

Quantitative Studies on the Rate of Cell Production in the Thymolymphatic Organs

II. Estimation of the Daily mitotic Activity in Young Adult Albino Rats*†

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In the preceding paper of this series a simple method for determination of the mitotic index in suspension of cell nuclei, which had been prepared from the thymolymphatic organs, was described. The present work is an attempt to estimate the daily rate of mitotic activity in the thymolymphatic system of young adult albino rats by this method and by using the drug, colchicine, which, in proper dosage, arrests dividing cells at metaphase. Its final aim is to make possible a calculation of the total number of cells newly produced by mitosis per day in the thymolymphatic organs, using the results of estimation of total cellular numbers in these organs by means of DNA determination (MONDEN, 1958).

MATERIAL AND METHODS

Male albino rats of a subline of the WISTAR strain, weighing approximately 200 g, were chosen as the standard animals to be examined, as in the earlier studies (MONDEN, 1955; 1958a, b; and Ito, 1959). They were fed on a standard laboratory diet, which consisted chiefly of unpolished rice, pressed barley and dried small sardine with a small amount of cod liver oil and minerals, supplemented once a week with cabbage and other vegetables.

The animals were divided into three groups. The first group consisted of 21 rats which served as controls. The second group, consisting of 27 rats, was used for a preliminary experiment to test the possibility of assessing the mitotic activity over a period of 2 to 8 hours after injection of colchicine. In the third group, which also comprised 27 rats, an attempt was made to assess the daily rate of mitosis, according

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to the technique of BERTALANFFY and LEBLOND (1953), as will be mentioned below. All rats had been starved since the previous evening.

The animals of the first group were further divided into three subgroups (Controls I-III). Control I consisted of 7 normal untreated rats, Control II 5 adrenalectomized rats which were examined immediately after operation, and Control III 9 adrenalectomized rats which were examined 4-8 hours after operation (cf. Table 1). The animals of Controls I and II were killed at about 10:00 A. M. and those of Control III between 2:00 P. M. and 6:00 P. M. Each rat of the second group was given a subcutaneous injection of 0.10 mg of colchicine per 100g of body weight at 10:00 A.M. The animals of this group were killed at successive intervals from 2 to 8 hours after injection (cf. Table 2). The rats of the third group were further divided into 4 subgroups consisting of 7 animals each. Each rat was also given a subcutaneous injection of colchicine in the same dosage as in the second group. The rats of the first subgroup received the injection at 10:00 A.M., those of the second at 4:00 P.M., those of the third at 10:00 P.M. on the same day, and finally those of the fourth subgroup at 4:00 A.M. on the next day. All animals were killed 6 hours after injection (cf. Table 3). In this way the same experiment was repeated over 4 successive 6-hour periods, thus covering a total of 24 hours. This method was adopted because some diurnal variations were to be seen in the mitotic activity in the thymolymphatic organs.

Immediately after the animals had been killed by cervical dislocation and bleeding, the thymolymphatic organs to be examined (the thymus, mesenteric lymph nodes and other lymph nodes*) were removed and weighed. The greater part of each of the excised organs was used for mitotic counts in suspension of nuclei and the remaining small portion for histological examination after fixing in ZENKER-formol.

The methods used for preparation of suspensions of nuclei from the thymolymphatic organs and for mitotic counts in this suspension have been described in detail in the preceding paper (ITO, 1959). Since colchicine produces the typical phenomena of acute stress, i.e., extensive destruction of proliferating lymphocytes in the lymphatic nodules and other parts of the lymphoid tissues, the adrenal glands of the animals had been removed immediately before colchicine injection. Adrenalectomy was also performed on 14 animals of the control group (Controls II-III), with the aim to disclose the effect of the adrenalectomy itself on the mitotic activity in the thymolymphatic organs.

RESULTS

The results of mitotic counts on 7 normal untreated rats (Control I) are given in

* The spleen and Peyer's patches were not included in the present study.

Table 1, together with those on 5 rats immediately after adrenalectomy and with those on 9 rats from 4 to 8 hours after adrenalectomy. All figures in the table are expressed in percentage of the total nucleated cells counted. Normal values of the mitotic index in young adult albino rats were found to be $1.13 \pm 0.06\%$ in the thymus, $0.79 \pm 0.06\%$ in the mesenteric lymph node, and $0.75 \pm 0.06\%$ in the other lymph nodes. It should be noticed here that the values of the mitotic index were much greater in the thymus than in the lymph nodes.

Table 1. Values of the mitotic index expressed in percentage of the total nucleated cells in the thymus, mesenteric and other lymph nodes of normal and adrenalectomized rats without colchicine treatment (Controls I-III).

No. of rats examined	Thymus*			Mesenteric lymph nodes			Other lymph nodes		
	M \pm m	σ	V	M \pm m	σ	V	M \pm m	σ	V
Control I (normal rats without operation)									
7	1.13 \pm 0.06	0.16	14.1	0.79 \pm 0.06	0.16	20.2	0.75 \pm 0.06	0.16	21.3
Control II (immediately after adrenalectomy)									
5	1.16 \pm 0.19	0.42	36.2	0.79 \pm 0.04	0.09	11.4	0.72 \pm 0.03	0.07	9.7
Control III (4-8 hours after adrenalectomy)									
9	0.74 \pm 0.07	0.21	28.4	0.54 \pm 0.05	0.15	27.8	0.53 \pm 0.06	0.18	34.0

* M \pm m: mean \pm standard error; σ : standard deviation; V: coefficient of variation.

Table 2. Values of the mitotic index expressed in percentage of the total nucleated cells in the thymus, mesenteric and other lymph nodes of adrenalectomized rats at various time intervals after colchicine injection.

Time after colchicine injection	No. of rats examined	Thymus			Mesenteric lymph nodes			Other lymph nodes		
		M \pm m	σ	V	M \pm m	σ	V	M \pm m	σ	V
2 hours	7	2.32 \pm 0.06	0.16	6.9	1.66 \pm 0.19	0.50	30.1	1.63 \pm 0.17	0.45	27.0
4 hours	7	3.52 \pm 0.29	0.77	21.9	2.03 \pm 0.25	0.66	32.5	1.75 \pm 0.09	0.24	13.4
6 hours	7	5.50 \pm 0.42	1.11	20.2	2.50 \pm 0.16	0.69	27.6	2.12 \pm 0.10	0.26	12.3
8 hours	7	7.71 \pm 0.25	0.66	8.6	3.03 \pm 0.10	0.26	8.6	2.66 \pm 0.17	0.45	16.9

As regards the effect of the adrenalectomy itself on the mitotic activity in the thymolymphatic organs, a considerable reduction in the values of the mitotic index was observed from 4 to 8 hours after operation. This must be taken into consideration in the discussion of the results to be presented below.

After injection of colchicine in a dose of 0.10 mg per 100 g of body weight into adrenalectomized rats immediately after operation, there was an almost linear increase in the percentage of mitoses in the thymus, mesenteric lymph nodes and other lymph nodes until the 8th hour (cf. Fig. 1 and Table 2). It is important to emphasize here that the rate of increase in the number of mitoses after colchicine injection

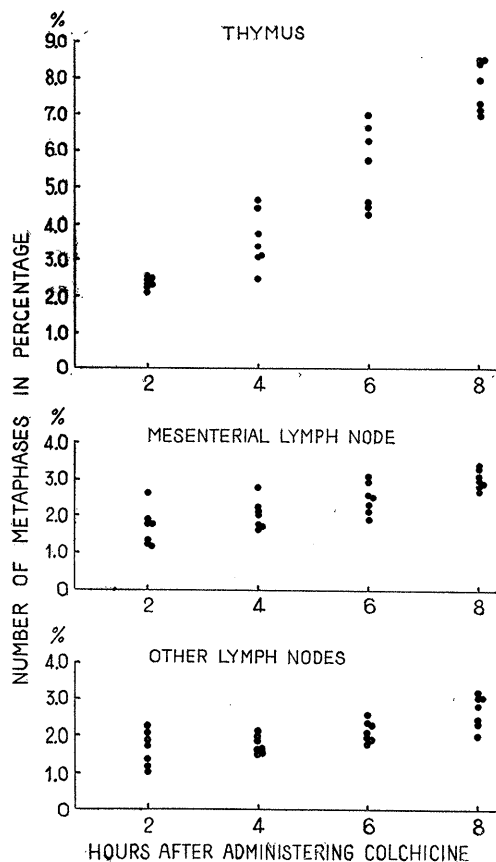


Fig. 1. Number of arrested metaphases (expressed in percentage of the total nucleated cells counted) in the thymus, mesenteric lymph nodes and other lymph nodes at various time intervals after colchicine administration. Note that the number of metaphases continues to increase for 8 hours after injection.

was much greater in the thymus than in the lymph nodes.

Since some diurnal variations were to be seen in the mitotic activity in the thymolymphatic organs, the total percentage of mitoses per day in each organ was computed by adding the mean values which were obtained at intervals of 6 hours from seven samples each, as shown in Table 3. The colchicine-treatment for a 6-hour period was adopted here because many of the metaphases arrested by colchicine began to degenerate by nuclear pyknosis and fragmentation from the 6th or 8th hour on (Fig. 2). In this way the daily mitotic rate in each organ was found to be $22.23 \pm 1.47\%$ in the thymus, $7.85 \pm 0.70\%$ in the mesenteric lymph nodes, and $6.65 \pm 0.51\%$ in the other lymph nodes.

In regard to diurnal variations in the mitotic activity in the thymolymphatic

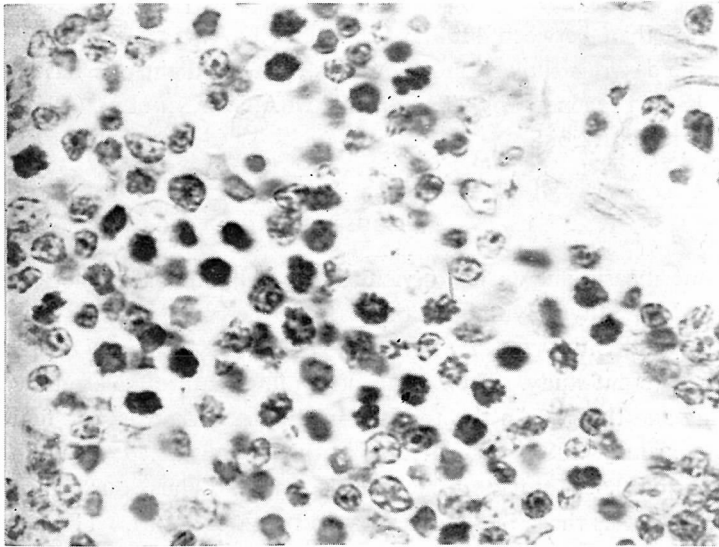


Fig. 2. Accumulation of numerous mitotic figures arrested at metaphase in the cortex of the thymus 8 hours after injection of colchicine. Many of the arrested metaphases are degenerating by nuclear pyknosis and a few of them by nuclear fragmentation. $\times 1000$.

Table 3. Diurnal variation in values of the mitotic index expressed in percentage of to the total nucleated cells for a 6-hour period, and the total percentage of mitoses per day in lymphoid organs.

Group of experiment	Number of animals	Thymus			Mesenteric lymph nodes			Other lymph nodes		
		M \pm m	σ	V	M \pm m	σ	V	M \pm m	σ	V
I (10:00 A.M.— 4:00 P.M.)	7	5.50 \pm 0.42	1.11	20.2	2.50 \pm 0.16	0.42	16.8	2.12 \pm 0.10	0.26	12.3
II (4:00 P.M.—10:00 P.M.)	7	5.06 \pm 0.40	1.06	21.0	1.64 \pm 0.10	0.26	15.9	1.59 \pm 0.16	0.42	26.4
III (10:00 P.M.— 4:00 A.M.)	7	5.06 \pm 0.48	1.27	25.1	1.67 \pm 0.26	0.69	41.3	1.27 \pm 0.13	0.34	26.8
IV (4:00 A.M.—10:00 A.M.)	7	6.61 \pm 0.17	0.45	6.82	2.04 \pm 0.18	0.48	23.5	1.67 \pm 0.12	0.32	19.2
Total per 24 hours	—	22.23 \pm 0.77	—	—	7.85 \pm 0.37	—	—	6.65 \pm 0.26	—	—

Table 4. Values of the mitotic index* in different regions of the sections from the thymus and mesenteric lymph nodes (unpublished observations of KAWAMURA).

Time after Colchicine injection	Thymus			Mesenteric lymph nodes			
	Superficial cortex	Deep cortex	Medulla	Germinal center	Dark-staining mantle	Internodular region	Medullary cords
2 hours	2.29	2.16	0.45	4.35	0.62	1.06	0.96
4 hours	4.11	3.03	1.27	6.95	0.78	1.36	1.93
6 hours	7.73	4.75	2.32	8.40	0.81	1.35	2.75
8 hours	6.49	4.55	1.85	5.64	0.58	1.82	2.54

* Expressed in percentage of the total nucleated cells.

organs, greater mitotic activity was observed during the period between 4:00 A.M. and 4:00 P.M. than between 4:00 P.M. and 4:00 A.M. (cf. Table 3). A similar finding that the day-rats showed more mitoses than the night-rats has been reported by JAFFE (1954) in regenerating liver and by MÜHLEMANN et al. (1956) in the oral epithelium and the thyroid.

DISCUSSION

The present measurements have yielded generally much higher values of the mitotic index in the thymolymphatic tissues than the corresponding figures reported by ANDREASEN and CHRISTENSEN (1949) who employed essentially the same methods as used in the present study. Their figures in young mature male rats were 0.216% in the thymus and 0.058% in the total lymph nodes; whereas our figures in male rats weighing around 200 g were 1.13% in the thymus, 0.79% in the mesenteric lymph nodes, and 0.65% in the other lymph nodes. Thus, our figures are about 5-13 times as great as those of ANDREASEN and CHRISTENSEN. The reason for this discrepancy is not apparent. However, it is probable that strain differences and variations in diet are partly responsible. In addition, it should be taken into account that the method for mitotic counts employed in the present study tends to yield higher values of the mitotic index than a similar method of CHRISTENSEN (1950), as has previously been emphasized by ITO (1959).

Of particular interest in this connection are the values of the mitotic index determined in sections from the different regions of the thymus and mesenteric lymph nodes in young mature albino rats of the same strain as used in the present study. As shown in Table 4, the mitotic activity is unexpectedly high in certain regions of the thymolymphatic organs, notably in the germinal centers of the lymph node and in the cortex of the thymus. In his extensive quantitative studies on the thymolymphatic tissues of young adult albino rats on the basis of mitotic counts in tissue sections, KINDRED (1942, 1955) also reported relatively high values comparable to our figures. From the above consideration it can be stated that the relative high values of the mitotic index obtained in the present study for the thymolymphatic tissues is not quite unreasonable.

Another point of interest is that in young mature albino rats the mitotic activity in the thymus was definitely greater than that in the lymph node. A similar finding has previously been reported by KINDRED (1942, 1955), ANDREASEN and OTTESEN (1945), and ANDREASEN and CHRISTENSEN (1949). In view of the morphological and physiological identity of the thymocyte with the lymphocyte, these authors considered the thymus of the growing rat to be the most active lymphocytopoietic organ. The present author also agrees with this view and regards the thymus as being one of the most important seats of lymphocyte production.

The principal objective of the present study is to estimate the daily rate of mitotic

activity in the thymolymphatic organs by the colchicine method, using the results of estimation of total cellular numbers in these organs by means of DNA determination (MONDEN, 1958 a). It was found that the daily mitotic rate was as great as $22.23 \pm 0.77\%$ in the thymus, $7.85 \pm 0.37\%$ in the mesenteric lymph nodes, and $6.65 \pm 0.26\%$ in the other lymph nodes, as shown in Table 3. The figures for the total number of lymphocytes in the thymolymphatic organs of standard rats have been estimated by MONDEN (1958 a) to be $(1,071 \pm 145) \times 10^6$ in the thymus, $(684 \pm 123) \times 10^6$ in the mesenteric lymph nodes, $(1,031 \pm 106) \times 10^6$ in the other lymph nodes, $(1,100 \pm 169) \times 10^6$ in the spleen, and $(382 \pm 34) \times 10^6$ in the PEYER's patches; and $(4,268 \pm 259) \times 10^6$ in the whole thymolymphatic organs. If we assume that the mitotic rate is the same for both the lymphocytic and the non-lymphocytic elements, and that the daily mitotic rate in the spleen and PEYER's patches is equal to that in the lymph nodes other than the mesenteric lymph nodes, the number of lymphocyte mitoses per day in the thymolymphatic organs may easily be calculated. The figures obtained were as follows: $(238 \pm 33.3) \times 10^6$ in the thymus; $(54 \pm 9.9) \times 10^6$ in the mesenteric lymph nodes; $(69 \pm 75) \times 10^6$ in the other lymph nodes; $(73 \pm 11.5) \times 10^6$ in the spleen; $(26 \pm 1.8) \times 10^6$ in the PEYER's patches; totaling $(460 \pm 37.4) \times 10^6$ in the whole thymolymphatic organs.

The number of lymphocytes needed for daily growth of the thymolymphatic organs was roughly estimated to total 26.6×10^6 in the whole lymphoid organs, assuming the rate of growth of the tissue to be 0.26% per day according to KINDRED (1942). Subtracting this figure from the number of total lymphocyte mitoses per day in the whole thymolymphatic organs, the average number of excess mitoses of lymphocytes per day in the thymolymphatic system was found to be 432×10^6 . This is 3.3 times as great as the number of lymphocytes present in the circulating blood. 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 b).

The data on the daily mitotic rate in the thymolymphatic organs given in Table 3 allows a direct calculation of the turnover time of the lymphocytes in these organs. Here, the term "turnover time" denotes the time taken for the replacement of a number of cells equal to that in the whole population (LEBLOND and WALKER, 1956). Since in the thymus the daily mitotic rate was estimated to be 22.23% on the average, the turnover time of the lymphocytes in this organ is then calculated to be 4.5 days, dividing 100 by 22.23. Likewise, the corresponding figures in lymph nodes are calculated to be 12.8 days in the mesenteric nodes and 15.0 days in the other nodes.

An indirect measurement of turnover time is also possible on the basis of the data obtained in the present study. If the duration time of mitosis or mitotic time (MT) and the mitotic index (MI) are known, the intermitotic time (IT) of tissue can be calculated from HOFFMAN's formula (HOFFMAN, 1947, 1953):

$$IT = MT/MI.$$

Although, strictly speaking, the turnover time is the sum of the intermitotic time plus the mitotic time, the turnover time may be regarded as being approximately equal to the intermitotic time in the greater majority of tissue cells. This is valid because the mitotic time is usually a small fraction, of the order of a few percent at most, of the intermitotic time. Since, as shown in Table 1, normal values of the mitotic index in the thymolymphatic tissues are already known, only the mitotic time remains to be estimated for the calculation of turnover time.

The mitotic time may be estimated from the rate of accumulation of mitotic figures after injection of colchicine, using DUSTIN's formula*:

$$A = \frac{M \cdot t}{X}$$

where A equals the calculated duration of mitosis, M is the original mitotic index, and X is the observed mitotic index after t hours of exposure to colchicine. The figures obtained after exposure to colchicine for from 2 to 8 hours are given in Table 5.

Table 5. Values of the mitotic time of dividing cells in the thymolymphatic organs estimated after exposure to colchicine for from 2 to 8 hours.

Time of exposure to colchicine in hours	Thymus (hour)	Mesenteric lymph nodes (hour)	Other lymph nodes (hour)
2	0.97	0.95	0.92
4	1.28	1.55	1.71
6	1.23	1.89	2.14
8	1.17	2.52	2.25

As seen from the table, the mitotic time varies from 0.92 to 2.52 hours in length. However, the figures estimated by the colchicine technique tend to be much greater than—often about twice as great as—those obtained by other methods (LUSHBAUGH, 1956), probably due to the toxic action of the drug on cell division. It is generally conceded that the rate of entrance into mitosis is somewhat diminished by the colchicine and, in addition, that not all the mitoses are arrested at metaphase. In view of these facts the author assumed the average mitotic time of dividing cells in the thymolymphatic tissues to be about one hour. Besides, a mitotic cycle of 30-minute duration was also considered possible. In his extensive quantitative study of the thymolymphatic organs of rats, KINDRED (1942) also assumed the mitotic time to be of either 1-hour or 30-minute duration.

On the basis of the assumptions noted above, the turnover time of the lymphocytes in the thymolymphatic organs was calculated, using the normal values of the mitotic

* Cited from LUSHBAUGH (1956).

Table 6. Values of the turnover time or intermitotic time of the lymphocytes in the thymolymphatic organs estimated by the direct and indirect methods.

Organ	Direct method (Colchicine method)	Indirect method*	
		1-hour duration of mitosis	30-minute duration of mitosis
Thymus	4.5 days	3.68 days	1.84 days
Mesenteric lymph nodes	12.8 days	5.26 days	2.63 days
Other lymph nodes	15.0 days	5.56 days	2.87 days

* Assumed that the mitotic time is of 1-hour and of 30-minute duration.

index listed in Table 1. The results are given in Table 6, together with the figures estimated directly by using colchicine method. It is seen from the table that the figures obtained by the indirect calculations generally give much lower values of the turnover time than those estimated directly by the colchicine method. Because of the toxic action of the drug mentioned above, the figure estimated by the colchicine method would probably be in the direction of an overestimate.* On the contrary, the calculation based on mitotic time of 30-minute duration would perhaps result in an underestimate, since the mitotic time of dividing cells in the thymolymphatic organs appears to be longer than 30 minutes.

SUMMARY

1. Using male albino rats of a subline of the Wistar strain weighing around 200 g, a series of quantitative measurements on the mitotic activity in the thymolymphatic organs have been made, with the aim to make possible a calculation of the total number of lymphocytes produced by mitosis per day in the thymolymphatic system. Mitotic counts were performed after suspensions of cell nuclei had been prepared from the thymolymphatic organs according to the technique described in the preceding paper (ITO, 1959).

2. Normal values of the mitotic index were found to be $1.13 \pm 0.06\%$ in the thymus, $0.79 \pm 0.06\%$ in the mesenteric lymph nodes, and $0.75 \pm 0.06\%$ in the other lymph nodes.

3. The total percentage of mitoses per day in each organ of the thymolymphatic system was estimated to be $22.23 \pm 0.77\%$ in the thymus, $7.85 \pm 0.37\%$ in the mesenteric lymph nodes, and $6.65 \pm 0.26\%$ in the other lymph nodes. These figures were obtained after repeating the same experiment with colchicine over 4 successive 6-hour periods so as to cover a total of 24 hours.

* In this connection, the effect of adrenalectomy on the mitotic activity in the thymolymphatic tissues, that is, a considerable reduction in the values of the mitotic index observed from 4 to 8 hours after operation, must also be taken into account.

4. Using the data of MONDEN (1958 a) on the total number of lymphocytes contained in the thymolymphatic organs, the average number of excess mitoses of lymphocytes in the whole thymolymphatic organs was calculated to be 432×10^6 . This is 3.3 times as great as the number of total lymphocytes present in the circulating blood.

5. By a direct calculation from the figures obtained for the daily mitotic rate, the turnover time of the lymphocytes in each organ of the thymolymphatic system was estimated to be 4.5 days in the thymus, 12.8 days in the mesenteric lymph nodes, and 15.0 days in the other lymph nodes.

6. Besides, an indirect calculation of the turnover time was made from HOFFMAN's formula, assuming that the mitotic time of dividing cells in the thymolymphatic tissue is of 1-hour and of 30-minute duration.

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