Survival of Mouse-Grown Rat Erythrocytes. (23961)

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Results of several lines of investigation have shown that irradiated mice injected with foreign hematopoietic cells accept the new tissue to the extent that this tissue implants, proliferates, and functions in the irradiated recipient (see review by Congdon,1). For example, in one experimental situation, Makinodan(2) using immunohematological technics, observed that the erythrocytes of X-irradiated mice injected with rat bone marrow were of rat origin. Furthermore, certain physicochemical properties of these mouse-grown rat erythrocytes were more like those of a rat than those of a mouse(3). To investigate a physiological characteristic of the mouse-grown rat erythrocyte, we used $Na_2Cr^{51}O_4$ as the label to compare survival time of these transplanted cells with survival time of normal rat and normal mouse erythrocytes.

Materials and methods. In all the experiments, (101 X C3H)F_1 mice and Sprague-Dawley rats were used. Mouse-grown rat erythrocytes were obtained from mice that had received 950 r of total-body X radiation followed by an injection of rat bone marrow cells. Erythrocytes of mice that survived at least 60 days were tested against species-specific antirat serum, and only those mice with 100% circulating rat erythrocytes, according to the test, were used. The $Na_2Cr^{51}O_4$, obtained from Abbott Laboratories, had a specific activity of 20-40 mc/mg. Blood for transfusions was aspirated from the heart of donor animals into heparinized syringes. The $Na_2Cr^{51}O_4$ was added to the blood and the mixture incubated at room temperature for 1 hour. Whole blood containing the labeled erythrocytes was injected into the jugular or tail vein of the recipient animal. The initial 24-hour blood sample was obtained from the tail vein or the heart, the erythrocytes lysed in 1.0 ml of distilled water, and radioactivity of the sample determined in a well-type scintillation counter. The 24-hour sample was taken as 100% and radioactivity of subsequent samples plotted as the percentage of this activity. Survival times in rats of normal rat, normal mouse, and mouse-grown rat erythrocytes were determined. These erythrocytes were labeled with $Na_2Cr^{51}O_4$ (8-15 μc/ml of whole blood), and 1 ml of the labeled blood was injected into recipient rats. Tail blood (20-μl samples) was obtained from each of these rats at 24 hours and subsequently at various intervals up to 60 days. Hematocrits were determined concomitantly. Two methods were used in studying the survival time in mice of normal rat, normal mouse, and mouse-grown rat erythrocytes. In one method, recipient mice were injected with 0.3 ml each of whole blood containing erythrocytes labeled with $Na_2Cr^{51}O_4$ at concentrations of 10 or 30 μc/ml of whole blood. The usual serial sampling method was then used whereby small amounts of blood (10 μl) were obtained from the tail of each mouse at various intervals after injection of the labeled erythrocytes. The other method involved injection of 25-35 mice with 0.1 ml each of labeled whole blood (incubated with 4.8-8.0 μc of $Na_2Cr^{51}O_4$/ml of whole blood). At various intervals thereafter a group of 4 or 5 of these mice was sacrificed to obtain 0.5 ml of heart blood from each animal. When this group method was used on mice of the same age, sex, and weight (within 2 g of each other), variation in blood-sample radioactivity among mice within any one group was less than 10%. Two points on each curve were used as a measure of erythrocyte survival. The time when 50% of the initial radioactivity remained is referred to as the 50% survival, and the intercept of the curve with the 5% radioactivity level was taken as an estimate of the maximum erythrocyte life span. We felt that, for our purposes, the 5% radioactivity level was better than the inter-

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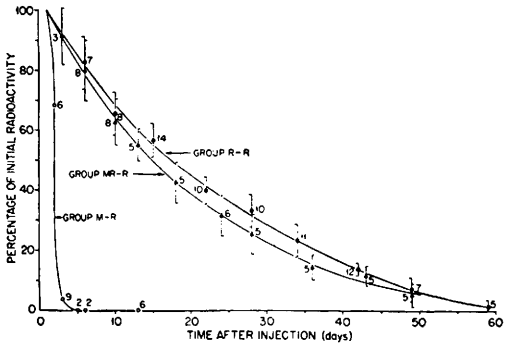

FIG. 1. Survival of normal rat, mouse-grown rat, and normal mouse erythrocytes in the rat, as determined by Cr^{51} labeling. R–R = rat RBC in rat; MR–R = mouse-grown rat RBC in rat; M–R = mouse RBC in rat. Vertical bars indicate ± one stand. dev. Figures beside points indicate No. of animals.

cept of the curve with the abscissa because of the asymptotic approach of the curve to the zero radioactivity level.

Results. Erythrocyte survival in rats (Fig. 1). The 50% survival for normal rat erythrocytes in the rat (group R-R) was 18 ± 2.5 days,‡ and the 5% survival was 52 ± 4.5 days. The 50% survival for mouse-grown rat erythrocytes in the rat (group MR-R) was 15 ± 2.5 days, and the 5% survival was 51 ± 4.5 days. The 50% survival for normal mouse erythrocytes in the rat (group M-R), however, was approximately 2 days, and the 5% survival was approximately 3 days; no detectable radioactivity was found on the 13th day. Although the 50% survival of group R-R was slightly greater than that of group MR-R, a comparison of the 2 slopes (log plot of the radioactivities from day 1 to day 49) of the groups indicated that there was no significant difference between the 2 survival curves. The slopes and standard errors of groups R-R, MR-R, and M-R were − 0.020 ± 0.003, − 0.023 ± 0.004, and − 0.248 ± 0.125 respectively. The slope for group M-R was significantly greater ($P <$ 0.001) than the slopes for groups R-R and MR-R. Hematocrits for all recipient rats remained at approximately control levels throughout the course of the experiments.

Erythrocyte survival in mice (Fig. 2). The data from both the serial and the group sampling methods were combined to construct the curves. The 50% survival for normal mouse erythrocytes in the mouse (group M-M) was 20 ± 2.5 days, and the 5% survival was 49 ± 5.0 days. The 50% survival for normal rat erythrocytes in the mouse (group R-M) or mouse-grown rat erythrocytes in the mouse (group MR-M) was approximately 2 days, and the 5% survival was 3-4 days. In groups R-M and MR-M no radioactivity was detected in samples obtained on days 9 and 16. Hematocrits of the recipient mice were essentially unchanged during the course of the experiments.

Discussion. The survival time of the mouse-grown rat erythrocytes in the rat was not significantly different from that of normal rat erythrocytes. This indicates that, by this physiological criterion, the transplanted erythrocytes are compatible with a rat environment and are therefore of rat origin. If they were of mouse type, their maximum life span would approach or be equal to that of the normal mouse erythrocyte in the rat; namely, 3 days. These results confirm those reported by Makinodan(2) and Vos *et al.*(4), who found that the mouse-grown rat erythrocytes agglutinate with antirat erythrocyte serum but not with antimouse erythrocyte serum.

When the mouse was used as a recipient for the labeled cells, we observed that the maximum life span of mouse-grown rat erythrocytes was the same as the maximum life span of normal rat erythrocytes; namely, 3-4 days.

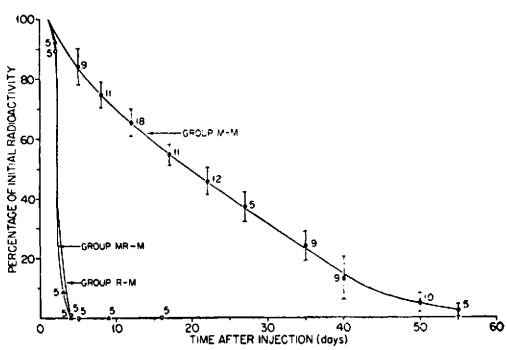

FIG. 2. Survival of normal mouse, mouse-grown rat, and normal rat erythrocytes in the mouse, as determined by Cr^{51} labeling. M–M = mouse RBC in mouse; MR–M = mouse-grown rat RBC in mouse; R–M = rat RBC in mouse. Vertical bars indicate ± one stand. dev. Figures beside points indicate No. of animals.

‡ The figures after survival times are 1 stand. dev.

These results further show that the mouse-grown rat erythrocytes are of rat type; if they were of mouse origin, their survival time would be similar to that of normal mouse erythrocytes.

In these experiments, the shapes of the survival curves for the compatible erythrocytes were curvilinear. Using the Cr^{51} method, other investigators have obtained similarly shaped curves for erythrocytes of man and several laboratory animals(5-9). Factors that might contribute to the shape of a survival curve include the elution of Cr^{51} from erythrocytes, normal or abnormal random destruction of the erythrocytes, and a relatively short mean survival time coupled with a large variation in survival of individual erythrocytes (see reviews by Eadie and Brown, 10 and Dornhorst, 11). Reutilization of the isotope could also affect the shape of a survival curve, but the work of Ebaugh *et al.* (5) indicates that Cr^{51} does not reincorporate into circulating human erythrocytes to an appreciable extent. It is difficult to ascertain the influence of these factors on erythrocyte survival curves. The *in vivo* elution of Cr^{51} from erythrocytes can be implicated if the Cr^{51} method is compared with a second method known to produce a linear line resulting from senescence of the erythrocytes. This comparison can be made for human erythrocytes by simultaneous use of the Ashby and Cr^{51} technics(5,12).

Comparison of the shapes of the survival curves for normal mouse and normal rat erythrocytes shows less curvilinearity for the mouse than for the rat erythrocytes. Several factors could contribute to this difference: (a) elution rate of Cr^{51} from mouse erythrocytes may be less than that for rat erythrocytes, (b) variation in the life span of individual mouse erythrocytes may be smaller than variation in life span of individual rat erythrocytes, (c) the mice used were more inbred than the rats and, therefore, transfused erythrocytes from one mouse to another might be more compatible than transfused erythrocytes from one rat to another, and (d) random destruction of erythrocytes may be greater in the rat than in the mouse.

Summary. A study was made of survival of Cr^{51}-labeled erythrocytes from irradiated mice that had been given rat bone marrow. Survival of these erythrocytes (mouse-grown rat erythrocytes) in both mice and rats was compared with survival of normal rat and normal mouse erythrocytes in mice and rats. The 50% survival time, *i.e.* the time when 50% of the initial radioactivity remained, of mouse-grown rat erythrocytes in the rat was 15 ± 2.5 days, and the 5% survival time, *i.e.* the time when 5% of the initial radioactivity remained, was 51 ± 4.5 days. Respective values for survival of normal rat erythrocytes in the rat were 18 ± 2.5 and 52 ± 4.5 days and for normal mouse erythrocytes in the rat, 2 and 3 days. The 50% survival time of mouse-grown rat and of normal rat erythrocytes in the mouse was 2 days and the 5% survival time was 3-4 days. Respective values for the survival of normal mouse erythrocytes in the mouse were 20 ± 2.5 and 49 ± 5.0 days. The data show that, by one physiological criterion, erythrocytes of X-irradiated mice (950 r) given rat bone marrow are of rat origin since their survival time in the rat is essentially the same as the survival time of normal rat erythrocytes in the rat.

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Changes in Cerebral Potassium During Insulin Hypoglycemia.* (23962)

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Little is known about metabolism of cerebral potassium during insulin hypoglycemia. Most recently La Fran *et al.*(1) have postulated that electroencephalographic changes and alterations of consciousness observed in both acute and prolonged insulin coma are in part due to shifts in electrolytes and water within the brain. They have postulated a decrease in intracellular potassium as a result of the deposition of brain glycogen. This results in a distortion of the intra- and extracellular relationship of potassium and sodium giving rise to extracellular edema. Several investigations are not in accord with this hypothesis, since it has been shown that there is an increase in intracellular potassium in liver, muscle, and erythrocytes during hypoglycemia(2-6). Further, Himwich has demonstrated a drop in cerebral glycogen content during hypoglycemia(7). Finally, *in vitro* and *in vivo* studies of the effect of insulin on cerebral metabolism have not demonstrated increased glucose utilization(8-10). These studies would tend to refute the arguments offered by La Fran *et al.*(1) to support their hypothesis, and would indicate that the changes observed electroencephalographically and clinically are primary or secondary results of a lowered level of metabolic substrate. This study was undertaken in an effort to clarify if possible, the changes that might be observed in the total brain potassium following a marked lowering of the blood sugar.

Method. 12 adult white male rats of the Osborne-Mendel strain were given 50 units of 80 units/cc crystalline regular insulin intravenously in a tail vein. The rats were then sacrificed by decapitation at 1½ to 5½ hours after injection, and in varying depths of hypoglycemic reaction ranging from stupor to coma. The whole brain including cerebellum was then analyzed for potassium by modification of the method of Elliott and Jasper(11). Following determination of wet and dry weights, samples were ashed in an electric furnace at 650°C for 4 to 5 hours and remained overnight at 350° to 400°C(11). The ashed weight was determined. The samples were then dissolved in 10 cc of concentrated hydrochloric acid, diluted to 100 cc with distilled water. Potassium was determined using an internal standard flame photometer (12). 12 control rats were lightly anesthetized with ether, decapitated, and the above procedure was carried out.

Results. In comparing brains obtained from experimental and control groups, there appeared to be no observable edema in either group; on close inspection, however, it was noted that in the insulin-treated group the brains appeared to be more friable, hyperemic and glistening. Also, the meninges were more adherent to the surface of the cortex.

There was no statistically significant increase in water content of brains of hypoglycemic rats. The values obtained were: controls 78.16%, hypoglycemic 78.38% ($p = > .50$).

Comparison of the amount of potassium on the basis of wet brain showed no statistically significant difference. The values were: control 92.89 meq. potassium/kg, hypoglycemic

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