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Erythrocyte Survival in Guinea Pigs. (27916)

L. H. SMITH AND T. W. MCKINLEY, JR.

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

Using calculations based on appearance of Fe^{59}-labeled erythrocytes in peripheral blood, Everett and Yoffey(1) estimated erythrocyte life span for the guinea pig to be 83 days. From a survival curve of erythrocytes labeled *in vivo* with Cr^{51}, Grönroos(2) concluded that 65 days was a good estimate of the life span of guinea pig erythrocytes. In previous studies of erythrocyte survival of mice and rats we used the technic whereby cells are labeled *in vitro* with Cr^{51}. The present series of experiments was performed to obtain comparative data for guinea pigs and to determine survival of autologous, isologous, and homologous erythrocytes of this species.

Methods. Male and female strain 2 and 13† guinea pigs weighing 700-800 g and 2-3 months old at the beginning of each experiment were used. From a punctured ear vein, 1-2 ml of donor blood were allowed to flow freely into a glass test tube containing about 100 IU of heparin. To each ml of blood 10-20 μc of Cr^{51} (as $Na_2Cr^{51}O_4$) were added. Concentration of chromium metal ranged from 0.04-0.09 μg/ml of blood. Chromation time at room temperature was 60-90 minutes, during which period the mixture was agitated frequently by tube inversion. Forty volumes of 0.9% NaCl were added and the blood was then centrifuged. The supernatant was discarded and sufficient saline added to the labeled cells to reconstitute to original blood volume. One ml of this preparation was injected into the external jugular vein of each anesthetized (Nembutal-ether) recipient. The first blood sample (0.1 ml) was collected 24 hours later from a punctured ear vein, and the sample discharged into 1.0 ml of water. Subsequent blood samples were collected likewise, at 7-day intervals for 10 weeks. Blood for duplicate microhematocrits was also obtained at each period. Radioactivity was measured in a well-type scintillation counter and triplicate 5000-count determinations were made for each sample. Counts were corrected for background, physical decay of isotope, and hematocrit. The 24-hour count was considered 100% and values for subsequent samples were expressed as percent of the initial 24-hour activity.

To determine the proportion of labeled cells, P, on day t, we used the following

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

† Both strains are maintained at this laboratory by brother-sister mating. Original breeders were obtained from Nat. Inst. of Health.

model that is similar to the one described by Eadie and Brown(3):

$$P = (1 - k_1 t)\, e^{-k_2 t}, \quad t \leq \frac{1}{k_1} \qquad (1)$$

In this model, $(1-k_1t)$ represents Cr^{51} loss resulting from senescence (linear) where k_1 is the constant daily change in the proportion of surviving cells. The rate of loss attributed to elution of Cr^{51} from erythrocytes plus possible random destruction of erythrocytes is jointly represented by the factor e^{-k_2t}. Estimates of parameters k_1 and k_2 were obtained from experimental data by minimizing the squares of the differences between proportions observed, P_O, and proportions estimated, P_E, since such estimates are unbiased. The estimates k_1 and k_2 are substituted in the model to obtain an estimate of the proportion of cells surviving at time t:

$$P_E = (1 - k_1 t)\, e^{-k_2 t}. \qquad (2)$$

That is, k_1 and k_2 were found such that the sum of squares of deviations, $\Sigma\ (P_O - P_E)^2$, was minimized. Since the parameters enter the model in a nonlinear manner, a computing procedure known as nonlinear least squares was used(4,5). Starting with initial estimates, a linear correction is made to each estimate of the parameters and P_E and $\Sigma\ (P_O - P_E)^2$ are computed. Successive corrections are made until the decrease in consecutive values of $\Sigma\ (P_O - P_E)^2$ is less than a predetermined small number. Given good initial estimates, the procedure will converge to the exact least squares solution. In addition, standard errors of the estimates of the parameters are obtained.

The radioactivity counts were made so that the number of counts was approximately constant regardless of t. However, variability among individual animals in proportion to cells surviving still decreased as t increased. Therefore, each observed proportion at time t was weighted by the inverse of the variance of the proportions among animals at time t.

Results. Autologous and isologous (strain 2) series. Rate constants with their standard errors derived from equation 1 were as follows: for autologous erythrocytes, $k_1 =$ 0.0089 $\pm$ 0.0019 and $k_2 =$ 0.0317 $\pm$ 0.0033; and for isologous erythrocytes $k_1 =$ 0.0098 $\pm$ 0.0017 and $k_2 =$ 0.0331 $\pm$ 0.0034. There were no significant differences ($P = 0.77$) between respective parameters for the 2 survival curves. On the basis of compatibility, differences in survival between the 2 types of erythrocytes were not expected since our breeding stock, maintained by strict sib mating, originated from isogenic breeders.

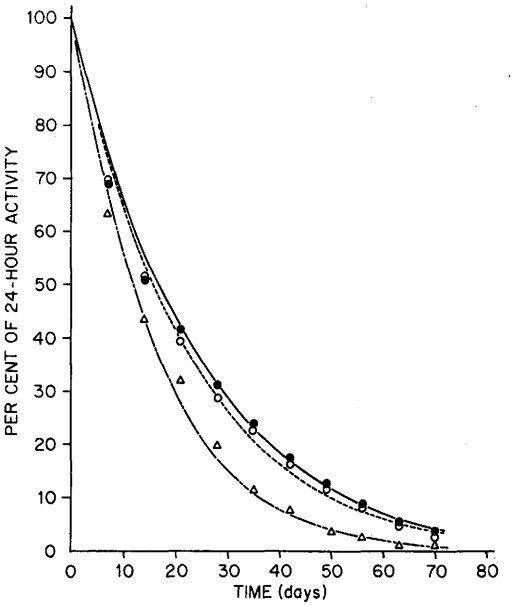

FIG. 1. Survival of Cr^{51}-labeled guinea pig erythrocytes. Autologous, ●——●; isologous, ○– – –○; homologous, △–•–△. Each point represents the mean of 10 animals. The curves were fitted by a nonlinear least squares method as described in text.

The time when 50% of the original activity remained ($T_{1/2}$) was about 16 days for both autologous and isologous erythrocytes (Fig. 1). Using the Cr^{51} method, we reported an apparent half-time value for the rat of 17-19 days(6), and for the mouse of 15-20 days(7). The time when 5% of the original activity remained was about 64 days for guinea pig erythrocytes compared to 59 and 49 days for rat and mouse.

Although Berlin *et al.*(8) have pointed out that the extinction point is probably the most acceptable datum obtainable from the Cr^{51} method, this point is often difficult to determine accurately by visual inspection of the survival curve, especially when the exponential component is large. In this and

past experiments, we have terminated sampling when activity reached the 3-4% level and considered this level to be slightly less than the extinction point. Accordingly, from the present data, 80-90 days would seem to be a reasonable estimate of this value (Fig. 1). Such an estimate would agree well with that reported by Everett and Yoffey(1). From the linear appearance rate of Fe^{59}-labeled erythrocytes in peripheral blood of guinea pigs for the first 7 days after isotope injection, they estimated that 1.2% newly formed red cells enter the circulation each day. This would give a life span of 83 days, assuming negligible random loss. Grönroos (2) obtained a survival curve for guinea pig erythrocytes labeled *in vivo* with Cr^{51} that indicated a T½ of 20 days, about 4 days longer than that obtained from our results. Compared to *in vitro* labeling, *in vivo* labeling may result in a slower disappearance rate because isotope is available for continuous uptake by newly formed erythrocytes. The 5% level, namely 57 days, estimated from the results of Grönroos, is about 9 days shorter than we obtained; and although this difference is not great, our visual estimates of the extinction points from the two curves would be different.

It is possible by use of equation 1 to calculate the value $P = O$ and, for example, obtain an extrapolated extinction time of 112 days for autologous erythrocytes. As expected, the 95% confidence interval (74-230 days) is extremely wide, and therefore the reliability of the extrapolation is questionable.

Homologous series. As previously stated, differences between autologous and isologous survival were not observed. Disappearance rate of strain 13 erythrocytes in strain 2 recipients, however, was moderately greater than normal (Fig. 1), and is related to a greater exponential rate loss as reflected by a significantly larger ($P<.01$) k_2. Rate constants with their standard errors derived from equation 1 were as follows: $k_1 = 0.0110 \pm 0.0019$ and $k_2 = 0.0494 \pm 0.0041$. The T½ was about 12 days and the 5% level was reached on day 46. As a control to the homologous series, autologous erythrocyte survival was studied in 4 strain 13 animals, and the curve was found to be identical to that obtained with autologous strain 2 erythrocytes. Thus, strain 13 erythrocytes do not inherently have a shorter life span than erythrocytes of strain 2. It could be postulated that elution of Cr^{51} from erythrocytes which probably occurs in all species and the magnitude of which cannot be assessed in absence of data reflecting senescent loss only (linear) is faster than normal in the homologous condition. We are not aware, however, of evidence supporting this interpretation. The most plausible implication of these results is that a moderate incompatibility exists between host and homologous erythrocytes, although the curve does not indicate immune clearance of erythrocytes as can be observed, for example, when mouse erythrocytes are transfused into a rat(9). We have not been able to detect natural agglutinins in concentrated serum of either strain against homologous erythrocytes. Homologous incompatibilities based on survival of Cr^{51}-labeled erythrocytes also have been observed in mice(7).

Summary. Disappearance curves of autologous, isologous, and homologous guinea pig erythrocytes labeled with Cr^{51} were compared. The T½ for autologous and isologous cells was about 16 days, and 5% of the activity remained on day 66. Calculations from a model used to describe survival curves indicated that disappearance rates of autologous and isologous erythrocytes were not significantly different. From the data, a value of 80-90 days was suggested as an estimate of life span for guinea pig erythrocytes. The T½ for homologous erythrocytes was 12 days, and 5% of the activity remained on day 46. Although the survival curve did not indicate immune clearance characteristics, homologous erythrocytes disappeared at a moderately but significantly faster rate than normal.

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Respiration of Gamma Irradiated *Brucella abortus* and *Mycobacterium tuberculosis*.* (27917)

TAI HEW AHN, HISAKO NISHIHARA, CHARLES M. CARPENTER AND GEORGE V. TAPLIN

Harry N. Falk Research Laboratory, Departments of Infectious Diseases and of Biophysics and Nuclear Medicine, School of Medicine, University of California, Los Angeles

Although extensive investigations of the oxidative metabolism of both *Brucella abortus*(1-4) and of *Mycobacterium tuberculosis* (5-6) on various substrates have been carried out, only limited studies of the effect of gamma irradiation on their metabolic activity have been published(7). The preliminary observations demonstrated that bacterial cells may be irradiated with gamma rays in such a manner that they neither multiply on appropriate culture media nor produce disease in experimental animals, yet continue to consume oxygen in the presence of suitable substrates. Herewith are reported additional oxygen uptake studies on gamma irradiated *Br. abortus* and on *M. tuberculosis*.

Method. A gamma irradiated vaccine – G.I.V. – was prepared from a commercially lyophilized and vacuum sealed *Br. abortus* 19.† Some of the vials were equilibrated with oxygen or nitrogen or with air prior to irradiation. For comparative studies heat and chemically inactivated *Br. abortus* vaccines were prepared from 48-hour cultures on Albimi agar. Heat inactivation was carried out in a water bath at 60°C for 10 minutes, and the 5 chemically inactivated vaccines were prepared by 72-hour exposure at 5°C to 50% ether, 5% formalin, 2% phenol, 50% alcohol, and 50% acetone, respectively. In the case of the vaccine from *M. tuberculosis* H37RV, the cells were cultured on Proskauer-Beck medium for one month, washed and resuspended in 5% Dubos albumin for lyophilization and irradiation.

Irradiation of the lyophilized cells was carried out by exposure to a cobalt-60 source, ranging from 2×10^4, 10^5, 2.5×10^5, 5×10^5, and 10^6 r. All vaccines were tested for inactivation by culture and by guinea pig inoculation.

Manometric measurements of oxygen uptake were fashioned after the standard method of Umbreit(8). The procedure of Cameron and Meyers(1-4) was followed closely for the *Brucella* respiration studies, and with slight modification in the case of *M. tuberculosis*. A 20 mg per ml suspension of the tubercle bacillus was employed instead of the 0.7 mg of cellular nitrogen recommended for the *Brucella*. One per cent solutions of D-alanine, L-asparagine, L-glutamic acid, and glucose were used as substrates for *Brucella*. Five-tenths molar solutions of acetic acid, glycerin, lactic acid, and pyruvic acid, the substrates reported to be most vigorously metabolized by *M. tuberculosis* were used(1-5). Both endogenous and exogenous oxygen uptake rates were determined, the latter in duplicate.

Results. Cells of *Br. abortus* and *M. tu-*

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