Neutrophil Kinetics in the Dog

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

From the Division of Hematology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT The production of neutrophils in dogs has been estimated from the number of postmitotic neutrophils in the marrow and the transit time of a [^{3}H]-thymidine pulse. The number of postmitotic neutrophils was derived from the erythron iron turnover measurement of erythroid number and the neutrophil-erythroid ratio in bone marrow sections. The mean value for marrow postmitotic neutrophils in dogs was $5.61 \pm 0.56 \times 10^9$ cells/kg. The mean transit time of these neutrophils was calculated to be 82.1 h. A marrow production of 1.65×10^9 neutrophils/kg/day was calculated from these data.

The turnover of circulating neutrophils was measured by [^{3}H]thymidine and [^{32}P]diisopropylphosphofluoridate ($DF^{32}P$) labeling of blood neutrophils. [^{3}H]-Thymidine labeling gave a calculated recovery of 65%, a $t_{\frac{1}{2}}$ disappearance time of 6.7 h, and a calculated turnover of 1.66×10^9 cells/kg/day. Corresponding results with $DF^{32}P$ tagging were 51%, 5.4 h, and 2.89×10^9 cells/kg/day. The discrepancy between these two tags persisted in doubly tagged cells and was considered to be due to elution of $DF^{32}P$.

INTRODUCTION

The disappearance from the circulation of neutrophils labeled with [^{32}P]diisopropylphosphofluoridate ($DF^{32}P$)[1] has served as a basis for calculating the turnover of circulating neutrophils in both dogs (1) and man (2). These studies provide estimates of effective neutrophil production. In other studies using tritiated thymidine attempts to determine total marrow neutrophil production have been made (3, 4). However, these latter studies have been of limited usefulness because of the inadequate methods available for measuring marrow cellularity. In an accompanying paper, a method for determining the number of erythroid and neutrophilic cells in dog marrow has been described (5). In the present paper this measurement of marrow postmitotic neutrophils (PMN) is coupled with the measurement by [^{3}H]thymidine of neutrophil marrow transit time (MTT) to determine total neutrophil production. The results of such measurements are compared with blood neutrophil turnover as measured by [^{3}H]thymidine- and $DF^{32}P$-labeled granulocytes, thereby comparing estimates of *total* marrow neutrophil production and *effective* neutrophil production in the dog.

METHODS

Animals studied. Male and female mongrel dogs weighing 10–12 kg, immunized against canine distempter, and treated for parasites, were studied after an observation period of at least 3 wk. Basal hematologic studies on all animals showed hematocits greater than 40%, white cell counts ranging between 4,000 and 8,000/μl, and plasma iron values in the normal range of 1.0–2.5 μg/ml. In one group of animals marrow neutrophil turnover was derived from measurements of the number of PMN and their rate of transit. In other normal animals the turnover of circulating neutrophils[2] was determined with [^{3}H]thymidine- and $DF^{32}P$-labeled cells.

Measurement of marrow neutrophils. Methodology involved in determining the number of marrow neutrophils includes the ferrokinetic measurement of the nucleated red cell (NRBC) mass (5), and the measurement of the PMN/erythroid ratio (PMN/E) in bone marrow sections. From

Dr. Harker is an Established Investigator of the American Heart Association. Drs. Deubelbeiss and Dancey were recipients of fellowship stipends from (respectively) the Swiss National Foundation and the Medical Research Council of Canada.

Dr. Deubelbeiss's present address: Division of Hematology, Inselspital, Bern, Switzerland. Dr. Dancey's present address: Division of Hematology, Montreal General Hospital, Quebec, Canada.

Received for publication 21 May 1974 and in revised form 22 November 1974.

[1] *Abbreviations used in this paper:* ACD, acid citrate dextrose; $DF^{32}P$, [^{32}P]diisopropylphosphofluoridate; EIT, erythron iron turnover; H-F, Hypaque®-Ficoll®; MTT, neutrophil marrow transit time; N/E, neutrophil-erythroid; NRBC, nucleated red blood cell; PIT, plasma iron turnover; PMN, postmitotic neutrophil; PMN/E, postmitotic-erythroid.

[2] The term "neutrophils" instead of "granulocytes" will be employed in this paper, although it is recognized that these isotope compounds tag all species of granulocytes. Thus, the calculation of neutrophil number and turnover will include about 4% of eosinophils and basophils whose kinetics are not as yet well defined.

these two determinations the marrow neutrophil mass is calculated.

Plasma iron was measured by a modification of the method of Bothwell and Mallett (6). Erythron iron turnover (EIT) was calculated by subtracting the nonerythroid component (serum iron [μg/100 ml] $\times$ plasmatocrit $\times$ 0.0035) from the plasma iron turnover (PIT) (7). The total number of NRBC per kilogram was caculated from the following formula:

$$\text{NRBC (cells} \times 10^9/\text{kg)} = \text{EIT(mg Fe/100 ml/day)} \times \frac{5.48 \times 10^9}{0.90} \quad (1)$$

where 5.48×10^9 represents the number of NRBC per kilogram in normal dogs and 0.90 represents the mean basal EIT in mg Fe/100 ml whole blood/day (5).

A bone marrow biopsy obtained from the posterior iliac crest with an electrical drill was fixed in formalin and embedded in methacrylate as previously described (5). Sections 3 μm in thickness were stained with naphthol-ASD chloroacetate esterase to permit identification of primary granules of the neutrophils and was counterstained with 1% toluidine blue. Neutrophil-erythroid (N/E) ratios were based on the counts of 10,000 neutrophilic and erythroid cells in each of two random marrow sections. The number of PMN in the marrow was then determined according to the following formula:

$$\text{PMN (cells} \times 10^9/\text{kg)} = \text{NRBC (cells} \times 10^9/\text{kg)} \times \text{ratio} \frac{\text{PMN}}{\text{E}} \quad (2)$$

where the term PMN/E represents the ratio between PMN and NRBC.

Measurement of PMN transit time. The transit time of PMN through the marrow was determined from the appearance curve of radioactivity in circulation after the intravenous injection of 100 μCi/kg of tritiated thymidine ([*methyl*-H^3]thymidine, sp act: 40–60 Ci/mmol, sterile aqueous solution, New England Nuclear, Boston, Mass.) diluted with 0.9% NaCl. Duplicate 5-ml samples were collected in 0.75 ml acid citrate dextrose (ACD) (NIH formula A) 48 h later and every 8 h thereafter for a total of 6 days. Neutrophils were isolated for counting by Hypaque®-Ficoll® (H-F) sedimentation (8) in the following manner. Blood (5 ml diluted with 15 ml normal saline) was carefully layered over 9 ml H-F solution (10 parts 33.9% Hypaque® [Hypaque sodium 50% brand of sodium diatrizoate USC, Winthrop Laboratories, New York] mixed with 24 parts of 9% Ficoll® solution [Pharmacia Fine Chemicals AB., Uppsala, Sweden]) which had been adjusted to specific gravity of 1.077–1.078. The blood was then centrifuged at 400 *g* for 40 min at room temperature, and the material above the red cell/granulocyte layer was discarded. 6 ml of 3% dextran (mol wt: 200,000–300,000, ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland) was added and after 30 min the granulocyte-rich supernate was removed; 7 ml of 0.87% NH_4Cl was added to lyse residual red cells. The tubes were centrifuged at 220 *g* for 5 min and the supernate was discarded after which the neutrophils were resuspended in 12 ml of 0.87% NH_4Cl. A sample was removed for cell count and smear, and 10 ml of the neutrophil suspension was spun for 10 min at 2,500 *g*. The supernate was discarded and the residual button was solublized overnight in 1 ml NCS (NCS Tissue Solubilizer, Amersham/Searle Corp., Arlington Heights, Ill.). After the addition of 16 ml scintillation solution (toluene 1,000 ml, 2,5-diphenyloxazole [PPO] 5 g, 1,4-bis[2-(5-phenyloxazolyl)]benzene [POPOP] 0.3 g, Packard Instrument Co., Inc., Downers Grove, Ill.), radioactivity was measured in a liquid scintillation spectrometer (Packard Instrument Co., Inc.) (counting efficiency 38%) and was expressed as dpm per 10^6 cells (external standard correction for quenching). The samples were counted to the 3% level of statistical accuracy.

Differential counts of 400 cells were done on 60 random cell buttons, yielding a mean differential of 97.0±2.6% granulocytes, 0.9±1.5% red cells, and 1.9±1.5% mononuclear cells. In 20 subjects direct counts of platelets removed during H-F sedimentation amounted to 99±8% of the number originally counted in the sample.

The MTT was defined as the interval between the appearance of half of the activity derived from the last myelocyte generation into the postmitotic pool and the subsequent appearance of radioactivity in circulating blood. The former was assumed to be 5 h on the basis of previously published data (9), a value in accord with our own similar studies. The appearance of radioactivity in the peripheral blood was plotted through its intial rise and until it began to fall. Minimal isotope appearance time in peripheral blood was represented by extrapolation of the linear portion of this curve to the baseline. Since the decrease in the first wave of radioactivity was obscured by the appearance of tagged cells in subsequent generations, mean appearance time was taken to be the time of maximum entry of labeled neutrophils into the circulation, determined as follows:

$$F(t) = D(t) + S \cdot V(t) \quad (3)$$

where $F(t)$ represents the rate of entry of labeled neutrophils at time t, $D(t)$ the slope of the curve at time t, and $V(t)$ the mean observed values for neutrophil radioactivity in circulation at time t. S is a decay constant ln 2 divided by the half-time disappearance of [^{3}H]thymidine-labeled neutrophils in normal dogs. The mean figure for $t_{\frac{1}{2}}$ of 7.1 h obtained in 12 normal dogs was employed. The MTT was derived from the time of maximal rate of entry by subtracting the 5 h required for incorporation of 50% of radioactivity into the metamyelocyte compartment after [^{3}H]thymidine injection. The daily turnover of nonmitotable marrow neutrophils was calculated from the number of PMN in the marrow divided by the estimated MTT in days.

Measurement of blood neutrophil kinetics. The turnover of circulating neutrophils was measured in three different ways. The survival of [^{3}H]thymidine-labeled isologous neutrophils was measured by transfusing 100 ml ACD whole blood from an unmatched donor who had received 300 μCi/kg of [^{3}H]thymidine intravenously 4 days previously. 100 ml of blood was removed immediately before the infusion of tagged blood. Duplicate 7.5-ml ACD-anticoagulated blood samples were collected at 5 min and 1, 2, 3, 4, 6, 8, 11, and 24 h postinfusion. Neutrophils were isolated for counting as described above. A standard was similarly prepared from the labeled blood perceding transfusion. Radioactivity levels reached 20 times background in the 5-min blood sample and fell to background by 24 h. Reproductibility in determining the specific activity of blood neutrophils was assessed by counting 40 separately processed, random, duplicate samples; the coefficient of variation was 5.5%.

To determine the degree of tritium labeling of other blood cellular elements, the specific activities of mononuclear cells,

erythrocytes, platelets, and neutrophils were compared (Table I). Platelet-rich plasma was obtained by centrifugation at 200 *g* for 10 min; contaminating lymphocytes were then removed by a second spin at 500 *g* for 5 min. After two saline washes, platelet buttons were obtained by spinning at 1,300 *g* for 30 min. Mononuclear cells and neutrophils were separated by H-F sedimentation into their respective layers. The mononuclear cell layer was washed with saline and then spun at 1,500 *g* for 10 min. Red cells were prepared by sedimenting the granulocyte red cell fraction after H-F sedimentation twice in 3% dextran and washing the red cell sediment with saline. To obtain a similar degree of quenching among these buttons, 100 μl of 60% perchloric acid and 200 μl of 30% H_2O_2 were added to each sample (10). After digestion at 80°C for 60 min in a shaking water bath, 5 ml of ethoxyethanol and 10 ml of scintillation fluid (toluene 1,000 ml, 2,5-diphenyloxazole [PPO] 5 g, Packard Instrument Co., Inc.) were added. Tritium activity in a typical experiment showed 110 cpm/10^6 neutrophils, 25/10^6 mononuclear cells, 0.1/10^6 platelets, and 0.04/10^6 erythrocytes. On the basis of their respective proportions in the blood, nonneutrophilic cells contained less than 1% of the sample activity. Radioactivity in the plasma 96 h after the injection of [^{3}H]thymidine was about 20 times that of the granulocytes per milliliter of transfused blood. However, when unlabeled dog blood cells were incubated in labeled plasma and subsequently separated, the neutrophils had no significant activity over background. Also, to exclude the possibility that donor plasma would label recipient neutrophils in vivo, an untreated dog was injected with 70 ml of plasma from a dog given [^{3}H]thymidine 4 days previously. No detectable radioactivity was found in neutrophils harvested from the recipient animal over the next 24 h.

The survival of DF^{32}P-labeled autologous neutrophils was measured in six dogs after incubation of 100 ml ACD blood for 45–60 min with DF^{32}P (Amersham/Searle Corp.) at a final concentration not exceeding 1.0 μg DFP/ml of blood. In this first series of survival studies, a modified nylon fiber column method of neutrophil isolation was used (11). The samples were heparinized (20 U heparin/ml whole blood), reconstituted with Ca^{++} and Mg^{++} ions (both 0.01 M final concentration), and drawn into 5-ml disposable syringes. They were then passed through nylon fiber columns at a constant flow rate of 35 μl/min by using a Harvard infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) with a multiple syringe adapter set. The columns were constructed by packing 0.2 g of nylon wool (Fenwal Laboratories, Inc., Morton Grove, Ill.) tightly into Silastic® medical grade tubing (Dow Corning Corp., Midland, Mich.) (0.104 inch internal diameter) which was attached to a syringe. Total white cell and differential counts were done on the blood before and after its passage through the columns, and the total number of neutrophils retained was calculated. White cells were diluted in cetrimide solution (12) for red cell lysis and counted in an electronic particle counter. After the columns were washed with 4 ml of 0.87% NH_4Cl at a flow rate of 68 μl/min, they were cut open; and the wool was placed in a scintillating counting vial and air dried. Finally, 16 ml of scintillation fluid was added and the sample was counted for radioactivity.

Isologous neutrophil survival measurements were also carried out with labeling by both [^{3}H]thymidine and DF^{32}P. In these studies blood of a donor previously injected intravenously with 300 μCi/kg of [^{3}H]thymidine was incubated with DF^{32}P as described above. The doubly labeled neutrophils were then injected into a recipient animal, and blood drawn at the times specified for single labeling studies was processed by H-F centrifugation and dextran sedimentation. Radioactivity was determined in separate channels with appropriate correction for cross counting. 1.6% of the ^{32}P activity was noted in the tritium channel while no ^{3}H activity was observed in the ^{32}P channel. Counting efficiency was 36% for ^{3}H and 80% for ^{32}P.

The number of circulating neutrophils in the recipient was calculated from an assumed blood volume of 74 ml/kg (13) and the neutrophil concentration per milliliter blood. The

TABLE I

Relative Labeling of Blood Cells 4 Days after [^{3}H]Thymidine Injections

	Sp act
	cpm/10^6 cells
Neutrophils	110.04
Mononuclear cells	25.12
Platelets	0.118
Erythrocytes	0.042

TABLE II

Neutrophil Number, Postmitotic Transit Time, and Total Neutrophil Production

Dog	EIT	Erythroblasts	N/E ratio		Neutrophil marrow mass		Neutrophil marrow transit time	Total neutrophil production
			Total	Postmitotic	Total	Postmitotic		
	mg/100 ml whole blood/day	*×10^9/kg*			*×10^9/kg*		*days*	*×10^9/kg/day*
1	0.76	4.75	1.27	1.09	6.04	5.18	3.12	1.68
2	0.78	4.87	1.47	1.26	7.14	6.13	3.85	1.59
3	0.70	4.37	1.63	1.39	7.11	6.09	3.44	1.77
4	0.96	6.00	1.17	1.01	7.01	6.04	3.23	1.87
5	0.92	5.60	1.16	0.97	6.49	5.40	3.54	1.53
6	1.05	6.56	0.99	0.73	6.51	4.82	3.33	1.45
Mean	0.86	5.36	1.28	1.07	6.72	5.61	3.42	1.65
SD	0.14	0.84	0.23	0.23	0.44	0.56	0.19	0.11

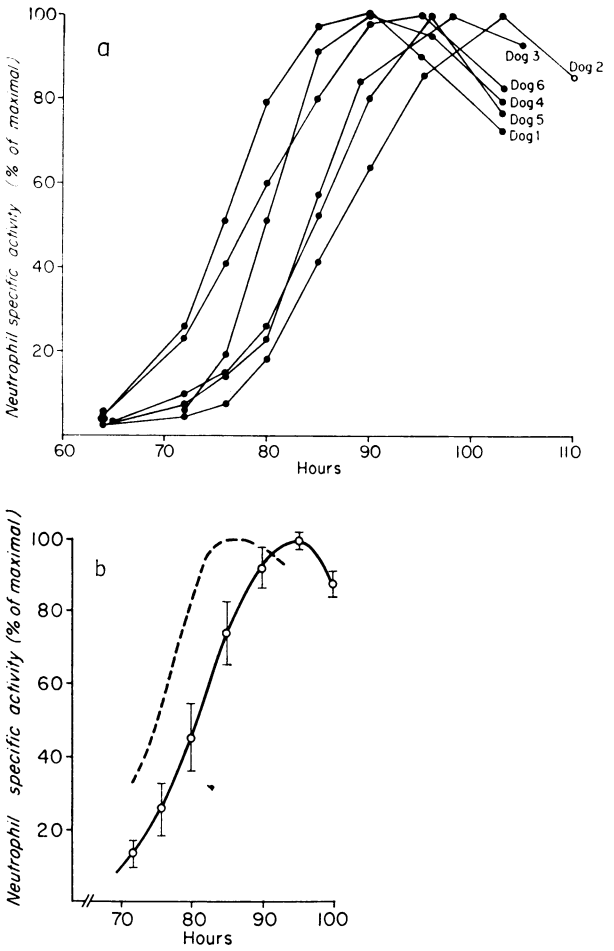

FIGURE 1 (*a*) The appearance of radioactivity in circulating neutrophils after the intravenous injection of [^{3}H]-thymidine. (*b*) The mean appearance curve of neutrophil specific activity in blood is shown by the solid line. The scale has been adjusted to a maximum value of 100% and vertical lines at each point indicate standard error. The dotted line represents appearance rate, corrected for disappearance (see Eq. 3 in Methods).

difference between neutrophil radioactivity injected and the extrapolated zero time value of neutrophilic activity in circulating blood was taken to represent the marginal pool. To avoid any bias, the $t_{\frac{1}{2}}$ disappearance time was determined by the method of least squares. Neutrophil turnover rate was calculated in the usual way, with the assumption that both circulating and marginal pools behave similarly (14).

RESULTS

Marrow neutrophil number. The data used for determining marrow neutrophil number are summarized in Table II. The mean EIT of 0.86±0.14 mg Fe/100 ml whole blood/day was similar to that measured in previous studies of normal animals in which the turnover was 0.90±0.11 (5). The number of NRBC was calculated to be 5.36±0.84 × 10^9 cells/kg. The total marrow N/E ratio was 1.28±0.23, and the postmitotic N/E ratio was 1.07±0.23. From these figures the total number of neutrophils was calculated to be 6.72±0.44 × 10^9/kg and the postmitotic cells 5.61±0.56 × 10^9/kg.

Transit time and turnover of marrow neutrophils. The appearance time of ^{3}H-labeled neutrophils in circulation is shown in Fig. 1 *a* and *b*. No measurable activity was found during the first 60 h after injection of the label. Thereafter, the increase in radioactivity appeared nearly linear between 20 and 70% of maximal activity. The peak neutrophil specific activity was reached 95 h after injection. A minimal MTT of 72 h was determined by extrapolating the ascending straight part of the line to baseline. The maximum rate of entrance of radioactivity, corrected for loss of activity from circulation was 87.1 h. Allowing for an entrance time into the PMN pool of 5 h, the true transit time was 82.1 h. With this and a PMN pool of 5.61 × 10^9/kg, the daily neutrophil turnover within the marrow was estimated to be 1.65 × 10^9 cells/kg/day.

Survival and turnover of circulating neutrophils. The turnover of circulating neutrophils was determined from blood labeled with DF^{32}P alone, [^{3}H]thymidine alone, and both isotopes simultaneously. In six autologous neutrophil survivals with DF^{32}P-tagged cells, the mean recovery (neutrophil activity extrapolated to 0 time) was 51±5%; and the half-time disappearance was 5.4 ±0.9 h. Mean values for total blood neutrophil pool, circulating pool and marginal pool, expressed as cells × 10^9/kg, were 0.93±0.11, 0.48±0.07, and 0.45±0.08, respectively (Table III). The neutrophil turnover per kilogram per day was calculated to be 2.89±0.57 × 10^9. The results of six isologous neutrophil survivals with ^{3}H-tagged cells are summarized in Table IV. The mean recovery was 65±9.7% and the mean half-time disappearance was 6.7±0.8 h. Total blood neutrophil pool

TABLE III
Turnover of DF^{32}P-Labeled Autologous Neutrophils in Dogs

Dog	Total blood neutrophil pool	Circulating pool	Marginated pool	$t_{\frac{1}{2}}$	Neutrophil turnover rate
		×10^9/kg		h	×10^9/kg/day
1	0.88	0.49	0.39	4.1	3.47
2	0.98	0.57	0.41	4.5	3.61
3	0.94	0.41	0.53	5.7	2.73
4	1.04	0.53	0.51	6.7	2.58
5	0.70	0.38	0.32	6.1	1.91
6	1.03	0.48	0.55	5.5	3.09
Mean	0.93	0.48	0.45	5.4	2.89
SD	0.11	0.07	0.08	0.9	0.57

and circulating and marginal pools expressed as neutrophils $\times 10^9$/kg were 0.67±0.12, 0.43±0.08, and 0.24 ±0.08. Granulocyte turnover was 1.66±0.23 $\times 10^9$ cells/kg/day. A comparison of $DF^{32}P$ and ^3H labeling studies (Tables III and IV) showed that the latter gave significantly lower values for total blood neutrophil pool ($P < 0.005$), marginal pool ($P < 0.005$), and neutrophil turnover rate ($P < 0.001$) and a significantly higher value for $t_{\frac{1}{2}}$ ($P < 0.05$).

This discrepancy was further examined with data obtained from neutrophils labeled with both isotopes (Table V and Figs. 2 and 3). The previously noted differences between total neutrophil pool, marginal pool, disappearance $t_{\frac{1}{2}}$, and neutrophil turnover rate were reproduced precisely; when tested by the method of paired comparisons (15) these differences were highly significant ($P < 0.005$, 0.005, 0.05, and 0.001).

TABLE IV

Turnover of [^3H]Thymidine-Labeled Isologous Blood Neutrophils in Dogs

Dog	Total blood neutrophil pool	Circulating pool	Marginated pool	$t_{\frac{1}{2}}$	Neutrophil turnover rate
		$\times 10^9$/kg		h	$\times 10^9$/kg/day
1	0.55	0.44	0.11	6.4	1.43
2	0.55	0.32	0.23	6.2	1.49
3	0.89	0.55	0.34	8.2	1.82
4	0.60	0.45	0.15	7.2	1.39
5	0.68	0.35	0.33	5.8	1.92
6	0.74	0.49	0.25	6.3	1.92
Mean	0.67	0.43	0.24	6.7	1.66
SD	0.12	0.08	0.08	0.8	0.23

DISCUSSION

Characterization of blood cell kinetics requires definition of both the generating marrow mass and the behavior of circulating cells. In the erythrocyte and thrombocyte systems, measurements of erythroid and megakaryocyte mass reflect the proliferative status of the marrow and accordingly are referred to as total erythropoiesis and thrombopoiesis. When cell production and destruction are at the same constant rate, the delivery of viable cells from the marrow to the circulating blood, i.e. effective production, is determined by dividing the concentration of circulating cells by their survival time. Such measurements of total and effective production have been useful for studying quantitatively the pathophysiology of clinical disorders affecting red cells and platelets. The present studies were designed to establish similar measurements for the neutrophil system.

The quantitation of marrow neutrophil cellularity from the NRBC number and the N/E ratio has been described in detail elsewhere (2). The total number of erythroid precursors was derived from the EIT, assuming a constant relationship between the number of immature erythroid forms and the iron they assimilate. Erythroid cellularity in normal dogs, previously determined directly by an isotope dilution technique (5, 16), was found to be 5.48±0.78 $\times 10^9$ nucleated erythroid cells/kg; and the EIT was 0.90±0.11 mg Fe/100 ml whole blood/day. In the present study the number of marrow NRBC was calculated from the EIT and found to average 5.36±0.84 $\times 10^9$ NRBC/kg in six dogs. The postmitotic marrow neutrophil/erythroid ratio and the postmitotic marrow neutrophil pool size were 1.07 ±0.23 and 5.61 $\times 10^9$ neutrophils/kg compared with 0.96±0.18 and 5.23±0.61 $\times 10^9$ neutrophils/kg found previously.

TABLE V

Turnover Studies of Isologous Neutrophils Doubly Labeled with $DF^{32}P$ and [^3H]Thymidine

		^3H				$DF^{32}P$			
Dog	Circulating pool	Total blood neutrophil pool	Marginated pool	$t_{\frac{1}{2}}$	Turnover rate	Total blood neutrophil pool	Marginated pool	$t_{\frac{1}{2}}$	Turnover rate
	$\times 10^9$/kg	$\times 10^9$/kg		h	$\times 10^9$/kg/day	$\times 10^9$/kg		h	$\times 10^9$/kg/da
1	0.62	0.85	0.24	7.1	1.97	1.01	0.40	6.0	2.80
2	0.43	0.66	0.24	8.4	1.31	0.81	0.39	5.7	2.37
3	0.50	0.71	0.21	6.7	1.77	0.95	0.45	4.4	3.58
4	0.39	0.78	0.39	7.9	1.64	0.84	0.45	5.0	2.80
5	0.39	0.59	0.20	6.0	1.63	0.80	0.41	4.5	2.95
6	0.50	0.89	0.39	9.3	1.59	1.00	0.50	6.0	2.76
Mean	0.47	0.75	0.23	7.6	1.65	0.90	0.43	5.2	2.88
SD	0.08	0.11	0.08	1.2	0.22	0.10	0.04	0.7	0.40

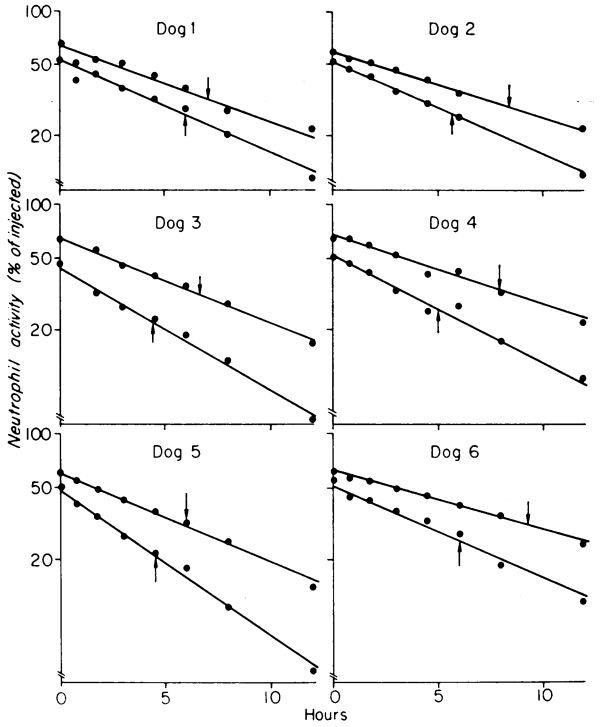

FIGURE 2 Disappearance of blood radioactivities after intravenous injection of doubly labeled neutrophils. Neutrophils were labeled in vivo with [^{3}H]thymidine and the same cells were labeled in vitro with DF^{32}P. In each instance the higher activity is [^{3}H]thymidine and the lower activity is DF^{32}P.

To calculate the turnover of postmitotic marrow neutrophils, a measurement of the transit time through this pool was also required. As a label, [^{3}H]thymidine was chosen because of its rapid incorporation into mitotable cells during S phase. This activity appears in the postmitotic pool in about 5 h (17). The emergence pattern of [^{3}H]thymidine-labeled granulocytes in circulation is influenced by the degree of synchrony during storage pool transit and the rate of disappearance of tagged cells from the blood. An assumption was made that the mean time of exit from the marrow would approximate the time of maximal entrance of activity into the blood which was found to be 82.1 h. This figure is reasonably consistent with other measurements in dogs by a variety of techniques in which peak activity was reached at 3–5 days (18–21).

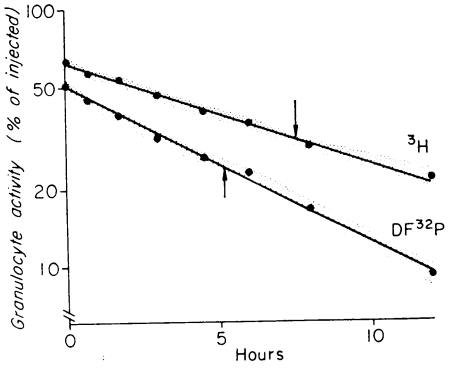

FIGURE 3 Blood activity (mean of six dogs) for [^{3}H]-thymidine and DF^{32}P. Shaded area represents standard error and arrows mark the $t_{\frac{1}{2}}$ disappearance time.

The turnover of marrow neutrophils in normal dogs, determined by dividing the number of marrow neutrophils composing the postmitotic pool by the transit time through that compartment, was $1.65 \pm 0.11 \times 10^9$ cells/kg/day for a group of six normal dogs (Table II).

Correlated studies of blood neutrophil turnover were performed with [^{3}H]thymidine-labeled isologous neutrophils (Table IV). In these studies the half-time disappearance averaged 6.7 ± 0.8 h, and 64% of the labeled cells were recovered in circulation. A value of $1.66 \pm 0.23 \times 10^9$ cells/kg/day was obtained for the turnover of circulating neutrophils. It is therefore evident that essentially all neutrophils leaving the marrow appear in the circulation. (A small difference exists relative to the cell population measured: the PMN turnover in the marrow measures only neutrophils, whereas, the measurement of circulating leukocytes includes all granulocytes). These data further suggest that measurements of marrow and peripheral blood neutrophil turnover are of sufficient precision to permit recognition of excessive cell destruction in the marrow.

Since the measurements of both marrow and circulating neutrophil turnover obtained with [^{3}H]thymidine were considerably less than previous reports employing DF^{32}P labeling, additional measurements were carried out comparing the [^{3}H]thymidine and DF^{32}P labeling of blood neutrophils. Results obtained with DF^{32}P (Table III) agreed closely with previous studies (1) but did not agree with the [^{3}H]thymidine data (Table IV). Since the results of the single isotope studies were precisely reproduced and since DF^{32}P tagging did not cause any change in [^{3}H]thymidine recovery as would occur with cell loss, it appeared that the DF^{32}P label was being lost from the neutrophils (Fig. 2). Although elution was not detected neither could it be entirely excluded by in vitro studies of tagged neutrophils (22); however, DF^{32}P elution has been consistently found with red cell tagging (23). Since the nature and consistency of this elution are not yet defined, the usefulness of neutrophil kinetic studies using DF^{32}P is at present in question.

These studies establish quantitative relationships between marrow and circulating neutrophil compartments in the dog. Of the 6.7×10^9/kg neutrophilic marrow cells, about 17% are in the proliferative compartment while the remaining 83% comprise the matu-

ration-storage compartment; the marrow delivers about 30% of the available storage cells to the peripheral blood daily. Of the 0.75×10^9 neutrophils/kg in the total blood granulocyte pool, two-thirds are in the vascular compartment and one-third is marginated in the microvasculature. The storage pool is approximately 7½ times the blood compartment and represents a 3–4 day supply of neutrophils. The application of these techniques to man to characterize kinetically the quantitative abnormalities of neutrophils will provide a pathophysiological basis for a systematic approach to neutropenia and neutrophilia.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Loretta Willis and Jean Greenaway, and the assistance of Dr. Eloise Giblett in reviewing the manuscript.

This work was supported by research grants (HL-06242 and HL-11775) and a training grant (AM-05130) from the National Institutes of Health and by the Washington State Heart Association.

REFERENCES

1. Raab, S. O., J. W. Athens, O. P. Haab, D. R. Boggs, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1964. Granulokinetics in normal dogs. *Am. J. Physiol.* **206**: 83–88.
2. Athens, J. W., O. P. Haab, S. O. Raab, A. M. Mauer, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1961. Leukokinetic studies. IV. The total blood, circulating, and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. *J. Clin. Invest.* **40**: 989–995.
3. Constable, T. B., and N. M. Blackett. 1972. The cell population kinetics of neutrophilic cells. *Cell Tissue Kinet.* **5**: 289–302.
4. Cronkite, E. P., and P. C. Vincent. 1969. Granulocytopoiesis. *Scr. Haematol.* **4**: 3–43.
5. 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.
6. Bothwell, T. H., and B. Mallett. 1955. Diurnal variation in the turnover of iron through the plasma. *Clin. Sci. (Oxf.)*. **14**: 235–239.
7. 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.
8. 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. Lab. Invest.* **21** (*Suppl. 97*): 77–82.
9. Maloney, M. A., C. L. Weber, and H. M. Patt. 1963. Myelocyte-metamyelocyte transition in the bone marrow of the dog. *Nature (Lond.)*. **197**: 150–152.
10. Mahin, D. T., and R. T. Lofberg. 1966. A simplified method of sample preparation for determination of tritium, carbon-14, or sulfur-35 in blood or tissue by liquid scintillation counting. *Anal. Biochem.* **16**: 500–509.
11. Deinard, A. S., and A. R. Page. 1970. An improved method for performing neutrophil survival studies. *Blood J. Hematol.* **36**: 98–110.
12. Cartwright, G. E. 1968. Diagnostic Laboratory Hematology. Grune & Stratton, Inc., New York. 4th edition. 67.
13. Rapaport, E., H. Kuida, F. W. Haynes, and L. Dexter. 1956. Pulmonary red cell and plasma volumes and pulmonary hematocrit in the normal dog. *Am. J. Physiol.* **185**: 127–132.
14. Athens, J. W., S. O. Raab, O. P. Haab, A. M. Mauer, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1961. Leukokinetic studies. III. The distribution of granulocytes in the blood of normal subjects. *J. Clin. Invest.* **40**: 159–164.
15. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames. 6th edition.
16. Finch, C. A., K. Deubelbeiss, J. D. Cook, J. W. Eschbach, L. A. Harker, D. D. Funk, G. Marsaglia, R. S. Hillman, S. Slichter, J. W. Adamson, A. Ganzoni, and E. R. Giblett. 1970. Ferrokinetics in man. *Medicine (Baltimore)*. **49**: 17–53.
17. Maloney, M. A., and H. M. Patt. 1968. Granulocyte transit from bone marrow to blood. *Blood J. Hematol.* **31**: 195–201.
18. Ohkita, T. 1967. The kinetics of granulocytopoiesis. *Acta Haematol. Jap.* **30**: 507–524.
19. Craddock, C. G., Jr., S. Perry, and J. S. Lawrence. 1956. The dynamics of leukopoiesis and leukocytosis, as studied by leukopheresis and isotopic techniques. *J. Clin. Invest.* **35**: 285–296.
20. Boggs, D. R., J. W. Athens, G. E. Cartwright, and M. M. Wintrobe. 1965. Leukokinetic studies. IX. Experimental evaluation of a model of granulopoiesis. *J. Clin. Invest.* **44**: 643–656.
21. Boggs, D. R., J. W. Athens, O. P. Haab, P. A. Cancilla, S. O. Raab, G. E. Cartwright, and M. M. Wintrobe. 1964. Leukokinetic studies VII. Morphology of the bone marrow and blood of dogs given vinblastine sulfate. *Blood J. Hematol.* **23**: 53–67.
22. Athens, J. W., A. M. Mauer, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1959. Leukokinetic studies. I. A method for labeling leukocytes with diisopropylfluorophosphate (DFP^{32}). *Blood J. Hematol.* **14**: 303–333.
23. Hjort, P. F., H. Paputchis, and B. Cheney. 1960. Labeling of red blood cells with radioactive diisopropylfluorophosphate (DFP^{32}): evidence for an initial release of label. *J. Lab. Clin. Med.* **55**: 416–424.