THE EFFECTS OF PROLONGED HYPOTHERMIA ON BLOOD VOLUME PARAMETERS OF THE ALLIGATOR, ALLIGATOR MISSISSIPPIENSIS

A Thesis

Presented to

The Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Richard Angelo Percoco

January 1965

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THE EFFECTS OF PROLONGED HYPOTHERMIA ON BLOOD VOLUME PARAMETERS OF THE ALLIGATOR, ALLIGATOR MISSISSIPPIENSIS

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ABSTRACT

The radioactive isotope dilution technique was used to measure blood volume parameters of young alligators, Alligator mississippiensis (Daudin), subjected to prolonged hypothermia. Specimens weighed 700-2400 grams and measured 66-86 centimeters in length. I^{131} tagged serum albumin (RISA) and Cr^{51} labeled red cells were used to measure the plasma volume and red cell volume, respectively. Also these isotopes were used to determine the blood volume of various organs. Further, the disappearance of isotopes from the circulation over a 48 hour period was measured. Hematocrits were determined by a micromethod (venous hematocrit) and by calculation (circulatory hematocrit). All determinations were made both at room temperature (24-25°C) and at a low temperature (3-4°C).

Cooling the alligator resulted in significantly lower venous and circulatory hematocrits and circulating red cell volume. The circulating plasma volume remained unchanged. The total visceral red cell volume increased; individual organs showing significant differences were the spleen, the liver, the lung, the stomach, and the intestine. The red cell volumes of the heart, the kidney, muscle, and skin remained unchanged. The total visceral plasma volume was also higher, with significant increases in the heart,

the spleen, and the kidney. Total blood volumes of visceral organs were, therefore, higher, particularly those of the heart, spleen, liver, lung, intestine, and kidney. The calculated hematocrits for most of the organs taken from the hypothermic group were slightly higher than those of the control group; cold materially increased the hematocrit of the spleen from 49.6% to 70.5%.

The percent of I^{131} serum albumin remaining in the circulation at the end of the experimental period was $43.3 \pm 2.24\%$ with no significant difference between the room temperature and cold room groups. For Cr^{51} tagged red blood cells the percent retained in the circulation after 48 hours was $80.1 \pm 2.04\%$. There was no significant difference between the room temperature and cold room groups.

I^{131} excreted in the urine in 48 hours was $1.05 \pm 0.19\%$ of the total counts injected while Cr^{51} excreted was $1.73 \pm 0.35\%$ of the original dosage. Thyroid uptake of I^{131} for the 48 hour period was $0.35 \pm 0.83\%$ of the original dosage.

PART I

INTRODUCTION

Review of the Literature

In the previous studies carried out in this laboratory (Huggins, 1961 and Huggins, PHGH-4318) it was shown that when alligators were warmed or cooled for a period of 30 minutes a number of changes in red blood cell and plasma volume parameters occur. Alligators whose body temperatures were increased to 30°C responded with a 20% increase in total plasma volume but the venous hematocrit and the red cell volume remained unchanged. Thus, there was a significant drop in the circulatory hematocrit (CH= CV/PV+CV where CH is the circulatory hematocrit, CV is the measured red cell volume, and PV is the measured plasma volume). The volume of the residual plasma (residual plasma= BV_1-BV_2 where BV_1 is the measured plasma volume plus the measured cell volume, and BV_2 is the cell volume/venous hematocrit) increased with the rise in body temperature. Significant increases in the organ plasma volumes were noted in the liver, the lungs, the intestine, the kidney, and the skin while significant decreases were observed in the organ cell volumes of the intestine and the kidney. Reflecting these specific changes the total visceral plasma volume increased while the total visceral

cell volume decreased, and there was a marked fall in visceral hematocrit.

Alligators studied at body temperatures of 5°C had a significantly lower venous hematocrit than those at room temperature. No significant change in plasma volume or in the cell volume occurred in a 30 minute cooling period and therefore the calculated blood volume was unchanged. The change in the hematocrit could be accounted for in one of two ways: a discharge of residual plasma from organ reservoirs or a sequestration of red blood cells. The significant increase in the red cell volume of the spleen and liver taken together with the apparent increase in the total visceral cell volume indicated that the sequestration of red blood cells rather than the release of plasma from organ reservoirs probably accounted for the fall in the venous hematocrit.

Other investigators (Rodbard, Saiki, Molin, and Young, 1951) using chicks and rabbits, cooled 15°C and 10°C respectively below their normal body temperature, found increases of 3% in the chicks' hematocrit and 3-15% in the rabbits' hematocrit. They also noted a one third reduction in the plasma volumes of both the chicks and rabbits. It was postulated by the above investigators that these changes were the result of the opening and closing of the various vascular areas. Further, it was suggested that the

control of this opening and closing of these vascular areas was under the control of the central nervous system.

Other investigators (D'Amato and Hegnauer, 1953), using dogs subjected to hypothermia (to 20°C), found that the plasma volume decreased by about 12% but that the red cell volume was not altered. It was suggested that the plasma unaccounted for might be trapped in the peripheral vasculature or it might have entered the extravascular spaces. Rodbard et al. (1951) reported a decrease in the thiocyanate space, supporting the former suggestion.

In dogs cooled to a rectal temperature of 27°C, Farrand and Horvath (1959) reported that the blood volume increased during the initial cooling period (three hours) and remained at the increased level during the period of study (three hours). During the initial period there was an increased hematocrit and red cell volume while the plasma volume remained unchanged. In addition there was no change in the extracellular or intracellular fluid volume during any period of the study as measured by antipyrine.

Cooling dogs to 15-20°C, Kahler et al. (1962), found a significant decrease in the total peripheral vascular resistance and a significant augmentation of the blood volume. Decreases in "actively" circulating blood volume were measured (indicator dye method) despite the

observed increase in the total systemic blood volume. The hematocrit increases averaged 1% during the period of cooling. To account for these observations it was postulated that in hypothermia arteriolar dilation occurred and that substantial volumes of blood were "trapped" in the peripheral vascular "tree". By an indicator dilution method the systemic blood volume showed a decline. It was suggested that a large volume of blood was sequestered in the vascular areas and that during the dye's first passage it did not disperse well into the vascular areas. No pooling was found to occur in either the spleen or the liver and therefore it was proposed that the pool of sequestered blood was distributed throughout the vascular areas. Furthermore, the decrease in venous return as well as the observed increase in total systemic blood volume might well be explained by the trapping of blood in the vascular areas.

Fedor and Fisher (1959) found that in lightly anesthetized dogs with a rectal temperature of 23-24°C, the red cell volume measured with Cr^{51} remained unchanged and that the plasma volume, measured with T-1824, decreased during the experimental period. These workers suggested that the increased hematocrit occurred because of the loss of plasma rather than fluid.

Willard and Horvath (1961) reported that in young rats (Wister strain) with hypothermically induced cardiac

arrest (60-70 minutes, body temperature 8-9°C) there were increases in red cell volume in the liver, lung, and spleen. There were no significant differences in hematocrits, blood volumes, or body weights between normal and hypothermic animals. The largest changes in the blood volume occurred in the spleen, which increased markedly over the normothermic animals. Hypothermia, therefore, tended to induce redistribution of red cells toward the visceral areas of the animal.

Beaton (1961) reported that rats subjected to body temperatures of 15°C had increased hematocrits, mean red cell corpuscular volumes and serum protein levels. The increased hematocrit was thought to be a consequence of both a decrease in plasma volume (rise in serum protein level and a fall in water plasma content) and an increase in mean corpuscular volume of erythrocytes. Orme and Beaton (1962), working with hamsters and rats, reported that in hypothermic hamsters (body temperature 15°C) there was increased hematocrit and erythrocyte volume, but that the mean corpuscular volume, hemoglobin content, and the serum protein levels were not altered. These findings suggest that in hypothermic hamsters, unlike hypothermic rats, the increased hematocrit resulted from an increased release or decreased destruction of erythrocytes, rather than an increase in the size of the red cell and decreased

plasma volume. Strong supporting evidence was the finding of an increased production of reticulocytes in the blood.

Disappearance of Isotopes

Erlanger (1921) was the first to suggest that the dye T-1824 disappeared from the circulation at a nearly constant rate after mixing. He suggested, therefore, that it was possible to extrapolate from the measured values on semi-log paper to zero time and obtain the plasma volume at that time.

Fine and Seligman (1944), using dogs, observed that 10-25% of the I^{131} tagged serum albumin left the circulation within the first hour after injection. Supporting this observation, Gitlin (1957) reported a very rapid fall in concentration of labeled serum albumin in the plasma during the first hour, then a slower rate and finally at a still slower rate that was constant. Huggins, et al. (submitted for publication) measured the rate of albumin exchange between the circulation and the extravascular spaces and suggested that disappearance of this protein from the vascular space probably occurs at three rates: a rapid loss during the first eight hours, a slower rate lasting 3-4 days, and finally a still slower rate until degradation of the albumin is completed at approximately two weeks.

Sears, Allen and Gregersen (1954), using dogs, reported 60-65% disappearance of I^{131} labeled albumin from the circulation after 48 hours at room temperature. Armstrong, et al. (1960), using rabbits, suggested that the albumin "leaked" into the gastrointestinal areas where it was hydrolyzed.

I^{131}, when detached (Cohen, et al. 1955) from the albumin, was rapidly and almost completely excreted in the urine. A measure of I^{131} in the urine could, therefore, provide an accurate measure of the elimination rate for albumin. It was also reported that no detectable re-incorporation of I^{131} was found in newly synthesized albumin. Earlier work by Berson, et al. (1953) reported that the radioactivity released by degradation of the labeled albumin was almost completely accounted for by the renal excretion in the presence of thyroid blockage.

The substitution of I^{131} in the molecule of albumin, as discussed by McFarlane (1956), takes place predominantly in the tyrosine ring and was associated with little or no change in the physical properties of the protein. (At higher combining ratios other parts of the protein were iodinated and an abnormal sedimentation rate was observed, suggesting that the structure of the protein was altered in some way). Sterling and Gray (1950) reported that when blood was incubated with radioactive

sodium chromate the unbound chromate was reduced by ascorbic acid. This prevented further binding by the red blood cells and the residue was removed by washing several times with saline. Ebaugh and Ross (1953) reported that the bonding of Cr^{51} to red blood cells was very rapid and quite stable; the rate of chromate elution from the red cells had a half time of 77±12 days as compared with a half time of 12-39 days for Fe^{59} labeled red cells. The chromium released from the donor cells as a result of elution or hemolysis was cleared rapidly from the circulation and excreted in the urine and feces; none reappeared in the recipient's erythrocytes. Talbot(1955), working with seven healthy men, reported that red blood cells labeled with Cr^{51} disappeared from the circulation at a constant rate which could be expressed as a straight line on semi-logarithmic plots. During the first forty-eight hours, in some cases, an acceleration of disappearance of the red blood cells was noted. Mollison and Veall (1955) suggested that the elution half time varied from 45 to 77 days and that the loss of Cr^{51} from the circulation was more rapid at first than it was subsequently. Jones and Mollison (1956) found no significant evidence for an increased loss or disappearance of Cr^{51} tagged red blood cells during the first forty-eight hours. These workers also showed that washing the tagged red cells with isotonic

saline did not alter or interfere with their subsequent survival.

Cline and Waldman (1962) reported that the frog's red cell life span at 24-26°C was approximately 200 days and at 4°C it was apparently even longer. The mean hematocrit for these amphibians at 4°C was lower by about 18% than those frogs kept at 24-26°C. The erythrocyte survival time at 4°C was longer but there was a significant decrease of erythropoesis at low temperatures. Working with alligators, Cline and Waldman (1962-b) reported that in these animals, whose metabolism, like frogs, was temperature dependent, the red cell showed an increased longevity at lower temperatures. In alligators and frogs the erythrocyte life span was therefore inversely related to the environmental temperature. These workers also reported that when alligators were maintained at 30°C the red cell life span was about 184-412 days. Subjecting these alligators to temperatures of 16°C (cloacal temperature) for seven months, there was a loss of body weight and an increased red cell life span to 1200 days. From the data reported these workers suggest that alligators subjected to hypothermia show significant decreases in red cell synthesis and that the alligator erythrocyte survival time is temperature dependent.

Statement of the Problem

In the studies of blood volume parameters that have been reviewed so far, the magnitude and the direction of these parameters have not followed any set pattern in response to cold stress.

The questions asked by us were two: Was there a significant change in the alligator's blood volume parameters when subjected to deep hypothermia? If so, how similar or different were these changes from those of warm blooded animals subjected to hypothermia? The specific parameters studied were the plasma volume, red cell volume, circulatory and venous hematocrits, organ cell volumes, plasma volumes, and organ hematocrits.

In general, animals exhibit two ways of adjusting to hypothermia: homeotherms regulate their internal core temperature in such a way as to avoid extremes of temperature in most tissues while poikilotherms adjust by avoidance of extreme temperatures or if subjected directly to cold their body temperature follows the environmental temperature and their metabolic rate falls. Under certain conditions homeotherms either lose or have not developed the ability to control their body temperature and behave more like poikilotherms.

With the current interest in deep hypothermia as an adjunct to surgery and as a potential therapeutic tool,

a better understanding of the physiology of hypothermia has become a matter of great importance. The clarification of the response to cold made by a poikilotherm may lead to a better understanding of the physiological emergency imposed upon the homeotherm by the lowered body temperature. In the cold blooded animal a greater body temperature change can be effected without imposing upon the animal as great a stress as on the homeotherm thus making it possible to study with relative ease the changes in blood volume.

PART II

MATERIALS AND METHODS

General Procedure

The animals used for this study were young alligators, Alligator mississippiensis (Daudin), weighing 700-2400 grams and measuring 66-86 centimeters in length. The animals were obtained from a commercial source, were usually kept in a shallow tank without food, and used within three weeks of the time they arrived. If the animals were kept for a longer period of time they were placed in a large cage containing water pans and were fed chopped fish or crayfish. Approximately an equal number of males and females randomly selected were used. A previous study (Huggins, 1960) found that there was no significant difference between sexes with regard to the parameters to be studied.

All alligators were lightly anesthetized using ether. The time required to produce the desired level of anesthesia was 5-10 minutes. An earlier study (Harris, 1960 unpublished) investigating several anesthetics found that ether was most suitable for alligators which were to be subjected to hypothermia.

Cr^{51} tagged red blood cells, prepared according to the technique of Gray and Sterling (1950) modified by

Deavers, Smith and Huggins (1960), were used to determine cell volume. I^{131} tagged serum albumin (RISA Abbott laboratories) was used to measure plasma volume. The red cells used were obtained from donor alligators (see below).

All alligators were weighed and measured, their sex was recorded, and a blood sample was taken for a microhematocrit determination. All bleeding and injections were done through an indwelling cardiac needle (number 22 gauge) introduced by puncture (Huggins, 1960). In some cases additional blood samples were withdrawn for investigations of the numbers and types of blood cells present and for electrophoretic analysis of plasma proteins. (The latter determinations were carried out by other investigators).

The hematocrits were determined by a micromethod using heparinized capillary tubes (13mm by 75mm) centrifuged for three minutes in an International Microcapillary Centrifuge, Model M.B., and read on an International Capillary Microhematocrit reader, Model C.R. These hematocrits are subsequently called venous hematocrits although they may represent either or both arterial and venous blood, due to the structure of the heart and the position of the intracardial needle.

Specific Procedures

Determination of cell volume

In the Cr^{51} series the procedure was as follows:

a donor alligator was anesthetized and 14-16 milliliters of blood was removed and tagged according to the technique of Deavers, Smith and Huggins (1960) as follows: The 14 ml. of blood was incubated at room temperature with sodium radiochromate (Cr^{51}) for thirty minutes. At the end of this period 2 ml. of a 2.5% solution of ascorbic acid was added to reduce the remaining unbound chromate to chromic chloride. Donahue and workers (1955) suggested that the anionic hexavalent chromium penetrated the red blood cells, was reduced to trivalent chromium, and bound to the protein of the cell, probably the globin of the hemoglobin. The blood was then centrifuged for ten minutes, the plasma decanted, and the cells washed with 0.9% saline solution. This procedure was repeated twice and the tagged cells were then suspended in enough saline to approximate the original volume of the blood. A 1 ml. sample of this suspension was set aside to serve as a control and was corrected for geometry by diluting with 1 ml. of anticoagulant. The anticoagulant used in all cases was polyanhydromanuronic acid sulfate (Manuronate - Wyeth Laboratories).

Two alligators were lightly anesthetized and the following procedure was used for each: a) An intracardial needle was introduced. b) Five milliliters of blood was withdrawn for microhematocrits and other determinatioss. c) Five milliliters of the suspension of tagged red cells

was injected followed by one milliliter of saline. The needle was left in place for five minutes to reduce bleeding from the cardiac puncture. After the needle was removed the alligator was released. Both alligators were marked and allowed to recover at room temperature for 24 hours before being subjected to prolonged hypothermia. A previous study (Harris, 1960 unpublished) indicated that a recovery period of 24 hours was necessary if the previously anesthetized animals were to survive the experimental cold period. After this period, one alligator was left at room temperature (24-25°C), while the other was subjected to hypothermic conditions (3-4°C) for 24 hours by being placed in a large cold room. In subsequent sections of this paper these will be referred to as room temperature and cold or hypothermic animals, respectively.

After the 24 hour experimental period, the alligators were again lightly anesthetized and cloacal temperatures were determined. Since both animals could not be operated on at the same time for terminal dissections, the procedure was alternated for each experiment. One milliliter of blood was withdrawn from the heart and placed in a vial with an equal amount of manuronate for radioactive monitoring. Additional blood was withdrawn for microhematocrit readings. The animal was then killed by rapid exsanguination followed by pithing. This bleeding yielded about 20-24

milliliters of blood which was made available for differential counts of blood cells and for determination of plasma proteins. The organs excised were: heart, spleen, liver, lungs, stomach, intestine, and kidney. Also removed were samples of the muscle and skin from the regions of the tail, forelimb, hindlimb, and chest. Each of the organs and tissue samples was removed as quickly as possible, blotted, and weighed. They were placed in beakers and digested with concentrated HNO_3 for a period of 24 hours. The resulting solutions were measured and 2 milliliter aliquots of each organ or tissue were placed in vials for radioactive counting.

Determination of plasma volume

In the I^{131} plasma albumin series one milliliter of the solution appropriately diluted in saline was injected via an intracardial needle puncture after a similar volume of blood had been withdrawn for the initial hematocrit determination and cell counts. The same wash-in technique was used as in the Cr^{51} series. From the tagged albumin solution one milliliter was used for the standard and to this one milliliter of distilled water was added to correct the geometry of the sample. The same procedure was followed as in the Cr^{51} series, as far as the number of animals, time of experimental period, organs and tissues excised, volume of blood removed, etc., except that the organs and

tissues were digested in 40% KOH and this usually required 48 hours.

All samples, including the organ samples, muscle and skin samples, blood samples and standards of both the control and the hypothermic animals were monitored for radioactivity at the same time using a deep well scintillation counter (Tracer-lab 1000 Scaler). All counts of Cr^{51} and I^{131} were made for two minutes at 1100 volts. Each count was repeated once and the mean of the two counts was used in subsequent calculations.

Data from each animal were corrected to a standard total body count of one million and a standard body weight of one thousand grams.

Determination of the disappearance for I^{131} tagged albumin

A total of 14 alligators, weighing 700-1400 grams, selected at random, divided into groups of two, one for a control to be held at room temperature, one for the hypothermic condition, were used. Each was lightly anesthetized with ether and the usual procedure for determining plasma volume was followed. At the end of 24 hours one animal was placed in a cold room (3-4°C) for 24 hours, the other was left at room temperature (24-25°C) for the same period of time. Beginning one half hour after the initial injection one half milliliter samples of blood plus microhematocrit samples were taken. The second sample was drawn

PART III

RESULTS

The Disappearance of I^{131} Tagged Albumin

The data related to the disappearance of I^{131} tagged serum albumin for 8 animals at room temperature (24-25°C) and for 8 animals in the cold room (3-4°C) are presented in Table I.

After 48 hours the animals kept at room temperature retained in the circulation 45.1±2.46%[1] of the counts present at the end of the half hour mixing period while the percent retained by the hypothermic group was 41.5±3.82%. The difference between the room temperature and the hypothermic groups was not statistically significant and therefore the mean value for the entire group, 43.3±2.24%, was used as a correction factor. Figure I presents these data graphically.

The Disappearance of Cr^{51} Tagged Red Blood Cells

The data related to the disappearance of Cr^{51} tagged red blood cells for 10 animals kept at room temperature (24-25°C) and for 10 animals kept in the cold room (3-4°C) are presented in Table II.

The percentage of Cr^{51} tagged red blood cells re-

[1]Standard error is given in each case unless otherwise noted.

after four hours, the third at 6 hours, and then samples were taken at 14 hours, 24 hours, 28 hours, 36 hours, and 48 hours. At each period one half milliliter of saline was reinjected to maintain approximately the standard blood volume. Blood loss at each period was minimal. Urine and feces were collected, the total volume recorded, and a two milliliter sample was placed in a vial for counting. All samples including the original standard were brought up to a 2 milliliter volume with saline to correct the geometry of the samples and were then counted as previously stated.

Determination of the disappearance for Cr^{51} tagged red blood cells from the circulation

The procedure for determination of the disappearance of Cr^{51} tagged red blood cells from the circulation was similar to the determination of the I^{131} loss except for the tagging procedure and the number of animals used. There were 10 pairs of animals used in this experiment.

The data for isotope remaining in the circulation after 48 hours were used to correct the calculated plasma and red cell volumes obtained in previous experiments.

TABLE I

I^{131} Tagged Serum Albumin Disappearance from the Circulation of the Alligator, with Standard Errors

Time	Room Temp. Group (24-25°C) %	#Obs.	Cold Room Group (3-4°C) %	#Obs.	Difference (rt-cr) %	Significance p-value	Average value %
½ hr.	100	8	100	8	0	0	100.0
1 hr.	93.8 ±2.49	7	90.0 ±3.70	8	3.8	n.s.	91.9 ±1.53
4 hr.	78.7 ±4.34	4	71.8 ±6.06	4	6.9	n.s.	75.3 ±4.05
6 hr.	76.0 ±6.54	6	67.8 ±4.09	7	8.2	n.s.	71.9 ±3.98
14 hr.	64.0 ±6.20	8	57.7 ±5.60	8	6.3	n.s.	61.3 ±4.41
24 hr.	60.4 ±5.14	4	44.0 ±5.61	4	16.4	n.s.	53.2 ±1.65
28 hr.	54.4 ±3.84	6	40.4 ±3.67	6	14.5	.05 .02	47.4 ±3.28
36 hr.	47.3 ±4.28	7	45.5 ±6.26	7	1.8	n.s.	46.4 ±2.68
48 hr.	45.1 ±2.46	8	41.5 ±3.82	8	3.6	n.s.	43.3 ±2.24

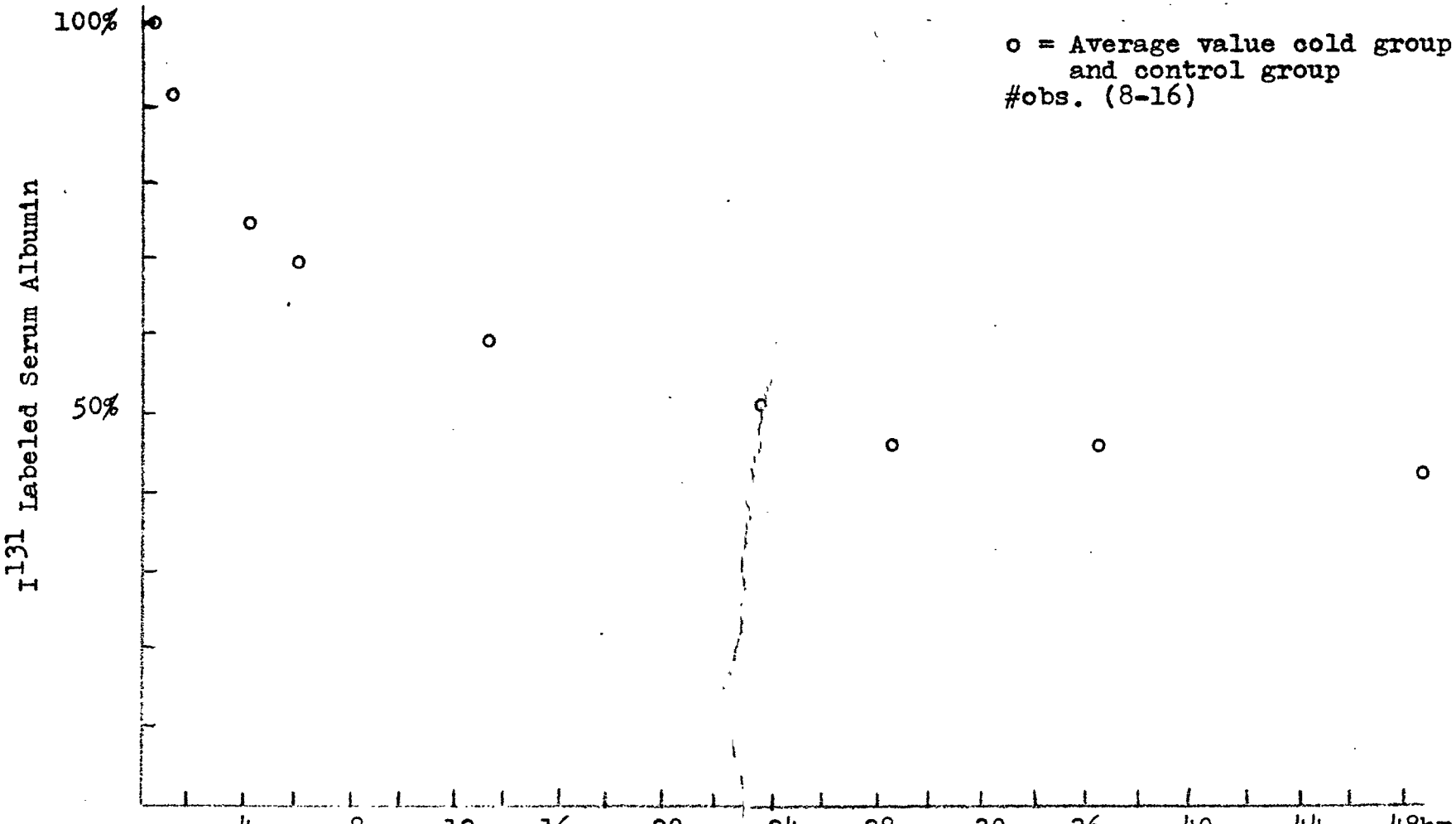

FIGURE I

Disappearance of I^{131} Labeled Albumin from the Circulation

TABLE II

Cr^{51} Tagged Red Blood Cell Disappearance from the Circulation of the Alligator, with Standard Errors

Time	Room Temp. Group (24-25°C) %	#Obs.	Cold Room Group (3-4°C) %	#Obs.	Difference (rt-cr) %	Significance p-value	Average value %
½ hr.	100.0	11	100.0	11	0.0	n.s.	100.0
1 hr.	87.6 ±2.87	10	91.2 ±1.25	11	3.6	n.s.	89.7 ±1.43
4 hr.	85.7 ±3.96	6	86.4 ±1.44	6	0.7	n.s.	86.1 ±2.01
6 hr.	85.9 ±2.17	5	89.5 ±4.39	5	3.6	n.s.	87.7 ±2.43
14 hr.	83.1 ±3.55	11	85.6 ±2.78	11	2.5	n.s.	84.4 ±2.19
24 hr.	79.0 ±3.42	6	86.0 ±3.10	6	7.0	n.s.	82.5 ±2.49
28 hr.	80.9 ±3.09	10	80.8 ±3.50	10	0.1	n.s.	80.9 ±2.48
36 hr.	78.4 ±3.44	10	79.4 ±4.29	10	1.0	n.s.	78.9 ±2.67
48 hr.	83.0 ±2.39	10	78.7 ±2.95	10	4.3	n.s.	80.9 ±2.04

maining in the circulation of the group of alligators kept at room temperature at the end of the experimental period was 83.0±2.39% while that remaining in the hypothermic group was 78.7±2.95%. The difference in these values was not statistically significant. The mean value for the entire group was 80.9±2.04% (Figure II), and this figure was used as a correction factor. Cr^{51} tagged red blood cell disappearance, according to Silver (1962), is exponential for the first 40 days if the first 24 hours are excluded. About 10% of the Cr^{51} tagged red blood cells disappear during the first 48 hours. In the case of the alligators studied here 10% was lost in the first hour but thereafter the rate of disappearance was slow and nearly constant.

Plasma and Cell Volumes of Room Temperature Group

The average plasma volume for nineteen alligators kept at room temperature was 58.0±2.70cc per 1000g while the average cell volume was 13.2±0.59cc per 1000g. Thus, the total blood volume, as calculated from the plasma volume plus the cell volume, was 71.2cc per 1000g. These values are comparable to those determined earlier in this laboratory. The circulatory hematocrit,

$$\text{Circulatory Hematocrit} = \frac{\text{Cell Volume}}{\text{Cell Volume + Plasma Volume}} \times 100,$$

was 18.5% and lower than the average measured venous

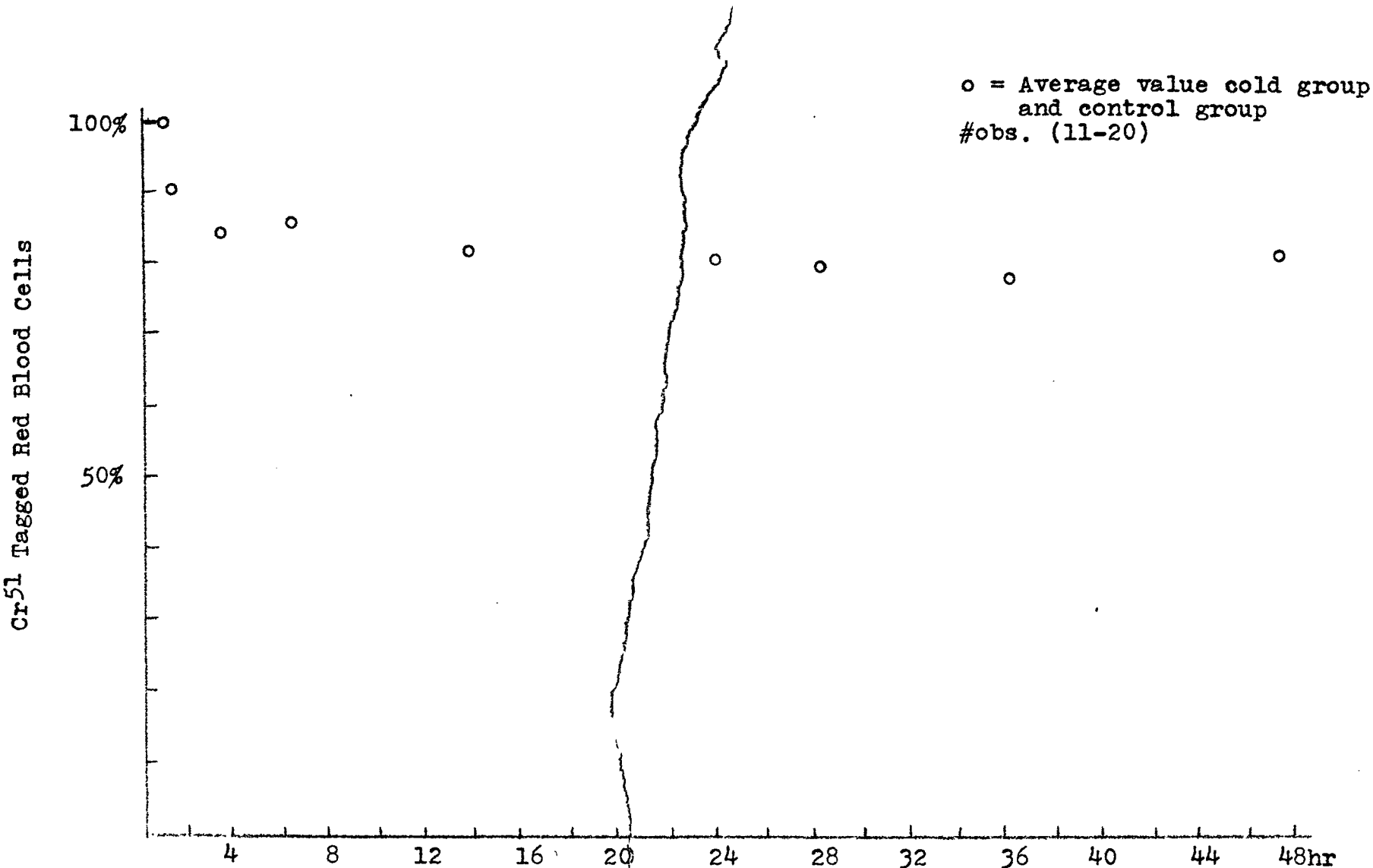

FIGURE II

Disappearance of Cr^{51} Tagged Red Blood Cells from the Circulation

hematocrit of 22.2% for the same group. Tables III and IV present these and other blood volume parameters for the alligator.

The blood volume calculated from the cell volume/venous hematocrit was 59cc per 1000g leaving a residual plasma volume of 11.8cc per 1000g.

The average initial venous hematocrit for all the alligators to be used as room temperature controls was 26.4±0.41%; this changed to 22.2±0.50% at the end of the 48 hour period. The difference between the initial hematocrit for the room temperature group and the 48 hour hematocrit of the same group was statistically significant (P .001). The average initial venous hematocrit for the alligators scheduled for hypothermia was 25.9±0.43%. The difference between the two groups was not, however, statistically significant (Table V).

Plasma and Cell Volumes of the Hypothermic Group

The average plasma volume for eighteen alligators subjected to hypothermia was 61.9±2.62cc per 1000g and the average cell volume for the same group was 11.4±0.45cc per 1000g. Therefore, the total circulating blood volume, calculated from the plasma volume and the cell volume, was 73.3cc per 1000g. The plasma volume appeared to be slightly greater in the hypothermic group than in the room temperature group but this difference was not statistically

TABLE III

MEAN MEASURED AND CALCULATED BLOOD VOLUME PARAMETERS FOR THE ALLIGATOR

	Room Temp. Group (24-25°C)	#obs.	Cold Room Group (3-4°C)	#obs.	Difference (rt-cr)	Significance p-value
Plasma Volume cc/kg	58.03 ±2.70	19	61.93 ±2.62	18	3.90	n.s.
Red Cell Volume cc/kg	13.19 ±0.59	14	11.37 ±0.45	17	1.82	.05 .02
Circulatory Hematocrit (CV/PV+CVx100)	18.5%	33	15.5%	35	3.0	

R.T.= Room Temperature Group
C.R.= Cold Room Group
C.V.= Cell Volume
P.V.= Plasma Volume
n.s.= Not Significant

TABLE IV

MEAN MEASURED AND CALCULATED BLOOD VOLUME PARAMETERS FOR THE ALLIGATOR

Treatment Group	Blood Volume$_1$ (CV+PV)		Blood Volume$_2$ (CV/VH)		BVR Cells (CH/VH)	Residual Plasma ($BV_1 - BV_2$)	BVR Plasma (100-CH/100-VH)
units	cc/kg	#obs.	cc/kg	#obs.		cc/kg	
Room Temp. Group (24-25°C)	71.2	33	59.5	19	0.84	11.8	1.116
Cold Room Group (3-4°C)	73.3	35	60.6	18	0.82	12.7	1.091

CV= Cell Volume
PV= Plasma Volume
VH= Venous Hematocrit
CH= Circulatory Hematocrit
BV_1= Blood Volume$_1$
BV_2= Blood Volume$_2$

BVR Cells= Blood Volume Ratio Cells
BVR Plasma= Blood Volume Ratio Plasma

TABLE V

VENOUS HEMATOCRIT VALUES FOR THE ALLIGATOR

	Room Temp. Group (24-25°C) %	#obs.	Cold Room Group (3-4°C) %	#obs.	Difference Between Means	Significance p-value
Initial Period	26.4 **±0.41	36	*25.9 ±0.43	36	0.5	n.s.
48 Hour Period	22.2 ±0.50	34	18.8 ±0.47	37	3.4	.001

*Initial period cold room group at 24-25°C
**Standard Error

significant. The cell volume of the cold room group was significantly lower than that of the alligators kept at room temperature. Thus, although the total blood volume was approximately the same in the two groups (Table III and IV), the circulatory hematocrit of the hypothermic group of alligators was definitely lower, being 15.5%.

The blood volume, calculated from the cell volume/venous hematocrit, was 60.6cc per 1000g leaving a residual plasma volume of 12.7cc. This was slightly (0.9cc) greater than the residual plasma volume of the room temperature group; this difference may not be significant.

The average initial venous hematocrit for the hypothermic group was 25.9±0.43% and this changed significantly to 18.8±0.47% at the end of the experimental period. The final hematocrit of the hypothermic group was also significantly lower than that of the room temperature group (18.8±0.47% vs. 22.2±0.50%) for the same period (See Table V for the venous hematocrits of the alligator).

Organ and Tissue Volumes for the Control and Hypothermic Groups

The total visceral plasma volume, the sum of the plasma volume of the seven organs studied, was greater for the hypothermic group than for the room temperature group.

Significant differences in the plasma volume between the hypothermic and the room temperature animals

were noted for the heart, the spleen, and the kidneys; all three organs had a higher plasma volume under hypothermia (Table VII).

Significant differences in the cell volumes were noted for the spleen, the liver, the lung, the stomach, and the intestine; all the above organs had a higher volume of cells under the hypothermic state. The spleen showed the greatest increase in red cells followed by the liver and the lung, respectively. The total visceral red cell volume increased during the 24 hour cold period. During the same period the muscle and skin appeared to lose red cells; however, the loss was not significant (Table VIII).

The total organ blood volumes were the same for the stomach while the heart, the spleen, the liver, the lung, the kidney and the skin showed increased blood volumes in the hypothermic state. A loss of total blood volume was noted for muscle. The total visceral blood volume increased during hypothermia (Table IX).

The total visceral hematocrit for the above organs at room temperature was 10.8% while under hypothermia it was 15.9% accounting, in part, for the lower venous and circulatory hematocrits of the hypothermic group (Table VI).

Hypothermia did not alter the calculated hematocrits of the muscle and skin. Hematocrits of the heart and the kidney decreased in the hypothermic state while

TABLE VI

CELL VOLUMES, PLASMA VOLUMES AND HEMATOCRITS FOR ORGANS AND TISSUES (cc/kg)

Room Temp. Group	Heart	Spleen	Liver	Lung	Stomach	Intestine	Kidney	Total Viscera	Muscle cc/100g	Skin cc/100g
Plasma Volume	0.3240 ±.007* (17)	0.1366 ±.010 (18)	1.5009 ±.080 (18)	1.0642 ±.141 (17)	1.3562 ±.098 (17)	1.7765 ±.147 (17)	0.4205 ±.028 (18)	6.5789	2.6200 ±.130 (16)	4.6000 ±.475 (17)
Cell Volume	0.0241 ±.003	0.1347 ±.017	0.2913 ±.018	0.2056 ±0021	0.0509 ±.002	0.0478 ±.004	0.0383 ±.004	0.7927	0.1480 ±.022	0.1240 ±.016
Hematocrit	7.0%	49.6%	16.2%	16.2%	3.7%	2.6%	8.4%	10.8%	3.6%	2.5%
Cold Room Group										
Plasma Volume	0.3907 ±.006 (18)	0.1810 ±.019 (18)	1.6472 ±.151 (18)	1.1415 ±.114 (18)	1.3362 ±.099 (18)	1.7601 ±.180 (18)	0.5620 ±.054 (18)	7.0187	2.2400 ±.280 (18)	5.0700 ±.704 (18)
Cell Volume	0.0215 ±.003 (15)	0.4326 ±.047 (14)	0.3882 ±.032 (14)	0.2791 ±.034 (13)	0.0675 ±.002 (15)	0.0868 ±.007 (16)	0.0439 ±.002 (13)	1.3196	0.1010 ±.010 (11)	0.1140 ±.016 (12)
Hematocrit	3.9%	70.5%	19.0%	19.6%	4.8%	4.6%	7.2%	15.8%	3.6%	2.4%

( )= Number of observations

$$\text{Hematocrit} = \frac{\text{Cell Volume}}{\text{Plasma Volume + Cell Volume}} \times 100$$

*Standard Error

TABLE VII

PLASMA VOLUME FOR ORGANS AND TISSUES
FOR THE ALLIGATOR, WITH STANDARD ERRORS

Organ	Room Temp. Group (24-25°C)	#obs.	Cold Temp. Group (3-4°C)	#obs.	Difference Between Means	Significance p-value
Heart cc/kg	0.3240 ±.007	17	0.3907 ±.006	18	+0.0667	>.001
Spleen cc/kg	0.1336 ±.010	18	0.1810 ±.019	18	+0.0444	>.05
Liver cc/kg	1.5009 ±.083	18	1.6472 ±.151	18	+0.1464	n.s.
Lung cc/kg	1.0642 ±.141	17	1.1415 ±.114	18	+.0773	n.s.
Stomach cc/kg	1.3567 ±.098	17	1.3362 ±.099	18	-0.0200	n.s.
Intestine cc/kg	1.7765 ±.146	17	1.7601 ±.180	18	-0.0164	n.s.
Kidney cc/kg	0.4205 ±.028	18	0.5620 ±.054	18	+0.1514	.05 .02
Total Viscera	6.5789		7.0187		+0.4398	n.s.
Muscle cc/100g	2.6200 ±.130	16	2.2400 ±.280	18	-0.3800	n.s.
Skin cc/100g	4.6000 ±.475	17	5.0700 ±.704	18	+0.3700	n.s.

TABLE VIII

RED BLOOD CELL VOLUME FOR ORGANS AND TISSUES
FOR THE ALLIGATOR, WITH STANDARD ERRORS

Organ	Room Temp. Group	#obs.	Cold Temp. Group	#obs.	Difference Between Means	Significance p-value
Heart cc/kg	0.0241 ±.003	15	0.0215 ±.003	15	-0.0026	n.s.
Spleen cc/kg	0.1347 ±.017	14	0.4326 ±.047	14	+0.2952	>.001
Liver cc/kg	0.2913 ±.018	13	0.3882 ±.032	14	+0.0969	.02>.05
Lung cc/kg	0.2056 ±.021	14	0.2791 ±.034	13	+0.0735	.1 >.05
Stomach cc/kg	0.0507 ±.002	13	0.0675 ±.002	15	+0.0166	.05>.02
Intestine cc/kg	0.0478 ±.004	14	0.0868 ±.007	16	+0.0390	>.001
Kidney cc/kg	0.0383 ±.004	13	0.0439 ±.002	13	+0.0056	n.s.
Total Viscera cc/kg	0.7929		1.3196		+0.5296	___
Muscle cc/100g	0.1480 ±.022	11	0.1010 ±.010	11	-0.0470	n.s.
Skin cc/100g	0.1240 ±.016	12	0.1140 ±.016	12	-0.0100	n.s.

TABLE IX

ORGAN AND TISSUE BLOOD VOLUME
FOR THE ALLIGATOR

	Heart cc/kg	Spleen cc/kg	Liver cc/kg	Lung cc/kg	Stomach cc/kg	Intestine cc/kg	Kidney cc/kg	Total Viscera	Muscle cc/100g	Skin cc/100g
Room Temp. Group (24-25°C)	0.3481	0.2713	1.7922	1.2698	1.4971	1.8243	0.4588	7.3716	2.7680	4.7240
Col Room Group (3-4°C)	0.4122	0.6136	2.0354	1.4206	1.4037	1.8469	0.6059	8.3383	2.3410	5.1840
Difference between cold and room temp. groups	+.0641	+.3423	+.2432	+.1508	-.0035	+.0226	+.1471	+.9667	+.4270	+.5600

Blood Volume = Cell Volume + Plasma Volume
Total Viscera = Sum Total of Organ Volumes

those of the spleen, the liver, the lung, the stomach, and the intestine increased. The spleen showed the greatest increase in the hematocrit during hypothermia and autopsies of cold animals always showed this organ to be very large, dark, and firm. The average weight of the spleen for the hypothermic group was 1.6±0.02g per 1000g which was significantly greater than that of the room temperature group which was 1.1±0.02g per 1000g.

I^{131} Excretion in the Urine

The average volume of urine excreted by the room temperature group was 5.46±1.50cc per 1000g. The amount of I^{131} excreted in the urine for this period averaged 1.02 ±0.19% of the total counts injected at the beginning of the experimental period. Urine and fecal material were collected from 5 of the cooled animals. These alligators excreted an average of 7.94±2.26cc of urine per 1000g with 1.07±0.07% of the original activity; of this amount 2.82cc were secreted during the 24 hours of hypothermia. The difference between the two groups in I^{131} excretion was not significant.

Cr^{51} Excretion in the Urine

Urine and fecal material were collected from six Cr^{51} treated animals kept at room temperature and from six Cr^{51} treated animals kept in the cold room. An average of 6.89 ±1.09cc of urine per 1000g was collected from the room temperature group. Cr^{51} excretion in the urine averaged

1.90±0.22% of the total counts injected at the beginning of the experiment. The 6 animals kept in the cold room excreted an average of 7.05±0.89cc of urine per 1000g of alligator. Of this volume a total of 3.05cc per 1000g was excreted while the animals were in the cold room. Cr^{51} excretion in the urine during this period was slightly less than for the room temperature group, averaging 1.50±0.46% of the original dosage. The difference between the two groups in Cr^{51} excretion was not significant.

Thyroid Uptake of I^{131}

An attempt was made to locate the thyroid gland of the alligator and to measure its uptake of I^{131}. In alligators this gland is located ventral to the trachea immediately in front of the pericardium. The 4 thyroid glands examined showed an average uptake of 0.35±0.05% of the total dosage injected.

PART IV

DISCUSSION AND SUMMARY

Discussion

After 48 hours the disappearance of I^{131} labeled albumin from the circulation of alligators subjected to hypothermia was not significantly different from that of those kept at room temperature. Therefore, the two values were averaged (See Figure I) and used as a correction factor for the plasma volumes.

The disappearance of Cr^{51} labeled red blood cells from the circulation of the hypothermic alligators was not significantly different from the control group. Therefore, an average value for the disappearance of Cr^{51} labeled red blood cells was calculated (See Figure II) and was used as a correction factor for determination of red cell volumes.

When alligators were subjected to hypothermia (3-4°C) for 24 hours the plasma volume appeared to increase; however, this increase was not significant. Huggins (1961) also found no significant difference in the plasma volume of alligators cooled rapidly (within 30 minutes) to 5°C. Comparable data for other species of poikilotherms are not available. When data from investigations in which homeothermic animals were subjected to reduction of body temperature are considered, conflicting data are found. A

decreased plasma volume was found by the following investigators: Rodbard et al., 1951, chicks and rabbits; Beaton 1961, rats; D'Amato and Heagnauer 1953, and Fedor and Fisher 1959, dogs. However, Farrand and Horvath (1959) using dogs and Orme and Beaton (1962) using hamsters found no significant change.

The average red cell volume for the alligators under hypothermic conditions for 24 hours was significantly lower than for those at room temperature. Huggins (1961) found that the cell volume in alligators, after rapid cooling (30 minutes) to 5°C, was the same as the control value, even though the venous hematocrit dropped sharply. It would seem, therefore, that prolonged deep hypothermia produced a definite decrease in the circulating red cell volume of the alligator. In homeothermic animals with lowered body temperature the red cell volume was found by some investigators (Farrand and Horvath, 1959) to increase while other investigators (Fedor and Fisher, 1959, and D'Amato and Hegnauer, 1953) all working with dogs, found no significant change.

Because of the slight but not statistically significant increase in plasma volume of hypothermic animals, the total blood volume, calculated from the plasma and cell volumes, remained the same. The average venous hematocrits for both the room temperature and the cold groups decreased

significantly from the initial value. The decrease was, however, larger in the cold group than in the room temperature group, and this difference was significant, reflecting the definite decrease in red cell volume and circulatory hematocrit. Huggins (1961) observed a significant decrease in the venous hematocrit, but the circulatory hematocrit remained the same. It was postulated that the change in the venous hematocrit resulted from the "trapping" or sequestering of red blood cells by the spleen and the liver. This proposal was supported by the finding that the red cell volume significantly increased for both organs. This was borne out by the present study at least insofar as the spleen is concerned.

In some studies of homeothermic animals, where the body temperature was lowered by cold exposure, there was an increased total blood volume and venous hematocrit (Farrand and Horvath 1959, Kahler 1962, dogs; Lynch 1963, squirrels). Others reported increases only in hematocrits (Beaton 1961, rats; Orme and Beaton 1962, hamsters).

The average total visceral plasma volume of cooled alligators was greater than that of alligators kept at room temperature. An increase in the red cell volume was also noted. However, the red cell volume of the visceral organs increased to a greater degree than the plasma volume; therefore the visceral hematocrit increased.

Redistribution of the blood in the alligator during prolonged hypothermia appeared in areas usually thought of as blood reservoirs. Significant increases in red cell volume were noted for the spleen, the liver, the lung, the stomach, and the intestine. These organs also showed increased hematocrits. Significant increases in plasma volume were noted for the heart, the spleen, and the kidney. The hematocrit of the heart and kidney decreased but that of the spleen, as noted above, showed a marked increase. It was therefore apparent that cold stress affected both the red cell and plasma distribution in the alligator. Further, in general, there was a greater shift in the visceral red blood cell volume than in the visceral plasma volume. This was paralleled by a decrease in red blood cells in the systemic circulation, but not by a significant loss in the systemic plasma volume.

Everett and Matson (1961), working with rats subjected to cold temperatures but with body temperatures remaining near normal, found that the somatic parts of the animals show an increased total blood volume while the visceral areas show a decreased volume.

It should be pointed out that in hypothermic alligators, whose hematocrit and circulatory red cell volume decreased, there was higher red cell volume in the spleen and liver as well as an increased spleen weight while in

cold exposed homeothermic animals, rats, whose body temperature remained near normal, there was a significant decrease in the cell volume of the spleen and an increased hematocrit. It would seem that the physiological stress of cold increased the hematocrit in homeotherms by releasing red cells from the spleen while in poikilotherms the spleen stores red blood cells in response to cold.

In homeotherms in which the body temperature was lowered, there was no correlation between changes in the plasma volume and decrease in body temperature. This generalization also appears to apply to the red cell volume. In alligators there was a decrease in the cell volume and venous and circulatory hematocrits in response to cold stress but either no change or an increase in cell volume and hematocrits for homeotherms under similar stress.

So far the results have shown that the blood volume shifts in response to prolonged hypothermia in alligators are not like those made by homeotherms whose body temperatures are reduced. It seems that the adjustments in red cell volume and hematocrit were in opposite directions to those of homeotherms and it might be possible to explain these differences by the general decrease in metabolic requirements of the alligator under hypothermia. Future investigations should include comparative studies between poikilotherms and homeotherms using similar techniques and

methods. Also the shifts of water, proteins, and electrolytes between the various body compartments should be investigated under hypothermia and changes that might occur in individual red cell volumes should be considered.

Summary

I^{131} tagged serum albumin and Cr^{51} labeled red cells were used to measure plasma and red cell volume, respectively. Also the blood volume of a number of organs were determined. In addition the disappearance of I^{131} labeled albumin and Cr^{51} tagged red blood cells from the circulation of the alligator were measured. Determinations were made both at room temperature (24-25°C) and after 24 hours at low temperature (3-4°C). Hematocrits were determined by a micromethod (venous hematocrit) and by calculation (circulatory hematocrit).

Cooling the alligator for 24 hours to 3-4°C resulted in a significantly lower venous hematocrit and circulatory hematocrit as well as circulating red cell volume but no significant change in the plasma volume. The total visceral red cell volume and plasma volume tended to increase, there being a greater increase in the red cell volume. There was a significant increase in the red cell volume for the spleen, the liver, the lung, the stomach, and the intestine. The heart, muscle and skin showed decreased red blood cell volumes. The plasma volume increased significantly in the

heart, the spleen, and the kidney. The plasma volume in the stomach, intestine, and muscle appeared to decrease, however, not significantly.

For most organs the hematocrits increased in response to cold. The spleen hematocrit showed the greatest increase. Only the heart and kidney hematocrits decreased. Blood volume tended to increase in all organs and tissues studied except for the stomach and muscle. Therefore, the total visceral areas of the alligator increased in blood volume on prolonged exposure to hypothermia.

The disappearance of I^{131} labeled albumin from the circulation in 48 hours was the same for the hypothermic group and the control group, $43.3 \pm 2.24\%$. Cr^{51} tagged red blood cells disappeared from the circulation of the hypothermic group at the same rate as from the control group; after 48 hours this value was $80.9 \pm 2.04\%$.

I^{131} excretion in the urine in 48 hours was $1.05 \pm 0.19\%$ of the total counts injected while Cr^{51} excretion was $1.73 \pm 0.35\%$ of the original dosage. Thyroid uptake of I^{131} for the 48 hour period was $0.35 \pm 0.08\%$ of the original dosage.

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