Erythrocyte Survival in the Cat as Determined by Glycine-2-C^{14}. (31602)

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The erythrocyte survival times of only a few species of small animals have been determined by the labeling of heme with glycine-2-C^{14}. The relatively large sample volumes required in the usual glycine-2-C^{14} methods have made its use and the interpretation of the radioactivity curves difficult. Valentine *et al*(1) corrected for a bleeding loss of 10% of blood volume per sample and estimated the erythrocyte life span to range between 72½ and 81½ days in cats. Berlin *et al*(2) used more animals, took only a few samples from each at different time intervals and graphed a composite radioactivity curve for their group of 10 rats. This report describes the results obtained in 4 cats by a direct method using glycine-2-C^{14} which would be applicable for determination of the erythrocyte life span in very small animals.

Materials and methods. Four adult domestic short haired cats (2 male and 2 female) were used in this study. Data concerning their weight, hematology and isotope injected are given in Table I. Glycine-2-C^{14} was injected intraperitoneally at a dose of 70 μc/kg in 3 divided doses at 2-hour intervals. At intervals indicated in Fig. 1, approximately 0.25 ml blood was obtained in a heparinized hemoglobin pipette by incising a small ear vein and pipetting. The blood was washed 3 times by mixing with 5 ml of 0.85% saline solution and centrifuging at 500 $\times$ *g* for 15 minutes in the cold. The washed erythrocytes were then hemolyzed by adding 5 ml distilled water and centrifuged at 5000 *g* for 20 minutes in the cold to remove the stroma. The hemoglobin concentration was determined in a one ml aliquot of the clear solution by the cyanmethemoglobin method and another one ml aliquot was pipetted onto a 1¼″ stainless steel concentric ringed planchett and dried. All planchetts were counted in a micro-mil window flow-gas Geiger Counter† and the specific activity expressed as μc C^{14}/g hemoglobin. No correction for self-absorption was necessary if there was less than 1.5 mg of hemoglobin per planchett. The medium survival times ($t_{½}$) of the erythrocytes were calculated from their survival probability functions, p(t) where $p(t_{½}) = ½$ by methods previously described(3) and used by us to determine the survival times in a variety of animals(4).

Results and discussion. The hemoglobin specific activity time curves of each cat are given in Fig. 1. The survival probability functions which were calculated from these curves are not shown in Fig. 1 for the sake of clarity. For each cat, a mean plateau hemoglobin specific activity level was calculated for the time interval 11 to 53 days and used for the calculation of the median survival times given in Table I. It is apparent in Fig. 1, however, that during this time the specific activity-time curve of cat 66-1 is decreasing, cat 66-4 shows a slight increase while 66-2 and 66-3 remain relatively constant. This would indicate that changes in blood volume were occurring in these cats. A clinical pneumonitis with high temperature was first noted in all cats on the 60th day post-injection and we would attribute the rise in specific activity to associated changes in blood volume. On the other hand, a plot of the mean values between 11 and 53 days post-injection resulted in a plateau and therefore use of a mean plateau value in each of the individual calculations was considered valid. The mean median erythrocyte survival time in these 4 cats is 72.6 days, a value only slightly shorter than the 77 days previously reported using N^{15}-glycine(1). In the present studies, bleeding losses amounted to only about 1-2 ml per cat during the entire 101-day period including the samples taken for microhematocrit and hemoglobin determination. This amounted to about 1% of the blood volume calculated on the basis of 7% of body weight and, therefore,

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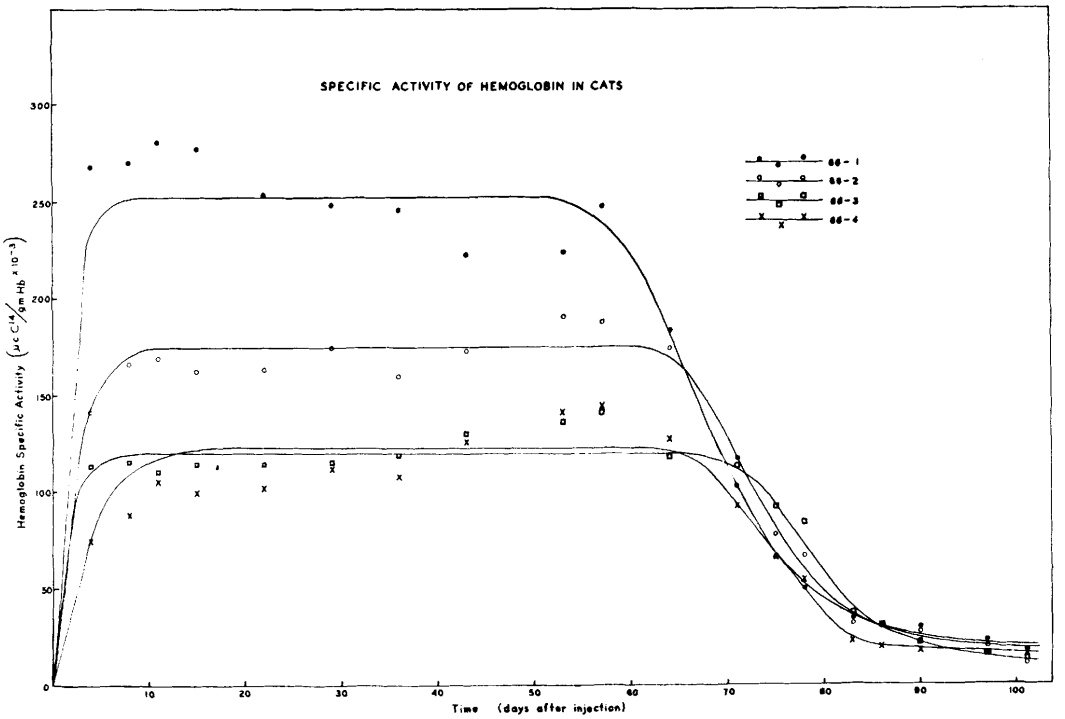

FIG. 1. Specific activities of hemoglobin in 4 cats following injections of glycine-2-C^{14}. Survival probability functions were calculated from these curves.

TABLE I. Median Erythrocyte Survival Times of Normal Mature Cats Using Glycine-2-C^{14}.

Cat	Sex	Wt (kg)	Packed cell vol (%)	Hemoglobin (g/100 ml)	Injected dose (μc)	Median survival time (days)
66-1	♂	4.10	37	12.3	287	66.4
66-2	♀	3.18	40	14.1	223	72.3
66-3	♀	4.10	35	12.8	287	78.8
66-4	♂	5.46	39	13.6	382	73.0

no correction for bleeding loss of isotope was applied.

The dosages of C^{14} (70 μc/kg) required in the present studies were considerably higher than that previously used (2-5 μc/kg) in the heme isolation techniques(5). There was, however, no evidence of illness in these cats at one year after the cessation of the study.

Summary. A method for determination of the median erythrocyte survival times applicable to small laboratory animals is described. The mean median survival times of the erythrocytes of 4 cats using this method was found to be 72.6 days.

1. Valentine, W. M., Pearce, M. L., Riley, R. F., Richter, E., Lawrence, J. S., Proc. Soc. Exp. Biol. and Med., 1951, v77, 244.

2. Berlin, N. I., Meyer, L. M., Lazarus, M., Am. J. Physiol., 1951, v165, 565.

3. Cornelius, C. E., Kaneko, J. J., Benson, D. C., Am. J. Vet. Res., 1959, v20, 917.

4. Berlin, N. I., in Bishop, C., Surgenor, D. M., The Red Blood Cell, Academic Press, New York, 1964, p423.

5. Kaneko, J. J., Cornelius, C. E., Baker, N. F., Proc. Soc. Exp. Biol. and Med., 1961, v107, 923.

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