old were inoculated with the Jensen sarcoma and the phagocytic index of the reticuloendothelial system was measured at various stages of tumor growth. The older animals bearing the Jensen tumor produced more and an earlier stimulation in the rate of carbon clearance than the younger tumor-bearing animals.

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Platelet Size in Relation to Platelet Age. (29006)

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In a study of the *in vivo* aging of rat blood platelets, the relative sizes of platelets of young, old, and normal populations were compared by measuring with a cathetometer the volumes of packed platelets in microhematocrit tubes; it was found that platelet size decreases with age(1). The purpose of this study was to investigate the frequency distributions of platelet sizes in populations of varying age using a Coulter Electronic Particle Counter with a particle size distribution plotter attached(2,3).

Methods. Blood was drawn from the abdominal aorta of anesthetized male Sprague-Dawley rats into cold, siliconed 20-ml syringes containing 1 ml of a 1% solution of filtered ethylenediaminetetraäcetic acid (EDTA) in 0.7% saline. All diluents used were filtered through a filter having 0.45 μ pore size. A standard volume of 10 ml of blood was collected from each rat and was diluted with 6.25 ml of filtered saline to obtain a better yield of platelets. The blood-saline mixture of each rat was equally divided into two 15-ml conical centrifuge tubes, and the platelets were separated by differential centrifugation in a refrigerated centrifuge (5°C). After a slow centrifugation of the blood at 107 $\times$ *g* (750 rpm in an International Refrigerated Centrifuge, model PR-2) for 40 minutes, the platelet-rich plasma-saline was removed and spun more rapidly at 760 $\times$ *g* (2,000 rpm) for 30 minutes to obtain a platelet button. The platelets were then resuspended in 0.9% saline to give concentrations of 9,747 to 37,030 platelets/50 μl. (The standard volume of suspension pulled through the counting aperture was 50 μl when making numerical counts.) Platelets that had been separated from blood and resuspended in saline were used in these experiments rather than diluted platelet-rich plasma because our own work and that of others(4) has indicated that, in addition to platelets, the latter may contain unidentified small particles that affect platelet counts and possibly size distribution curves.

Populations of young platelets were obtained from rats that were recovering from thrombocytopenia induced with antiplatelet serum(1). Four hours after injection of the antiplatelet serum there were no circulating platelets. At 24 hours the platelet count was approximately 4,000/mm^3, which was too few for satisfactory collection. Platelets were collected and sized 2, 3, 4, 5, 6, and 7 days after antiserum injections. Platelets collected 2 days after antiserum injection, therefore, had an age range of 0-2 days.

Populations of old platelets were prepared

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

by incubating normal platelets in rats made platelet-deficient as a result of the bone marrow aplasia produced by 900 r of whole-body X-radiation. On the 6th day after irradiation when platelet counts averaged 168,000/mm^3, 11.78 $\times$ 10^9 freshly prepared platelets in 2 ml of normal rat serum were injected into the jugular vein. Two days later these platelets were collected. At this time, platelet counts of the transfused rats averaged 178,000 platelets/mm^3 ($N = 6$), and counts of rats similarly irradiated but not transfused averaged 31,700 platelets/mm^3 ($N = 6$). Therefore, the host may have contributed about 18% of the collected platelets, probably largely "old" platelets, while the remainder were donor platelets, the youngest of which were 2 days old.

Platelet sizes were measured with a Coulter Electronic Particle Counter, model B, coupled with a particle size distribution plotter. The heart of the counter is a small hole or aperture in a glass tube that is immersed in a conducting solution (saline) and across which a current is applied by means of an electrode on each side. Passage of a particle (cell), which is a poor conductor, through the aperture causes a change in the current that is visualized as a spike on an oscilloscope and recorded as a pulse on a scaler. The aperture must be large enough in diameter that it does not frequently become clogged. On the other hand, reduction in size of the aperture reduces the coincidence experienced because of simultaneous passage of more than one particle. Coincidence is also reduced by greater dilution of the particles. A 50 μ aperture was found suitable for these experiments. Pulse height is proportional to particle size and the particle size plotter records the relative number of pulses occurring in a standard length of time (4 seconds) in 25 successive 4-unit pulse-height windows. Thus particles of increasing size are counted in successive windows. Window number can be translated to platelet size in μ^3 by applying a conversion factor, but in this report window number has been used to designate relative platelet size. The relative number of platelets per window is plotted directly in graph form(4), and a frequency distribution curve of particle size can be obtained by joining the peaks plotted in each window. The relative frequencies were converted to percents of total counts for comparison purposes. Amplification and aperture current (multipliers) are set to place the mean pulse height of the particles being measured near the middle window; in these experiments, they were each set at ½.

After sizing was accomplished, the plotter was disengaged and the number of particles per 50 μl was determined. When counting numbers of platelets without the plotter, the lower threshold was set at 5 and the upper threshold at its maximum, 100. (In subsequent experiments, the lower threshold has been set at 8 to eliminate a small increase in electronic background noise of the instrument.)

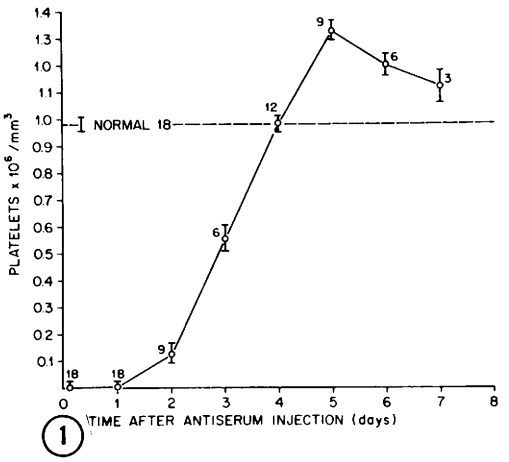
The concentrations of platelets in the samples measured were great enough to result in some coincidence counting, which would tend to increase the skewing of the particle size distribution curves to the right. In addition, the range of platelet concentration of the samples may have introduced some variability in curves of size distribution. However, although stringent control of platelet concentration might reduce variability due to experimental procedure, the standard errors of the mean in our experiments were small.

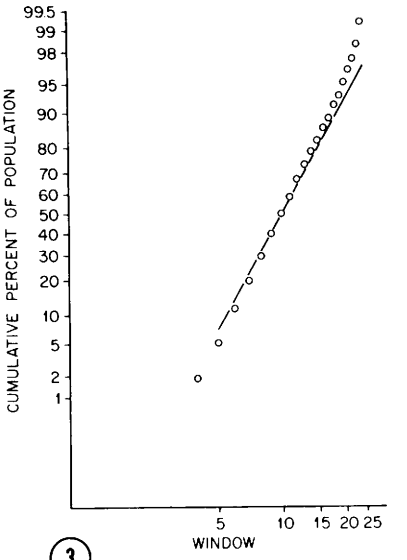
Peripheral platelet counts were made visually on blood taken directly from the leg vein into red cell pipettes using the hemocytometer and the phase microscope method(5).

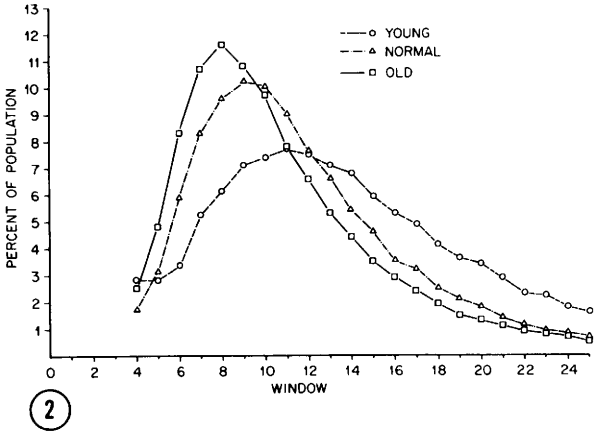
Results. Injection of platelet-specific antiserum into rats reduced the peripheral platelet count essentially to zero within 4 hours. Two days later the count began to rise sharply, reaching normal levels by day 4, overshooting on day 5, and then declining toward normal levels (Fig. 1).

Size distribution curves of young platelets, collected 2 days after injection of platelet-specific antiserum, were shifted to the right (larger) of curves of a normal platelet population. Curves of old platelets were shifted to the left (smaller) as illustrated in Fig. 2. The window numbers were converted to loga-

rithms to transform the skewed distributions to distributions that were approximately Gaussian(2). Statistical analysis by Duncan's Multiple Range Test(6,7) of the results of 2 sets of experiments, presented in Table I, showed that young platelets differed signifi-







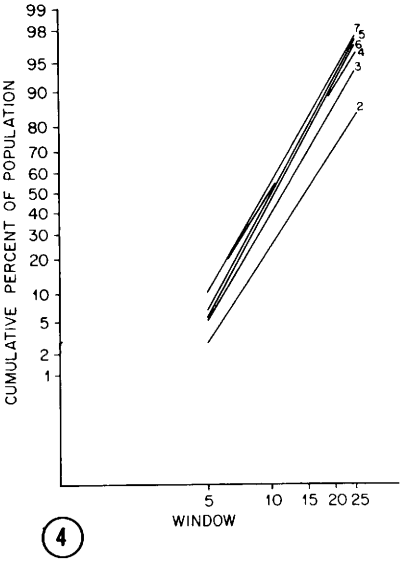


FIG. 1. Peripheral platelet counts of rats after injection of platelet-specific antiserum. Numbers indicate number of rats at each point, and bars denote standard errors of mean. The mean of normal (untreated) platelet populations is shown by a horizontal dashed line.

FIG. 2. Size distributions of platelets of young, normal, and old populations. Curves of young and old platelets are the means of 3 rats, and of normal platelets, 2 rats. Size is shown as window number rather than in μ^3.

FIG. 3. Cumulative frequency of size distribution of a population of normal platelets (one rat) plotted on a Gaussian probability scale against the logarithm of the window number. Open circles denote experimental points before correction for truncation.

FIG. 4. Cumulative frequencies of size distributions of platelets plotted on a Gaussian probability scale against the logarithms of platelet size (window number). Numbers on lines indicate the day after injection of platelet-specific antiserum on which platelets were collected. Each line represents the mean of 3 rats, except at 2 days when N = 2.

TABLE I. Comparison of Platelet Size (Mean Window) of Young, Normal, and Old Populations of Platelets.

	Exp 1				Exp 2			
	No. of rats	Mean window	Log mean window	S.E. of mean of preceding column	No. of rats	Mean window	Log mean window	S.E. of mean of preceding column
Young	3	12.96[a]	1.112[a]	.0105	2	14.65[a]	1.166[a]	.0164
Normal	2	10.27	1.012	.0127	5	8.78	.942	.0105
Old	3	9.32	.969	.0105	3	8.32	.920	.0134

[a] Mean with superscript "a" is significantly different from means without superscript "a," according to Duncan's Multiple Range Test at the 1% level of significance.

cantly from normal and old. The difference between old and normal platelets was nearly significant statistically at the 5% level in the 1st experiment and was not different by this criterion in the 2nd experiment. Differences of mean window values and thus size between corresponding populations in the 2 experiments may be attributable to a variation in technique, such as a difference in the pH of the diluent, which can affect platelet volume. The same procedures were followed within each experiment but a different lot of saline was used in the two experiments.

Windows 1, 2 and 3 were omitted from the size distributions since they contained counts due to background noise. Also, counts were not possible on sizes corresponding to window numbers greater than 25, and a statistical method for truncated data(8) was used to obtain unbiased estimates of the mean and variance of the size distributions. The points on the observed size distribution curve of 1 animal (normal) and the curve corrected for truncation are plotted in Fig. 3. The cumulative frequency of the size distribution is plotted on a Gaussian probability scale against the logarithm of the platelet size (window number). The curvature and steeper slope of the points are assumed to be due to truncation. The distribution curves of young platelets showed the greatest deviation from linearity plotted in this manner. This may result in part from the fact that the upper end of the distribution curves of populations of young platelets were not included in the plots at the aperture current and amplification settings used in these experiments. For the old platelets the truncation is not as apparent and the plots are nearly linear indicating that the logarithmic transformation of platelet size converts the skewed distributions into distributions which are approximately Gaussian(2).

During the recovery period after injection of platelet-specific antiserum, as the number of platelets in the peripheral circulation approached normal values, the size distribution curves approached those of normal populations. In Fig. 4 the cumulative frequencies of the size distributions corrected for truncation are plotted on a Gaussian probability scale against the logarithms of the platelet size (window number). Table II presents the results of a statistical comparison of the mean windows of the platelet distribution curves, which are directly proportional to mean platelet volumes. The mean platelet volume on day 2 was considerably greater than on any other day measured, and then declined.

TABLE II. Comparison of Platelet Size (Mean Window) of Populations of Platelets Collected at Intervals After Injection of Platelet-Specific Antiserum.

Day	No. of rats	Mean window	Log mean window	S.E. of mean of preceding column
2	2	14.65[a]	1.166[a]	.0164
3	3	11.67[b]	1.066[b]	.0134
4	3	9.94[c]	.997[c]	.0134
5	3	10.09[c]	1.004[c]	.0134
6	3	10.35[c]	1.015[c]	.0134
7	3	9.63[c]	.984[c]	.0134

[a] Mean with superscript "a" is significantly different from means without superscript "a," according to Duncan's Multiple Range Test at the 5% level of significance.

[b,c] Likewise, means with superscript "b" are different from those without superscript "b," and means with superscript "c" are different from those without superscript "c."

Discussion. The results of these experiments are in agreement with earlier work(1) that suggested a diminution in size of platelets during their survival time in the peripheral circulation.

It should be noted that the young platelets utilized in these experiments were obtained from rats that had been completely depleted of platelets and at time of collection were producing platelets at a rate that was probably more rapid than normal. The peripheral platelet count rose from about 10% of normal on the 2nd day after injection of platelet-specific antiserum to about 135% of normal on the 5th day (Fig. 1). Thus at least 40% of the 5-day population was produced each day, without allowance for any platelet loss during these 3 days. It is therefore possible that the larger size of the "young" platelets may be conditioned, at least in part, by the rapid rate of production rather than by their youth. This question cannot be resolved with the results available at present.

Although the difference in size (volume) between old and normal platelets was not statistically significant in these and earlier experiments, the old platelets have consistently had a smaller size than that of normal populations. The old platelets in these experiments were derived from normal populations and were thus not produced under abnormal circumstances. It is conceivable that they might have been altered in an unusual way by incubation for 2 days in an irradiated host, although no evidence is now available that suggests such an occurrence. While not conclusive, the results of the experiments on old platelets support the concept of a decrease in size of platelets with age.

What changes in platelets are responsible for a decrease in size with length of survival, and their significance in terms of platelet function, remain to be determined. Results of Detwiler and coworkers(1) suggested that the content of ATP decreases with age which may imply a gradual loss of functional factors with age.

The decrease in size and change in chemical composition suggest that platelets undergo a process of maturation or senescence while circulating in the blood stream. Whether or not platelets have a mean life span or are randomly lost from the circulation remains an open question, although it appears likely that both alternatives are operative, the relative importance of each depending on physiological circumstances.

Studies by Spertzel *et al*(9) in dogs after whole-body irradiation demonstrated differences in the size distribution of platelets that may be interpreted to indicate a correlation between age and size. In the first week after exposure, there was a slight progressive decrease in number of larger platelets, which we interpret to indicate an increase in the average platelet age due to inhibition of new platelet production by the radiation. When the dogs were sampled again on the 28th day during recovery, mean platelet size was greater than normal (newly produced platelets) followed by a return toward a normal size distribution in the following weeks.

Summary. The frequency distribution of platelet sizes in platelet populations of various ages was investigated using a Coulter Electronic Particle Counter, model B, and a particle size distribution plotter. Platelets were obtained from male Sprague-Dawley rats, young platelets from rats recovering from thrombocytopenia induced with platelet-specific antiserum, and old platelets after 2 days' *in vivo* incubation of normal platelets in irradiated, thrombopenic rats. The size distribution curves demonstrated a shift toward larger than normal size in the case of young platelets and smaller size for old platelets. Statistical analysis of the curves gave unbiased estimates of mean sizes and variances which were used to test hypotheses concerning the effect of age on platelet size.

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Observations on Erythrocytic Inclusion Bodies from Mice Infected with Rabies Virus. (29007)

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It has long been argued that there exists no demonstrable viremic phase in clinical rabies. Koprowski and Cox(1) cite numerous reports on both affirmative and negative attempts to isolate rabies virus from the blood of several species of rabid animals. None of our several attempts to induce rabies experimentally in new-born mice after injection of the blood of rabid animals has been successful. It seems logical, however, that at some time in the progression of rabies, virus would be present in the peripheral blood. Wong and Freund(2) found rabies virus in the blood shortly after intracerebral injection. Reports have shown rabies virus to be present in such non-nervous tissue sites as renal tubular epithelium, mammary gland, pancreas, brown fat and respiratory mucosal epithelium of infected animals(3-10). The thought that virulent virus could proceed to these sites solely by way of the nervous system seems tenuous at best. There is also mounting evidence of the transmission of rabies virus via aerosol from flying bats, presumably arising from inflight urination(11).

In studies with canine distemper virus it has been shown that intracytoplasmic inclusion bodies are present in conjunctival, respiratory and urinary bladder epithelial cells in addition to the cells of the central nervous system(12-15). Recent work has shown that neutrophils and monocytes from peripheral blood as well are found to contain distemper inclusions when specifically stained. Fluorescent antibody studies have proven their specificity(13,14,16,17).

This report concerns an investigation as to whether the peripheral blood of laboratory mice would yield similar structures during the course of rabies.

A pilot study was made by randomly sampling the peripheral blood of mice in various stages of clinical rabies induced with several sylvatic strains of virus. During this preliminary study, several of the stained smears showed erythrocytes with inclusion bodies strikingly similar to those seen in leukocytes and epithelial cells from dogs with clinical distemper. Following this initial discovery, a systematic approach was taken to find just when these bodies first appeared.

Materials and methods. Webster-Swiss mice 21 days old were divided into groups of 12 each with 6 mice per cage. Equal numbers of males and females were present but with the sexes separate. Group I was the control. Group II consisted of 12 mice inoculated with virulent rabies originally isolated from a rabid coyote (no. L488, mouse pool 4), and subsequently identified by serum neutralization, fluorescent antibody studies and Seller's staining for Negri bodies. They were inoculated intramuscularly in the right hind leg with 0.1 ml of a 10^{-1} dilution of mouse brain suspension with an intramuscular titer of $10^{-1.75}$(18). Following inoculation, one-half of each group (6 animals) was bled on alternate days by clipping the tail tip to obtain free flowing blood to prepare film pairs. The slides thus prepared were fixed while still wet in absolute alcohol anhydrous diethyl ether, equal parts, and allowed to remain