Red cell life span in the turtle and toad

PAUL D. ALTLAND AND KIRKLAND C. BRACE
National Institute of Arthritis and Metabolic Diseases and National Cancer Institute, National Institutes of Health, Bethesda, Maryland

ALTLAND, PAUL D., AND KIRKLAND C. BRACE. Red cell life span in the turtle and toad. Am. J. Physiol. 203(6): 1188-1190. 1962.—Estimates of the mean life span of the red blood cells of box turtles and South American giant toads were made with glycine-2-C14. The mean life span of the turtle red cells probably lies between 600 and 800 days and that of the toad between 700 and 1,400 days. The body weight, hematocrit, and hemoglobin values remained at relatively normal levels for 7 years in turtles and nearly 2 years in toads. These results indicate that the low metabolic rate, characteristic of poikilothermalms, is correlated with a long red cell life span.

Our discovery (1) that the life span of the red cell of the turtle is greater than 11 months is so far the only published study of this type on reptiles. Continued study has provided data showing the life span of box turtle red cells to be between 600 and 800 days. As pointed out in a recent review (2) the long red cell span suggested by the preliminary work is unusual and certainly requires further study in this and other species. Accordingly, we have also used the same method to study the red cell life span of the South American giant toad and found evidence of a long life span, probably in excess of 700 days.

METHODS

Twenty-seven adult turtles (Terrapene carolina carolina) were collected in Montgomery County, Maryland, and housed in indoor cages at room temperature (24 ± 2°C). Adequate amounts of lean horse meat, tomatoes, lettuce, cantaloupe, bread, and water were supplied. The turtles were sacrificed at intervals to obtain single blood samples. Fifteen adult South American giant toads (Bufo marinus) were obtained from Ross Allen, Miami, Florida. The toads were housed at room temperature in groups of two in 10- or 15-gal aquariums one-fourth filled with wet sphagnum moss. They were hand-fed one bolus of lean horse meat weekly. Approximately 1 cc of blood was obtained by cardiac puncture, and each toad was sampled only at scattered intervals. Body weights, hematocrits, and hemoglobins were determined in order to help establish the general physical condition of the toads. The hemoglobins were determined by a method previously described (3).

All the animals were injected intraperitoneally with 35 μC of glycine-2-C14/kg body wt. Blood samples containing 0.1 ml packed cells were used for each determination. The cells were washed three times with normal saline and the protein precipitated with 10% trichloroacetic acid. The precipitate was washed, plated on a copper planchet, and counted in a gas flow proportional counter. Each sample was counted to 5,000 counts or for 1 hr, whichever came first. With this technique duplicate samples were readily reproducible to ±5%.

RESULTS

Figure 1 shows the levels of activity for the 23 turtles sampled between the 1st and the 1,180th day. It is evident that there is considerable variability in the activity, but even the lowest values are several times background. Since each point represents the value determined from a single animal, it is evident a meaningful survival curve cannot be obtained. High levels of activity were observed in all animals for the first 600 days. It had not been anticipated that such prolonged activity would be encountered, and only five turtles remained for sampling after the 600th day. These all showed some activity, but it was significantly lower than the values observed before this time. The comparatively low values from the 820th day, later including two samples taken at 2,590 days (not shown in Fig. 1), suggest that the life span of the turtle erythrocyte probably lies between 600 and 800 days.

The average weight of the box turtles at the onset of the experiment was 368 g (range 287–492 g). There was relatively little change in body weights, even after more than 7 years in the laboratory. The average hematocrit was 24.8 (range 19–29) and the average hemoglobin was 5.8 mg/100 ml (range 5.1–7.1), which are normal values for this species.

Figure 2 shows that the levels of activity observed in the toad erythrocytes are not as variable as those of the turtle and that all animals showed a substantial uptake of isotope. The open circles represent the values from animals bled only once, and the points with other symbols represent sequential samples taken from indi-
TURTLE AND TOAD RED CELL LIFE SPAN

FIG. 1. Radioactivity of turtle erythrocyte (in counts/min of packed red cells) after a single labeling with C14. Solid points are 100-day means; open circles represent samples of individual animals.

FIG. 2. Radioactivity of toad erythrocyte (in counts/min of packed red cells) after a single labeling with C14. Open circles represent animals sampled only once; other symbols show repeated sampling of same animal.

individual animals. Uniformly high levels of activity were observed up to the 700th day. Only one animal survived beyond this period. Sometime during the 4th year there was a decline in the red cell activity to a value approximately that found at 200 days (dark triangles, Fig. 2). This toad expired on the 1,520th day so it is not possible to report any low activities. The apparent life span of these cells certainly exceeds 700 days and may be close to 1,500 days.

The average body weight of the toads was 229 g (range 120–438 g) at the start of the study. Many of the toads gained weight for a considerable period, some as much as 136 g. However, during the latter half of the 2nd year the number of toads was reduced by disease. The average hematocrit value was 24.8 (range 14–29), and the average hemoglobin value was 6.2 mg/100 ml (range 3.5–7.6).

DISCUSSION

The presence of primitive erythroblasts in the extensive bone and shell marrow of the turtle and reticulocytes in the peripheral blood (3, 4) could readily account for the active uptake of C14 in turtle cells. It has been shown previously (1) that the circulating cells actively incorporate the glycine-2-C14 in vitro. In a study of the nucleated red cells of the bird (5) it was found the C14 labeling is satisfactory for determination of red cell life span despite the fact that these cells also actively metabolize during their stay in the circulating blood. If the incorporation of the isotope takes place in the mature cells we would expect extensive reutilization and a gradual decline in the activity and there should not be a prolonged period of high activity. The avian red cell showed a short period of high activity followed by a rapid decline and little evidence of reutilization. A few scattered measurements of the activity of the turtle plasma showed relatively high levels of activity up until the 50th day and uniformly low activities after the 150th day (1). These findings, plus the rapid initial incorporation observed, suggest that the labeled hemoglobin precursors do not persist in the circulation for prolonged periods. The single-sample technique used in these small species does not permit the presentation of a typical survival curve. Furthermore, the limited number of samples obtained during the decline in activity makes it very difficult to evaluate the extent of reutilization if it does occur. However, the sustained high levels of activity for at least 2 years is indicative of a long life span.

Our studies indicate that the mean life span of the turtle red blood cell is somewhere between 600 and 800 days and the red cell life span of the toad certainly may exceed 700 days. The use of C14-labeled glycine has been reported to be a satisfactory method for determination of red cell life span in mammals (2) and birds (5). We previously reported (1) that this method is apparently satisfactory for study of red cell longevity in reptiles. There is additional evidence for a long life span of the turtle red cell (6) by use of the isotope Na2Cr5104. The senescent decay of the red cells of Pseudemys had an extinction point as high as 678.9 days (personal communication, F. G. Ebaugh, Jr.).

Recently it has been found (7) that the use of 32P-labeled diisopropylfluorophosphonate (DFP32) in a study of the frog (Rana catesbiana) provided evidence of an average red blood cell life span of 24 days. Our results indicate that the longevity of the red blood cells of the box turtle is greater than any mammal or bird studied and six to eight times greater than those of man.
Due to the disparate values of longevity in the two species of amphibians reported, further work is needed in this class.

A possible explanation for these apparently long life spans is that they may be the product of the lower metabolic activity of the poikilotherms. A review of the literature (6) has shown that there is a positive correlation between metabolic rate and red cell turnover, with the animals with the lowest basal heat production showing the longest life span. In marmots maintained in a cold room the life span is longer than when they are maintained at room temperature (8). Hibernation also increased the life span of the frog red blood cells (7). Since box turtles normally hibernate it is quite likely that the life span of the turtle cells in the field would be longer than in those maintained in the laboratory. Since we have found that the life span of the toad cells appear to be longer than the turtle cells, it is possible that there may be a correlation between the life span of the red cell and the phylogenetic position of the species. Further study of this problem in the Pisces, Elasmobranchii, and Cyclostomata might be fruitful.

We wish to thank Milton Parker for technical assistance.

REFERENCES