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SURVIVAL OF BLOOD PLATELETS LABELED WITH CHROMIUM^{51 1, 2}

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Various criteria exist for the evaluation of the integrity, viability and physiological activity of human blood platelets. Alterations in platelet morphology, clot promoting and clot retracting activities, oxygen consumption, clinical effect on bleeding disorders, and the life span of platelets in thrombocytopenic recipients have been used as measurements of platelet function. The transfusion studies in thrombocytopenic recipients indicate that platelets have a life span of three to seven days and have been helpful in the classification of certain pathological disorders (2). These observations have been dependent upon obtaining donors with high platelet counts and accepting the errors associated with platelet counting. The difficulty in procuring adequate donors and the desirability of measurements other than platelet enumeration have initiated numerous studies of the *in vivo* survival of platelets labeled with radioactive isotopes.

The earlier studies concerning measurement of the life span of blood platelets with isotopes prior to 1955 have been reviewed by Odell, Tausche and Furth (3). In Table I the various isotope techniques previously reported are tabulated for comparison. The recent report by Leeksa and Cohen (13) has demonstrated a life span of labeled platelets of eight to nine days. These investigators followed the activity of radioactive phosphorus in platelets after the recipient had received diisopropylfluorophosphonate (DFP³²) parenterally. Reisner, Keating, Friesen and

Loeffler (14) have noted that radioactive sodium chromate could be bound to platelets *in vitro*. When these platelets were transfused to recipients, a survival period of five to eight days could be measured. Similarly, transfusions of *in vivo* labeled P³² platelets from polycythemic donors have had a life span of seven days in normal recipients (15).

The present investigation was in progress at the time of the observations by Reisner and co-workers (14). A workable technique is presented for the *in vitro* labeling of human blood platelets with radioactive chromium and evaluating their survival after transfusion to normal recipients. Comments regarding details of the method will be presented.

METHODS

Material. Whole blood donations were obtained from volunteer medical staff and patients. In many instances *in vitro* studies were done using blood obtained from therapeutic phlebotomies.

Preparation of platelet suspension. The following technique was derived from past experience in the preparation of platelet suspensions using plastic bag equipment. Five hundred ml. of whole blood is collected by gravity into polyvinyl chloride plastic bags (Fenwal Laboratories) containing 50 ml. of 1.5 per cent disodium ethylenediaminetetracetic dihydrate (Na₂EDTA) in saline. As noted previously (16) Arquad® 4-coated 15 gauge male-hub needles are used. The plastic tubing leading from the needle to the plastic bag has a "Y" type division that allows 10 ml. of blood to be withdrawn to exclude tissue juice contamination and to evaluate venous blood flow. The whole blood is centrifuged at 2° C. for 15 minutes at 1,300 rpm. (325 G) in an International Centrifuge, Model PR-2. After centrifugation, the platelet-rich plasma is transferred to another plastic bag containing 15 ml. of 2 per cent Triton®⁵ in saline using a piercing

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² A preliminary report was presented at The Sixth Congress of The International Society of Blood Transfusion, 1956 (1).

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⁴ Arquad® is a trade-mark of Armour Chemical Division, Armour and Co., Chicago, for Tris (2-hydroxyethyl) dodecylamine and N,N'(2,3-dihydroxypropyl)-N-alkyl-trimethylenediamine (A-15).

⁵ Triton® is a trade-mark of Rohm & Haas Co., Philadelphia, for oxyethylated tertiary octyl phenol formalde-

TABLE I
Summary of isotope platelet survival studies

Author	Year	Isotope	Mode of tagging	Species	Indicated life span
Julliard and associates (4)	1952	P ³²	<i>In vitro</i>	Human	2 hours
Mueller (5)	1952	P ³²	<i>In vitro</i>	Rabbit	2 hours
Odell and associates (6)	1953	C ¹⁴	<i>In vivo</i>	Rat	3-4 days
Morgan and associates (7)	1954	I ¹³¹	<i>In vitro</i>	Rabbit	1-2 hours
Robertson and associates (8)	1954	Cr ⁵¹ Cl ₃	<i>In vitro</i>	Rat	2 days
Odell and associates (9)	1954	C ¹⁴ + S ³⁵	<i>In vivo</i>	Rat	5-6 days
Odell and associates (10)	1955	C ¹⁴	<i>In vitro</i>	Rat	1-3 days
Odell and associates (10)	1955	S ³⁵	<i>In vivo</i>	Rat	4-5 days
Desai and associates (11)	1955	P ³²	<i>In vitro</i>	Human	Apparent 1/2-survival time 35-50 hours
Morgan and associates (12)	1955	Na ₂ Cr ⁵¹ O ₄	<i>In vitro</i>	Rabbit	3-4 days
Leeksa and Cohen (13)	1956	DFP ³²	<i>In vivo</i>	Human	8-9 days
Reisner and associates (14)	1956	Na ₂ Cr ⁵¹ O ₄	<i>In vitro</i>	Human	5-8 days
Adelson and associates (15)	1957	P ³²	<i>In vivo</i>	Human	7 days

coupler (16). A positive pressure pneumatic press is helpful in allowing careful separation of plasma from the red cells and buffy coat. The plastic bag containing the platelet-rich plasma is distended with air to prevent platelet trapping in folds of the bag during centrifugation and is then centrifuged at 2° C. for 30 minutes at 2,300 rpm. (1,000 G). The platelets separate out as a creamy button at the bottom of the bag. The platelet-poor supernatant plasma is withdrawn under sterile conditions and saved, leaving approximately 10 ml. of residual plasma with the platelet button.

Labeling of platelets. Through the plastic inlet tubing attached to the bag, 10 ml. of 0.2 per cent Triton® in saline solution is injected by syringe. The bag is kneaded between the fingers to resuspend the platelet button until no macroscopic clumps are visible. Three hundred microcuries of sterile radioactive sodium chromate with a specific activity of 20 microcuries Cr⁵¹ per microgram is injected into the bag through the plastic tubing and directed into the platelet suspension. The chromate solution is mixed and the platelet suspension is incubated for 15 minutes at room temperature (20 to 22° C.). Thereafter, the suspension is centrifuged again at 2° C. for 30 minutes at 2,300 rpm. (1,000 G), and the plasma-Triton® solution is drained from the platelet button by gravity and saved to determine its residual Cr⁵¹ activity. Fifteen ml. of the sterile platelet-poor plasma, previously saved, and 15 ml. of 0.2 per cent Triton®-saline solution are added to the platelet button and the platelets resuspended. One hundred milligrams of a 5 per cent ascorbic acid solution is then added to the platelet suspension to prevent red cell binding in the recipient by excess Na₂Cr⁵¹O₄. The platelets are dispersed by kneading, and about 100 ml. of air is allowed to enter the bag to facilitate complete infusion of the platelet suspension. A short piece of plastic tubing is attached to the plastic bag using a piercing coupler to decrease loss of platelets adhering to the tub-

ing. Usually a saline infusion has been started in the recipient to assure the rapid injection of the 30 to 35 ml. volume of platelet suspension. From the average donor, the platelet concentrate in a 35 ml. volume usually has a 3.5×10^6 cu. mm. platelet count. The entire procedure requires about two hours from the phlebotomy to the infusion of labeled platelets.

Comments on method

1. **Anticoagulant.** Plasma preparations prepared with acid citrate dextrose (ACD) solution (National Institutes of Health formula) have numerous microscopic aggregations of platelets that do not resuspend after centrifugation and labeling with Cr⁵¹. Earlier studies have demonstrated that platelets are discrete and not clumped when Na₂EDTA was used as the anticoagulant (16, 17). No evaluation was made in this present study to determine if microscopic clumping of platelets influenced viability after labeling and infusion.

2. **Differential centrifugation.** The speeds listed have been derived by arbitrary studies to achieve the maximum yield of platelets in platelet-rich plasma (16). About 70 per cent of the platelets are recovered from whole blood by this procedure.

3. **Platelet suspension.** Irreversible platelet agglutination has been controlled by the use of surface-active agents, especially Triton® (WR-1339) (18), which allows rapid suspension of platelets and is an important factor in decreasing the time interval of handling platelets *in vitro*.

Platelet radioactivity in the recipient. To follow the fate of the transfused Cr⁵¹ labeled platelets in the recipient, 20 ml. blood samples are drawn from the recipient at one-half, two and four hours, and daily thereafter, until no further radioactivity can be detected in the recipient's platelets. The blood is collected using Arquad®-coated needles and silicone-coated syringes, and is placed in 50 ml. silicone-coated tubes containing 2.0 ml. of 3 per cent Na₂EDTA solution in saline. Two ml. of

hyde polymer and supplied as entsufon (WR-1339) by Winthrop Laboratories, New York.

whole blood is removed to determine radioactivity of the whole blood sample. The tube is then centrifuged at 2° C. for 15 minutes at 1,000 rpm (175 G). The platelet-rich plasma is removed from the red cells with a silicone-coated pipette and transferred to a 10 ml. silicone-coated test tube which is centrifuged at 3,000 rpm (1,200 G) for 30 minutes at 2° C. The platelet-poor plasma is decanted and the platelet "button" at the bottom of the tube is resuspended in 10 ml. of 0.2 per cent Triton® saline solution and recentrifuged. The supernatant saline solution is decanted and saved to determine its radioactivity, while the platelet button remains in the test tube for determination of its radioactivity. The red cell mass and buffy coat from the initial centrifugation are eluted twice with 10 ml. aliquots of 0.2 per cent Triton® saline solution. The supernatant saline solutions from the two centrifugations are pooled and saved to determine radioactivity. Platelet counts of the whole blood, platelet-rich plasma, platelet-poor plasma, red cell mass and red cell eluates ("wash solution") are determined by phase contrast microscopy (19). Two ml. aliquots of whole blood, platelet-poor plasma, platelet "wash solution," red cell mass and red cell "wash solution" are used to determine radioactivity in these respective separations.

Daily 24 hour urine collections were obtained from four recipients for 22 days to determine excretion of Cr⁵¹ activity. These specimens are counted in a thallium-activated sodium iodide well-type scintillation counter (Tracerlab Model P-20A) with three-inch lead shielding. All samples are counted to maintain a counting error of less than 5 per cent. Values followed for more than 24 hours are corrected for physical decay.

Determination of Cr⁵¹ labeling of platelets. One ml. of the labeled platelet suspension is withdrawn from the plastic bag before infusion to determine the total radioactivity of the volume transfused. One ml. of the plasma-Triton® solution is removed before suspension of the labeled platelets in the platelet-poor plasma and is measured to determine the radioactivity remaining in plasma-Triton® solution that was not bound to platelets. The summation of the radioactivity is considered to be the total Cr⁵¹ that was available to label the platelets from the 300 microcuries injected into the bag. Inasmuch as some platelets will adhere to the plastic bag surface, the injected labeling dose of chromate (300 microcuries) is not used to determine the percentage of activity bound to the platelets.

$$\frac{\text{Radioactivity of final platelet suspension}}{\text{Radioactivity platelet-poor plasma}} + \frac{\text{Radioactivity of final platelet suspension}}{\text{Cr}^{51}\text{-labeled platelets}} \times 100 = \text{per cent}$$

Previous studies have indicated that one may anticipate by platelet counting 50 to 60 per cent initial recovery of the transfused concentrated platelet suspension in the recipient's blood volume (16). These studies involved the platelet suspension in only one rapid centrifugation

(1,000 G). The present method of labeling involves centrifuging the platelets twice at high speed. A single platelet recovery study has been done without Cr⁵¹ labeling to evaluate such a manipulation of the platelets. In this instance 46 per cent of the theoretical platelet yield was counted in a thrombocytopenic recipient. The 50 per cent loss of the transfused platelets has been attributed to viability changes during manipulation.

In vitro studies. To determine the optimal conditions for labeling platelets with radioactive chromium, numerous *in vitro* observations were performed. Whole blood was collected in 500 and 125 ml. plastic bags and centrifuged to prepare the platelet suspensions as described previously. Platelet suspensions in 10 ml. of plasma were incubated with radioactive sodium chromate at 3° C., 20 to 22° C. (room temperature), and 37° C. The suspensions were centrifuged and the residual radioactivity of the platelets was expressed as the percentage of the total radioactivity of the platelet suspension before centrifugation. Approximately 1 per cent of the Cr⁵¹ activity remained with the platelet mass at 3° C. and 37° C., while 3.6 per cent remained at 22° C.

The lower percentage at 37° C. has been attributed to elution of the radioactivity into the surrounding plasma media. Likewise, the Cr⁵¹ bound to the platelets was not increased beyond an incubation period of 15 minutes. Labeled platelet preparations used for clinical studies were incubated for 15 minutes at room temperature.

Relationship of Cr⁵¹ concentration. To determine the efficiency of binding Cr⁵¹ to platelets, a platelet-plasma suspension was divided into 12 ml. aliquots in silicone-coated test tubes. The platelet count was 323,000 cu. mm. Varying amounts of Na₂Cr⁵¹O₄ from 3 microcuries to 25 microcuries diluted to equal volumes in saline were added to the platelet suspension, and the samples were incubated 15 minutes at room temperature. The tubes were then centrifuged at 1,000 G at 3° C. and the platelet button measured for radioactivity. A linear relationship between the amount of Cr⁵¹ introduced into the platelet suspension and the radioactivity bound to the platelets was observed. Similarly, if the same experiment is repeated with the Na₂Cr⁵¹O₄ remaining constant and the platelet count varied by plasma dilution, more radioactivity is found in the platelet button with the highest platelet count. In essence, the platelets will be labeled with Cr⁵¹ in direct relationship to the number of platelets in the suspension.

Suspending medium. When platelet-rich plasma is utilized as the medium in which to label platelets, 1 to 3 per cent of the total radioactive sodium chromate is bound to the platelets. Such suspensions usually had platelet counts of 300,000 cu. mm. Platelet concentrates suspended in 10 ml. of plasma (platelet count 2 million cu. mm.) averaged 9 per cent labeling, and when such concentrates were prepared from donor patients with polycythemia vera (platelet counts 5 to 6 million cu. mm.), 15 to 25 per cent of the radioactive chromate was bound to the platelets. The average platelet concentrate suspended in a saline-Triton® solution had 15 per cent of the radioactivity bound to the platelets.

Previous studies have emphasized the selective binding of chromic chloride to plasma proteins (20). Less than 1 per cent of Cr^{51} is bound to the platelets if $\text{Cr}^{51}\text{Cl}_3$ is used in platelet-rich plasma suspensions while platelet concentrates in 10 ml. of plasma bound 1 to 2 per cent of $\text{Cr}^{51}\text{Cl}_3$. Higher residual radioactivity in the platelet concentrate is obtained by the removal of plasma and suspension of platelets in saline-Triton® solution. However, use of saline-Triton® solution requires additional centrifugation of the platelets and a prolonged time of preparation. Likewise, phase microscopy studies reveal marked morphological changes in platelets suspended in saline. Therefore, to hasten the preparation of platelets for *in vivo* studies and to prevent effects of saline contact, the smaller percentage of bound Cr^{51} activity in plasma concentrates has been preferred in these studies.

Cr^{51} elution from platelets. The addition of ascorbic acid to the $\text{Na}_2\text{Cr}^{51}\text{O}_4$ labeled platelet concentrate reduces the chromium to trivalent form. *In vitro* incubation of the ascorbic acid-treated labeled platelets with red cells demonstrated no transfer of Cr^{51} to the red cells, and similar platelet suspensions do not label buffy coat preparations. One hour incubation at room temperature of the Cr^{51} labeled platelets in 40 ml. of platelet-poor plasma results in elution of 40 to 70 per cent of the

radioactivity into the plasma, and ascorbic acid does not alter this rate of elution. Cr^{51} activity from the $\text{Na}_2\text{Cr}^{51}\text{O}_4$ labeled platelets is eluted to a similar degree in saline-Triton® solution, but platelets labeled with $\text{Cr}^{51}\text{Cl}_3$ have less elution (20 to 25 per cent) when incubated in plasma or saline. This rapid elution of Cr^{51} from the platelets has not been observed after infusion and may reflect improvement *in vivo* of platelet viability and metabolic function.

The release of Cr^{51} from the platelet membrane should not be confused with the binding of chromium to the plastic material. When $\text{Cr}^{51}\text{Cl}_3$ saline solution was introduced into a plastic bag with no platelets, 12 per cent of the injected radioactivity remained on the plastic surface even after repeated washing. Depending upon the length of the inlet tubing used to introduce the $\text{Cr}^{51}\text{Cl}_3$ into the bag, as much as 21 per cent of the injected dose may bind to the tubing and not be eluted despite repeated washing. In contrast $\text{Na}_2\text{Cr}^{51}\text{O}_4$ is associated with negligible adherence of radioactivity to plastic; namely, 0.1 per cent in the plastic bags and 0.04 per cent in the plastic inlet tubing. Similar observations have been noted in other applications of chromium salts (21).

It may be concluded from these *in vitro* observations that chromium⁵¹ chloride and sodium chromate⁵¹ can be used to label platelets. Sodium chromate offers greater ease in the preparation of the labeled platelets and less difficulty in the evaluation and measurement of radioactivity.

RESULTS

In vivo observations

Radioactive sodium chromate labeled platelets were transfused to 30 recipients with normal platelet counts. Heterologous or autologous labeled platelets were used with no untoward reactions such as pain, fever, urticaria or phlebitis. Inasmuch as each volunteer recipient received only one labeled platelet transfusion, there was no concern that platelet sensitization would develop to alter the survival period (2). In these studies all radioactive components of the recipient's venous blood samples were followed to evaluate localization of radioactivity. The term "wash water" was used to denote the saline washings of red cells and platelets, and the radioactivity of these components is graphed in Figure 1. The peak of the Cr^{51} activity of the platelet "button" at 24 hours after infusion is about 30 to 50 per cent of the whole blood radioactivity. The separated platelet button in this study indicates that the labeled platelets had a life span of 10 to 11 days. A parallel decline in whole blood activity is associated with the loss of platelet activity. The radioactivity of the separated platelet-poor plasma represents: 1) un-

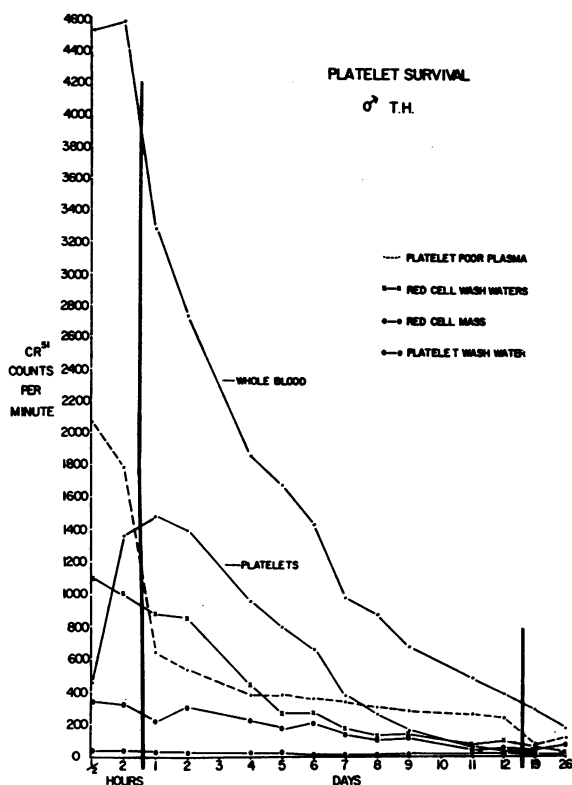


FIG. 1. MEASUREMENTS OF Cr^{51} ACTIVITY IN $\text{Na}_2\text{Cr}^{51}\text{O}_4$ LABELED PLATELETS AND SEPARATED BLOOD COMPONENTS AFTER INFUSION IN A NORMAL VOLUNTEER RECIPIENT.

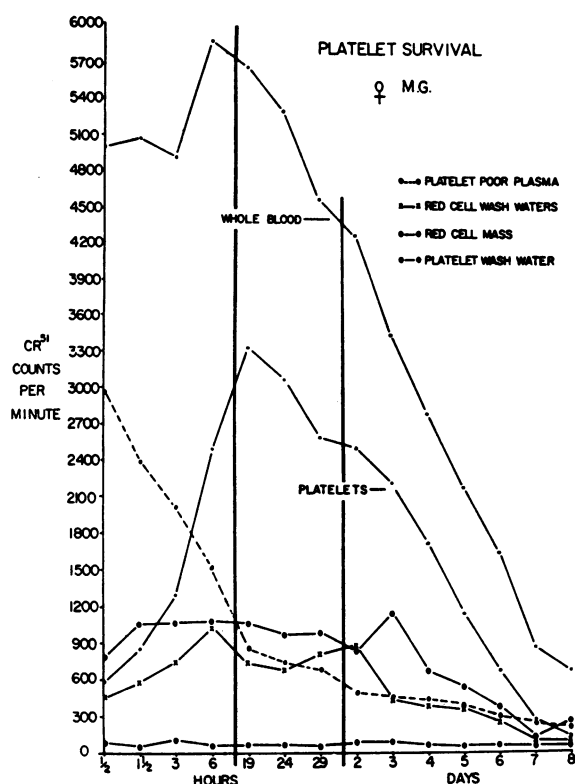


FIG. 2. $\text{Na}_2\text{Cr}^{51}\text{O}_4$ LABELED PLATELETS IN A NORMAL VOLUNTEER RECIPIENT

The maximum Cr^{51} activity was observed at 19 hours after infusion.

bound Cr^{51} in the plasma of the platelet concentrate that was not removed completely before the labeled platelets were resuspended in sterile platelet-poor plasma for infusion; and 2) Cr^{51} from nonviable platelets that constitute the 50 per cent of platelets not accounted for in the recipient after infusion. The radioactivity of the platelet-poor plasma declines rapidly during the first 24 hours after infusion, although some Cr^{51} may be found in the plasma for a month indicating minimal labeling of plasma proteins by $\text{Na}_2\text{Cr}^{51}\text{O}_4$. The red cells are not tagged in this procedure aside from the few that remain in the platelet-rich plasma during preparation. The radioactivity in the red cell mass during the first 24 hours after the transfusion is derived from entrapped plasma. Thereafter the small residual radioactivity in the centrifuged red cells is due to platelets. The radioactivity in the saline washings (wash water) of the platelet button is negligible, about 1 per cent of the whole blood radioactivity.

The maximum radioactivity of the separated labeled platelets is always noted about 24 hours after the infusion. The platelet radioactivity was followed at frequent time intervals in one recipient to determine the maximum radioactivity which in this instance occurred at 19 hours (Figure 2). Probably the time intervals vary among recipients depending upon when the maximum numbers of labeled platelets reappear in the circulation. The maximum platelet activity observed 24 hours after infusion has been used to compare the per cent of labeled platelets appearing in the circulation during the first few hours. This progressive increase in Cr^{51} activity of the platelet "button" has been graphed in Figure 3. The marked variability of platelet activity half an hour after transfusion is startling. The delayed appearance of circulating platelets after transfusion of platelet concentrates into thrombocytopenic recipients has been noted previously (15, 16), (Figure 9).

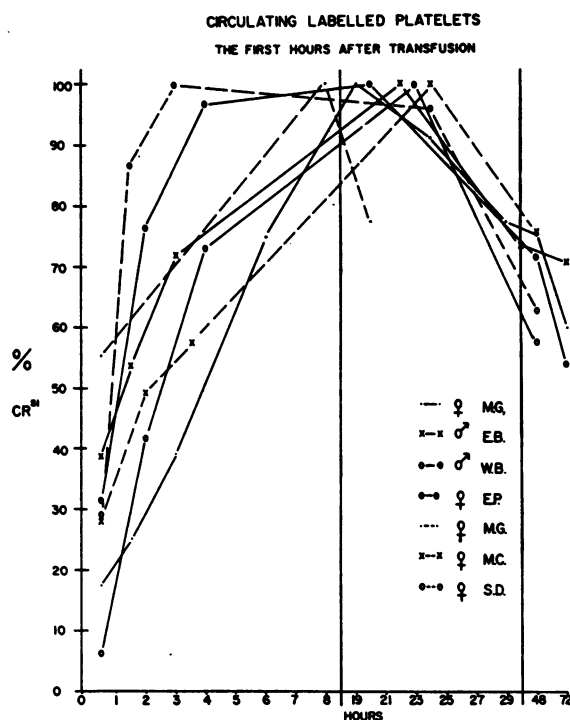


FIG. 3. PLATELET RADIOACTIVITY WITH TIME

The observed maximum Cr^{51} activity of the separated platelets is plotted as the 100 per cent value. The percentage of Cr^{51} during the initial period after infusion is related to the maximum value which is observed about 20 hours later.

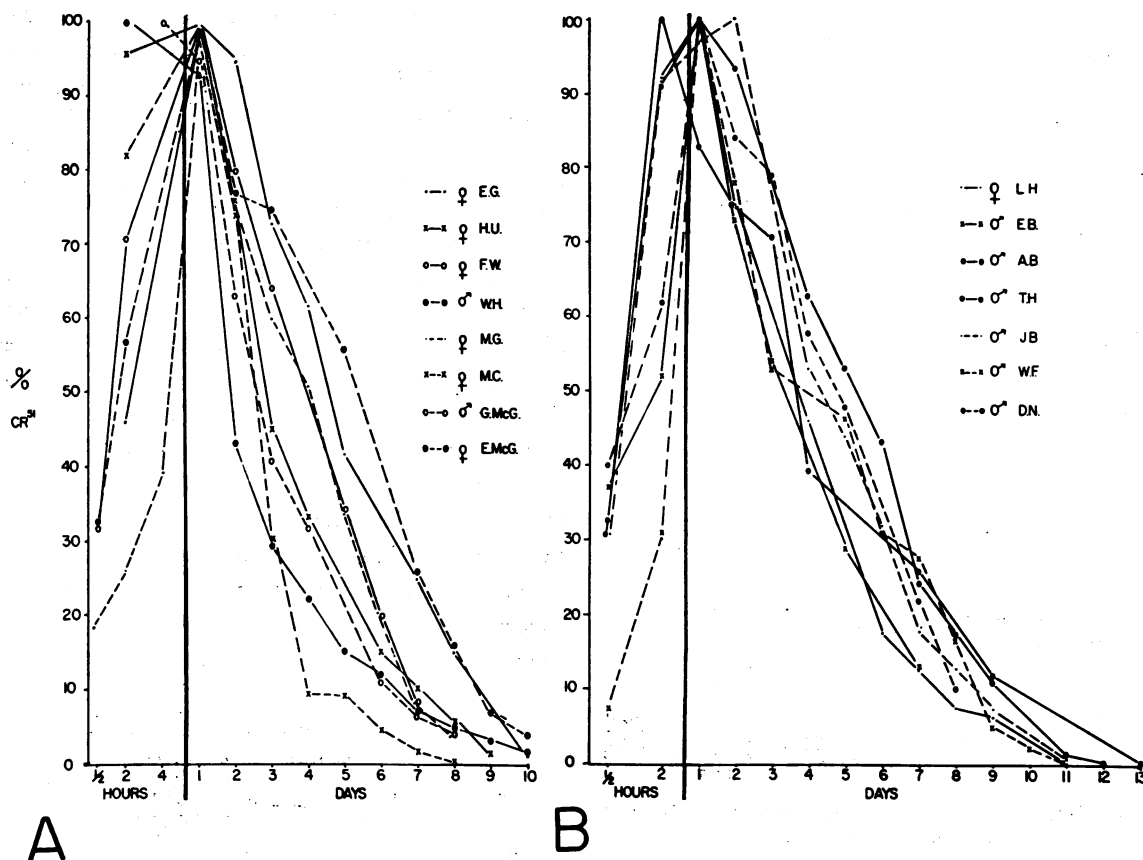


FIG. 4. PLATELET SURVIVAL IN NORMALS

A. The initial studies are shown in normal volunteer recipients by the technique described. The measurement of the Cr^{51} labeled platelets suggests an exponential curve of the platelet survival *in vivo*. B. The last seven studies performed with technical improvement indicate a linear loss of the labeled platelets from the circulation. Platelet radioactivity is plotted as in Figure 3.

In all the platelet survival studies done with the described procedure, the radioactivity of the labeled platelets has been followed for 9 to 11 days. The Cr^{51} activity of the platelet button observed during this period suggests a linear loss of the labeled platelets, indicating that platelets have disappeared from the circulation by senescence rather than random destruction. The plotted values have a more linear relationship during the first eight days, while alterations in platelet viability during the labeling procedure probably enhanced the exponential appearance of the plotted curves during the last three days that radioactivity was measured. The influence of the manipulations of the initial platelet concentrate may be appreciated by comparison of the survival curves in Figure 4. The eight platelet curves to the left

represent values obtained during the early phase of the investigation, while the seven curves to the right represent the last seven recipients studied. The more linear loss of Cr^{51} platelet activity in the later studies reflects improved handling of the platelets during the labeling procedure.

Modifications of transfusion procedure

Previous studies have emphasized the value of ascorbic acid to prevent further binding of sodium chromate to the red cell membrane. One study was performed to determine whether ascorbic acid could be omitted from the technique (Figure 5). While the platelet button radioactivity could be measured, the Cr^{51} activity of the whole blood was 10 times as high as anticipated due to the labeling of the red cells *per se*. In certain patho-

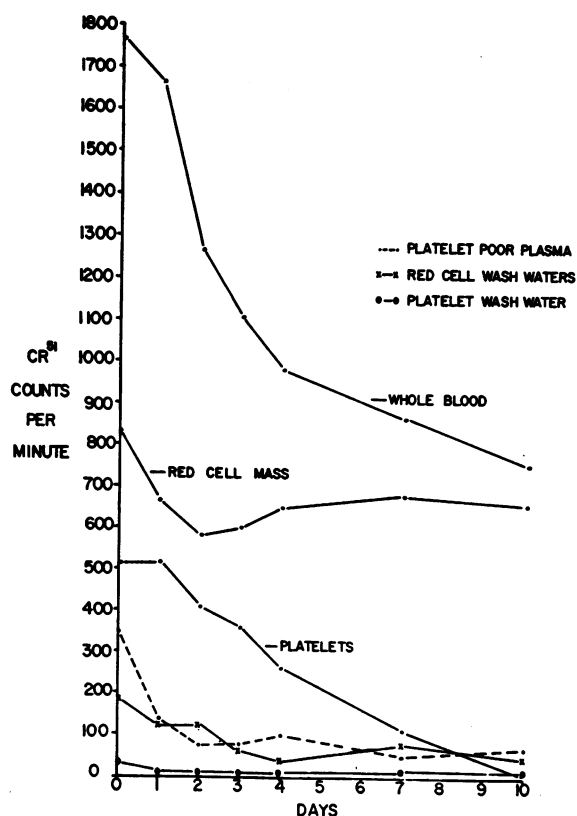


FIG. 5. PLATELET SURVIVAL WITHOUT ASCORBIC ACID—FEMALE, M. W.

The omission of ascorbic acid from the platelet concentrate before infusion does not influence the interpretation of the labeled platelet survival period. The labeling of red cells by the hexavalent chromium prolongs the period of circulating radioactivity and limits repeated observations in the recipient.

logical states the Cr^{51} bound to red cells would be cumulative if repeated observations were made to evaluate therapeutic responses in thrombocytopenic recipients.

In the present procedure the platelets are centrifuged after the initial labeling with $\text{Na}_2\text{Cr}^{51}\text{O}_4$ to remove residual Cr^{51} in the surrounding plasma. About 80 to 90 per cent of the Cr^{51} mixed with the platelet concentrate remains in the plasma. Hence it is desirable to remove the 240 to 270 microcuries of Cr^{51} from the suspension. However, this additional centrifugation may lessen platelet viability. Therefore, one study was done to determine whether omission of the last centrifugation in the preparation of the platelets for infusion would alter the observed survival period.

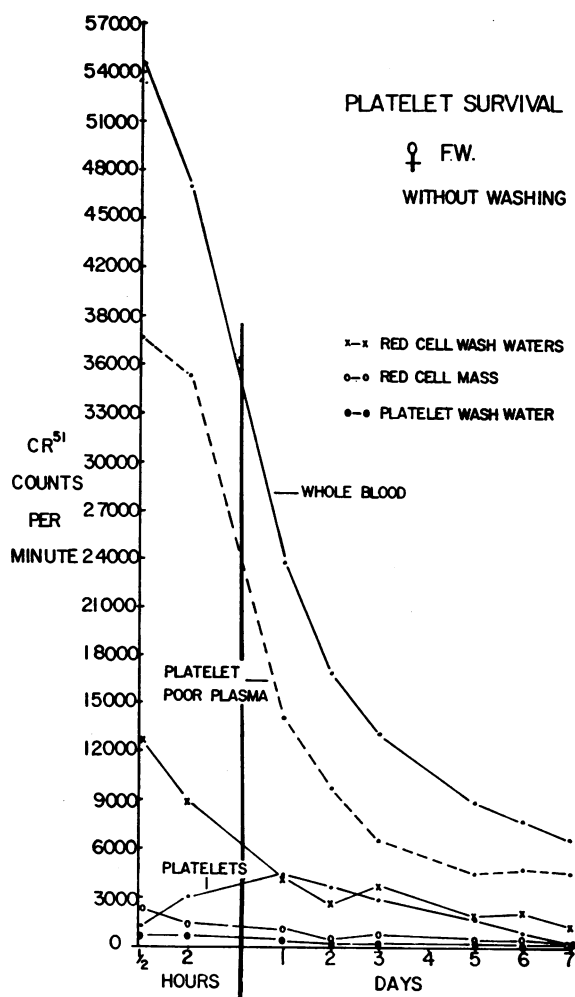


FIG. 6. INFUSION OF Cr^{51} LABELED PLATELETS WITHOUT REMOVAL OF EXCESS $\text{Na}_2\text{Cr}^{51}\text{O}_4$ IN PLASMA BY CENTRIFUGATION

There is no improvement or alteration of platelet values by elimination of this additional centrifugation. Measurements were discontinued after seven days, although adequate Cr^{51} activity was present in the platelet button for a longer period of observation.

In Figure 6 the survival period indicates no prolongation of the Cr^{51} platelet activity, and furthermore the higher dosage of Cr^{51} would limit the evaluation of platelets to one study and would not allow repeated observations in thrombocytopenic recipients.

The *in vitro* studies with $\text{Cr}^{51}\text{Cl}_3$ indicate that platelet concentrates could be labeled although the larger portion of radioactivity resides in the plasma of the final platelet concentrate. One

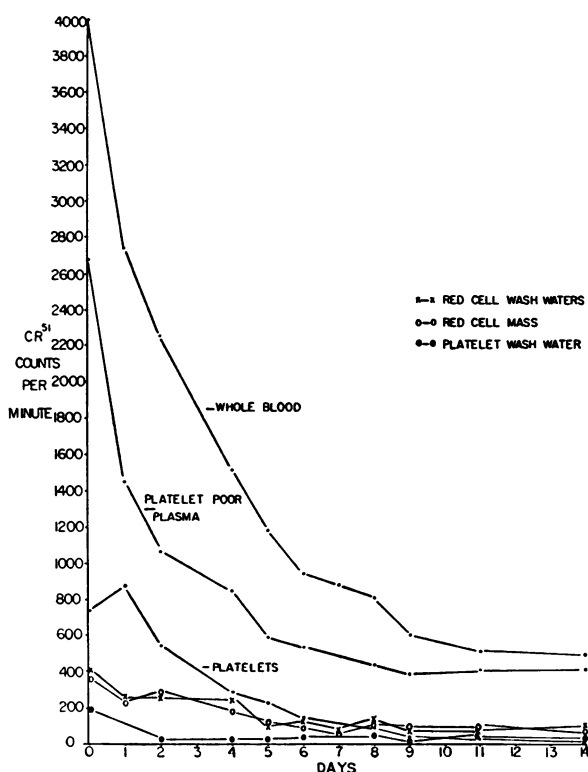


FIG. 7. PLATELET SURVIVAL OF $\text{Cr}^{51}\text{Cl}_3$ —FEMALE, B. G.

$\text{Cr}^{51}\text{Cl}_3$ labeled platelet concentrates may be followed for nine days in this study. This chromium salt offers no advantage over $\text{Na}_2\text{Cr}^{51}\text{O}_4$ and prolongs circulating radioactivity by the associated binding of plasma proteins.

recipient received $\text{Cr}^{51}\text{Cl}_3$ labeled platelets, and the results are plotted in Figure 7, showing that the radioactivity of the Cr^{51} platelet button could be followed for nine days. However, the high residual plasma activity emphasizes the value of $\text{Na}_2\text{Cr}^{51}\text{O}_4$ for *in vitro* labeling.

The selective separation of platelets by centrifugation was demonstrated in one recipient who inadvertently received a test dose of KI^{131} for evaluation of thyroid function. Despite the marked increment of radioactivity in the whole blood on the sixth day of study, a linear survival curve of Cr^{51} platelet activity could be followed for 10 days (Figure 8).

Specificity of platelet labeling

Observation of the plotted curves of Cr^{51} activity in the recipients indicates that there is no binding of the recipient's platelets. Measurable

radioactivity of the platelet button disappears between 9 to 11 days, and no residual activity will be found in the separated platelets thereafter. Platelet-poor plasma, separated red cells, and the white cell buffy coat suspended in platelet-poor plasma, were labeled with 30 microcuries of $\text{Na}_2\text{Cr}^{51}\text{O}_4$, and after incubation ascorbic acid was added to each preparation. These suspensions were then transfused into normal recipients. Blood samples from the recipients showed no radioactivity in the separated platelet buttons.

Urinary excretion of Cr^{51}

The Cr^{51} urinary excretion was measured during the studies of $\text{Na}_2\text{Cr}^{51}\text{O}_4$ labeled platelets in four normal recipients (Table II). The urinary radioactivity is expressed as the percentage of total counts of Cr^{51} in the infused platelet concentrate. A large portion of the Cr^{51} activity infused into the recipient is excreted during the first 24 hours after the platelet transfusion. The variability in the initial 24 hour excretion reflects the efficiency of platelet labeling and is a measure of the residual Cr^{51} in the surrounding plasma that was not removed completely after labeling. The excretion was followed for 22 days in each instance, and the percentage of Cr^{51} left within the body ranged from 19 to 35 per cent of the injected dose. In Table III the absolute values of Cr^{51} derived from the 300 microcurie labeling dose are tabulated on the basis of the per cent of platelets labeled. After 22 days, 6 to 18 microcuries of Cr^{51} activity remained. Assuming a continued daily excretion of 0.5 per cent of the original Cr^{51} activity, the infused Cr^{51} will be excreted in 80 to 90 days.

TABLE II

Radioactivity excreted in the urine and remaining in the body expressed as per cent of the radioactivity infused in the platelet concentrate

Recipient	Excreted first 24 hours	Excreted first 22 days	Remaining after 22 days
	%	%	%
W. F.	16.8	38.6	34.5
T. H.	45.0	65.0	19.7
D. N.	37.6	61.0	22.0
J. B.	24.0	48.0	29.0

DISCUSSION

The life span of Cr^{51} labeled human blood platelets is 9 to 11 days according to these studies. The manipulation of blood in plastic bag containers has continued to offer the most satisfactory method for preparing sterile platelet concentrates. The elimination of air phase exposure in the separation procedures assures rapid separation of plasma components after centrifugation, and with practice the labeled platelet concentrates may be prepared in less than two hours.

Recent studies by Campbell, Small and Dame-shek (22) have emphasized alterations of the metabolic function of human platelets during storage. Platelets labeled with P^{32} demonstrated leakage of phosphorus into plasma during storage; a lesion similar to changes noted in the red cell during preservation studies. These metabolic changes emphasize the difficulties associated with *in vitro* studies, for the platelet function begins to deteriorate to some extent when the blood sample is obtained. The rapid elution of Cr^{51} activity from the labeled platelets *in vitro* must be evaluated in relation to the time involved in making the observation. Certainly, after the infusion of labeled platelets into the recipient, elution cannot be observed by the present methods.

Throughout the present technique the platelets have been maintained in a plasma medium, since platelets washed in physiologic saline show morphological alterations. Oxidase activity of platelets and the ability to promote clot retraction diminish rapidly if platelets are suspended in saline (22). During the present investigation one transfusion was done with Cr^{51} labeled platelets suspended in physiological saline instead of

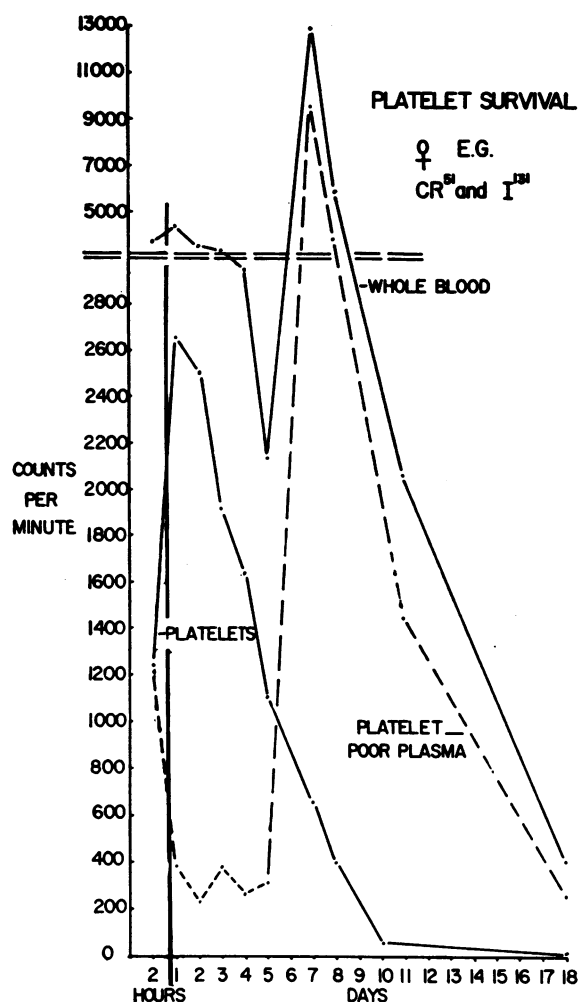


FIG. 8. FAILURE OF KI^{131} TO AFFECT PLATELET SURVIVAL CURVE

On the sixth day of this study, the recipient received KI^{131} orally to evaluate thyroid function. The separated platelet button radioactivity was not influenced by the circulating I^{131} . Although the measurements are plotted as Cr^{51} activity, the whole blood and plasma reflect the presence of Cr^{51} and I^{131} .

plasma. Although a normal life span of platelets could be followed in the recipient's circulation, the low radioactivity of the platelet button (15 per cent of the whole blood activity) suggested that the majority of platelets had suffered irreversible damage in the saline solution.

The low Cr^{51} activity of the separated platelets in the recipient during the first few hours after infusion has not been explained completely. A review of the platelet counts of thrombocytopenic

TABLE III
The radioactivity infused in the platelet concentrate and left in the body

Recipient	Radioactivity of infused platelet concentrate expressed as per cent of the initial tagging dose (300 $\mu\text{c.}$)	Radio-activity infused	Radio-activity remaining in body 22 days after infusion
	%	$\mu\text{c.}$	$\mu\text{c.}$
W. F.	16	48	17
T. H.	22	66	13
D. N.	9	27	6
J. B.	21	63	18

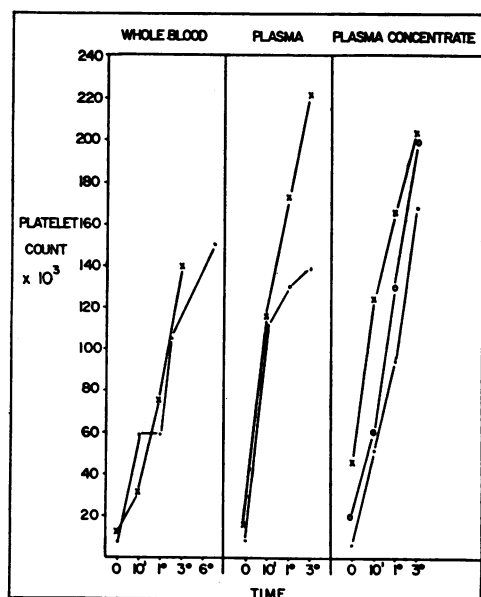


FIG. 9. TABULATION OF PLATELET COUNTS IN THROMBOCYTOPENIC RECIPIENTS AFTER RECEIVING DONOR PLATELETS COLLECTED IN PLASTIC BAG EQUIPMENT WITH Na_2EDTA AS THE ANTICOAGULANT

The platelet counts after infusion are listed for 10 minutes, 1, 3 and 6 hours. The infusion of whole blood, platelet-rich plasma, and platelet concentrates is associated with a delayed mixing for several hours.

recipients after transfusions from polycythemic donors with thrombocytosis has demonstrated a similar phenomenon. The gradual rise in circulating platelets in the peripheral blood has been plotted in Figure 9. In each instance Na_2EDTA was used as an anticoagulant. Despite the rapid collection of whole blood in plastic bags and reinfusion without manipulation, a delayed appearance of circulating platelets was noted. These changes were not noted if whole blood was collected and infused in small aliquots with silicone-coated syringes. The mere collection of platelets in an anticoagulant appears to alter the membrane. In addition, centrifugation of the platelets for concentration and labeling may further effect platelet viability. In thrombocytopenic recipients only about 50 per cent of the theoretical platelet yield has been recovered. In the present study there has been no opportunity to compare directly the Cr^{51} platelet activity with platelet yield, for such a study must be done in a recipient with amegakaryocytic thrombocytopenia.

Preliminary investigations by body surface-counting with a scintillation probe have suggested transient increased radioactivity over the lungs and liver areas. Possibly alterations in the platelet membrane during the labeling procedure produce a sticky surface with transient sequestration in the reticulo-endothelial tissues. With nutritional and environmental equilibration, the platelet regains normal function and is released into the circulation. Separate populations of labeled platelets do not appear as may be noted in the curves of peripheral Cr^{51} platelet activity. The "peaking" of platelet activity during the first 24 hours has been observed in all of the transfusion studies. Preliminary observations suggest that this 24 hour peak disappears in the presence of thrombolytic plasma factors as noted in idiopathic thrombocytopenic purpura and in platelet immunization from multiple transfusion (2).

A review of the ABO blood groups in these studies indicates that incompatible transfusions also had 9 to 11 day survival periods. Although the blood platelets appear to have grouping parallel to the red cell ABO classification (23), individual platelet transfusion studies are not affected by the incompatibilities. There is inadequate evidence to demonstrate that shortened platelet survival periods are related to ABO blood group sensitization.

The measurement of labeled platelet life span in the early transfusions of this study suggested an exponential curve when the values were plotted. Random destruction was assumed to exist following metabolic alteration of the donor platelets during the labeling procedure. With improved proficiency in the preparation of the platelet concentrates, the labeled platelet activity appears to be linear, to suggest that the platelets have disappeared by senescence. This linear loss of platelet radioactivity during the first nine days is helpful evidence to exclude any pathological effect of metallic chromium. During the last 48 hours that the labeled platelets may be followed in the peripheral circulation, the decline in Cr^{51} activity levels off. There is no adequate explanation for this observation, although this phenomenon may suggest changes in the binding of the Cr^{51} to the platelet membrane and consequent elution. The full analysis of these changes may be evaluated by simultaneous study of platelets labeled with

$\text{Na}_2\text{Cr}^{51}\text{O}_4$ and DFP³². In such instances the DFP³² incorporation *in vivo* would be the life span platelet reference, much as the Ashby differential agglutination survival period has been used to compare $\text{Na}_2\text{Cr}^{51}\text{O}_4$ life span of red cells.

The present procedure with $\text{Na}_2\text{Cr}^{51}\text{O}_4$ offers a reproducible method as observed in 30 normal adult subjects. The ease of preparing labeled platelets will allow more complete interpretation of thrombocytopenic states. At the present time the method offers no therapeutic advantage over direct transfusion of platelet-rich whole blood or plasma concentrates. However, labeled platelets may be prepared with ease from the average donor without seeking polycythemic donors with thrombocytosis. The life span of platelets measured with DFP³² has the most reasonable physiologic application for the normal recipient, but large volumes of venous blood are required to separate an adequate number of platelets. The amount of P³² activity in the labeled platelets of thrombocytopenic subjects would not allow reproducible determinations.

SUMMARY

A standardized technique has been developed to evaluate the life span of transfused blood platelets in normal recipients. *In vitro* measurements indicate that radioactive sodium chromate will bind to human blood platelets suspended in plasma. The factors related to time, temperature and concentration of sodium chromate for labeling platelets *in vitro* have been presented. $\text{Na}_2\text{Cr}^{51}\text{O}_4$ labeled platelets have been infused into 30 normal recipients. Determination of the radioactivity in the recipient's platelets separated by differential centrifugation indicates that the transfused platelets have a survival period of 9 to 11 days. No relabeling of the recipient's platelets or other blood elements was observed. The platelet radioactivity in the recipient declines for the most part in a linear fashion to suggest that this method does measure the life span of the transfused platelet.

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