A Quantitative Study on the Relationship of Lymphocytopoiesis to Granulo- and Erythrocytopoiesis in Young Mature Albino Rats*

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As is well known, the hematopoietic system is among the most actively proliferating tissues. Concerning the rate of cell proliferation in this system, however, direct quantitative measurements have seldom been made probably because of technical difficulties. So far as the writer is aware, Kindred is the first who has conducted a number of systematic studies along this line, using rats as material.[1-3] The methods employed in these studies are based on cell counts and mitotic counts of given areas in sections of the hematopoietic organs. Such procedures meet with difficulties particularly in lymph nodes, because variations in cellular density and irregular distribution of mitotic figures in the tissue complicate the quantitative approach in sections and may, for this reason, cause uncertainty in the results.

In an attempt to gain more reliable data which make possible a calculation of the total number of blood cells newly produced by mitosis per day in the hematopoietic sysem, a series of quantitative measurements have been conducted in our laboratory in collaboration with Drs. K. AWAYA, Y. MONDEN, H. ITO and M. OKADA, using young mature albino rats; (1) for evaluation of total cellular numbers in the circulating blood, in the thymolymphatic organs and in the bone marrow, and (2) for estimation of the daily rate of mitotic activity in the thymolymphatic organs and in the bone marrow.

This paper will describe briefly the chief results obtained, with special emphasis on the relationship of lymphocytopoiesis to granulo- and erythrocytopoiesis.

MATERIALS AND METHODS

As the standard animals to be examined, male albino rats from a subline of the Wistar strain, weighing around 200 Gm., were used. They were maintained on a standard laboratory diet.[4]

The total number of nucleated cells in the thymolymphatic organs was estimated by chemical determination of DNA-P content of the whole tissue and of each nu-

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cleus.[4] For estimation of total cellular numbers of bone marrow, a weighed sample of femoral marrow was mixed with known volume of homologous serum in a small vial and an even suspension was obtained by the gentle aspiration and expulsion of the mixture with a rubber-nippled pipette, following which the sample was taken for hemocytometer counts.[5] This is a modification of the technic described by FRUHMAN and GORDON.[6] The bone marrow volume was taken as 1.65 Gm. per 100 Gm. of body weight, according to HASHIMOTO[7] and WATANABE.[8] The daily rate of mitosis in individual cell populations was estimated by determining the mitotic index at intervals of 4–6 hours after injecting 0.10 Gm. of colchicine per 100 Gm. of body weight subcutaneously.[9,10] The injection of colchicine was performed at the same time of the day, nemely at 10 : 00 a. m. in most instances. After injection there was an almost linear increase in the percentage of mitoses in the hematopoietic tissues until the 8th hour. The circulating blood volume was measured by the method of BERLIN et al.,[11] using radioactive phosphorus.

RESULTS

1. *Cell Populations in the Circulating Blood*

On the basis of measurements of the circulating blood volume in 10 rats and the results of blood cell counts in 50 rats,[12] the total number of each type of blood cells present in the circulating blood was calculated. The results obtained are listed in Table 1.

Table 1. Total Numbers of Different Types of Blood Cells in the Circulating Blood

Cell type	Average number per mm^3 of blood	Total number in circulating blood* $\times 10^{-6}$
Erythrocytes	$(10.37 \pm 0.45) \times 10^6$	$114,400 \pm 9,380$
Total white cells	$17,100 \pm 630$	189 ± 14.1
Lymphocytes	$11,970 \pm 640$	132 ± 12.0
Total granulocytes	$4,400 \pm 388$	48.5 ± 6.1
Neutrophils	$4,234 \pm 350$	46.7 ± 5.6
Eosinophils	159 ± 35	1.75 ± 0.45
Monocytes	722 ± 71	7.96 ± 1.06

* Circulating blood volume in standard rats was estimated to be 11.03 ± 0.44 ml.

2. *Lymphocyte Populations in the Thymolymphatic Organs*

The total number of lymphocytes contained in the thymolymphatic system was estimated by chemical determination of DNA-P content of the whole tissue and of each nucleus, using 10 standard rats. The computations showed that the number of total lymphocytes in this system amounted to $(4,268 \pm 259) \times 10^6$ (Table 2). Since the average number of total lymphocytes in the circulating blood has been found to

Table 2. Average Number of Lymphocyte Mitoses per Day in the Thymolymphatic Organs

Organ	Total number of lymphocytes $\times 10^{-6}$	Total percentage of mitoses per day	Total number of mitoses per day $\times 10^{-6}$
Thymus	1,071±145	22.23±0.77	283±33.3
Mesenteric lymph nodes	684±123	7.85±0.37	54±9.9
Other lymph nodes	1,031±106	6.65±0.26	69±7.5
Spleen	1,100±169	6.65±0.26*	73±11.5
Peyer's patches	382± 34	6.65±0.26*	25±1.8
Total thymolymphatic organs	4,268±259		459±37.4

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

be $(132\pm12.0)\times10^6$ (Table 1), the ratio of this value to the number of total lymphocytes in the thymolymhatic system is calculated to be about one to thirty-two. This indicates that the lymphocyte reserve in this system is about 32 times as great as the number of lymphocytes present in the blood.

3. *Daily Rate of Mitotic Activity in the Thymolymphatic System*

The daily rate of mitotic activity in the thymolymphatic system was estimated on the basis of colchicine treatment for a 6-hour period. The mitotic index was determined in suspension of cell nuclei which had been prepared from individual lymphoid organs. Since some diurnal variations were to be seen in the mitotic activity of lymphocytes, the total percentage of mitoses of these cells per day was computed by adding the mean values of the mitotic index which were obtained at intervals of 6 hours from seven samples each. Thus, the daily rate of mitosis of lymphocytes was found to be 22.23 ± 0.77 per cent in the thymus, 7.85 ± 0.37 per cent in the mesenteric lymph nodes, and 6.65 ± 0.26 per cent in other lymph nodes (Table 2).

From the data obtained, the total sum of lymphocyte mitoses per day was calculated to be $(459\pm37.4)\times10^6$. The number of lymphocytes needed for daily growth of the lymphoid tissue was roughly estimated to total 26.6×10^6, assuming the daily rate of growth of the tissues to be 0.624 per cent, according to KINDRED.[3] It was then found that the number of excess mitoses of lymphocytes per day totaled 432×10^6. This is about 3.3 times as great as the number of total lymphocytes present in the circulating blood.

If we assume that all excess lymphocytes newly produced by mitosis enter into the general circulation, the turnover time of lymphocytes in the circulating blood may be estimated to be 0.3 day.

Using the data for the total percentage of mitoses of lymphocytes per day, the turnover time of these cells was computed to be 4.5 days in the thymus and 12.8–15.0 days in the lymph nodes.

4. *Cell Populations in the Bone Marrow*

Using the data of total number of nucleated cells in the bone marrow of 41 standard rats and the results of bone marrow differential counts in 5 standard rats, the number of nucleated cells of each series within the whole bone marrow was computed. The results are given in Table 3.

Table 3. Average Number of Mitoses of Different Types of Blood Cells in the Whole Marrow

Cell type	Total cellular number $\times 10^{-6}$	Total percentage of mitoses per day		Total number of mitoses per day $\times 10^{-6}$	
		Total	Late forms*	Total	Late forms*
Erythrocytic series	2,458±133	134.8±6.9 (151.6±13.4)†	105.4±5.5 (122.5±11.4)†	3,104±239 (3,190±354)†	2,418±193 (2,579±206)†
Granulocytic series	2,476±180	23.9±1.4 (25.4±1.6)†	10.6±0.5 (11.3±1.9)†	664±47.4 (641±41.6)†	294±29.4 (282±48.8)†
Neutrophils	2,183±236	26.0±2.0 (28.0±1.8)†	11.6±0.9 (12.3±2.1)†	647±52.8 (616±40.0)†	291±29.1 (271±49.6)†
Eosinophils	249±58.8	6.6±4.7 (8.3±2.5)†	—	16.8±9.6 (25.2±6.8)†	—
Lymphocytes	683±16.7	—	—	—	—
Monocytes	15.7±6.5	—	—	—	—

* The term "late forms" denotes polychromatic and orthochromatic erythroblasts in the erythrocytic series and half-mature and mature myelocytes in the granulocytic series, respectively.

† Figures in parenthesis indicate the values calculated on the basis of 6-hour treatment with colchicine.

When the values obtained for the bone marrow are contrasted with the corresponding values for the circulating blood (Table 1), the ratios between cells in the bone marrow and those in the blood are found to be: 1 : 47 for the erythrocytic series and 51:1 for the granulocytic series, 47:1 for the neutrophils, 142:1 for the eosinophils, 5:1 for the lymphocytes, and 2:1 for the monocytes. It is of especial interest that the granulocytic cell reserve within the whole marrow was estimated to be 50 times as great as the number of total granulocytes present in the circulating blood.

5. *Daily Rate of Mitotic Activity in the Bone Marrow*

The total number of mitoses of each type of blood cells per day in the whole marrow was calculated on the basis of colchicine treatment either for a 4-hour period or for a 6-hour period. The mitotic index was determined in imprint preparations stained with MAY-GIEMSA. Diurnal variations in mitotic rates were not taken into consideration. For calculation of the turnover times of the erythrocytes and the granulocytes in the blood, special attention was directed to the number of mitoses of the late forms, such as polychromatic and orthochromatic erythroblasts in the erythrocytic series, and half-mature and mature myelocytes in the granulocy-

tic series, because only these forms may be regarded as the direct supply source of the circulating blood cells.

On the basis of the data obtained, the total number of mitoses of each series of blood cells per day was calculated to be : $(3,104 \pm 239) \times 10^6$ or $(3,190 \pm 354) \times 10^6$ for the erythrocytic series and $(664 \pm 47.4) \times 10^6$ or $(641 \pm 41.6) \times 10^6$ for the granulocytic series. The corresponding values for late formes of each series were $(2,148 \pm 193) \times 10^6$ or $(2,579 \pm 206) \times 10^6$ and $(294 \pm 29.4) \times 10^6$ or $(282 \pm 48.8) \times 10^6$, respectively (Table 3).

When these values are contrasted with the total cellular numbers in the blood, the turnover time of each type of cells may easily be calculated. The results of calculations are summarized in Table 4, together with the values for the "Daily Replacement Factor" of YOFFEY,[13] which indicates how many times the cell populations in the blood are daily replaced by newly formed cells.

One of the assumptions upon which our calculations are based is that all the cells newly produced by mitosis enter into the general circulation through the normal

Table 4. Values for Turnover Time and "Daily Replacement Factor*" of Each Type of Blood Cells in the Blood and Blood-Forming Tissues, Calculated Either from Total Mitoses or from Those of Late Forms.

	Turnover time in days		Daily Replacement Factor	
	Total[1]	Late forms[2]	Total[1]	Late forms[2]
Blood				
Lymphocytes	0. 30		3. 3	
Total granulocytes	0. 073 – 0. 076	0. 165 – 0. 169	13. 2 – 13. 6	5. 9 – 6. 1
Neutrophils	0. 072 – 0. 076	0. 161 – 0. 172	13. 2 – 13. 9	5. 8 – 6. 2
Eosinophils	0. 069 – 0. 104	0. 125 – 0. 250	9. 0 – 14. 4	4. 0 – 8. 0
Erythrocytes	36 – 37	44 – 47	0. 026 – 0. 028	0. 021 – 0. 022
Thymolymphatic organs				
Lymphocytes in thymus	4. 5			
Lymphocytes in lymph nodes	12. 8 – 15. 0			
Bone Marrow				
Total granulocytes	3. 94 – 4. 19 (2. 3)**			
Neutrophils	3. 56 – 3. 84 (1. 9)**			
Eosinophils	12 – 15			
Nucleated red cells	0. 66 – 0. 74			

* This factor indicates how many times the cell population in the blood are daily replaced by the newly formed cells; it is expressed as the reciprocal of the turnover time in days.

** Turnover time when mature granulocytes with filamented or segmented nucleus are excluded.

1 Calculation is based on the total number of mitoses per day, including those of the early forms.

2 Calculation is based on the number of mitoses of the late forms per day, excluding those of the early forms.

process of cell maturation. Strictly speaking, this assumption is not correct. Since only the late forms may be regarded as the direct supply source of the circulating blood cells, the calculation of the turnover times of cell populations in the blood should be based on the mitotic activity of the late forms alone.

The turnover times of cell populations in the bone marrow were calculated to be about 4 days for the total granulocytes and about 0.7 day for the nucleated red cells. When mature granulocytes with filamented or segmented nucleus are excluded, the turnover time of the total granulocytes turns out to be 2.3 days.

CONCLUDING REMARKS

A comparison of the values for the turnover time, or intravascular life span, of the lymphocytes, granulocytes and erythrocytes estimated by different methods is shown in Table 5.

It is generally believed that the true life span of the lymphocyte and the granulocyte of the human is of the order of a few days or longer[18-20,23-24] and that of the erythrocyte of the rat is of the order of 60–70 days.[26-27] The values of the turnover time of these cells in the circulating blood, which were estimated by mitotic counts in the hematopoietic tissues using colchicine, are generally smaller than the corresponding figures noted above. This indicates that not only lymphocytes but also granulocytes and erythrocytes are being produced by mitosis in their parental tissues in sufficiently great numbers when contrasted with the estimated needs of the blood.

As regards the relationship of lymphocytopoiesis to granulo- and erythrocytopoiesis, YOFFEY and his associates[30] still maintain an old hypothesis of JORDAN[31] and KINDRED[3] that lymphocytes produced in excess in the thymolymphatic system are filtered off in the bone marrow to become stem cells for the development of granulocytes and/or erythrocytes. Such a hypothesis infers that the bone marrow cannot produce sufficient blood cells to meet the demands of the blood.

The results of the present study are not in accord with the above mentioned hypothesis. There is no necessity for assuming that the cell populations of the bone marrow must receive from some outside source in order to produce sufficient blood cells to meet the demands of the blood.

Furthermore, it should be emphasized that both lymphocytes and granulocytes are being produced by mitosis in their parental tissues in much greater numbers than are needed by the blood, if we assume that the true life span of these cells is of the order of a few days or longer. The overproduction of lymphocytes is not consistent with the concept of either large scale recirculation or reutilization of these cells.[32-34]

SUMMARY

Using male albino rats of the Wistar strain weighing around 200 Gm., an attempt

Table 5.— Comparison of the Values for Turnover Time (Intravascular Life Span or Age) of the Lymphocytes, Granulocytes and Erythrocytes in the Circulating Blood Estimated by Different Methods

Cell Population	Species	Turnover time days	Method	Reference
Lymphocytes	Rat	0. 30	Mitotic counts by colchicine method	Table 4, this paper
	Rat	0. 25 —0. 61*	Mitotic counts in sections	Kindred[3]
	Rat	0. 49	Cannulation of thoratic duct	Reinhardt[14]
	Rat	0. 17 —0. 20	Cannulation of thoratic and cervical ducts	Mann & Higgins[15]
	Rat	0. 056—0. 081	Cannulation of thoratic duct	Whaler & Widdicombe[16]
	Guinea-pig	0. 23	Cannulation of thoratic and cervical ducts	Reinhardt & Yoffey[17]
	Human	30	^{32}P incorporation in DNA	Osgood et al.[18]
	Human	3—4**	^{32}P incorporation in DNA	Ottesen[19]
	Human	85	Adenine-8-^{14}C incorporation in DNA	Hamilton[20]
Granulocytes	Rat	0. 161—0. 169	Mitotic counts by colchicine method	Table 4, this paper
	Rat	0. 033—0. 071	Mitotic counts in sections	Kindred[3]
	Rat	0. 12	Parabiosis	Van Dyke & Huff[21]
	Human	0. 021—0. 062	Transfusion	White[22]
	Human	8. 8	^{32}P incorporation in DNA	Kline & Cliffton[23]
	Human	2. 7—3. 4	^{32}P incorporation in DNA	Ottesen[19]
	Human	2	^{32}P incorporation in DNA	Osgood & Krippaehne[24]
	Human	2. 3	^{3}H-Thymidine incorporation in DNA	Bond et al.[25]
Erythrocytes	Rat	44—47	Mitotic counts by colchicine method	Table 4, this paper
	Rat	382—790*	Mitotic counts in sections	Kindred[3]
	Rat	70	^{14}C incorporation in hemoglobin	Berlin & Lotz[26]
	Rat	59±2	Labelling, ^{59}Fe and ^{51}Cr	Belcher & Harris[27]
	Human	120	Labelling, ^{51}Cr	Hyman et al.[28]
	Rabbit	64	Glycine ^{15}N incorporation in heme	Neuberger & Nieven[29]

* Calculated by OSOGOE from the data given in the paper of KINDRED.

** According to OTTESEN, the lymphocytes form two groups, one younger than 10 days with a mean age of about 3 to 4 days, the other having a mean age of about 100 to 200 days, and the shortliving fraction represents 11 or 22 per cent and the long-living 78 or 89 per cent of the blood lymphocytes.

was made to make possible a calculation of the total number of blood cells newly produced by mitosis per day in the hematopoietic system. The results obtained indicated that not only lymphocytes but also granulocytes and erythrocytes are being produced by mitosis in their parental tissues in sufficiently great numbers when contrasted with the estimated needs of the blood. There is no necessity for assuming that lymphocytes produced in excess in the thymolymphaic system are filtered off in the bone marrow to become stem cells for the development of granulocytes and/or erythrocytes.

REFERENCES

1) KINDRED, H. E.: A quantitative study of the lymphoid organs of the albino rat. *Am. J. Anat.* **62**: 453-473, 1938.
2) ————: A quantitative study of the hematopoietic organs of young albino rats. *Am. J. Anat.* **67**: 99-149, 1940.
3) ————: A quantitative study of the hematopoietic organs of young adult albino rats. *Am. J. Anat.* **71**: 207-243, 1942.
4) MONDEN, Y.: Quantitative evaluation of total cellular number and cellular density in the thymolymphatic organs of young adult albino rats by means of DNA determination. *Okajimas Fol. anat. jap.* **32**: 193-206, 1959.
5) AWAYA, K., FUJII, H., TANAKA, Y., and OKADA, M.: Estimation of total number of nucleated cells in the bone marrow of young adult aldino rats. *Arch. hist. jap.* **18**: 473-477, 1960.
6) FRUHMAN, G. J., and GORDON. A. S.: Quantitative evaluation of total cellular numbers of bone marrow. *Anat. Rec.* **117**: 603, 1953 (abstract).
7) HASHIMOTO, M.: Histological structure of the bone marrow. Symposium on Hematology **5**: 114-172, 1953 (in Japanese).
8) WATANABE, S.: The weight of bone marrow. *J. Kyushu Soc. Hematol.* **5**: 132-157, 1955 (in Japanese).
9) ITO. H.: Quantitative studies on the rate of cell production in the thymolymphatic organs. II. Estimation of the daily mitotic activity in young adult albino rats. *Okajimas Fol. anat. jap.* **34**: 13-25, 1959.
10) OKADA, M.: A quantitative study on the rate of cell production in the bone marrow of young mature albino rats. *Okajimas Fol. anat. jap.* **35**: 375-402, 1960.
11) BERLIN, N. T., HUFF, R. L., VAN DYKE, D. C., and HENNESSY, R. F.: The blood volume of the adult rat, as determined by Fe^{59} and P^{32} labelled cell. *Proc. Soc. Exp. Biol. & Med.* **17**: 175-178, 1949.
12) MONDEN, Y.: A quantitative study of cell populations in the circulating blood of young adult albino rats. *Okajimas Fol. anat. jap.* **32**: 207-213, 1959.
13) YOFFEY, J. M.: Bone marrow. *Brit. Med. J.* **2**: 193-204, 1954.
14) REINHARDT, W. O.: Growth of lymph nodes, thymus and spleen, and output of thoracic duct lymphocytes in the normal rat. *Anat. Rec.* **94**: 197-211, 1946.
15) MANN, J. D., and HIGGINS, G. M.: Lymphocytes in the thoracic duct, intestinal and hepatic lymph. *Blood* **5**: 177-190, 1950.
16) WHALER, B. C., and WIDDICOMBE, J. G.: The blood life-span of the lymphocyte in rabbits and rats. *J. Physiol.* **732**: 41-42, 1956.
17) REINHARDT, W. O., and YOFFEY, J. M.: Lymphocyte content of lymph from the thoracic and cervical ducts in the guinea-pig. *J. Physiol.* **136**: 227-234, 1957.
18) OSGOOD. E. E.: Number and distribution of human hemic cells. *Blood* **9**: 1141-1154, 1954.
19) OTTESEN J.: On the age of human white cells in peripheral blood. *Acta physiol. scand.* **32**: 75-93, 1954.
20) HAMILTON, L. D.: Nucleic acid turnover studies in human leukemic cells and the function of lymphocytes. *Nature* **178**: 597-599, 1957.
21) VAN DYKE, D. C., and HUFF, R. L.: Life span of white blood cells as measured in irradiated parabiotic rats. *Am. J. Physiol.* **165**: 341-347, 1957.
22) WHITE, L. P.: The intravascular life span of transfused leukocytes tagged with atabrine. *Blood* **9**: 73-82, 1954.
23) KLINE, D. L., and Cliffton, E. E.: The life span of leukocytes in the human. *Science* **115**: 9-11, 1952.

24) OSGOOD, E. E., and Krippaehne, M. L.: Comparison of the life span of leukemic and non-leukemic neutrophils. *Acta hematol.* **13**: 153–160, 1955.
25) BOND, V. P., FLIEDNER, T. M., CRONKITE, E. P., RUBINI, J. R., and ROBERTSON, J. S.: Cell turnover in blood and blood-forming tissues studied with tritiated thymidine. In *The Kinetics of Cellular Proliferation.* F. Stohlman, Jr., ed. New York, Grune & Stratton, 1959, pp. 188–200.
26) BERLIN, N. T., and LOTZ, C.: Life span of red blood cells of the rat following acute hemorrhage. *Proc. Soc. Exp. Biol. & Med.* **79**: 788–790, 1951.
27) BELCHER, E. H., and HARRIS, E. B.: Studies of red cell life span in the rat. *J. Physiol.* **146**: 217–234, 1959.
28) HYMAN, G. A., GELLHORN, A., and HARVEY, J. L.: Studies on the anemia of disseminated malignant neoplastic disease. II. Study of life span of the erythrocyte. *Blood* **11**: 618–631, 1956.
29) NEUBERGER, A., and NIEVEN, J. F. S.: Haemoglobin formation in rabbits. *J. Physiol.* **112**: 293–310, 1951.
30) YOFFEY, J. M., HANKS, C. A., and KELLY, L.: Some problem of lymphocyte production. *Ann. New York. Acad. Sci.* **73**(1): 47–78, 1958.
31) JORDAN, H. E.: The relation of lymphoid tissue to the process of blood production in the bone marrow. *Am. J. Anat.* **59**: 249–297, 1936.
32) GOWANS, J. L.: The recirculation of lymphocytes from blood to lymph in the rat. *J. Physiol.* **146**: 54–69, 1959.
33) TROWELL, O. A.: Re-utilization of lymphocytes in lymphopoiesis. *J. Biophys. & Biochem. Cytol.* **3**: 317–318, 1957.
34) HAMILTON, L. D.: Control and function of the lymphocyte. *Ann. New York Acad. Sci.* **73**(1): 39–46, 1958.