1 5
	14 days	Ke18, 2.0kg, ♂ Ke19, 2.1kg, ♂	5 6	2 0
	21 days	Ke15, 2.1kg, ♂ Ke16, 1.8kg, ♀	9 7	3 2
Daily injection of 50mg of ovalbumin	3 days	Ke34, 1.9kg, ♂ Ke35, 2.0kg, ♂	15 18	12 10
	5 days	Ke32, 2.0kg, ♂ Ke33, 2.0kg, ♂	14 8	5 1
	7 days	Ke30, 2.1kg, ♂ Ke31, 2.1kg, ♂	5 10	9 12
	14 days	Ke12, 2.4kg, ♀ Ke13, 2.2kg, ♂	13 11	5 0
	21 days	Ke11, 2.2kg, ♀ Ke14, 2.4kg, ♀	4 11	0 0

long before the immature Flemming's nodules begin to appear, and second, that while the immature Flemming's nodules are foci of active proliferation of medium-sized lymphocytes, the solid and pseudo-secondary nodules usually do not contain such foci. Ehrich (1929) is of opinion that the solid and pseudo-secondary nodules are the places in which small lymphocytes are produced. Gyllensten (1950) has demonstrated that in guinea-pigs these nodules develop without the stimulation of toxins and in this way they differ from the Flemming's nodules. The present observations support the view of Gyllensten.

C. Reactions in the spleen.

Following the ovalbumin administration, the white pulp underwent a

marked hypertrophy with new formation of Flemming's secondary nodules as in the mesenterial lymph nodes. The red pulp also participated in the reaction, and was greatly enlarged when ovalbumin had been injected daily more than 14 times. In such instances, the spleen weighed several times its normal weight, and there appeared many large foci of plasmocytes in the Billroth's cords of the enlarged red pulp. The significance of occurrence of these foci will be discussed elsewhere.

The mode of new formation of secondary nodules of Flemmings's type in the white pulp was substantially the same as observed in the mesenterial lymph nodes. However, the early Flemming's nodules in the spleen differ in some respects from those in the lymph nodes. At first they also appear as small isolated masses of medium-sized lymphocytes which are very frequently in mitosis in the dense lymphatic tissues surrounding the central arteries. This occurs in the same manner as in the lymph nodes. Yet, the masses of medium-sized lymphocytes are not so compact as in the lymph nodes, and, being enclosed in the dense lymphatic tissue, do not assume the appearance of "bare germinal centers" of Conway. Therefore, the early stages of development of Flemming's nodules in the spleen can hardly be classified into three types (+, ++ and

TABLE VII

Variations in the average number of secondary nodules in different phases of activity occurring in 1 cm^2 of sections of the spleen of normal rabbits at various ages.

Age in weeks or months	Rabbit No., body weight and sex	Phases of activity			Total
		active	half-inactive	inactive	
1 week	1Wa, 168g, ♂	0	0	0	0
	1Wb, 170g, ♂	0	0	0	0
2 weeks	2Wa, 278g, ♂	0	0	0	0
	2Wb, 158g, ♂	0	0	0	0
3 weeks	3Wa, 564g, ♂	0	0	0	0
	3Wb, 238g, ♂	0	0	0	0
1 month	1Ma, 450g, ♂	0	0	0	0
	1Mb, 588g, ♂	0	0	0	0
	1Mc, 610g, ♂	0	0	0	0
2 months	2Ma, 925g, ♀	0	0	0	0
	2Mb, 743g, ♂	6	0	0	6
3 months	3Ma, 1.5kg, ♀	16	8	56	80
	3Mb, 1.4kg, ♀	35	0	0	35
4 months	4Ma, 1.6kg, ♀	61	24	12	97
	4Mb, 1.6kg, ♀	14	7	34	55
6 months	6Ma, 2.5kg, ♀	0	0	30	30
	6Mb, 2.6kg, ♀	0	11	27	38
8 months	8Ma, 3.1kg, ♂	0	0	0	0
	8Mb, 3.3kg, ♀	0	0	0	0
12 months	12M, 3.8kg, ♂	0	3	13	16
18 months	18M, 3.6kg, ♀	0	0	14	14

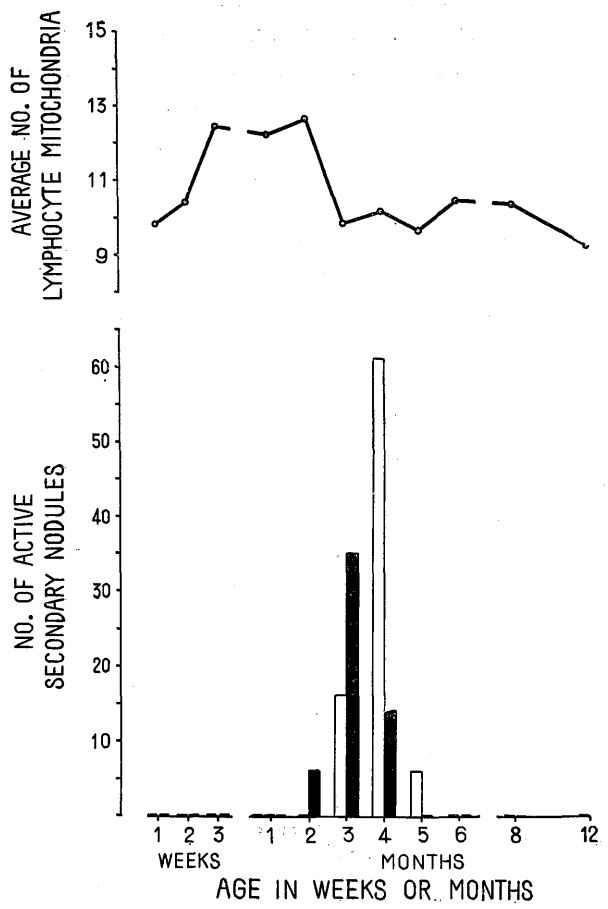


Fig. 9. Variations in the average number of active secodary nodules occurring per 1 cm^2 of sections of the spleen and in the average number of mitochondria per lymphocyte in peripheral blood in normal rabbits at different ages.

+ + + types) as in the lymph nodes, because this classification is chiefly based on whether the nodules are bare or surrounded by a lymphocyte jacket. For this reason no distinction was made between immature and mature secondary nodules. Instead, the secondary nodules of spleen were divided into three types, "active", "half-inactive" and "inactive", according to the cellular constitution of thier light-staining centers, regardless of whether they are in earlier or later stages of development. The "active" secondary nodules denote those having centers which are chiefly composed of actively proliferating, medium-sized

lymphocytes; the "half-inactive" nodules those having centers which are half filled with such medium-sized lymphocytes. The nodules with centers almost devoid of such medium-sized lymphocytes and chiefly consisting of reticular cells with scattered small lymphocytes were disignated "inactive". The inactive nodules correspond to the "reaction centers" of Hellman.

In normal rabbits, the average numbers of these three types of secondary nodules occurring per unit area of sections of spleen vary considerably with age. The data from animal, from one week to 18 months of age, are listed in Table VII. It is seen that secondary nodules first appear at two months of age and become most numerous during the period from three to five months after birth, thereafter abruptly decreasing in number. Therefore, the time of first appearance of Flemming's nodules in the spleen is somewhat delayed as compared

TABLE VIII

Changes in the average number of secondary nodules in different phases of activity occurring in 1 cm^2 of sections of the spleen after administering ovalbumin.

Groups	Time after the initial injection	Rabbit No., body weight and sex	Phases of activity			Total
			active	half-inactive	inactive	
Single injection of 100mg of ovalbumin	12 hours	Ke28, 2.0kg, ♂	0	0	30	30
		Ke29, 2.1kg, ♀	0	0	3	3
	1 day	Ke26, 2.2kg, ♀	0	0	14	14
		Ke27, 2.2kg, ♀	9	9	60	78
	3 days	Ke24, 2.0kg ♀	5	0	5	10
		Ke25, 2.1kg, ♀	10	0	13	23
	5 days	Ke22, 2.0kg, ♂	26	20	65	111
		Ke23, 2.0kg. ♂	37	24	12	73
	7 days	Ke20, 2.0kg, ♂	28	7	56	91
		Ke21, 1.8kg, ♂	24	8	96	130
	14 days	Ke18, 2.0kg, ♂	12	12	9	33
		Ke19, 2.1kg, ♂	15	8	15	38
	21 days	Ke15, 2.1kg, ♂	10	0	35	45
		Ke16, 1.8kg, ♀	7	0	44	51
Daily injection of 50 mg of ovalbumin	3 days	Ke34, 1.9kg, ♂	22	11	22	55
		Ke35, 2.0kg, ♂	3	0	9	12
	5 days	Ke32, 2.0kg, ♂	15	19	53	87
		Ke33, 2.0kg, ♂	17	26	26	69
	7 days	Ke30, 2.1kg, ♂	60	16	16	92
		Ke31, 2.1kg, ♂	48	6	0	54
	14 days	Ke12, 2.4kg, ♀	53	0	0	53
		Ke13, 2.2kg, ♂	36	4	6	46
	21 days	Ke10, 3.0kg, ♀	17	17	9	43
		Ke11, 2.2kg, ♀	23	10	4	37
		Ke14, 2.4kg, ♀	12	10	4	26

with that in the mesenterial nodes. (Compare Figs. 3 and 9.) It should be noticed here that at the beginning of their appearance the active nodules preponderate over the half-inactive and inactive forms. Since many of active nodules in the spleen are in earlier stages of development comparable to the

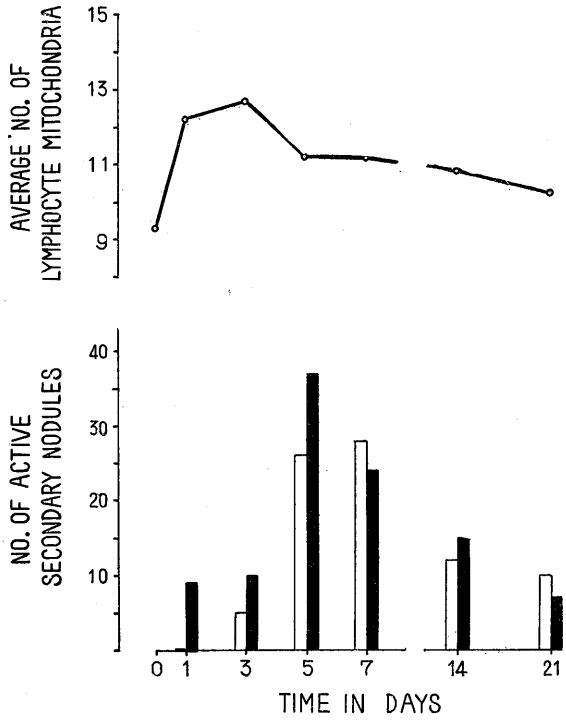


Fig. 10. Changes in the average number of active secodary nodules occurring per 1cm^2 of sections of the spleen and in the average number of mitochondria per lymphocyte in peripheral blood after a single injection of 100 mg of ovalbumin.

immature secondary nodules in the lymph node, a greater significance must be attached to an increase of active nodules than to that of other types of nodules. As seen in Fig. 9, a maximal increase in number of active nodules was observed at three or 4 months of age, whereas a maximal increase in the mitochondrial content in circulating lymphocytes occurs at 2 months of age. Thus in the spleen some delay was observed as regards the time of maximal increase in number of active secondary nodules.

Following the ovalbumin administration, the active secondary nodules also greatly increased in number, the maximal value per unit area of sections being attained on the 5th day in the case of single injection and on the 7th day in the case of daily injection, respectively (Table 8). Thereafter these nodules gradually decreased in number. It is evident therefore that the increase in the number of active secondary nodules occurs almost simultaneously with a marked rise in the mitochondrial content in circulating lymphocytes (Figs. 10–11). The half-inactive and inactive secondary nodules were also increased in number

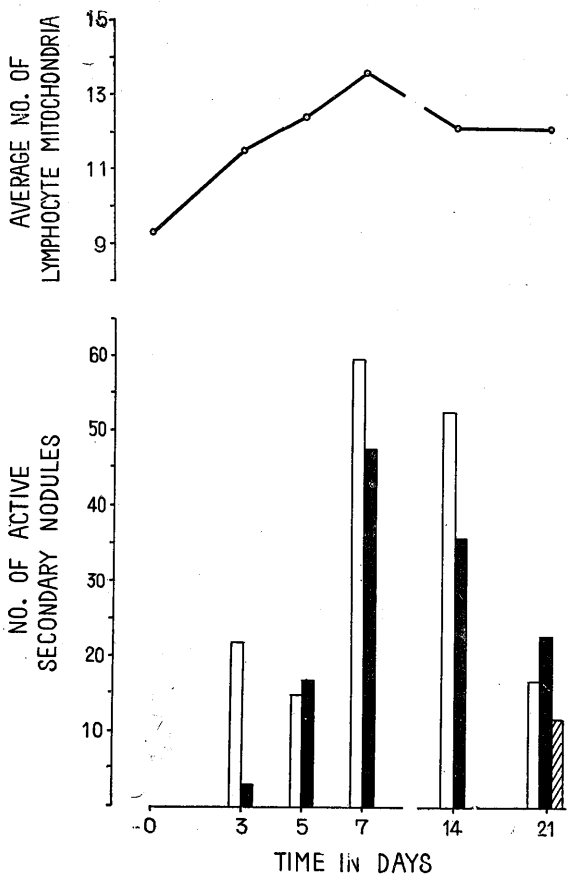


Fig. 11. Changes in the average number of active secondary nodules occurring per 1 cm^2 of sections of the spleen and in the average number of mitochondria per lymphocyte in peripheral blood in the course of daily injection of 50 mg of ovalbumin.

at the same periods. The results agree fairly well with those in the mesenterial nodes.

On the basis of the above-described observations, it can be stated that the lymphatic tissue of spleen responds to the stimulation of ovalbumin almost in the same way as that of the mesenterial lymph nodes. It should be noticed, however, that the early Flemming's nodules in the spleen differ in some respects from those in the lymph nodes, and that in the normal course of postnatal development, the time of first appearance and that of maximal increase of immature Flemming's nodules are somewhat delayed in the spleen as compared with that in the lymph nodes.

D. Reactions in the popliteal lymph nodes, Peyer's patches of small intestine and vermiform appendix.

In some instances, the popliteal lymph nodes also underwent a more or less hyperplasia of lymphatic tissue with new formation of immature secondary nodules; but in many others such reactions could not be recognized. The Peyer's paches of small intestine and vermiform appendix showed no consistent changes. It appears that the lymphatic tissues other than those of the mesenterial lymph nodes and spleen scarcely respond to the stimulation of ovalbumin introduced intravenously.

E. Reactions in the liver and bone marrow

Following the single injection of ovalbumin, no alteration was observed in the histological picture of these organs, except for a slight relative increase of myelocytes in the bone marrow. However, when its daily injection had been repeated more than 14 times, some remarkable changes have appeared.

The most striking change in the liver was new formation of lymphocyte aggregations in the periportal spaces, which was first recognized 14 days after the initial injection. In the later period, however, the lymphocytes aggregated in the periportal spaces were gradually replaced by plasmocytes. The bone marrow parenchyma was depleted to a large extent by the repeated injections of ovalbumin and there appeared small or large foci of plasmocytes in large numbers. Thus, a marked plasmocytic reaction was observed in the liver and bone marrow, as well as in the spleen as already mentioned. The significance of such reaction in relation to the lymphatic response will be discussed in another paper.

IV. Discussion

The present experiments have shown that in response to the stimulation of ovalbumin, the lymphatic tissue of chief lymphoid organs of adult rabbits, such as the mesenterial lymph nodes and spleen, undergo a marked hyperplasia with an extensive new formation of Flemming's nodules, and that the Flemming's nodules first appear as foci of active proliferation of medium-sized lymphocytes, being accompanied by a significant rise in the mitochondrial content in circulating lymphocytes. Since in the present research chief attention was directed to the relationships between the reactions of circulating lymphocytes and those of limphatic tissue to ovalbumin, the author wishes to consider first the significance of a rise in the average number of mitochondria in circulating lymphocytes.

It has been repeatedly stated that younger lymphocytes possess more abundant and basophilic cytoplasm containing a greater number of mitochondria

than the mature and older forms (Wiseman, 1931b; Miller and Taylor, 1948; Fukase, 1949; and Ackerman and Bellios, 1955). Fukase (1949) demonstrated by using the method of supravital staining with Janus green B and neutral red that, in normal rabbits, the average number of mitochondria in lymphocytes is highest in the lymph nodes, intermediate in the efferent lymph and lowest in the peripheral blood. In mice inoculated with transplantable lymphoid leukemia, Miller and Taylor (1948) who also used the supravital technic, observed a marked increase in the number of mitocohndria in circulating lymphocytes as compared with normal mice. In view of the above-mentioned observations by previous workers, it can be stated that a rise in the average number of mitochondria in circulating lymphocytes takes place as a consequence of increased delivery into the blood stream of younger lymphocytes, which contain in their cytoplasm more numerous mitochondria than the mature and older forms.

We shall next consider mode of new formation of Flemming's secondary nodules and its relation to a rise in the mitochondrial content in circulating lymphocytes. It was pointed out by Conway (1937) that the Flemming's nodules first appear as small isolated masses of densely packed, rapidly dividing, medium-sized lymphocytes without peripheral mantle of small lymphocytes. She called such nodules "bare germinal centers". Gyllensten (1950) also noticed in his study of the postnatal differentiation processes of the guinea-pig lymphatic tissue that the early Flemming's nodules assume the appearance of "bare germinal centers" of Conway. In our present study, we were able to confirm that the early Flemming's nodules are lymphocytopoietic center, consisting mainly of densely packed, actively proliferating, medium-sized lymphocytes without demarcating peripheral mantle of small lymphocytes. These nodules are termed "immature secondary nodules" because they give rise to typical Flemming's nodules having pale-staining centers surrounded by dark-staining mantles in later stages.

Regarding the relationships between new formation of secondary nodules in the lymphatic tissue and alterations in the lymphocytic picture of peripheral blood, it is of especial interest that a marked increase in number of immature secondary nodules and corresponding early Flemming's nodules in the lymphatic tissue of chief lymphoid organs is accompanied by a significant rise in the mitochondrial content in circulating lymphocytes. Since mitochondrial increase in circulating lymphocytes signifies, as already mentioned, an increased delivery of younger lymphocytes, the above finding indicates that the newly formed, early Flemming's nodules are important sites of production and delivery of younger lymphocytes. This inference is supported by the fact that the early Flemming's nodules are devoid of demarcating, peripheral mantles consisting

of densely packed small lymphocytes, for this condition favors mobilization of lymphocytes newly formed in the nodules.

Concerning the source of delivery of lymphocytes, however, other changes in the lymphatic tissue, particularly of the mesenterial lymph nodes, must also be taken into consideration. It has been shown that diffuse proliferation of larger lymphocytes in the medullary cords as well as in the intermediary sinuses is markedly intensified by the stimulation of ovalbumin, coincident with new formation of Flemming's nodules. It follows from these findings that the mode of reaction of the lymphatic system to ovalbumin is characterized by an extensive, generalized proliferation of larger lymphocyte, the medium-sized lymphocytes in particular, which leads to a new formation of Flemming's nodules, on the one hand, and to a diffuse hyperplasia of larger lymphocytes in other parts of lymphatic tissue without formation of Flemming's nodules, on the other. As a direct factor to effect an increased delivery of lymphocytes, a diffuse hyperplasia of larger lymphocytes in the lymphatic tissue other than the secondary nodules appears to be of greater significance.

Nevertheless, it must again be emphasized here that the Flemming's secondary nodules, the early forms in particular, play an important rôle in lymphocyte production of lymphatic tissue. It is indisputable that the early Flemming's nodules are foci of active proliferation of medium-sized lymphocytes, because mitotic figures are numerously found in such nodules and because most of the nuclei in mitosis are approximately equal in size to those of medium-sized lymphocytes and are moreover very frequently in prophase. Another series of experiments with adult albino rats revealed that, when mitoses are arrested at metaphase by the injection of colchicine, metaphases occur in the secondary nodules often in a much greater number than in other parts of the lymphatic tissue (Osogoe and Ito, unpublished observation).

The lymphocytopoietic function of Flemming's secondary nodules has been refuted by Hellman and his associates (Hellman, 1938, 1943; A. and H. Sjövall, 1930; Rudebeck, 1932), who investigated the reaction of lymphatic tissue to bacteria or bacterial toxins. These investigators believe that pale-staining centers of secondary nodules are chiefly composed of reticular cells, having nothing to do with lymphocytopoiesis. This view is correct so far as the late, inactive Flemming's nodules are concerned. Since, however, Hellman and his associates failed to notice the early stages of development of the Flemming's nodules, their concept cannot generally be accepted. In this connection it is worthy of notice that ovalbumin does not contain toxin. It is conceivable therefore that the mode of reaction of lymphatic tissue to ovalbumin would differ considerably from that to bacteria or bacterial toxins. However, final certainty on this point must await further experimentation.

Finally, some mention should be made of the responses of solid secondary nodules of Groll and Krampf and of pseudo-secondary nodules of Ehrich to the stimulation by ovalbumin. As far as the present experimental conditions are concerned, neither solid secondary nodules nor pseudo-secondary nodules were significantly increased in number. The result indicates that these nodules differ from the Flemmlng's nodules as regards the mode of reaction to ovalbumin.

V. Summary

In adult rabbits, the modes of reaction of circulating lymphocytes and of lymphatic tissue of chief lymphoid organs, particularly the mesenterial lymph nodes and spleen, were investigated. Ovalbumin (E. Merck) was given intravenously either in a single large dose (100 mg) or daily in smaller doses (50mg each).

Following the ovalbumin administration, a significant transient rise in the mitochondrial content of circulating lymphocytes was produced within several days. This fact signifies a markedly increased delivery into the blood stream of younger lymphocytes, which contain in their cytoplasm a greater number of mitochondoria than the mature and older forms.

In the mesenterial lymph nodes, there occurred an extensive new formation of secondary nodules of Flemming's type. The Flemming's nodules first appeared as isolated round masses of densely packed, rapidly dividing, medium-sized lymphocytes without demarcating, peripheral mantles of small lymphocytes. Such early Flemming's nodules (here termed "immature secondary nodules") greatly inceased in number within several days, almost coincident with a significant mitochondrial increase of the circulating lymphocytes. These secondary nodules decreased rapidly thereafter, giving rise to typical text-book secondary nodules consisting of pale-staining centers and surrounding dark-staining mantles. Neither solid secondary nodules nor pseudo-secondary nodules were noticeably increased in number.

The lymphatic tissue of spleen showed an almost similar response to ovalbumin with an extensive new formation of Flmming's nodules, almost simultaneously with a pronounced rise in the mitochondrial content in circulating lymphocytes. In the spleen, the early Flemming's nodules also appeared as isolated masses of actively proliferating, medium-sized lymphocytes, but the masses were less compact than in the lymph nodes.

The popliteal lymph nodes, Peyer's patches of small intestine and vermiform appendix showed little or no reaction to ovalbumin introduced intravenously.

In the present research, our attention was focused on the early phases of reaction of circulating lymphocytes and of lymphatic tissue, and a considerable

emphasis is laid on the facts that the immature Flemming's nodules are foci of active proliferation of medium-sized lymphocytes, and that a marked increase in number of such nodules in lymphatic tissue of the chief lymphoid organs is accompanied by a significant rise in the mitochondrial content in circulating lymphocytes, indicating that the newly formed, early Flemming's nodules are important sites of production and delivery of younger lymphocytes responsible for the alteration in the lymphocytic blood picture. This inference is supported by the fact that the immature Flemming's nodules are devoid of demarcating, peripheral mantles consisting of densely packed small lymphocytes, for this condition favors mobilization of lymphocytes newly formed in the nodules.

Literature Cited

ACKERMAN, A. AND N. C. BELLIOS. 1955. A study of the morphology of the living cells of blood and bone marrow in vital films with the phase contrast microscope. I. Normal blood and bone marrow. *Blood*, **10**: 3–16.

CONWAY, E. A. 1937. Cyclic changes in lymphatic nodules. *Anat. Rec.*, **69**: 487–513.

EHRICH, W.E. 1929. (a) Studies of the lymphatic tissue. 1. The anatomy of the secondary nodules and some remarks on the lymphatic and lymphoid tissue. *Amer. J. Anat.*, **43**: 347–383. (b) Studies of the lymphatic tissue. II. The first appearance of the secondary nodules in the embryology of the lymphatic tissue. *Amer. J. Anat.*, **43**: 384–401.

EHRICH, W.E. 1946. The rôle of the lymphocyte in the circulation of the lymph. *Ann. New York Acad. Sci.*, **66**, Art 8: 823–858.

FLEMMING, W. 1885. Studien über die Regeneration der Gewebe. *Arch. Mikr. Anat.*, **24**: 50 a. 355.

FUKASE, M. 1949. (a) Experimental studies on the lymphocyte mitochondria. 1. Comparison of mitochondrial content in lymphocytes of the lymph node, lymph and blood. II. Variations in mitochondrial content in lymphocytes of the lymph in vitro. *Acta Haemat. Jap.*, **12**: 47–63. (b) Experimental studies on the lymphocyte mitochondria. III. Variations in mitochondrial content of lymphocytes in the case of lymphocytosis. IV. Variations in mitochondrial content of lymphocytes in the case of lymphocytopenia induced by cachexia. *Acta Haemat. Jap.*, **12**: 111–125.

GLIMSTEDT, G. 1936. Bakterienfreie Meerschweinchen, Aufzucht, Lebensfähigkeit und Wachstum, nebst Untersuchungen über das lymphatische Gewebe. *Acta path. et microbiol. Scandinav.*, Suppl. XXX.

GROLL, H. and F. KRAMPF. 1920–21. Involutionsvorgänge an den Milzfollikeln. *Zbl. Path.*, **31**: 145–159.

GYLLENSTEN, L. 1950. The postnatal histogenesis of the lymphatic system in guinea-pigs. *Acta Anat.*, **10**:130–160.

HELLMAN, T. 1938–39. Die Reaktionszentren des lymphatischen Gewebes. *Verh. anat. Ges. Leipzig*, 1938. *Erg.-Heft. Anat. Anz.*, **89**: 132–151.

HELLMAN, T. 1943. Lymphgefässe, Lymphknötchen und Lymphknoten. in *W. v. Möllendorffs Handbuch d. mikro. Anat. d. Mensch.*, VI/4:173–261.

HORII, I. 1938. Über die Zellproduction der Lymphdrüse. 1. Teil, Zellzahl. *A.A. Kyoto Serie D. H.* **7**: 121–136.

KARASAWA, K. 1954. Experimental studies on the chief source of blood lymphocytes in adult rabbits. *Folia anat. jap.*, **26**: 309–325.

MILLER, R. A., and TAYLOR, M.J. 1948. A concomitant change in mitochondria and virulence of a transplanted lymphoid leukemia. *Proc. Soc. Exp. Biol. &. Med.*, **68**: 336–339.

OSOGOE, B., L. C. CHANG, K. AWAYA a. K. KARASAWA. 1953. Variation in mitochondrial content of lymphocytes in the peripheral blood in relation to postnatal development of lymphoid organs in the rabbit. *Bull. Yamaguchi Med. School*, **1**: 72–78; *Acta Haemat. Jap.*, **16**: 293–298 (in Japanese).

RÖHLICH, K. 1930. Beitrag zur Cytologie der Keimzentren der Lymphknoten. *Z. mikrosk.-anat. Forsch.*, **20**: 287–297.

RUDEBECK, J. 1932. Experimentelle Untersuchungen über die Sekundärknötchen in den Kniekehlenlymphknoten des Kaninchens bei Staphylokokkeninfektion. *Virchows Arch.*, **284**: 504–517.

SJÖVALL, A. and H. SJÖVALL. 1930. Experimentelle Studien über die Sekundärknötchen in den Kniekehlenlymphknoten des Kaninchens bei Bacillus Pyocyaneus-Infektion. *Virchows Arch.*, **278**: 258–283.

WISEMAN, B. K. 1931a. The induction of lymphocytosis and lymphatic hyperplasia by means of paraenterally administered protein. *J. Exp. Med.*, **53**: 499–510.

WISEMAN, B. K. 1931b. Criteria of the age of lymphocytes in the peripheral blood. *J. Exp. Med.*, **54**: 271–294.

WISEMAN, B. K., C. A. DOAN and L. A. ERF. 1936. A fundamental, reciprocal relationship between myeloid and lymphoid tissues. *J.A.M.A.*, **106**: 609–615.

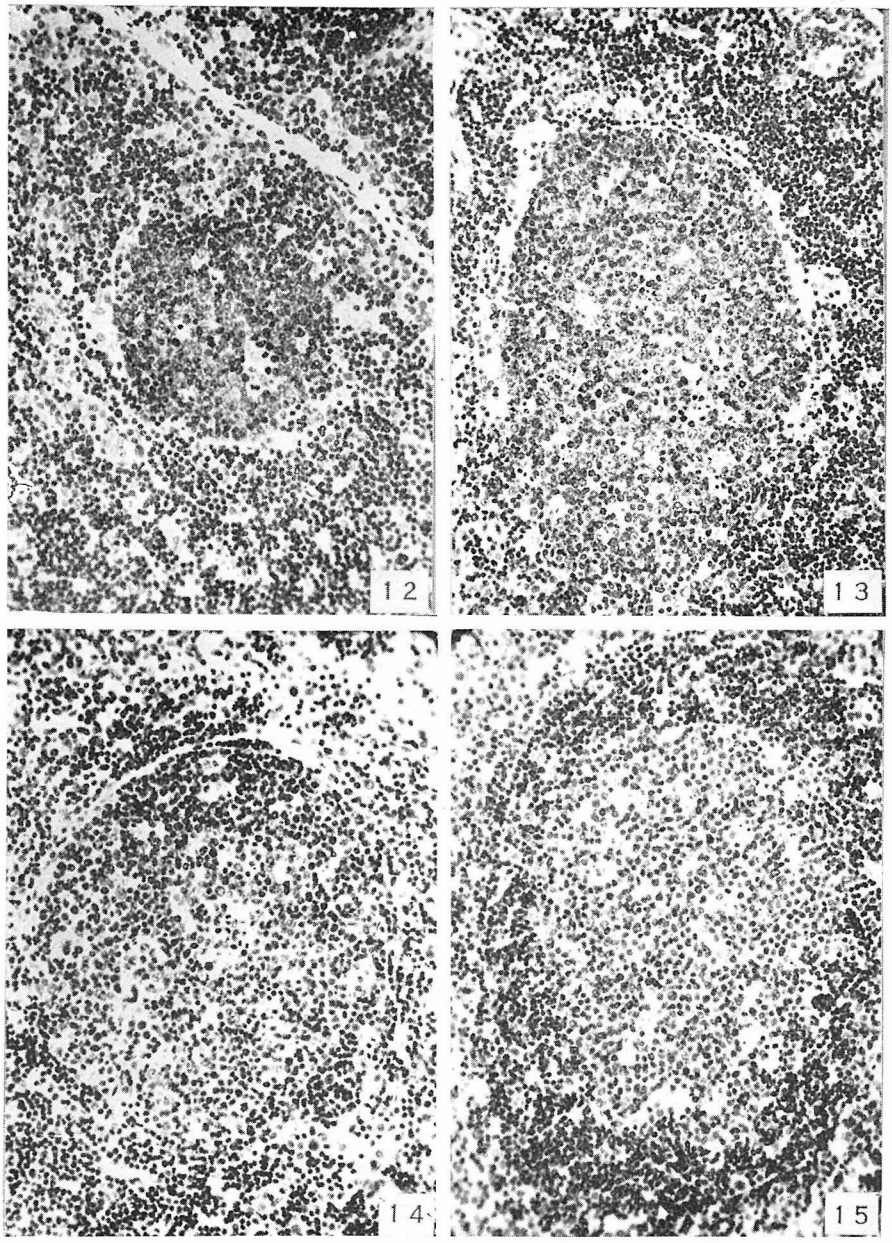
EXPLANATION OF PLATE I.

Fig. 12. A immature secondary nodule of *type* +, consisting nearly exclusively of medium-sized lymphocytes. Neither demarcating fibrilar capsule nor outer zone of small lymphocytes is discernible around the nodule. × 150.

Fig. 13. A immature secondary nodules of *type* ++, consisting chiefly of medium-sized lymphocytes. The upper part of this nodule is demarcated partially by a thin fibrilar capsules, but without outer zone of small lymphocytes. × 150.

Fig. 14. A immature secondary nodule representing *type* +++. This nodule is demarcated almost completely by a thin fibrilar capsule and surrounded moreover partially by a lymphocyte jacket (the upper left part of the nodule). In this nodule the cellular density of medium-sized lymphocytes is markedly reduced by an increase in reticular cells and small lymphocytes. × 150.

Fig. 15. A fully developed secondary nodule (mature secondary nodule of *type* ++++). × 150

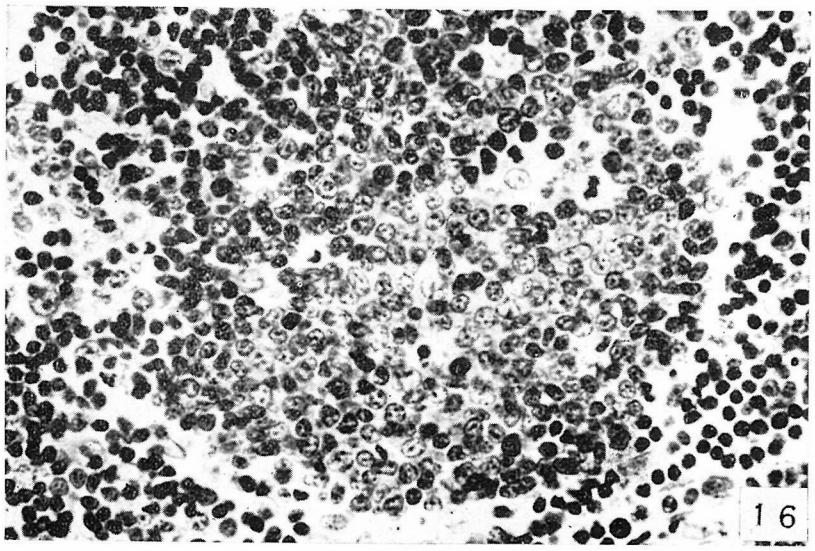


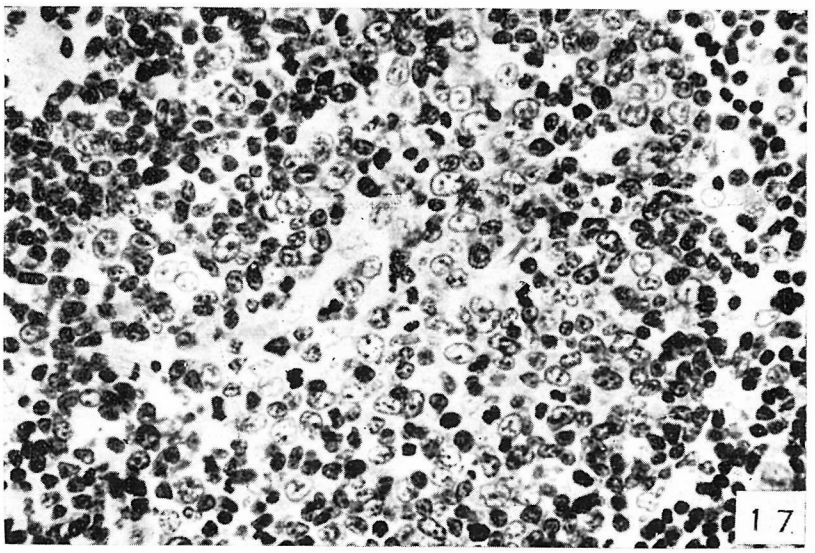
Kazuhiko Awaya

EXPLANATION OF PLATE II.

Fig. 16. Greater magnification of a immature secondary nodule of *type* +. Notice densely packed medium-sized lymphocytes, some of which are in mitosis. ×400.

Fig. 17. Greater magnification of a immature secondary nodule of *type* ++. Notice numerous mitotic figures. Most of dividing nuclei are approximately equal in size to those of medium-sized lymphocytes.


16


17