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Survival of CR^{51} Labeled Erythrocytes in Swine.* (28108)

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Survival study of transfused erythrocytes is of general use both as a diagnostic aid and as a research tool in man and several domestic animals. Several technics have been used for detecting erythrocyte survival time in swine. Bush *et al.*(1) using C^{14} labeled glycine, reported a mean survival time of 62 days in 5 growing pigs. Jensen *et al.*(2), reported an "apparent" life span of 63 days in 18 normal growing pigs using Fe^{59}. Bush *et al.*(3), using Cr^{51} determined the mean erythrocyte half-life to be 17 days in 4 normal growing pigs while Hansard and Kincaid(4) using Cr^{51} found the "average" survival time for mature swine to be 71 days.

In all previous studies the erythrocyte life span in swine has been deduced from the results of cross-transfusion experiments (homologous transfusions). However, the use of this method presupposes that the "new environment" for the injected cells will have no influence upon their survival time. Such an assumption seems not justified for various reasons. Hence, an "a priori" better method is estimation of the life span of the individual's own erythrocytes in its own circulation (autologous transfusions). This report concerns comparison of these 2 methods.

Materials and methods. Fifteen pigs varying in age from 10 to 12 weeks at the beginning of the study were used. Four of the animals were Durocs from 2 litters, 4 were Hampshires from 2 litters and the remaining 7 were Landrace-Poland crossbreeds from one litter. The pigs were fed a commercial grower ration and water *ad libitum* throughout the study.

Erythrocyte survival time was estimated by labeling the erythrocytes with Cr^{51}. Four of the animals received injections of autologous labeled cells while the other 11 received homologous labeled cells from a donor pig. Blood was withdrawn from a donor pig or from the pig to be studied and placed in special formula ACD solution.† Two microcuries of radioactive sodium chromate† were added for every 1 ml of blood obtained and the mixture incubated at 37°C with intermittent agi-

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tation. After 30 minutes of incubation 2 mg of ascorbic acid were added for every 1 ml of blood present. The recipient animal was then anesthetized lightly with ether and 15 ml of the Cr^{51} labeled blood were injected into an ear vein.

Oxalated blood samples were obtained 15 minutes after the injection. Additional samples were taken once daily for the first week, on alternate days for the next 12 days and every fourth day thereafter until 40 days had elapsed after the injection. The packed cell volume (PCV) was determined on each blood sample by the microhematocrit method. One ml of blood was pipetted into a tube for radioactive counting and hemolyzed with 2 ml of distilled water. All samples except the 15 minute sample, which was counted for radioactivity immediately for determination of the red cell volume, were refrigerated for counting later. All samples from the same animal were counted on the same day to eliminate correction for radioactive decay and counting apparatus variation.

The samples were counted in a well-type, thallium activated sodium iodide crystal scintillation counter utilizing a radiation analyzer to reduce the influence of natural radiation (background). The activity present in each sample was counted to a preset count of 10,000 and the activity recorded as counts per minute per ml of red cells by the appropriate calculation from the PCV. (The PCV was corrected by a factor of 0.97 which has been shown to correct for the amount of plasma trapped in the packed cell fraction(5).) In order not to bias the curve with the early loss of chromium which is probably due to leakage of loosely-bound chromium to intact red cells(6), the sample obtained at 24 hours after injection was considered to contain 100% of the activity and this day was taken to be day 0. The activity in later samples was calculated as per cent of the activity present at time 0.

Since the animals were growing during the experiment, the concentration of the isotope was diluted by the expanding blood volume. To correct for the changing blood volume, red cell volume at the beginning of the study was calculated from amount of activity injected and amount in the 15 minute sample. At the end of the study, plasma volume was estimated using the T-1824 technic and red cell volume calculated from total blood volume and PCV. Total blood volume was calculated from the following formula:

$$\text{Blood Volume} = [(\text{Plasma Volume})/(100 - (\text{PCV} \times 0.72))] \times 100.$$

0.72 is a correction factor for swine which has been shown to correct the blood volume for overestimation when the plasma-PCV method is used.

To correct for the changing cell volume during the study period, the determined proportion of the Cr^{51} labeled cells remaining in the circulation was correctd by the following formula: $(RBC_t^{Act.} \times CV_t)/CV_o = RBC_c^{Act.}$ where:

$RBC_t^{Act.}$ is proportion of Cr^{51} remaining at time t

CV_t is the calculated cell volume at time t (a linear increase was assumed between the 2 blood volume determinations)

CV_o is cell volume on day 0

$RBC_c^{Act.}$ is proportion of Cr^{51} remaining after correction for an increase in cell volume.

The corrected amounts of Cr^{51} remaining were expressed as the logarithm of the per cent remaining and analyzed by regression technics(7) as well as plotted on graph paper against time for each animal. From the regression calculations the half-life (50% survival time) was calculated. The means for the 2 groups were tested for significant difference by the Student t-test(7).

Results and discussion. In Fig. 1, the disappearance curve of radiochromium from the blood of one pig of each group is plotted on a semi-logarithmic scale. The data closely approximated a straight line in all of the experimental animals and were verified by testing the goodness of fit of the experimental values to the regression line by use of the t-test. In no case were the values significant at the 5% or less level and thus can be interpreted as sampling variations from the calculated regression line. Differing opinions appear in the literature concerning whether Cr^{51} red cell survival curves are simple exponential or linear with respect to time. Practically speaking, the disappearance of radiochromium from

the blood stream of swine represents both cell destruction and chromium elution. Bush *et al.*(1), have interpreted from their C^{14} glycine data that most of the red blood cells from swine are destroyed in a random manner. To the authors' knowledge the rate of Cr elution from swine erythrocytes has not been investigated. If the elution process is exponential in swine it would theoretically be possible for the aging process to be linear and still have a logarithmic curve when the two are combined. Regardless of whether the cell destruction process itself is linear or logarithmic, the disappearance of Cr^{51} from the blood stream, when not corrected for Cr elution, represents a logarithmic process quite similar to that reported by Bush *et al.*(3).

The results concerning mean half-lives of erythrocytes in swine are presented in Table I. The mean half-life for the autologous injections of labeled red cells was 28.0 days while that for homologous injections was only 13.8 days. These values are quite different from those reported by Bush *et al.*(3) who found a mean half-life of 17.0 days in 4 normal growing swine who had received Cr^{51} labeled homologous cells. According to Mollison(8), differences in time taken for 50% of the chromium to leave the circulation of man after injecting Cr^{51} labeled red cells are due to a large extent to differences in rate of elution of Cr, depending upon the medium in which the red cells are suspended at the time of labeling and whether they have been washed before labeling. Since all transfused cells in these studies were treated similarly, differences due to these factors can be ruled out and a true difference said to exist between the life spans of autologous and homologous injections of erythrocytes in swine. The cause of the differences seen in this study is not clear.

TABLE I. Erythrocyte Half-Life Determined by Cr^{51} Technic.

Type of cells injected	No. of pigs	Half-life* Mean (days)	Half-life* S.D.† (days)
Autologous	4	28.0	4.0
Homologous	11	13.8	5.7

* t-value for difference between means = 12.56, $P < .001$.
† S.D. = standard deviation.

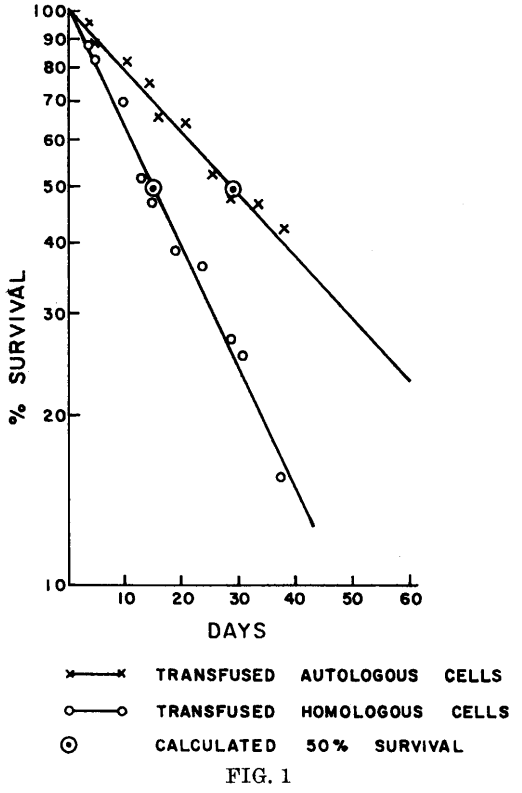

FIG. 1

The values reported herein for autologous injected cells indicate that the life span of swine erythrocytes can be considerably longer than values previously reported and may even approximate the 25-30 day $t_{1/2}$ value reported for man, even though porcine cells appear to be destroyed by a random process.

Summary. The 50% survival time of Cr^{51} labeled porcine erythrocytes was found to be 28.0 ± 4.0 days when autologous injections of cells were used and 13.8 ± 5.7 days when homologous injections of cells were used. Based on these data, swine erythrocyte survival times may actually be longer than the values previously reported.

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Hesperidin, Phagocytosis, and O_2 and CO_2 Tensions in Subcutaneous Gas Pockets.* (28109)

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Gas from artificially-induced subcutaneous pockets in unanesthetized rats can be used to study the relation of local blood flow to local metabolism of the subcutaneous tissue(1). Actions of various agents in a circumscribed region can be observed by administration of drugs or chemicals directly into these pockets.

Pocket tensions of O_2 and CO_2 reflect tissue tensions, and are expected to change in a reciprocal manner—high local blood flow increases pO_2 at the same time that it decreases pCO_2; low blood flow or high aerobic metabolism decreases pO_2 while increasing pCO_2. However, it is shown below that when the flavonoid hesperidin was injected into pockets, CO_2 increased but O_2 did not uniformly decrease; many times both pO_2 and pCO_2 were higher than in saline-injected control pockets. To investigate some of the explanations which occurred to us, we injected particulate substances, acid, and a vasoconstrictor agent into pockets.

Methods. Pockets were formed in the subcutaneous tissue on the backs of female rats (200 g, NMRI Long-Evans strain) by injection of 20 ml of wet air with hypodermic needle and syringe(1). Additional injections every 3 or 4 days replaced gas absorbed into the tissues. Samples of pocket gas aspirated into syringes were analyzed for O_2 and CO_2 (2). No attempt was made to use sterile technic, and there were no indications of infection in the rats or their pockets. Some rats were sacrificed and tissue from the gas pocket wall was fixed for histological examination.

Preparations injected into the pockets were phosphorylated hesperidin, 10 mg/ml; commercial India ink, 0.1 ml/ml; trypan blue, 10 mg/ml; epinephrine, 1/50,000; and lactic acid, 5%. The solvent or suspending medium was saline in each case except for lactic acid when water was used. Pure saline served as a control injection.

Results. Each of the 61 points in Fig. 1 represents the pO_2 and pCO_2 of a single analysis of pocket gas in one of 33 rats receiving an intrapocket injection of 1 ml per day of the phosphorylated hesperidin preparation during a 5 day period. The shaded box contains 95% of 68 samples obtained from 28 saline-injected or untreated controls. (The black rectangle is mean of the control samples, the dotted line a regression line of pCO_2 on pO_2, the oblique boundaries are 2 SE from the regression line, and the vertical boundaries are range for the pO_2 values.) Most of the points with both O_2 and CO_2 above the control mean occurred after 3 days of treatment.

Fig. 2 shows samples obtained from 12 rats, 3 rats per treatment, during a 3 day pe-

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting views of Navy Department or naval service at large.

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