SURVIVAL AND SEQUESTRATION OF ^{51}Cr- AND $^{99m}TcO_4$-LABELED PLATELETS

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A new method by which platelets can be labeled with ^{99m}Tc and ^{51}Cr has been developed by the authors and is presented here. Platelets separated by Aster's acid-citrate method were incubated with ^{99m}Tc and ^{51}Cr followed by reduction with stannous chloride ($SnCl_2 \cdot 2H_2O$) and ascorbic acid. After washing with physiologic saline, labeled platelets were infused into human subjects. Platelet survival and turnover and body surface counting were done. Organ distribution of ^{99m}Tc-labeled platelets was observed by scintillation camera.

Technetium-99m fulfilled all requirements of an ideal cell label. Platelets can be labeled for a brief period of time and there is no evidence that the label damages the platelets or is eluted from platelets under the conditions of the present study.

In 13 out of 16 patients, sequestration observed by body surface counting coincided with sites of sequestration observed using a scintillation camera. In three cases in which platelet destruction rather than sequestration was present, the results using both techniques were different.

It appeared that camera images obtained with ^{99m}Tc-labeled platelets would improve the limited collimation of a small part of the organ obtained by body surface counting and make possible quantitative measurement of the sites of platelet sequestration.

The suitability of ^{51}Cr as a platelet label for survival study of platelets has been well established (*1*). However precise quantitative measurement of sequestration and destruction of platelets has been limited by the methodology available. Splenic and hepatic surface counting is inadequate for quantitative measurement of the sequestration and destruction sites, as only a small part of the liver or spleen can be viewed at one time.

This paper describes the procedures for measuring sequestration and destruction of platelets labeled by ^{99m}Tc-pertechnetate and platelet survival by ^{51}Cr, and presents the kinetic patterns in patients with platelet disorders.

MATERIALS AND METHODS

Two normal individuals and 14 others with various platelet disorders were the subjects investigated. They were informed about the procedure and a signed consent was obtained. Hematologic data in patients with platelet disorders are shown in Table 1. Red cell survival and ferrokinetic studies were performed by the radioactive chromium (*2*) and Huff's method (*3*), respectively. Spleen scintigraphy was done following injection of the patient's own ^{51}Cr-tagged red cells, which had been heated. Splenic size was expressed as the ratio of splenic volume to the normal value (223 cm^3), which was evaluated from the product of posterior or anterior scan area (Ap or Aa) and lateral area (Al) divided by their common axis (r), as previously described (*4*):

$$\text{Splenic volume} = (\text{Ap or Aa}) \times \text{Al}/\text{r}$$

These studies were carried out after completion of the platelet kinetic study.

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TABLE 1. HEMATOLOGIC DATA ON PATIENTS INVESTIGATED*

Case (No.)	Age, Sex	Diagnosis	Platelets $\times 10^4/\mu l$	RBC $\times 10^4/\mu l$	Hb (gm/dl)	WBC/μl	RBC $T_{1/2}$ (days)	PID $T_{1/2}$ (min)	Spleen (size)	Bone marrow (appearance)
1	20 F	Normal	25.0	475	11.9	8,400	24.0	ND	2.0	Normal
2	62 M	Normal	25.0	425	13.3	8,200	ND	ND	ND	Normal
3	26 F	ITP	8.2	352	10.1	8,600	22.0	43.0	2.8	MG and EH
4	24 M	ITP	1.0	290	9.0	5,700	20.5	37.5	1.8	ND
5	14 F	ITP	7.0	470	11.3	4,900	ND	ND	3.0	Normal
6	23 F	ITP	18.4	434	13.7	12,500	ND	ND	ND	Normal
7	29 F	ITP	7.9	398	11.2	6,800	ND	ND	ND	MG
8	19 M	AML	3.2	280	9.2	2,900	18.0	130.0	2.7	IM and hBM
9	62 M	BMF	4.2	235	7.2	8,400	18.5	61.0	ND	hBM
10	44 F	CS	3.6	407	11.4	1,300	23.0	64.0	7.0	EH
11	40 F	CC	1.7	350	7.0	6,000	ND	ND	2.0	MG and HBM
12	69 M	PV	60.0	729	13.4	34,000	30.0	10.0	11.3	MG and HBM
13	54 M	PV	32.0	703	15.0	19,200	26.0	11.0	3.4	MG and HBM
14	61 M	PT	230.0	535	15.3	19,600	26.0	51.0	3.5	MG and HBM
15	21 F	PT	100.0	421	12.4	11,600	ND	58.0	3.5	MG
16	42 M	PT	168.0	520	15.1	12,000	ND	ND	2.4	MG

* RBC $T_{1/2}$, Red cell survival; PID $T_{1/2}$, plasma iron disappearance $T_{1/2}$; ITP, idiopathic thrombocytopenic purpura; AML, acute myelocytic leukemia; BMF, bone marrow failure; CS, congestive splenomegaly; CC, consumption coagulopathy; PV, polycythemia vera; PT, primary thrombocythemia; ND, no data; MG, increased megakaryocyte number; EH, erythroid hyperplasia; HBM, hypercellular bone marrow; hBM, hypocellular bone marrow; IM, atypical immature cell.

The labeling was performed under sterile conditions using a pyrogen-free plastic bag. The complete procedure utilized for platelet labeling is as follows:

1. Draw 250 ml of blood from the patient into a plastic bag (Fenwal Laboratories) by the use of the acid-citrate method (*5*).
2. Centrifuge the blood at 1500 rpm for 15 min (4°C) and transfer the platelet-rich plasma (PRP) into a transfer pack.
3. Centrifuge the PRP at 1500 rpm for 3 min to remove contaminating red cells and express the PRP into the second transfer pack.
4. Centrifuge the PRP at 2300 rpm for 15 min and resuspend the concentrated platelets into 5 ml of physiologic saline in a siliconized sterile centrifuge tube.
5. Add 0.1 ml of $^{99m}TcO_4$ saline solution (1–3 mCi) and 300 μCi of $Na_2{}^{51}CrO_4$.
6. Incubate for 15 min at room temperature (15°–25°C).
7. Add 0.1 ml of 100 μg/ml $SnCl_2 \cdot 2H_2O$ in acid citrate dextrose (ACD) solution and 50 mg of ascorbic acid as reducing agent. ($SnCl_2 \cdot 2H_2O$ solution was prepared as needed and filtered through a 0.22-micron Millipore filter.)
8. Mix gently at room temperature for 10 min.
9. Wash one time and resuspend the cells in 20 ml of physiologic saline.
10. Transfuse the labeled platelets to the subject through the antecubital vein.

TABLE 2. IN VITRO STUDIES CONCERNING PLATELET LABELING BY ^{99m}Tc-PERTECHNETATE

^{99m}Tc incubation		Elution				Incubation*							
						90 min				24 hr			
Time (min)	Labeling (%)	Washing times	A	B	C	Washing times	A	B	C	Washing times	A	B	C
5	58.3	0	100	100	100	6	55	53	53	8	53	51	51
10	56.4	1	61	59	59	7	54	53	52	9	53	51	50
30	56.0	2	58	56	56								
		3	57	55	55								
		4	56	55	54								
		5	56	54	54								

* Elution of label during in vitro incubation was examined. White cell suspension was incubated for 90 min or 24 hr and labeling yields before and after incubation were compared.

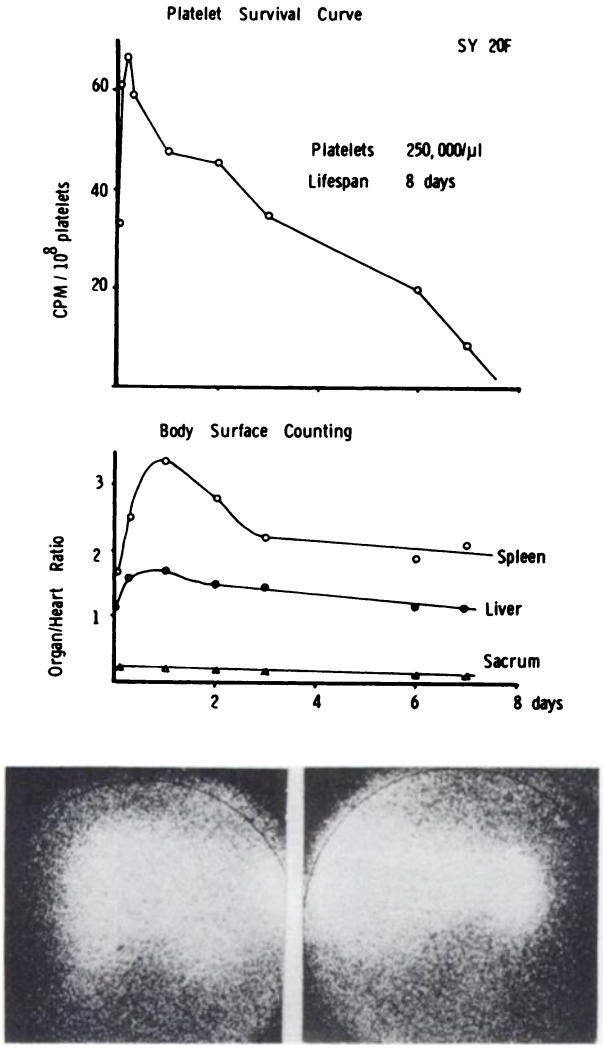


FIG. 1. Twenty-year-old woman (Case 1). This hematologically normal patient had platelet counts of 250,000/μl and a lifespan of 8 days. Splenic sequestration is seen in body surface counting with ^{51}Cr-labeled platelets. Right side of camera images using ^{99m}Tc-labeled platelets shows spleen. Gross cpm of splenic area number 28,637. Left side is liver and gross cpm of hepatic area number 19,112. Sequestration pattern of ^{51}Cr-labeled platelets coincided with that of ^{99m}Tc.

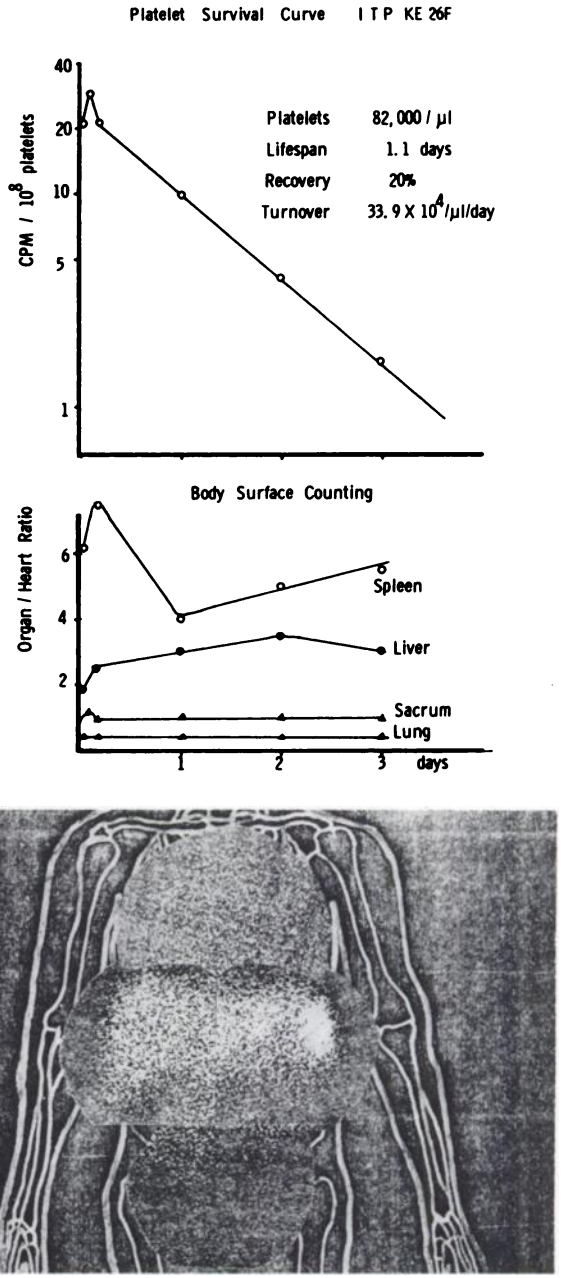


FIG. 2. Twenty-six-year-old woman with idiopathic thrombocytopenic purpura (ITP) (Case 3). Markedly shortened survival with single exponential decrease and increased turnover are evident. Splenic destruction pattern is noted by both body surface counting of ^{51}Cr-labeled platelets and camera image of ^{99m}Tc-labeled platelets. Gross cpm of splenic area number 35,500 and that of hepatic area 19,860.

The radioactivity of platelets that were separated by Aster's technique (*6*) was measured by means of an automatic well scintillation counter. Platelet survival was determined from the linear disappearance curves by extrapolating to zero activity. When an exponential curve was obtained, the mean survival was calculated from the half-time divided by the natural logarithm of 2. The proportion of ^{51}Cr-labeled platelets remaining in the systemic circulation immediately after infusion which is called "recovery" and platelet turnover per microliter per day were calculated in the usual way (*7*). The blood volume was estimated from the ^{51}Cr-labeled red cells (*8*).

Using a NaI(Tl) scintillation detecter, counts were obtained over the spleen, liver, lung, and heart. The organ-to-heart ratio of ^{51}Cr-labeled platelets (Spleen/Heart, Liver/Heart, and Lung/Heart) were calculated for each time period.

A Searle Radiographics Pho/Gamma scintillation camera was used for immediate imaging of ^{99m}Tc-labeled platelets. Scintiphotographs of the

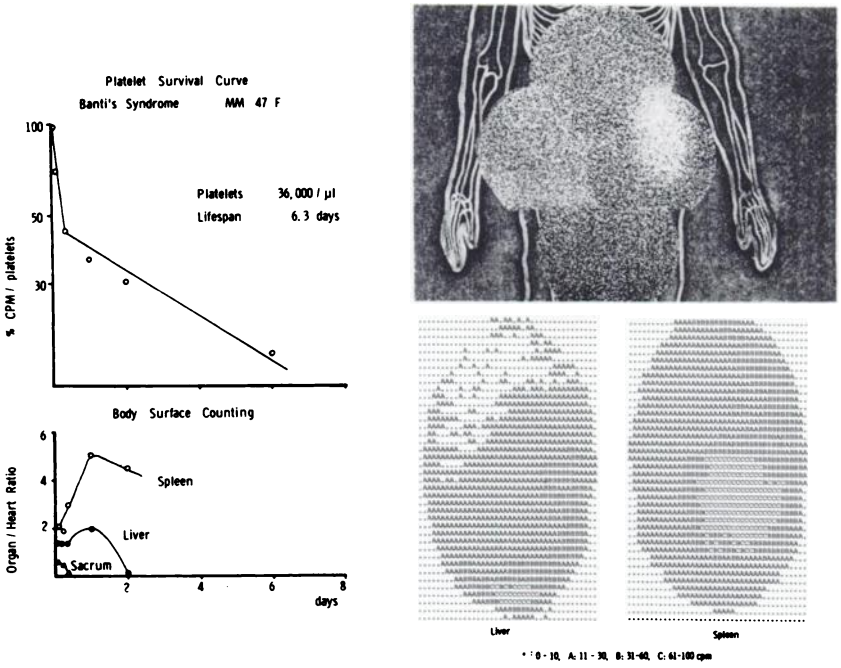


FIG. 3. Forty-four-year-old woman with congestive splenomegaly (Case 10). Lifespan of platelets is 6.3 days and recovery is less than 10%. Splenic sequestration was evident in body surface counting. Camera image and digital scintigram show accumulation of ^{99m}Tc-labeled platelets only in spleen. With splenomegaly, an increased proportion of platelets are pooled in spleen, resulting in decreased concentration of platelets in general circulation.

whole body were taken 1 to 4 hr after the completion of the infusion. Counts per unit area were collected in a 1600-channel multichannel analyzer and stored in the memory bank. Quantitative comparison of the radioactivity over the spleen and liver was expressed by using count levels in each matrix designated by the letters A, B, and C.

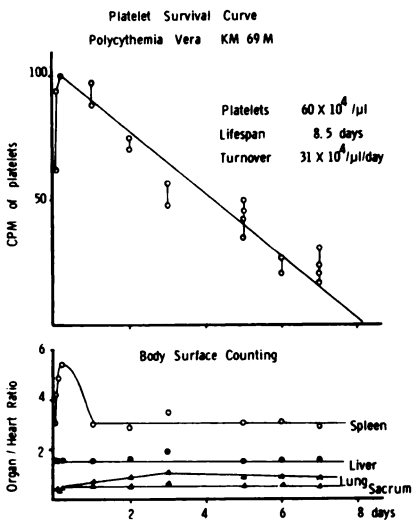
RESULTS

In vitro studies. Platelets separated from 100 ml of blood from patients with polycythemia vera were used in these studies. Platelet incorporation of ^{99m}Tc during incubation reached a maximum at 5 min (Table 2). No platelet was labeled without stannous chloride being present. A concentration of 0.1 ml of 100 μg/ml $SnCl_2 \cdot 2H_2O$ in ACD solution was sufficient. Labeling yields, i.e., the ratio of radioactivity of labeled platelets after washing to that of ^{99m}Tc utilized, reached 56% within 5 min. Repeated washes of labeled platelets were done to measure

TABLE 3. PLATELET KINETIC DATA ON PATIENTS INVESTIGATED

			^{51}Cr labeling method				^{99m}Tc labeling method		
Case (No.)	Life-span (days)	Recov-ery (%)	Turnover (× 10^4/ μl/day)	S/H ratio	L/H ratio	Sequestration site	Splenic gross cpm	Hepatic gross cpm	Sequestration site
1	8.0	57.3	4.9	1.3–3.5*	1.0–1.6*	splenic	28,637	19,112	splenic
2	10.5	65.0	3.3	0.9–1.8	0.9–1.5	hepatosplenic	34,589	28,632	hepatosplenic
3	1.1	20.2	33.9	3.4–7.6	2.1–3.9	splenic	35,500	19,860	splenic
4	0.7	55.3	54.2	1.4–3.5	1.9–4.9	hepatosplenic	10,640	14,280	hepatosplenic
5	0.2	—	—	0.6–2.6	0.6–2.6	hepatosplenic	25,632	19,191	hepatosplenic
6	1.2	10.5	137.1	0.8–5.5	0.8–3.6	splenic	33,147	33,274	hepatosplenic
7	0.5	35.3	36.1	0.9–8.4	1.2–3.2	splenic	14,129	19,031	hepatosplenic
8	0.4	14.1	47.5	1.2–4.6	0.9–2.0	splenic	21,010	13,771	splenic
9	0.3	21.2	59.4	1.0–7.8	4.3–7.2	hepatosplenic	11,789	12,459	hepatosplenic
10	6.3	<10.0	—	1.8–9.8	0.8–2.3	splenic	31,930	11,520	splenic
11	1.0	<10.0	—	1.5–2.8	1.1–2.0	hepatosplenic	29,025	22,444	hepatosplenic
12	8.5	19.4	31.0	2.7–5.4	1.4–2.2	splenic	61,872	36,863	splenic
13	9.7	15.0	19.8	1.5–3.4	1.2–3.6	hepatosplenic	47,312	46,021	heptatosplenic
14	9.0	30.4	74.8	1.1–5.2	1.0–3.1	splenic	44,334	39,646	hepatosplenic
15	8.0	55.5	20.4	2.5–5.4	1.0–1.7	splenic	42,322	28,408	splenic
16	10.2	68.1	21.8	0.7–2.4	0.8–2.4	hepatosplenic	56,430	42,770	hepatosplenic

Abbreviations: S/H ratio: spleen/heart ratio, L/H ratio: liver/heart ratio. <10.0: less than 10.0 percent.
* The range of S/H or L/H ratio during the period of observation is shown.



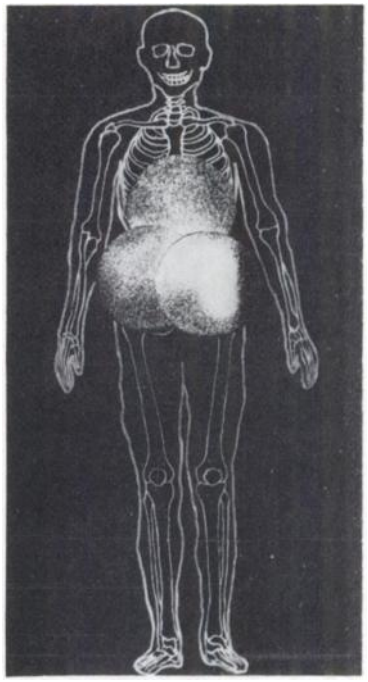


FIG. 4. Sixty-five-year-old man with polycythemia vera (Case 12). Normal lifespan and low recovery, but increased turnover are demonstrated. Platelets are sequestered within spleen, as shown in camera images and body surface counting.

elution of the label. As is shown in Table 2, loss of radioactivity from the labeled platelets as a result of each wash was less than 3% of the radioactivity after free ^{99m}Tc had been washed for the first time.

In vivo studies. The results of platelet kinetic patterns in 16 subjects are shown in Table 3. The range of spleen-to-heart and liver-to-heart count ratios for ^{51}Cr labeling during the period of observation are expressed in this table. Total counts per minute of ^{99m}Tc over splenic or hepatic area of camera images are tabulated. Splenic sequestration pattern was defined as having a spleen-to-liver ratio of 1.5 or greater.

In normal individuals, ^{51}Cr-labeled platelets showed linear disappearance. The surface countings and camera images revealed that platelet sequestration takes place in both the spleen and liver. Disappearance curve, body surface counting, and camera images of Case 1 are shown in Fig. 1. Sequestration patterns by surface counting of ^{51}Cr-labeled platelets coincided with camera images of the ^{99m}Tc-labeled platelets.

In patients with ITP, platelet survival was markedly shortened. Platelets labeled with ^{51}Cr showed exponential disappearance and platelet turnover increased. Two patterns of splenic and hepatosplenic sequestration were recognized. Body surface counting of ^{51}Cr-labeled platelets and camera images of ^{99m}Tc-labeled platelets coincided except for Cases 6 and 7. In these patients, platelets were sequestered within the spleen and liver on the first day, and thereafter the spleen-to-liver ratios gradually increased, which suggested the destruction of platelets in the spleen by an immune mechanism. The platelet kinetic pattern in Case 3 with ITP is shown in Fig. 2.

In two cases of hypomegakaryocytic bone marrow associated with acute leukemia (Case 8) and possible drug-induced marrow failure (Case 9), decreased platelet survival, increased turnover and splenic and hepatosplenic sequestration were observed.

In Case 10 with congestive splenomegaly, where the spleen was seven times greater than normal, platelet survival was 6.3 days and recovery was less than 10%. Marked splenic sequestration was recognized by both methods. Platelet disappearance curve, body surface counting, and camera images are shown in Fig. 3. Quantitative comparison of the radioactivity over the spleen and liver is expressed on a statistical basis of the counting-rate ranges by utilizing a 1600-channel multichannel analyzer. Display of the splenic and hepatic images is shown in Fig. 3.

In Case 11 with consumption coagulopathy, shortened platelet survival was observed. Sequestration pattern in this patient was of the hepatosplenic type.

In patients with myeloproliferative disorders such as polycythemia vera and primary thrombocythemia, normal platelet survival and increased turnover were observed. Splenic or hepatosplenic sequestration was detected by body surface counting and the scintillation camera. The results of platelet kinetics in Case 12 with polycythemia vera are shown in Fig. 4. Splenic sequestration was markedly apparent in this patient. The camera image and the quantitative analysis in Case 15 with primary thrombocythemia

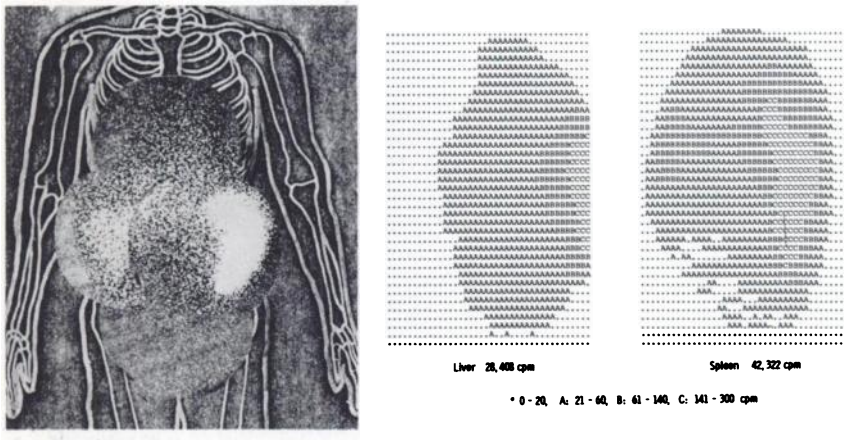


FIG. 5. Twenty-one-year-old woman with primary thrombocythemia (Case 15). Normal lifespan, recovery, and increased turnover are evident in this patient. Camera image and digital scintigram reveal splenic sequestration.

are shown in Fig. 5. Splenic sequestration was detected quantitatively.

DISCUSSION

From the data presented, it is apparent that platelets can be labeled in vitro with ^{99m}Tc-pertechnetate. Technetium-99m labeled the platelets immediately and reutilization of ^{99m}Tc was negligible because of the short half-life. Evidence that ^{99m}Tc damaged platelets was lacking as platelets labeled by ^{99m}Tc and ^{51}Cr, when given to normal subjects, disappeared in linear fashion with survival of 8–10 days and recovery of 55–65%. The possible toxic effects on platelets of 10 μg of stannous chloride was not noted from survival studies and insignificant elution was shown by repeated washes of ^{99m}Tc-labeled platelets. These results thus indicate that ^{99m}Tc is a satisfactory platelet-labeling agent.

The methods used for separating and labeling platelets with ^{51}Cr were those reported by Aster and coworkers (*5,6*). Some modifications were necessary for platelet label by ^{99m}Tc. The presence of plasma in concentrated platelet suspension prevents labeling by ^{99m}Tc. Instead of ACD plasma, physiologic saline was used and the amount of ^{51}Cr taken up by platelets was two- to threefold greater than that obtained in ACD plasma (*9*).

Platelet survival and patterns of organ surface radioactivity in normal subjects have been reported by many authors (*6,7*). The present results were in accordance with these reports. Major sites of platelet sequestration were indicated to be the spleen and liver by Aster (*10*). Methods used for body surface counting are crude and cannot be utilized for organs with abnormal size (*6*). A more difficult problem is encountered with the liver as only a small volume could be viewed. These disadvantages are removed partially when using the scintillation camera and digital scintigram of ^{99m}Tc-labeled platelets. The major sites of platelet sequestration in normal subjects were the liver and spleen as observed when using this technique.

Camera images revealed no accumulation of the radioactivity over the thyroid gland, lungs, stomach, kidneys, or bone marrow. The colloid particles of ^{99m}Tc, such as ^{99m}Tc-sulfur colloid, are found to accumulate in the spleen, liver, and bone marrow. In this study, ^{99m}Tc was not visualized in the bone marrow and was seen only in the spleen in some cases.

In patients with ITP, markedly shortened survival and increased turnover were characteristic. There were two patterns of splenic and hepatosplenic sequestrations. Najean classified the sequestration patterns into hepatic, hepatosplenic, splenic, and diffuse types (*11*). Aster described the liver and spleen as the sites of platelet destruction (*12*). In Cases 6 and 7 spleen-to-liver ratios on the first day were minimum whereas those of the final day were maximum. There was also a difference in the destruction sites shown by means of body surface counting and the scintillation camera in these patients. These results plus the short survival suggest that platelets in patients with ITP are destroyed rather than sequestered in the spleen and/or liver.

In patients with congestive splenomegaly, platelets were sequestered within the spleen rather than destroyed as the survival rate was almost normal and more than 90% of the platelets were found to have accumulated in the spleen after the completion of infusion of labeled cells. In this patient, camera images by scintillation camera coincided with the results by body surface counting. This would be analogous to the increased marginal granulocyte pool in the spleen in such subjects with neutropenia (*4,13*).

In patients with myeloproliferative disorders, lifespan was normal and turnover increased. "Recovery" was normal or slight. The sites for platelet sequestra-

tion were splenic or hepatosplenic. These results suggest megakaryocyte proliferation of the bone marrow with increased platelet production and splenic sequestration of the increased platelets in the peripheral blood.

In thrombokinetic studies, the double-tracer techniques with ^{99m}Tc and ^{51}Cr offer several advantages. Quantitative measurement of the sites of platelet sequestration and destruction would be possible, thus avoiding the limited viewing of only part of an organ in body surface counting by ^{51}Cr-labeled platelets. Organ distribution by ^{99m}Tc-labeled platelets could be visualized in a brief period of time. These camera images may in turn contribute to the indications for splenectomy and diagnosis of the sites of platelet consumption in patients with consumption coagulopathy.

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