VARIATION IN MITOCHONDRIAL CONTENT OF LYMPHOCYTES IN THE PERIPHERAL BLOOD IN RELATION TO POSTNATAL DEVELOPMENT OF LYMPHOID ORGANS IN THE RABBIT *

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INTRODUCTION

Since Arneth's monumental work[1] it is well known that the number of nuclear segments is an indication of the age of the polymorphonuclear leucocyte. In the lymphocyte, however, a similar criterion of the age of the cell according to the nuclear configuration has been seldom used, although Arneth attached maturative significance to the nuclear identations.

The criteria of the age of lymphocytes have been reviewed by Wiseman (1931)[2]. According to him, basophilia of the cytoplasm and mitochondrial content, especially the former, can serve as reliable indicator of the age of lymphocytes, the youngest cells being the most basophilic with most numerous mitochondria; while other features, such as motility, vacuoles, chromatin content of the nucleus, proportion of nucleus to cytoplasm, shape of nucleus (identations), and azur granulations, have minor significance in this relation.

In their clinical investigations of infectious processes, Reich and Reich (1933)[3] classified the lymphocytes according to the degree of basophilia and obtained a lymphocytic hemogram, which indicated the participation of lymphoid tissue in the process. The method, however, was found less helpful than Schiling's index in determing the severity of the disease.

Recently our collaborator, Fukase (1949)[4], has elaborated another method for the classification of lymphocytes, in which these forms were arranged according to the number of mitochondria stained by supravital technique. He demonstrated by this method that, in normal rabbits, the mitochondrial number in lymphocytes is highest in the lymph nodes, intermediate in the efferent lymph, and lowest in the peripheral blood. It was also shown that, if the lymphatic system is stimulated by daily intravenous injections of heterogenous protein, e. g., egg albumin or serum albumin, the mitochondrial content of lymphocytes in the

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peripheral blood is markedly increased within several days after the initial injection, approaching the picture of the normal lymph nodes[5]. On the basis of these observations, Fukase claims that a high mitochondrial content is linked with youth in the lymphocyte. In mice inoculated with transplantable lymphoid leukemias, Miller and Taylor (1948)[6] who also used the supravital technique, observed a marked increase in the number of mitochondria in lymphocytes as compared with normal mice.

It should be noticed, however, that the supravital technique does not permit an accurate estimation of the mitochondrial content, partly because of physical difficulties involved in counting these bodies and partly because of the fact that some of the mitochondria occasionally fail to take the dye, while some faiding of the stain occurs. Nevertheless, the number of mitochondria in lymphocytes estimated by this technique, as mentioned above, seems to show the general trend with respect to the age of lymphocytes and to the lymphocytopoietic activity. With these facts in mind, the present study was undertaken to determine what changes if any do occur in mitochondrial content of lymphocytes of the blood during the normal course of the postnatal development of the lymphoid organs.

Material and Methods

Sixty six normal healthy rabbits of both sexes were used, which were widely diversified among various age groups from 1 week to 12 months after birth, as shown in Table I. For the younger groups, from 1 week up to 1 month of age, 6 to 10 rabbits were represented, and for the older groups 3 to 5 rabbits.

In blood examinations, virtually the same procedures were used as in the earlier studies by Fukase[4][5]. Mitochondria stained with Janus green B were counted in 100 lymphocytes from each rabbit and in a total of 300 to 1000 lymphocytes from each age group. A mixed solution of Janus green B and neutral red in absolute alcohol was used for preparing slides. Each 10 ml of this solution contained 20 drops of Janus green B (Grübler) and 30 drops of neutral red (Grübler), both saturated in absolute alcohol. A drop of blood from the marginal veins of rabbits 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.

The lymphocytes thus stained were classified in the same order as in the earlier studies by Fukase[4][5] as follows:

Classes of lymphocytes	I	II	III	IV	V	VI
No. of mitochondria	0	1–5	6–10	11–15	16–20	21–

Lymphocytes containing more than 25 mitochondria were occasionally met with but only in small numbers, and hence they were included in Class VI,

representing cells with more than 21 mitochondria. Besides the average number of mitochondria per cell, an age index of the lymphocytes from each rabbit was calculated according to the method of Yokota (1950)[7]. For example, it may be calculated, using the data presented in the upper row of Table II, in the following manner:

$$\text{Age index} = \frac{1\times 4.7+2\times 22.0+3\times 33.0+4\times 23.4+5\times 8.7+6\times 8.2}{4.7+22.0+33.0+23.4+8.7+8.2} = 3.340$$

Besides, the lymphocytes were classified according to cell size into 3 types: small lymphocytes with a diameter of less than 7μ, medium-sized lymphocytes with a diameter of 8 to 9μ, and large lymphocytes with a diameter of more than

TABLE I

Average Number of Mitochondria and Age Index of Lymphocyte in the Peripheral Blood of Normal Rabbits at Different Ages

Age in weeks or months	No. of rabbits	Body wt	RBC $\times 10^4$	HB gms/dl	WBC	Abs. No. of lymphocytes	Total No. of lymphocytes counted	Avg. No. of mitochondria per lymphocyte	Age index of lymphocyte	P (Age index)
1 W	9	142	400	8.6	2280	1570	900	9.8	3.340	
2 W	9	2 32	452	9.1	2740	2020	900	10.4	3.471	>0.05
3 W	10	319	479	9.4	4330	2720	1000	12.4	3.874	<0.01
1 M	6	320	495	8.6	7800	4650	600	12.2	3.847	<0.05*
2 M	5	748	498	9.8	7980	4620	500	12.6	3.882	<0.05*
3 M	5	992	466	9.8	5820	4250	500	9.8	3.356	>0.05
4 M	4	1530	538	8.3	6680	4810	400	10.1	3.450	
5 M	5	1820	555	9.2	6740	4070	500	9.6	3.312	
6 M	5	2000	541	8.1	9100	5250	500	10.4	3.450	
8 M	5	2300	604	8.8	8750	5250	500	10.3	3.062	
12 M	3	2700	551	9.3	7500	5990	300	9.2	3.207	

* Because of a relatively small number of cells counted, the value is statistically less significant than the value at 3 weeks, as compared with the initial value at 1 week.

10μ. The diameter of lymphocytes was measured with a calibrated ocular micrometer in blood smears stained with Giemsa. If the lymphocytes were oval rather than round, the diameter was assumed to be the average of the length and breadth.

Two or three animals from each age group were sacrificed by exsanguination or injection of air and autopsied As a representative of lymphoid organs the mesentarial lymph nodes (pancreas Aselli) were studied, together with other tissues, such as lung, liver, spleen, small and large intestines, kidney, adrenal and bone marrow. The tissues were fixed in Zenker formol and embedded in paraffin. Sections were made at 6–8μ and stained with Mayer's acid hemalum and

eosin or with eosin-azur II.

Results and Discussion

In the rabbit, the gestation period is so brief (averaging 31 days) that the hematopoietic organs of the newborn are at considerably immature stages of development (Ōmura and Yamasowa, 1952)[8]. The blood picture of the newborn and infantile animals reflects such immaturity of the hematopoietic organs.

As seen in Table I, the blood cell counts, especially the leucocyte counts, are at considerable low levels until 3 weeks after birth. At 1 month of age, however, the number of white cells rises abruptly and reaches almost the maximal level of approximately 8,000 cells per cubic millimeter. Thereafter, the number fluctuates about this level, showing a slight tendency towards increase. The absolute number of lymphocytes increases almost parallel to the total white cell count (Fig. 1).

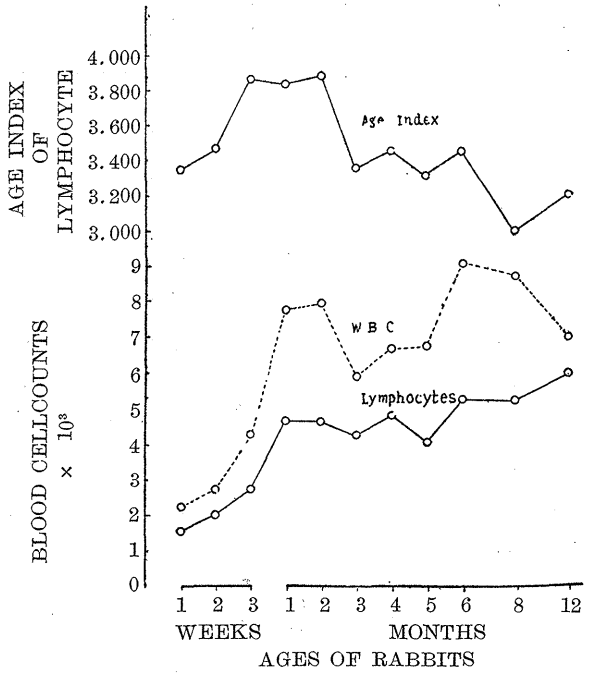

Fig. 1 Variations in "age index" of the lymphocyte, total leucocyte count and lymphocyte count of the peripheral blood with age.

The number of mitochondria in lymphocytes, on the other hand, varies in a different manner. As shown in Table I, both the average number of mitochondria per cell and the age index are markedly increased at 3 weeks of age as compared with the initial value, and maintain at a similar high level until 2 months after birth. After 3 months, however, both values fall to about the initial level and do not rise again. In rabbits older than 8 months both values appear to decrease

beneath the initial level (Fig. 1).

Because of physical difficulties involved in counting mitochondria, the age index, which is based upon a rough estimation of the mitochondrial content, seems to be of more practical value than the seemingly more accurate mean number of mitochondria per cell. Accordingly, in the present study the statistical treatment was confined to the variations in age index (Table I). It was found that the rise of the age index at 3 weeks above the intial value at 1 week is significant ($P<0.01$). Similar high values at 1 and 2 months are also significant ($P<0.05$) as compared with the initial value. Because of a relatively small number of cells counted (less than 600 cells), the latter values are statistically less significant. Other variations in age index were found to be statistically insignificant.

TABLE II

Differential Counts (%) of Lymphocytes in the Peripheral Blood of Normal Rabbits at Varying Ages According to Mitochondrial Content and Cell Size

Age in weeks or months	No. of lymphocytes counted	Mitochondrial Content of Lymphocyte						Cell Size of Lymphocyte		
		I 0	II 1–5	III 6–10	IV 11–15	V 16–20	VI 21–	Small L.	Medium-sized L.	Large L.
1 W	900	4.7	22.0	33.0	23.4	8.7	8.2	81.1	14.7	4.2
2 W	900	3.8	19.1	32.8	24.5	10.4	9.4	84.4	12.6	3.0
3 W	1000	3.0	9.5	29.1	28.6	15.1	14.7	73.5	18.6	7.9
1 M	600	1.3	8.8	31.2	32.8	14.2	11.7	79.5	14.7	5.8
2 M	500	2.4	10.2	29.8	27.0	15.6	15.0	72.2	18.2	9.6
3 M	500	3.0	21.6	36.6	21.8	9.6	7.4	82.8	11.8	5.4
4 M	400	2.0	17.0	40.0	23.0	11.0	7.0	84.8	10.2	5.0
5 M	500	3.6	20.2	40.2	19.6	8.6	7.4	84.6	10.8	4.6
6 M	500	5.0	16.2	37.4	21.0	11.0	9.4	84.6	9.8	5.6
8 M	500	6.4	25.4	40.1	16.6	7.0	4.5	85.7	9.3	5.0
12 M	300	5.3	24.7	33.7	21.0	9.0	6.3	86.6	7.7	5.7

The classification of lymphocytes according to mitochondrial content and cell size for different age groups is shown in Table II. The modes of the frequency distributions of mitochondria per lymphocyte differ in the order of the values of the age index. The proportions of small, medium-sized, and large lymphocytes also differ according to the order of the values of age index but to a much lesser extent.

With regard to the morphology of the mitochondria no change could be detected among different age groups. Most of the mitochondria in lymphocytes are small distinct granules but there are some rods and, though more infrequent, also filaments. These bodies are either scattered around the nucleus or grouped

in a portion adjacent to the nuclear identation.

Histological examination of the mesenteric lymph nodes revealed that, coincident with the period in which the values of age index are markedly elevated above the intial value, that is, during the period between 3 weeks and 2 months after birth, there can be seen a marked change in the histological differentiation of the nodes.

Up to the age of 2 weeks, the parenchyma of the mesenteric lymph nodes is composed of diffuse lymphatic tissue, with no definite differentiation into nodular masses, except for Ehrich's pseudosecondary nodules[8)9)] and solid secondary nodules[10)]. The cortex preponderates greatly over the medulla until 2 weeks after birth. In older groups, the relative proportion of medulla to cortex is gradually increased with advancing age.

At the age of 3 weeks, the cellular density of lymphocytes is markedly increased in both cortex and medulla, especially in the outer zone of the former. The most striking change at this age is the appearance of Conway's "bare germinal centers"[11)], most frequently at the cortico-medullary junction and occasionally in the outer zone of the cortex. Such areas appear as small isolated masses of densely packed, rapidly dividing, medium-sized lymphocytes in the diffuse lymphatic tissue, without demarcating peripheral zones of smaller lymphocytes. Apparently, such areas give rise to typical secondary nodules in later stages, and by the 3rd or 4th months after birth differentiation of secondary nodules with pale centers surrounded by peripheral, dark staining mantles have been accomplished.

It is worthy of notice that the period during which a remarkably higher level of mitochondrial content in the lymphocytes of the peripheral blood is maintained coincides with the period in which the differentiation of secondary nodules in lymph nodes proceeds, and that after the differentiation of secondary nodules in the nodes has been accomplished, the mitochondrial content in the lymphocytes decreases and does not rise again. These facts suggest that a marked rise in mitochondrial content in the lymphocytes of the peripheral blood is linked with a new formation of secondary nodules in the node.

Summary

1. During the normal course of postnatal development of lymph nodes in the rabbit, a remarkable elevation of the mitochondrial content in the lymphocytes of the peripheral blood was observed during the period from 3 weeks to 2 months after birth.

2. This period coincides with the period in which the differentiation of secondary nodules proceeds in the nodes.

3. After the differentiation of secondary nodules in the nodes has been accomplished, the number of mitochondria in the lymphocytes of the peripheral

blood decreases and does not rise again.

4. These facts suggest that a marked rise in mitochondrial content in the lymphocytes of the peripheral blood is linked with a new formation of secondary nodules in the node.

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