Life Span of Mouse Blood Platelets. (26252)

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Little information about mouse blood platelets is available even though the mouse is widely used for mammalian experimentation, including hematological research. In this study we undertook to determine the life span of mouse blood platelets by a radioisotopic method previously used in rats(1).

Methods. Male BDF_1 mice weighing 23-28 g were injected intraperitoneally daily throughout each experiment with 1 μc of $Na_2S^{35}O_4$/g of body weight, except that at the first injection they received 135 or 115% of this dose. The purpose of daily injections was to maintain a relatively constant day-to-day pool of radioisotopic label for incorporation into platelets. Injections were made on the basis of daily body weights, except in the first experiment where initial weight was used. In some experiments carrier sulfate (added as Na_2SO_4), usually 0.6 μg SO_4/μc of S^{35}, was added to the radioactive sulfate to facilitate its distribution throughout the blood. Mice were killed at intervals after first injection of sulfate and always 24 hours after last injection. In some experiments, peripheral platelet counts were taken at this time. The mice were injected intraperitoneally with 0.5 ml of a heparin-Nembutal-saline solution† and were bled about 10 minutes later from the heart into siliconed syringes containing 0.05 ml of 1.0% disodium ethylenediaminetetraacetate dihydrate in 0.7% saline. The blood of each mouse was expressed into a 60 × 10 mm siliconed tube and mixed with an equal volume of 3.5% PVP,‡ a plasma expander. Platelets were then separated from the blood by differential centrifugation, first by centrifuging slowly to obtain a plasma-PVP-platelet layer, then by decanting this into another tube and centrifuging at a more rapid rate to obtain a platelet button in the bottom of the tube. Platelets of each mouse were washed twice with 0.5 ml of saline and resuspended in approximately 0.2 ml of saline. Numerical white blood cell and platelet counts were made on each platelet suspension. One 100 μl sample was placed on a planchet and dried for counting in a windowless gas flow counter. The radioactivity of two 100 μl samples of the platelet-free plasma-PVP component and of one 200 μl sample of each wash was also determined. Very few to no white blood cells were present in the platelet suspensions. Radioactivity per average platelet was determined by dividing radioactivity of a platelet sample by total number of platelets in that sample; the resulting values were multiplied by 10^7 for use in Fig. 1. Actual radioactivity counts of samples of platelets were about

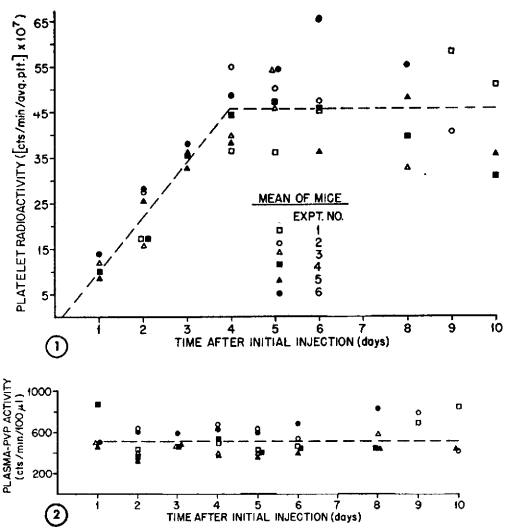

FIG. 1. Radioactivity of mouse blood platelets during a period of daily inj. of $Na_2S^{35}O_4$. Symbols denote mean platelet activity of mice (3 or 4) in each experiment at each interval. Total No. of mice was 136. For dashed line see text.

FIG. 2. Radioactivity of plasma-PVP during period of daily inj. of $Na_2S^{35}O_4$. Symbols denote means in each experiment at each interval. Dashed line represents grand mean of all mice.

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.

† 1 cc heparin (1000 U.S.P. units); 1 cc Nembutal (60 mg); 10 cc saline.

‡ PVP: Polyvinylpyrrolidone or Vinisil was kindly supplied by Abbott Laboratories.

300 to 2000 cpm (background was 25 cpm). Total number of platelets in 100 μl samples counted for radioactivity was in the range of 1.5 to 3.5 $\times$ 10^8.

Results. The radioactivity of platelets increased rapidly during the first 4 days, and was widely variable thereafter, although apparently without definite upward or downward trends. Mean platelet activity of the mice in each of 6 experiments is presented in Fig. 1. The rising dashed line was drawn by eye through the means obtained on days 1-3, and the horizontal dashed line was determined by averaging platelet activities of all 67 mice on days 5-10. Rate of increase toward the plateau value during the initial period was about 26% per day. Although variation among mice was large, mean plasma activity was quite constant throughout the 10-day period (Fig. 2). Radioactivity of 200 μl samples of the second platelet washes was low, in the general range of once to twice background. The amount of S^{35} injected was less than that which produces detectable hematologic(2) or histologic(3) damage. In one of our experiments no damage was evident in the bone marrow of sections of femurs of mice given 8 or 10 daily injections.

Discussion. Incorporation of sulfate into platelets appears to take place primarily through their precursors, the megakaryocytes. In rats this was suggested by a gradual, rather than immediate, increase in S^{35} activity in platelets of peripheral blood over a 3-day period after a single injection of $Na_2S^{35}O_4$, and by occurrence of heaviest labeling of the megakaryocytes, as seen in autoradiograms of bone marrow smears, about 2 days prior to peak activity of peripheral platelets(4). The sulfate becomes incorporated in platelets into a sulfated mucopolysaccharide(5).

Survival time of platelets in the peripheral blood of rats was previously studied essentially by the method used here, and 3 possible interpretations of the experimental data were considered: 1) that rat blood platelets have a life span; 2) that platelets are removed from circulation by random destruction; and 3) that both kinds of removal occur(1). On the basis of degree of correspondence of the experimental points to a straight (life span) or a curved (random loss) line it was concluded that the data were most consistent with a life span concept. We believe that a similar situation exists in mice, although the greater amount of variation in the mouse data results in a somewhat greater deviation of the experimental points from the lines in Fig. 1. By our interpretation, the rate of increase of platelet radioactivity toward the plateau level, 26% per day, indicates a life span of platelets in the peripheral circulation of BDF_1 mice of approximately 4 days. The mouse platelet survival is about the same or slightly shorter than that of rats.

Summary. In male BDF_1 mice injected daily with $Na_2S^{35}O_4$, radioactivity of the circulating platelets increased at a rate of 26% per day, reaching a plateau in 4 days. From the time required to attain maximum labeling of the circulating platelet population, the life span of mouse platelets is estimated to be approximately 4 days.

We thank Dr. M. Asano for examining the bone marrow sections.

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Received August 31, 1960. P.S.E.B.M., 1961, v106.