Life Span of Rat Erythrocytes as Determined by Cr^{51} and Differential Agglutination Methods. (24512)

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(Introduced by C. C. Congdon)
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Estimates of life span of rat erythrocytes have ranged from 4 to 100 days. The 2 most common methods used to obtain these estimates are measurement of return of circulating erythrocytes to normal levels after hemorrhage, induced erythropenia by chemical agents, or hypoxic erythremia, and disappearance of isotopically labeled erythrocytes from circulation. The literature cited by Hall *et al.* (1) indicates that in most cases the first method gave lower estimates (4 to 18 days) and the second, higher values (45 to 100 days). They, however, using Cr^{51} as erythrocyte label, reported average survival to be ~24 days with a half life of 8 days. A similar half life was reported by Aschkenasy *et al.*(2) who also used the Cr^{51} method. By using the Cr^{51} method, the survival time of Sprague-Dawley rat erythrocytes was ~60 days(3). Fairly reliable estimates of erythrocyte life span are obtainable by the Ashby technic(4), in which transfused cells are detected by differential agglutination or hemolysis. Although comparison of Cr^{51} and Ashby technics indicated that the former method is satisfactory for estimating erythrocyte life span in man and dog(5-8), a similar comparison has not been made in the rat. Owen's experiments(9) indicated that a differential agglutination technic could be used to determine life span of rat erythrocytes. He followed the rate of disappearance of exchanged cells after rats of distinguishable serotype had been disjointed from parabiosis. We used the same markers in the CD strain furnished by Owen and found that it is possible to transfuse a rat possessing D type erythrocytes with C cells and, at various intervals thereafter, test the blood for percentage of C type cells by means of anti-C serum (agglutinin) produced in rabbits. In this paper we compared rat erythrocyte survival curves obtained by the differential agglutination and Cr^{51} methods.

Methods. Blood was withdrawn from the abdominal aorta of C donors into heparin-containing syringes and was incubated with $Na_2Cr^{51}O_4$† (5.0 μc/ml of whole blood) for ~ 1 hr at room temperature with frequent swirling. About a third of blood volume was withdrawn from the jugular vein of D rats, which were then immediately transfused with a restorative amount of whole C blood‡ containing Cr^{51}-labeled erythrocytes. Two tail blood samples were obtained at 24 hrs and thereafter at 6 intervals up to the 62nd day. One sample (~20 μl) was tested with anti-C serum for percentage of C type cells as described (10). The other sample (20 or 50 μl) was taken for radioactivity determination. Erythrocytes obtained for radioactivity determination at 24 hrs were washed with 100 volumes of saline, centrifuged, and resuspended in 2 ml of distilled water. Subsequent blood samples were not washed but were discharged directly into test tubes containing 2 ml of distilled water. A well-type scintillation counter was used to determine radioactivity of the samples. Counting efficiency was about 35% and counting error about 2%. Considerable variation was present in percentages derived from the agglutination method because this technic is subject to errors inherent in any procedure involving erythrocyte counting. In addition, the smaller the number of agglutinable cells present, the larger the possible error. On the other hand, a C cell concentration as small as 2% can be detected among D cells as was determined on dilution series of known mixtures of the 2 kinds of cells.

Results. In individual survival curves of C

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† Abbott Labs., specific activity 20 μc/μg.

‡ Total blood vol. was calculated as 5½% of body weight; donor blood was not pooled.

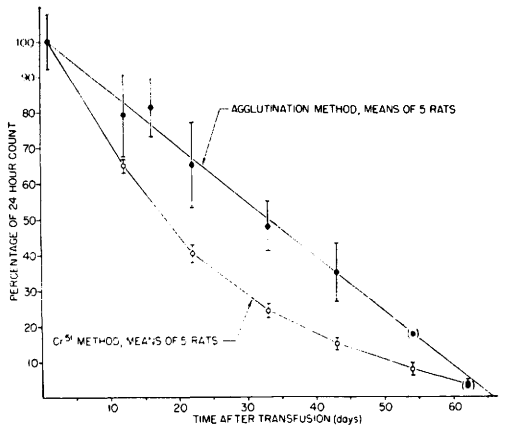

FIG. 1. Survival of rat erythrocytes simultaneously determined by agglutination and Cr^{51}-labeling technics. Vertical bars indicate $\pm$ one stand. error of the mean [S.E. $= \sqrt{\Sigma(d^2/n(n-1)}$]. Agglutination points in parentheses are estimates based on observations in counting chamber rather than on numerical counts. Counting of C and D cells in artificial mixtures showed that 2% C cells was just detectable. When agglutination was stronger but still not countable (<10%) the value of 5% was arbitrarily assigned.

erythrocytes in 5 D recipients, determined by agglutination technic, the C cells disappeared linearly from circulation of recipients until they were just detectable 61 days after injection. At this time they comprised about 2 to 5% of the circulating population in each rat. No C cells were detected on the 68th day. One day later the same 5 D rats were injected with C erythrocytes labeled with Cr^{51}. Fig. 1 gives survival curves obtained both from agglutination and Cr^{51} methods. C type erythrocytes again disappeared in a linear fashion from the recipients' circulation and were just detectable on the 62nd day. On about the 33rd day 50% of the initial population of C type erythrocytes remained. An estimate of the extinction time was 65.7 days based on a fitted line calculated from the agglutination data obtained up to day 43. Although the 95% confidence interval for this estimate was wide (42.5 to 114.1 days), the estimated percentage of C type cells on days 54 and 62 indicated that the extinction time was close to 65 days.

In contrast, the survival curve obtained for Cr^{51}-labeled C erythrocytes was curvilinear rather than linear. By day 62 ~3% of the initial radioactivity was present (Fig. 1). On day 17 half of the initial radioactivity was present. Radioactivity values ploted on semilogarithmic paper gave a straight line for the first 45 days.

Discussion. Donor erythrocytes disappear curvilinearly from a recipient's circulation when factors producing random destruction are present and disappear linearly in absence of such factors(11). Brown and Eadie(12) reported that random destruction occurred in healthy cats and rabbits and occasionally in dogs when blood was tagged with Fe^{55}. Our results suggest that C type cells in D recipients disappeared in a linear manner, indicating that random destruction of rat erythrocytes does not normally occur. The disappearance curve of the Cr^{51}-labeled erythrocytes was curvilinear and showed time relations similar to those obtained for Sprague-Dawley rats(3). Although the 50% survival time obtained by the isotope and agglutination methods differed, the extinction points derived from these methods both occurred at about 65 days. Similarly, extinction time as derived from both methods is about the same for erythrocytes of man(5-7), and the dog(8). In the absence of random destruction, the curvilinear line obtained by plotting the Cr^{51} data reflects elution of the Cr^{51} from the erythrocytes. Calculation of the elution rate according to a formula used by Stohlman and Schneiderman(8) indicates that the isotope was lost from rat erythrocytes at the rate of 1 to 2% per day for the first 45 days, a rate that compares favorably with those obtained by others in dog and man. Precise estimate of erythrocyte life span is, however, precluded by variations in the elution rate. Cr^{51} apparently does not have a toxic effect on erythrocytes, since, according to agglutination data, similar survival patterns were obtained whether or not the transfused cells were labeled.

Recipient D rats we used were transfused on 3 different occasions with C type erythrocytes whose survival was determined by means of differential agglutination. Results of second and third experiments have been discussed (Fig. 1). In the first experiment, how-

ever, transfused cells in 3 rats had reached low levels by 15th day. Immunization against C erythrocytes was not indicated since a secondary response was not elicited by a second or third transfusion of C type cells. The CD strain of rats also segregate for independently inherited red cell antigens (E and F) both of which are isoantigenic (R. D. Owen, personal communication). It is possible that shortened survival in the 3 rats of the first experiment resulted from production of antibodies against these or other unidentified red cell antigens. Failure to observe "immune clearance" in the second and third transfusion may then reflect the absence of corresponding isoantigens in donors.

Our data suggest that the Cr^{51} method gives a reliable estimate of life span of rat erythrocytes when the extinction point is used, although the method gives a falsely shortened 50% survival time because of chromium elution. The life span we obtained compares well with that reported by Berlin *et al.*(13) and Owen (personal communication) but is considerably longer than that reported by Hall *et al.*(1). Differences in technic could partly account for the variance since these workers obtained the first blood sample 1 hr after transfusion, whereas we used a 24-hr period. The relative merits of each method are discussed by Eadie and Brown(11).

Summary. Survival of rat erythrocytes has been studied by simultaneous use of 2 methods, Cr^{51}-labeling and differential agglutination. Results suggest that random destruction of rat erythrocytes did not occur and that the extinction of radioactivity of Cr^{51}-labeled cells is a valid criterion for determination of erythrocyte life span. Both methods suggested a life span of about 65 days.

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Immunologic Treatment of Tumors.*† (24513)

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We have prepared anticancer antibodies by injection of tumor cells into animals of a different species, and have injected the antibody solution into host bearing a tumor. Antibodies prepared in rabbits against Walker 256 rat carcinosarcoma were injected into rats at the same time they were inoculated with 10,000 Walker 256 cells. The recent work of Hiramoto and Nungester(1), Korngold and van Leenwen(2) among others showed the feasi-

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