JCI The Journal of Clinical Investigation

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J Clin Invest. 1967;46(12):1943-1953. https://doi.org/10.1172/JCI105684.

Research Article

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The Journal of Clinical Investigation
Vol. 46, No. 12, 1967

Neutrophil Kinetics in Acute Infection *

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Abstract. Neutrophil kinetics of acute experimental infection were studied with diisopropylfluorophosphate-^{32}P labeling in 31 dogs inoculated intrabronchially with pneumococci. In vitro neutrophil labeling indicated a rapid transit time through the blood in early infections, with an elevated marginal granulocyte pool sometimes preceding an elevation of the circulating granulocyte pool. 13 hr after infection, the circulating and total blood granulocyte pools were increased but the rate of neutrophil transit through the blood was normal. During the recovery from infection there was a marked prolongation of neutrophil blood transit time, suggesting virtually complete cessation of bone marrow release of neutrophils into the blood. Labeling of neutrophils in vivo indicated an increased rate of emptying of the bone marrow storage pool proportional to the severity of infection as measured by the fever index. The change in the blood ratio of nonsegmented to segmented neutrophils was a much more accurate index of the severity of infection than the blood granulocyte concentration, correlating significantly with the fever index.

Introduction

Studies of neutrophil kinetics in patients with chronic infections have been reported by several workers (1–4) but the effect of acute infection upon the kinetics of isotopically labeled neutrophils has not been studied previously. Such data are difficult to obtain during the acute phases of naturally occurring infections in man because patients rarely consult a physician until infection is well established and therapy is begun shortly thereafter. For this reason and since studies in normal dogs suggest that neutrophil kinetics in this species are qualitatively similar to those of normal humans, we elected to induce acute infection in dogs and study the attendant changes in neutrophil kinetics.

Pneumococcal pneumonia was induced in dogs by the method of Terrell, Robertson, and Coggeshall (5), a model of infection which was explored in detail by those workers and by Robertson and Fox (6). We induced infections of varying severity, and just before infection and at various times thereafter initiated leukokinetic studies employing radioactive diisopropylfluorophosphate (DF^{32}P) as a label for neutrophils. Data were obtained regarding the total number and distribution of neutrophils in the blood, the rate of disappearance of neutrophils from the blood, and the transit time of neutrophils through the bone marrow.

Methods

Mongrel dogs of either sex, weighing from 11 to 32 kg, were given antihelminthic therapy and distemper vaccine and observed for at least 10 days before use.

Induction of infection. Through the courtesy of Dr. W. B. Wood, Jr., Johns Hopkins University School of Medicine, the strain of *D. pneumoniae,* type I(A5), that was used by Terrell and coworkers (5) was obtained. It was maintained in a synthetic dialyzable broth culture

* Received for publication 16 June 1967 and in revised form 2 August 1967.

Supported by a research grant (AM-04489) and a graduate training grant (AM-5098) from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md. Presented in part at the Ninth Annual Meeting of the American Society of Hematology, 6 December 1966.

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medium (7) and for inoculation, from 10^4 to 10^{15} viable units were suspended in 10 ml of medium to which was added 2.5 g of starch (Difco Laboratories, Detroit, Mich.). Dogs were anesthetized by intravenous injection of pentobarbital (30 mg/kg) or thiopental (15 mg/kg). The bacterial suspension was injected through a number 12 French, Levin tube which was lodged as far distally as possible in the tracheobronchial tree through a bronchoscope. After injection of the bacteria, air was forced through the tube to flush as much of the suspension as possible into the bronchi.

Labeling procedure. A brief section noting the general method employed and the significance of normal labeling curves is included in appropriate sections in Results. The activity of the $DF^{32}P$ (Radiochemical Centre, Amersham, England) was approximately 200 μc/ml and the concentration of $DF^{32}P$ in propylene glycol diluent varied from 0.4 to 0.8 mg/ml. Each batch of $DF^{32}P$ was assayed in our laboratory (8). For in vivo labeling 3.5 to 5.0 ml of $DF^{32}P$ was injected intravenously and for in vitro labeling 0.3 ml was added to approximately 400 ml of whole blood. All blood samples for determination of leukocyte specific activity, leukocyte counts, and blood smears were obtained by jugular puncture.

The previously reported (9) technique for separating dog granulocytes from whole blood and for determining the specific activity of the granulocytes (counts per minute per milligram of leukocyte nitrogen) was used with the following modifications: 20 ml of blood was added to 40 ml of 3% dextran (molecular weight 215,000; Pharmachem Corporation, Bethlehem, Pa.) in 0.9% saline containing 0.1% potassium EDTA. The leukocyte button remaining after hemolysis was washed four times with cold isotonic saline. All samples for determination of granulocyte specific activity were obtained and processed in duplicate. In the in vivo labeling studies, samples were obtained daily for 10 days. In the in vitro labeling studies, samples were obtained 3, 6, 12, and 24

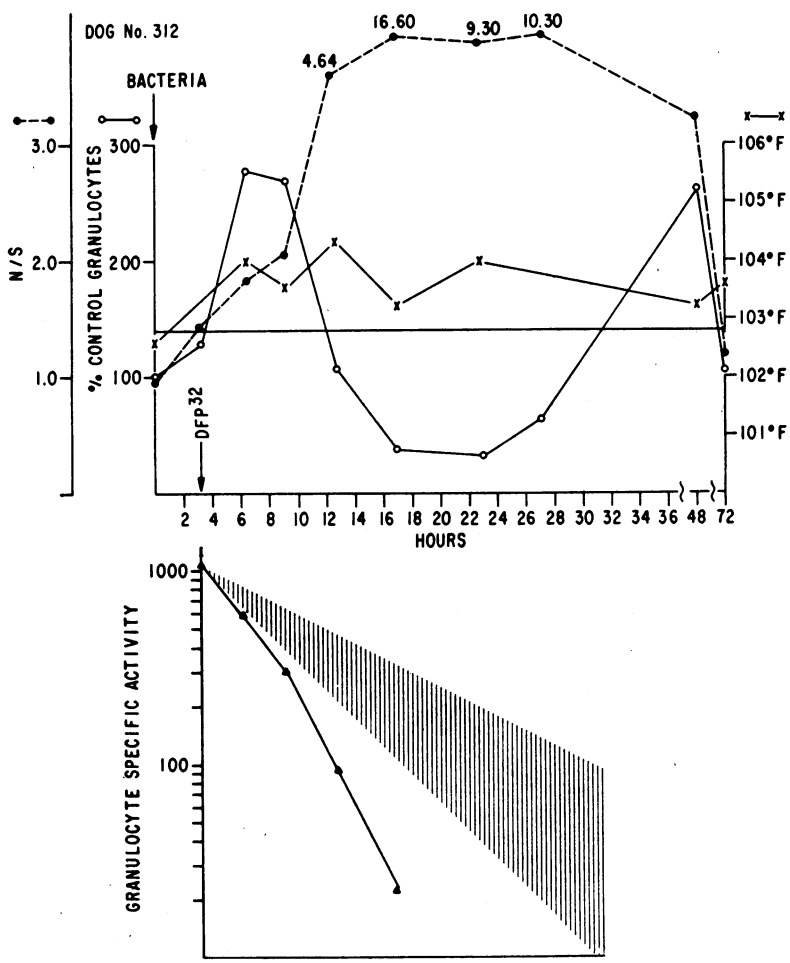

FIG. 1. THE COURSE OF INFECTION (TOP) AND BLOOD GRANULOCYTE SPECIFIC ACTIVITY CURVE (BOTTOM) IN EARLY INFECTION. Granulocytes were labeled in vitro and infused 4 hr after infection. The shaded area indicates the normal range of specific activity. *N/S*, nonsegmented/segmented blood neutrophils.

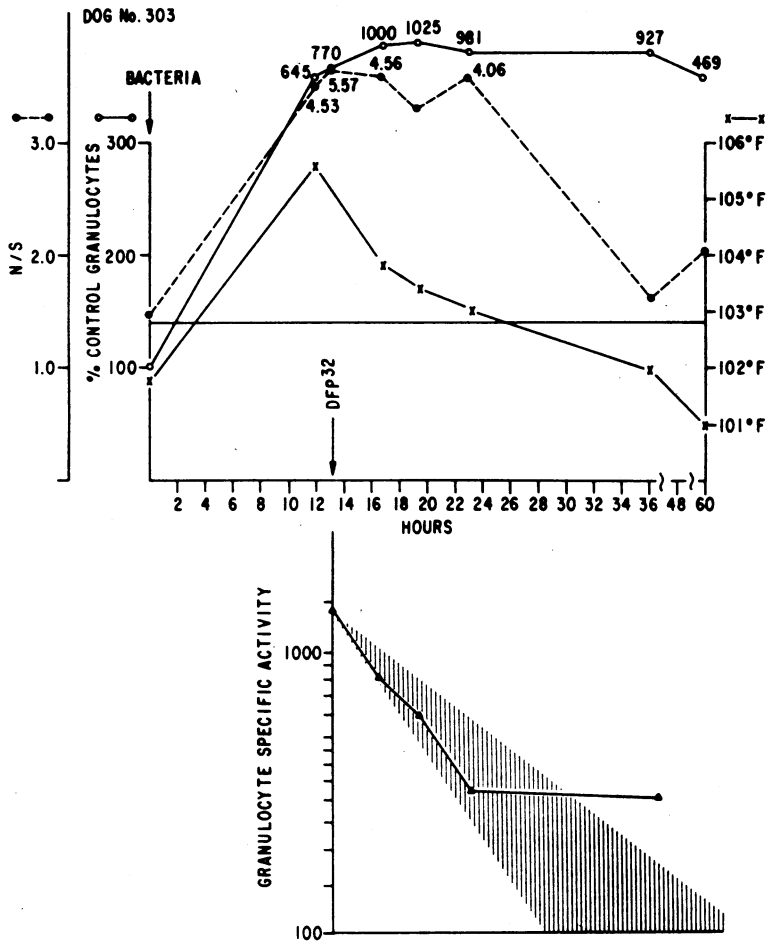

FIG. 2. THE COURSE OF INFECTION (TOP) AND BLOOD GRANULOCYTE SPECIFIC ACTIVITY CURVE (BOTTOM) IN ESTABLISHED INFECTION. Granulocytes were labeled in vitro and infused 13 hr after infection. The shaded area indicates the normal range of specific activity. *N/S*, nonsegmented/segmented blood neutrophils.

hr after reinfusion of labeled blood and in some experiments more frequently.

Clinical course of infections. Blood for leukocyte counts was obtained hourly for the first 6 hr after infection and then daily for the next 10 days in the in vivo studies, and at 6-hr intervals during the 1st day of the in vitro studies. Total leukocyte counts were performed electronically (Coulter Counter, Model B, Coulter Electronics, Hialeah, Fla.) and 200 cell differential counts were done on all blood smears. Blood neutrophils were classified as segmented or nonsegmented on the basis of whether or not a thread-like filament was demonstrable between nuclear lobes.

It is noteworthy that normal dog peripheral blood contains a much higher number of band, or nonsegmented forms than is found in human blood. The median control ratio of nonsegmented to segmented forms was 1.0 with a range of 0.42–2.55. The maximum change in this ratio was chosen, therefore, as the most easily comparable index of increasing granulocyte immaturity rather than the actual percentage of nonsegmented forms.

Bone marrow was aspirated from the sternum before and 24 hr after pneumococci were inoculated intrabronchially. Differential cell counts of 500 cells were done on the marrow smears.

Rectal temperature was recorded and a physical examination was performed each time a blood sample was obtained. Rectal temperatures were plotted against time for each individual study and the area of the curve which exceeded 102.8°F was used as a "fever index." The highest rectal temperature recorded in 14 normal dogs was 102.8°F. Area measurements were made with a compensating polar planimeter. (Model 4236 M, Keuffel & Esser Co., San Francisco, Calif.).

Blood samples obtained at 6-hr intervals during the 1st day of infection were cultured on a tryptic digest of

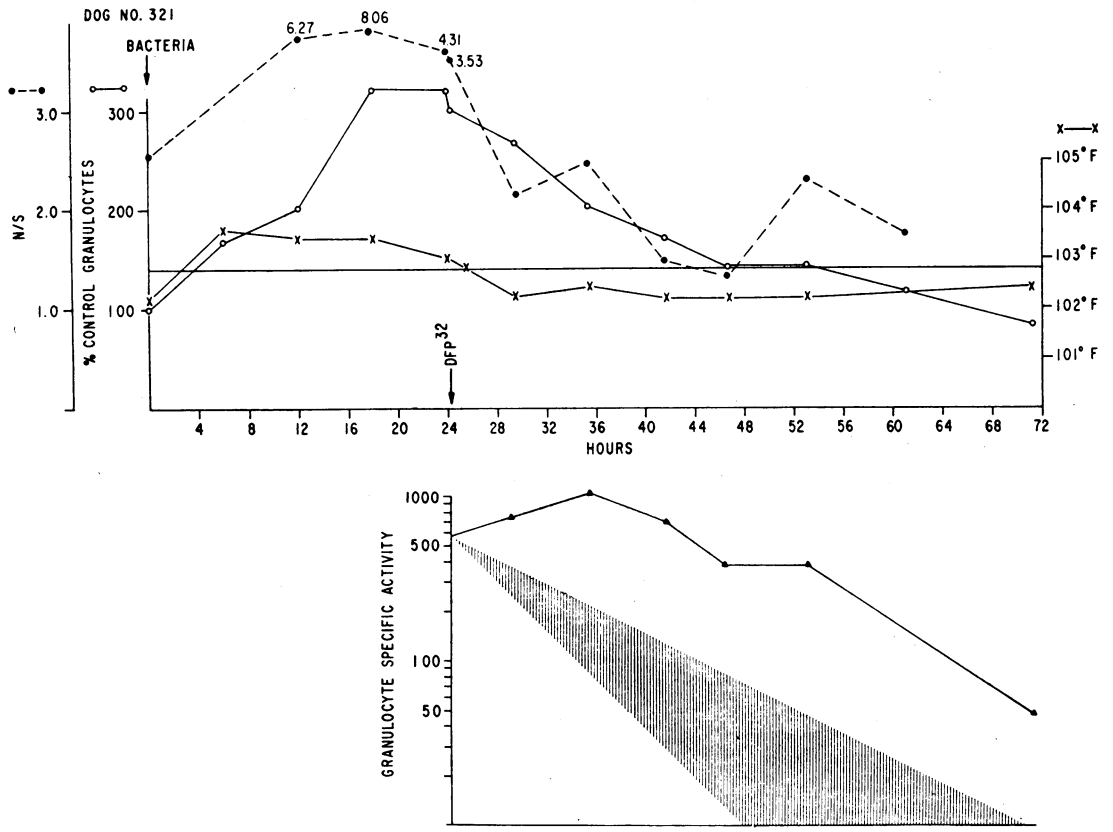

FIG. 3. THE COURSE OF INFECTION (TOP) AND BLOOD GRANULOCYTE SPECIFIC ACTIVITY CURVE IN LATE INFECTION (BOTTOM). Granulocytes were labeled in vitro and infused 25 hr after infection. The shaded area indicates the normal range of specific activity. *N/S*, nonsegmented/segmented blood neutrophils.

casein-papaic digest of soy broth (Baltimore Biological Laboratories, Baltimore Md.).

Statistical analyses consisted of correlation coefficients and *t* tests (10).

Results

Course of infection

The induced pneumococcal infections varied in severity from very mild to fatal. No attempt was made to study in detail the bacteriological and clinical course of pneumococcal infection in dogs as was done by earlier workers (5, 6). The typical infection observed herein was characterized by rapid onset of fever, neutrophilia, and a marked "shift to the left" as measured by changes in the ratio of nonsegmented to segmented (N/S) blood neutrophils. These findings were evident within 4 hr of bacterial inoculation, persisted for about 24 hr, and then subsided (Figs. 1–3).

Of 39 dogs inoculated with bacteria, eight are not considered further in this report. Four of these died within 24 hr of overwhelming infection. One was autopsied and bilateral consolidation of the lungs was found. Many Gram-positive diplococci and neutrophils were evident on a stained touch preparation from a consolidated area of one lung. Since the leukocytes of these dogs had been labeled in vivo, no useful kinetic data were obtained since several days of study are needed. In another study employing in vivo labeling of four other dogs, results were uninterpretable because of technical problems of leukocyte isolation.

Interpretable data were obtained in 31 dogs, all but one of which survived. Eight were afebrile throughout the study, 13 developed moderate fever, and 10 were severely febrile (Table I). Positive blood cultures were noted in four dogs, all of which were febrile. Bronchial breathing was present in one of these for 2 days but disappeared shortly after a single injection of penicillin. This was the only instance in which anti-

TABLE I

Fever indexes, blood, and bone marrow changes in infected dogs

Group	No. of dogs	Fever index*	ΔN/S*‡	ΔCGP*§	Bone marrow aspirate (24 hr) ΔM:E‖	Bone marrow aspirate (24 hr) ΔI/P¶
		U				
1. No fever	8	0	0.88 (0.16–2.21)	2.69 (1.59–6.10)	−0.28**	+0.09**
2. Moderate fever	13	104 (30–270)	2.13 (0.59–5.01)	3.41 (1.46–8.81)	+0.08‡‡	+0.03‡‡
3. Severe fever	10	594 (394–1290)	7.57 (1.38–15.67)	3.47 (2.11–10.25)	−1.44§§	+0.23§§

* Mean values for group. Numbers in parentheses refer to ranges.
‡ Maximum increase in ratio of nonsegmented to segmented neutrophils in blood.
§ Ratio of maximum granulocyte concentration to control.
‖ Mean change in marrow myeloid: erythroid ratio.
¶ Mean change in ratio of marrow immature granulocytes (myeloblasts, promyelocytes, and myelocytes) to postmitotic granulocytes (metamyelocytes, mature neutrophils).
** Four dogs in group.
‡‡ Five dogs in group.
§§ Six dogs in group.

bacterial therapy was administered. In another dog, decreased breath sounds were observed for 1 day. Aside from lethargy, occasional wheezes, and coarse breath sounds, no other abnormal physical findings were noted. Most dogs with fever became afebrile within 3 days of bacterial inoculation.

In dogs without fever, little change was noted in N/S whereas in those with marked fever this ratio was markedly increased (Table I). There was a significant correlation between the fever index and N/S in febrile dogs ($r = +0.62$, $P = < 0.01$). There was no significant correlation between the fever index and the maximum change in granulocyte concentration. Although there was frequently a marked increase in nonsegmented cells, these were virtually all band forms. Myelocytes were not found and young metamyelocytes were observed rarely.

15 bone marrow aspirates obtained 24 hr after infection was initiated were analyzed and compared to control aspirates obtained from the same dogs (Table I). In severely febrile dogs the myeloid to erythroid ratio was decreased significantly and the ratio of potentially mitotic neutrophils to postmitotic neutrophils was increased.

Leukocytes labeled in vitro

Within 4–12 hr of initiating infection, changes in neutrophil concentration and N/S were apparent (Figs. 1–3). Accordingly at 4, 13, and 25 hr after bacterial inoculation, studies of neutrophil kinetics employing neutrophils labeled in vitro were initiated.

In this type of study, freshly withdrawn blood is incubated with $DF^{32}P$ for an hour and returned to the circulation of the donor. Thus, only those neutrophils which were infused are labeled. The total blood granulocyte pool (TBGP) is calculated by noting the degree of labeled cell dilution 5 min after the completion of the infusion (T_0). The number of circulating neutrophils is calculated from the neutrophil concentration in venous blood and the blood volume, and is termed the circulating granulocyte pool (CGP). Subtraction of the CGP from the TBGP yields an estimate of the number of marginal leukocytes, this value being termed the marginal granulocyte pool (MGP). The rate of decline of blood granulocyte specific activity (BGSA) is then determined. In normal dogs in a steady state, the BGSA declines exponentially with a $t_{\frac{1}{2}}$ of 4–8 hr (9).

The neutrophils of infected dogs were labeled in vitro at approximately 4 hr (early infection, group A), 13 hr (established infection, group B), and 25 hr (late infection, group C) after inoculation of pneumococci (Table II). None of the infected dogs can be considered to have been in a steady state, so calculation of the granulocyte turnover rate is unjustified. It must be under-

TABLE II

Fever indexes and blood granulocyte kinetic values in infected dogs whose leukocytes were labeled in vitro

Group	No. of dogs	Fever index*	TBGP*‡	CGP*‡	MGP*‡	$t_{\frac{1}{2}}$*‡
		U	*10^7 G/kg*	*10^7 G/kg*	*10^7 G/kg*	*hr*
Normal (ref. 9)	31		102 (48–168)	54 (27–98)	48 (10–86)	5.6 (4–8)
Infected						
A. Labeled at 4 hr						
No fever	3	0	118 (68–184)	67 (47–100)	51 (13–85)	3.8§ (3.0–4.2)
Fever	4	306 (95–530)	191§ (104–337)	78§ (68–83)	115§ (31–255)	2.3§ (1.8–3.2)
B. Labeled at 13 hr						
Fever	4	304 (35–695)	168§‖ (143–202)	90§ (34–121)	60‖ (36–82)	5.1 (3.6–6.1)
C. Labeled at 25 hr						
Fever	4	351 (176–428)	475§ (94–1027)	146§ (65–269)	329§ (11–861)	37§¶ (11–65)

* Mean values for group. Numbers in parentheses refer to ranges.

‡ G, granulocytes; TBGP, total blood granulocyte pool; CGP, circulating granulocyte pool; MGP, marginal granulocyte pool; $t_{\frac{1}{2}}$, half disappearance time of blood granulocyte specific activity.

§ Differs from normal with $P = < 0.05$ by t test.

‖ Only three animals in group.

¶ Calculated from first 24 hr of curve (other $t_{\frac{1}{2}}$ values in Table calculated from first 12 hr).

stood that under these circumstances $t_{\frac{1}{2}}$ values read from different portions of the BGSA curve are only approximations but do yield a simple estimate of whether labeled cells were declining in the blood at an abnormal or normal rate.

Leukokinetics of early infection. Seven dogs were studied in the early stage of infection (group A, Table II). Blood was withdrawn for labeling 2.5 hr after inoculation of bacteria, incubated with $DF^{32}P$ for 1 hr and reinfused, so that T_0 was approximately 4 hr after infection.

Three dogs in this group did not become febrile but did have a slight rise in N/S. In one, a slight increase in TBGP was noted but the pool sizes of these three dogs as a group were not significantly increased. In the dog with the largest increase in N/S, an abnormally short $t_{\frac{1}{2}}$ value (3 hr) was found, while those for the other two were at the lower limit of normal. There was a significant decrease in $t_{\frac{1}{2}}$, as measured during the first 12 hr of the BGSA curve, in these dogs as compared to normal dogs.

The course of the temperature, granulocyte count, and N/S, as well as BGSA curve of one of the most severely ill dogs, is illustrated in Fig. 1. After a brief period of neutrophilia, the dog became neutropenic as N/S rose to 16 times the control value. BGSA declined at an abnormally rapid rate ($t_{\frac{1}{2}}$ of 2.3 hr) as leukopenia developed. In this dog pool sizes were normal. In another severely febrile dog, however, the TBGP was increased, reflecting a large MGP, for the CGP was normal. The $t_{\frac{1}{2}}$ was abnormally short (1.8 hr) during the first 12 hr of study in this dog also.

Statistically significant increases in mean pool sizes and shortening of the $t_{\frac{1}{2}}$ of the BGSA were present in the entire febrile group studied in the early hours of infection. Abnormally high values of MGP in the presence of normal values for CGP were noted in two of the four febrile dogs. The mean CGP was significantly higher than the normal mean although no values were outside the normal range. An abnormally low value for $t_{\frac{1}{2}}$ was noted in each of the four febrile dogs.

Leukokinetics of established infection. All four dogs in which labeling studies were initiated 13 hr after bacteria were introduced were febrile. An example of one such study is shown in Fig. 2 and the results for the group are summarized in Table II.

With established infection the CGP and TBGP were significantly increased but the MGP was normal. During the first 10–12 hr the $t_{\frac{1}{2}}$ was of

normal duration but in all four dogs the rate of decline of BGSA slowed and then plateaued between 23 and 37 hr after initiation of infection.

Leukokinetics of late infection. The four dogs in which labeled neutrophils were infused 25 hr after infection was initiated were all febrile (Fig. 3, Table II).

Blood pool sizes were variable in this group. The TBGP and CGP were larger than normal in three, two of which also had an enlarged MGP. Curves of BGSA were quite complex in all dogs (the example in Fig. 3 is representative of the group). The $t_{\frac{1}{2}}$ values listed in Table II are approximations of the curves during the first 24 hr after labeled cells were infused. In three of the dogs, no decline in BGSA occurred during the first 12 hr of study and this plateau was associated with stable or decreasing neutrophil concentrations and with a decreasing N/S. The range of BGSA 24 hr after labeling the neutrophils of normal dogs is 1–12% of the T_0 value. In these four dogs the 24 hr BGSA was 65, 40, 39, and 17% of the T_0 value. 29 hr after labeling (54 hr after initiation of infection) the slopes of BGSA decline returned to normal. By this time neutrophil concentration and N/S had returned to values similar to the control.

Leukocytes labeled in vivo

The increase in N/S in the blood, decrease in marrow myeloid to erythroid ratio, and decrease in the proportion of postmitotic marrow neutrophils suggested an increased rate of release of neutrophils from the bone marrow into the blood. The results of the in vivo labeling studies confirmed this impression.

Intravenously administered $DF^{32}P$ labels blood neutrophils, the myelocytes of the bone marrow, and marrow neutrophils more mature than myelocytes (11, 12). Promyelocytes and myeloblasts, lymphocytes, eosinophils, basophils, and monocytes are not labeled detectably. Myelocytes are labeled approximately twice as heavily as more mature marrow neutrophils. Consequently, the progeny of the first myelocyte division after labeling are labeled to the same degree as initially labeled postmitotic neutrophils. BGSA therefore changes little until the progeny of the second myelocyte division after labeling begin to enter the blood. The resultant initial plateau in BGSA has been termed phase I + II and lasts an average of 4.8 days in normal dogs (Fig. 4) (12). Thus, in the normal dog, the average time required for a myelocyte to complete DNA synthesis, and divide, and for the progeny of this division to traverse the maturation and storage pool of the marrow and traverse the blood is approximately 4.8 days. As subsequent myelocyte divisions occur, the label is diluted so that the BGSA falls (phase III) as the progeny of the divisions reach the blood. Phase I is a decline of BGSA on the 1st day of labeling due to dilution of relatively more heavily labeled blood neutrophils by less heavily labeled marrow neutrophils and phase IV is due to reutilization of isotope (12).

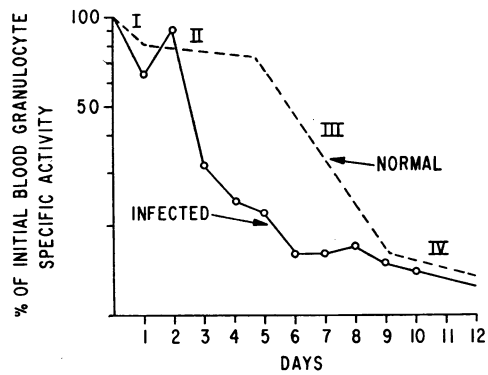

FIG. 4. BLOOD GRANULOCYTE SPECIFIC ACTIVITY CURVE OF AN INFECTED DOG COMPARED TO THE MEAN CURVE FOR 14 NORMAL DOGS (reference 12). Granulocytes were labeled in vivo 1 hr before infection.

16 dogs were injected with $DF^{32}P$ 1 hr before pneumococci were introduced intrabronchially (Table III). The mean BGSA curve from 14 normal dogs given $DF^{32}P$ intravenously (12) is shown in Fig. 4 for comparison with that of one of the severely febrile dogs. A shortening of phase I + II and a decrease in half disappearance time ($t_{\frac{1}{2}}$) of phase III is apparent in the infected animal.

There was a significant decrease in the duration of phase I + II in the infected group as a whole and the degree of decrease paralleled the severity of fever (Table III). The duration of phase I + II was within normal limits in the dogs that failed to develop fever (group 1), shorter than normal in all those with severe fever (group 3), and of intermediate duration in dogs with moderate fever (group 2). The decrease in phase I + II was statistically significant in both groups

TABLE III

Fever indexes, leukocyte changes, and phases of the blood granulocyte specific activity (BGSA) curves in infected dogs whose leukocytes were labeled in vivo

Group	No. of Dogs	Fever Index*	ΔN/S*‡	ΔCGP*§	Phases in BGSA curve	
					I+II duration*	III $t_{\frac{1}{2}}$*
		U			*days*	*hr*
Normal (ref. 12)	14				4.8 (3.0–7.1)	46 (32–60)
Infected						
1. No fever	5	0	1.15 (0.64–2.2)	3.03 (1.59–6.10)	4.6 (3.1–8.0)	41 (25–58)
2. Moderate fever	8	107 (30–270)	1.58 (0.59–3.57)	3.62 (1.46–8.81)	3.3‖ (2.2–4.0)	50 (30–75)
3. Severe fever	3	863 (500–1290)	9.15 (2.83–13.10)	2.80 (2.11–3.87)	2.3‖ (2.0–2.8)	30‖ (24–35)

* Mean values for group. Numbers in parentheses refer to ranges.
‡ Maximum increase in ratio of nonsegmented to segmented neutrophils in blood.
§ Ratio of maximum granulocyte concentration to control.
‖ Differs from normal with $P = <0.05$ by t test.

of febrile dogs, in which none of the values was larger than 4 days.

The decrease in duration of phase I + II was accompanied by a progressive increase in the maximum change in N/S in the blood although a statistically significant direct correlation was not present ($r = -0.28$, $P = > 0.30$). No correlation was observed between the duration of phase I + II and the degree of neutrophilia. The slope of phase III of the BGSA was significantly shortened ($P = < 0.02$) in the most severely febrile dogs (group 3) and in a few other dogs as well (Table III). However, there was no demonstrable correlation between the duration of phase I + II and the slope of phase III in the infected group as a whole ($r = -0.09$, $P = > 0.70$).

Discussion

These studies confirm the concept of acceleration of bone marrow release of neutrophils in response to infection and provide evidence for a new principle of neutrophil kinetics, namely total cessation of marrow release of neutrophils during recovery from the leukocytosis of infection.

It should be kept in mind that the rate of decline of the blood granulocyte specific activity (BGSA) curve obtained in the in vitro labeling method is primarily a function of the bone marrow release of neutrophils in the blood (13). A very rapid $t_{\frac{1}{2}}$ value suggests an acceleration of marrow neutrophil release.

Within 4 hr of bacterial inoculation, an accelerated rate of release of neutrophils from bone marrow to blood was observed as evidenced by an increase in N/S. At times this was detected before an increase in the concentration of neutrophils in the venous blood samples was detected. An increased rate of release was also indicated by an abnormally fast rate of decline of the BGSA curve in the in vitro labeling studies. This is quite similar to the sequence of events following the administration of bacterial endotoxin (14). In two dogs, the marginal granulocyte pool (MGP) was enlarged during the period of accelerated release even though the circulating granulocyte pool (CGP) was not. This is similar to our previous observations of "masked" granulocytosis in normal human subjects in whom small skin exudates had been induced (15). In these studies the MGP was enlarged selectively so that neutrophilia was undetected by enumeration of neutrophils in venous blood samples. The CGP never became enlarged in the human subjects, whereas in the present studies "masked" granulocytosis was followed by CGP enlargement. The response to bacterial endotoxin is also associated with an enlargement of the MGP before that of the CGP (14). When the bone marrow responds to a depletion of the blood neutrophil pool by leukopheresis, the lag period before rise of the blood neutrophil count has been interpreted as a replenishment of the MGP before the CGP (16).

From these observations one may speculate that infection initiates the following chain of kinetic events. Infection in tissues presents increased demand for egress of neutrophils from the blood. The cells must leave the blood by diapedesis from a marginal position so that an increase in the size of the MGP develops. A feed-back loop, probably mediated by a plasma factor (17), is initiated which accelerates the rate at which cells are released from the marrow. The CGP does not enlarge until acceleration of release has exceeded the rate of acceleration of cell egress from the blood.

The magnitude of the acceleration of marrow release was reflected in the degree of shortening of phase I and II of the BGSA curve after in vivo labeling as dogs were infected. The duration of this portion of the curve in the normal dog (mean of 4.8 days) reflects the temporal size of the postmitotic maturation and storage pool of the marrow (approximately 3.5 days) (12), the myelocyte generation time (less than 24 hr) (18), and the blood neutrophil transit time [approximately 6 hr (9)]. The duration of phase I + II was reduced markedly, in some instances to less than one-half the normal value, in most febrile dogs, suggesting that most mature or relatively mature neutrophils had been released from the storage pool. Differential counts of marrow aspirates obtained 1 day after bacterial inoculation were compatible with this interpretation. Similar shortening of phase I + II has been observed after administration of bacterial endotoxin and in two dogs with spontaneous infection (12). The early appearance of radioactive leukocytes in the blood after the intravenous administration of radioactive sodium phosphate was observed in two patients with acute infection by Perry, Craddock, and Lawrence (19). These workers suggested that cells entered not only at a greater rate than usual but at an earlier period of cell maturation, as indicated by the methyl-green pyronin stain (20).

One dog developed severe neutropenia in response to infection after first developing transient neutrophilia (Fig. 1). The development of neutropenia was associated with an abnormally rapid rate of decrease of BGSA after in vitro labeling and with a marked increase in N/S. This suggests that in this dog release of cells from the marrow was not able to keep up with the accelerated cell loss from the blood. Data from kinetic observations of labeled cells, the loss of mature neutrophils from marrow aspirates, and the increase in blood N/S as neutropenia developed suggest that this dog may well have exhausted his marrow granulocyte reserve. If the usual mechanism of neutropenia in severe bacterial infections represents exhaustion of the marrow granulocyte reserve, then the poor prognostic implications of such neutropenia (21) is easily explained. There is no evidence from our studies to support the concept that infection depresses marrow release of neutrophils.

The degree of neutrophilia, as measured by neutrophil concentration in venous blood samples, proved to be a poor index of severity of infection. However, N/S changes correlated reasonably well with the extent of fever. This is compatible with the clinical observation that in pneumococcal pneumonia the degree of "shift to the left" is a better index of the severity of infection than is the degree of neutrophilia (6, 21). The size of the CGP represents a balance of three factors: the rate at which cells are lost to the tissues, the rate of release from marrow, and the proportion of blood cells that are in the MGP. The variability of CGP/MGP ratios observed during the different stages of infection suggests that the proportion of marginated cells may be an important factor in the variability of the degree of neutrophilia as measured by the CGP. Conversely, the primary factor that influences the N/S is the rate of release from marrow.

The studies of dogs in which neutrophils were labeled in vitro during the short period in which the infection appeared relatively stable are similar to reported studies of chronic infection in man (3, 4). In such dogs, the mean total blood granulocyte pool (TBGP) size was increased as was the CGP, but the MGP was normal. The rate of decline of the BGSA for the group was normal. This resembles the pattern which has been observed in human subjects with subacute and chronic infections, in whom the CGP was more frequently elevated than the MGP and in whom the $t_{\frac{1}{2}}$ of BGSA was normal or slightly prolonged (3). Such patients have also been noted to have abnormally short marrow transit times as measured by labeling studies employing tritiated thymidine (22) or $DF^{32}P$ (4). This suggests that

in chronic infections the marrow storage pool is, at least from a temporal viewpoint, decreased in size. Whether a decrease in myelocyte generation time is also partly responsible for shortening of these curves is conjectural. Myelocyte generation time contributes more significantly to the slope of phase III of the in vivo $DF^{32}P$ curve than it does to the duration of phase I + II (12). In certain severely infected dogs the slope of phase III was steeper than normal. However, factors other than the myelocyte generation time influence the slope of this curve, such as blood transit time, label reutilization, and most importantly, variation in the transit time of cells through the postmitotic compartment (12). Since the onset of phase III in the infected dogs occurred 2–5 days after the infection, it is unlikely that the blood transit time (t_i of in vitro curves), which was normal or prolonged at that time, can account for the observed decrease. From earlier studies, there is no evidence to suggest that reutilization of isotope plays any role (12). A reduction in the variation in postmitotic transit time, which may explain the acceleration of phase III induced by endotoxin injection (12), may well be equally responsible for the accelerated phase III curves associated with pneumococcal infection.

These dogs appeared to recover from the induced neutrophilia by a virtual cessation of release of neutrophils from the marrow to the blood. This mechanism was suggested by the configuration of the blood granulocyte specific activity (BGSA) curves in animals recovering from infection. As fever disappeared, as N/S declined, and as the degree of neutrophilia either declined or stabilized, a plateau appeared in the BGSA curve derived from in vitro labeling studies. The failure of BGSA to decline after in vitro labeling can be accomplished only if no new unlabeled cells are entering the blood from the marrow or if labeled cells are returning to the blood from the tissues. In the latter event, a rise in granulocyte count would be expected, just the opposite of what was observed. There is abundant evidence against the return of neutrophils from the tissues to the blood. Irradiated dogs subjected to leukopheresis fail to show reentry of granulocytes into the blood from infected areas (23). Granulocytes labeled with ^{32}P do not reenter the circulation from the tissues in response to leukopheresis (24) or typhoid vaccine (25). No interchange of labeled granulocytes between a peritoneal exudate and the blood was demonstrable (16). The lobe count of blood neutrophils during the recovery from leukopenia induced by antineutrophilic serum suggests that the source of neutrophils is the marrow, not the tissues (26). Direct observations have repeatedly shown neutrophils leaving the blood but not returning to it (27).

Although the slow fall in BGSA is similar to that seen in leukokinetic studies in patients with chronic granulocyte leukemia in relapse (2), such patients have large numbers of immature granulocytes which are capable of mitotic division in the blood. Such immature cells were not observed in our studies and extravascular sequestration and reentry, or intravascular division are not a likely explanation for the BGSA curves observed.

The fall in N/S during the recovery phase suggests that maturation of nonsegmented cells to segmented ones was occurring in the blood. Cessation of marrow neutrophil release represents an efficient mechanism for rapid replenishing of the depleted marrow storage pool.

The demonstration of a flat BGSA curve after infusion of in vitro labeled cells is also of some interest with respect to the usefulness of the in vitro $DF^{32}P$ labeling method. In previously published studies significant elution of $DF^{32}P$ from labeled neutrophils or damage to such cells was not detected (28). If $DF^{32}P$ labeled cells present in the blood at the completion of the infusion were subject to elution of the label or were in any way damaged so that they behaved differently from unlabeled cells, a flat BGSA curve would not result.

Acknowledgments

We are grateful to Mr. George Trappett and Mr. Dale Chlarson for animal husbandry, Dr. Richard Parker for advice concerning the bacteriology, Mrs. Vreni Bithell, Mrs. Janice Anderson, Mrs. Barbara Saxon, and Miss Lona Bindbeutel, M.T. (ASCP) for technical assistance, and to Dr. John W. Athens for many helpful suggestions.

References

1. Athens, J. W., A. M. Mauer, S. O. Raab, O. P. Haab, and G. E. Cartwright. 1960. Studies of granulocyte kinetics. *J. Clin. Invest.* 39: 969.
2. Cartwright, G. E., J. W. Athens, O. P. Haab, D. R. Boggs, and M. M. Wintrobe. 1964. Blood granu-

locyte kinetics in conditions associated with granulocytosis. *Ann. N. Y. Acad. Sci.* **113**: 963.

3. Athens, J. W., O. P. Haab, S. O. Raab, D. R. Boggs, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1965. Leukokinetic studies. XI. Blood granulocyte kinetics in polycythemia vera, infection, and myelofibrosis. *J. Clin. Invest.* **44**: 778.
4. Sacchetti, C., A. Ponassi, P. Boccaccio, and L. Morra. 1964. Osservazioni sulla dinamica dei granulociti in alcune condizioni di leucocitosi neutrofila. *Haematol. Latina. (Milan).* **49**: 1231.
5. Terrell, E. E., O. H. Robertson, and L. T. Coggeshall. 1933. Experimental pneumococcus lobar pneumonia in the dog. I. Method of production and course of the disease. *J. Clin. Invest.* **12**: 393.
6. Robertson, O. H., and J. P. Fox. 1939. The relationship of infecting dosage, leukocytic response, bacteremia, and extent of pulmonary involvement to the outcome of experimental lobar pneumonia in the dog. *J. Exptl. Med.* **69**: 229.
7. Hoeprich, P. D. 1957. Evaluation of an improved chemically defined medium for the culture of Diplococcus Pneumoniae. *J. Bacteriol.* **74**: 587.
8. Marsh, D. J., and E. Neale. 1956. A colorimetric method for the detection and determination of certain acid halide and acid anhydride compounds. *Chem. Ind. (London).* **22**: 494.
9. 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.
10. Croxton, F. E. 1959. Elementary Statistics with Applications in Medicine and the Biological Sciences. Dover Publications Inc., New York. 126, 238.
11. Kurth, D., J. W. Athens, E. P. Cronkite, G. E. Cartwright, and M. M. Wintrobe. 1961. Leukokinetic studies. V. Uptake of tritiated diisopropylfluorophosphate by leukocytes. *Proc. Soc. Exptl. Biol. Med.* **107**: 422.
12. 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.
13. Cartwright, G. E., J. W. Athens, and M. M. Wintrobe. 1964. The kinetics of granulopoiesis in normal man. *Blood.* **24**: 780.
14. 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.
15. Boggs, D. R., J. W. Athens, G. E. Cartwright, and M. M. Wintrobe. 1965. "Masked" granulocytosis. *Proc. Soc. Exptl. Biol. Med.* **118**: 753.
16. Craddock, C. G., Jr., S. Perry, and J. S. Lawrence. 1959. Control of the steady state proliferation of leukocytes. *In* The Kinetics of Cellular Proliferation. F. Stohlman, Jr., editor. Grune & Stratton Inc., New York. 242.
17. Boggs, D. R., G. E. Cartwright, and M. M. Wintrobe. 1966. Neutrophilia-inducing activity in plasma of dogs recovering from drug-induced myelotoxicity. *Am. J. Physiol.* **211**: 51.
18. Lala, P. K., M. A. Maloney, and H. M. Patt. 1964. A comparison of two markers of cell proliferation in bone marrow. *Acta Haematol.* **31**: 1.
19. Perry, S., C. G. Craddock, Jr., and J. S. Lawrence. 1958. Rates of appearance and disappearance of white blood cells in normal and in various disease states. *J. Lab. Clin. Med.* **51**: 501.
20. Perry, S., and J. Reynolds. 1956. Methyl-green-pyronin as a differential nucleic acid stain for peripheral blood smears. *Blood.* **11**: 1132.
21. Painton, J. F., and H. J. Ulrich. 1937. Lobar pneumonia: an analysis of 1298 cases. *Ann. Internal Med.* **10**: 1345.
22. Fliedner, T. M., E. P. Cronkite, S. A. Killmann, and V. P. Bond. 1964. Granulocytopoiesis. II. Emergence and pattern of labeling of neutrophilic granulocytes in humans. *Blood.* **24**: 683.
23. Craddock, C. G., Jr. W. S. Adams, S. Perry, W. A. Skoog, and J. S. Lawrence. 1955. Studies of leukopoiesis. *J. Lab. Clin. Med.* **45**: 881.
24. Craddock, C. G., Jr., S. Perry, and J. W. Lawrence. 1956. The dynamics of leukopoiesis and leukocytosis, as studied by leukopheresis and isotopic techniques. *J. Clin. Invest.* **35**: 285.
25. Perry, S., I. M. Weinstein, C. G. Craddock, Jr., and J. S. Lawrence. 1957. The combined use of typhoid vaccine and P^{32} labeling to assess myelopoiesis. *Blood.* **12**: 549.
26. Patt, H. A., M. A. Maloney, and E. M. Jackson. 1957. Recovery of blood neutrophils after acute peripheral depletion. *Am. J. Physiol.* **188**: 585.
27. Algire, G. cited as personal communication by Cronkite, E. P., T. M. Fliedner, V. P. Bond, and J. S. Robertson. 1959. Anatomic and physiologic facts and hypotheses about hemopoietic proliferating systems. *In* The Kinetics of Cellular Proliferation. F. Stohlman, Jr., editor. Grune & Stratton Inc., New York. 242.
28. 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.* **14**: 303.