Platelet Life Span in Normal, Splenectomized and Hypersplenic Rats

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THE PLATELETS are produced in the bone marrow, circulate in the blood for a number of days, and then disappear. Their eventual fate is not known. Conceivably, the spleen might be an important site of platelet destruction, and the increased platelet level following splenectomy might be due to a decreased destruction of platelets. Likewise, the decreased level in hypersplenism might be due to an increased destruction.

One approach to this problem is to label platelets in vitro, and then study their localization in the normal animal. The spleen contains a considerable amount of the label in such experiments,[1-4] apparently supporting the concept that the spleen normally destroys a large fraction of platelets. However, this evidence may be criticized on the grounds that in vitro labeling and handling may damage the platelets and lead to abnormal tissue localization. The body may handle its own intact platelets differently.

Another approach to the problem is to study the possible influence of the spleen on the platelet life span by in vivo tagging. If the spleen is the main destructor of platelets, their life span might be prolonged after splenectomy, and shortened in hypersplenism. Following this approach, we have found the platelet life span to be the same in normal, splenectomized, and hypersplenic rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats were used. The average weight was 375 Gm. (290 to 485 Gm.) and was the same in the normal as in the abnormal groups. Splenectomy was done under ether anesthesia, and all rats recovered promptly without suppuration. Sham-splenectomy was done on control animals in a similar manner, a piece of omentum being removed instead of the spleen. Hypersplenism was produced by injections of methyl-cellulose.[5] Each rat was given 1.95 Gm. of methylcellulose over 24 weeks; 39 intraperitoneal injections of 2 ml. 2.5 per cent Methocel* (400 centipoise). No rats died, but toward the end of the experiment they lost weight and appeared chronically ill.

Labeling of platelets.—The platelets were labeled in vivo by an intramuscular injection of radioactive diisopropylfluorophosphate (DFP[32])[†6] in water-free propylene glycol. The specific activity at the time of injection was 165 to 188 μc. per mg. The injected dose was planned to be 400 μg. of DFP[32] per Kg. rat, but in retrospect the dose was estimated to be at most 130 μg. per Kg. The reason for this discrepancy is that our analyses of the DFP[32] preparations differed from those of the producer. We measured the DFP content by a

modification of the method of Marsh and Neale,[7] and correlated the results with the total phosphorus in the preparations. These two determinations agreed closely, and indicated that the preparations contained only one-third or less of the stated DFP content. The purity in terms of phosphorus was 78 per cent or better.

Counting of platelets.—The method of Brecher et al.[8] was used and each sample was counted in duplicate. The coefficient of variation as determined from 306 duplicate counts was 6.8 per cent.

Glassware.—All glassware was freshly coated with silicone.*

Collection of blood.—The rat was anesthetized with ether. As it stopped breathing, the abdomen was opened and blood collected from the vena cava. A 10 ml. syringe with a 21 gage needle coated with Monocote E† was used. The syringe contained 0.4 ml. of a 5 per cent solution of disodium versenate‡ in water, adjusted to pH 6.5 The blood was collected by slow suction for about 20 seconds; the average amount obtained was 8.1 ml. (5.4 to 11.1 ml.). With slower aspiration, more blood could be obtained, but this led to clumping of the platelets.

Isolation of platelets.—DFP is not a specific platelet label: 24 hours after the injection the platelets carried only about 0.3 per cent of the total activity in whole blood. The isolation procedure must therefore give a high yield of uncontaminated, but not necessarily viable, platelets. By differential centrifugation of undiluted rat blood only 20 to 30 per cent of the platelets were isolated. Dextran increased the yield, but also the contamination with red cells. Dilution with saline proved to be the most convenient way to facilitate the separation of platelets, and the following method was used.

The blood collected was transferred into two 15 ml. graduated tubes, containing 3 to 5 ml. each. Two to three ml. of 0.9 per cent saline were added to each tube, and the tubes were centrifuged at 800 rpm (150× g.) for 20 minutes at 20 C. The platelet-rich supernatant was collected with a capillary pipet. Gentle suction was used, and care was taken not to disturb the red cells. However, the loose upper layer of the buffy coat was collected, since it contained masses of platelets and only occasional white cells. Another 2 to 3 ml. of saline were then added to the remaining red cells, and the tubes were centrifuged at 650 rpm (100× g.) for 20 minutes at 20 C. The supernatant was collected in the same manner and added to the first supernatant. The volume of the combined supernatants was measured, and the number of platelets, red and white cells counted. The platelet-rich mixture of plasma and saline was then centrifuged at 3000 rpm. (2100× g.) for 30 minutes at 20 C. in a 40 ml. centrifuge tube, and the supernatant decanted. The supernatant was practically free of platelets. The platelet button was washed three times in 10 ml. portions of saline.

The centrifuge tubes must contain about the same amount of blood each time, otherwise the effective radius and hence the centrifugal force will vary too much. An optimal amount of saline must be used (2.5 to 3 ml. saline to 4 ml. blood), since too little saline decreased the yield, and too much increased the contamination with red cells. If low temperature is used, the centrifugal force must be increased. To insure a constant high yield, it was necessary to carry out two successive separations. In one series, for instance, the first separation gave an average yield of 63 per cent (44 to 85 per cent). The platelets from the second separation brought the yield to 74 per cent (61 to 88 per cent). Red and white cell contamination was consistently low, provided the pipetting was carefully done (table 1). The contamination with plasma was studied by measuring the radioactivity of the wash waters. The fourth wash water was always free of radioactivity, and three washings were therefore considered adequate.

Measurement of the radioactivity.—The platelets were finally resuspended in three drops of saline, hydrolyzed for 30 minutes at 100 C. in 0.3 ml. of 30 per cent sodium hydroxide, and transferred quantitatively to a counting planchet. One ml. nonradioactive

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*SC–87 Dri Film, General Electric.
†Armour Company, Chicago, Ill.
‡Abbott Laboratories, North Chicago, Ill.

TABLE 1.—*Results of 103 Consecutive Isolations of Platelets from Rat Blood*

	Average	Range
Yield of platelets %:	70	50–91
Contamination with red cells (No. of red cells per 100,000 platelets):	1.2	0–14
Contamination with white cells (No. of white cells per 100,000 platelets):	0.9	0–4

blood was added, the sample was dried at room temperature and counted in an end-window Greiger Mueller counter. A total of 4096 counts was compiled for each sample. The background radioactivity was 24 to 26 counts per minute. Twenty-four hours after the injection of DFP, the platelet samples gave 72 to 170 net counts per minute. The radioactivity was related to the total number of platelets in the combined supernatants. No correction was made for the contamination with red and white cells, since on an average there was only one red cell and one white cell per 100,000 platelets (see table 1).

RESULTS

Platelet life span in normal and splenectomized rats.—Thirty-six rats were used, one-half of which were splenectomized and the other half sham-operated. The rats were operated upon 14 days prior to the injection of DFP[32]. At intervals after the injection of DFP[32], two rats were killed in each group, and the radioactivity of the platelets was measured.

The number of platelets in whole blood was slightly, but significantly, higher ($0.025 < P < 0.05$) in the splenectomized group: 1,286,000 platelets per cu.mm. (1,032,000 to 1,590,000) as compared to 1,191,000 (1,031,000 to 1,329,000) in the normal group.

The platelet radioactivity decreased from day to day in both groups. The points fit an exponential curve better than a straight line (fig. 1). In this diagram each point corresponds to one rat. In figure 2, each point represents the average of a pair of rats, and the two lines are fitted by the method of least squares. There is no significant difference between the slopes of these two lines ($P = 0.65$), suggesting that the platelet life span is essentially the same in both groups.

Platelet life span in normal and hypersplenic rats.—Forty rats were used; one-half was made hypersplenic, and the other was untreated. At the time of sacrifice the weight was similar in both groups, but the spleens were on an average six times heavier in the hypersplenic group: 4.42 Gm. (3.15 to 6.31 Gm.) versus 0.69 Gm. (0.46 to 0.93 Gm.). The hypersplenic rats were anemic; their average hematocrit was 37.7 (33.0 to 42.0) versus 45.9 (43.8 to 50.0) in the normal group. They also had a slight, but significant ($P < 0.01$) thrombocytopenia: 780,000 platelets per cu.mm. (542,000 to 1,046,000) compared to 966,000 (721,000 to 1,144,000) in the normal group.

Both groups were injected with DFP[32] at the same time, and two rats in each group were killed at intervals after the injection. Figure 3 gives the platelet radioactivity of the two groups. The data are plotted as in figure 2, each point is the average of two rats, and the lines are fitted by the method of least squares. Again, there is no significant difference in the slope of
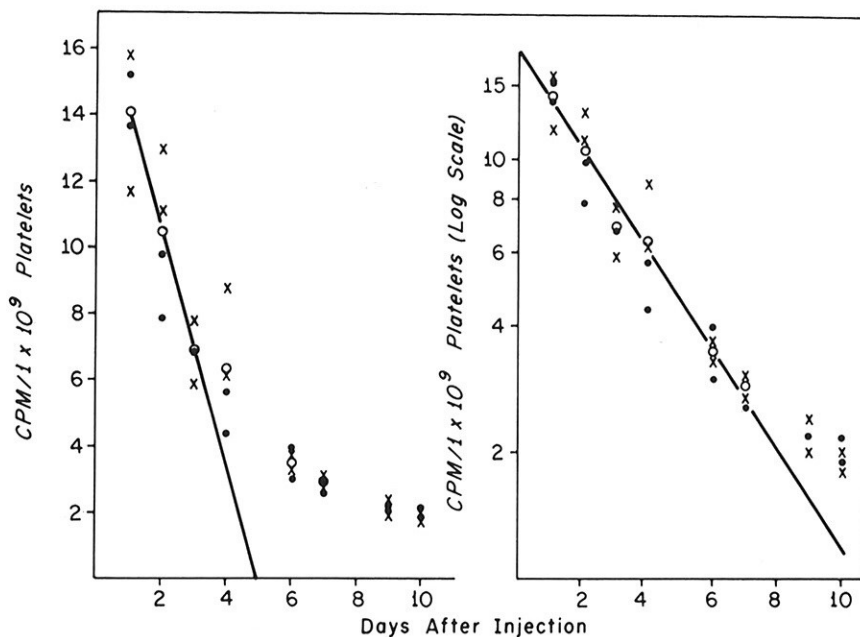
Fig. 1.—Platelet radioactivity after injection of DFP[32] in normal and splenectomized rats. The same data are plotted on regular paper (*left*) and on semilogarithmic paper (*right*). Each point represents one rat; • = normal rats, and × = splenectomized rats. The open circles give the average for the four daily determinations.

the two lines (P = 0.17), suggesting that the platelet life span is the same in both groups.

DISCUSSION

The disappearance curve for labeled platelets is most often reported as a straight line, suggesting a disappearance by senescence.[8-11] However, some workers find an exponential curve, suggesting a random destruction.[12]

Figure 1 shows that our data do not exactly fit either of these two types, since the curves level off at an activity equal to 10 to 20 per cent of the 24 hour value. Other workers[6,13] also mention such residual activity. This might be explained either by contamination, or by a prolonged period of labeling. The residual activity is not due to contamination with plasma, since plasma is virtually inactive eight days after the injection; and contamination with red and white cells was ruled out by microscopic examinations. Therefore, we believe that there is a prolonged period of labeling. This is not due to a slow release of DFP[32] from the site of injection, since we found similar curves after intraperitoneal injections. A reutilization of the label is held to be impossible,[14] and we have confirmed that diisopropyl phosphate (DIP[32]) does not label blood cells. Therefore, we believe that the prolonged labeling is due to a gradual release of labeled platelets, probably from megakaryocytes.

If this is so, only the first parts of our curves reflect the life span of the platelets initially labeled in the circulation. Figure 1 shows that the data fit a
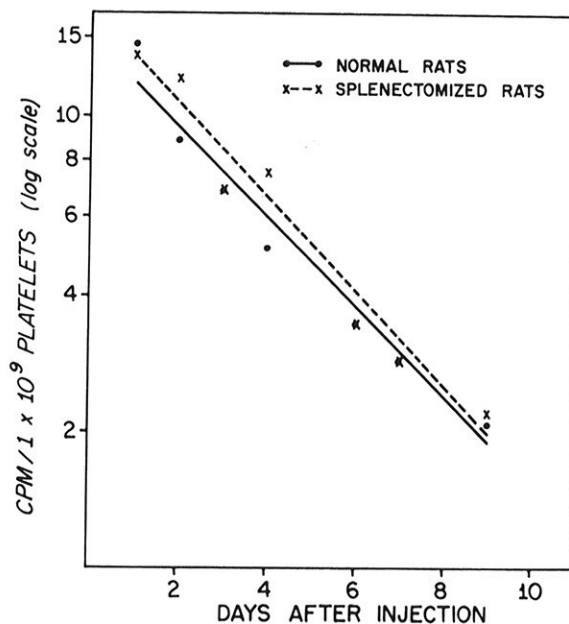
FIG. 2.—Platelet radioactivity after injection of DFP[32] into normal and splenectomized rats. Each point represents the average of two rats. The lines are fitted by the method of least squares. In the normal group, the slope of the line corresponds to a half-life of 3.0 days, with 2.4 to 4.0 days as 95 per cent confidence limits. In the splenectomized group, the half-life was 2.8 days.

straight or an exponential curve, depending on whether the observations from the first three or from the first seven days are used. Obviously, our data do not allow conclusions regarding the type of the life span curve, but we have drawn our curves on semilogarithmic paper to facilitate comparison of the two groups in each experiment.

The platelet activity is higher in figure 3 than in figure 2. The dose was 130 μg. of DFP[32] per Kg. rat in figure 3. It was probably considerably lower in figure 2, since this experiment was done before we had started to control the DFP[32] preparations with our own assay. Subsequent experiments with different doses of DFP[32] have confirmed that higher doses result in increased platelet activity and curves which level off at a higher activity.

Our experiments show no difference in the platelet life span in normal and splenectomized rats, indicating that destruction of platelets proceeds at a normal rate in other areas than the spleen following removal of that organ, and that the spleen therefore must exert its effects on the production of platelets rather than on their destruction. Consistent with our observation are the previous reports of Leeksma and Cohen[6] and of Gardner et al.,[9] while Reisner et al.[2] found an average life span of nine days in splenectomized patients versus nearly seven days in normals.

In hypersplenic thrombocytopenia both a normal[9] and a short[6] platelet life span has been reported. Our hypersplenic rats had spleens which were six times larger than normal, but they had only a mild thrombocytopenia.
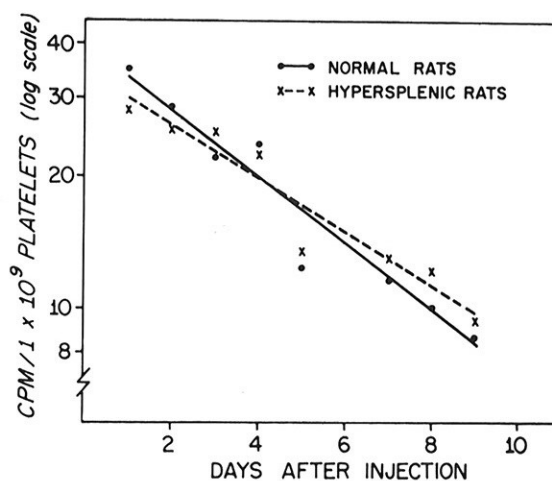
Fig. 3.—Platelet radioactivity after injection of DFP[32] into normal and hypersplenic rats. Each point represents the average of two rats. The lines are fitted by the method of least squares. In the normal group the slope of the line corresponds to a half-life of 4.0 days, with 3.1 to 5.6 days as 95 per cent confidence limits. In the hypersplenic group the half-life was 5.0 days.

However, to make an analogy, any significant fall of red cell concentration produces a marked increase in the erythropoietic rate. Thus, a mild degree of anemia in hereditary spherocytosis is associated with erythropoietic rates of five to six times normal,[15] and mild anemia induced by bleeding results in increases of three times normal.[16] Our studies show that no such increased state of platelet turnover is present in the hypersplenic rats with thrombocytopenia. Since platelet production *can* be increased both in normal and in splenectomized methylcellulose-treated rats,[17] our results suggest that the thrombocytopenia was caused by a splenic marrow depression, rather than by increased destruction.

SUMMARY

1. A method is described for the isolation of rat platelets. The method gives high yields of platelets with negligible white and red cell contamination.

2. Rat platelets were labeled in vivo with radioactive diisopropylfluorophosphate (DFP[32]). The platelet life span was the same in normal, splenectomized and hypersplenic rats.

3. Some problems involved in the use of DFP[32] as a blood cell label are discussed.

SUMMARIO IN INTERLINGUA

1. Es describite un methodo pro le isolation de plachettas de ratto. Illo resulta in un alter rendimento de plachettas, con negligibile grados de contamination leuco- e erythrocytic.

2. Plachettas de ratto esseva marcate in vivo con biisopropylfluorophosphato

a phosphoro radioactive (BFP³²). Le duration del vita de plachettas ab rattos normal, rattos splenectomisate, rattos hypersplenic esseva le mesme.

3. Es discutite certe problemas inherente in le uso de BFP³² como marca pro cellulas de sanguine.

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