The Journal of Clinical Investigation

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Leukokinetic Studies.   X. Blood Granulocyte Kinetics in Chronic Myelocytic Leukemia *

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By the transfusion of granulocytes labeled *in vitro* with diisopropyl fluorophosphate (DFP^{32}), it is possible to measure the size of the total blood granulocyte pool (TBGP), the distribution of granulocytes in the two subcompartments thereof, the blood granulocyte half disappearance time ($t_{\frac{1}{2}}$), and the blood granulocyte turnover rate (GTR) (1–3).   In preliminary reports from this laboratory (4–6) and in reports from other laboratories (7, 8) an increase in TBGP size, prolongation of blood granulocyte $t_{\frac{1}{2}}$, and an increase in GTR have been described in a few patients with chronic myelocytic leukemia.   In the present report 43 studies carried out on 29 patients with chronic myelocytic leukemia (CML) in various stages of their disease have been analyzed in an attempt to gain an understanding of these deviations from normal granulocyte kinetics.   In addition, several studies designed to evaluate the cause of the long $t_{\frac{1}{2}}$ values in patients with CML are presented.   Similar studies of granulocyte kinetics in patients with polycythemia vera, subacute or chronic infections, myelofibrosis, or diseases of other kinds are presented in a companion paper.

Methods

In vitro labeled (1), autologous granulocytes were infused in 43 studies on patients with CML.   Of these, 23 studies were performed on patients with a blood granulocyte concentration greater than 30,000 per mm^3

(Table I), 8 studies were carried out on patients with granulocyte concentrations between 10,000 and 30,000 per mm^3 (Table II), and 12 were made on patients with granulocyte concentrations less than 10,000 per mm^3 (Table III).   These groups roughly correspond to patients with CML in relapse, partial relapse (or remission), and remission, respectively, though the classification is obviously an arbitrary one.   Immature granulocytes (myeloblasts, promyelocytes, myelocytes, and metamyelocytes) were present in the blood of most of the patients (Tables I, II, and III), but were generally present in lower numbers in patients in remission.

Repeated leukocyte and 200 cell differential counts were done on each patient while under study.   Throughout the course of each study the granulocyte count remained stable.   Fourteen studies were carried out while patients were receiving therapy with busulfan or colcemide, and these are indicated in the tables.

TBGP size was measured by means of the isotope dilution principle and calculated in two ways.   The "determined" TBGP was calculated from the mean of triplicate blood granulocyte radioactivity values obtained 5 minutes after completion of the infusion (t_0).   The "extrapolated" TBGP was calculated from the blood granulocyte radioactivity value obtained by extrapolating the blood granulocyte radioactivity curve back to the ordinate.   For reasons that will be discussed below, the determined TBGP is believed to be the more accurate value, and therefore only determined TBGP values are given in the tables.   In autologous studies the patient received his own labeled blood, whereas in the isologous transfusions labeled blood from a compatible donor was infused.

In the labeled bone marrow infusion studies marrow was aspirated from the iliac crest and suspended by agitation in 10 ml of acid-citrate-dextrose (ACD) solution, DFP^{32} was added to give a concentration of about 3 μg per ml, and the mixture was incubated for 1 hour at room temperature.   The labeled marrow cells were then transfused intravenously from a standard recipient set (Fenwal HD-92D), the filter of which removed any large marrow particles.

Results

Blood granulocyte radioactivity disappearance curves.   In 24 studies a single exponential blood

TABLE I

Blood granulocyte kinetic values in patients with chronic myelocytic leukemia in
*relapse compared with those in normal subjects**

	Study no.	G	Blasts	Pro	Myelo	Meta	Band	Seg	TBGP	CGP	t½	GTR	Type of curve	Treatment
		per mm³	%	%	%	%	%	%	$\times 10^7$ G/kg		*hrs*	G/kg/day $\times 10^7$		
Mean 56 normals		4,650							62.5	31.4	6.7	163		
95% limits		2,250–6,600							(14–108)	(19–44)	(4–10)	(50–340)		
	V-136	486,550	0.5	5.5	12.5	10.0	40.5	28.0	9,514	3,694	78	2,029	A	4 mg Bu
	II-26	298,680	7.5	6.0	13.0	13.5	28.0	22.5	5,453	2,114	89	1,019	B	0
	V-48	249,240	2.0	3.5	7.5	8.0	38.5	33.5	4,592	1,816	76	1,004	A	0
	VI-12	494,680	4.5	10.5	26.0	12.5	30.5	7.5	4,124	3,724	†		C	0
	V-106	222,700	2.5	6.0	10.0	7.5	36.5	31.5	2,589	1,463	57	755	A	0
	II-84	197,350	Blood smear not available						2,430	1,285	72	561	B	0
	V-66	150,075	0.5	3.0	7.0	12.0	36.5	35.5	2,247	1,074	60	623	A	2 mg Bu
	III-126	88,970	6.0	3.5	4.5	8.0	41.5	26.5	2,009	657	34	983	A	0
	III-102	81,288	1.5	3.0	10.0	11.0	42.0	19.0	1,853	587	46	670	B	0
	III-106	108,113	1.0	2.5	4.5	5.5	39.5	31.0	1,733	809	76	379	A	0
	V-10	59,508	2.5	2.5	11.0	9.0	28.0	38.0	1,513	388	78	559	A	0
	I-159	54,735	1.0	1.5	11.5	11.0	29.0	36.0	1,435	376	26	918	A	0
	VI-42	70,110	1.0	1.0	12.5	7.5	46.0	20.5	1,181	524	†		C	4 mg Bu
	II-82	89,775	1.5	1.5	7.5	10.5	37.0	35.5	1,045	561	32	543	A	0
	VII-88	84,430			14.5	15.0	27.5	35.5	1,012	591	†		C	0
	IV-96	56,088	2.5	4.0	9.5	16.5	38.5	24.0	909	399	67	226	B	0
	VII-48	69,501	2.5	1.5	15.0	12.0	38.0	13.0	890	547	55	269	A	0
	V-124	44,550	1.5	0.5	3.0	5.0	39.0	39.0	849	322	25	565	A	4 mg Bu
	I-200	59,833		0.5	11.0	11.0	30.5	36.5	762	376	40	317	B	0
	III-172	47,499			8.0	13.0	41.0	26.0	695	333	†		C	0
	VII-74	35,363	1.0	3.5	4.5	10.0	25.5	35.0	689	279	†		C	0
	IV-92	73,515	1.0	2.0	12.5	10.0	33.5	32.0	681	520	†		C	6 mg Bu
	III-128	49,855	2.5	2.5	4.5	2.0	28.0	36.0	680	377	78	145	A	0

* G = granulocytes; Blasts = myeloblasts; Pro = promyelocytes; Myelo = myelocytes; Meta = metamyelocytes; Seg = segmented neutrophils; TBGP = total blood granulocyte pool; CGP = circulating granulocyte pool; GTR = granulocyte turnover rate; Bu = busulfan.

† No single t½ value measurable but blood granulocyte radioactivity remained elevated for considerably longer than in normal subjects (see Figure 1).

granulocyte specific activity (BGSA)[1] curve was obtained. These curves are designated type "A" (Figure 1). In five of the studies on patients in relapse and in two patients in remission a rapid fall in blood granulocyte radioactivity took place during the first 3 hours after infusion of the labeled blood. Thereafter a single exponential disappearance curve was found. Such curves are designated type "B" in Figure 1 and Tables I and III. In twelve studies a changing granulocyte disappearance rate was observed, and a single value for t½ could not be obtained. These curves are referred to as type "C" in Figure 1 and Tables I, II, and III.

In type A curves the determined and extrapolated TBGP values were the same. In type B curves the values obtained by extrapolating the second phase of the curve to the ordinate, the extrapolated TBGP, resulted in a TBGP value

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[1] Expressed as counts per minute per milligram granulocyte nitrogen.

considerably larger than the determined values. In type C curves no extrapolated TBGP could be calculated.

Effect of cell damage on BGSA curve. To evaluate cell damage as a possible explanation for the type B and C BGSA curves just described, blood was withdrawn from normal subjects, incubated for time periods of from 2 to 24 hours, labeled with DFP[32], and then returned to the circulation of the donor. The configuration of the BGSA curve and the size of the determined and extrapolated TBGP in these subjects are summarized in Table IV.

When blood was incubated for 2 hours, labeled with DFP[32], and infused, type A curves were obtained. When blood was incubated for 8 or 16 hours predominantly type B curves resulted, and when blood was incubated for 24 hours the BGSA was so low several hours after completion of the infusion that often no BGSA curve could be obtained. No type C curves were encountered in

TABLE II

*Chronic myelocytic leukemia in partial relapse or remission**

Study no.	G	Blasts	Pro	Myelo	Meta	Band	Seg	TBGP	CGP	t½	GTR	Type of curve	Treatment
	per mm³	%	%	%	%	%	%	× 10⁷ G/kg		hrs	G/kg/day ×10⁷		
III-32	20,792	1.0	2.0	7.5	4.5	30.0	37.5	644	135	30.0	357	A	8 mg Bu
I-163	26,522	5.0	2.5	5.0	4.5	25.5	44.0	620	182	36.0	286	A	0
VII-66	15,770	0.5	0.5	5.0	11.0	30.5	36.5	413	130			C	0
V-180	18,426	0.5	1.0	10.0	11.5	24.5	35.5	304	114			C	4 mg Bu
III-104	16,632		4.0	5.5	7.5	7.5	56.5	272	117	10.6	325	A	0
V-186	22,605	1.5	3.0	5.0	5.5	20.0	54.0	263	165			C	0
V-130	12,549		1.0	6.0	4.5	17.0	47.0	187	86			C	0
V-148	11,106		1.5	9.5	7.5	10.0	56.5	128	81	7.1	92	A	4 mg Bu

* See Table I for ionsabbreviat and footnotes.

any of these studies. In addition, when blood was incubated for 8, 16, or 24 hours, labeled, and infused, a significant portion of the labeled cells was apparently removed from circulation during the infusion since the determined TBGP values were unusually large for normal subjects with normal granulocyte counts. The removal of damaged cells from the circulation apparently continued for several hours as indicated by the initial rapid fall in type B curves and the extremely large extrapolated TBGP values in such studies. There was no cell clumping or other obvious reason for the rapid removal of these cells from the circulation.

Relationship between granulocyte count and TBGP size. There was good correlation between granulocyte concentration and determined TBGP size in the 43 studies on patients with CML $(r = +0.76, p = <0.001)$. This relationship

for the 31 studies on patients in relapse and in partial relapse is shown in Figure 2.

In all patients with granulocyte counts greater than 10,000 per mm³ the TBGP was increased in size, and in four of the eight patients with granulocyte counts in the normal range [less than 7,500 per mm³ (9)] the TBGP was greater than the upper limit of normal.

In the seven studies in which type B curves were obtained the TBGP calculated from the determined t_0 values was in good agreement with that expected from the granulocyte count (Figure 2), whereas the TBGP size calculated from the extrapolated t_0 value was unusually large.

Distribution of granulocytes in the TBGP. The distribution of cells in the TBGP of patients with chronic myelocytic leukemia (CGP/TBGP, circulating:total blood granulocyte pool ratio) was

TABLE III

*Chronic myelocytic leukemia in remission**

Study no.	G	Blasts	Pro	Myelo	Meta	Band	Seg	TBGP	CGP	t½	GTR	Type of cruve	Treatment
	per mm³	%	%	%	%	%	%	×10⁷ G/kg		hrs	G/kg/day ×10⁷		
II-190	9,116		1.5	2.0	1.0	15.0	59.0	252	65	7.8	537	A	0
V-8	6,799			5.5	1.5	4.0	73.0	136	49	5.4	419	A	0
II-172	6,391	1.0				5.5	74.0	128	39	6.1	349	A	Colcemide
III-140	5,472			5.5	5.0	10.5	60.5	118	35	10.3	191	A	0
V-164	9,173		1.0	4.0	10.0	25.0	32.5	106	67	†		C	0
II-188	5,720	0.5	1.5	2.0	1.0	5.5	60.5	101	45	8.6	195	A	0
III-174	7,703	1.5	1.0	3.5	7.5	20.0	44.5	85	57	9.6	147	A	4 mg Bu
III-114	6,645			4.5	1.5	18.0	56.5	82	64	7.5	182	A	Colcemide
II-186	4,635	1.0		2.0	3.0	17.5	41.5	71	31	7.0	169	A	0
VII-82	1,943				4.0	13.0	41.5	42	13	†		C	0
IV-76	7,052	2.0	1.5	6.0	11.0	27.0	29.5	242	52	40.5	99	B	‡
III-38	7,224	2.5		3.0	9.0	57.5	18.5	88	51	45.0	33	B	‡

* See Table I for abbreviations.
† No single t½ value measurable.
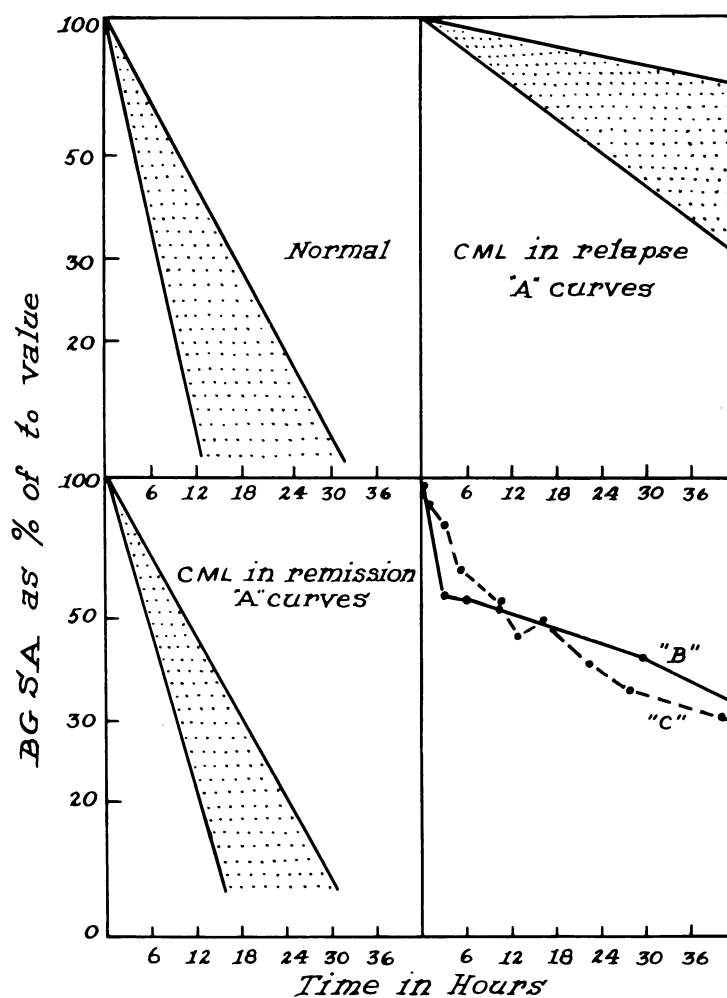‡ Off therapy 3 days.

FIG. 1. BLOOD GRANULOCYTE SPECIFIC ACTIVITY (BGSA) CURVES OBTAINED
IN PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA (CML) IN RELAPSE AND
REMISSION COMPARED WITH THOSE IN NORMAL SUBJECTS (FIRST THREE
PANELS). Examples of type "B" and "C" curves are shown in the last panel.

TABLE IV

*Effect of time of incubation of blood on the configuration of the blood granulocyte radioactivity
curve and on TBGP size in normal subjects**

Incubation time	No. of studies	Curve type	Determined TBGP	Extrapolated TBGP
hrs			$\times$ *10⁷ G/kg*	
2	4	4 Type A	113 (48–179)	113 (48–179)
8	5	1 Type A 4 Type B	167 (62–404)	560 (164–610)
16	5	1 Type A 4 Type B	349 (160–820)	1,415 (124–1,927)
24	8	2 Type B 6 Radioactivity too low to give a curve	676 (319–1,168)	

* See Table I for abbreviations.

compared with the distribution found in normal subjects (Figure 3). There was no difference between these groups. This is in contrast to the finding in myelofibrosis and in some cases of polycythemia vera where a shift of cells into marginal sites (MGP) was noted whenever the TBGP was found to be increased in size (10).

Four patients were given epinephrine infusions (0.5 mg diluted in 50 ml of isotonic saline injected over 5 to 7 minutes) to determine whether granulocytes in the MGP could be mobilized as readily in patients with chronic myelocytic leukemia as in normal subjects (2). An increase in granulocyte count resulted from the infusion of epinephrine (Table V). No alteration in differential count or blood granulocyte radioactivity accompanied the mobilization of 45 to 71% of the cells in the MGP of these patients.
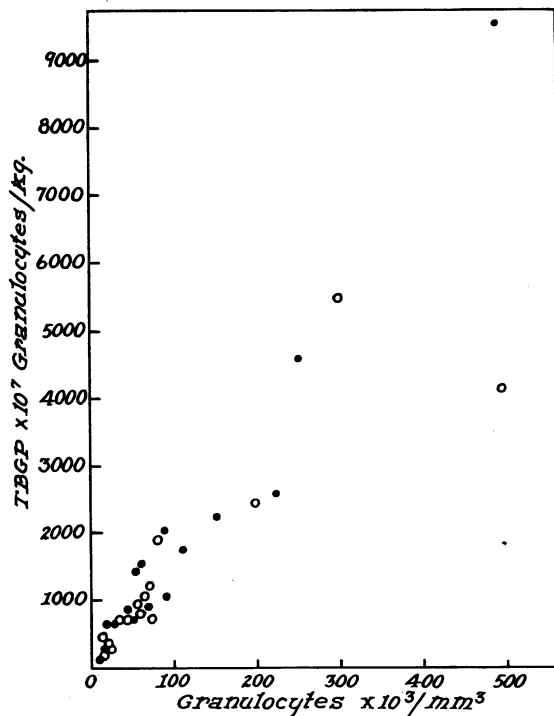
FIG. 2. RELATIONSHIP BETWEEN BLOOD GRANULOCYTE COUNT AND TOTAL BLOOD GRANULOCYTE POOL (TBGP) SIZE IN PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA. The values on patients in remission are not plotted because of the congestion that would result in the lower left corner of the graph. The solid circles (●) represent the values obtained in patients with type "A" curves. The hollow circles (O) are the TBGP values (calculated from "determined" rather than "extrapolated" t₀ radioactivity) obtained in patients with type "B" and "C" curves.
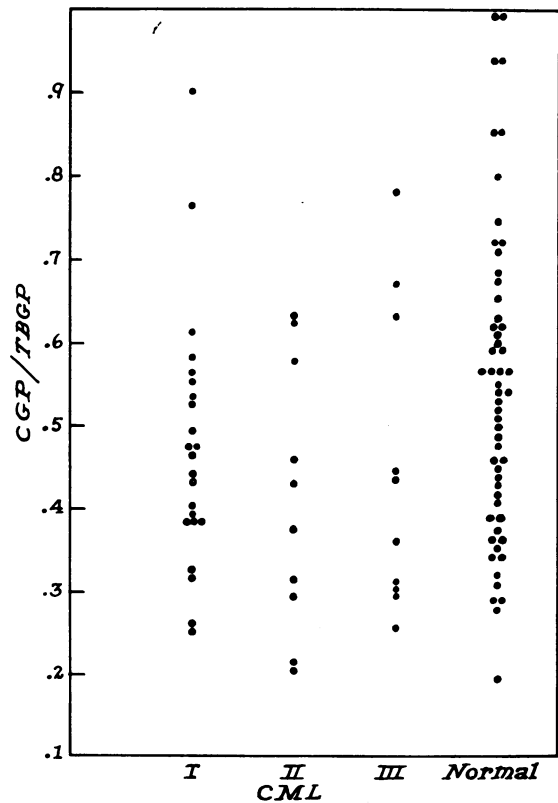

FIG. 3. DISTRIBUTION OF GRANULOCYTES AS REPRESENTED BY THE RATIO, CIRCULATING GRANULOCYTE POOL TO TOTAL BLOOD GRANULOCYTE POOL (CGP/TBGP), IN THE TBGP OF PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA (CML) IN RELAPSE (I), PARTIAL RELAPSE OR REMISSION (II), AND IN REMISSION (III), AS COMPARED TO NORMAL SUBJECTS.

Granulocyte disappearance curve. The curves of BGSA encountered in these studies are illustrated in Figure 1. Regardless of curve shape the BGSA remained elevated for a prolonged period in all patients with chronic myelocytic leukemia in relapse (Table I and Figure 1). Among patients with granulocyte counts less than 10,000 per mm³, 8 of the 10 $t_{\frac{1}{2}}$ values were normal in spite of the persistence of a few immature granulocytes in the blood (Table III and Figure 1). The two striking exceptions to this were patients IV-76 and III-38, both of whom had just completed their fourth and fifth courses of therapy, respectively, and soon thereafter entered the terminal phase of their disease. In the patients in partial relapse (or remission) $t_{\frac{1}{2}}$ values intermediate between those encountered in the two groups just mentioned were observed (Table II).

TABLE V

*Influence of epinephrine infusion on blood granulocyte count and granulocyte radioactivity in patients with chronic myelocytic leukemia**

Study no.	Time of epinephrine infusion†	G per mm³		% of MGP mobilized	G Radioactivity	
		Before	After		Before	After
III-32	t_0	20,790	70,600	64		
III-172	t_{52}	56,980	101,130	71	76	81
IV-76	t_{29}	8,672	22,869	45	250	242
V-130	t_{47}	11,914	20,604	62	159	169

* See Table I for abbreviations.   MGP = marginal granulocyte pool.
† The values t_0, t_{52}, etc., refer to the time after completion of the infusion of autologous, DFP³²-labeled blood that the epinephrine infusions were given.

Since the $t_{\frac{1}{2}}$ was markedly prolonged in CML patients with high granulocyte concentrations and large TBGP, the relationship between the $t_{\frac{1}{2}}$ and TBGP was examined. We have previously reported poor correlation between the $t_{\frac{1}{2}}$ and TBGP size, as calculated from extrapolated t_0 values (6). In contrast, the correlation between the $t_{\frac{1}{2}}$ and

the TBGP calculated from determined t_0 values was + 0.67 (p = < 0.001). Patients IV-76 and III-38 (Table III), mentioned above, were excluded from this calculation.

Because the $t_{\frac{1}{2}}$ values in patients with CML in relapse were unusually long as compared to those in normal subjects and in patients with other types of neutrophilia without increased numbers of immature granulocytes in the blood (10), the relationship between the $t_{\frac{1}{2}}$ and the proportion of immature granulocytes in the blood was examined. In the 28 patients with $t_{\frac{1}{2}}$ values and differential counts (Tables I, II, and III) the correlations between $t_{\frac{1}{2}}$ values and the proportion of different types of neutrophilic granulocytes were calculated (Patients IV-76, III-38, and II-84 were excluded). The correlation between the $t_{\frac{1}{2}}$ and the proportion of myelocytes was r = + 0.58, p = < 0.005, and between the $t_{\frac{1}{2}}$ and the sum of myelocyte and metamyelocyte percentages it was r = + 0.61, p = < 0.001. Since promyelocytes and myeloblasts are not labeled with DFP³² (11), no attempt was made to correlate $t_{\frac{1}{2}}$ and the proportion of these cell forms present in the blood.

Relationship between the TBGP and GTR. In patients in relapse the GTR was usually moderately increased above normal (Table I and Figure 4). In patients in remission the GTR values were normal or nearly normal (Table III), whereas in patients in partial relapse the values overlapped the other two groups. The correlation between GTR and TBGP was + 0.90 (p = < 0.001).

Isologous transfusions. In an attempt to discover the reason for the slow decrease in blood granulocyte radioactivity in patients with CML, normal blood was labeled with DFP³² and trans-
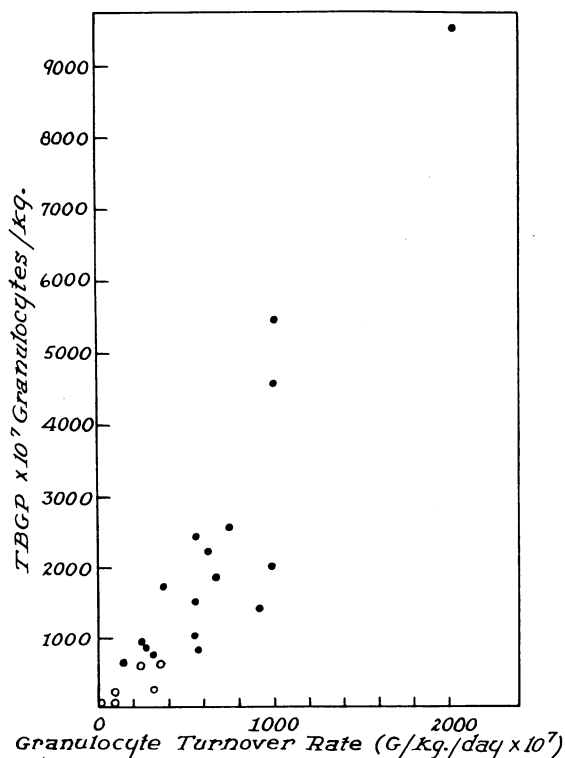

FIG. 4. RELATIONSHIP BETWEEN THE GRANULOCYTE TURNOVER RATE (GTR) AND THE TOTAL BLOOD GRANULOCYTE POOL (TBGP) IN PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA IN RELAPSE AND PARTIAL RELAPSE. The solid circles (●) represent patients in relapse. The hollow circles (○) refer to patients in partial relapse.

TABLE VI

*Isologous transfusion studies: normal blood to patients with chronic myelocytic leukemia**

Study no.	Recipient	TBGP	CGP	$t_{\frac{1}{2}}$	Type of curve
	G per mm³	$\times 10^7$ *G/kg*		*hrs*	
II-162	23,250	274	148	10.7	A
III-8	24,840	623	178	8.0	A
VI-52	29,205	522	207	6.8	A
VI-72	49,940	724	363	7.7	A

* See Table I for abbreviations.

fused into compatible recipients with CML (in relapse and partial relapse), and blood from patients with CML in relapse was labeled with DFP[32] and transfused into hematologically normal, compatible recipients with incurable carcinomas.

In four of five patients with CML given normal cells, the normal neutrophils left the blood in a random manner, $t_{\frac{1}{2}}$ values being normal (Table VI). The fifth study was technically unsatisfactory.

In three hematologically normal subjects given labeled blood from patients with CML (Table VII) the blood granulocyte radioactivity curves were complex (Figure 5, curves 1, 2, and 3). Nevertheless, the blood granulocyte radioactivity remained elevated for considerably longer periods than when normal blood was given to isologous recipients (1). This was observed in spite of the fact that in no instance was a recipient transfused with more than about half as many granulocytes as were calculated to be present in his TBGP.

Infusion of DFP[32]-labeled autologous bone marrow. To further evaluate the role of immaturity of leukocytes as a possible reason for the slow decrease in BGSA in patients with CML, two subjects were given infusions of DFP[32]-la-

beled, autologous bone marrow cell suspensions. One study was unsatisfactory because of low radioactivity values. In the second study the labeled cells left the blood in a complex manner (Figure 5, curve 4), but the granulocyte radioactivity was still elevated after 8 days. This subject received 700 million labeled granulocytes, a number equal to about 3% of his TBGP.

Patients in whom several studies were carried out. In two patients three and four studies respectively were carried out at various stages of their disease. When the $t_{\frac{1}{2}}$ obtained in the several studies on each patient was compared with the TBGP size (Figure 6), it was evident that at low cell counts and thus smaller TBGP the $t_{\frac{1}{2}}$ was shorter than when the cell count was higher and the TBGP larger. The blood differential count changed very little during the course of therapy in Patient V-48, whereas in patient III-102 the proportion of myelocytes plus metamyelocytes decreased from 21 to 3% and the juvenile plus polymorphonuclear percentage increased from 61 to 74.

Discussion

A ten- to 150-fold increase in TBGP size has been demonstrated in patients with CML in re-

TABLE VII

*Isologous transfusion studies: chronic myelocytic leukemia blood to normal subjects**

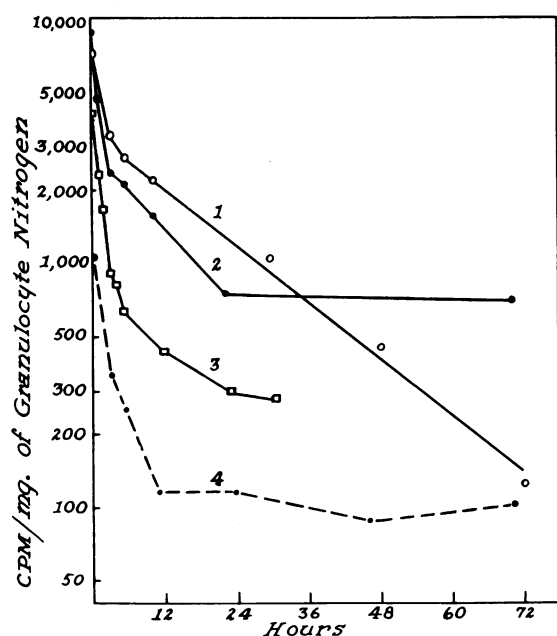| Study no.	Donor	Recipient			
		TBGP	CGP	G radioactivity disappearance	Type of curve
	G per mm³	$\times 10^7$ *G/kg*			
II-234	57,024	219	35	Prolonged	B
IV-4	50,030	75	22	Prolonged	B
V-12	51,238	123	28	Prolonged	C

* See Table I for abbreviations.

FIG. 5. CURVES FOR BLOOD GRANULOCYTE SPECIFIC AC-
TIVITY OBSERVED WHEN ISOLOGOUS BLOOD FROM PATIENTS
WITH CHRONIC MYELOCYTIC LEUKEMIA WAS TRANSFUSED
INTO HEMATOLOGICALLY NORMAL HUMAN SUBJECTS
(CURVES 1, 2, 3). Curve 4 was obtained after the infu-
sion of autologous normal bone marrow.

lapse. These data together with the three com-
parable studies reported by Mauer and Jarrold
(7) comprise the only quantitative information
on the size of the blood granulocyte pool (TBGP)
in this disease. We observed higher TBGP values
than did Mauer and Jarrold. This is probably
due to the fact that a few patients with much
higher blood granulocyte counts were studied by
us than by these authors. They raised some ques-
tion as to the validity of pool size estimations in
CML. However, several findings in the present
study lead us to believe that the size of the TBGP
can be measured as accurately in this disease as in
normal subjects. Thus, in the epinephrine stud-
ies (Table V) no change in differential cell counts
or BGSA accompanied the mobilization of large
numbers of granulocytes from the MGP. This
indicates that cells in the CGP and MGP are in
equilibrium, as is the case in normal subjects
(2), and that there is no selective sequestration of
immature cell forms. In addition, good correla-
tion was found between granulocyte count and
TBGP size when single exponential BGSA curves

were obtained (Figure 1). It is true that TBGP
values calculated from extrapolated zero time (t_0)
values were unusually high in subjects with type
B curves (Figure 3). However, TBGP calcu-
lated from the determined t_0 values (the mean of
triplicate samples was used) almost invariably gave
values consistent with those expected from the
granulocyte count (Figure 1). It therefore seems
that the TBGP calculated from the determined t_0
value gives a good approximation of TBGP size
in most instances regardless of the shape of the
subsequent BGSA curve.

The distribution of cells in the CGP and MGP
of patients in relapse was similar to that in normal
subjects (Figure 2). This is in contrast to the
findings in patients with polycythemia vera and
myelofibrosis in whom, as the TBGP increased in
size, an increasing proportion of granulocytes ac-
cumulated in the MGP (10).

In all patients with CML in relapse the disap-
pearance of labeled cells from the blood was much
slower than in normal subjects or in patients with
most other types of neutrophilia (10). This was
true in patients with complex BGSA curves (types
B and C) as well as in patients with single ex-
ponential BGSA curves (type A). This finding,
coupled with the demonstration that as patients
were brought into remission the BGSA curve
returned toward and finally reached normal (Ta-
bles II and III and Figure 6), is most provocative.
Several possible explanations for this have been
considered, but no single one seems entirely ade-
quate.

One possible explanation for the slow decrease
in BGSA might be the presence of increased num-
bers of labeled myelocytes and metamyelocytes in
the blood of patients with CML in relapse as
compared with the blood of normal persons.
[Myeloblasts and promyelocytes do not accept
the label (11)]. It is of interest that t_i values
similar to those found in CML have been en-
countered in only several other studies, all in pa-
tients thought to have myelofibrosis but with dif-
ferential leukocyte counts similar to that seen in
CML (10).

Craddock postulated that immature cells may
circulate longer than mature forms (12). Jaffé
(13) demonstrated that young cell forms do not
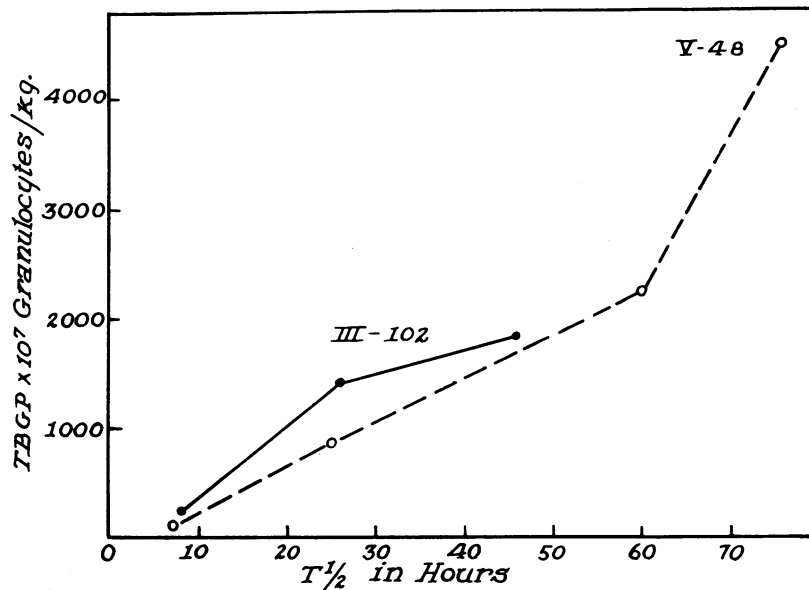enter the exudates associated with fatal infections

FIG. 6. RELATIONSHIP BETWEEN TOTAL BLOOD GRANULOCYTE POOL (TBGP) SIZE AND T½ IN TWO PATIENTS STUDIED AT SEVERAL PERIODS DURING THE COURSE OF THEIR DISEASE. The measurements made at different times are joined by a continuous and an interrupted line in these patients.

in patients with leukemia. Boggs (14) and Perillie and Finch (15) confirmed these findings by producing experimental inflammations. They demonstrated that myelocytes do not leave the blood to enter sites of inflammation and metamyelocytes do so only in small numbers. If, as such studies suggest, the rate of efflux of granulocytes from the blood is related to their stage of maturity, the slow decline of blood granulocyte radioactivity in CML is understandable. The correlation found between the percentage of immature cells and the $t_{\frac{1}{2}}$ ($r = +0.58$ to $+0.61$), the slow decrease in blood granulocyte radioactivity seen in the hematologically normal subjects given labeled CML blood and in the subject given labeled bone marrow (Figure 5), and the normal $t_{\frac{1}{2}}$ encountered when patients with CML were transfused with isologous normal blood (Table V) are compatible with this hypothesis. The last mentioned observations, however, must be interpreted with caution since it appears from preliminary studies that infused isologous granulocytes are different kinetically from autologous granulocytes (16).

It is necessary to consider whether the slow decrease in BGSA can be attributed to other causes than the prolonged circulation of immature forms of granulocytes. Since a single exponential BGSA curve was encountered in 12 of 23 studies on patients in relapse it would appear that mature and immature cells left the blood at the same rate in these subjects. Furthermore, in some patients in remission but with considerable numbers of immature cells present in their blood, a normal rate of decrease in BGSA was observed (Table III). Neither of these observations is compatible with the hypothesis that a slow decrease in BGSA in patients with CML in relapse is due solely to the continued circulation of labeled myelocytes and metamyelocytes.

A slow decrease in BGSA could also be explained on the basis of a large pool (TBGP) with a smaller than normal fractional turnover rate. The correlation between TBGP size and the $t_{\frac{1}{2}}$ ($r = +0.67$, $p = <0.001$) is compatible with this thesis. This hypothesis seems especially attractive since, as the disease is brought under control, the TBGP and $t_{\frac{1}{2}}$ return to normal values even in patients who exhibit no appreciable change in differential cell count (Patient V-48 in Figure 6). However, that TBGP size alone does not determine the $t_{\frac{1}{2}}$ is indicated by the findings in
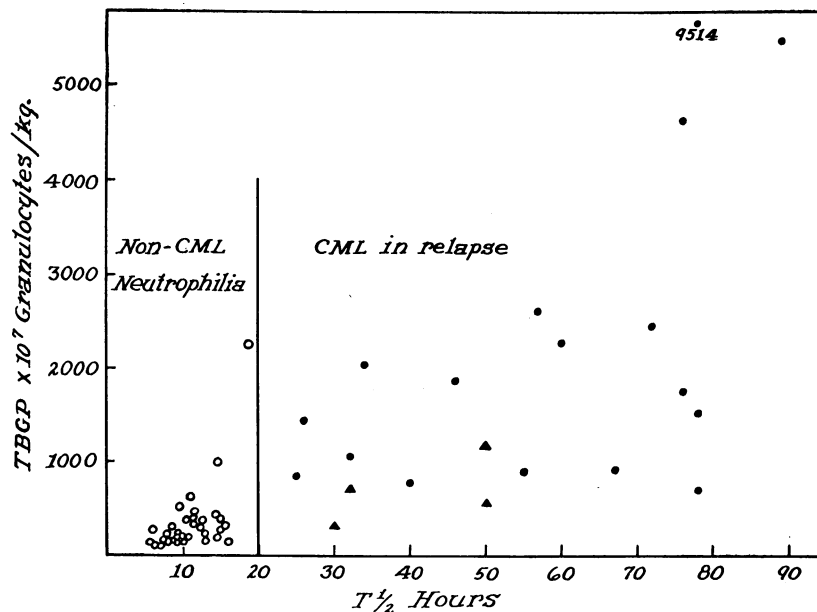
FIG. 7. RELATIONSHIP OF TOTAL BLOOD GRANULOCYTE POOL (TBGP) TO $t_{\frac{1}{2}}$ IN PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA (CML) IN RELAPSE AS COMPARED WITH PATIENTS WITH NEUTROPHILIA OF OTHER TYPES (POLYCYTHEMIA VERA, SUB- ACUTE AND CHRONIC INFECTION, AND MISCELLANEOUS DISORDERS). Solid circles (●) refer to patients with CML in relapse. Hollow circles (○) represent non- CML neutrophilias. The triangles (▲) depict studies in patients with myelofibro- sis but with myelocytes and metamyelocytes in the blood in about the same numbers as in patients with CML in relapse.

Patients IV-76 and III-38 (Table III), in whom the TBGP size was normal but the $t_{\frac{1}{2}}$ was mark- edly prolonged, and from the TBGP size and $t_{\frac{1}{2}}$ values encountered in patients with CML as com- pared with those in patients with other diseases (Figure 7) (10). At comparable TBGP sizes, patients with CML have considerably longer $t_{\frac{1}{2}}$ values than do patients with other forms of neu- trophilia not characterized by the presence of im- mature granulocytes in the blood. In addition, if a long $t_{\frac{1}{2}}$ occurred only as a result of large TBGP size, normal compatible cells given to patients with CML would be expected to have a long $t_{\frac{1}{2}}$. This was not the case (Table V).

The fact that myelocytes bind more DFP[32] per cell than more mature forms exposed to the same concentrations of label (11) and the demonstration by Monti, Maloney, Weber, and Patt (17) that at least some of the immature cell forms in the blood of patients with CML divide and mature into segmented neutrophils add to the difficulty of interpreting the decline in the BGSA curve and

permit one to postulate still other explanations for the long $t_{\frac{1}{2}}$ in these patients. These are being explored.

The inability of immature cell forms to enter inflammatory exudates (14, 15) suggests that the rate of egress of granulocytes from the blood may vary with their stage of maturity. However, it may be argued that such variability in cell disap- pearance rates should result in a biphasic or more complex curve, not in a single exponential BGSA curve. In this respect, it is noteworthy that it has not yet been demonstrated that blood granulocytes disappear from the blood only to enter sites of tis- sue injury and inflammation or that such egress determines the blood granulocyte turnover rate. Not only is there evidence that granulocytes are normally lost via such sites as the lungs (18), oral cavity (18, 19), and urinary tract (20), in the ab- sence of any evidence of infection, but, in addition, it is recognized that immature as well as mature granulocytes are found in many tissues in patients with CML and perhaps reached such sites via the

blood. Although the validity of the calculation of GTR in CML cannot be accepted without reservation as pointed out previously (7), since in 12 of the 23 studies on patients in relapse a single exponential BGSA curve was obtained, and since the sum of a spectrum of first order granulocyte disappearance rates could not give a single exponential curve (21), the calculation of GTR in these subjects perhaps is valid. If we assume this to be the case, GTR values in CML range from normal to 12 times the normal mean value (Table I).

Unfortunately, our own GTR values and those of Mauer and Jarrold cannot be compared with similar values obtained by other methods since none are available at present. Nevertheless, if urinary uric acid excretion values have any significance as an indication of cellular turnover, they support the hypothesis that there is a moderate increase in cell turnover in CML (22–24).

There is good evidence for differences in cell enzyme concentration (25) and chromosome make-up (26) in CML leukocytes as compared to normal granulocytes. However, several patients thought to have myelofibrosis and with immature granulocytes in their blood showed a kinetic pattern similar to that seen in CML (10). Detailed leukocyte enzyme analyses and chromosome studies are not available for these patients. However, the leukocyte alkaline phosphatase values in several of them were in the normal range (10). This fact and the prolonged elevation of BGSA after the infusion of normal bone marrow granulocytes (Figure 5) make us reluctant at this time to accept the hypothesis that CML granulocytes are kinetically different from normal cells of the same morphologic type.

The several different types of curves (Figure 1, panels 2 and 4) encountered in these studies deserve further comment. The first order or random disappearance of granulocytes from the blood in over one-half of these studies is the same as was encountered in 56 of 59 normal subjects and denotes a single granulocyte disappearance rate. The seven studies in which a rapid decrease in BGSA occurred during the first several hours after transfusion and was followed by a single exponential curve (Figure 1, curve B) can be explained in several ways. Two populations of

cells, one with a rapid $t_{\frac{1}{2}}$ and the other with a slow $t_{\frac{1}{2}}$ could give such a curve. A more likely explanation is that in some studies a portion of the labeled cells was damaged while the remainder was not, and after infusion the damaged cells were removed from the blood while the undamaged cells remained. The finding that in subjects with type B curves the TBGP calculated from the determined t_0 value was consistent with the granulocyte count (Figure 2) whereas the extrapolated t_0 value resulted in unusually high TBGP values, suggests this possibility.

A pertinent question remains, however. Why are several hours required for the removal of the damaged cells? The only answer we can give is to cite our experience with the removal of purposely damaged, DFP^{32}-labeled granulocytes (Table IV). In eight of ten studies in which granulocytes were damaged by incubation for 8 or 16 hours outside the body, and were then labeled with DFP^{32} and infused, the BGSA curve was of the type B configuration. The mean TBGP calculated from the determined t_0 radioactivity values was twice normal in the 8-hour group and six times normal in the 16-hour group, and the extrapolated TBGP values were extremely large. In eight studies in which the cells were incubated for 24 hours, labeled, and infused, two curves were of the B configuration, whereas in the remaining six the radioactivity values were too low to obtain a curve. The mean determined TBGP in the group was ten times normal. From these results it seems that granulocytes damaged during incubation are removed from the blood and that the rate of removal may be related to the degree of damage, less severely damaged cells remaining in the circulation for up to several hours.

It is possible that type C curves may also reflect cell damage in some way. However, no such curves were encountered in the studies just cited in which cells were intentionally damaged. Other explanations such as an equilibration of blood granulocytes with a large mass of cells in extravascular sites could explain type C curves. However, since type C curves were seen in patients with CML in remission as well as in relapse, in patients with other diseases (10), and in occasional normal subjects, it seems more likely that these curves reflect a nonsteady state that is not

evident in the blood granulocyte concentration. One finding in favor of this suggestion is that in patients in whom skin inflammations were produced, type C curves were common. In these patients there was an increased inflow of cells into the blood from the bone marrow, but no changes in granulocyte concentration were observed. The TBGP enlarged but the enlargement was in the MGP not the CGP (27). It is also possible that type C curves reflect the presence of several cell populations in the blood, each with a different disappearance rate. This possibility has been discussed already.

Summary

Blood granulocyte kinetics in chronic myelocytic leukemia has been evaluated by means of the diisopropyl fluorophosphate (DFP^{32}) *in vitro* technic. Forty-three studies were carried out in 29 patients in all phases of their disease. Chronic myelocytic leukemia in relapse was characterized by marked enlargement of the total blood granulocyte pool (10 to 150 times normal) and a prolonged blood granulocyte disappearance time (4 to 12 times normal). With appropriate therapy these kinetic parameters returned to normal. The blood granulocyte count was a good index of the degree of blood granulocyte pool enlargement since both the circulating and marginal granulocyte pools were enlarged and there was no selective sequestration of immature granulocytes in marginal sites. The prolonged blood granulocyte disappearance time appeared to be related to the presence of immature granulocytes in the blood and the size of the blood granulocyte pool and may have been influenced by other unknown factors as well. No support was found for the hypothesis that the granulocytes of chronic myelocytic leukemia are different kinetically from normal granulocytes of the same morphologic type.

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