Neutrophil Kinetics in Man

JOHN T. DANCEY, KURT A. DEUBELBEISS, LAURENCE A. HARKER, and CLEMENT A. FINCH

From the Division of Hematology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98105

ABSTRACT A method has been developed for measuring neutrophil cellularity in normal human bone marrow, in which the neutrophil-erythroid ratio was determined from marrow sections and marrow normoblasts were estimated by the erythron iron turnover. Neutrophil maturational categories, defined by morphologic criteria, were supported by autoradiographs of marrow flashed-labeled with ^{3}H-thymidine. Correction for multiple counting error was empirically derived by counting serial sections through cells of each maturational category. The normal neutrophil-erythroid ratio in 13 normal human subjects was 1.5±0.07. The mean number of normoblasts in the same subjects was estimated to be 5.07±0.84 × 10^9 cells/kg. Total marrow neutrophils (× 10^9 cells/kg) were 7.70±1.20, the postmitotic pool (metamyelocytes, bands, and segmented forms) was 5.59±0.90 and the mitotic pool (promyelocytes + myelocytes) was 2.11±0.36.

Marrow neutrophil ("total") production has been determined from the number of neutrophils comprising the postmitotic marrow pool divided by their transit time. Transit time was derived from the appearance in circulating neutrophils of injected ^{3}H-thymidine. The postmitotic pool comprised 5.59±0.90 × 10^9 neutrophils/kg, and the transit time was 6.60±0.03 days. From these data marrow neutrophil production was calculated to be 0.85 × 10^9 cells/kg per day.

Effective production, measured as the turnover of circulating neutrophils labeled with ^{3}H-thymidine, was 0.87±0.13 × 10^9 cells/kg per day. This value correlated well with the calculation of marrow neutrophil production. A larger turnover of 1.62±0.46 × 10^9 cells/kg per day was obtained when diisopropylfluorophosphate-^{32}P was used to label circulating neutrophils. Studies using isologous cells doubly labeled with ^{3}H-thymidine and diisopropylfluorophosphate-^{32}P demonstrated a lower recovery and shorter $t_{\frac{1}{2}}$ of the ^{32}P label.

INTRODUCTION

The rate of neutrophil production can be measured as the turnover of neutrophils in either marrow or blood. Blood neutrophil turnover as determined with [^{32}P]diisopropylfluorophosphate (DFP) [1] cells has gained general acceptance: Cartwright et al. (1) in a study of 109 subjects, reported a mean turnover of 1.63 × 10^9 cells/kg per day, and a similar figure of 1.36 × 10^9/kg per day was reported by Galbraith et al. (2) who studied 18 subjects. Such studies have provided an estimate of blood neutrophil turnover in normal man. Attempts to measure marrow neutrophil production have been indirect in the absence of reliable measurements of marrow cellularity. In this paper we describe a method for determining the number of marrow neutrophils and report measurements of basal rates of total production (marrow turnover) and effective production (blood turnover) of neutrophils in man.

METHODS

Measurements of marrow neutrophils were carried out in 13 normal subjects with normal body temperature and no history of any infective process within the previous week or during the study. Their hematocrits were normal, reticulocyte counts were less than 1.5%, and platelet, total leukocyte, and neutrophil counts were within normal limits (3). The erythrocyte sedimentation rate was less than 20 mm/h, and the leukocyte alkaline phosphatase score was less than 100 (4). All subjects were white, the ages ranged from 18 to 55 yr, and 5 of the 13 were women.

Dr. Dancey's present address is Division of Hematology, Montreal General Hospital, Montreal, Quebec, Canada.

Dr. Deubelbeiss' present address is Division of Hematology, Inselspital, Bern, Switzerland.

Received for publication 27 February 1975 and in revised form 24 May 1976.

[1] *Abbreviations used in this paper:* DFP, diisopropylfluorophosphate; EIT, erythron iron turnover; Hct, hematocrit; NE, neutrophil-erythroid; PIT, plasma iron turnover; tdr, thymidine; WB, whole blood.

In one group of 13 subjects ferrokinetic measurements and marrow biopsies were carried out to determine marrow neutrophil cellularity. In a second group of eight subjects, the marrow transit time of postmitotic neutrophils was measured, and in a third group of five subjects, the circulating neutrophil turnover was determined. In three subjects, plasma iron turnover measurements, marrow transit time determinations, and marrow biopsies were all performed.

Neutrophil-erythroid (NE) ratios were obtained by comparing counts of nucleated erythroid cells with those of neutrophil granulocytes in marrow sections. Marrow biopsies were obtained from the posterior iliac spine using either a Westerman-Jensen (5) or a Jamshidi needle (6). In preparing marrow sections (7) the biopsy cores were placed promptly in 20 ml of acrolein (distilled acrolein, E. M. grade, N_2 sealed, Polysciences Inc., Paul Valley Industrial Park, Warrington, Pa.) fixative solution (5% vol/vol in distilled water) and incubated for 24 h in a 37°C waterbath. Without prior decalcification, the fixed tissues were dehydrated for successive 2-h periods in 50, 70, 85, and 95% ethanol followed by two 1-h exposures to fresh absolute ethanol. The dehydrated tissues were transferred from absolute ethanol to methacrylate monomer, two changes of 1 h each, then placed in a 1:1 mixture of monomer and slurry (methyl methacrylate slurry: 150 ml methacrylate monomer, Polysciences, Inc.; 25 g of polyethylene glycol distearate mol wt 1,540, Kessler Chemical Co., Ltd., Philadelphia, Pa.; 1.2 g benzoyl peroxide, Eastman Organic Chemicals, Rochester, N. Y.); 500 mg methyl methacrylate polymer VS-100, Rohm and Haas Co., Philadelphia, Pa.; 18 ml dibutyl phthalate, Polysciences Inc.) for at least 2 h. The cores were then transferred to plastic embedding molds, covered with slurry, and placed under vacuum for 2 h. Polymerization was carried out at room temperature under ultraviolet light and was complete by 24 h. Sections were cut at 2-μm by using a Leitz L-1212 Minot rotary microtome (Technical Instrument Co., San Francisco, Calif.) with steel knives and mounted on methanol-cleaned albumin-subbed slides. The slides were left on a slide warming stage at 55°C for at least 4 h. After removal of the plastic matrix by a 20-min immersion in acetone, the sections were brought through ethanol to distilled water. After 5 min in distilled water, the sections were held in 1% eosine-Y for 4 min, rinsed in tap water, and placed in 0.1% toluidine blue for 5 min. The stain was differentiated briefly in 95% acid-isopropanol (2 ml glacial acetic acid in 200 ml 97% isopropyl alcohol), dehydrated in 99% isopropanol, and cleared in three changes of zylol. The stained sections were air dried, and a coverslip was applied over a drop of immersion oil. Neutrophil primary granules are stained by this method, permitting recognition of marrow neutrophils as early as the promyelocyte.

Nucleated erythroid cells were assigned to three maturational categories according to their size and the degree of nuclear chromatin condensation. In cells of the earliest category, the chromatin was finely divided and evenly dispersed throughout the nucleus; in cells of the intermediate category prominent clumps were evident; in late normoblasts, chromatin masses occupied most of the nucleus.

Neutrophils were identified as containing azurophilic cytoplasmic granules. They were classified according to their stage of maturation employing the following criteria. Cells designated as promyelocytes were large with usually round nuclei displaying fine nuclear chromatin, minimal chromatin clumping limited to the nuclear membrane and large, usually multiple red nucleoli. Myelocytes were defined as large cells with predominantly round nuclei showing clumps of chromatin along the nuclear membrane, and sometimes containing one or two small nucleoli. Metamyelocytes were recognized as smaller cells with round or indented nuclei devoid of nucleoli and with prominent clumping of chromatin throughout. Band forms were distinguished from metamyelocytes by the characteristic elongate chromatin-dense nucleus or, in transverse cuts, by a nuclear diameter one-third the diameter of the cell. Segmented neutrophils were defined by two or more distinct dense nuclear masses with interlobar filaments, or by a single nuclear mass with a diameter less than one-third that of the entire cell. Where there was uncertainty regarding the degree of cell maturation, the cell was assigned to the more mature category. Promyelocytes and myelocytes identified by the above criteria were designated the mitotic pool, and metamyelocytes bands and segmented neutrophils were designated the postmitotic pool.

The criteria used to identify and exclude cells of the mitotic pool were examined by preparing autoradiographs of normal marrow sections labeled with tritiated thymidine. 100 μCi ^{3}H thymidine (^{3}Htdr) (^{3}H-methyl thymidine S.A 40–60 Ci/mmol aqueous solution, New England Nuclear, Boston, Mass.) was injected into the posterior iliac marrow of a normal postmenopausal volunteer (8). 30 min later a biopsy was obtained from the same site and was fixed and embedded as described above. Mounted sections were coated with Kodak NTB-2 (Eastman Kodak Co., Rochester, N. Y.) autoradiographic emulsion and exposed for 2 wk. Developed slides were stained with eosine-Y and toluidine blue, and 200 consecutive labeled neutrophils were classified. Only cells classified as promyelocytes or myelocytes were found to have assimilated ^{3}Htdr; no metamyelocytes, bands, or segmented neutrophils were labeled. Marrow sampling 30 min after injection of label ensured that no label would have appeared in the nonmitotable progeny of the last myelocyte stage. This approach provided direct support for the criteria used to distinguish between mitotable and nonmitotable cells.

Because any single cell is represented in more than one section, cell counts from sectioned marrow overestimate the actual number of cells by a factor related to nuclear diameter and thickness of the section (9). The "multiple counting" factor for each cell type was empirically determined by counting the number of serial 2-μm sections in which nucleated profiles of the same cell could be identified. For this purpose, cells of each morphologic type were examined in black and white photomicrographic enlargements of given areas on serial sections. Size correction factors for normoblasts were 4.30, 3.50, and 2.11, respectively, for the early, intermediate, and late maturational stages. The percentage distribution, determined by grading 1,000 normoblasts on sections from three normal subjects, was 4.1, 37.3 and 58.6%, respectively. From these figures a composite normoblast correction factor of 2.72 was calculated (Table I). The actual cell count was then derived by dividing the raw section count by the composite size correction factor. The "multiple counting" factors for the neutrophil maturational categories were similarly determined and were found to be, respectively, 4.70 and 3.81 for promyelocytes and myelocytes, and 3.44, 3.24, and 3.23 for metamyelocytes, bands, and segmented neutrophils (Table I).

For each subject neutrophils and normoblasts were counted by using a Howard ocular grid among 10,000 sequential cells of all types on each of two sections cut at least 10 μm apart. When 10,000 cells were counted the

TABLE I

Size Correction Factors for Human Marrow Cells in Tissue Sections

Category	Total sections examined	Number of cells examined	Correction factor
Normoblasts:			
Early	99	23	4.30
Intermediate	193	55	3.50
Late	217	103	2.11
Composite*			2.72
Neutrophils:			
Promyelocytes	146	31	4.70
Myelocytes	324	85	3.81
Metamyelocytes	62	18	3.44
Bands	253	78	3.24
Segmented neutrophils	258	80	3.23

* The composite erythroid correction factor is based on percent distributions of 4.1, 37.3, and 58.6 for early, intermediate, and late normoblasts, respectively, determined from marrow sections.

coefficient of variation in the NE ratio lay between 5.5 and 8.5% for all neutrophil categories. The reliability of the NE ratio was further evaluated by comparing duplicate counts of 10,000 cells from each of two random sections from 13 normal subjects. The error in the NE ratio, calculated by the method of Copeland (10) between duplicate sections relative to the mean NE ratio for each neutrophil category was between 8.3 and 13.3% (Table II). The precision of the measurement of NE ratios was further assessed by comparing results obtained from biopsies taken from the right and left posterior ilium of one normal subject, from multiple sections through one biopsy from a second normal subject, and from two biopsies obtained at a 4-mo interval from a single individual in a steady state. Variation with time and site remained within 10%.

For the measurement of plasma iron turnover, 2 μCi of ^{59}Fe citrate were added to 10 ml of the subject's heparinized plasma and incubated for 15 min at room temperature on a rotating turntable to permit complete binding with transferrin. The labeled plasma was injected intravenously and eight 2 ml-plasma samples obtained at intervals during the ensuing 2 h. The plasma iron turnover (PIT), expressed as mg Fe/100 ml whole blood (WB)/day, was calculated as previously described (11) according to the formula:

$$\text{PIT (mg Fe/100 ml WB/day)} = \frac{\text{serum iron } (\mu g/100\,ml)}{t_{\frac{1}{2}} \text{ (minutes)}} \times \frac{100 - (\text{Hct} \times 0.9)}{100}$$

The erythron iron turnover was then derived from the plasma iron turnover by subtracting the nonerythroid fraction (12) as follows:

$$\text{EIT (mg Fe/100 ml WB/day)} = \text{PIT} - \text{serum iron } (\mu g/100\,ml) \times \text{plasmatocrit} \times 0.0035$$

Calculations of normoblast numbers were based on the subject's erythron iron turnover (EIT) and the normal number of nucleated erythrocytes, according to the formula:

$$\text{Subject's normoblasts} = \frac{\text{subject's EIT (mg/100 ml WB/day)}}{0.56 \text{ mg/100 ml WB/day}} \times 5.29 \times 10^9 \text{ normoblasts/kg}$$

where 5.29×10^9/kg represents the mean normal nucleated erythrocyte population[2] and 0.56 represents the mean normal EIT (11). The number of marrow normoblasts was determined by adjusting the normal value according to the subject's EIT. Absolute cell numbers were then calculated for total marrow neutrophils and for each maturational stage by multiplying the subject's NE ratio for that category by the subject's normoblast number.

Neutrophil marrow transit time was determined in eight normal volunteers over 50 yr of age. ^{3}Htdr (^{3}H-methyl thymidine SA-40–60 Ci/mmol sterile aqueous solution, New

[2] This number is derived from the following data: a daily circulating erythrocyte turnover of 2.56×10^9/kg, a marrow reticulocyte transit time of 65 h (13), a ratio between marrow reticulocytes and normoblasts of 1.55 (13, 14), and an assumption of 15% ineffective erythropoiesis (15). Erythrocyte turnover is based on a circulating erythrocyte concentration of $4.8 \times 10^6/\mu l$ (3), a body/venous hct ratio of 0.9 (16), a blood volume of 71 ml/kg (17, 18), and an erythrocyte life span of 120 days.

TABLE II

NE Ratio Variation in Duplicate Section Counting of Human Marrow

	NE ratio				
	Promyelocytes	Myelocytes	Metamyelocytes and bands	Segmented neutrophils	Total marrow neutrophils
Mean	0.21	0.41	0.79	0.53	1.94
SD	0.02	0.04	0.07	0.08	0.19
Coefficient of variation	8.3	10.3	9.91	13.3	9.8

NE ratios, uncorrected for multiple counting, were obtained from counts of 10,000 consecutive cells of all types in duplicate sections from 13 normal subjects. Variation about the mean for each neutrophil category was calculated by the method of duplicates as described by Copeland (10).

England Nuclear), 50 μCi/kg body weight (maximum activity injected was 3.5 mCi providing an estimated dose of 129 mrads to the marrow and 22 mrads total body) was diluted in 20 ml sterile normal saline and injected intravenously. On days 1–3 after injection, 20-ml blood samples were drawn at 24 h, and from days 4–8 blood was drawn at 12-h intervals. In two studies, an additional sample was drawn on day seven. A neutrophil fraction was prepared by using the following modification of Boyum's Ficoll-Hypaque technique (19). 10 ml acid-citrate (20) anticoagulated whole blood was layered over 3 ml Ficoll-Hypaque (sodium diatrizoate USC 50%, Winthrop Laboratories, N. Y.; Ficoll, Pharmacia Fine Chemicals, Uppsala, Sweden) in 13 mm diameter siliconized glass tubes in duplicate and centrifuged at 400 *g* for 35 min. The sediment containing erythrocytes, neutrophils, and eosinophils was mixed with 12 ml 3% dextran (mol wt 300,000) in 0.9% saline and allowed to sediment at room temperature until a clear line of demarcation appeared. The neutrophil-rich supernates from 20 ml blood were transferred to a single 40 ml siliconized round-bottomed glass centrifuge tube and centrifuged at 300 *g* for 10 min. The supernate was decanted and the button was suspended in 20 ml saponin solution (Saponin, Eastman Organic Chemicals, Rochester, N. Y. 0.0125% in 0.6% saline). Remaining erythrocytes were lysed at room temperature for 2 min. The suspension was centrifuged at 250 *g* for 8 min, the supernate was decanted, and the cells were washed once with acid citrate (250 g for 10 min). The neutrophils were then suspended in 15 ml acid citrate, and 1 ml was removed for cell count and preparation of a Wright's-stained smear. Microscopic examination of wet preparations and stained films revealed at least 95% granulocytes of which less than 5% were eosinophils. The yield of neutrophils varied between 65 and 80% of those present in the original WB. The remaining neutrophil suspension was centrifuged at 1,000 *g* for 10 min, and the residual button of cells was dissolved in 2 ml NCS (Amersham Searle Corp., Arlington Heights, Ill.). After adding 16 ml of scintillation solution (PPO (Amersham Searle Corp.) 5 g, POPOP (Amersham Searle Corp.) 0.3 g dissolved in toluene to make a final volume of 1,000 ml) radioactivity was measured in a liquid scintillation spectrometer (Packard Instrument Co., Inc., Downer's Grove, Ill.) sufficient counts being obtained to reduce counting error to within 3%. Quenching was monitored by means of an external standard, and radioactivity was expressed as net dpm/10^7 neutrophils. Results were plotted as a function of time on arithmetic graph paper to obtain a curve representing net neutrophil radioactivity in circulation. From this curve, which represented the sum of influx from marrow and exit into tissues the time of maximum rate of entry of labeled neutrophils was determined using the formula:

$$g(t) = cH(t) + H'(t)$$[3]

[3] The neutrophil is considered to spend a period of time, X, in the marrow, then is released into the circulating blood where it spends an additional amount of time, Y, before leaving the blood. If $H(t)$ is the fraction of injected radioactivity that is in the blood at time t, then:

$$H(t) = \text{Prob } X < t \text{ and } X + Y > t$$

If X has density function $g(x)$, and Y has density ce^{-cy}

$$H(t) = \int_o^t \int_{t-x}^{\infty} ce^{-cy} g(x)dydx = \int_o^t e^{-c(t-x)} g(x)dx$$

where $g(t)$ represents the rate of entry of labeled neutrophils at time t, $H(t)$ the slope of the curve at time t, and $H'(t)$ the observed value for neutrophil radioactivity in circulation at time t. The decay constant, c, is ln 2/7.6, determined from the mean half-disappearance time of ^{3}Htdr-labeled neutrophils in normal subjects. Neutrophil marrow transit time was derived from the time of maximum rate of entry by subtracting the average time required for 50% of the last myelocyte generation to enter the metamyelocyte compartment. This interval was assumed to be 8 h on the basis of the data of Cronkite et al (21). The turnover of neutrophils in the postmitotic pool was then calculated by dividing the number of postmitotic neutrophils by the marrow transit time.

Blood neutrophil turnover was measured using isologous whole blood labeled in vivo with ^{3}Htdr. Five normal individuals over 50 yr of age with negative tests for syphilis and hepatitis B antigen were given an intravenous injection of ^{3}Htdr 50 μCi/kg body weight. Approximately 500 ml of blood was withdrawn from each subject 7 days later at the expected zenith of neutrophil ^{3}H-specific activity and, after removal of a 30-ml standard, transfused into each of five normal men, aged 19–26, with no transfusion history but who were established as positive for hepatitis B antigen by radioimmune assay (Austria II-125 Abbott Laboratories, Diagnostics Division North Chicago, Ill.). Donors and recipients were matched with respect to the presence or absence of the erythrocyte antigens, D, c, and K, and serological compatibility tests were negative. In three studies blood was also labeled in vitro with [^{32}P]DFP, (Amersham Searle Corp.), using a maximum dose of 50 μCi ^{32}P and 1 μg DFP/ml blood (22). Radioactivity of the two isotopes was measured simultaneously in separate channels. Correction for cross counting was 2.3% for ^{32}P in the ^{3}H channel. Counting efficiency was 80% for ^{32}P and 35% for ^{3}H. In two subjects, blood neutrophil turnover was measured with [^{32}P]DFP labeled autologous cells 2 wk after the ^{3}Htdr study. For rapid infusion of this labeled blood, a 14-gauge needle was used; only 7 min were required to complete the transfusion, which was preceded by a 500-ml phlebotomy. Neutrophil preparations were made by the method described above. Radioactivity, expressed as net dpm/10 ml blood, was determined on triplicate 10-ml standard samples drawn from the bag before transfusion and on 40-ml samples drawn from the recipient at 5 min and 1, 3, 5, 7, 9, and 12 h after transfusion. Sufficient counts were obtained to reduce statistical error in the case of both isotopes to within 3%. Disappearance curves were drawn by using the method of least squares. Recovery was calculated from the extrapolated radioactivity at zero time (22) and the estimated blood volume (23). Total blood neutrophils, marginal pool size, and turnover rate were calculated as described by Mauer et al. (22).

The magnitude of error in measuring neutrophil specific activity due to other labeled blood components was assessed by two methods. Error due to combined plasma and erythrocyte radioactivity was measured by examining the filtrate

Thus

$$e^{ct}H(t) = \int_o^t e^{cx} g(x)dx$$

and differentiating both sides with respect to t gives

$$ce^{ct}H(t) + e^{ct}H'(t) = e^{ct}g(t)$$

or

$$g(t) = cH(t) + H'(t)$$

TABLE III

Marrow Neutrophil Numbers in 13 Normal Human Subjects

Subjects	Sex and age	Serum iron	EIT	Marrow normoblasts	Marrow neutrophils: Promyelocytes NE	Promyelocytes no.*	Myelocytes NE	Myelocytes no.	Metamyelocytes and bands NE	Metamyelocytes and bands no.	Segmented neutrophils NE	Segmented neutrophils no.	Total marrow neutrophils NE	Total marrow neutrophils no.
		mg/100 ml	*mg/100 ml per day*											
1	M, 26	93	0.59	5.57	0.12	0.67	0.30	1.67	0.59	3.29	0.46	2.56	1.47	7.63
2	M, 25	135	0.48	4.53	0.13	0.59	0.32	1.45	0.64	2.90	0.52	2.36	1.61	8.35
3	M, 22	108	0.65	6.14	0.14	0.86	0.33	2.03	0.67	4.11	0.46	2.82	1.60	8.30
4	F, 21	77	0.68	6.33	0.12	0.76	0.28	1.77	0.63	3.99	0.52	3.29	1.55	8.04
5	F, 30	66	0.49	4.53	0.10	0.45	0.25	1.13	0.66	2.99	0.51	2.31	1.52	7.90
6	M, 21	145	0.47	4.43	0.13	0.58	0.31	1.37	0.67	2.97	0.42	1.86	1.53	7.94
7	F, 18	90	0.48	4.53	0.13	0.59	0.32	1.45	0.63	2.85	0.47	2.13	1.55	8.04
8	M, 45	83	0.45	4.16	0.12	0.50	0.28	1.16	0.76	3.16	0.41	1.71	1.57	8.15
9	F, 28	121	0.43	4.06	0.12	0.49	0.29	1.18	0.60	2.44	0.49	1.99	1.50	7.78
10	F, 52	114	0.48	4.44	0.14	0.62	0.28	1.24	0.62	2.75	0.44	1.95	1.48	7.67
11	M, 55	93	0.62	5.84	0.11	0.64	0.27	1.58	0.60	3.52	0.38	2.23	1.36	7.06
12	M, 50	90	0.53	4.90	0.14	0.69	0.32	1.57	0.74	3.63	0.39	1.92	1.59	8.24
13	M, 53	60	0.70	6.51	0.11	0.72	0.25	1.63	0.72	4.69	0.35	2.28	1.43	7.43
Mean		98.1	0.54	5.07	0.12	0.63	0.29	1.48	0.66	3.33	0.45	2.26	1.52	7.70
SD		25.4	0.09	0.84	0.01	0.11	0.03	0.26	0.05	0.61	0.05	0.42	0.07	1.20

* All data for cell numbers are expressed as the number $\times 10^9$/kg body weight.

from eight 5-ml volumes of heparinized blood passed over nylon fiber columns (Nylon wool 0.1 g (Fenwall Laboratories, Inc., Morton Grove, Ill.) Packed tightly in silastic packed medical grade tubing, 0.104 inch internal diameter (Dow Corning Corp., Midland, Mich.) 4.5 cm in length.) at a constant rate of 30 μl/min (Harvard Infusion Pump, Harvard Apparatus Co., Ltd., Millis, Mass.). Rare platelets and lymphocytes and no neutrophils were observed microscopically. After adding [^{32}P]DFP (1 μg/ml), the filtrate was divided into four 10-ml portions and processed by the Ficoll-Hypaque method described above. The residual radioactivity amounted to 0.07% of the mean radioactivity in 19 standards prepared for neutrophil survival studies.

The magnitude of error due to contamination of the neutrophil fraction by platelets and other leukocytes was assessed as follows: 120 ml acid citrate-anticoagulated blood from a normal subject was divided into two equal portions. One portion was labeled with [^{32}P]DFP as described above, and the other was set aside. The labeled blood was divided into six samples of 10 ml each. Neutrophil fractions were prepared as described for measurement of neutrophil specific activity. A suspension of labeled platelets, lymphocytes, and monocytes was prepared by combining the supernates after Ficoll-Hypaque sedimentation. The suspension was centrifuged at 1,000 *g* for 15 min, and the cell button was resuspended in 5 ml unlabeled autologous plasma. Radioactivity was measured in a 0.5-ml sample designated the standard. The second portion of blood was divided into six 10-ml samples. To each was added 0.5 ml of the mixed cell suspension prepared from the labeled portion. Neutrophil specific activity was measured after sample processing. Radioactivity in the six samples to which the labeled cell suspension had been added was 5.32±2.53 dpm, 0.05% of the radioactivity in the standard. Mean neutrophil specific activity in these samples was 5.08±2.50 dpm/10^7 cells, 0.06% of the mean neutrophil specific activity (8933.6±477.0 dpm/10^7 cells) in the samples of labeled blood.

Leukocyte concentration was measured with a Coulter model B electronic particle counter (Coulter Electronics, Inc., Hialeah, Fla.) using centrimide (24) for erythrocyte lysis. Blood films were made on coverslips with a blood film centrifuge (Platt General Co., Ltd., Feasterville, Pa.), air-dried, and stained with Wright's stain. Differential counts were made on 400 consecutive cells by traversing the length of the middle third of the coverslip.

RESULTS

In sections from marrow biopsies prepared as described both erythroid and neutrophil elements were easily recognized (Fig. 1). Beginning with promyelocytes, all neutrophils were clearly identified by the presence of azurophilic cytoplasmic granulation. Maturational stages were determined by nuclear configuration.

Marrow neutrophil cellularity was calculated for each subject from the NE ratio and ferrokinetic determination of normoblast numbers. The results for each maturational category are shown in Table III. The mean and SD for promyelocytes was 0.63±0.11, for myelocytes 1.48±0.26, for metamyelocytes and bands 3.33± 0.61 and for segmented neutrophils 2.26±0.42 $\times 10^9$ cells/kg. The total number of marrow neutrophils in thirteen normal human volunteers was 7.70±1.20 $\times 10^9$ cells/kg body weight. The average number of postmitotic neutrophils was calculated to be 5.59±0.90 $\times 10^9$ cells/kg.

Neutrophil marrow transit time was determined in eight normal subjects (Fig. 2). A composite curve representing appearance in circulation of ^{3}H-labeled neutrophils is shown as the solid line in Fig. 3. Low levels of radioactivity were found during the first 4 days after injection of ^{3}Htdr. After 5½ days neutrophil specific activity rose in a linear fashion over a 42-h

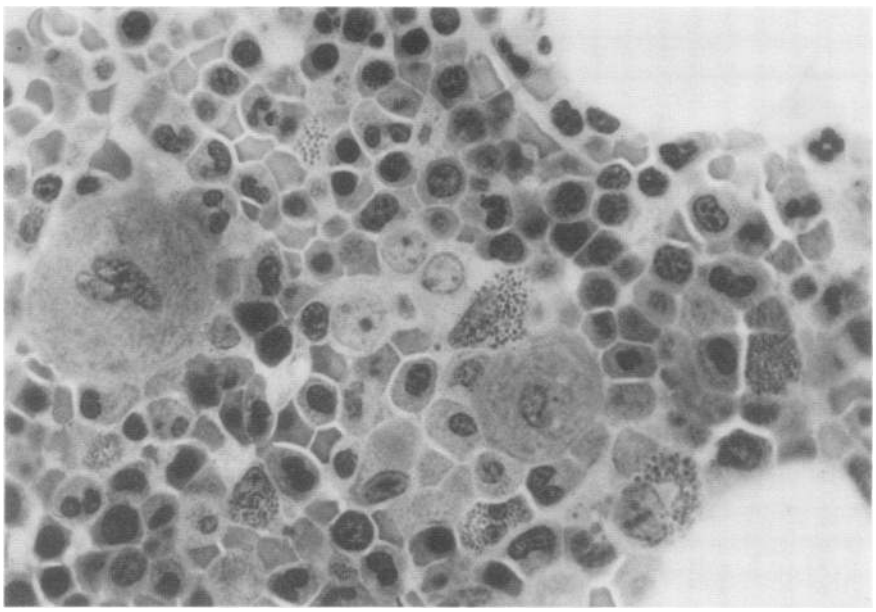

FIGURE 1 Stained section of normal marrow biopsy × 400.

period. Because the measured activity in circulation was the result of input from the marrow and egress into tissues, the data for each subject were corrected for a simultaneous random removal, assuming a half-disappearance time of 7.6 h (See Table V). The interrupted curve shown in Fig. 3 represents the calculated rate of neutrophil release from marrow into circulation. The time of maximum neutrophil appearance occurred 6.9 days±0.03 after injection of the isotope. When corrected for the 50% entrance time into the postmitotic pool of 8 h, the postmitotic transit time was 6.6 days±0.03.

The rate of marrow neutrophil production in normal subjects, determined by dividing the postmitotic neutrophils (5.59×10^9 cells/kg) by the transit time across the postmitotic pool (6.6 days), was 0.85×10^9 cells/kg per day. Similar results were obtained from three additional subjects in whom measurements of EIT, NE ratio, postmitotic pool mass, and marrow transit time were all performed (Table IV).

Initial studies of neutrophil turnover in circulation were carried out using autologous cells labeled with [^{32}P]DFP. In 19 normal volunteers the mean and stan-

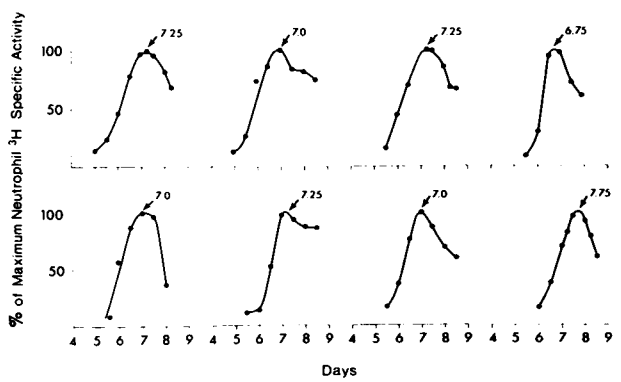

FIGURE 2 Appearance of neutrophil activity in circulating blood. ^{3}H-tdr 50 μCi/kg was injected intravenously in eight normal subjects and neutrophil specific activity measured at intervals over eight days. The data for each subject were expressed as percent of maximum activity.

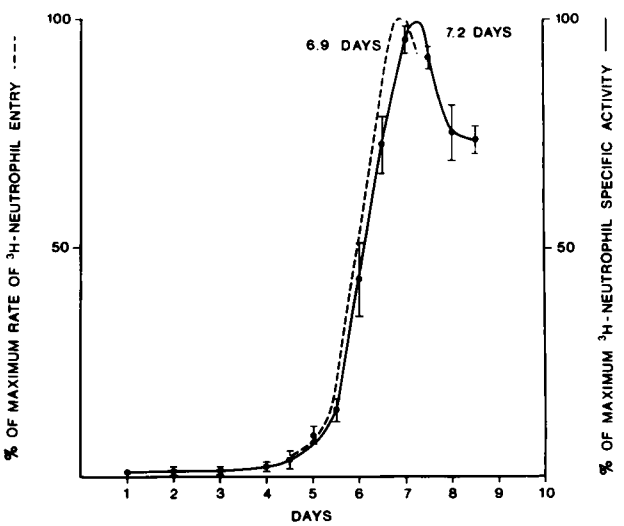

FIGURE 3 Blood appearance time of ^{3}H-tdr labeled neutrophils. The solid line was drawn through points representing ^{3}H-tdr neutrophil activity in circulation (mean±SE of eight determinations). The interrupted line represents the rate of entry of ^{3}H-tdr labeled neutrophils and was drawn by correcting each observed point for simultaneous egress of labeled cells from circulation as described in Methods.

TABLE IV

Marrow Neutrophil Production

Subject	EIT	Normoblasts	NE* ratio	Postmitotic neutrophil numbers	Marrow transit time	Marrow neutrophil turnover
	mg/100 ml per day	*10^9/kg*		*10^9/kg*	*days*	*10^9/kg per day*
V. G.	0.69	6.51	1.07	6.96	6.7	1.04
R. B.	0.62	5.84	0.98	5.72	6.6	0.87
E. S.	0.52	4.90	1.12	5.49	6.7	0.82
Mean	0.61	5.75	1.06	6.06	6.7	0.91

* Ratio of postmitotic marrow neutrophils to normoblasts.

dard deviation for total blood neutrophils was 0.61± 0.18 × 10^9 cells/kg; marginal pool, 0.41±0.17 × 10^9 cells/kg; half disappearance time 5.7±1.2 h; and neutrophil turnover rate, 1.73±0.36 × 10^9 cells/kg per day. Survival curves were obtained in five normal subjects where neutrophils were labeled in vivo with ^{3}Htdr and in vitro with [^{32}P]DFP. ^{32}P count rates at zero time ranged from 1,050 to 8,000 net cpm; ^{3}H count rates lay between 340 and 2,780 net cpm at zero time.

In Table V are given the measured half-disappearance times and percent recoveries and the calculated blood pool sizes and neutrophil turnover rates for both isotope labels. Mean recovery for [^{32}P]DFP was 47.9± 19.6%; half-disappearance time was 5.4±1.2 h, and neutrophil turnover was 1.62±0.46 × 10^9 cells/kg per day. Corresponding data based on the ^{3}Htdr label were 58.5±12.9%, 7.6±1.8 h and 0.87±0.13 × 10^9 cells/kg per day. In the three subjects in whom the two labels were applied to the same population of cells blood neutrophil turnover was 2.09, 1.18, and 1.96 × 10^9 cells/kg per day in the case of [^{32}P]DFP, and 0.80, 0.69, and 1.00 × 10^9 cells/kg per day in the case of ^{3}Htdr. A comparison of the half disappearance times of [^{32}P]DFP and ^{3}Htdr labeled cells by an analysis of regression lines showed that the slopes were significantly different in four of the five subjects ($P < 0.01$, < 0.05, < 0.001, and < 0.05); the recovery calculations were significantly different in three of the five subjects ($P < 0.05$, < 0.005, and < 0.05). Mean neutrophil turnover rates calculated for the two labels also differed significantly ($P < 0.02$).

Composite curves comparing mean recovery and survival of neutrophils labeled with ^{3}Htdr and [^{32}P]DFP are shown in Fig. 4.

DISCUSSION

To define neutrophil kinetics, production by the marrow was compared with neutrophil turnover in the circulation. Previous measurements of marrow erythrocyte production (total erythropoiesis) and erythrocyte turnover in the blood (effective erythropoiesis) (25), and comparable measurements of megakaryocytopoiesis and platelet turnover (26) indicate equivalent rates of total and effective production in normal subjects for these two cell systems. It was therefore anticipated that similar measurements of neutrophil production would provide an internal assessment of their quantitative validity.

The measurement of marrow neutrophil cellularity is derived from the NE ratio and the calculated number of marrow normoblasts. The accuracy depends on both the

TABLE V

Comparison of [^{32}P]DFP *and* ^{3}*Htdr as Neutrophil Labels*

Subjects	Recovery		$t_{\frac{1}{2}}$		Circulating neutrophils		Marginal pool		Total blood neutrophils		Neutrophil turnover through circulation	
	^{3}H	[^{32}P]DFP	^{3}H	[^{32}P]DFP	^{3}H	[^{32}P]DFP	^{3}H	[^{32}P]DFP	^{3}H	[^{32}P]DFP	^{3}H	[^{32}P]DFP
	%		*h*		*10^9/kg*						*10^9/kg/day*	
1*	63.6	39.9	9.6	5.8	0.29	0.29	0.17	0.44	0.46	0.74	0.80	2.09
2*	72.7	75.7	9.1	5.1	0.27	0.27	0.10	0.09	0.38	0.36	0.69	1.18
3*	45.6	42.8	7.9	4.3	0.21	0.21	0.26	0.29	0.48	0.51	1.00	1.96
4‡	66.6	23.6	5.4	7.5	0.17	0.18	0.12	0.61	0.30	0.80	0.92	1.78
5‡	44.0	57.3	6.2	4.5	0.15	0.16	0.20	0.13	0.36	0.29	0.95	1.08
Mean	58.5	47.9	7.6	5.4	0.22	0.22	0.17	0.31	0.40	0.54	0.87	1.62
SD	12.9	19.6	1.8	1.2	0.05	0.54	0.08	0.21	0.07	0.22	0.13	0.46

* Studied using isologous blood labeled simultaneously with ^{3}Htdr and [^{32}P]DFP.

‡ Autologous blood used for [^{32}P]DFP survival study, isologous blood for ^{3}Htdr study.

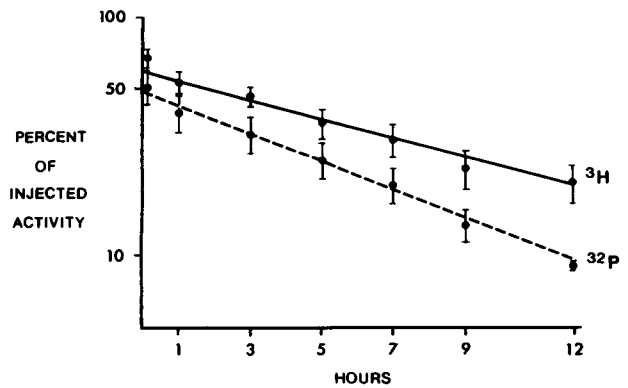

FIGURE 4 Blood neutrophil ^{3}H and ^{32}P activity. Each point represents percent of activity injected. The solid and interrupted lines were drawn by the method of least squares through points representing mean circulating ^{3}H and ^{32}P activity, respectively. Vertical bars represent 1 SEM.

quantitative evaluation of marrow sections and the determination of normoblast cellularity. The ratio of neutrophils to normoblasts in the marrow has traditionally been determined from marrow aspirates (27) or suspensions (28). Reported values for this ratio obtained from aspirates from normal subjects have demonstrated considerable variation but generally are in the order of 3:1 (25, 27, 29). These methods are subjects to both cell destruction and sampling errors and are considered too imprecise for accurate quantitative work. In marrow aspirates variable numbers of cells are devoid of cytoplasma and therefore unclassifiable. Reiff et al. (30) used radioiron as a cytoplasmic marker, and found that as much as 40% of normoblast hemoglobin was lost during preparation of marrow suspensions from normal subjects. Furthermore, sinusoidal blood may contribute to the number of mature neutrophils counted in aspirates, and aspiration may harvest a selected population of cells. Since neutrophil deformability bears a relationship to cell maturity (31), a disproportionately greater number of segmented neutrophils, bands, and metamyelocytes might appear in aspirated preparations. The section technique employed to determine the NE ratio is believed to eliminate error due to cell breakage or selection. Size correction is applied to derive true cell counts from the raw section counts, since cells are variably over-counted in thin sections according to cell diameter and section thickness (9). Use of a factor for multiple counting established absolute ratios between cell types. Counting neutrophils and normoblasts among 10,000 consecutive cells of all types in duplicate reduces variation to less than±10%.

The validity of the criteria for assigning cells in section to the mitotic pool was tested by examining the morphologic characteristics of marrow neutrophils labeled *in situ* with ^{3}Htdr. Marrow sampling 30 min after local injection of thymidine ensured that no label would have appeared in the nonmitotable progeny of the last myelocyte stage.

The representative nature of a single marrow biopsy may also be questioned. Although Kindred (32) suggested a 10% difference in relative numbers of nucleated erythrocytes in long bones of rats as compared with central marrow, the marrow section NE ratio in dogs was found to be consistent at various sites in both axial and peripheral skeleton (33, 34). Furthermore, Donohue et al. (28) found insignificant variation in differential counts carried out on marrow from two or more areas in man, consistent with the present report. In these studies uniform ratios were obtained from opposite sides of the pelvis and within the same biopsy.

The absolute number of nucleated red cells has been derived from ferrokinetic measurements in which certain assumptions were required concerning nonheme iron exchange, the size of the normal marrow reticulocyte pool and constant iron uptake per erythrocyte (35). The direct relationship between iron turnover and total erythropoiesis in normal subjects is established (36). The ferrokinetic determination of normoblast numbers has been previously validated by demonstrating a direct relationship between normoblast estimates derived from the PIT on the one hand and from measurement of platelet turnover and normoblast-megakaryocyte ratios on the other (11). While the EIT and normoblast mass are correlated, the absolute number of normoblasts must be derived from other data. The value of 5.29×10^{9} normoblasts/kg employed in this study is based upon the daily erythrocyte turnover, the marrow reticulocyte transit time (13), the ratio of marrow reticulocytes to normoblasts (13, 14), and an assumed 15% ineffective erythropoiesis (15). The figure of 5.29×10^{9}/kg is in general agreement with published figures for normoblast mass in the dog of 5.48 (33) and 6.7 (37), in the rat of 5.55 (38), in the rabbit of 5.80 (38), and in the guinea pig of 6.7×10^{9}/kg (39). It also corresponds to the value in man of 5.6×10^{9}/kg reported by Donohue et al. (38).

Previous estimates of marrow neutrophil number in normal man have been widely disparate. Osgood, from the turnover of circulating erythrocytes, an assumed maturation time of normoblasts, and the NE ratio, estimated that the marrow contained 25.7×10^{9} neutrophils/kg (40). Patt, by using the erythrocyte production rate, the NE ratio, and available data for the neutrophil mitotic index and mitosis time, arrived at an estimate of 8.3×10^{9} (37). Cartwright et al. (1) used the "myelocyte-blood transit time" measured in vivo with [^{32}P]DFP and the "granulocyte turnover rate" calculated from in vitro [^{32}P]DFP granulocyte survival

data to calculate 18.6×10^9 total marrow granulocytes/kg.

Donohue et al. (28) applied a radioiron dilution technique to obtain a more direct measurement of human marrow cellularity. The number of marrow neutrophils was calculated by multiplying total marrow cellularity by the proportion of neutrophils in suspensions of rib marrow (38); the mean value for neutrophil mass was found to be 11.4×10^9/kg. This measurement of marrow cellularity depended on a questionable (9, 41) correction of 29% for normoblast breakage. By omitting this correction (41), neutrophil numbers are reduced to 8.09×10^9/kg, very close to our measurement of 7.70×10^9/kg. Donohue's original figures of 8.8 for the postmitotic pool and 2.6×10^9/kg for myelocytes (38) then become 6.2 and 1.8×10^9/kg, in agreement with our measurements of 5.6 and 1.5×10^9/kg.

A variety of nuclear and cytoplasmic tags have been employed to estimate marrow transit time (42). ^{3}Htdr was chosen for this study because its uptake is confined to the phase of DNA synthesis, and because of its limited reutilization. An assumption was made that the time of maximum emergence from marrow was a valid measure of mean transit time. The appearance curve of label in circulating neutrophils reaches a sharp peak permitting objective definition of the time of maximum circulating activity (Fig. 2). Allowance has been made for entrance time into the postmitotic pool and for the effect of the rate of departure of the tagged cells from circulation. Our measurement of 6.6 days for the mean marrow transit time is in agreement with those of Perry et al. (43). Employing this value for postmitotic pool transit time and the pool number of 5.59×10^9 cells/kg, marrow neutrophil turnover was calculated to be 0.85×10^9 cells/kg per day. This figure is lower than production rates calculated by others. For example, based on the postmitotic pool previously determined by Donohue et al. (8.88×10^9 cells/kg) the turnover rate is calculated to be 1.34×10^9 cells/kg per day. On the basis of [^{32}P]DFP studies, Cartwright et al. calculated neutrophil turnover to be 1.63×10^9 cells/kg per day (1).

Initial studies on marrow quantitation were carried out in the dog (33). Particular attention was directed to quantitation of neutrophil precursors in the marrow by relating the number of neutrophils in marrow sections to total marrow neutrophils through a marrow radioiron label. The relationship between marrow cellularity and EIT was then established so that marrow cellularity could be determined by measuring plasma iron kinetics. The validity of these studies was established in two ways: (*1*) a comparison of neutrophil production in the marrow determined from the number of postmitotic neutrophils and their transit times was found to be in close agreement with the ^{3}Htdr turnover of circulating neutrophils (44); and (*2*) in leukopheresis studies, the sum of the deficit induced in the postmitotic pool and the calculated loss from circulation accurately corresponded to the number of neutrophils removed (33). In the present study data has been collected which permits the first comparison of total and effective neutrophil production in man.

There was a discrepancy between [^{32}P]DFP measurements and ^{3}Htdr measurements of blood neutrophil turnover of the same magnitude as previously documented in the dog (33, 44). Our [^{32}P]DFP data in man were similar to those previously published; approximately 50% of the isotope disappeared at once and the subsequent $t_{\frac{1}{2}}$ was 5.7 h. Cartwright et al. have reported studies of 109 normal subjects showing a mean recovery deficit of 56% of injected activity and a $t_{\frac{1}{2}}$ of 6.7 h (1). When ^{3}Htdr was used as the neutrophil label, however, the marginal pool was only 43% of injected activity, the half-disappearance time was 7.6 h. Furthermore, when a single population of cells was tagged with both isotopes in three studies, neutrophil turnovers calculated from ^{32}P recoveries and half-disappearance times were 2.6, 1.7, and 2.0-fold greater than those calculated from measurements based on the ^{3}H label. The differences in immediate recovery and $t_{\frac{1}{2}}$ disappearance rate between these two isotopes resulted in a calculated daily neutrophil turnover twice as great with [^{32}P]DFP as with ^{3}Htdr (1.73 vs. 0.87×10^9 cells/kg per day).

The turnover of blood neutrophils labeled with ^{3}Htdr is in accord with the turnover of postmitotic neutrophils in the marrow. The higher turnover obtained with [^{32}P]DFP labeled neutrophils observed in studies using doubly labeled neutrophils in man (subjects 1, 2, and 3, Table V) and dogs (44) may be due to elution, as occurs with both erythrocytes and platelets (45, 46). Hansen (47) has suggested that a linear relationship exists between turnover of neutrophils and turnover of their lysozyme content. In five normal subjects, the lysozyme turnover rate was 0.75×10^9 cells/kg per day in contrast with 1.42×10^9 cells/kg per day obtained with [^{32}P]DFP labeled cells. Thus, the lysozyme turnover rate corresponds with the rate we obtained with ^{3}Htdr and is clearly discrepant with Hansen's (and our) [^{32}P]DFP turnover data.

From the present study some idea of the dimensions and kinetics of the neutrophil series may be obtained. Within the marrow, 0.85×10^9 postmitotic neutrophils per kg body weight are generated daily. The postmitotic pool contains sufficient maturing and storage neutrophils to supply the basal turnover of 0.85×10^9 cells/kg per day for 6.6 days. Turnover of circulating neutrophils was observed to be 0.87×10^9 cells/kg per day; thus, it appears that in normal individuals there is no appreciable wastage at the level of the postmitotic pool during

neutrophil production. Parallel data have been obtained in dogs where effective production matched total production (44).

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Jean Greenaway, Marylin Winkler, and Connie Vandeventer, and the assistance of Dr. Eloise Giblett in reviewing the manuscript. We are indebted to Dr. George Marsaglia for his interest and advice concerning the mathematics described in Methods.

This work was supported by research grants (HL-06242 and HL-11775) and training grant (AM-05130) from the National Institutes of Health. Fellowship stipend support was provided by the Medical Research Council of Canada (Dr. Dancey) and by the Swiss National Foundation (Dr. Deubelbeiss). A portion of this work was conducted through the Clinical Research Center's facility of the University of Washington with the support of grants (FR-37 and FR-133) from the National Institutes of Health.

REFERENCES

1. Cartwright, G. E., J. W. Athens, and M. M. Wintrobe. 1964. The kinetics of granulopoiesis in normal man. *Blood.* **24**: 780–803.
2. Galbraith, P. R., L. S. Valberg, and M. Brown. 1965. Patterns of granulocyte kinetics in health, infection and in carcinoma. *Blood.* **25**: 683–692.
3. Williams, W. J. and A. S. Schneider. 1972. Examination of the peripheral blood. *In* Hematology. W. J. Williams, E. Beutler, A. J. Erslev, and R. W. Rundles, editors. McGraw Hill Book Co., N. Y. 11. Table 2-1.
4. Rutenberg, A. M., C. L. Rosales, and J. M. Bennett. 1965. An improved histochemical method for the demonstration of leukocyte alkaline phosphatase activity: Clinical application. *J. Lab. Clin. Med.* **65**: 698–705.
5. Ellis, L. D., W. N. Jensen, and M. P. Westerman. 1964. Needle biopsy of bone marrow. An experience with 1,445 biopsies. *Arch. Intern. Med.* **114**: 213–221.
6. Jamshidi, K., and W. R. Swaim. 1971. Bone marrow biopsy with unaltered architecture: A new biopsy device. *J. Lab. Clin. Med.* **77**: 335–342.
7. Dancey, J. T., K. A. Deubelbeiss, and L. A. Harker. 1976. Section preparation of human marrow for light microscopy. *J. Clin. Pathol. (Lond.).* In press.
8. Goodell, B. W., and V. Devita. 1970. Rapid *in vivo* cell kinetic studies in human acute leukemia by intramarrow double labelling with ^{3}H and ^{14}C-thymidine. *Proc. Am. Assoc. Cancer Res.* **11**, 120a: 31.
9. Harker, L. A. 1968. Megakaryocyte quantitation. *J. Clin. Invest.* **47**: 452–457.
10. Copeland, B. E. 1957. Standard deviation. A practical means for the measurement and control of the precision of clinical laboratory determinations. *Am. J. Clin. Pathol.* **27**: 551–558.
11. Finch, C. A., K. Deubelbeiss, J. D. Cook, J. W. Eschbach, L. A. Harker, D. D. Funk, G. Marsaglia, R. S. Hillman, S. J. Slichter, J. W. Adamson, A. Ganzoni, and E. R. Giblett. 1970. Ferrokinetics in man. *Medicine. (Baltimore).* **49**: 17–53.
12. Cook, J. D., G. Marsaglia, J. W. Eschbach, D. D. Funk, and C. A. Finch. 1970. Ferrokinetics: a biologic model for plasma iron exchange in man. *J. Clin. Invest.* **49**: 197–205.
13. Labardini, J., T. Papayannopoulou, J. D. Cook, J. D. Adamson, R. D. Woodson, J. W. Eschbach, R. S. Hillman, and C. A. Finch. 1973. Marrow radioiron kinetics. *Haematologia.* **7**: 301–312.
14. Seip, M. 1953. Reticulocyte studies. The liberation of red blood corpuscles from the bone marrow into the peripheral blood and production of erythrocytes elucidated by reticulocyte investigations. *Acta Med. Scand. Suppl.* 282, **146**: 1–164.
15. London, I. M., R. West, D. Shemin, and D. Rittenberg. 1950. On the origin of bile pigment in normal man. *J. Biol. Chem.* **184**: 351–358.
16. Chaplin, H., Jr., P. L. Mollison, and H. Vetter. 1953. The body/venous hematocrit ratio: Its constancy over a wide hematocrit range. *J. Clin. Invest.* **32**: 1309–1316.
17. Reeve, E. B., and M. Veall. 1949. A simplified method for the determination of circulating red-cell volume with radioactive phosphorus. *J. Physiol. (Lond.).* **108**: 12–23.
18. Wadsworth, G. R. 1954. The blood volume of normal women. *Blood.* **9**: 1205–1207.
19. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. and Lab. Invest.* **21**(Suppl. 97): 77–89.
20. Rothstein, G., C. R. Bishop, J. W. Athens, and H. E. Ashenbrucker. 1971. A method for leucokinetic study in the nonsteady state. *Blood.* **38**: 302–311.
21. Cronkite, E. P., V. P. Bond, T. M. Fliedner, and S. A. Killman. 1960. The use of tritiated thymidine in the study of haemopoietic cell proliferation. Ciba Foundation Symposium on Hemopoiesis. No. 13. 70–92.
22. Mauer, A. M., J. W. Athens, H. Ashenbrucker, G. E. Cartwright and M. M. Wintrobe. 1960. Leukokinetic studies. II. A method for labeling granulocytes *in vitro* with radioactive diisopropylfluorophosphate [^{32}P]DFP. *J. Clin. Invest.* **39**: 1481–1486.
23. Nadler, S. B., J. U. Hildalgo, and T. Bloch. 1962. Prediction of blood volume in normal human adults. *Surgery (St. Louis).* **51**: 224–232.
24. Gagon, T. E., J. W. Athens, D. R. Boggs, and G. E. Cartwright. 1966. An evaluation of the variance of the leukocyte counts as performed with the hemocytometer, Coulter, and Fisher instruments. *Am. J. Clin. Pathol.* **46**: 684–691.
25. Giblett, E. R., D. H. Coleman, G. Pirzio-Biroli, D. M. Donohue, A. G. Motulsky, and C. A. Finch. 1956. Erythrokinetics: Quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. *Blood.* **11**: 291–309.
26. Harker, L. A., and C. A. Finch. 1969. Thrombokinetics in man. *J. Clin. Invest.* **48**: 963–974.
27. Williams, W. J. 1972. Examination of the bone marrow. *In* Hematology. W. J. Williams, E. Beutler, A. J. Erslev, and R. W. Rundles, editors. McGraw Hill Book Co., N. Y. 26. Table 3-1.
28. Donohue, D. M., B. W. Gabrio, and C. A. Finch. 1958. Quantitative measurement of hematopoietic cells of the marrow. *J. Clin. Invest.* **37**: 1564–1570.
29. Cartwright, G. E. 1963. Diagnostic laboratory hematology. 3rd edition. Grune and Stratton, N. Y. 237. Table 66.
30. Reiff, R. H., J. Y. Nutter, D. M. Donohue, and C. A. Finch. 1958. The relative number of marrow reticulocytes. *Am. J. Clin. Pathol.* **30**: 199–203.
31. Lichtman, M. A., and R. I. Weed. 1972. Alteration of the cell periphery during granulocyte maturation: Relationship to cell function. *Blood.* **39**: 301–316.

32. Kindred, J. E. 1942. A quantitative study of the hemopoietic organs of young adult albino rats. *Am. J. Anat.* **71**: 207–243.
33. Deubelbeiss, K. A., J. T. Dancey, L. A. Harker, B. Cheney, and C. A. Finch. 1975. Marrow erythroid and neutrophil cellularity in the dog. *J. Clin. Invest.* **55**: 825–832.
34. Reckers, P. E., and M. Coulter. 1948. A hematological and histological study of the bone marrow and peripheral blood of the adult dog. *Am. J. Med. Sci.* **216**: 643–655.
35. Deubelbeiss, K. A., J. D. Cook, L. A. Harker, and C. A. Finch. 1971. The ferrokinetic measurement of marrow cellularity. *In* Platelet Kinetics: Radioisotopic, Cytological, Mathematical and Clinical Aspects. J. M. Paulus, editor. North Holland Publishing Co., Amsterdam. 161–166.
36. Hillman, R. S. 1969. Characteristics of marrow production and reticulocyte maturation in normal man in response to anemia. *J. Clin. Invest.* **48**: 443–453.
37. Patt, H. M. 1957. A consideration of myeloid-erythroid balance in man. *Blood.* **12**: 777–787.
38. Donohue, D. M., R. H. Reiff, M. L. Hanson, Y. Betson, and C. A. Finch. 1958. Quantitative measurement of the erythrocytic and granulocytic cells of the marrow and blood. *J. Clin. Invest.* **37**: 1571–1576.
39. Yoffey, J. M. 1954. Bone marrow. *Brit. Med. J.* **2**: 193–197.
40. Osgood, E. E. 1954. Number and distribution of human hemic cells. *Blood.* **9**: 1141–1154.
41. Harrison, W. J. 1962. The total cellularity of the bone marrow in man. *J. Clin. Pathol. (Lond.).* **15**: 254–259.
42. Dresch, C., A. Faille, J. Bauchet, and Y. Najean. 1973. Granulopoïèse: Comparaison de différentes méthods d'étude de la durée de maturation et des réserves médullaires. *Nouv. Rev. Fr. Hematol.* **13**: 5–22.
43. Perry, S., J. H. Moxley III, G. H. Weiss, and M. Zelen. 1966. Studies of leukocyte kinetics by liquid scintillation counting in normal individuals and in patients with chronic myelocytic leukemia. *J. Clin. Invest.* **45**: 1388–1399.
44. Deubelbeiss, K. A., J. T. Dancey, L. A. Harker, and C. A. Finch. 1975. Neutrophil kinetics in the dog. *J. Clin. Invest.* **55**: 833–839.
45. Hjort, P. F., H. Paputchis, and B. Cheney. 1960. Labelling of red blood cells with radioactive diiso propyl fluoro-phosphate ($DF^{32}P$), evidence for an initial release of label. *J. Lab. Clin. Med.* **55**: 416–424.
46. Bithell, T. C., J. W. Athens, G. E. Cartwright, and M. M. Wintrobe. 1967. Radioactive diiso propyl fluoro phosphate as a platelet label; an evaluation of in vitro and in vivo labelling techniques. *Blood.* **29**: 354–372.
47. Hansen, N. E. 1973. The relationship between the turnover rate of neutrophilic granulocytes and plasma lysozyme levels. *Br. J. Haematol.* **25**: 771–782.