Survival of Washed Rabbit Platelets *in Vivo*[1] (37212)

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It is possible to prepare suspensions of washed platelets from rabbit blood which respond normally to stimuli that cause platelet aggregation and the release reaction (1). These platelets are disc-shaped and appear to retain their electron density and organelles (1). The purpose of the study reported in this paper was to determine whether these platelets survived normally *in vivo* by comparing their survival with that of platelets labeled *in vivo*.

Materials and Methods. Twice-washed rabbit platelets were prepared by the method of Ardlie *et al.* (1). Blood from 3 rabbits anesthetized with sodium pentobarbital was collected by carotid artery cannulation. The platelets were labeled in the first washing solution by incubation for 1 min with 5 μCi tritiated diisopropylfluorophosphate (^{3}H-DFP; specific activity 3.3 Ci/mmole; Amersham/Searle Corp., Des Plaines, Ill.) in 0.1 ml propylene glycol. The platelet count in the final suspension averaged 4.6 $\times$ 10^6/mm^3. The specific activities of the platelet preparations were between 3,000 and 4,000 cpm/mg washed and dried platelets. Approximately 10^{10} washed platelets in 2.0–2.5 ml of platelet suspending medium, corresponding to about 15% of the total number of platelets in the recipient rabbit were injected into a marginal ear vein of each of 4 animals. In each experiment a control group of 4 rabbits received 30 μCi ^{3}H-DFP in 0.03 ml propylene glycol/kg iv. The ^{3}H-DFP was diluted 1:20 in 0.85% saline just before administration.

A total of 16 male New Zealand rabbits weighing 3,160 $\pm$ 640 g ($\bar{x} \pm$ SD) were used. There was no statistical difference in the body weight between the experimental groups. The rabbits received a standard rabbit chow and water *ad libitum*. In two experiments 4 rabbits were injected with platelets labeled with ^{3}H-DFP *in vitro* and 4 other rabbits were injected intravenously at the same time with ^{3}H-DFP. The first blood sample was taken 8 hr after the injection of either *in vitro* labeled platelets or ^{3}H-DFP. Blood samples were drawn daily thereafter for four days.

For each determination of platelet radioactivity 6 ml of blood was drawn from an ear vein into a plastic syringe containing 1 ml acid–citrate–dextrose. Platelets were counted by the method of Brecher and Cronkite (2). Platelets were isolated for radioactivity determination by the method described previously (3, 4). Ten microliters of water was added to the dried and weighed (Cahn electrobalance, Cahn Motor Co., Paramount, Calif.) platelets in an aluminum boat followed ten minutes later by 0.2 ml of NCS solubilizer (Amersham/Searle). After 30 min incubation at room temperature 10 ml of toluene-fluor solution was added and the radioactivity determined in a Philips liquid scintillation analyzer (efficiency 25%). Each sample was counted for 10,000 counts or 100 min. The specific activities of the platelets were expressed as cpm/mg dry weight.

Results were evaluated by two methods (3). In the first method it is assumed that the disappearance of labeled platelets is exponential. A line is fitted by unweighted least squares to the plot of the logarithm of radioactivity/milligram platelet against time. The slope of the regression curve divided into -0.301, *i.e.*, $-\log_{10} 2$, is the estimated half-life (PHL_e). The mean life span (MLS) is obtained by dividing $\log_e 2$ ($=0.693$) into PHL_e. Platelet turnover is calculated by

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dividing the platelet count/mm^3 of blood by the mean life span.

In the second method it is assumed that the disappearance curve is approximately linear with time over most of the period of study. Therefore, the best straight line is fitted through the points by the method of least squares. This line cuts the baseline at the estimated mean survival time (Gaussian model). Platelet turnover is again calculated by dividing the mean survival time into the platelet count/mm^3 of blood.

The dry weight of the sum of all the platelets in the animal was calculated from the total blood volume of the rabbit, the platelet count during the period of the survival study, and the number of platelets/milligram dry weight. Experiments with ^{51}Cr-labeled red cells showed that the blood volume was 58 ml/kg. To estimate the total number of platelets, the average platelet count during the period of the study was used, after statistical analysis had shown that there was no significant change in the platelet concentration between Day 1 and Day 5 of the experiments. It was found that 1 mg dry weight of rabbit platelets contained 477×10^6 platelets. By dividing the calculated total dry weight (mg) of platelets in the rabbit into the total injected platelet-bound radioactivity, the theoretical specific activity for 100% recovery was obtained (cpm/mg). The percentage of the reinjected labeled platelets that was circulating at each time was calculated as

$$\frac{\text{cpm/mg platelet (time } t)}{\text{cpm/mg platelet (theoretical 100\%)}} \times 100$$

Platelet survival curves were extrapolated to zero time using the exponential as well as the Gaussian model and the recovery at zero time was estimated.

Platelet aggregation was measured as described previously (5). ADP (Sigma Chemical Co., St. Louis, Mo.) and crude bovine thrombin (Parke Davis & Co., Detroit, Mich.) were dissolved in Tyrode solution. Acid soluble collagen was prepared as described elsewhere (6).

Results. Washed rabbit platelets used in these experiments showed a similar sensitivity to ADP-induced aggregation and collagen-induced aggregation as platelets in citrated platelet-rich plasma (PRP) (Fig. 1). In contrast, the suspension of washed rabbit platelets was more sensitive to thrombin than platelets in citrated PRP. This is not surprising in view of the antithrombin in plasma.

Figure 2 shows the survival curves for one set of experiments comparing the survival of platelets washed and labeled *in vitro* with platelets labeled *in vivo*. The pattern of disappearance of the platelets labeled *in vivo* was similar to that of platelets that had been washed twice and reinfused into normal rabbits.

The data in Table I compare washed platelets with platelets labeled *in vivo*. No significant difference was observed in platelet survival, platelet half-life, platelet count, or platelet turnover between the two groups.

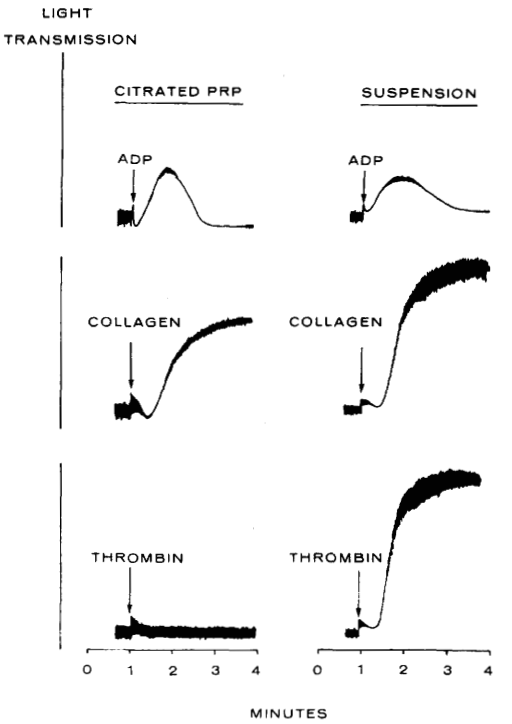

FIG. 1. Comparison of the platelet aggregation response of platelets in platelet-rich plasma and of washed platelets labeled with ^{3}H-DFP. The platelet counts were adjusted to 300,000/mm^3. The following final concentrations were used: ADP 9.1×10^{-7} *M*; thrombin 0.045 U/ml; acid soluble collagen 1:1000 (in saline).

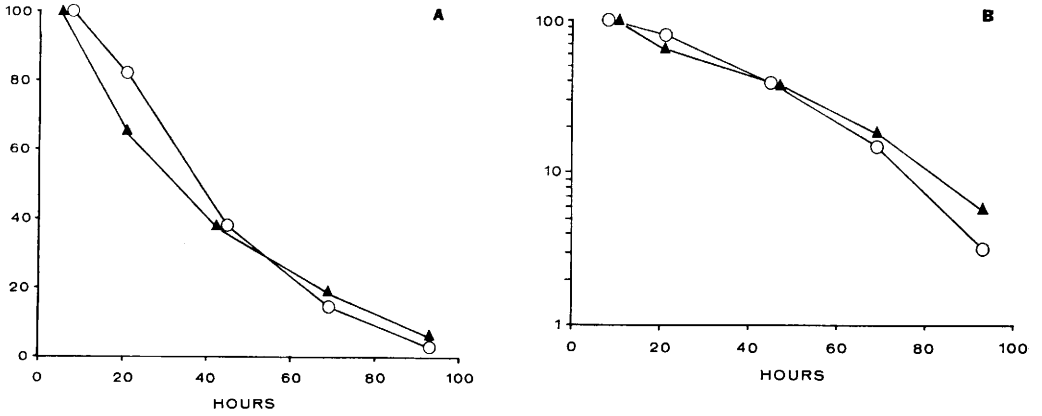

FIG. 2. (A) Survival of *in vitro* labeled platelets (○) and *in vivo* labeled platelets (▲) for one set of experiments. Each point represents the mean of four experiments. Blood sampling was commenced 8 hr after either injection of the labeled washed platelets or ^{3}H-DFP. On the ordinate the specific activity of the platelets (cpm/mg platelet dry weight) was plotted as percentage of the initial value at 8 hr. (B) The same data on a semilogarithmic plot.

Discussion. The results of these experiments show that that more than 80% of rabbit platelets washed twice, resuspended by the procedure described by Ardlie *et al.* (1) and reinfused, are found in the circulation and have the same survival time as platelets labeled *in vivo*. Thus it appears that the washing procedure does not alter the platelets sufficiently to cause their removal from the circulation (7). If this method of preparing washed platelets were selecting a young or an old platelet population, one would expect a difference in their survival compared to platelets labeled *in vivo* (8). Since such a difference was not observed, it seems likely that the washed platelet suspensions are not comprised of a selected platelet population.

These studies indicate that the method of preparing washed platelets leads to satisfactory suspensions as far as platelet sur-

TABLE I. *In Vivo* Survival of Rabbit Platelets Washed and Labeled *in Vitro* and of Platelets Labeled *in Vivo*.[a]

	In vitro ($n = 8$)	*In vivo* ($n = 8$)	p
Mean platelet survival time (Gaussian model) in hours	88.9 ± 3.8	86.5 ± 4.8	<0.30
Half-life PHL_e (exponential model) in hours	18.7 ± 3.5	18.2 ± 4.5	<0.80
Platelet count in blood no./mm^3	367,000 ± 69,000	328,000 ± 97,000	<0.40
Turnover of platelets (Gaussian model) no./mm^3/day	99,000 ± 17,600	91,000 ± 23,500	<0.50
Turnover of platelets (exponential model) no./mm^3/day	326,000 ± 55,000	307,000 ± 66,000	<0.60
Estimated recovery at zero time in percent			
— Gaussian model	82 ± 20	—	—
— exponential model	110 ± 27	—	—

[a] First blood sample 8 hr after the injection of *in vitro* labeled platelets or injection of ^{3}H-DFP. Mean values and standard deviations are given.

vival is concerned. The data do not show whether these platelets function normally *in vivo* in such processes as hemostasis.

Summary. Survival of rabbit platelets washed twice and labeled with ^{3}H-DFP *in vitro* was compared to the survival of rabbit platelets labeled *in vivo* with ^{3}H-DFP. There was no significant difference in the platelet survival between these two groups.

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