

Pooling of Platelets in the Spleen: Role in the Pathogenesis of "Hypersplenic" Thrombocytopenia *

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Reduction in one or more of the circulating elements of the blood often attends splenic enlargement. Anemia has been attributed to hemolysis plus pooling of red blood cells in the spleen (3-6). The causes of the leukopenia and thrombocytopenia seen in splenomegalic subjects are less well understood. Thrombocytopenia has been ascribed both to bone marrow suppression through a humoral mechanism (7-9) and to premature destruction of cells in the enlarged spleen (10-11). The present work explores still a third possibility: that the general circulation is depleted of platelets by their entry into a large exchangeable splenic pool.

Methods

Labeling of platelets with ^{51}Cr and calculation of platelet recoveries after transfusion were carried out as described previously (12-13). The value used for platelet recovery was the per cent of transfused platelets remaining in the general circulation for 2 hours. From 1 to 4 hours after infusion of ^{51}Cr -labeled platelets into normal or splenomegalic subjects there was no significant variation in circulating ^{51}Cr . Duplicate determinations of platelet recovery in the same subject never varied from each other by more than 10%.

In most studies ^{51}Cr -labeled autogenous platelets were employed. Where it was necessary to use homologous platelets, these were obtained from ABO, Rh-compatible

donors. In the absence of isoimmunity from pregnancy or previous transfusion, homologous platelets behave like autogenous cells (13-14).

Surface scanning of body organs was done with a directional scintillation counter using a radiation spectrometer to reduce background to less than 100 cpm.¹ Normal-sized spleens were scanned with the subject lying on the right side. The orifice of the collimator was placed on the skin surface in the midaxillary line with its center directly over the line of diaphragmatic dullness (midinspiration). Maximal splenic surface activity was nearly always obtained at this site. The solid angle encompassed by the scanning device was sufficient to include the entire normal spleen. Deep inspiration reduced surface counts per minute over the spleen, but the counting rate was not affected by casual breathing. With enlarged spleens that extended into the abdomen, an anterior site was chosen for scanning. The anterior of the liver was scanned with the medial margin of the collimator at the midline and the superior margin $\frac{1}{2}$ inch above the area of diaphragmatic dullness. The anterior of the lung was scanned over the right upper lobe. Surface counts per minute were divided by ten times the number of microcuries of ^{51}Cr given so as to make values independent of the dose of radioactivity. The number of counts per minute to be expected over various sites on the basis of blood content alone was determined by scanning normal subjects 1 hour after transfusion of ^{51}Cr -labeled red blood cells. With the particular scanning device used, liver, spleen, and lung counts per minute were 50 to 75% of the precordial values, whereas spleen:liver ratios averaged 1:1 in agreement with the observations of Jandl, Greenberg, Yonemoto, and Castle (15). In asplenic subjects, "spleen":liver ratios ranged from 0.5 to 0.8. A significant deviation from these values after transfusion of platelets indicated that platelet- ^{51}Cr was concentrated (relative to erythrocytes) in a particular site. Previous studies have suggested that actual total radioactivity in normal-sized organs can be crudely estimated from external scanning with various standards for comparison (12, 16-18). Direct assay of ^{51}Cr in three surgically removed normal-sized spleens (idiopathic thrombocytopenic

* Submitted for publication April 2, 1965; accepted January 4, 1966.

Supported in part by grants HE-07652-02 and T1-AM-5391-02 from the U. S. Public Health Service and by research grant FR-76 from the Division of Research Facilities and Resources, National Institutes of Health, Bethesda, Md.

Part of this work was presented previously in preliminary form (1, 2), and at the Seventy-eighth Annual Meeting of the Association of American Physicians, Atlantic City, N. J., May 4-5, 1965.

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¹ Nuclear-Chicago DS-301 scintillation detector with 3-inch diameter crystal and collimator 3 inches wide and 3 inches deep and 132B spectrometer-scaler with window at 100 kv. Counts over various body sites could be reproduced with an error of less than 5%.

purpura) showed that estimates of radioactivity obtained with external scanning and previously derived conversion factors were in error by only 15, 20, and 22%, respectively (19). The obvious limitations of such measurements have been noted by a number of observers (15-19). Jandl and co-workers (15) as well as Hughes Jones, Mollison, and Veall (17) emphasized the geometrical complexities that make quantitation difficult. Wolf and Fischer (20), who used a realistic plastic "phantom," also advised caution in calculating organ radioactivity from external scanning data. In the present work, ratios of radioactivity over one site as compared to another were relied upon in studying organ distribution of platelets. When ^{51}Cr was released from the spleen by epinephrine injection, surface values before and after drug infusion were compared with each other so as to utilize the patient as his own control. Estimates of absolute organ radioactivity made from external scanning were consistent with, but not crucial for, the conclusions reached.

Platelets were counted by the method of Brecher and Cronkite (21) with blood obtained from an arm vein and anticoagulated with EDTA. When platelet levels were less than 100,000 per mm^3 , dilution was 1:20 rather than 1:100 in order to improve counting accuracy.

Epinephrine infusion. Epinephrine hydrochloride diluted 1:125,000 in 5% dextrose was given intravenously over a 20- to 25-minute period at a constant rate. Baseline platelet levels were determined in duplicate, and counts were obtained at 5 minutes, at 15 minutes, and at the end of the epinephrine infusion. A total of about 7 μg

epinephrine per kg of body weight was given (0.49 mg for a 70-kg subject), and pulse and blood pressure were monitored during the infusion. Smaller doses were used in persons over 50 years of age. Patients with known cardiovascular disease were excluded from this study. Pallor was observed within 2 to 3 minutes after the start of infusion, and palpitation was noted for the first few minutes by all the recipients. Systolic blood pressure increased approximately 30 to 50 mm Hg above base-line levels. Rarely, premature ventricular contractions occurred. In general, however, side effects with slow, controlled infusion were less than with subcutaneous injections of the same amount of adrenalin and disappeared within 3 to 5 minutes after terminating the infusion. No adverse reactions occurred in any subjects.

Normal subjects comprised medical investigators or informed convalescent patients hospitalized on the medical wards for illnesses unrelated to blood or blood-forming organs.

Results

Transfusion of ^{51}Cr -labeled platelets to normal subjects. Previous studies have shown that when platelets are labeled in acid citrate and transfused to normal persons, 50 to 80% are recovered in the peripheral blood 1 to 2 hours after transfusion (average, 62%). The remaining platelet- ^{51}Cr is concentrated largely in the spleen (12, 13). A normal disappearance curve of ^{51}Cr -labeled plate-

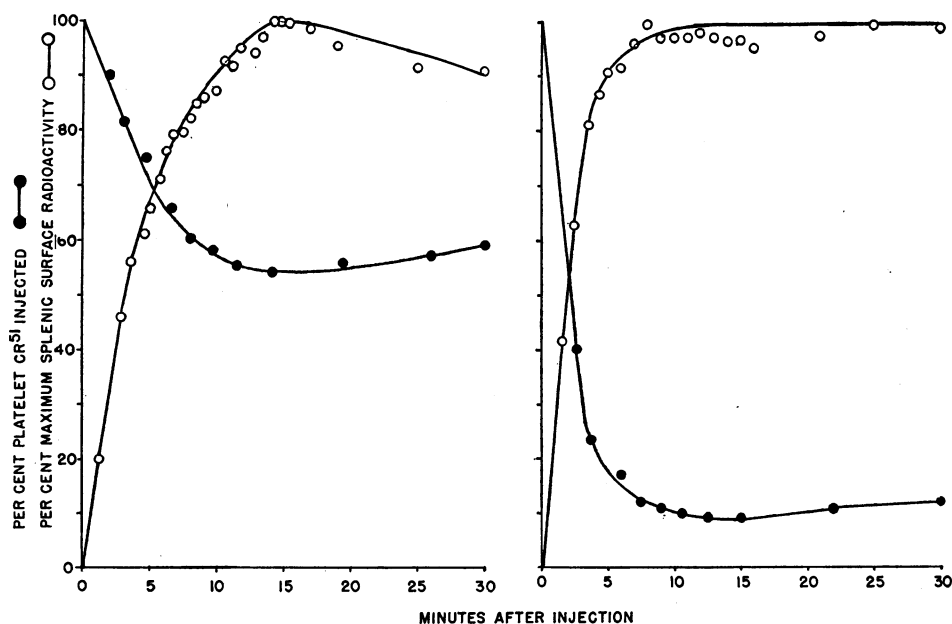


FIG. 1. ACUTE DISAPPEARANCE FROM THE CIRCULATION (DESCENDING CURVES) OF ^{51}Cr -LABELED AUTOGENOUS PLATELETS TRANSFUSED TO A NORMAL SUBJECT (LEFT) AND A PATIENT WITH SPLENOMEGALY (RIGHT). Splenic surface radioactivity as per cent of maximal value is shown in the ascending curves.

lets typical of four such studies in which multiple determinations were made during the first 30 minutes after transfusion is given in Figure 1 (left). The initial rate of platelet disappearance was about 5% per minute. In normal subjects, circulating platelet ^{51}Cr was minimum at about 10 minutes, after which there was often an increase of about 5% to a level that remained steady for several hours. In all cases, splenic surface radioactivity increased in direct proportion to the rate at which ^{51}Cr -labeled cells disappeared from the blood. In contrast, there was a decrease in radioactivity over liver, lung, and precordium consistent with the amount of ^{51}Cr leaving the bloodstream during the same time interval.

After the platelet- ^{51}Cr level had stabilized in the peripheral blood, the ratio of spleen:liver and spleen:precordial radioactivity averaged 5:1 and 4:1, respectively, as compared to average values of about 1:1 and 2:3, respectively, after injection of ^{51}Cr -labeled red blood cells.

Transfusion of ^{51}Cr -labeled platelets to asplenic subjects. Survival of autogenous ^{51}Cr -labeled platelets was studied in six subjects splenectomized for a variety of reasons 2 to 12 weeks previously (four for traumatic rupture, one for "spontaneous rupture," one for splenic tuberculosis). Recoveries of transfused platelets were higher than in normal persons, ranging from 80 to 100% and averaging 91% (Figure 2). In the asplenic subjects, labeled platelets disappeared over 8 to 10 days at a rate approximately equal to that of normal subjects. Each point on the disappearance curves was about 50% higher than the corresponding point on the curve of an average normal subject.

Transfusion of ^{51}Cr -labeled platelets to splenomegalic subjects. Platelet survival was studied in eleven patients with congestive splenomegaly secondary to Laennec's cirrhosis, of whom three had ascites or jaundice or both. There was no evidence of bleeding at the time of study. Five patients were studied in whom splenic enlargement was primarily of a proliferative type: two chronic lymphatic leukemia, two polycythemia vera, one infectious mononucleosis. In five instances homologous platelets were used. None of the patients receiving homologous platelets had been transfused more than three times previously, and no significant differences were observed in the recovery and survival of homologous, as opposed to

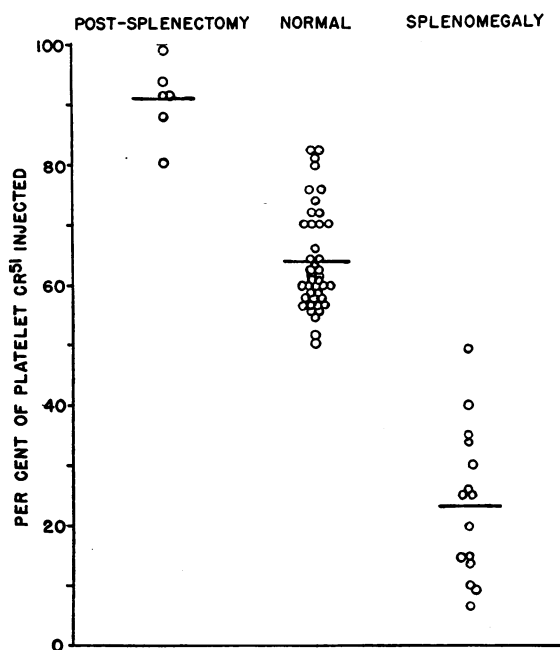


FIG. 2. RECOVERY IN THE GENERAL CIRCULATION OF ^{51}Cr -LABELED PLATELETS TRANSFUSED TO ASPLENIC, NORMAL, AND SPLENOMEGALIC SUBJECTS. "Recovery" is the percentage of platelets remaining in the general circulation 2 hours after injection.

autogenous, cells in the splenomegalic group. Platelet levels ranged from 27,000 to 300,000 per mm^3 .

After transfusion to splenomegalic subjects, most ^{51}Cr -labeled platelets were rapidly cleared from the general circulation (Figure 1, right). The disappearance rate averaged 13% per minute for the first few minutes after transfusion as compared with 5% per minute in normal subjects. After 8 or 9 minutes, a relatively steady level of ^{51}Cr activity in the peripheral blood was maintained for the next few hours. After circulating platelet ^{51}Cr had stabilized, surface radioactivity over the liver and lung was that which would be expected from normal blood flow through these areas, i.e., there was no evidence for concentration of ^{51}Cr in either site. In contrast, ^{51}Cr accumulated in the spleen, the ratio of spleen:liver radioactivity ranging from 7:1 to 15:1 (range of 20 normal subjects, 2:1 to 6:1).

Platelet recoveries (platelet- ^{51}Cr remaining in the general circulation 2 hours after transfusion) were uniformly low in the splenomegalic patients, averaging 23% (Figure 2). In general, lower

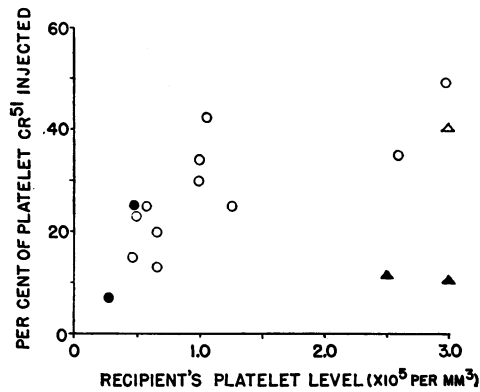


FIG. 3. RELATIONSHIP BETWEEN PLATELET LEVELS AND RECOVERY IN THE GENERAL CIRCULATION OF ^{51}Cr -LABELED PLATELETS INJECTED INTO SPLENOMEGALIC PATIENTS. \circ = congestive splenomegaly secondary to cirrhosis, \bullet = chronic lymphatic leukemia, \triangle = infectious mononucleosis, and \blacktriangle = polycythemia vera.

recoveries were obtained in patients with more severe thrombocytopenia (platelets less than 60,000 per mm^3) (Figure 3). Lowest recoveries were obtained in patients with the largest spleens, as estimated by palpation. In two splenomegaly pa-

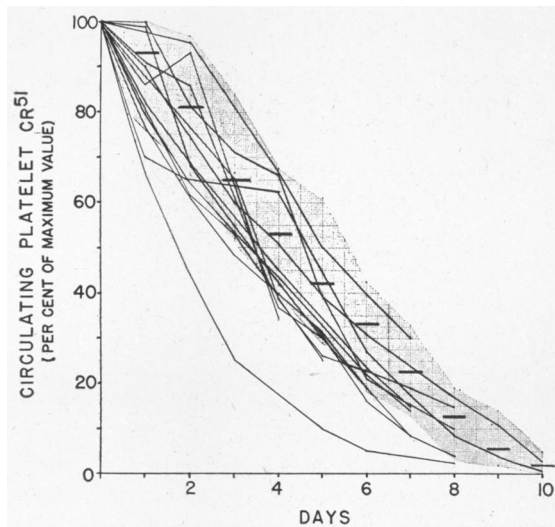


FIG. 4. SURVIVAL OF ^{51}Cr -LABELED PLATELETS IN 13 SPLENOMEGALIC PATIENTS. Platelet ^{51}Cr present in the general circulation 2 hours after infusion of labeled platelets was taken as the 100% value, and platelets remaining on subsequent days were related to this. Shaded area represents the range and horizontal lines the average values for 30 normal subjects 1 to 10 days after infusion of platelets. Although initial platelet recoveries were low in splenomegaly patients (Figure 3), the survival time of platelets remaining in the general circulation was nearly normal.

tients originally given autogenous cells, repeat experiments with homologous platelets resulted in similar recoveries: 18% and 34% as compared to 20% and 30%, respectively, for autogenous platelets. In a third patient labeled platelets were divided into two portions: one was returned to the donor, and the other was transfused into a normal recipient. Recovery was 15% in the splenomegaly patient and 50% in the normal. Approximately the same value (55%) was obtained in the normal subject with autogenous cells.

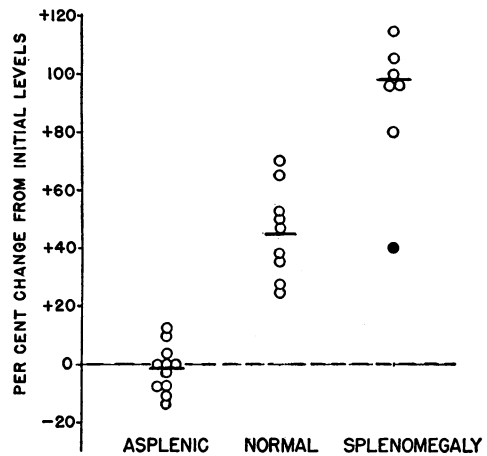


FIG. 5. PER CENT CHANGE IN PLATELET LEVELS AFTER INFUSION OF EPINEPHRINE TO ASPLENIC AND NORMAL SUBJECTS AND SPLENOMEGALIC PATIENTS WITH THROMBOCYTOPENIA (PLATELETS 40,000 TO 120,000 PER MM^3). The maximum of three platelet determinations obtained during epinephrine infusion was used. Dark circle denotes per cent increase in a patient with splenomegaly (infectious mononucleosis) but normal platelet level (300,000 per mm^3). Although percentage increase in platelets was greater in splenomegaly patients than in normal subjects, the absolute increase was equal to or less than that in normals, since initial platelet concentrations were lower.

Life-span curves of platelets that remained in the general circulation are shown in Figure 4. The average time required for circulating ^{51}Cr to diminish to 50%, 25%, and 12.5% of the initial values was 3.65, 5.83, and 7.63 days, respectively. These figures were 85%, 87%, and 95% of the comparable normal values.

Effect of epinephrine on platelet levels in normal subjects. It is well known that pharmacologic doses of epinephrine cause an increase in circulating platelets (22-24), not accounted for by the small associated changes in blood volume (25, 26),

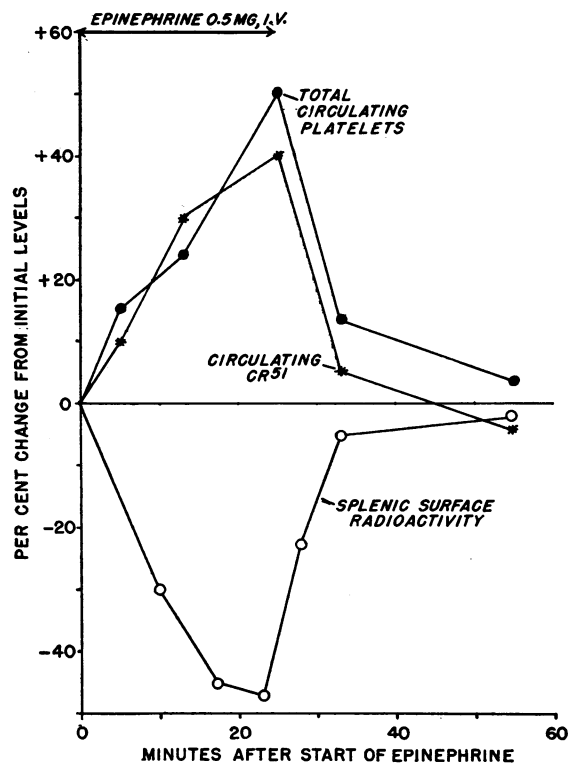


FIG. 6. PER CENT CHANGE IN PLATELET LEVELS, CIRCULATING PLATELET ⁵¹Cr, AND SPLENIC SURFACE RADIOACTIVITY AFTER INFUSION OF EPINEPHRINE TO A NORMAL SUBJECT 4 HOURS AFTER TRANSFUSION OF LABELED PLATELETS. As platelet concentrations and ⁵¹Cr increased in the peripheral blood, there was a decrease in splenic surface radioactivity.

but the source of these cells has not been determined. When 0.5 to 1.0 mg of epinephrine was injected subcutaneously or intramuscularly into normal subjects, platelets usually increased, but the responses (and the intensity of side effects) were quite variable. With slow intravenous infusion of epinephrine (see Methods) a much more uniform platelet response was achieved. A steady increase in platelet levels followed infusion of epinephrine, reaching a peak after about 15 minutes. Platelet levels remained elevated as long as the infusion was continued and returned to base line or slightly below within 10 to 15 minutes after it was terminated. After infusion of 4.0 to 9.0 μ g epinephrine per kg (average, 6.0 μ g per kg), platelet increases in nine normal subjects (recorded in Figure 5) ranged from 25 to 70%, averaging 45%. On four occasions, epinephrine was given to normal subjects 2 to 24 hours after transfusion of ⁵¹Cr-

labeled autogenous platelets. Increases in platelet levels of 27 to 45% above base line were observed and were accompanied by proportional increases in circulating platelet-⁵¹Cr so that the specific activity of circulating platelets remained nearly constant. Splenic surface radioactivity declined steadily during the period of epinephrine infusion (Figure 6). After termination of the infusion, radioactivity in the peripheral blood and over the spleen returned to base-line levels in 10 to 15 minutes. Figure 6 shows the effect of epinephrine in a typical subject. Ten days after transfusion, ⁵¹Cr-labeled platelets had disappeared from the bloodstream, but considerable radioactivity remained in the spleen (12). When epinephrine was given at this time, no change in surface radioactivity or circulating ⁵¹Cr resulted.

In three normal subjects, the effects of epinephrine on the acute disappearance of transfused ⁵¹Cr-labeled platelets from the circulation were

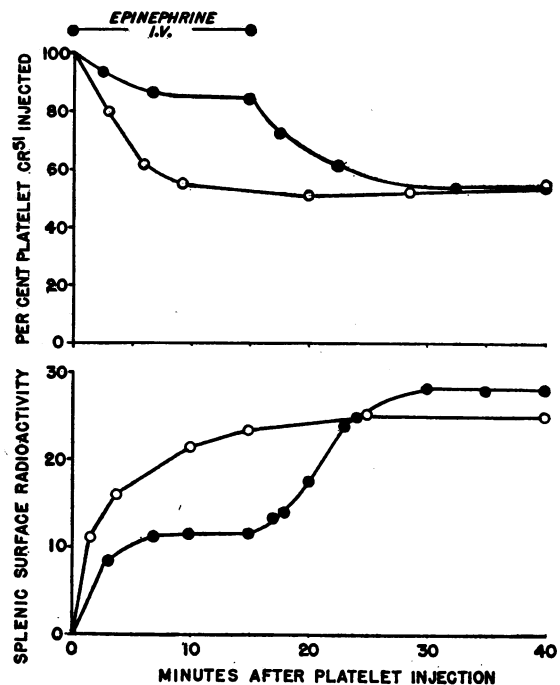


FIG. 7. DISAPPEARANCE FROM THE GENERAL CIRCULATION OF ⁵¹Cr-LABELED PLATELETS TRANSFUSED TO A NORMAL SUBJECT AND ASSOCIATED CHANGES IN SPLENIC SURFACE RADIOACTIVITY WITH (●—●) AND WITHOUT (○—○) SIMULTANEOUS INFUSION OF EPINEPHRINE. With epinephrine there was a delay both in the disappearance of ⁵¹Cr from the blood and in the expected rise in splenic surface radioactivity.

studied. When epinephrine infusion was begun before platelet transfusion, ^{51}Cr disappearance and splenic ^{51}Cr uptake curves were radically altered. During epinephrine infusion only about 15% of platelets entered the spleen, after which a steady state was established; 85% of transfused platelets were thus left in the peripheral circulation. After epinephrine was stopped, there was a further increase in splenic radioactivity and a fall in circulating platelet- ^{51}Cr as additional platelets entered the organ. Figure 7 illustrates this sequence in a typical subject, comparing platelet disappearance curves obtained with and without epinephrine infusion.

Epinephrine stimulation in asplenic subjects. On 12 occasions, amounts of epinephrine that invariably produced significant platelet increases in normal persons were given to asplenic subjects. Indications for splenectomy included hereditary spherocytosis (two patients) and trauma (six patients). The time interval between splenectomy and adrenalin testing ranged from 3 days to 12 years. All subjects but one had normal or increased platelet levels at the time of testing. During a 20- to 25-minute period of epinephrine infusion during which at least three blood samples were obtained, no significant increase in platelet levels occurred (Figure 5). In three subjects, platelets were labeled with ^{51}Cr before epinephrine testing. No increase in circulating ^{51}Cr resulted from the epinephrine infusion. Total amounts of epinephrine administered ranged from 3.5 to 7.7 μg per kg (average, 5.2).

Epinephrine stimulation in splenomegalic subjects. Epinephrine infusions were given to six

subjects with congestive splenomegaly and thrombocytopenia (40,000 to 120,000 platelets per mm^3). Total amounts of epinephrine administered ranged from 3.1 to 6.5 μg per kg (average 4.6). Because of the age and poor health of some of the patients studied, the dose was less than that used in normal individuals. The percentage increases in platelets above base-line levels were greater (average two-fold) than in normal subjects (Figure 5). Since initial blood platelet levels were low, however, the absolute magnitude of the increase was equal to or less than that observed in normal persons. In three of the six subjects, the spleen decreased in size during epinephrine infusion. The estimated reduction in spleen size did not correlate closely with the platelet increase observed in the peripheral blood.

In four of the splenomegalic patients, platelets were labeled with ^{51}Cr 1 day before epinephrine testing. As in normal subjects, an increase in circulating ^{51}Cr occurred that was proportional to the total platelet increase. Simultaneous decreases in splenic radioactivity were observed (15 to 30% below base line).

One patient with splenomegaly secondary to infectious mononucleosis who had a normal platelet level showed a lesser percentage increase in circulating platelets after epinephrine than did splenomegalic patients with thrombocytopenia (Figure 5).

Studies on surgically removed spleens. Platelet levels were determined on splenic blood from five surgically removed spleens. This was done on blood drained from the spleen or by making incisions through the splenic capsule at several sites

TABLE I
Comparison of platelet concentrations in peripheral and splenic blood

Patient	Diagnosis	Spleen Weight	Platelets		Spleen pool	
			Peripheral blood	Splenic blood	Total platelets*	% of total body platelet mass†
		g	/ mm^3			
1	Hereditary spherocytosis	745	150,000	650,000	2.4×10^{11}	26
2	Hereditary spherocytosis	420	121,000	880,000	1.9×10^{11}	24
3	Hereditary spherocytosis	428	315,000	960,000	2.1×10^{11}	17
4	Idiopathic thrombocytopenic purpura	155	70,000	1,000,000	7.7×10^{10}	31
5	Congestive splenomegaly	1,800	75,000	466,000	4.2×10^{11}	62

* Assuming 50% of spleen weight to represent blood.

† Blood volume in general circulation estimated as 7% of body weight.

and depths and drawing blood into diluting pipettes directly from the incision. Table I shows that in all cases, platelet concentration was much higher in splenic blood than in peripheral blood.² The platelet concentrations in splenic blood probably represent minimal values, because some fibrin formation was present. In one patient with thrombocytopenia and splenomegaly secondary to post-necrotic cirrhosis, the enlarged spleen was injected with heparin immediately after surgical removal. Blood was drained from the spleen and coagulation was minimal. The spleen was then perfused with 100 ml isotonic NaCl, and the perfusate was pooled with the venous drainage; 3.5×10^{11} platelets were recovered from the organ. If transfused to the peripheral circulation of the same patient, this quantity of platelets would have been sufficient to increase her platelet level from 75,000 to 180,000 per mm³. Removal of red blood cells from the spleen by drainage and perfusion was about 90% effective. Scrapings taken from cut sections of the perfused organ showed, however, that many platelets were not removed. It is probable that the total number of platelets in the enlarged spleen would, if transferred to the peripheral circulation, have increased this patient's platelet level to normal or above.

Discussion

The human spleen normally contains less than 2% of the red cell mass, although a larger proportion may be pooled there in certain pathologic states characterized by splenomegaly. The total red cell mass present in the organ even in extreme splenomegaly rarely exceeds 20% of the total (5, 28-30). On the other hand, the total body plate-

let mass is quite small (about 5 to 10 ml) so that the spleen could readily retain a large percentage of total platelets if factors operated to concentrate them there. The following considerations suggest that even the normal spleen does indeed contain a sizeable fraction of the total body platelet mass in the form of an exchangeable pool and that sufficient platelets may be concentrated in enlarged spleens to cause thrombocytopenia.

In both the normal and splenomegalic subjects there was a reciprocal relationship between the disappearance of ⁵¹Cr-labeled platelets from the circulation during the first 10 minutes after transfusion and the increase in splenic radioactivity. In contrast, very few platelets (average 9% of those infused) disappeared from the general circulation acutely after transfusion to asplenic subjects (Figure 2). It was apparent from surface scanning in normal subjects that ⁵¹Cr was concentrated in the spleen far out of proportion to its volume of blood, since spleen:liver and spleen:precordium ratios averaged 5:1 and 4:1, respectively, as compared to average values of about 1:1 and 2:3, respectively, after infusion of ⁵¹Cr-labeled red blood cells. In splenomegalic patients, the discrepancy was even more striking, with spleen:liver ratios ranging from 7:1 to 15:1 shortly after infusion of ⁵¹Cr-labeled platelets. In both normal and splenomegalic subjects radioactivity of lung and liver relative to precordium was consistent with that anticipated on the basis of the blood content of these regions. No concentration of ⁵¹Cr could be detected over any body site other than the spleen, nor was any ⁵¹Cr released in detectable form into the plasma. Estimates of total radioactivity in the spleens of normal subjects derived from previous external scanning data (16), although only approximations, correlated within limits of error with the amount of ⁵¹Cr that left the bloodstream. As noted earlier (15), total ⁵¹Cr is underestimated by scanning over enlarged spleens because a smaller segment of the organ is covered by the orifice of the scanning device. This fact would tend to decrease surface counts per minute as compared with the same amount of radioactivity concentrated in a normal-sized spleen. Thus, the disproportion between ⁵¹Cr in the spleen and liver of splenomegalic subjects after transfusion of labeled platelets is even greater than the increased spleen:liver ratio would imply. Taken together,

² Estimates of total splenic platelet content on the basis of platelet counts done on splenic pulp or blood drained from the spleen are probably only rough approximations, since the volume of platelet distribution in the spleen is not precisely known. The volume of red blood cells contained in the normal spleen has been estimated at 20 to 40 ml (5, 27), which would correspond to 27 to 54% blood by weight in a 150-g spleen. In a patient with congestive splenomegaly, we were able to recover 50% of organ weight as whole blood despite probable loss by the organ of some blood content as a result of tying off the splenic artery before the vein at operation. In calculating total platelet content of the enlarged spleens (Table I), it was assumed that platelets were distributed through 50% of the organ.

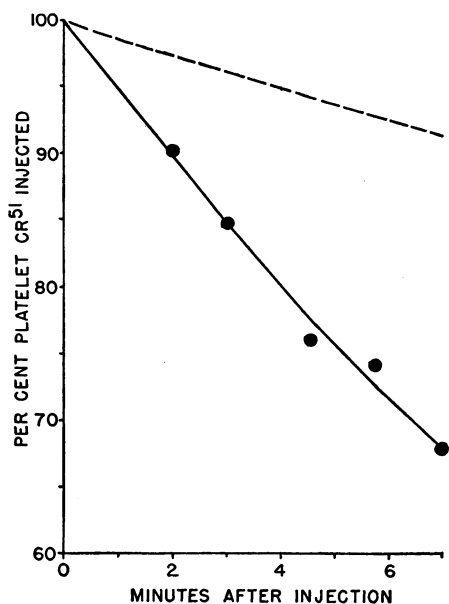


FIG. 8. ACUTE DISAPPEARANCE FROM THE CIRCULATION OF ^{51}Cr -LABELED PLATELETS TRANSFUSED TO A NORMAL SUBJECT (\bullet — \bullet). Final platelet recovery at 2 hours was 65%. The dashed curve represents the expected disappearance rate if 35% of platelets were damaged in the labeling process and destined to be removed from the circulation on first passage through the spleen. In deriving the theoretical curve, it was assumed that cardiac output equals one blood volume per minute and that splenic blood flow represents 4% of cardiac output. The percentage of platelets remaining in the circulation t minutes after injection is given by $100 (.65 + .35e^{-.04t})$.

the data strongly suggest that ^{51}Cr -labeled platelets that leave the circulation acutely after transfusion enter the spleen.

The rate at which ^{51}Cr -labeled platelets leave the bloodstream after transfusion is consistent with estimates of splenic blood flow at rest.³ If all these platelets do enter the spleen, then during the first 8 minutes or so after transfusion most labeled cells entering the spleen are, at least temporarily, retained there. This could be due either to mixing

³ Splenic blood flow in man has not been measured directly, but observations by Hughes Jones and associates (17) on splenic sequestration of red blood cells sensitized with incomplete antibodies suggest a minimal normal value of 3 to 4% of cardiac output. Using a similar technique, Kaplan and Jandl (31) showed that 15% of cardiac output was cleared of cells by the spleen in a patient with hypersplenism. Wagner and co-workers found that enlarged spleens removed damaged red blood cells from the circulation three to four times as rapidly as did normal spleens (32).

of cells with an exchangeable "pool" or to permanent (nonexchangeable) sequestration. After 7 to 8 minutes, peripheral blood and splenic radioactivity remained relatively constant (Figure 1), implying that destruction of labeled cells stops rather abruptly or, alternatively, that labeled platelets have fully mixed with unlabeled platelets in the hypothetical pool. The data are not consistent with the possibility that splenic concentration of ^{51}Cr is due solely to damage of a fixed fraction (say 35%) of cells during labeling so that these cells are destined to be sequestered when they reach the spleen, for in such a case, disappearance of ^{51}Cr from the circulation would follow a simple exponential curve dependent on the fraction of cardiac output that comprises splenic blood flow. It may be seen from Figure 8 that the observed disappearance of platelet ^{51}Cr is much more rapid than the rate expected on the basis of this type of destructive mechanism. For the theoretical curve to approximate the observed one even during the first 1 to 2 minutes, normal splenic blood flow must be assumed equal to 15% of cardiac output, a figure at least three times greater than even the most liberal estimates. If platelet injury is responsible for splenic ^{51}Cr uptake after transfusion, the rapidity with which labeled cells enter the spleen makes it necessary then to postulate a uniform injury to all the platelets, which is reversible after the cells have spent 7 to 8 minutes in the recipient's circulation. That such is not the case is suggested by the observation that when entry of transfused cells into the spleen is delayed 10 to 15 minutes by infusion of epinephrine, final platelet recovery is not increased (four observations), despite the fact that transfused platelets circulate freely from the time of their injection (Figure 7). Moreover, infusion of epinephrine results in release of at least part of the splenic ^{51}Cr into the general circulation (Figure 6), even when given 1 day after the original transfusion. Since platelet levels did not increase in asplenic persons given epinephrine, it seems probable that virtually all the increase in circulating platelets after adrenergic stimulation in normal subjects is derived from the spleen. Less than a 50% reduction of splenic surface radioactivity could be achieved with epinephrine, but some of the initial surface activity was derived from underlying structures such as liver.

As splenic radioactivity diminished and radioactivity in other regions such as the liver increased, the contribution from extrasplenic sites became relatively greater. Thus the data are consistent with the possibility that more than 50% of splenic radioactivity was mobilized, albeit a precise estimation is not possible.

These observations are subject to various interpretations. For example, it could be postulated that platelets are mobilized from the lung (33), liver, or the marginal vascular pool (34) after epinephrine infusion. It would then be necessary, however, to explain why the platelet levels of asplenic subjects do not change during adrenergic stimulation. Moreover, the consistent decrease in radioactivity over the spleen after epinephrine and the small but definite increase over the liver and lungs are inconsistent with these possibilities. A splenic platelet pool exchanging with the general circulation and sensitive to epinephrine can, on the other hand, readily explain the observed data. The finding that asplenic subjects do not increase platelet levels in response to epinephrine confirms the recent report of Griffoni, Scaltrini, Confalonieri, and Conigliaro (35), but contrasts with the earlier findings of Chatterjea, Dameshek, and Stefanini (36). Possibly, the indirect counting technique used by the latter workers accounts for the discrepancy. Others have found that indirect platelet counts may show apparent fluctuations in platelet levels not confirmed by direct counting of cells (37).

Disappearance curves of platelet ^{51}Cr from the circulation (Figure 1) suggest that about 8 minutes is required for a steady state to be established in the normal spleen. In contrast, labeled red blood cells establish equilibrium in the spleen in less than 2 minutes (38, 39). This suggests that platelets may enter a compartment of the spleen, pass through the compartment serially, and then enter the splenic vein. If this is so, then the size of the pool would be proportional to the product of splenic blood flow (approximately 4% of total blood volume per minute) and platelet transit time through the spleen. An average value of 8 minutes for transit time would produce a "pool" constituting about 32% of the total platelet mass. This figure is consistent with the time required for splenic radioactivity to stabilize after injection of labeled cells and with the difference in platelet re-

covery observed between asplenic and normal subjects (Figures 1 and 2). It is of interest that Björkman has shown that particles less than $3\ \mu$ in diameter enter the pulp cords rather than sinuses when injected into the splenic artery, whereas the reverse is true of larger particles (40). If platelets ($< 2\ \mu$ in diameter) are required to traverse the more complex system of splenic cords, their transit time through the organ might well be prolonged relative to red blood cells, leukocytes, and plasma. Such a process could be compared on a macro scale to the "molecular sieve" type of column utilized for molecular separations in analytical chemistry.

Doubtless the model suggested by the data is an oversimplification, since other types of pools could be made to "fit" the observed curves. Moreover, it is probable that some of the ^{51}Cr that accumulates in the spleen after transfusion is a result of permanent sequestration of cells damaged *in vitro* or that the entire pool is not mobilized by epinephrine or both. For these reasons the exact size of the splenic platelet pool cannot be estimated with precision from the available data. It seems highly probable, however, that such an exchangeable pool exists and that it constitutes between 20 and 40% of the total platelet mass. Direct determination of platelet numbers in surgically removed normal spleens may be helpful in determining the spleen's platelet content more precisely. Such spleens are not in a normal state, however, and it is not possible to be certain from such data that the platelets encountered are exchangeable with those in the bloodstream.

Since human platelets are present in excess of the amount required for hemostasis, it is difficult to assign an important physiologic role to the splenic platelet pool. Certain implications are suggested, however. For example, estimates of human platelet production based on platelet lifespan and circulating platelet levels should probably be increased. At least part of the thrombocytosis that follows splenectomy may be attributed to loss of the splenic pooling function. In experimental and clinical studies involving transfusion of fresh or preserved platelets the calculation of recovery should take into consideration obligatory splenic platelet pooling. Thus, an apparent recovery in venous blood of 65 to 75% of transfused cells may indicate nearly 100% viability. In ear-

lier studies from this laboratory it was presumed that the high uptake of ^{51}Cr by the spleen after transfusion of labeled platelets represented permanent sequestration by this organ of cells injured in the labeling process (12). During the 8- to 10-day period during which ^{51}Cr -labeled platelets disappeared from the blood stream, no further rise in ^{51}Cr in the spleen was observed. Since elimination of ^{51}Cr from the spleen after permanent sequestration of platelets was rather slow (about 3% per day), it appeared that relatively few platelets were destroyed in the organ under normal conditions. With splenic pooling recognized as the primary cause of the initial splenic surface radioactivity, it appears likely that a number of effete platelets at least equivalent to the pool size are eventually removed from the circulation by the spleen and destroyed therein.

Splenic pooling as a possible cause of hypersplenic thrombocytopenia. If the mechanism for splenic platelet pooling postulated above is correct, it is to be expected that factors which increase splenic blood flow or prolong platelet transit time through the spleen will increase the pool size. In splenomegalic patients platelet recoveries in the peripheral blood were markedly reduced (Figure 3). For reasons stated above, apparently all or nearly all of the ^{51}Cr leaving the bloodstream acutely after transfusion accumulated in the spleens of these individuals. It is unlikely that any defect intrinsic to platelets themselves accounted for the reduced recoveries, for similar results were obtained with homologous and autogenous cells. Moreover, platelets from one splenomegalic subject gave 15% recovery in the donor but normal recovery in a normal recipient. It is also improb-

able that a so-called "platelet debt" contributed significantly to decreased recovery, for normal platelet recoveries are often observed in patients with much more severe degrees of thrombocytopenia (19, 41). Furthermore, transfusion of 4.5×10^{11} homologous platelets (equivalent to 5 U of whole blood) to one of our splenomegalic recipients resulted in about the same recovery (18%) as did a much smaller number of autogenous cells (1.5×10^{10}) (20% recovery). As with normal individuals, then, it appears necessary to choose between permanent destruction in the spleen of cells damaged *in vitro* and splenic pooling or a combination of these two factors to explain the very low platelet recoveries observed in splenomegalic subjects. If cell injury is responsible, it is likely for reasons stated above to be of a reversible nature, since peripheral blood ^{51}Cr was relatively stable after about 7 to 8 minutes as with normal persons (Figure 1). In terms of total platelets mobilized, splenomegalic patients responded no better and usually less well to epinephrine stimulation than did normal subjects, although the percentage platelet increase was greater (Figure 5). If one assumes that splenic pool size is represented by platelets mobilized by epinephrine and that the fraction of platelets "released" by a given dose of epinephrine is unrelated to spleen size, then the low recovery of platelets transfused to splenomegalic subjects cannot be accounted for entirely by pooling, and the initial high splenic ^{51}Cr uptake may be due in part to *in vitro* cell injury. Reversible platelet injury is, however, conjectural, and there is no information as to the relative effectiveness of epinephrine in pathological spleens. Indeed, there is no reason to think that infusion of epinephrine for 15 to 20 minutes necessarily mobilizes all platelets from an enlarged spleen, and the doses of epinephrine given were less than those administered to normal subjects. Even though shrinkage of several spleens was observed, the compartment containing platelets, which can occupy a total packed volume of only 5 or 10 ml, need not necessarily have been affected in proportion to the gross reduction in spleen size, which presumably reflects mainly a shift of the red cell pool.

The data in Figure 3 relating circulating platelet levels to the fraction of cells that remains in the general circulation after transfusion can be

TABLE II
Maximal platelet mass calculated from dilution of injected ^{51}Cr -labeled platelets

Diagnosis	No. of subjects	Maximal platelet mass*	
		Average	Range
		<i>platelets/kg body wt</i>	
Normal	30	2.97×10^{10}	1.80- 5.20
Congestive splenomegaly	11	2.75×10^{10}	2.17- 6.12
Chronic lymphatic leukemia with splenomegaly	2	2.89×10^{10}	1.92- 3.86
Infectious mononucleosis	1	6.10×10^{10}	
Polycythemia vera with splenomegaly	2	24.0×10^{10}	20.8-27.2

* Maximal platelet mass = [platelets per milliliter venous blood/per cent recovery of transfused patients (venous blood)] \times (70 ml/kilograms body weight).

used to calculate total body platelet mass by isotope dilution. The values obtained are maximal ones based on the assumptions that transfused cells mix completely with the total circulating platelet mass and that none are permanently sequestered in the spleen as a consequence of injury due to *in vitro* manipulation alone. Table II compares the platelet mass of splenomegalic patients calculated in this way with that of normal subjects. The average platelet mass of all splenomegalic subjects excluding two patients with polycythemia vera was about the same as for 30 normal subjects. The range of values in the two groups was also comparable.

Although the sources of error referred to above leave some uncertainty as to the exact fraction of total body platelets that is present in the spleens of splenomegalic patients, it seems evident that the enlarged spleens do concentrate platelets to a degree disproportionate to the amount of blood they contain. Average platelet life-span in the 16 splenomegalic patients studied was nearly normal (Figure 4), in confirmation of studies by Cohen, Gardner, and Barnett (14). If the total platelet mass is approximately normal (Tables I and II), it follows that platelet production, which is proportional to the ratio platelet mass:platelet life-span, is also normal in these individuals, as might be expected from the number of megakaryocytes in their marrow. It has been suggested that in hypersplenic states the margins of the megakaryocytes appear smooth and inactive (9), but there is not uniform agreement on this point. Moreover, it has been shown experimentally that similar alterations in megakaryocyte morphology follow acute platelet depletion despite a normal rate of platelet production (42-43). The "nonproductive" appearance of the megakaryocyte has been most strongly emphasized in idiopathic thrombocytopenic purpura (44), a disease now known to be characterized in almost all instances by normal or even increased platelet production rates (45). Apparently the smooth appearance of megakaryocytes is, in most instances, a physiological reflection of immaturity of the cell, rather than of a pathological failure to function properly.

That thrombocytopenia in the peripheral blood of patients with hypersplenism is chiefly a consequence of splenic platelet pooling was suggested earlier by Reimann, Erdogan, and Ulagay (46),

who found platelet concentrations to be higher in blood from enlarged spleens than in the general circulation. Wright, Doan, Bouroncle, and Zollinger (11) and Chatterjea and co-workers (36) showed that injection of epinephrine directly into the splenic artery of patients with congestive splenomegaly results in greatly increased platelet levels in the splenic vein, demonstrating concentration of platelets in the enlarged spleens. Penny, Rozenberg, and Firkin, using a perfusion technique, recovered 11×10^{11} platelets that appeared normal from a patient with thrombocytopenia and splenomegaly secondary to cirrhosis (47). Thirteen other pathologic spleens perfused by these workers all contained platelets far in excess of the amount to be expected on the basis of splenic blood volume alone. This was true whether or not the subjects had thrombocytopenia. Our experience with a limited number of surgical specimens was similar (Table I). On the basis of a limited number of observations (Figure 3) it would appear that pooling is as pronounced in spleens enlarged by proliferative disease as in spleens enlarged as a result of congestion in the splanchnic vascular bed. More studies of patients with proliferative splenomegaly will be required, however, particularly in subjects with hereditary spherocytosis, a disease which is rarely complicated by thrombocytopenia and in which selective red cell trapping is a primary event. If the spleens of these subjects pool platelets to a significant degree, as suggested by Penny and associates (47), then platelet production must be increased to maintain normal platelet levels. In our two patients with polycythemia vera, the pathological increase in platelet production known to be characteristic of this disease probably accounted for normal platelet levels despite the presence of a very large splenic platelet pool.

In conclusion, the evidence indicates that pooling of platelets in an enlarged spleen accounts for the thrombocytopenia of the hypersplenic state. A slightly increased rate of platelet destruction plays an additive, but relatively minor, role. Until more precise estimates can be made of the pool size and its exchangeability with the general circulation, however, it is not possible to state with certainty that these two mechanisms are totally responsible for reduced platelet levels in splenomegalic patients.

Even though platelet production appears to be normal in most individuals with hypersplenism, the possibility that enlarged spleens exert humoral control over the marrow is not altogether excluded. Indeed, the fact that most patients with congestive splenomegaly appear unable to increase platelet production so as to compensate for platelet pooling could be attributed to an inhibitory effect. On the other hand, the failure of these subjects to compensate may be a consequence of the limited capacity of normal marrow to increase platelet production in response to platelet depletion (42-43). Alternatively, the apparent "failure" of marrow to respond to the low blood platelet levels that result from pooling raises the prospect that the marrow reacts to a product of net platelet destruction rather than to absolute platelet levels in the peripheral blood.

Summary

The distribution of human platelets in the body was studied with ^{51}Cr -labeled platelets, external scintillation scanning, epinephrine stimulation, and platelet counts on the blood of surgically removed spleens.

Evidence is presented which indicates that normally about one-third of the total platelet mass is concentrated in the spleen, exchanging with the remaining two-thirds of platelets, which are evenly distributed throughout the rest of the vascular system. Pulmonary or "marginal" pools do not appear to contain a significant fraction of total platelets in man.

In splenomegaly, the splenic platelet pool may be greatly increased, so that 50 to 90% of platelets are in the spleen at any given time. This redistribution of cells from the peripheral circulation to the spleen appears sufficient to produce thrombocytopenia despite unimpaired platelet production, normal total body platelet mass, and nearly normal platelet life-span.

The protracted thrombocytosis that usually follows splenectomy may be due, in large part, to removal of the splenic platelet pool.

The present studies are compatible with the possibility that platelet production is governed by the rate of platelet destruction rather than by the platelet concentration of the blood.

Acknowledgments

I am indebted to Dr. James Jandl for reviewing the manuscript and for many helpful suggestions made during the course of this study, and to Dr. William McDermott for providing a surgically removed spleen and several patients for investigation. The technical assistance of Miss Nancy Mann, Miss Barbara Mink, and Miss Hollister Nash is gratefully acknowledged.

Addendum

Since this manuscript was prepared, Penny, Rozenberg, and Firkin have determined the platelet content of 14 surgically removed spleens by a direct perfusion technique (*Blood* 1966, 17, 1). Estimates of splenic pool size obtained by this method and the data here presented on the magnitude and dynamics of the pool appear to correlate closely.

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