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Blood Volume Parameters of a Poikilothermal Animal in Hypo- and Hyperthermia.* (26900)

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With the current interest in blood volume changes in warm-blooded animals produced by alteration in body temperature (particularly hypothermia) it appears logical that these phenomena be investigated in a poikilothermal animal as a possible source of insight into the nature of physiologic adjustment of blood volume to changes in body temperatures. Several common cold-blooded laboratory animals were considered for this study but due to ease of maintenance, size, and phylogenetic "highness" the alligator is the animal of choice.

Methods. Animals used in this study were 66 American Alligators, *Alligator mississippiensis,* weighing 615-1300 g and measuring 66.5-82.0 cm in length. The animals were obtained from Louisiana, kept in a shallow tank without food, and used within 2 weeks of the time they arrived but not during the first 3 days. Approximately an equal number of males and females were used, and no significant difference between sexes was noted with regard to the parameters studied.

The alligators were anesthetized using 30 mg/kg sodium pentobarbital injected intraperitoneally; anesthesia administered approximately 1½ hours before the experiments were begun proved to be most satisfactory. Cr^{51} tagged red cells were used to determine cell volumes, and I^{131} tagged serum albumin (RISA) was used to measure plasma volumes. The red cells to be tagged were obtained from donor alligators early in the day and tagged according to the technic of Gray & Sterling(1). Donor alligators were used only once although some recovered from this relatively massive bleeding (approximately 18 cc). A suspension of 5 cc of washed, tagged red cells suspended in enough 0.9% saline to approximate the normal hematocrit was given to each animal in the Cr^{51} series immediately after removal of a like volume of their own blood. The tagged blood-cell suspension was washed in with 1 cc of saline and an additional 1 cc of saline was injected slowly during the experimental period (about one hour).

Both withdrawal and injection, as well as subsequent bleeding, were done through an indwelling cardiac needle introduced by puncture. An initial hematocrit determination was made for each animal using the first blood drawn from the heart; for this determination micro-technics were used. Hematocrits were also measured for donor alligators. These hematocrits are subsequently called venous hematocrits although they may represent mixed venous and arterial blood.

In the I^{131} series one cc of a solution of RISA appropriately diluted in 0.9% saline was injected after blood had been withdrawn for hematocrit determinations. The same washing-in technic was used as in the case

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of the Cr^{51} series. The anti-coagulant used in all cases was polyanhydromanuronic acid sulfate (Manuronate—Wyeth Laboratories). One half-hour, found by previous experiments, was allowed for mixing of the isotopes. Each experimental group consisted of 3 animals. At the close of the mixing period cloacal temperatures were ascertained. After this one animal was set aside to continue at room temperature (22.5°C-26.5°C-av. 24°C) until termination of the experiment 30 minutes later; one animal was enclosed in a warming blanket and warmed as rapidly as possible to a cloacal temperature of 35°C; and the third animal was packed in crushed ice and chilled to a cloacal temperature of 5°C. The intention was to reach the experimental temperatures in 30 minutes. The actual warming time ranged from 25-60 minutes, averaging 40 minutes. Animals were bled rapidly and pithed when the desired temperature was reached but not earlier than 30 minutes. The terminal temperature in 80% of the animals was 35°C, in 5%–36°C, and in 15%–37°C. Cooling required 20-60 minutes, averaging 36 minutes. These animals were killed when body temperature reached 5°C. The terminal temperatures were 5°C for 70% of the animals, 4°C for 25%, and 3°C for 5%. After killing, the alligators were dissected as rapidly as possible; the organs were blotted, weighed, and digested in nitric acid or KOH as was appropriate to the tagging technic used. Radioactivity was determined for blood samples and aliquots of the solutions of organs.

Considerable effort was made to keep trauma and blood loss minimal. A previous series of 42 animals(2) had been subjected to the open chest technic with relatively high blood losses, and precautions were taken to avoid excessive blood loss in this series. However, the results obtained in the 2 series of animals seem to be in substantial agreement. Data for each animal were corrected to a standard total body count of one million and a standard total body weight of 1000 g. Tables I and II include the principal data obtained; methods used in calculating specific blood volume parameters and level of significance of the data are included within the tables.

Results and discussion. The average plasma volume of alligators kept at room temperature is 60.1 cc with a standard error of ±3.88 cc per 1000 g; average cell volume for this same group of animals is 12.6 cc ± 0.34 cc per 1000 g. Thus total circulating blood volume is 72.7 cc/1000 g of body weight. The circulatory hematocrit,

$$\text{Circulatory hematocrit} = \frac{\text{cell volume}}{\text{cell volume} + \text{plasma volume}} \times 100,$$

TABLE I. Mean Measured and Calculated Blood Volume Parameters, with Standard Errors, for the Alligator.

Treatment group	PV, ml/kg	CV, ml/kg	VH, %	CH,* %	BVR cells†	BV_1,‡ ml/kg	BV_2,§ ml/kg	Residual plasma,‖ ml/kg	BVR plasma¶
A Room temp. 25°C	60.1 ± 3.88	12.6 ±.34	22.6 ±.64	17.3	.76	72.7	55.9	16.8	1.07
B Chilled 5°C	56.6 ± 4.11	12.4 ±.12	19.8 ±.69	17.9	.90	69.0	62.3	6.7	1.02
C Warmed 35°C	72.8 ± 3.77	12.6 ±.82	23.0 ±.52	14.8	.64	85.4	54.8	30.6	1.11

P values for differences between means

$PV_A - PV_B = 3.43$	not significant
$PV_A - PV_C = 12.68$	<.05
$PV_B - PV_C = 16.11$	<.02
$VH_A - VH_B = 2.47$	<.02
$VH_C - VH_A = .66$	not significant
$VH_C - VH_B = 3.13$	<.01

PV = Plasma vol
CV = Cell vol
VH = Venous hematocrit
CH = Circulatory hematocrit

* Circulatory hematocrit = CV/PV + CV × 100.
† BVR cells = Blood vol ratio cells = CH/VH.
‡ $BV_1 = CV + PV$.
§ $BV_2 = CV/VH$.
‖ Residual plasma = $BV_1 - BV_2$.
¶ BVR plasma = Blood vol ratio plasma = 100–CH/100–VH.

TABLE II. Cell and Plasma Volumes (ml/kg), with Standard Errors, for Organs of Alligators.

	Heart	Spleen	Liver	Lungs	Stomach	Intestine	Kidney	Total viscera	Muscle	Skin
A—Room temp.										
PV	.710	.211	2.973	1.221	1.114	1.377	.880	8.486	1.121	1.417
	±.049	±.015	±.195	±.041	±.103	±.122	±.095		±.163	±.181
CV	.097	.089	.301	.326	.193	.237	.110	1.353	.286	.292
	±.017	±.023	±.017	±.022	±.021	±.028	±.005		±.022	±.026
Hematocrit	12.0%	29.8%	9.2%	21.1%	14.7%	14.6%	11.1%	13.7%	20.3%	17.0%
B—Cold										
PV	.677	.266	2.870	1.237	1.057	1.334	1.010	8.451	.841	1.406
	±.027	±.012	±.161	±.065	±.056	±.090	±.085		±.043	±.075
CV	.089	.399	.417	.323	.152	.234	.101	1.715	.233	.281
	±.007	±.037	±.022	±.016	±.011	±.011	±.007		±.014	±.027
Hematocrit	11.5%	60.0%	12.6%	20.7%	12.6%	14.9%	9.1%	16.8%	21.6%	16.6%
C—Warm										
PV	.821	.243	3.943	1.866	1.190	1.952	1.302	11.317	1.219	2.886
	±.055	±.011	±.274	±.190	±.133	±.129	±.138		±.104	±.296
CV	.060	.070	.307	.337	.146	.141	.076	1.137	.261	.292
	±.005	±.014	±.046	±.029	±.021	±.016	±.011		±.036	±.024
Hematocrit	6.8%	22.4%	7.2%	15.3%	10.9%	6.7%	5.4%	9.1%	17.6%	9.1%
	P values for differences between means									
$PV_A - PV_B$	—	—	—	—	—	—	—		—	—
$PV_C - PV_A$	—	—	<.05	<.01	—	<.01	<.05		—	<.01
$PV_C - PV_B$	<.05	—	<.01	<.01	—	<.01	—		<.01	<.001
$CV_B - CV_A$	—	<.001	<.01	—	—	—	—		—	—
$CV_A - CV_C$	—	—	—	—	—	<.05	<.05		—	—
$CV_B - CV_C$	—	<.001	<.05	—	—	<.001	—		—	—

PV = Plasma vol CV = Cell vol

is therefore 17.3 compared with a venous hematocrit of 22.5 ± 0.64. However, if blood volume is calculated from cell volume and venous hematocrit, it is only 55.9/1000 g giving a residual plasma volume(3) of 16.8 cc/1000 g not in the venous circulation but retained in the organs. This viewpoint is reinforced by the data on organ hematocrits, which, with the exception of the spleen, are lower than the venous hematocrits, most of them significantly so; skin and muscle hematocrits are also lower than venous hematocrit. Only the spleen, lungs, and muscles have hematocrits higher than circulatory hematocrit.

Comparing these data from a cold-blooded animal with those from a warm-blooded animal, the dog, shows several differences. The dog's plasma volume was 9.9/1000 g of tissue less than that of the alligator. The difference is significant at the <.01 level. Venous and circulatory hematocrits for dogs are higher than those of alligators; Deavers *et al.*(4) reported a venous hematocrit of 45.2% ± 0.73 and a circulatory hematocrit of 40.2% with a BVR cells ratio of 0.89.

$$\text{BVR cells} = \frac{\text{circulatory hematocrit}}{\text{venous hematocrit}}$$

For alligators this same ratio is 0.76. Finally total circulating blood volume is higher in dogs, 88.7 cc/1000 g, *i.e.*, 16.0 cc/1000 g greater than that of the alligator.

When alligators are cooled rapidly to a body temperature of 5°C there is usually a drop in venous hematocrit and the difference between the average hematocrit of these animals (19.8 ± 0.69) and of those kept at room temperature for the same length of time is 2.7%. This is in contradistinction to the reaction of the dog where cooling raises the hematocrit. Average plasma volume (56.6 ± 0.12), however, remains relatively constant; and therefore the circulatory hematocrit is not affected significantly, being 17.9%. Total visceral plasma volume (the

sum of volumes for the 7 organs studied) for the cooled group remains the same and total visceral cell volume increases slightly; however, the only statistically significant difference is the increase in cell volume of the spleen. The change in venous hematocrit could be explained if there were a "trapping" or sequestering of red cells by the spleen and liver, and to some extent by a fall in total muscle plasma volume. Total residual plasma volume, however, decreases from an initial volume of 16.8 cc to a level of 6.71 cc/1000 g in cooled alligators. Other changes must be postulated in addition to the cell volume changes in spleen and liver to explain the magnitude of the fall in venous hematocrit; possibly the "lost" plasma of the muscles is added to the venous circulation.

Rapid warming of alligators to 35°C, approximately 10°C higher than the temperature to which they had been acclimated, caused no significant change in venous hematocrit (22.9 ± 0.52) although there was an upward trend in the hematocrits. There was a significant increase in total plasma volume to 72.8 ± 3.77 cc/1000 g but cell volume remained constant. As a result, the circulatory hematocrit fell to 14.75 and the volume residual plasma increased to 30.5/1000 g. Significant increases in organ plasma volumes were noted in the liver, lungs, intestine, kidney, and skin. Significant decreases in cell volumes were noted for intestine and kidneys, and, in addition, total visceral cell volume fell. Thus, total visceral plasma volume increased, while total visceral cell volume decreased. The venous hematocrit, therefore, may increase sightly in a majority of normal animals in spite of widespread increases in plasma volume because of release of red cells from viscera, particularly the intestines and kidneys. At the same time circulatory hematocrit falls because of the increases in plasma volume presumably coming from fluid released by extra-circulatory sources into the small vessels of the viscera, skin and possibly the muscles, but more specifically into the liver, lungs, intestines, kidneys, and skin.

Summary. Cooling the alligator rapidly to 5°C results in a lowered venous hematocrit but does not cause significant change in circulatory hematocrit. Total visceral cell volume tends to increase, although of the individual organs only one, the spleen, has a statistically significant increase in cell volume. Rapid warming of alligators to temperature of 35°C results in no significant change in venous hematocrit but in a significant fall in circulatory hematocrit. Organ cell volumes in general are lower, that of the intestine and kidneys significantly so. This release of cells must account for the maintenance of a constant venous hematocrit in spite of a great increase in plasma volume not accounted for solely by the significant increases in organ plasma volumes.

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Antigenic Properties of Liver Cell Fractions Derived from Laying Hens and Estrogenized Chickens. (26901)

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It is known that there are phosphoproteins in the sera of laying hens and estrogenized chickens, but not in the sera of non-laying hens, cocks and immature chickens, and that they are produced in the liver by the effects of estrogens(1,2,3,4). The fluctation of