Reprinted from The American Journal of Veterinary Research, Vol. 39, No. 2

Blood Volume Measurements in Gopher Snakes, Using Autologous ^{51}Cr-Labeled Red Blood Cells

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SUMMARY

Blood volume determinations were performed in 5 anesthetized gopher snakes (*Pituophis melanoleucus catenifer*) by means of a ^{51}Cr-labeled red blood cell (RBC) method. The mean blood volume was 52.8 ml/kg of body weight (± 6.21 SE). Previous blood volume measurements have not been reported for this species.

The RBC survival rate was estimated to be > 660 days. The RBC survival rate is long, but it cannot be determined accurately by this method.

Whole blood volume measurements have been reported in reptilian species *Alligator mississippiensis*[1] and *Chrysemys scripta elegans*,[1] but not in snakes. Because investigators are now using snakes in their studies, blood volume information would be helpful in determining the amount of blood to withdraw and the frequency of sampling. Blood volume measurements also would aid veterinarians in clinically treating and performing surgical procedures on snakes.

The purpose of this study was to determine the whole blood volume in 5 gopher snakes (*Pituophis melanoleucus catenifer*[2]).

Materials and Methods

Snakes (n = 5) were obtained from a commercial source, and each was housed in a 38-L aquarium tank at an environmental temperature of 24 C. They were fed mice each week and water was available ad lib.

Received for publication June 7, 1977.

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TABLE 1—Blood Volume of 5 Gopher Snakes Determined with ^{51}Cr-Labeled Erythrocytes

Snake No.	Sex	Weight (kg)	Hematocrit (%)	Erythrocyte volume (ml/kg)	Whole blood volume (ml/kg)
1	♀	1.26	32.0	11.2	35
3	♂	1.03	33.0	16.5	50
5	♂	0.72	33.0	24.8	75
6	♀	0.34	29.0	15.4	53
8	♀	0.49	30.0	15.3	51
$\bar{X}$ ± SE			31.4 ± 0.81	16.6 ± 2.23	52.8 ± 6.21

The procedure used to collect blood has been previously described.[3] Blood samples were obtained from the exposed caudal vena cava of snakes which were anesthetized with halothane,[a] nitrous oxide, and oxygen.

For blood volume determinations, 2 ml of heparinized blood was collected from the snakes, and separated erythrocytes (RBC) were labeled with ^{51}Cr by a modification of the method reported by Zehr et al.[4] The blood sample was placed in a small glass tube and centrifuged to separate the RBC. A plasma-saline solution was prepared by adding 0.2 ml of plasma to 10 ml of sterilized 0.9% saline solution. The remainder of the plasma was discarded. Approximately 2 to 3 μCi of [^{51}Cr] Na_2 CrO_4 in a volume of 1 ml of isotonic saline solution was then added to the packed RBC, and the contents were mixed by inversion. Cells were incubated with the isotope at 30 C for 30 minutes and then were washed twice with the plasma-saline solution. Washed cells were diluted with the plasma-saline solution to an approximate volume of 2.5 ml. A 0.5-ml aliquot of the labeled blood was put into a glass vial to be counted. At time zero, 2.0 ml of the labeled blood was injected intravenously into the snake for determination of total injected counts. The entire labeling and injection procedure was performed under sterile conditions.

Heparinized blood samples (approximately 1.5 ml) were obtained at 3 hours, 22 hours, and then at various intervals of time for the following 54 days.

Blood samples (1 ml) were counted in a gamma counter[b] to a total accumulated count of 3,000. The background was 30 counts per minute (cpm). The counting efficiency for ^{51}Cr was 3%. An aliquot of a sample of the injected, ^{51}Cr-labeled cells was counted each time in order to correct for radioactive decay and to allow the calculation of the total counts injected.

The blood volume was calculated from blood samples collected at 3 hours and 22 hours by means of the equation:

$$V = Q/C,$$

where Q is the total count of ^{51}Cr injected, as determined by the standard sample, C is the cpm/ml of sampled blood, and V is the circulating (whole) blood volume. The RBC volume was calculated by multiplying the circulating (whole) blood volume by the hematocrit value.

An estimation of the RBC survival was made on data corrected for the amount of isotope and blood removed at each sampling.

Results

The mean whole blood volume for the 5 anesthetized gopher snakes was 52.8 ml/kg of body weight (± 6.21 SE; Table 1). The average hematocrit value was 31.4% (± 0.81 SE), and the average RBC volume was 16.6 ml/kg of body weight (± 2.23 SE).

The RBC survival rate was studied in 4 of the 5 gopher snakes. After 54 days, there was no marked decline in the ^{51}Cr activity; hence, little elution of the ^{51}Cr from the RBC could have occurred. Assuming a 5% overall error in techniques and given the 50-day sample period with no decline in specific activity of the RBC, the RBC sur-

[a] Fluothane, Ayerst Laboratories, New York, NY.
[b] Nuclear Chicago Corporation, Searle Analytic Inc, Des Plaines, Ill.

vival rate must have been > 660 days in these snakes.

Discussion

Range of measurements of the whole blood volume of 5 gopher snakes was 35 to 75 ml/kg of body weight. It is not known whether this large variation is a result of the methods used or of biological differences between the snakes. In order to explain the results properly, a further study involving at least 20 snakes is necessary, and blood volume determinations using other methods, such as ^{59}Fe-incorporation, would be helpful.

There are some limitations in blood volume measurements obtained using hematocrit methods, but we do not feel that they markedly affected our results. The RBC of the snake are elliptic-shaped cells which do not pack well and which may trap considerably more plasma, thereby altering RBC and plasma volumes. It is also possible that some fraction of the RBC may have been removed rapidly from circulation during the first 30 minutes. However, all blood volume determinations were performed using the same standard set of conditions.

Although there is large variation in whole blood volumes, we feel that the mean value of 52.8 ml/kg of body weight is accurate.

Results are in agreement with the blood volume value of 55.9 ml/kg of body weight reported in *A mississippiensis*.[5] Using the ^{51}Cr-labeled RBC method, the average RBC volume for *A mississippiensis* (at 24 C, weighing between 0.62 and 1.3 kg, and anesthetized with sodium pentobarbitol) was 12.6 ml/kg of body weight (± 0.34 SE).[5] The average RBC volume of the gopher snakes was 16.6 ml/kg of body weight (± 2.23 SE), which is not markedly different. The mean venous hematocrit of the *A mississippiensis* was 22.6% (± 0.64 SE), whereas the mean venous hematocrit of the gopher snakes was 31.4% (± 0.81 SE).

In comparing blood volume measurements, the method used for calculating the value should be known, because blood volume measured by the RBC tracer method underestimates the total volume by 10%.[6] For example, the blood volume calculated by the RBC volume and hematocrit in *A mississippiensis* was 55.9 ml/kg of body weight.[5] However, using the RBC volume and the plasma volume values, the blood volume was 72.7 ml/kg.[1,5] Differences in the blood volume values were due to the retention of plasma in the organs.

Using a plasma volume determination method and hematocrit, the blood volume of *C scripta elegans* was estimated to be 90.8 ml/kg of body weight.[1] However, this value may not be compared directly to the blood volume values determined for the gopher snakes by the ^{51}Cr-labeled RBC method.

Marked differences in the calculated blood volume results between the 3-hour and the 22-hour samples were not detected. Therefore, we can assume that adequate mixing and equilibration of the ^{51}Cr-labeled RBC occurred within 3 hours.

After sampling over a 50-day period, we could not detect any marked decline in the ^{51}Cr bound to the circulating RBC. We estimated that the RBC survival rate must be > 660 days, assuming a 5% overall error. A long life of RBC also is reported in turtles (600 to 800 days).[7]

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