Comparative Studies of Platelet Survival by Different Methods in the Rabbit

By Shirley Ebbe, Mario Baldini and Janet Donovan

The ability of platelets to survive in the circulation appears to be their most labile property. Platelets may function normally in blood coagulation in the presence of damage severe enough to prevent their circulation when transfused to compatible recipients. After storage for 2 weeks, platelets still exhibit clot retraction, one of the most sensitive in vitro tests of platelet damage, but they would be destroyed immediately if transfused after this period of storage. Antiplatelet antibodies may destroy platelets when present in amounts too small to be detected by in vitro tests. Since transfused platelets must survive in the circulation to be effective hemostatically, measurement of their survival is the most direct method for studying their usefulness.

The present studies were designed to evaluate several methods for measuring platelet survival in rabbits and the applicability of such measurements to human platelets. Platelet survival has been measured in rabbits with Cr\textsuperscript{51}-labeled platelets\textsuperscript{7-10} and with P\textsuperscript{32}-labeled platelets transfused as whole blood\textsuperscript{11}. These studies have generally indicated a survival time of 3 or 4 days for rabbit platelets, with extremes of 2 and 6 days. Four methods for determining survival of homologous platelets were compared: (1) transfusion of non-labeled platelets to thrombocytopenic recipients, (2) transfusion of concentrates of platelets labeled in vitro with Cr\textsuperscript{51}-sodium chromate, (3) transfusion of concentrates of platelets labeled in vivo with P\textsuperscript{32}-orthophosphate and (4) transfusion of whole blood labeled in vivo with P\textsuperscript{32}-orthophosphate.

Materials and Methods

All experiments were performed in New Zealand white rabbits, either male or female, weighing from 2.1 to 4.5 Kg. Glassware and needles were siliconized. Blood and platelet suspensions were centrifuged in a refrigerated centrifuge at 4 C. Platelets were counted in duplicate by the method of Brecher and Cronkite\textsuperscript{12} using 1 per cent ammonium oxalate as diluent for whole blood and EDTA\textsuperscript{§} for platelet-rich plasma (FRP). The platelet counts ranged from 282,500 to 1,067,500 mm\textsuperscript{3} with an average of...

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|Siliclad, Clay-Adams, Inc., New York, N. Y.


§The abbreviation "EDTA" throughout the paper refers to 1.5 per cent disodium ethylenediaminetetraacetate in 0.7 per cent sodium chloride.

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STUDIES OF PLATELET SURVIVAL IN THE RABBIT

562,163/mm.\(^3\) in 100 normal rabbits. Platelet concentrates were prepared by a modification of the method of Dillard, Brecher and Cronkite:\(^{13}\) 24–29 ml. of cardiac blood were aspirated into a syringe containing 6 ml. of EDTA. Multiple donors were bled and the blood pooled in a plastic bag\(^*\) cooled in ice and water. The bag was centrifuged at 1000 rpm (225 g.) for 25 minutes. The supernatant PRP, which contained 61–95 per cent of the platelets, was transferred to another plastic bag, to which 2 per cent Triton\(^\dagger\) in saline had been added in the amount of 1 ml. per 100 ml. of donor blood, and centrifuged at 2500–3000 rpm (1500–2000 g.) for 30 minutes. The platelet button thus obtained was resuspended by massage of the bag in small volumes of plasma and saline. These platelet concentrates were transfused as fresh preparations unless otherwise specified.

Survival of Platelets Transfused in Thrombocytopenic Rabbits

Rabbits were made thrombocytopenic by 2 weekly intravenous injections of \(^{32}\)P-sodium phosphates 1 mc./Kg. body weight in each injection. Three days after the 2nd injection, they were thrombocytopenic. The average platelet count in 23 animals was 46,800/mm.\(^3\). Each recipient received intravenously an average of 46.2 x 10\(^9\) platelets. Platelet counts were done on the injected sample and on blood taken from cut ear veins of the recipients before and at intervals after injection.

Transfusion of \(^{51}\)Cr-Labeled Platelets in Normal Rabbits

Platelets were labeled with \(^{51}\)Cr as previously described,\(^{14}\) and resuspended in non-radioactive platelet-poor plasma (PPP) and an equal volume of 0.02 per cent Triton in saline. This final platelet concentrate contained 48–78 per cent of the platelets from the blood. Immediately after preparation, the platelet suspension was injected into a marginal ear vein of the recipient rabbit. The platelet counts of the recipients had been determined before injection from blood obtained by slashing an ear vein. Each recipient received 3.5–10 ml. of platelet suspension containing 10–34 x 10\(^9\) platelets (average: 19 x 10\(^9\)). Infused platelet-bound radioactivity was determined from twice-washed aliquots of injected platelets. Approximately 2.5 hours elapsed from the beginning of blood collection to the time of injection of labeled platelets.

Samples of cardiac blood were taken from the recipient animals 30 minutes, 1, 4, 22 and 28 hours after injection and daily thereafter. To each 5 ml. of blood, 1 ml. of EDTA and 2.5 ml. saline were added. The tubes were centrifuged at 1000 rpm (250 g.) for 20 minutes. The supernatant PRP, containing 23–100 per cent of the platelets from the blood sample, was measured and its platelets were counted. The PRP was then centrifuged for 30 minutes at 3000 rpm (2000 g.). The PPP was poured off and the button drained by inversion of the tube. Radioactivity in the platelet button was determined.\(^\S\) The platelet radioactivity was expressed as counts per minute (cpm) per 10\(^9\) platelets, and a correction was made for radioactivity previously withdrawn in each sample after the first.

Transfusion of \(^{32}\)P-Labeled Platelet Concentrates

The survival of platelets labeled in vivo with \(^{32}\)P and transfused as concentrates was determined as previously described.\(^{15}\) Each recipient animal was transfused with 5.4–10 ml. containing a total of 12–32 x 10\(^9\) platelets (average: 24 x 10\(^9\)).

Transfusion of \(^{32}\)P-Labeled Whole Blood

The blood donors were injected with 100–200 \(\mu\)c. \(^{32}\)P per Kg. body weight 3 days before the experiment. The recipient animals were prepared by weighing, counting their

\(^*\)Fenwal Laboratories, Inc., Framingham, Massachusetts.
\(^\dagger\)Triton WR-1339, Rohm and Haas Co., Philadelphia, Pennsylvania.
\(^\S\)Phosphotope, E. R. Squibb and Sons, New York.
circulating platelets and prebleeding 20 ml. from a cut ear vein. Immediately after the prebleeding they were transfused with 18 ml. of radioactive donor blood which had just been collected by heart puncture into a siliconized syringe containing 2 ml. EDTA. The blood contained 5–25 x 10⁹ platelets. Injected platelet radioactivity was determined by separation and double washing of platelets from an aliquot of transfused blood. Samples from the recipients were taken at intervals and processed as previously described. Platelet-bound radioactivity was expressed as cpm/10⁹ platelets.

**Measurement of Platelet Survival**

Two parameters of platelet survival were measured, the "recovery" and the "survival time." The recovery was the value of circulating platelet specific activity expressed in per cent of that which would have been obtained if all the infused radioactive platelets had appeared in the recipient's circulation. The platelet survival curves were obtained by plotting the recovery values on a linear scale against the time of sampling. The survival time was expressed as the time, in hours after injection, at which only 10 per cent of the maximum observed platelet radioactivity was still present in the recipient's circulation.

For the survival of nonradioactive platelets infused in thrombocytopenic recipients, the recovery value was determined from the circulating platelet count expressed in per cent of the value which would have been obtained if all the infused platelets had appeared in the circulation. The platelet survival time was determined from the time at which the 10 per cent value of the maximum increase in platelet count was reached.

The value used for blood volume was 46.9 ml./Kg. body weight.

**RESULTS**

**1. Transfusion of Platelets into Thrombocytopenic Rabbits (Platelet Enumeration method)**

Eight experiments were done in which platelet concentrates were infused into thrombocytopenic recipients and their survival followed by platelet enumeration (fig. 1). In these recipients the preinjection platelet counts were between 1250 and 51,500/mm.³ After platelet infusion the platelet counts varied between 207,000 and 588,750/mm.³ The maximum recovery of transfused platelets was never observed later than 30 minutes after transfusion (the earliest time at which platelet counts were done). The observed increase in platelet count was 82–110 per cent (average: 92 per cent) of that possible; the survival time was 40–58 hours (average: 52 hours).

**2. Transfusion of Cr⁺¹¹-Labeled Platelets**

Ten platelet survival studies were done using concentrates of platelets labeled in vitro with Cr⁺¹¹ (fig. 2). The maximum recoveries varied from 44 to 83 per cent (average: 62 per cent) occurring at 30 minutes after injection in 1, at 1 hour in 7, and at 4 hours in 2. A brief period of platelet sequestration was, therefore, apparent in 9 of the 10 experiments. The survival time was from 72 to 98 hours (average: 83 hours).

**3. Transfusion of P⁺³²-Labeled Platelet Concentrates**

Thirteen platelet survival studies were done using concentrates of platelets which had been labeled in vivo with P⁺³² (fig. 3). The maximum recovery values of these platelets in the recipient's circulation were from 71 to 113 per cent (average: 86 per cent), occurring at 30 minutes in 3, at 1 hour in 4
4. Transfusion of P<sup>32</sup>-Labeled Whole Blood

Six platelet survival studies were done after the infusion of blood which had been labeled in vivo with P<sup>32</sup> (fig. 4). The maximum recoveries of transfused platelets in these recipients were variable, ranging from 72 to 154 per cent (average: 118 per cent). In 2 recipients there was no apparent temporary platelet sequestration, with the highest points of the curves occurring at 30 minutes; in the other 4, the maxima were between 1 and 4 hours. The survival time was 69 to 106 hours (average: 86 hours).
Fig. 2.—Survival of Cr$^{51}$-labeled platelets in 10 normal rabbits. The shaded area represents one standard deviation on either side of the mean.

There was marked variability in the observed maximum recoveries by this method, with over 100 per cent values being observed in 4 of the 6 experiments. The procedures and calculations for arriving at recovery values with this method were somewhat complex, and the brief period of hypovolemia in the recipient animals between prebleeding and transfusion may have resulted in alteration of circulatory dynamics and platelet distribution. These difficulties may have accounted for the variability in the results which was too great to make this a useful method of determining platelet viability in rabbits.

5. Influence of Red Cell Contamination on Calculation of Recovery Values

In 15 experiments with Cr$^{51}$-labeled platelets the recovery values were also calculated from radioactivity of aliquots of injected platelets from which red cells (1–40/1000 platelets) were completely removed by differential centrifugations. In only 2 instances was the recovery changed to 100 per cent by
Fig. 3.—Survival of P³²-labeled platelets transfused as platelet concentrates into 13 normal rabbits. The shaded area represents one standard deviation on either side of the mean.

this correction; in the other 13, it did increase somewhat, but the values remained within the range observed before correction. Four similar experiments were done with P³²-labeled platelets. In 2, the usual number of red cells was present (3 and 10/1000 platelets); in the other 2, the platelet suspensions were purposely contaminated with red cells (96 and 269/1000 platelets). The recovery in each case was found to be influenced by red cell radioactivity; when correction was made for the radioactivity bound to red cells the recovery values changed from 95 to 127 per cent, 78 to 137 per cent, 77 to 97 per cent and 53 to 106 per cent, respectively. However, the number of contaminating red cells could not be directly correlated with the difference between corrected and uncorrected recovery values, with either P³² or Cr⁵¹.
Fig. 4.—Survival of $^{32}$P-labeled platelets transfused as whole blood into 6 normal rabbits. To the right of the curves are shown the individual maximum recovery values for these platelets as compared to those for $^{32}$P-labeled platelets transfused as concentrates.

Thus, it appeared that, although red cells contaminating the infused radioactive platelet concentrates interfered with calculations of recovery values, this was not the only factor contributing to the relatively low recovery after transfusion of Cr$^{51}$-labeled platelets. On the contrary, for $^{32}$P-labeled platelet concentrates, the red cell contamination appeared to be the only factor preventing observation of 100 per cent of the injected radioactivity in the recipient’s circulating platelets.

6. Effect of the Chromium Labeling Procedure on Platelet Survival

The effect of the Cr$^{51}$-labeling procedures on platelet survival was evaluated by treating $^{32}$P-labeled platelets in a fashion identical to the Cr$^{51}$ procedure
Fig. 5.—Each curve represents the average of 4 platelet survival studies with P³²-labeled platelet concentrates. The curve labeled Cr⁵² was obtained with platelets which had been subjected to the chromium-labeling procedure with nonradioactive sodium chromate.

except that nonradioactive sodium chromate (Cr⁵²) in corresponding amounts was used. The average values obtained in 4 experiments are compared with the average values of 4 survival curves with P³² platelet concentrates prepared as usual in figure 5. Neither chromium nor the additional manipulations affected platelet survival.

7. Distribution of Radioactivity after Transfusion of Cr⁵¹-Labeled Platelets

After the injection of Cr⁵¹-labeled platelets, the amount of radioactivity was determined in the whole blood, platelets, red cells, white cells, and plasma of 2 recipient animals (fig. 6). The maximum amount of radioactivity in the circulating whole blood was observed 1 hour after injection and was 68 per cent of the total radioactivity injected. The platelet curve closely paralleled

*Leukocytes were separated from blood by the following procedure: (1) separation and elimination of platelets; (2) sedimentation of red cells and buffy coat for 1 hour in plasma-gelatin medium (3 per cent gelatin in saline); (3) centrifugation of supernatant at 250 g. for 15 minutes; (4) resuspension of button in 2 ml. 1 per cent acetic acid; (5) centrifugation of acetic acid suspension at 250 g. for 15 minutes.
Fig. 6.—The distribution of radioactivity in the recipients' whole blood, platelets, red cells, leukocytes and plasma after the transfusion of fresh Cr⁵¹-labeled platelets. Each point represents the average of 2 studies.

The whole blood curve; plasma, red cells and leukocytes contained only trace amounts of radioactivity.

To determine further if Cr⁵¹ released when the labeled platelets were destroyed could relabel other platelets in the circulation, a suspension of Cr⁵¹-labeled platelets was subjected to ultrasonic vibrations* for 25 minutes, after which only rare intact platelets could be seen under the phase-contrast microscope. After injection of 5 ml. of the suspension of disrupted platelets into each of 2 recipient animals, radioactivity of their whole blood, platelets, plasma and erythrocytes was determined. Thirty minutes after injection, only 22 per cent of the injected radioactivity was present in the circulation; all of it was in the platelet-poor plasma. The red cells and platelets did not become labeled with Cr⁵¹ and the radioactivity rapidly disappeared from the circulation. Thus, it appears that in vivo, as well as in vitro,⁹ Cr⁵¹ is released from destroyed platelets in a form incapable of attaching itself to other platelets.

*Sonic oscillator, Raytheon Manufacturing Co., Waltham, Massachusetts.
8. Comparison of Platelet Survival Values Obtained with Three Different Methods (Enumeration, Cr\textsuperscript{51}, P\textsuperscript{32})

Comparisons were made of survival values obtained with Cr\textsuperscript{51}- and P\textsuperscript{32}-labeled platelet concentrates in normal rabbits and nonlabeled platelet concentrates in thrombocytopenic rabbits from data presented in fig. 7. The values for maximum recovery obtained with the Cr\textsuperscript{51} method were lower than those obtained with the P\textsuperscript{32} or enumeration method. The average value of maximum recovery with Cr\textsuperscript{51} was 62 per cent, whereas by platelet enumeration it was 92 per cent; this difference was significant (\(p < 0.01\)). The maximum recovery values with the P\textsuperscript{32} technic were similar to those observed by the platelet enumeration method, although their average value was somewhat lower (86 per cent); this difference was not significant (\(p > 0.20\)). In 2 experiments, large numbers of Cr\textsuperscript{51}-labeled platelets were injected into rabbits which had been made thrombocytopenic by total body x-irradiation. In these 2 rabbits, 59 and 51 x 10\textsuperscript{9} Cr\textsuperscript{51}-labeled platelets were infused, respectively. Blood samples were obtained from the ear and recovery of the infused platelets was determined simultaneously from the changes in platelet counts and in total circulating platelet radioactivity; Cr\textsuperscript{51} recovery values in these 2 animals were corrected for changes in platelet count induced by platelet transfusion. The platelet suspensions contained minimal numbers of red cells (1 and 3/1000 platelets, respectively), so their influence on the calculation of the values of maximum recovery on the basis of platelet radioactivity was probably insignificant. By platelet enumeration, the values of maximum recovery were 110 per cent and 88 per cent, whereas by the Cr\textsuperscript{51} method they were, simultaneously, only 67 per cent and 57 per cent, respectively (fig. 7, A and B). These results indicated that the infused platelets were circulating in the recipient animals, but had lost approximately 30 per cent of the radioactivity that they contained before infusion.

The average values obtained in all experiments were plotted by expressing the peak values of the curves as 100 per cent (fig. 7, right portion) to compare the shapes of the survival curves. The survival curves with Cr\textsuperscript{51} and P\textsuperscript{32} were similar, whereas the enumeration technic gave a shorter platelet survival time. The fact that the P\textsuperscript{32} and Cr\textsuperscript{51} curves were identical would indicate either that no loss of the 2 radioactive labels occurred during survival of the platelets in the circulation, or that both labels were lost at the same rate. The latter seems less probable because the 2 labels are intrinsically different, P\textsuperscript{32} being metabolically incorporated into the platelets at the megakaryocytic stage,\textsuperscript{16,17} whereas Cr\textsuperscript{51} is an “adsorption” type of label.

9. Stability of the P\textsuperscript{32} Platelet Label

Since platelets have been demonstrated to exchange their phosphorus con-

\*Picker X-ray Corp., White Plain, N. Y. (250 Kvp., 15 mA., filters 0.5 mm. Cu and 1.0 mm. Al, exposure rate 50 r./55 sec., distance 50 cm., exposure time 16.5 min.) Ten days after receiving 900 r., the platelet counts were 49,000 and 31,000/mm.\textsuperscript{3}
Fig. 7—Comparison of the 3 parameters of platelet survival obtained from the transfusion of platelet concentrates. The curves on the right are the average of all studies with each method. Points A, B, and C represent the maximum recovery, survival, and survival time, respectively.
tent in vitro. Additional studies were done to investigate whether a similar process occurred to a significant extent in vivo. The course of plasma radioactivity was followed in animals after infusion of P32-platelet concentrates or P32-whole blood. Maximum plasma radioactivity was observed immediately after infusion and represented only a trace of the radioactivity in platelets. Plasma radioactivity declined rapidly during the first 24 hours as compared to the gradual fall of platelet activity.

After injection of two rabbits with P32-labeled plasma, containing about 10 times the radioactivity of platelet concentrates, a constant low level activity was seen in platelets for 4 days, representing either platelet labeling per se or adsorption. The degree of radioactivity was less than 5 per cent of the initial radioactivity seen in parallel experiments in which labeled platelets had been infused. In these studies, a considerable excess of P32 over that usually infused with platelet concentrates was injected, so it is probable that this "secondary" labeling does not importantly affect the early part of a platelet survival curve, but it may contribute to the "tail of the curve."

The transfusion of P32-labeled platelets which had been stored in saline for 24 hours at 4 C. resulted in no significant radioactivity in the recipients' platelets over a 24-hour period. Thus, the P32-labeled material from nonviable platelets did not transfer to the recipients' circulating platelets.

10. Stability of Cr1 and P32 Labels during Storage of Platelets
One of the objectives of this study was a useful animal model which would measure viability of platelets after storage. The Cr51 technic appeared to be the most applicable, so, to determine if the label was lost from platelets when they were damaged by storage, viabilities of damaged platelets by 2 methods, Cr51 labeling and platelet enumeration in thrombocytopenic recipients, were compared. Survival curves were plotted as "relative recovery" which was the observed recovery expressed as percentage of the average normal maximum recovery for the method involved; this calculation allowed comparison of 2 methods without further consideration of the differences inherent in the methods.

Practically identical survival curves were obtained with Cr51 and platelet enumeration after varying degrees of platelet damage induced by storage at 4 C. for 24 hours in various media or by the glycerol-freezing procedure (fig. 8). These results indicated that the Cr51 label was firmly attached to the platelets and was not released until the labeled platelets were destroyed (except for the early elution which was previously discussed).

When P32-labeled platelet concentrates were subjected to storage in saline or to freezing with glycerol, their viability was similar to that of nonlabeled platelets subjected to the same conditions and transfused into thrombocytopenic recipient animals. As for Cr51, the P32 label was, for practical purposes, stable under these conditions and did not leave the platelets until they were destroyed.

11. Comparative Platelet Survival Studies in Rabbits and Human Beings
To ascertain if results obtained with rabbit platelets in abnormal conditions
Fig. 8.—Comparison of survival of platelets by the enumeration and the Cr\(^{51}\) methods after varying degrees of damage to the platelets imposed by storage or glycerol-freezing. The results are plotted as relative recovery values.
STUDIES OF PLATELET SURVIVAL IN THE RABBIT

were applicable to human platelets, survival studies were made of rabbit and human Cr51-labeled platelets stored for 24 hours at 4 C. in various media. For comparative purposes the viability index was determined to compensate for differences in normal platelet survival in the 2 species. Rabbit platelets were stored as previously described. Human platelets were stored in identical media and their survival measured by the Cr51 method; the results were compared to the average normal survival curve for Cr51-labeled human platelets previously published from this laboratory.

As shown in figure 9, the viability indices in the 2 species for platelets stored for 24 hours under identical conditions were similar. Results obtained with rabbit platelets in storage studies were thus indicative of the behavior of human platelets. Studies of isoimmunity to blood platelets in rabbits and man also indicate similarities between the 2 species.

DISCUSSION

The transfusion of platelets to rabbits with amegakaryocytic thrombocytopenia was the most reliable method for measuring recovery of such platelets in the circulation. It was thereby established that virtually all the platelets in a platelet concentrate recirculated after transfusion. The survival time as measured by this method was less than that observed with isotopically labeled platelets transfused to normal recipients, and a similar difference has been seen in other species. Results with thrombocytopenic dogs suggest a platelet life span of 1–4 days whereas the survival of platelets labeled with P32
or Cr⁵¹ ²⁷ was about 7 days. In human beings with aplastic anemia enumeration of transfused platelets²⁸ suggested a survival time of 2–6 days, but longer survival times of 7–14 days have been observed with platelets labeled with DFP²⁸,²⁹,³⁰ P³²-orthophosphate,¹⁶ or Cr⁵¹.³¹,³²,³³ The average survival curves obtained by the 3 methods compared in the present study (fig. 7, right portion) indicated that values of platelet survival by the enumeration technic fell more abruptly even in the early portion of the curve, when the platelet count in the recipient animals was high. It is possible, therefore, that the major cause of a shorter survival of transfused platelets in thrombocytopenic animals was an increased platelet utilization.

Of the methods studied for quantification of platelet viability, the 2 involving transfusion of isotopically labeled platelet concentrates proved to be the most useful. No gross damage to the platelets seemed to occur in the preparation of concentrates. The survival curves obtained with platelet concentrates labeled either in vitro with Cr⁵¹ or in vivo with P³² were quite reproducible, nearly all the curves falling within a narrow band describing one standard deviation on either side of the average curve. Reutilization of these isotopic labels did not occur to any significant extent.

The usefulness of the Cr⁵¹-labeling method for human platelets has been amply demonstrated.³¹,³²,³³ This method is the only one of those studied which is adaptable for autologous platelets. It appeared, however, that approximately one-third of Cr⁵¹ which was not removed from the platelets by 2 saline washes in vitro was immediately eluted from them in the recipient’s circulation. This explained the consistently low recovery values obtained with the Cr⁵¹-labeling procedure in rabbits and may partially explain that seen with human platelets labeled with Cr⁵¹.³¹,³³

Except for the transfusion of Cr⁵¹-platelets to thrombocytopenic rabbits, recovery values for P³²- or Cr⁵¹-labeled platelets were not corrected for changes in the level of circulating platelets. The theoretical 100 per cent value was derived from division of the injected radioactivity by the number of circulating platelets before injection plus the number of injected platelets so that the latter were considered in the calculations. Some of the irregularities in individual survival curves may have been due to fluctuations in platelet count. However, in figure 6 it is apparent that platelet specific activity paralleled whole blood radioactivity suggesting that variations in platelet count do not seriously alter the survival curve. This parallelism also suggested the possibility of following only the whole blood radioactivity as a simple screening test for platelet survival.

While the P³²-labeling method gave good results in rabbits, it is not readily applicable to human beings. Although platelets have been demonstrated to exchange the phosphorous associated with glycolytic intermediates²⁰ and phospholipids¹⁸,²¹ in vitro, it would appear that in vivo the magnitude of this exchange is not such to alter significantly the platelet survival curve.

The shape of the normal platelet survival curves in rabbits was neither linear nor simple exponential, suggesting that probably the mode of platelet destruction is neither completely age-dependent nor random. In other species, platelet
destruction has been reported by some to be a linear function\textsuperscript{29-31,34} and by others to be an exponential function representing predominantly random loss.\textsuperscript{16,17,32,35-38} From our platelet survival curves which fit neither pattern, a combination of the two processes appears the most likely.

The rabbit as an experimental animal for study of platelet viability and factors which influence it has been extremely useful. In our experience, rabbit platelets behaved similarly to human platelets, when compared to their respective normal controls, with respect to their viability after storage in various media, their ability to induce isoimmunization, and their reduced survival in isoimmunized recipients. Thus, experiments in rabbits, reinforced by a few pertinent observations in humans, could supplant extensive human experiments.

**SUMMARY**

Four methods for measuring the survival of homologous platelets in rabbits were studied: (1) transfusion of nonradioactive platelet concentrates to thrombocytopenic recipients, (2) transfusion of concentrates of platelets labeled in vitro with Cr\textsuperscript{51}-sodium chromate, (3) transfusion of concentrates of platelets labeled in vivo with P\textsuperscript{32}-orthophosphate and (4) transfusion of whole blood labeled in vivo with P\textsuperscript{32}-orthophosphate. The survival time of platelets in normal rabbits was 3–4 days.

From comparison of the 3 methods using platelet concentrates, the following conclusions were drawn. (1) All the platelets in a platelet concentrate were capable of recirculating after transfusion. (2) Labeling with P\textsuperscript{32} or Cr\textsuperscript{51} did not damage platelets. (3) About one-third of the Cr\textsuperscript{51} was immediately eluted from viable platelets after they were transfused. (4) Further exchange of the label in vivo did not occur to a significant degree with either Cr\textsuperscript{51} or P\textsuperscript{32}. (5) Cr\textsuperscript{51} did not elute from platelets during storage of the platelets. (6) Studies of rabbit platelets had applicability in predicting the behavior of human platelets.

**SUMMARIO IN INTERLINGUA**

Esseva studiate quatro methodos pro mesurar le superviventia de homologe plachettas in le conilios: (1) Transfusion de non-radioactive concentratos de plachettas in recipientes thrombocytopenic, (2) transfusion de concentratos de plachettas marcate in vitro con chromato de natrium a Cr\textsuperscript{51}, (3) transfusion de concentratos de plachettas marcate in vivo con orthophosphato a P\textsuperscript{32}, e (4) transfusion de sanguine total marcate in vivo con orthophosphata a P\textsuperscript{32}. Le tempore del superviventia de plachettas in conilios normal esseva 3 a 4 dies.

A base de un comparation del tres methodos utilisante concentratos de plachettas, le sequente conclusiones esseva formulate. (1) Omne le plachettas in le concentratos de plachettas eseva capace a recircular post le transfusion. (2) Le marcation del plachettas con P\textsuperscript{32} o Cr\textsuperscript{51} non lesionava le plachettas. (3) Circa un terto del Cr\textsuperscript{51} eseva eluite immediatemente post le transfusion ab le viabile plachettas. (4) Le excambio additional que occurreva in vivo eseva non-significative in le caso de Cr\textsuperscript{51} e etiam in le caso de P\textsuperscript{32}. (5) Cr\textsuperscript{51}...
non esseva eluite ab le plachettas durante lor thesaurisation. (6) Le studios con plachettas de conilio habeva applicabilitate in le prediction del comportamento de plachettas human.

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