

QUANTITATIVE EVALUATION OF TOTAL CELLULAR NUMBER AND CELLULAR DENSITY IN THE THYMO-LYMPHATIC ORGANS OF YOUNG ADULT ALBINO RATS BY MEANS OF DNA DETERMINATION*†

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(Received March 10, 1959)

Attempts to estimate the total cellular number and the rate of cell production in the hemopoietic organs have been made in a series of studies on the rat by *Kindred* (1938, 1940, 1942 and 1955). The methods employed in these studies are based on cell counts and mitotic counts of given areas in sections of the hemopoietic organs of the rat. Such procedures, however, meet with difficulties particularly in sections of lymph node, because variations in cellular density and irregular distribution of mitotic figures in different regions of lymphatic organs complicate the quantitative approach in sections and may, for this reason, cause uncertainty in the results.

In the earlier communications from this laboratory, a simple method for estimating total cellular number in lymphoid organs by means of DNA determination of whole tissue and of each nucleus has been reported, together with some preliminary results (*Monden*, 1955; *Osogoe et al.*, 1957; *Osogoe*, 1958; and *Osogoe and Awaya*, 1958). The present paper makes a more detailed description of the results of measurements on normal adult rats that have been carried out thus far by this method.

MATERIAL AND METHODS

Male albino rats of a subline of the *Wistar* strain, weighing 200 g ($\pm 2\%$), served as the material. They were maintained on a standard laboratory diet¹ and water *ad libitum* supplemented once a week with cabbage or other vegetables. The chief reason why the albino rat was chosen for this work is that its diet and environment can readily be standardized.

* Supported in part by a grant to Professor *Bunsuke Osogoe* from the Ministry of Education (Grant-in-Aid for Fundamental Scientific Research, Cooperative).

† Reproduced from the *Okajimas Folia Anatomica Japonica* Vol. 32, No. 4 (February, 1959) under the permission of the editor.

¹ The diet consisted chiefly of unpolished rice, pressed naked barley and dried small sardines, with a small amount of cod liver oil and minerals (CaCO₃ + NaCl).

For the present type of measurement, it is essential to use animals of constant weight, for with advancing age the lymphoid organs, the thymus in particular, gradually undergo involution. In addition, sex differences in growth rate also

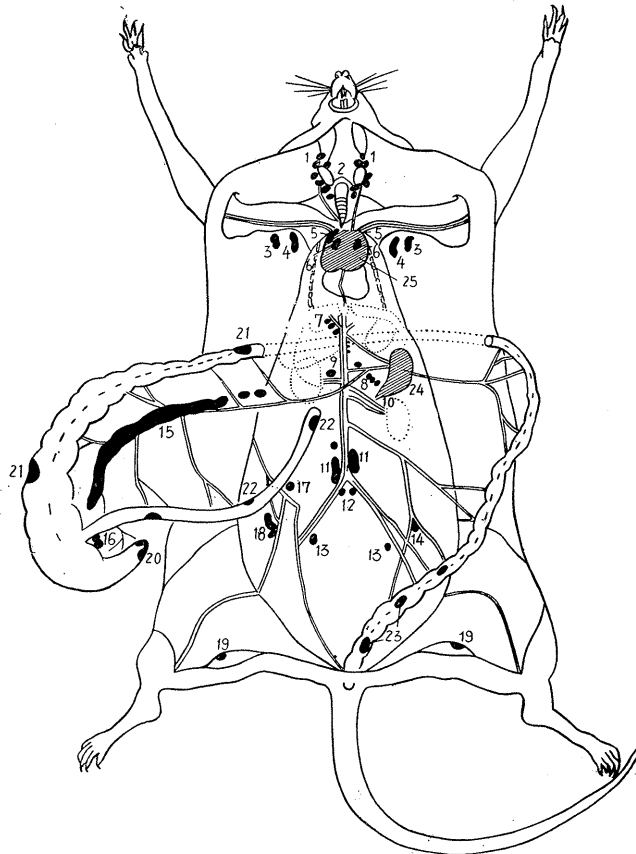


Fig. 1. Thymolympathic organs of the rat.

- | | |
|-----------------------------|--|
| 1. Superficial cervical. | 14. Mesocolic. |
| 2. Deep cervical. | 15. Mesenteric. |
| 3. Superficial axillary. | 16. Cecal. |
| 4. Deep axillary. | 17. Posterior abdominal wall. |
| 5. Superficial mediastinal. | 18. Inguinal. |
| 6. Deep mediastinal. | 19. Popliteal. |
| 7. Portal. | 20. <i>Peyer's</i> patch in the cecum. |
| 8. Postgastric. | 21. Ditto in large intestine. |
| 9. Right renal. | 22. Ditto in small intestine. |
| 10. Left renal. | 23. Ditto in rectum. |
| 11. Para-aortic. | 24. Spleen |
| 12. Sacral. | 25. Thymus. |
| 13. Iliac. | |

The terminology is the same as used in the paper of *Sanders and Florey* (1940)

must be considered. Taking these into account, young adult male rats weighing around 200g were employed as the standard animals to be examined in the present study.

(1) *Determination of the total DNA content in lymphoid organs*

All lymph nodes, thymus, spleen and *Peyer's* patches (Fig. 1) were rapidly taken out from a sacrificed animal and chilled with ice. After weighing the tissues, they were finely minced with a pair of scissors, and homogenized with 9 volume of ice-cold 2% citric acid in a *Potter Elvehjem*-type glass homogenizer and made up to a volume. An appropriate volume of this homogenate was dispensed for the *Schneider* method to determine, in duplicate, the total DNA content of the homogenate using diphenylamine reaction for the test of DNA. A sample of calf thymus DNA (sodium salt; N:P = 1.6; P content: 9.1%) served as the standard.

(2) *Determination of the average DNA content per nucleus in lymphoid organs*

This was made by a simplified procedure of the method employed by *Fukuda* and *Sibatani* (1953a) for determination of the average DNA content of rat liver nuclei. The bulk of the homogenate obtained in (1) was further diluted 5–10 fold and filtered through 8 layers of gauze to remove the fiber and clumps of unbroken cells. The dilution facilitated the sedimentation of nuclei on centrifugation. Then it was centrifuged at very low speed (500 rpm) for 3–4 minutes to sediment the last trace of unbroken cells along with a small amount of nuclei which formed a thin white layer on the top of the sediment. The supernatant (S_0) now contained only isolated nuclei and was quite homogeneous with respect to the distribution of nuclei. Counting of nuclei was made with S_0 . For this purpose duplicate 0.5–1.0 ml samples of S_0 were diluted 5–10 fold with ice-cold 2% citric acid (S_1). Nuclei were counted on S_1 in *Bürker* hemocytometer. Five 0.1 mm³ sections were counted twice and averaged. The final value of the number of nuclei was the mean of the two dilutions S_1 . Two 2 ml aliquots were taken from the original S_0 in separate tubes and centrifuged at 3000 rpm for 10 minutes to sediment all the nuclei. The sediment thus obtained was then analyzed for DNA as in (1). The number of nuclei in 2 ml of S_0 was calculated from S_1 , and the average DNA-P content per nucleus was obtained by dividing the total DNA-P content of S_0 by the corresponding number of nuclei.

(3) *Determination of the percentages of lymphocytes in lymphoid organs*

For this purpose, imprint preparations were made from various lymphoid organs

and stained with *Giemsa*. Because of rather irregular distributions of lymphocytes and other cellular elements in lymphoid organs, differential cell counts were made from different portions of the imprint preparations and averaged. In each slide at least 1000 nucleated cells were counted.

RESULTS AND DISCUSSION

The results of measurements of the mesenteric and other lymph nodes, thymus, spleen and *Peyer's* patches on 10 animals each are listed in Tables 1-5. From these data, the number of total nucleated cells in the individual and whole lymphoid organs can readily be calculated. The figures obtained were as follows: $(763 \pm 121) \times 10^6$ in the mesenteric lymph nodes; $(1,165 \pm 79) \times 10^6$ in the other lymph nodes; $(1,094 \pm 144) \times 10^6$ in the thymus; $(1,375 \pm 189) \times 10^6$ in the spleen; $(420 \pm 33) \times 10^6$ in the *Peyer's* patches; totaling $(4,823 \pm 234) \times 10^6$ in the whole lymphoid organs (Tables 1-6).

The proportions of lymphocytes to total nucleated cells in imprint preparations were found to be $89.7 \pm 2.0\%$ in the mesenteric lymph nodes, $88.5 \pm 2.3\%$ in the other lymph nodes, $97.9 \pm 0.4\%$ in the thymus, $80.7 \pm 1.5\%$ in the spleen, and $91.0 \pm 1.0\%$ in the *Peyer's* patches (Table 7).

Using the data noted above, the number of total lymphocytes in the individual and whole lymphoid organs was then computed. The computations showed that total lymphocytes numbered $(684 \pm 123) \times 10^6$ in the mesenteric lymph nodes, $(1,031 \pm 106) \times 10^6$ in the other lymph nodes, $(1,071 \pm 145) \times 10^6$ in the

Table 1. Total nucleated cell counts in the mesenteric lymph nodes.

Rat. No.	Weight of Organ (mg)	DNA-P Content per Organ (mg)	DNA-P Content per Nucleus ($\times 10^{-3}$ mg)	Total Nucleated Cells per Organ ($\times 10^6$)	Total Nucleated Cells per mm ³ of Tissue ($\times 10^6$)
1	380	0.37	0.70	528	1.39
2	280	0.34	0.62	548	1.96
3	630	0.80	0.58	1,383	2.20
4	490	0.54	0.59	915	1.87
5	290	0.30	0.92	326	1.12
6	380	0.65	0.72	902	2.37
7	400	0.55	0.52	1,057	2.64
8	400	0.54	0.83	650	1.63
9	350	0.58	0.79	734	2.10
10	300	0.47	0.80	587	1.96
Mean \pm S.E.*	390 \pm 33	0.51 \pm 0.05	0.71 \pm 0.04	763 \pm 121	1.92 \pm 0.15
σ	103	0.16	0.13	382	0.47
V	26.4%	31.4%	18.3%	50.1%	24.2%

* S. E. = Standard error.

Table 2. Total nucleated cell counts in the lymph nodes other than mesenteric lymph nodes.

Rat No.	Weight of Organ (mg)	DNA-P Content per Organ (mg)	DNA-P Content per Nucleus ($\times 10^{-9}$ mg)	Total Nucleated Cells per Organ ($\times 10^6$)	Total Nucleated Cells per mm^3 of Tissue ($\times 10^6$)
1	870	0.75	0.70	1,071	1.23
2	1,210	0.94	0.62	1,516	1.25
3	1,140	0.64	0.58	1,103	0.97
4	1,030	0.83	0.59	1,406	1.36
5	1,040	0.94	0.92	1,021	0.98
6	860	0.80	0.72	1,111	1.29
7	1,180	0.65	0.52	1,250	1.05
8	1,010	0.67	0.83	807	0.80
9	870	0.87	0.79	1,101	1.27
10	1,010	1.01	0.80	1,262	1.25
Mean \pm S.E.	1,020 \pm 45	0.81 \pm 0.04	0.71 \pm 0.04	1,165 \pm 79	1.15 \pm 0.06
σ	142	0.13	0.13	250	0.48
V	13.9%	16.0%	13.8%	21.4%	41.6%

Table 3. Total nucleated cell counts in the thymus.

Rat No.	Weight of Organ (mg)	DNA-P Content per Organ (mg)	DNA-P Content per Nucleus ($\times 10^{-9}$)	Total Nucleated Cells per Organ ($\times 10^6$)	Total Nucleated Cells per mm^3 of Tissue ($\times 10^6$)
1	410	0.63	0.83	760	1.84
2	380	0.51	0.89	571	1.50
3	360	0.75	0.54	1,390	3.85
4	300	0.91	0.85	1,070	3.57
5	370	0.82	0.75	1,093	2.95
6	470	0.55	0.62	887	1.89
7	430	1.08	0.88	1,227	2.85
8	450	1.17	0.72	1,625	3.61
9	450	0.87	0.68	1,280	2.85
10	550	0.69	0.66	1,045	1.90
Mean \pm S.E.	417 \pm 18	0.80 \pm 0.04	0.74 \pm 0.04	1,094 \pm 144	2.68 \pm 0.27
σ	57	0.13	0.13	455	0.85
V	13.8%	16.2%	17.6%	41.6%	31.2%

Table 4. Total nucleated cell counts in the spleen.

Rat No.	Weight of Organ (mg)	DNA-P Content per Organ (mg)	DNA-P Content per Nucleus ($\times 10^{-9}$ mg)	Total Nucleated Cells per Organ ($\times 10^6$)	Total Nucleated Cells per mm^3 of Tissue ($\times 10^6$)
1	1,050	0.93	0.83	1,120	1.07
2	1,580	1.58	0.53	2,981	1.89
3	750	0.84	0.80	1,050	1.40
4	850	0.90	0.84	1,072	1.26
5	600	0.92	0.82	1,121	1.86
6	890	1.41	0.85	1,658	1.86
7	1,200	0.94	0.80	1,175	0.98
8	600	0.92	0.76	1,210	2.02
9	1,030	0.80	0.79	1,012	0.97
10	850	0.91	0.67	1,350	1.60
Mean \pm S.E.	940 \pm 93	1.02 \pm 0.08	0.77 \pm 0.04	1,375 \pm 189	1.49 \pm 0.13
σ	294	0.25	0.13	597	0.41
V	31.2%	24.5%	16.8%	43.4%	27.5%

Table 5. Total nucleated cell counts in the Peyer's patches.

Rat No.	Weight of Organ (mg)	DNA-P Content per Organ (mg)	DNA-P Content per Nucleus ($\times 10^{-9}$ mg)	Total Nucleated Cells per Organ ($\times 10^6$)	Total Nucleated Cells per mm^3 of Tissue ($\times 10^6$)
1	220	0.28	0.58	483	2.19
2	220	0.28	0.53	528	2.40
3	200	0.19	0.75	253	1.27
4	250	0.26	0.78	333	1.33
5	280	0.31	0.59	525	1.88
6	400	0.42	0.84	500	1.25
7	340	0.29	0.59	492	1.45
8	370	0.21	0.74	284	0.77
9	300	0.28	0.66	424	1.41
10	220	0.28	0.72	388	1.76
Mean \pm S.E.	280 \pm 25	0.28 \pm 0.02	0.68 \pm 0.03	420 \pm 33	1.57 \pm 0.16
σ	73	0.06	0.10	104	0.51
V	26.9%	21.4%	14.7%	24.8%	32.5%

Table 6. Total weight and total number of nucleated cells of the whole lymphoid organs of normal rats weighing around 200 g.

Rat No.	Body Weight (g)	Total Weight of Lymphoid Organs (g)	Total Nucleated Cells ($\times 10^6$)
1	200	2.93	3,962
2	203	3.67	6,154
3	202	3.08	5,187
4	202	2.92	4,792
5	202	2.58	4,125
6	201	3.00	5,055
7	205	3.55	5,201
8	199	2.83	4,566
9	203	3.00	4,557
10	200	2.93	4,630
Mean \pm S. E.	201 \pm 0.6	3.049 \pm 0.103	4,823 \pm 234
σ	1.9	0.326	739
V	0.9%	10.6%	15.3%

Table 7. Percentages of lymphocytes in smears from different lymphoid organs of normal male rats weighing around 200g. Mean of 10 animals. Mean \pm S. E.

Cell Type	Lymph Nodes		Thymus	Spleen	<i>Peyer's</i> Patches
	Mesenteric Nodes	Other Nodes			
Lymphocytes	89.7 \pm 2.0	88.5 \pm 2.3	97.9 \pm 0.4	80.7 \pm 1.5	91.0 \pm 1.0
Non-lymphocytic Cells					
Plasma Cells	9.5 \pm 1.9	10.7 \pm 2.0	—	0.7 \pm 0.1	—
Reticuloendothels	0.6 \pm 0.2	0.5 \pm 0.1	1.8 \pm 0.4	1.8 \pm 0.3	8.0 \pm 0.5
Neutrophils	0.2 \pm 0.05	0.2 \pm 0.02	0.2 \pm 0.09	4.6 \pm 0.8	0.9 \pm 0.1
Eosinophils	0.1 \pm 0.02	0.1 \pm 0.04	0.1 \pm 0.05	1.2 \pm 0.1	0.1 \pm 0.03
Monocytes	—	—	—	0.3 \pm 0.1	—
Erythroblasts	—	—	—	10.6 \pm 2.0	—
Megakaryocytes	—	—	—	0.1 \pm 0.04	—

thymus, $(1,100 \pm 169) \times 10^6$ in the spleen, $(382 \pm 34) \times 10^6$ in the *Peyer's* patches, amounting to $(4,268 \pm 577)$ in the whole lymphoid organs² (Table 8).

² In the earlier communications (Monden, 1955; Osogoe et al., 1955; and Osogoe, 1958), the corresponding figure was computed to be $(4,120 \pm 601) \times 10^6$. A slight increase in the figure is due to increase in the number of observations and to more accurate calculations.

Table 8. Total number of lymphocytes present in the chief thymolymphatic organs of normal male rats weighing around 200g. Mean \pm S. E.

Organ	Total Number of Nucleated Cells $\times 10^6$	Percentage of Lymphocytes	Total Number of Lymphocytes
Mesenteric Lymph Nodes	763 \pm 121	89.7 \pm 2.0	684 \pm 123
Other Lymph Nodes	1,165 \pm 79	88.5 \pm 2.3	1,031 \pm 106
Thymus	1,094 \pm 114	97.9 \pm 0.4	1,071 \pm 145
Spleen	1,375 \pm 189	89.7 \pm 1.5	1,100 \pm 169
<i>Peyer's</i> Patches	429 \pm 33	91.0 \pm 1.0	383 \pm 34
Total Thymolymphatic Organs	4,823 \pm 234*		4,268 \pm 577

* Calculated from the figures for total nucleated cells present in the chief thymolymphatic organs of individual animals and not from the data given above in the same column (cf. Table VI).

Since the average number of total lymphocytes in the circulating blood has been found to be $(132 \pm 12.0) \times 10^6$ from a study of 50 normal male rats weighing around 200g (Monden, 1958), the ratio of this value to the number of total lymphocytes in the thymolymphatic system is calculated to be about one to thirty-two³. This indicates that the lymphocyte reserve in the thymolymphatic system is 32 times as great as the number of lymphocytes present in the blood.

The cellular density in lymphoid organs, that is, the number of nucleated cells per unit volume of the tissue, was then computed from the weight of organ and number of total nucleated cells per organ, assuming the specific gravity of the lymphoid tissue being approximately 1.00. The values obtained for total nucleated cells per 1mm^3 of tissue were as follows: $(1.92 \pm 0.15) \times 10^5$ in the mesenteric lymph nodes; $(1.15 \pm 0.06) \times 10^6$ in the other lymph nodes; $(2.68 \pm 0.27) \times 10^6$ in the thymus; $(1.49 \pm 0.13) \times 10^6$ in the spleen; and $(1.57 \pm 0.16) \times 10^6$ in the *Peyer's* patches (Tables 1-5). As previously expected, the cellular density is highest in the thymus and it decreases in the following order: mesenteric nodes, *Peyer's* patches, spleen, lymph nodes other than the mesenteric nodes.

It should be emphasized here that all of the numerical analyses made in the present study showed relatively small variations in the values obtained. As seen in Tables 1-6, the coefficient of variation in each measurement did not exceed 50% and lies in most instances between 16 and 43%. It is of interest to note that the values found for total lot of lymphoid organs showed less variations than those obtained for the individual lymphoid organs (cf. Tables 1-5 and Table 6).

It is generally believed that the amount of lymphoid tissue is subject to con-

³ In the earlier communications (Monden, 1955; Osogoe et al., 1957; and Csogoe, 1958), the corresponding ratio was estimated to be 1 : 36.

siderable individual variation even in the animals which are of the same weight or age class and are normal according to ordinary laboratory standards. In his extensive quantitative studies of the lymphoid organ by chemical determination of the nuclear substance (nucleic acid phosphorus), *Andreasen* (1943) demonstrated that, in adult rats, the coefficient of variation in the amount of lymphoid tissue was 44% for the total lymph nodes, whereas the corresponding values for the individual lymphoid organs greatly varied between 43 and 341%. The value for the thymus was 66%. (The corresponding figure is 31.2% in our material.)

That the variability of our material with regard to the amount of lymphoid tissue is generally much less than that of *Andreasen* is of particular interest. It must be borne in mind, however, that our material is too small in number to draw definite conclusions as regards the variability of the lymphoid organs. In order to gain further information on this point, an extensive study is now being made by *Sasaki* in our laboratory. The results obtained so far seem to confirm the present observations (*Sasaki*, unpublished observations).

The present method of evaluating total cellular number in the tissue is based principally on the constancy of the DNA content per nucleus in the diploid nuclei. The degree of accuracy or reliability of this method, therefore, depends upon whether the estimated values for the DNA content per nucleus are reasonable or not. *Thomson et al.* (1953), who made a number of accurate measurements on various tissues of rats weighing from 195 to 250g, claim that the average DNA-P content per nucleus from the tissues, such as spleen, kidney, pancreas, small intestine, leukocytes, bone marrow, thymus, heart, lung and salivary glands, lies between 0.65×10^{-9} mg and 0.70×10^{-9} mg. As regards the DNA-P content per nucleus in the rat liver, however, divergent values are reported in the literature as follows: 0.60×10^{-9} mg by *Cunningham et al.* (1951); 0.80×10^{-9} mg by *Mirsky and Ris* (1951); 0.86×10^{-9} mg by *Vendrely, R. and C. Vendrely* (1949, 1952); 0.90×10^{-9} mg by *Thomson et al.* (1953); and 1.01×10^{-9} mg by *Harrison* (1951). Of particular interest in this connection is the work of *Fukuda and Sibatani* (1953b), who have demonstrated that during the earlier course of the postnatal growth of the rat, the average DNA content of liver nuclei shows a certain age variation, and that this value is determined by the maximum body weight which the animals have attained irrespective of growth rate or nutritional conditions. This is one of the main reasons why the animals of constant weight must be used in the present type of measurements.

From the above considerations, it is expected that the values determined for average DNA-P content per nucleus in the different lymphoid tissue of rats of approximately constant weight would agree fairly well among each other. As a matter of fact, no significant difference are to be seen among the following figures obtained: $(0.71 \pm 0.04) \times 10^{-9}$ mg in the whole lymph nodes; $(0.74 \pm 0.04) \times 10^{-9}$ mg in the thymus; $(0.77 \pm 0.04) \times 10^{-9}$ in the spleen; and $(0.68 \pm 0.03) \times$

10^{-9} mg in the *Peyer's* patches (Tables 1-5). Furthermore, these values are in good agreement with the figures of *Thomson* et al. (1953), who claim that the DNA-P content per nucleus lies between 0.65×10^{-9} mg. and 0.70×10^{-9} mg in the rat tissues as already cited. Therefore, the values obtained in the present study for the DNA-P content per nucleus in the lymphoid organs may all be considered to be quite reasonable.

In the present study, the calculated figures for total lymphocytes in the individual as well as in the whole lymphoid organs are slightly greater than the corresponding figures reported in the preliminary communications (*Monden*, 1955; *Osogoe* et al., 1957; *Osogoe*, 1958a), owing to increase in the number of observations and to more accurate calculations. Hence, it seems desirable to make renewed calculations on the daily production of lymphocytes by mitosis in the thymolymphatic organs based on the present data. For the daily mitotic rates in the individual lymphoid organs the figures of *Ito* reported in the preliminary papers (*Osogoe* et al., 1957; *Osogoe*, 1958; and *Osogoe* and *Awaya*, 1958) were used, and the computations were made on the similar assumption as in the earlier studies that the mitotic rate is the same for both the lymphocytic and the non-lymphocytic elements (Table 9). The calculations showed that the number of

Table 9. Average number of lymphocyte mitoses per day in lymphoid organs of normal male rats weighing around 200g. Mean \pm S. E.

Organ	Total Number of Lymphocytes ($\times 10^6$)	Total Percentage of Mitoses per Day*	Number of Mitoses per Day ($\times 10^6$)
Thymus	1,071 \pm 145	22.23 \pm 1.47	238 \pm 47.6
Mesenteric Lymph Nodes	684 \pm 123	7.85 \pm 0.70	54 \pm 14.5
Other Lymph Nodes	1,031 \pm 106	6.65 \pm 0.51	69 \pm 12.4
Spleen	1,100 \pm 169	6.65 \pm 0.51**	73 \pm 11.8
<i>Peyer's</i> Patches	382 \pm 342	6.65 \pm 0.51**	26 \pm 2.5
Total Lymphoid Organs	4,268 \pm 577		460 \pm 88.8 (361 \pm 74.5***)

* The data from the observations of *Ito* reported in the preliminary communications (*Osogoe* et al., 1957; *Osogoe*, 1958; and *Osogoe* and *Awaya*, 1958).

** The daily mitotic rate in the spleen as well as in the *Peyer's* patches is assumed to be equal to that observed in the lymph nodes other than the mesenteric nodes.

*** The figures when the spleen and *Peyer's* patches are excluded.

mitoses per day amounted to $(238 \pm 47.6) \times 10^6$ in thymus, $(54 \pm 14.5) \times 10^6$ in the mesenteric lymph nodes, $(69 \pm 12.4) \times 10^6$ in the other lymph nodes, $(73 \pm 11.8) \times 10^6$ in the spleen and $(26 \pm 2.5) \times 10^6$ in the *Peyer's* patches, totaling $(460 \pm 88.8) \times 10^6$ in the whole lymphoid organs. Here, the daily mitotic rate of lymphocytes in the spleen and *Peyer's* patches, in which no mitotic counts has been made, was

assumed to be equal to that observed in the lymph nodes other than the mesenteric nodes.

The number of lymphocytes needed for daily growth of the lymphoid tissue was roughly estimated to total 26.6×10^6 in the whole lymphoid organs, assuming the rate of growth of the tissue to be 0.624% according to *Kindred* (1942). Subtracting this figure from the number of total lymphocyte mitoses per day in the whole lymphoid organs, the average number of excess mitoses per day in the whole thymolymphatic organs was found to be 433×10^6 . This is 3.3 times as great as the number of lymphocytes present in the circulating blood⁴.

If we assume that all excess lymphocytes newly produced by mitosis in the lymphoid organs enter into the general circulation, the turnover or renewal time of the lymphocyte in the circulating blood may be estimated to be 0.30 day or 7.2 hours and the "Daily Replacement Factor" (D.R.F.) of *Yoffey* (1954) for the blood lymphocyte to be 3.3. In the earlier studies (*Osogoe et al.*, 1957 and *Osogoe*, 1958), in which the spleen and *Peyer's* patches were not included, the D.R.F. was estimated to be 2.87 or 2.6.

SUMMARY

1. In a series of 10 male albino rats of the *Wistar* strain weighing approximately 200 g each, total cellular number and cellular density in the thymolymphatic organs were estimated chemically by determining DNA-P content of the whole tissue and of each nucleus, using the *Schneider* method and diphenylamine test.

2. The values obtained for total nucleated cells in the lymphoid organs were as follows: $(763 \pm 121) \times 10^6$ in the mesenteric lymph nodes; $(1,165 \pm 79) \times 10^6$ in the other lymph nodes; $(1,094 \pm 144) \times 10^6$ in the thymus; $(1,375 \pm 189) \times 10^6$ in the spleen; $(420 \pm 33) \times 10^6$ in the *Peyer's* patches; and $(4,823 \pm 234) \times 10^6$ in the whole thymolymphatic organs.

3. The proportion of lymphocytes to total nucleated cells in imprint preparations was found to be: $89.7 \pm 2.0\%$ in the mesenteric lymph nodes; $88.5 \pm 2.3\%$ in the other lymph nodes; $97.9 \pm 0.4\%$ in the thymus; $80.7 \pm 1.5\%$ in the spleen; and $91.0 \pm 1.0\%$ in the *Peyer's* patches.

4. The number of total lymphocytes was calculated to be: $(684 \pm 123) \times 10^6$ in the mesenteric lymph nodes; $(1,071 \pm 45) \times 10^6$ in the thymus; $(1,100 \pm 169) \times 10^6$ in the spleen; $(382 \pm 34) \times 10^6$ in the *Peyer's* patches; and $(4,268 \pm 577) \times 10^6$ in the whole thymolymphatic organs. It was estimated that the lymphocyte reserve in the whole thymolymphatic organs is 32 times as great as the number of

⁴ For this calculation an estimate of $(132 \pm 12.0) \times 10^6$ was used for the total number of lymphocytes present in the circulating blood (cf. *Monden*, 1958).

lymphocytes present in the circulating blood.

5. The estimated figures for total nucleated cells per 1 mm^3 of tissue (cellular density) were as follows: $(1.92 \pm 0.15) \times 10^6$ in the mesenteric lymph nodes; $(1.15 \pm 0.06) \times 10^6$ in the other lymph nodes; $(2.68 \pm 0.27) \times 10^6$ in the thymus; $(1.49 \pm 0.13) \times 10^6$ in the spleen; and (1.57 ± 0.15) in the Peyer's patches. Thus, the cellular density is highest in the thymus and it decreases in the following order: mesenteric nodes, Peyer's patches, spleen, lymph nodes other than the mesenteric nodes.

6. On the basis of the figures obtained, a renewed calculation was made of the daily rate of production of lymphocytes by mitosis in the whole thymolymphatic organs.

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