IN VITRO ^{51}Cr AND ^{32}P-DFP LABELING OF GRANULOCYTES IN MAN

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Several labels have been used for in vitro labeling of granulocytes. ^{32}P-diisopropylfluorophosphate (^{32}P-DFP) was originally used by Athens, et al in 1959 (*1*). These authors found a disappearance half-time from circulating blood of 6.6 hr (4–10 hr) for ^{32}P-DFP-labeled granulocytes. In 1966 Dresch and Najean (*2–4*) labeled normal granulocytes with ^{51}Cr, using the technique published by McCall (*5*) for leukemic cells, and found a disappearance half-time of 19 ± 4 hr for normal granulocytes.

In a previous paper (*2*) we proposed three hypotheses to explain the discrepancy between the results obtained with ^{51}Cr- and ^{32}P-DFP-labeled granulocytes:

1. Cells are uniformly labeled, regardless of age, by ^{51}Cr whereas ^{32}P-DFP labels older cells more readily. This hypothesis could explain a shorter disappearance half-time with ^{32}P-DFP labeling if granulocytes do not leave the circulation in a strictly random way.
2. The longer disappearance half-time in the circulation of ^{51}Cr-labeled granulocytes is due to metabolic damage which hampers their migration into the tissues but does not alter their intravascular survival time.
3. ^{32}P-DFP is eluted in vivo although there is no evidence for in vitro elution (*1*), or ^{32}P-DFP alters the labeled cells and causes a shorter life span. In both cases, the disappearance half-time of ^{32}P-labeled cells would be less than normal.

None of these hypotheses could be substantiated when we proposed them. However, in this paper we wish to present data on sequential and simultaneous in vitro labeling of granulocytes by ^{51}Cr and ^{32}P-DFP which explain the different results obtained with the two methods.

We have found a very good correlation between the disappearance half-time of granulocytes labeled separately by these two radionuclides. Such a correlation enabled us to use a double-labeling method to demonstrate a qualitative granulocyte anomaly in some cases of chronic leukopenia. Indeed, when one uses only one radionuclide, two separate labelings are needed, first of the patient's own granulocytes and, second, of homologous normal granulocytes (*3,6*). However, several consecutive tests are often difficult to complete, and their interpretation is always subject to controversy since in the interval between the tests a change in leukocyte kinetics may occur. Double labeling by ^{51}Cr of autologous granulocytes and by ^{32}P-DFP of homologous granulocytes would allow us to compare the life span of normal and pathological granulocytes under the same conditions if a consistent correlation exists between the disappearance half-time of cells labeled by these two tracers.

MATERIALS AND METHODS

Labeling methods. The method for ^{51}Cr labeling of granulocytes has already been described (*2*). Three hundred to 400 ml of blood are collected in 0.6% EDTA or in ACD in a siliconized glass bottle. We observed no difference when using EDTA or ACD in the disappearance half-time of ^{51}Cr-labeled granulocytes. From 1963 to 1966, 0.6% EDTA was used in 92 studies with a mean disappearance half-time for 32 normal subjects of 19.6 ± 3.8 hr; from 1966 to 1970, ACD (citric acid 1.76 gm, NaOH 0.68 gm, glucose 1.8 gm, distilled water 75 ml for 300–400 ml blood) was used in 70 studies, with a mean disappearance half-time for 27 normal subjects of 18.3 ± 2.8 hr. The difference between these two series is not statistically significant.

After sedimentation in five volumes of 2.5%

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plasmagel (gelatine 2.5%—Roger Bellon) in siliconized tubes (30 cm long and 4 cm wide) at 4°C for 45 min, the supernatant is centrifuged at low speed (800 rpm) for 10 min to concentrate the granulocytes and to eliminate most of the platelets. The supernatant plasma is then centrifuged at high speed (3,000 rpm) to obtain a cell-free plasma. White cells, contaminated by some red cells, are suspended in 20–30 ml of plasma and incubated at room temperature for 45 min with either (A) 600 μCi of ^{51}Cr (CEA, Saclay, specific activity 100 mCi/mg Na-chromate) or (B) 50 μCi (0.10–0.20 mg) of ^{32}P-DFP (Amersham, specific activity 150–200 μCi/ml, 0.46–0.64 mg/ml). White cells are then washed in 200 ml plasma and suspended in 20 ml plasma for injection. All manipulations are made in a previously U.V. sterilized chamber, and centrifugations are done at 4°C.

Sampling and measurements. Blood samples of 20–30 ml are withdrawn from the patient 10, 20, 30, and 60 min after infusion, and then every 90 min to 8 or 10 hr; samples are then withdrawn three times the following day, twice the third day, and once a day up to the fifth or sixth day. Each blood sample is withdrawn in a heparinized plastic or siliconized syringe. After sedimentation in a 1.5% dextran solution (molecular weight 200,000) for 45 min, five volumes of 5% acetic acid are added to the supernatant to hemolyze the red cells. The supernatant is then centrifuged at low speed (800 rpm) for 10 min. Cells are washed twice with saline and then counted for ^{51}Cr activity in a well scintillation counter with a spectrometer (Mesco, Tracerlab).

When ^{32}P-DFP is used, the white cells are dissolved in 0.5 ml of 1 *N* NaOH at 50°C; 1 ml of NCS solubilizer (Amersham/Searle, Nuclear-Chicago) is added to 0.2 ml of the solution. After 12–20 hr of incubation at room temperature, 15 ml of the scintillation mixture (Liquifluor, Nuclear-Chicago) is added, and the sample is counted for ^{32}P-DFP activity in a Beckman liquid scintillation counter. For well and liquid scintillation counting, window widths have been established so that no correction is required for the presence in the sample of the other nuclide.

Nitrogen is estimated in total white cells in single ^{51}Cr labeling or in the remaining white cells in double labeling studies using a Technicon Autoanalyzer. Radioactivity is expressed as counts/min/mg nitrogen in each sample. Results are plotted on semilogarithmic paper. With ^{51}Cr labeling, radioactivity on the fifth or sixth day is taken as contamination by labeled lymphocytes and subtracted from each previous point (*2*).

Subjects studied. Seven normal adults (four males and three females) had two separate studies: First, autologous ^{51}Cr-labeled granulocytes were infused. Then two to six months later, autologous granulocytes simultaneously labeled with ^{51}Cr and ^{32}P-DFP were infused for a second study. The blood granulocyte number was the same in both studies.

In addition, 25 adults were studied by a simultaneous double test. Their own granulocytes were labeled with ^{51}Cr, and homologous normal granulocytes were tagged with ^{32}P-DFP. The separately labeled autologous and homologous granulocytes were simultaneously injected. Of these 25 subjects, two were hematologically normal, 20 were leukopenic (marrow hypoplasia—4, idiopathic chronic leukopenia—10, rheumatoid arthritis—3, and toxic chronic leukopenia—3) and three had a chronic leukocytosis without any recognized etiology.

In vitro studies. For in vitro studies, normal granulocytes were labeled as described previously either with ^{51}Cr or with ^{32}P-DFP at 0.15, 0.30, and 0.60 mg/30 ml of cell suspension. The ^{51}Cr-labeled granulocytes were incubated with two concentrations of DFP (0.15 mg and 0.30 mg/30 ml), and these were compared with ^{51}Cr-labeled granulocytes incubated without DFP. Incubations were made in plasma at 37 and 4°C. At 1, 2, 3, and 4 hr, total supernatant radioactivity, protein-bound supernatant radioactivity, and granulocyte specific activity were determined.

RESULTS

Studies in normal subjects. Table 1 shows the results of the two different tests in seven normal subjects. Column 1 gives the $T_{1/2}$ of ^{51}Cr-labeled cells in the first study (single labeling of autologous granulocytes). Column 2 gives the $T_{1/2}$ of ^{51}Cr-labeled cells in the second study (simultaneous labeling by ^{51}Cr and ^{32}P-DFP of autologous granulocytes). Column 3 gives the $T_{1/2}$ of ^{32}P-DFP-labeled cells in the second study.

The kinetics of ^{51}Cr-labeled granulocytes has been described in previous papers (*2,4*). One typical curve is shown in Fig. 1. Distribution of the labeled leukocytes into two pools at equilibrium, circulating and marginal, of nearly equivalent size, is attained between half an hour and one hour. Thereafter the labeled cells disappear from the circulating pool with a $T_{1/2}$ of 18.9 ± 3.3 hr in 59 normal subjects. In the present experiment with seven normal subjects we found a disappearance half-time of 17.5 ± 1.5 hr (Table 1, Column 1). These results are in the normal range.

We took as normal the half-time values for ^{32}P-DFP labeling reported by Athens et al (*7–9*) (6.6 ± 1.16 hr in 45 normal subjects). Preliminary studies showed that in vitro labeling of isolated cells

TABLE 1. HALF DISAPPEARANCE TIME OF ^{51}Cr AND ^{32}P-DFP IN VITRO-LABELED GRANULOCYTES IN NORMAL SUBJECTS

Patient	First study (single labeling)	Second study (simultaneous double labeling)		^{32}P-DFP $T_{1/2}$ (3) / ^{51}Cr $T_{1/2}$ (1)
	^{51}Cr $T_{1/2}$ (hr) 1	^{51}Cr $T_{1/2}$ (hr) 2	^{32}P-DFP $T_{1/2}$ (hr) 3	
BOU	18	10	7	0.39
JAY	17	9	5	0.29
KOJ	19	11	5.5	0.29
HAC	17	13.5	8	0.47
MAG	17	8.5	5	0.29
MAT	15	6	4	0.27
KAI	20	6	4.5	0.23
Mean ± 1 s.d.	17.5 ± 1.5	9.4 ± 2.4	5.5 ± 1.3	0.32 ± 0.09
Normal values ± 1 s.d.	18.9 ± 3.3*		6.6 ± 1.16†	0.35 ± 0.12

* ^{51}Cr single labeling in our series of 59 normal subjects.
† ^{32}P-DFP single labeling from Athens et al (2) on 45 normal subjects.

gave the same results as in vitro labeling of whole blood. In the present experiment (Table 1, Column 3) in which granulocytes were simultaneously labeled with ^{51}Cr and ^{32}P-DFP, we found a disappearance half-time for ^{32}P-DFP-labeled granulocytes of 5.5 ± 1.3 hr. These results are in the normal range. When granulocytes are labeled simultaneously with ^{32}P-DFP and ^{51}Cr, the $T_{1/2}$ of ^{32}P-DFP is not changed.

Comparison of Columns 1, 2, and 3 in Table 1 shows that the ^{51}Cr disappearance half-time is always longer than the ^{32}P-DFP one. But when autologous granulocytes are labeled with ^{51}Cr alone (Column 1) or with ^{51}Cr in the presence of DFP (Column 2), the ^{51}Cr disappearance half-time is always shortened in the second case. The mean ratio $T_{1/2}$ ^{51}Cr (with DFP)/$T_{1/2}$ ^{51}Cr (alone) is 0.54 ± 0.14. Four experimental curves are given in Fig. 2.

To find out if this shortening of the ^{51}Cr disappearance half-time in the presence of ^{32}P-DFP is due to ^{32}P, autologous granulocytes labeled with ^{51}Cr in the presence of 0.15 mg of cold DFP per 30 ml of cell suspension were injected in three normal subjects. The disappearance half-time of ^{51}Cr-labeled granulocytes was 13–14 hr. Thus the shortening of the life-span of ^{51}Cr-labeled granulocytes by ^{32}P-DFP is neither due to the radioactivity nor to an eventual misinterpretation of the counting data, but to the DFP molecule.

In vitro studies with ^{51}Cr-labeled granulocytes were carried out to find whether DFP acts by shortening the life-span of the granulocytes or by increasing the ^{51}Cr elution. Figure 4 shows the results of these studies. Each point is the mean of four to six experimental results.

TABLE 2. EFFECT OF DFP ON RADIOACTIVITY OF SUPERNATANTS OBTAINED AFTER INCUBATING FOR 3 HR LEUKOCYTES LABELED IN VITRO WITH ^{51}Cr*

Incubation temperature (°C)	15 mg DFP/30 ml: Total supernatant radioactivity	15 mg DFP/30 ml: Protein-bound radioactivity	30 ml DFP/30 ml: Total supernatant radioactivity	30 ml DFP/30 ml: Protein-bound radioactivity
37	125	100	150	120
4	150	180	180	200

* Results, in percent of supernatant radioactivity in incubation without DFP at the same temperature, are the mean of six experimental values.

Two conclusions can be drawn from these experiments:

1. At 37°C the supernatant radioactivity is always greater than at 4°C, but protein-bound radioactivity does not increase in the same proportion. A slight decrease in granulocyte specific activity is also evidence of some ^{51}Cr elution from granulocytes in the presence of DFP at 37°C.
2. Increased concentrations of DFP are not clearly more effective at 37°C, but at 4°C there is a distinct increase of protein-bound activity compared with incubation without DFP (Table 2). At 4°C there is no decrease in granulocyte specific activity. The increase in protein-bound radioactivity gives evidence of cellular lysis in the presence of DFP.

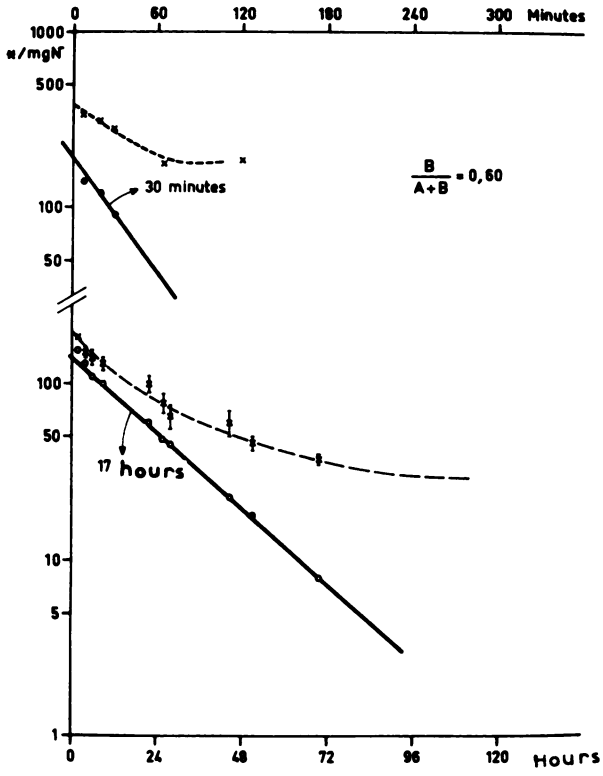

FIG. 1. ^{51}Cr single labeling in a normal subject.

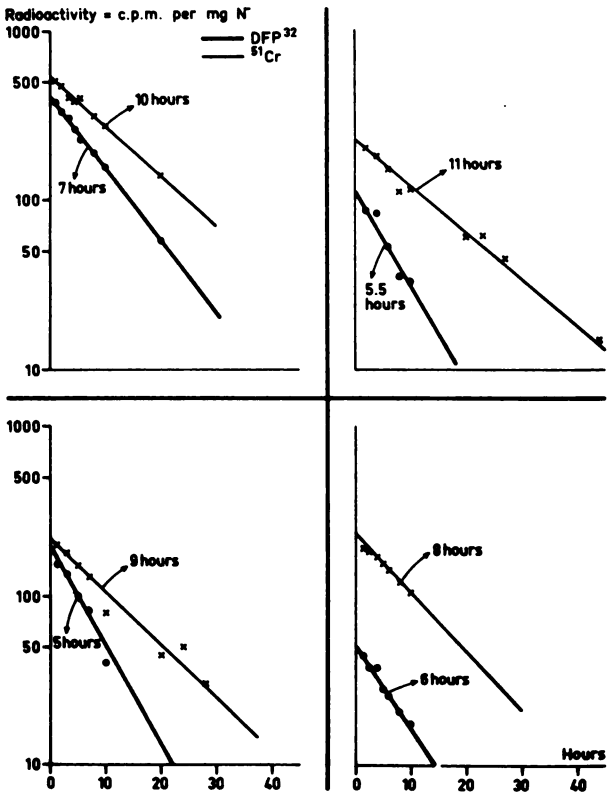

FIG. 3. ^{51}Cr and ^{32}P-DFP simultaneous labeling in four normals.

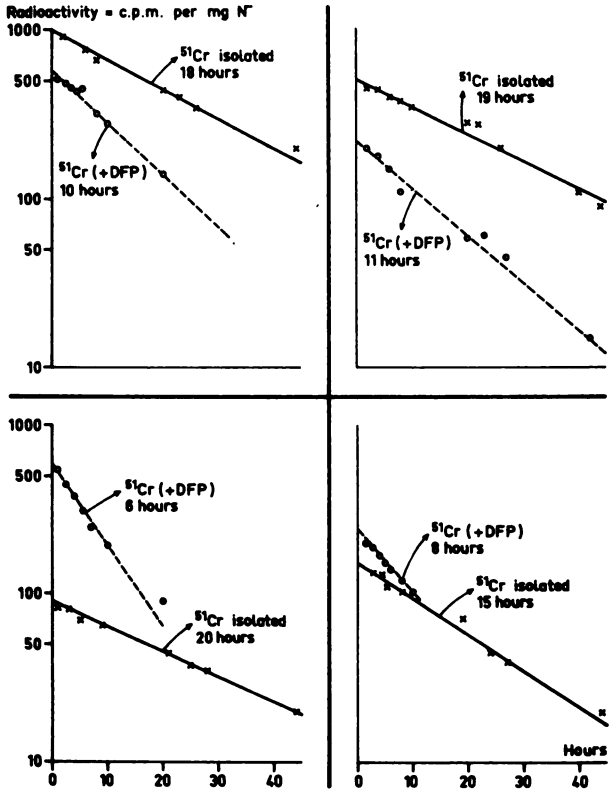

FIG. 2. ^{51}Cr isolated and ^{51}Cr + ^{32}P-DFP simultaneous labeling. Comparison between ^{51}Cr half-time of disappearance in four normal subjects.

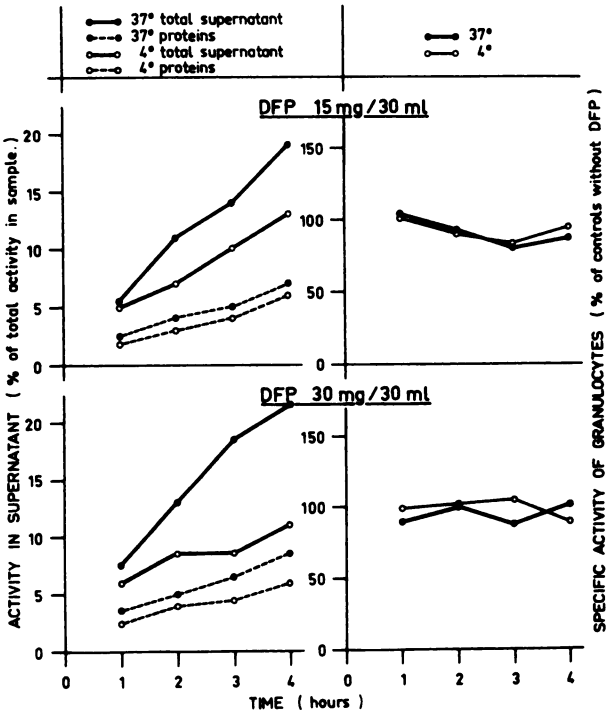

FIG. 4. In vitro incubation of ^{51}Cr-labeled granulocytes with two different concentrations of DFP (each point is mean of 4–6 different studies).

Results of in vitro studies of ^{32}P-DFP-labeled granulocytes are given in Fig. 5. With increased concentrations of ^{32}P-DFP, there is increased activity in the supernatant which is nearly all protein-bound. There is no significant difference between the 4 and 37°C non-protein-bound radioactivity which is always less than 4% of the total radioactivity of the sample. The granulocyte specific activity does not decrease except when high concentrations of DFP have been used.

Studies of pathological cases. Tables 3 and 4 show the disappearance half-time of the patient's own leukocytes labeled with ^{51}Cr and of ^{32}P-DFP-labeled normal homologous leukocytes injected at the same time. One experimental curve is shown in Fig. 6 (Patient PER in Table 3).

In most cases (ten cases of leukopenia and three of leukocytosis), the same ratio between the $T_{1/2}$ of ^{32}P-DFP-labeled homologous granulocytes and the $T_{1/2}$ of ^{51}Cr-labeled autologous granulocytes is present as that of ^{51}Cr and ^{32}P-DFP autologous granulocytes with separate labeling in normal subjects.

On the other hand, the ratio ^{32}P-DFP $T_{1/2}$/^{51}Cr $T_{1/2}$ is far above the normal range in three cases of Felty's syndrome and two cases of toxic granulopenia in which marrow and leukocyte studies suggested a qualitative leukocyte abnormality.

In four cases, the disappearance half-time of ^{32}P-DFP-labeled granulocytes is much more reduced than that of ^{51}Cr autologous granulocytes. In two of these cases, circulating antibodies were found by immunological studies.

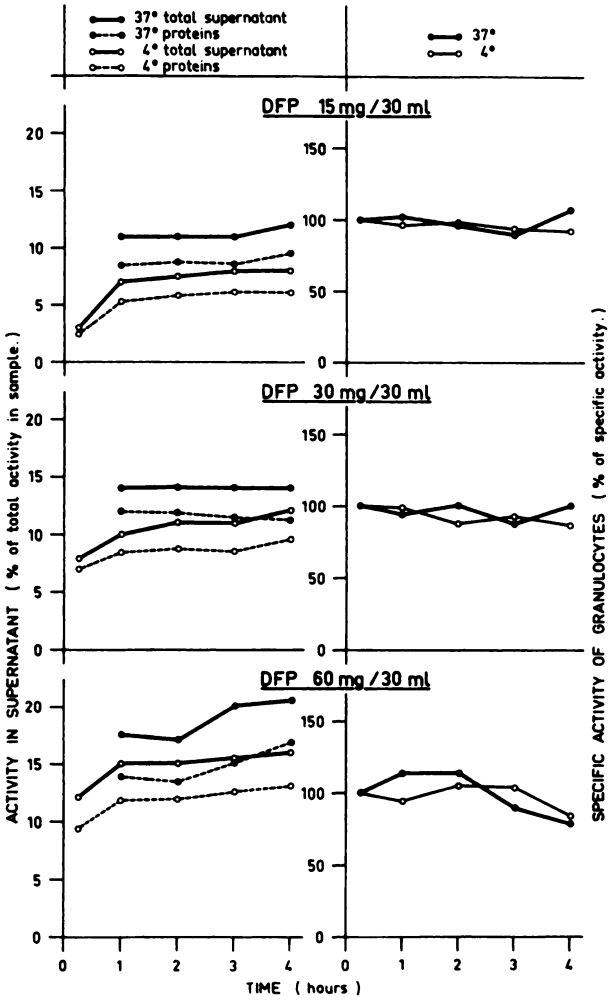

FIG. 5. In vitro incubation of granulocytes labeled with three different concentrations of ^{32}P-DFP (each point is mean of 4–6 different studies).

TABLE 3. HALF DISAPPEARANCE TIME OF ^{51}Cr (AUTOLOGOUS LEUKOCYTES) AND ^{32}P-DFP (HOMOLOGOUS LEUKOCYTES) IN LEUKOPENIC PATIENTS (SEPARATE LABELING AND SIMULTANEOUS INFUSION)

Cases	Name	^{51}Cr (autologous) $T_{1/2}$ (hr)	^{32}P-DFP (homologous) $T_{1/2}$ (hr)	^{32}P-DFP $T_{1/2}$ / ^{51}Cr $T_{1/2}$
Normal ± 1 s.d.		18.9 ± 3.3	6.6 ± 1.16	0.35 ± .12
Extra cellular destruction	MOR	10	3	0.30
	PAL	11.5	3	0.26
	MYR	8	2	0.25
	LAT	2	.6	0.30
	BER	13	3	0.25
	LEG	2.5	.9	0.36
Marrow hypoplasia	POM	20	7	0.35
	LEN	20	6	0.30
	LEC	21	8.5	0.40
	HAC	17	8	0.44
Qualitative abnormality of polymorphonuclear leukocytes	PER	9	6	0.67
	LAV	7	4.5	0.64
	RUI	8	6	0.75
	LED	8	6	0.75
	DES	10	6	0.60
Isoimmunisation	OLI	19	1	0.05
	GIL	14	1.5	0.11
	AMA	15	.8	0.05
	ANC	12	1	0.09
Auto immunisation	LED	1	1	1

TABLE 4. HALF DISAPPEARANCE TIME OF ^{51}Cr (AUTOLOGOUS LEUKOCYTES) AND ^{32}P-DFP (HOMOLOGOUS LEUKOCYTES) IN LEUKOCYTOSIS (SEPARATE LABELING AND SIMULTANEOUS INFUSION)

Cases	^{51}Cr (autologous) $T_{1/2}$ (hr)	^{32}P-DFP (homologous) $T_{1/2}$ (hr)	^{32}P-DFP $T_{1/2}$ / ^{51}Cr $T_{1/2}$
Normal ± 1 s.d.	18.9 ± 3.3	6.6 ± 1.16	0.35 ± .12
MUS	30	12.5	0.42
POD	26	6	0.23
LAC	30	7	0.23

In leukocytosis (Table 4), both autologous ^{51}Cr-labeled granulocytes and ^{32}P-DFP homologous granulocytes have a longer than normal disappearance half-time as already published for ^{32}P-DFP-labeled autologous granulocytes (*8,10*).

Correlation between the disappearance half-time of ^{32}P-DFP- and ^{51}Cr-labeled granulocytes. Figure 7 shows the dispersion of the results of 31 studies in which leukocytes were separately labeled with the two isotopes. In 22 cases the ratio ^{32}P-DFP $T_{1/2}$/ ^{51}Cr $T_{1/2}$ is similar:

1. Thirteen of these subjects had a normal granulocyte life-span [seven subjects were subjected to a double study with autologous leukocytes and six to a single study with ^{51}Cr-labeled autologous and ^{32}P-DFP-labeled homologous leukocytes (two normal subjects and four with bone marrow hypoplasia)].
2. Six other subjects had a reduced life-span of both autologous and homologous granulocytes in the same study (extracellular destruction).
3. Three subjects had a prolonged life-span of both autologous and homologous granulocytes in the same study (leukocytosis).

The correlation factor is $r = 0.86$. The 95% confidence limits are $0.68 < r < 0.94$.

The five cases of qualitative abnormality of granulocytes and the four cases of iso-immunization are outside the normal dispersion range.

DISCUSSION

After in vitro labeling, ^{32}P-DFP $T_{1/2}$ is always shorter than ^{51}Cr $T_{1/2}$. The method of in vitro ^{51}Cr

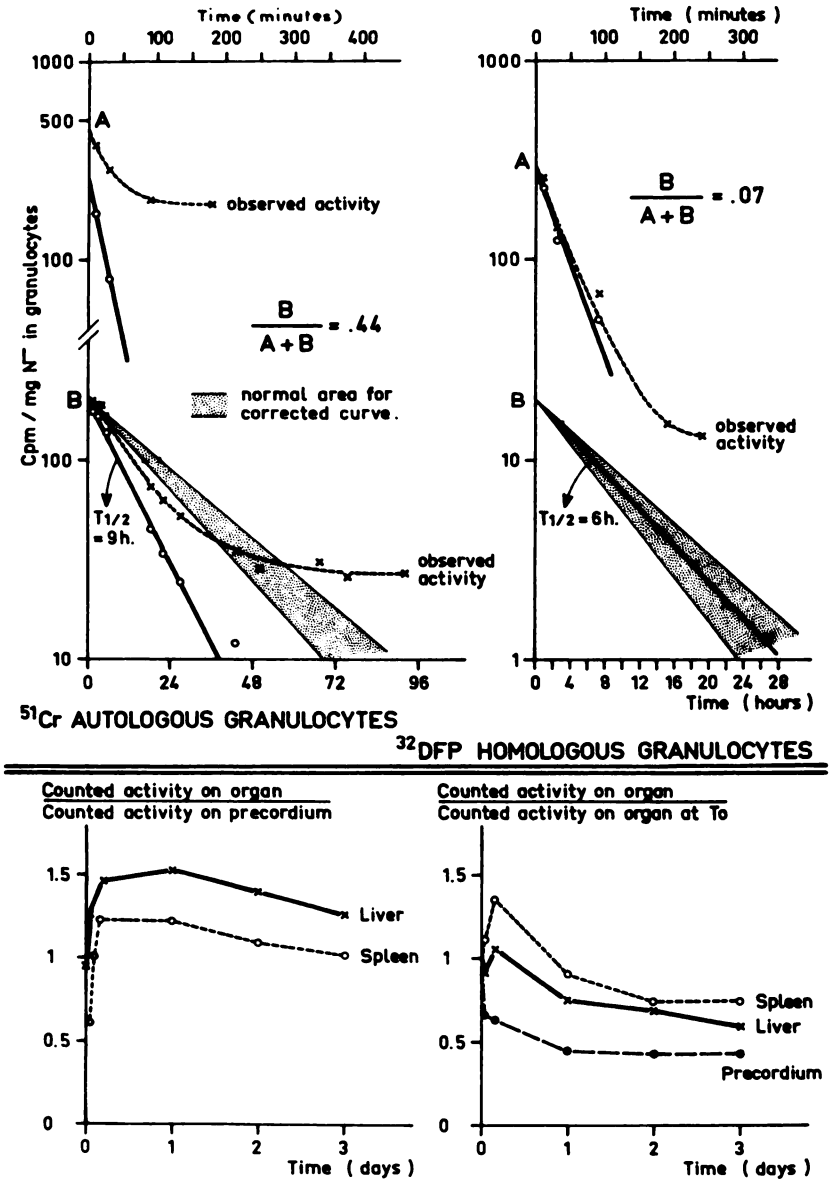

FIG. 6. ^{51}Cr-labeled autologous granulocytes and ^{32}P-DFP labeled homologous granulocytes in case of leukopenia with qualitative abnormality of granulocytes. Above: disappearance of ^{51}Cr on left and ^{32}P-DFP on right. Below: external counting of ^{51}Cr.

labeling has already been discussed in a previous paper (*2*). We have shown that the disappearance half-time calculated for in vitro ^{51}Cr-labeled leukocytes is that of granulocytes alone. After hemolysis with 5% acetic acid and two washes with 0.9% NaCl, there are neither red cells nor hemoglobin present in the white cell suspension. Platelet labeling does not contribute to any counted radioactivity because most platelets are eliminated by the low-speed centrifugations before and after labeling. Lymphocyte activity is very low in normal subjects (less than 0.05 of the initial circulating activity), but is more important in granulopenic patients. We found (*2*) that mature lymphocytes are labeled less by ^{51}Cr than mature granulocytes: the ratio of lymphocytes to granulocytes is 0.1 to 0.8 (mean 0.3). These results are in agreement with those of McMillan and Scott (*11*) who found a labeling ratio of lymphocytes to granulocytes of 0.4 to 0.9. However, Pfisterer (*12*) found a labeling ratio of lymphocytes to granulocytes of 5 to 25 and Eyre, et al, 2 to 3 (*13*). This point should be further investigated. The lymphocyte disappearance half-time is thought to be greater than ten days: 13 ± 2.6 days for Pfisterer (*14*), 12–34 days (mean 23 days) for Goswitz (*15*). The remaining white cell radioactivity after the fourth day can therefore be considered to be due to lymphocyte radioactivity, and this value can be used to correct the white cell curve.

A factor in estimating the disappearance half-time of labeled granulocytes is the variability from one subject to another of the delay before the equilibration between the circulating and the marginal pools is completed (*2,3*). The initial fall in granulocyte specific activity corresponding to this equilibration is identical with ^{51}Cr- and with ^{32}P-DFP-labeled cells (Fig. 8). Similar curves have been observed by Deinard (*16*) with ^{32}P-DFP-labeled cells in the samples taken soon after the injection. We think that the lack of equilibration, due to early blood collection, explains the double curves published by Duvall (*17*) and Perry (*18*) and some of the complex curves published by Athens (*10*). When it is impossible to have four blood samples in the first two hours after the infusion (in order to draw the curve of equilibration), the first blood sample to be taken into account has to be collected at least 2 hr after the infusion. Epinephrine injection before this delay showed (*2*) that the first exponential curve observed was due to the margination of the labeled leukocytes.

The difference of the disappearance half-time observed after in vitro labeling with ^{32}P-DFP and ^{51}Cr is not due to technical reasons (the same technique is used for both labels and an eventual cellular injury by manipulations should shorten the life span of the cells labeled with either isotope). In vitro ^{32}P-DFP labeling of granulocytes gave us the same results when whole blood or when isolated cells were labeled, and these are the same as those of Athens et al (*7*) who labeled whole blood.

A constant discrepancy is observed between the life span of ^{51}Cr- and ^{32}P-DFP-labeled granulocytes when the comparison is based on large series of normal subjects such as Athens' (*7,9*) and Sacchetti's (*19,20*) for ^{32}P-DFP, and ours (*2,3*) for ^{51}Cr. The present study confirms the longer survival time of

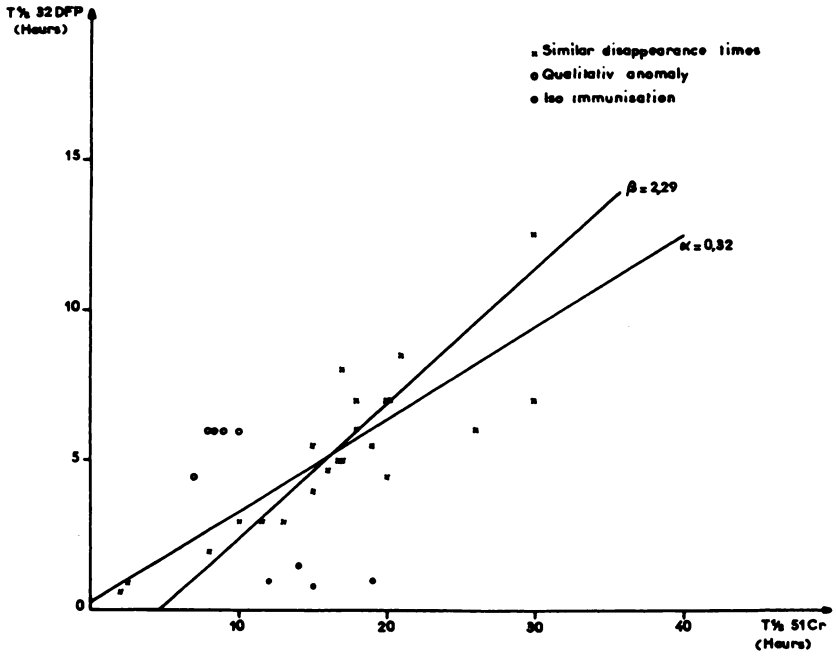

FIG. 7. Correlation between the half-times of disappearance of ^{32}P-DFP- and ^{51}Cr-labeled granulocytes (separate in vitro labeling).

^{51}Cr-labeled granulocytes in normal and pathological cases. These observations are in disagreement with those of McMillan and Scott (*11*) who found an identical disappearance half-time of granulocytes labeled with ^{32}P-DFP and ^{51}Cr (10.5 ± 3.3 hr), either simultaneously (five cases) or separately (five cases). It is very difficult to explain the discrepancy between their results and ours without having more data. We can only suggest some possible explanations which arise on examination of their report:

1. The granulocyte disappearance half-time, in a large series of cases, extends over a very wide range. It is possible, as the authors suggest, to explain the very long survival time of ^{32}P-DFP-labeled cells compared with Athens' data by saying the ten subjects studied were not strictly normal.
2. The very low specific activity of ^{51}Cr-labeled circulating granulocytes (3 to 8 cpm/mg N) could explain the fact that a late circulating activity was not observed. We usually find an initial specific activity for circulating leukocytes of 100–500 cpm/mg N with ^{51}Cr labeling, and 100–300 cpm/mg N with ^{32}P-DFP. More than 10% of the initial specific activity is found in the leukocytes circulating on the second day of the study. Duvall and Perry (*17*) also observed this late circulating activity with ^{51}Cr labeling of leukocytes in normal subjects. To ignore the circulating activity after 24 hr would lead one to set a shorter disappearance half-time for the ^{51}Cr-labeled cells.

Several hypotheses have been made to explain the discrepancy between the results of ^{51}Cr and ^{32}P-DFP labeling of granulocytes. It has been suggested that there is a difference of labeling according to the age of the cells between ^{51}Cr and ^{32}P-DFP. In fact, we have shown that in vitro ^{51}Cr labeling is homogeneous on young and old granulocytes either in chronic myelocytic leukemia or in myelofibrosis (*2*). Kurth et al (*21*) has shown that ^{3}H-DFP labels myelocytes more intensely than metamyelocytes and polymorphonuclears when leukocytes from chronic myelocytic leukemia are labeled in vitro. Even if there is some difference in young cell labeling between ^{51}Cr and ^{32}P-DFP, this cannot account for so large a difference in the disappearance half-time of granulocytes in normal subjects.

When the same cells are labeled simultaneously by ^{32}P-DFP and ^{51}Cr, the disappearance half-time of ^{51}Cr remains longer than that of ^{32}P-DFP (Table 1, Columns 2 and 3). This fact eliminates the second hypothesis suggested in a previous paper (*2*), namely that ^{51}Cr could damage the granulocytes sufficiently

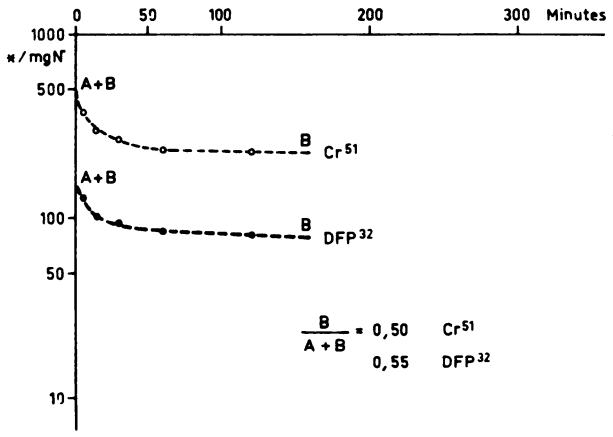

FIG. 8. Equilibration of ^{51}Cr and ^{32}P-DFP in vitro labeled granulocytes (separate labeling) between marginal and circulating pools.

to hamper their migration towards the tissues but not to shorten their survival time. If this were true, the $T_{1/2}$ of ^{32}P-DFP would be lengthened to the same value as the $T_{1/2}$ of ^{51}Cr in the case of simultaneous labeling of the same cells with both labels.

An elution of ^{32}P-DFP from the granulocytes is suggested by the difference between the disappearance half-time of ^{51}Cr and ^{32}P-DFP when granulocytes are labeled simultaneously by the two tracers. Both labels are on the same cells, and the discrepancy in the $T_{1/2}$ of each appears related to the nature of the label.

Early in vivo elution of ^{32}P-DFP has already been shown for red cells and platelet in vitro labeling (*22,23*). In vitro studies of ^{32}P-DFP labeled granulocytes are not entirely conclusive; incubation (Fig. 5) shows a constant but low non-protein-bound radioactivity in the supernatant and the specific activity of granulocytes does not decrease for usual concentrations of DFP. But as Athens, et al (*1*) emphasize in their study of this problem, it is difficult to know whether elution is more important in vivo than in vitro. This is suggested by the simultaneous double-labeling data (Table 1). After simultaneous double labeling by ^{51}Cr and ^{32}P-DFP of the same granulocytes, the in vivo disappearance time of ^{32}P-DFP is always shorter than that of ^{51}Cr. This fact can only be explained by an in vivo elution of ^{32}P from the labeled granulocytes.

A direct toxic effect of DFP on the granulocytes is suggested by the difference between the disappearance half-time of ^{51}Cr-labeled granulocytes incubated with or without DFP (Table 1, Columns 1 and 2). The mere presence of DFP during the in vitro labeling shortens the $T_{1/2}$ of the ^{51}Cr-labeled granulocytes by half. This effect can be due either to an increased elution of ^{51}Cr from the double-labeled granulocytes or to a toxicity of DFP for the granulocytes.

The ^{51}Cr elution of single-labeled granulocytes cannot be important in vivo. Significant elution would mean that the disappearance half-time of granulocytes from the circulation is longer than 24 hr, which differs from the evaluation of granulocyte survival time made by Osgood (*24*) and Cronkite (*25,26*). The disappearance half-time of normal granulocytes, as measured by ^{51}Cr labeling, seems to be corroborated by Yankee, et al (*27*) who find normal infused granulocytes in leukopenic patients 19 hr after the infusion. In vitro elution of ^{51}Cr-labeled granulocytes is not important, but it does exist as for red cells (*28*), as shown by our studies (Fig. 4) and Ronai's (*29*). Ronai suggests that ^{51}Cr labeling leads to a metabolic alteration in protein catabolism and the elimination from the cells of labeled peptides. The presence of DFP could enhance this process, as shown by in vitro studies of ^{51}Cr elution at 37°C in the presence of DFP (Fig. 4 and Table 2). But the fact that the specific activity of the granulocytes in in vitro incubation decreases only slightly is more in favor of cellular death.

Direct toxicity of DFP for granulocytes was not demonstrated conclusively in in vitro studies, as previously published (*1,2*), at usual concentrations of DFP and at 37°C. In vitro studies at 4°C (Figs. 4 and 5, Table 2) show that radioactivity in the supernatant of incubation is proportional to the DFP concentration, either with ^{51}Cr- or with ^{32}P-DFP-labeled cells. Most of this radioactivity is bound to proteins, and the granulocyte specific activity does not change. The toxicity of DFP for granulocytes is also demonstrated in vivo:

1. The infusion of ^{32}P-DFP (0.4–0.5 mg) in ten patients with normal leukocyte counts (in vivo labeling of red cells for studying hemolysis) showed between 4 and 20 hr a temporary decrease of one-third of the initial leukocyte count (−33% ± 13 in the leukocyte count, −32% ± 18 in the granulocyte count, −33% ± 24 in the lymphocyte count).
2. In three subjects, ^{32}P-DFP was injected intravenously on the second day after the infusion of ^{51}Cr in vitro labeled leukocytes. A rapid decrease in the specific activity of the circulating labeled leukocytes was observed, as shown in Fig. 9.
3. The concentration of ^{32}P-DFP used in the present in vitro labeling is ten times as large as that used by Athens, et al (*1*) for the whole blood labeling, but is the same as that used by McMillan and Scott (*11*) in a technique similar to ours. We have found the same survival time for ^{32}P-DFP-labeled granulocytes in normal subjects, either by whole-blood labeling or by leukocyte suspension labeling, up to 0.3 mg DFP/30 ml. But the disappearance half-time was shortened in three patients when the DFP concentration was increased (1–1.5 hr with a labeling concentration of 0.6–0.8 mg DFP/30 ml). With the same concentration, there is only a slight increase in cell death in in vitro studies (Fig. 5). Thus the toxic effect of DFP on granulocytes seems to be more important in vivo than in vitro.

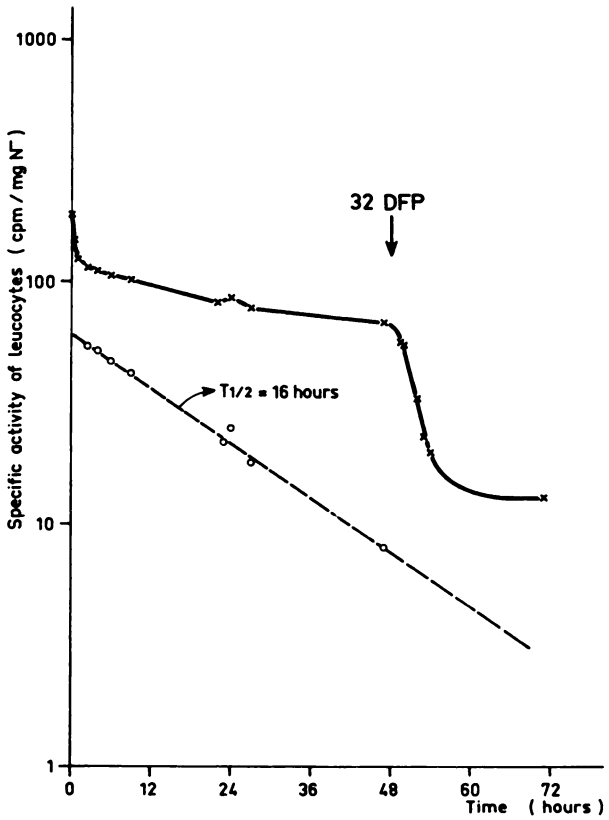

FIG. 9. Effect of intravenous injection of ^{32}P-DFP on specific activity of in vitro ^{51}Cr-labeled leukocytes.

Recently, Woodin and Wieneke (*30*) have shown that the DFP effect on leukocytes is not solely due to phosphorylating proteins. DFP alters the potassium pump of the cell membrane and operates as a non-ionic detergent. Also it enhances the action of bacterial compounds such as leucocidin. These effects could explain an in vivo fragility of ^{32}P-DFP labeled leukocytes.

To sum up, both mechanisms, toxicity and increased ^{51}Cr elution by DFP, exist as shown by in vitro studies. But these studies cannot reflect exactly what happens in the circulation, and in vivo studies indicate mainly a direct toxic effect of DFP on circulating leukocytes

There is a good correlation between ^{51}Cr- and ^{32}DFP-labeled granulocytes survival time in both

normal and abnormal subjects which allows one to use ^{32}P-DFP-labeled granulocytes in homologous infusion for a double labeling. We have already shown that there is no difference in the granulocyte life span of ^{51}Cr-labeled cells with homologous and autologous transfusion when the patient has not been previously transfused (*3*). We are in disagreement with Kauder et al (*31*) who found with ^{32}P-DFP labeling a shorter mean $T_{1/2}$ in homologous transfusion than in autologous transfusion. But on examination of their report, it is found that of 12 subjects studied with homologous transfusion, seven had a granulocyte disappearance half-time in the normal range and five well below the normal limits (0.6–2 hr—mean 1.4 hr, normal range 4–10 hr—mean 6.7 hr). We had the same results with ^{51}Cr labeling for homologous transfusion (*3*) (either normal values of disappearance half-time, or values of 0.5–2 hr when $T_{1/2}$ was short, without any intermediary value). Such results have already been observed for red cells and platelets after in vitro labeling. In our studies of leukopenias with double homologous and autologous granulocytes labeling, only four patients out of 30 had a shorter disappearance half-time of normal homologous leukocytes than the time corresponding to the disappearance half-time of ^{51}Cr autologous granulocytes. In two of these cases, circulating antibodies for the infused granulocytes could be found by immunological assays.

The good correlation between the disappearance half-time of ^{51}Cr- and ^{32}P-DFP-labeled granulocytes in normal and pathological conditions (Fig. 7) permits a double study to be performed simultaneously. This study is only valid if the $T_{1/2}$ of both labels are compared with the normal values for each method. In leukopenic patients, several mechanisms can be involved (*3*) and a shortened disappearance half-time of autologous granulocytes can be due to either corpuscular or extra-corpuscular destruction. The study of homologous normal granulocytes is, in these cases, essential. For reasons developed in the first part of this paper, it is difficult to perform two studies consecutively in a short time, unless one uses only ^{32}P-DFP (*6*). But ^{51}Cr, as a polymorphonuclear label, has the great advantage of allowing external counting, as described in a previous paper (*3*). The study is longer than with ^{32}P-DFP labeling and cannot be repeated until several weeks later, because of the lymphocyte activity if one wishes a study with homologous normal granulocytes. The double labeling, ^{51}Cr on autologous granulocytes and ^{32}P-DFP on homologous normal granulocytes, is a valuable method for studying granulocyte abnormalities. As we had already suggested (*3*), qualitative abnormalities of granulocytes are not an uncommon discovery in chronic leukopenias, either congenital or acquired. These abnormalities can only be demonstrated by comparing autologous and homologous granulocyte life span in the circulation.

SUMMARY

Comparison between the disappearance half-time of ^{51}Cr and ^{32}P-DFP in vitro labeled granulocytes shows that ^{51}Cr $T_{1/2}$ is always longer than ^{32}P-DFP $T_{1/2}$ in normal subjects. This discrepancy has been studied by double-labeling methods and is due, on the one hand, to an in vivo elution of ^{32}P-DFP and, on the other hand, to a direct toxicity of ^{32}P-DFP for the labeled granulocytes. However, there is a good correlation between the $T_{1/2}$ of granulocyte disappearance from the circulating pool as measured by the two tracers in separate labeling in normal subjects and in some pathological conditions. This fact makes possible a double-labeling method, ^{51}Cr on autologous granulocytes and ^{32}P-DFP on homologous normal granulocytes, for the study of leukocyte abnormalities, corpuscular, and extra-corpuscular destruction and leukocyte iso-immunization.

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