suppressed(10). No explanation has been advanced to account for this phenomenon, which contradicts evidence that the arthritis depends on an immunologic response of the delayed type(3,11). If a new type of response is involved, any explanation of the apparent competition by BSA must remain highly tentative. If the response is of the delayed type and occurs in thymectomized animals only because of the intensity of the stimulus provided by the (unknown) antigen, it is not clear why the BSA stimulus, insufficient itself to induce a response in these animals, can nevertheless, when it occurs, overwhelm the arthritis response.

Summary. Adjuvant arthritis is suppressed in sham-operated rats sensitized with adjuvant mixtures containing BSA. In neonatally thymectomized rats, in whom sensitization to BSA fails to occur, there is a moderate reduction in the intensity of the arthritis observed. In similar animals given lymph node cells at or just after the time of sensitization, the response to BSA is normal; yet the arthritis response is like that in animals not receiving cells.

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Blood Volume in Alligators During Prolonged Hypothermia.* (30270)

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In previous publications it was shown that lowering the body temperature of an alligator from 25°C to 5°C within a 30-minute period produced a small reduction in plasma volume, no change in cell volume, and a very slight increase in circulatory hematocrit. There was, however, in spite of the reduced plasma volume, a significant fall in the venous hematocrit. The ratio of circulatory (over-all cell percentage) to venous hematocrit, BVR_{cells}, increased while the volume of residual plasma decreased significantly. Therefore, as a result of the rapid cooling, there was a shift of cells from the large to small vessels within the circulation of the alligator. This was confirmed by tissue analysis, the spleen and liver showing a significant increase in the volume of red cells(1). Because these animals had only 30 minutes to equilibrate at the low temperature, it was decided to study animals subjected to deep hypothermia for a longer period to see whether or not the effects were intensified or compensated for with time.

Materials and methods. Young North American alligators, *Alligator mississippiensis* (Daudin), weighing 700-2400 g and measuring 66-86 cm in length were obtained from a commercial dealer and kept in a shallow tank. They were usually used within 3 weeks after their arrival, but those kept for a longer period were fed frozen fish scraps. Approximately equal numbers of males and females were selected for this study, although previous work in this laboratory has indicated that the sexes showed no significant differ-

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ences in the parameters studied.

Animals were lightly anesthetized using ether; the desired level of anesthesia was produced in 5-10 minutes. Preliminary investigation demonstrated that this was the most suitable procedure when deep hypothermia was to follow. Even so it was necessary to allow 24 hours to elapse before placing the animals in the cold chamber, since fixed anesthesia combined with cold exposure, or ether followed immediately by cold exposure, was almost invariably fatal.

Cell volumes were determined using Cr^{51}-tagged red cells according to the technique of Gray and Sterling(2) as modified by Deavers, Smith and Huggins(3). I^{131}-tagged serum albumin (RISA Abbott) was used to measure plasma volume. The red cells to be used were obtained from donor alligators. All injections and withdrawals were made through an intracardial needle, and whenever an anticoagulant was indicated polyanhydromanuronic acid (Manuronate, Wyeth) was used.

Alligators were used in pairs and the regimen for red cell volume determination was as follows: 5 ml of blood was withdrawn and replaced by 5 ml of a solution of tagged red cells suspended in enough saline to approximate the normal alligator hematocrit and washed in with 1 ml of saline. The needle was left in place for 5 minutes to allow for mixing and to reduce bleeding. Initial hematocrits were determined from blood withdrawn at the beginning of this procedure. The alligators were released and allowed to recover for 24 hours. At the end of this period one remained at normal room temperature (24-25°C) and the other was placed in a cold room (3-4°C). The animals are hereafter referred to as room temperature controls and cold or hypothermic animals, respectively. At the end of 24 hours the cloacal temperature approximated the environmental temperature. At this time animals were again anesthetized. One milliliter of blood was withdrawn for radioactive monitoring and a little additional blood was drawn for micro-hematocrit determinations. The animal was then killed by rapid exsanguination (20-24 ml) and pithing. Organs were excised rapidly, blotted, and weighed. Small samples of muscle and skin were taken from the tail, hind- and forelimbs, and chest. These were combined to represent "muscle" and "skin" in subsequent determinations. Each organ was digested in concentrated HNO_3, the solution measured, and aliquots monitored for radioactivity.

The I^{131}-tagged serum albumin used for measuring plasma volume was appropriately diluted in saline and injected in 1 ml amounts and washed in with 1 ml of saline into pairs of animals following withdrawal of a single milliliter of blood. Approximately the same procedure was then used as was followed for the Cr^{51} series. The only difference came in the process of tissue digestion; in the I^{131} series 40% KOH was used.

All samples, including organ samples and standard volumes of the original isotope solution, were monitored using a deep-well scintillation counter (Tracer Labs 1000).

Data from each animal were corrected to a standard total body count of one million and a standard body weight of 1,000 g.

It is known that during a 48-hour period substantial amounts of I^{131}-tagged serum albumin disappear from the blood of experimental animals(4), while the disappearance of Cr^{51} during the same period is negligible (5). To provide a correction factor for disappearance of isotopes, 2 additional series of animals were run, one for I^{131} and one for Cr^{51}. The procedure was similar to the main series except that 0.5 ml blood samples were taken at 6, 14, 24, 28, 36, and 48 hours. Replacement of each sample withdrawn was made with saline. Urine and feces were collected, the volumes recorded, and 2 ml aliquots counted.

Results and discussion. Data on the disappearance of I^{131}-tagged serum albumin and Cr^{51}-tagged red cells for the animals kept in the cold room and at room temperature are presented graphically in Fig. 1. The data for the 2 groups of animals were originally kept separate, but in neither the case of I^{131} nor Cr^{51} were the differences between room temperature animals and cold animals statistically significant. Therefore, the groups were combined and the correction factors determined. In the case of I^{131}, $43.3 \pm 2.24\%$ of

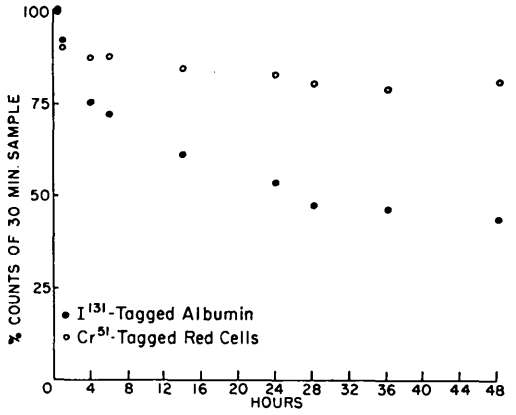

FIG. 1. Disappearance of radioisotope tags from circulation of alligators over a 48-hr period.

the original count remained in the circulation of the alligator at the end of 48 hours, and for Cr^{51} the value was 80.9 ± 2.04%.

The average red cell volume of the room temperature animals was 13.2 ± 0.59 ml/1000 g, and that of the cooled animals was 11.4 ± 0.45 ml/1000 g (Table I). This difference is significant ($p<.05>.02$). Also, the cell volume of the long-term cooled animals was significantly lower than that of the acutely cooled group.

The average plasma volume for alligators at room temperature was 58.0 ± 2.7 ml/1000 g, while that of the hypothermic group was 61.9 ± 2.62 ml/1000 g (Table I). This difference was not significant, but accounts for the fact that total blood volume was approximately the same for both groups despite a significant decrease in red cell volume, 71.2 ml/1000 g for the room temperature group and 73.3 ml/1000 g for the cold group. This suggests the possibility that red cells were stored and small amounts of plasma released as a consequence of the long period in the cold. The plasma volumes do not differ significantly from those of acutely cooled animals or their controls(1).

The circulatory hematocrits (cell volume × 100/cell volume + plasma volume) for the two groups differ significantly, being 18.5% for room temperature animals and 15.5% for cold animals. In both instances the circulatory hematocrits are lower than the venous hematocrits which are 22.2 ± 0.50% and 18.8 ± 0.47, respectively. While the significant difference between the hematocrits of the control and experimental group suggests a shift in cells or plasma, the final distribution of cells and plasma between the large and small vessels remained unchanged. This is reflected in the fact that there was no significant difference in the BVR_{cells} (0.84 and 0.82). Consequently, the residual plasma was the same for both groups (11.8 ml/kg and 12.7 ml/kg). Both circulatory and venous hematocrits were lower in the long-term cold group than they were for acutely cooled alligators(1).

Organ cell and plasma volumes were determined in order to explain the fall in hematocrit and lowering of red cell volume (Table II). The total visceral cell volume, the sum for 7 visceral organs studied, was increased in the cold-exposed group. The in-

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

	Room temperature group (24-25°C)		Cold room group (3-4°C)		Significance P value
Venous hematocrit (%)	22.2 ± .501	(34)*	18.8 ± .472	(37)	<.001
Plasma vol (ml/kg)	58.03 ± 2.70	(19)	61.93 ± 2.62	(18)	
Red cell vol (ml/kg)	13.19 ± .59	(14)	11.37 ± .45	(17)	<.05 >.02
Blood vol_1 (CV + PV) (ml/kg)	71.2	(33)	73.3	(35)	
Circulatory hematocrit (%) (CV/PV + CV × 100)	18.5%	(33)	15.5%	(35)	
Blood vol_2 (CV/VH) (ml/kg)	59.5	(19)	60.6	(18)	
BVR_{cells} (CH/VH)	.84		.82		
Residual plasma (BV_1—BV_2) (ml/kg)	11.8		12.7		

* No. of observations.

TABLE II. Cell Volumes, Plasma Volumes and Calculated Hematocrits for Organs and Tissues.

	Heart	Spleen	Liver	Lung	Stomach	Intestine	Kidney	Total viscera	Muscle	Skin
					A. Room temperature group					
Plasma vol (ml/kg)	.3240 ±.007 (17)*	.1366 ±.010 (18)	1.5009 ±.080 (18)	1.0642 ±.141 (17)	1.3562 ±.098 (17)	1.7765 ±.147 (17)	.4205 ±.028 (18)	6.5789	2.6200 ±.130 (16)	4.6000 ±.475 (17)
Cell vol (ml/kg)	.0241 ±.003 (15)	.1347 ±.017 (14)	.2913 ±.018 (13)	.2056 ±.021 (14)	.0509 ±.002 (13)	.0478 ±.004 (14)	.0383 ±.004 (13)	.7927	.1480 ±.022 (12)	.1240 ±.016 (12)
Hematocrit (%)	7.0	49.6	16.2	16.2	3.7	2.6	8.4	10.8	3.6	2.5
					B. Cold room group					
Plasma vol (ml/kg)	.3907 ±.006 (18)	.1810 ±.019 (18)	1.6472 ±.151 (18)	1.1415 ±.114 (18)	1.3362 ±.009 (18)	1.7601 ±.180 (18)	.5620 ±.054 (18)	7.0187	2.2400 ±.280 (18)	5.0700 ±.704 (18)
Cell vol (ml/kg)	.0215 ±.003 (15)	.4326 ±.047 (14)	.3882 ±.032 (14)	.2791 ±.034 (13)	.0675 ±.002 (15)	.0868 ±.007 (16)	.0439 ±.002 (13)	1.3196	.1010 ±.010 (11)	.1140 ±.016 (12)
Hematocrit (%)	3.9	70.5	19.0	19.6	4.8	4.6	7.2	15.8	3.6	2.4
					P values for differences between means					
PV_A—PV_B	<.001	<.05					<.05 >.02			
CV_A—CV_B		<.001	<.05 >.02	<.10 >.05	<.05 >.02	<.001				

* No. of observations.

$$\text{Hematocrit} = \frac{\text{Cell volume}}{\text{Plasma volume} + \text{cell volume}} \times 100.$$

crease accounts for approximately one-third of the cells missing from the circulation. The individual organs in which this difference was significant were the spleen, liver, lungs, stomach, and intestines. Skin and muscle showed no significant alteration, but the tendency was toward cell loss rather than gain.

The plasma volumes of spleen, heart, and kidney were significantly higher in the cold animals. In the latter two organs these differences were sufficient to lower the organ hematocrits. In the spleen the elevation in red cell volume was sufficient to more than offset the rise in plasma volume and the organ hematocrit rose sharply. Other organs showing a small but not significant increase in plasma volumes were liver and lungs, while the stomach and intestines showed a small decrease in plasma volume. Thus the "total viscera" showed overall a 7% increase in plasma volume. Muscle plasma volume fell, but not significantly, whereas skin plasma volume showed an apparent rise. While the primary effect of a short-term exposure to cold was a shift in the distribution of red cells from the large to the small blood vessels, during a prolonged exposure to cold this initial effect on red cell distribution disappears and the distribution returns to that found at room temperature.

In the past it was postulated(6) that the cold-blooded animal might differ materially in blood volume from the warm-blooded animal, thus accounting for the observed whiteness of meat, etc. In the light of our observations we conclude that it is the lower hematocrit principally, rather than the differences in blood volume which explains this appearance. The total calculated blood volume for dogs is about 80.00 ml/kg; that of alligators is about 72 ml/kg, or about 10% less. On the other hand, the hematocrit of unanesthetized dogs is about 44%(7), whereas that for unanesthetized alligators is about 22.7% (unpublished data from this laboratory). In alligators anesthetized with ether the hematocrit is about 26%, but in a large series of alligators anesthetized with pentobarbital the hematocrit is about 16%. This situation is reminiscent of what happens in dogs under the same conditions. Ether anesthesia in dogs causes contraction of the spleen with elevation of the hematocrit(8), while pentobarbital has the reverse effect; by relaxing the spleen and taking up red cells the venous hematocrit is significantly lowered(9).

Summary. Alligators cooled for 24 hours (3-4°C) show a significant drop in red cell volume and in venous and circulatory hematocrits when compared with animals held at room temperature (24-25°C). These parameters are lower than those observed in animals cooled acutely (in 30 minutes) to the same temperature. The principal organ taking up red cells is the spleen although other organs, liver, lungs, stomach, and intestines also take up extra cells. Shifts in plasma are small and for the most part not significant. In spite of these changes, the final distribution of cells and plasma in the circulation is not affected by prolonged cooling.

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