Effect of Repeated Erythrocyte Transfusions on Subsequent Survival of Homologous Erythrocytes in Chickens.* (27615)

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Whole blood or suspensions of erythrocytes given to an immunologically immature animal can cause a later failure of the immune response to erythrocytes that normally follows their administration to the mature animal. Embryonic parabiosis in fowl(1), repeated intraperitoneal injections of whole blood into newborn rabbits(2), and single, intravenous injections of whole blood into newly hatched chickens(3,4), suppress the formation of isoantibodies to the erythrocytes of the donor when the recipient is later challenged with these erythrocytes. Mitchison(5) showed that repeated intravenous injections of homologous erythrocytes into chickens, begun soon after hatching and repeated at 6-day intervals, resulted in a slightly longer than expected survival of this blood in the adult recipients. Mitchison's erythrocyte suspension was prepared by incubating whole blood for 15 minutes at 48° to 50° in an attempt to destroy the leukocytes present.

We have reported(8) that repeated transfusions of blood given to chicks prolonged the survival of erythrocytes from the same donor when the chicks were challenged at 10 weeks of age. The present experiment was done to determine the lifespan of transfused erythrocytes in mature chickens and test the effect on erythrocyte lifespan in chicks that had been given repeated transfusions of erythrocyte suspension from day of hatching.

Materials and methods. On the day of hatching, white Leghorn chickens were given injections intravenously of 0.3 ml of erythrocyte suspension from adult donors of the same breed. These suspensions were prepared by centrifuging heparinized whole blood in Wintrobe sedimentation rate tubes at 3000 rpm for 10 minutes, then aspirating and discarding the plasma and buffy coat. The cells were resuspended in ACD solution and the procedure repeated. At the end of the procedure, no leukocytes could be found in stained smears of cells from the upper layer of the red cell mass. Intravenous transfusions in gradually increasing amounts up to 1 ml of erythrocyte suspension from the same donors were given weekly thereafter to ensure that living homologous red cells were continuously present in the recipient. Ten-week-old chickens have been shown to be uniformly capable of antibody production in response to antigen(7). At this age, survival time of the donor blood was measured in these chicks and in control chicks that were hatched the same day but not inoculated. Survival time of the donor cells was also measured in the donor as an additional control measure to test both the efficiency of tagging and the degree of damage that might have occurred to the cells.

Survival time of the erythrocytes was measured by removing a sample of whole blood and incubating this blood at room temperature with radioactive sodium chromate in concentrations of 3 to 5 μc/ml. The blood cells were then washed in saline and 0.5- to 2-ml amounts of tagged cell suspension were injected intravenously back into the donor and into the other chickens in which survival time was to be measured. One-ml samples of blood were then drawn from each chicken at 1 hour and successively at 1- to 4-day intervals thereafter. The residual radioactivity in these samples was measured in a well scintillation counter. The level of radioactivity in the sample taken at 1 hour, when complete mixing of the tagged cells was assumed to have occurred, was assigned a value of 100%. The level of radioactivity on succeeding days was expressed as a percentage of the 1-hour value. The number of days that elapsed for the level of radioactivity to reach 50% of the 1-hour

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sample was expressed as the "apparent Cr^{51} half-life"(6) of the erythrocytes.

Results. Survival time of erythrocytes from the original donor was measured in the recipient chickens at 10 weeks of age. In every instance the transfused erythrocytes survived longer in these chickens than in previously uninoculated control chickens from the same hatching that were given injections intravenously with 1 ml of the same tagged blood. At the same time, samples of the same erythrocytes injected intravenously back into the donor had a longer lifespan than in either previously homotransfused chickens or previously uninoculated control chickens (Table I).

Each horizontal row of figures in Table I represents the erythrocyte half-life of an aliquot of chromium-tagged red cells injected into the donor (auto), an uninoculated homologous recipient (homo), and a homologous recipient that had received weekly intravenous injections of the same erythrocytes since the day of hatching (experimental).

The mean Cr^{51} half-life of donor cells in the previously inoculated chickens was 6.3 $\pm$ 0.7 (S.E.) days, while in a larger group of donors and uninoculated controls it was 10 $\pm$ 0.48 (S.E.) days and 2.75 $\pm$ 0.25 (S.E.) days, respectively (Fig. 1). The P value for the differences in all 3 groups is $<.001$.

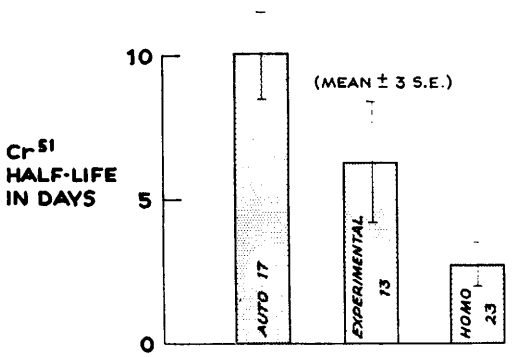

FIG. 1

Discussion. These results indicate that homologous erythrocytes introduced into and maintained in the vascular system of newly hatched chicks significantly alter the recipients' later behavior toward these erythrocytes. The chickens used in these experiments rapidly destroyed erythrocytes transfused from other members of the colony. Under the conditions of the experiment, transfused erythrocytes survived significantly ($P<.001$) longer in the animals that had been given transfusions previously. This prolonged survival is comparable to the tolerance to skin grafts in animals given grafts with homologous skin or other tissues from the future donor while still *in utero* or during the neonatal period. It has been postulated that the cellular antigens introduced into the immunologically immature animal destroy this animal's capacity to form antibodies to the same antigens in adult life. The continued presence of antigen has been shown to be necessary for the maintenance of immunological tolerance(5). The present study indicates that a similar chain of events occurs when washed erythrocytes are used first as a source of foreign antigen, then later as a means for testing cell survival. Erythrocytes are ideally suited for the latter purpose because their survival time can be measured accurately by the Cr^{51} technic.

The figures in Table I show a wide variation in erythrocyte survival time within each of the groups; survival time in the autotransfused controls varied from 7 to 13 days, and in the experimental group from 3 to 11 days. These differences most likely reflect varying degrees of damage inflicted on the erythrocytes during the tagging procedure. Longer survival time in the autotransfused control is generally associated with longer

TABLE I. Effect of Prior Transfusions of Red Cell Mass on Erythrocyte Survival.

RBC half-life in days		
Controls		Experimental
Auto	Homo	
13	1	9
13	1	11
13	1.5	9.5
13	1	8.5
7	3	3.5
7	1.5	3
9	4	5
7.5	3	3.5
8	2	7
8	2	6.5
8	2	5.5
8.5	3	6.5
8.5	3	4

survival in the experimental animal that has received the same blood. Each horizontal line in Table I represents a single experiment in which aliquots of the same donor blood have been used, and in each instance survival time in the experimental animal was longer than in the homotransfused control.

Summary. Repeated intravenous injections of homologous erythrocytes, begun in the early neonatal period, later resulted in the inability of the recipients to destroy rapidly the same homologous erythrocytes. This behavior contrasted with that of control chickens from the same hatching, which uniformly and rapidly destroyed the same donor blood.

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Thyroxine Secretion Rate of Missouri Valley Mole, *Scalopus aquaticus.* *† (27616)

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Thyroxine secretion rate (TSR) of the mole, *Scalopus aquaticus,* has not been reported. The present paper reports on TSR at room temperature (78°F) and 40°F. In captivity moles eat ½ their weight in food per day(1), and in the wild they are extremely active(2,3). High TSR would be one endocrine mechanism that would partially account for these activity patterns.

Materials and methods. Moles captured in Boone County, Missouri, between July and November, 1961 were maintained in No. 12 galvanized iron wash tubs in 3″ of dirt. They were fed freshly killed skinned rats each day and fresh water was supplied. The moles maintained or gained weight. The animal room was artificially illuminated during regular daylight hours and kept at uniform temperature (78°F ± 1°). The method of Grosvenor and Turner(4) was used to determine TSR on adult (mean body weight 101.6 g) and juvenile (young of the year with mean body weight 90.4 g) moles. Some modifications of this method were employed. On day 1, moles were injected i.p. with 5 μc of carrier-free I^{131}, and 48 hours were allowed for fixation by thyroids. External neck counts were made at this time by anaesthetizing each mole with ether, then placing it on its back with thyroid over scintillation probe containing 2″ NaI crystal. Each mole was injected on day 3 and 4 with 0.5 μg/100 g BW L-thyroxine (T_4) and 0.5 mg tapazole/100 g BW (goitrogen) which blocked recycling of I^{131}. T_4 dose was increased by 0.25 μg increments, each dose being injected for 2 consecutive days, with neck counts being taken on the day of each increase. The dose of T_4 which prevented further thyroidal-I^{131} output (95-100% previous count) was estimated as TSR.

TSR of 3 moles was determined after 14 days at 78°F. Moles were then placed in a 40°F cold room and TSR determined after 10 and 22 days exposure.

Resting O_2 consumption was determined on 4 moles in a post-absorptive state. Each was placed in a 22 mm desiccator containing anhy-

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