Hormonal and Metabolic Responses of Juvenile Alligators to Cold Shock

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ABSTRACT Juvenile alligators became completely immobile 5 min after immersion in ice water and remained in rigor for 40 min when removed from the water, but recovered righting responses within 5 min after immersion in tepid water. A blood sample was taken prior to the treatment, at 1 hr post-treatment and at 24 and 48 hr after recovery. Plasma norepinephrine, epinephrine, and dopamine were measured using high-pressure liquid chromatography (HPLC), and corticosterone by radioimmunoassay (RIA). Plasma ions, phosphate, and lipids were measured on an autoanalyzer and blood smears were taken for differential white cell counts. Norepinephrine and epinephrine were close to 4 ng/ml at the initial bleed: at 1 hr post-treatment epinephrine increased to 7 ng/ml and norepinephrine rose to over 40 ng/ml. Mean plasma dopamine was less than 0.7 ng/ml at the initial bleed and post-treatment means were as high as 10 ng/ml, but values were too variable to show statistical significance. Plasma corticosterone rose significantly at 1 hr and returned to levels not significantly different from initial at 24 and 48 hr. Despite the massive increase in catecholamines, plasma glucose did not change throughout the experiment. Plasma triglyceride increased significantly at 24 and 48 hr and plasma cholesterol decreased significantly at 24 and 48 hr. All other plasma components with the exception of calcium and sodium showed changes. Both lymphocytes and heterophils increased at 48 hr and other white cell types showed a decrease. Overall, these results suggest that short-term cold exposure is less stressful to alligators than simple restraint. *J. Exp. Zool. 283:566–572, 1999.* © 1999 Wiley-Liss, Inc.

The American alligator, *Alligator mississippiensis,* is the most northerly distributed of the extant crocodilians, ranging as far north as 35°. It is the only crocodilian known to inhabit regions that are subjected to occasional periods of freezing. Anecdotal reports (Barton, '55) and a number of field and behavioral studies (Brisbin et al., '82; Hagan et al., '83; Brandt and Mazzotti, '90; Lee et al., '97) have documented that the American alligator can survive sub-zero temperatures, even to the extent of being partially frozen in ice. A prolonged, record freeze in Louisiana did, however, result in the death of a large number of alligators, though no change in nesting density or population size the year following the freeze could be detected. Obviously most of the animals in the population survived (Joanen and McNease, '90). Brandt and Mazzotti ('90) reported that three of five caimans (*Caiman crocodylus*) in an outdoor pond died when the water temperature in the pond went down to 2.2°C. Seven alligators in the same pond were unaffected. A month later when the pond froze, seven out of a group of nine juvenile alligators survived. Four of the seven were trapped under the ice, but only the two smallest individuals died (Brandt and Mazzotti, '90). These observations would indicate that alligators are physiologically adapted to survive a sudden cold shock whereas caimans from a tropical environment are not. We investigated the effect of sudden cold shock on a number of hormonal and biochemical parameters in juvenile alligators and compared these with what is known in alligators subjected to other stresses. It is believed that a short exposure to cold results in serious immune suppression in reptiles (Boyer, '92). We therefore examined changes in white blood cells during the experiment to assess any major changes in the immune function. Part of this work appeared in an earlier review (Lance, '94).

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MATERIALS AND METHODS

Nine juvenile alligators raised under controlled conditions following hatch from artificially incubated eggs (Joanen and McNease, '87) were used in this study. Mean total length was 63.7 ± 1.0 cm, and the mean body mass was 709.6 ± 33.8 g. A heparinized blood sample (2 ml) was taken by heart puncture, centrifuged, and the red cells separated. The plasma was frozen at –20°C until assayed. A small aliquot of whole blood and a blood smear were taken for differential white cell counts. Immediately after the initial blood sample was taken, the alligators were weighed to the nearest gram and total length measured to the nearest cm, and a model identification tag (National Band and Tag Co., Newport, KY) placed on the web between the toes. The alligators were then placed in a bath of melted ice. After 20 min the alligators were removed from the ice bath and placed in tepid water until a full righting reflex was observed. A second blood sample was taken and the animals were returned to the heated tanks where they were maintained at 30°C. At 24 and at 48 hr additional blood samples were collected.

Norepinephrine, epinephrine and dopamine were measured in the laboratory of Dr. K.S. Matt, Tempe, Arizona, using high pressure liquid chromatography (HPLC) as described in Steger et al. ('85). Briefly, 1.5 ml of plasma was pipetted into a 5 ml conical extraction tube, and dihydroxybenzylamine (DHBA) added as an internal standard. Alumina (10 mg) was added and the tube shaken for 15 min. The plasma layer was discarded and the alumina washed three times with 0.2% Tris/EDTA. The catecholamines were then extracted from the alumina with 100 µl of a mixture of acetic acid/sodium disulfite/EDTA. Catecholamine concentrations were determined following separation on a C8 (5 µm) column using electrochemical detection with a glassy carbon electrode.

Corticosterone was measured by radioimmunoassay as previously described (Lance and Lauren, '84). Duplicate 100 µl aliquots of plasma were extracted with 20 vols of ethyl acetate:n-hexane (3:2), the solvent evaporated under a stream of nitrogen gas and the dried extract reconstituted in 500 µl of PBS buffer, pH 7.0. Antibody and tritiated corticosterone were added and the tubes held at 4°C overnight. Unbound steroids were separated from bound with dextran charcoal. Tritiated corticosterone was purchased from NEN (Boston, MA). Corticosterone antibody was purchased from ICN (Costa Mesa, CA). Plasma glucose was measured colorimetrically at 505 nm using the Trinder method (Sigma). Insufficient plasma was available to analyze all samples for plasma ions and lipids, but in all cases there were at least six samples for each sampling period. Sodium, potassium, magnesium, phosphorus, triglycerides, and total cholesterol were analysed using a Hitachi 911 autoanalyzer. Statistical analysis of the results was caried out using Staview software for the Mac. The catecholamine, corticosterone, glucose, and white cell data were analyzed using a repeated measure single factor ANOVA followed by Scheffe's multiple range test. The plasma chemistry results were analyzed using a single factor ANOVA.

RESULTS

The alligators immersed in melted ice struggled briefly, but in less than five minutes had lost all reflexes and assumed a catatonic rigor. The animals remained unresponsive for 40 min after they were removed from the ice bath, but began moving and exhibited a normal righting reflex after immersion in tepid water for five minutes. No unusual behavior was noted. Plasma dopamine and epinephrine levels at the four sampling periods are shown in Figure 1. Dopamine values were extremely variable and ranged from less than 0.40 ng/ml to over 30 ng/ml, but because of this variability, mean values did not change significantly during the experiment. Epinephrine increased significantly ($P < 0.05$) from a mean of 4.62 ng/ml at the initial bleed to a mean of 7.01 ng/ml at 1 hr post-treatment, then declined to levels significantly ($P < 0.05$) below the initial value at 24 and 48 hr. Plasma norepinephrine showed a massive increase at 1 hr post-treatment to a mean of over 40 ng/ml, almost tenfold greater that the increase in epinephrine ($P < 0.001$), but returned to levels no different from the initial at 24 and 48 hr (Fig. 2).

Corticosterone showed a significant increase at 1 hr post-treatment and declined at 24 and 48 hr (Table 1).

Plasma chemistries are presented in Table 1. Sodium, glucose, and calcium levels remained unchanged for the 48 hr of the experiment, but all other parameters measured showed significant differences. Chloride showed a significant increase only at 48 hr. Magnesium increased at 1 hr then returned to baseline at 24 hr. Potassium increased at 1 hr but returned to baseline at 48 hr. Plasma phosphate doubled at 1 hr then declined to slightly below baseline at 24 and 48 hr.

Cholesterol declined significantly ($P < 0.05$) from

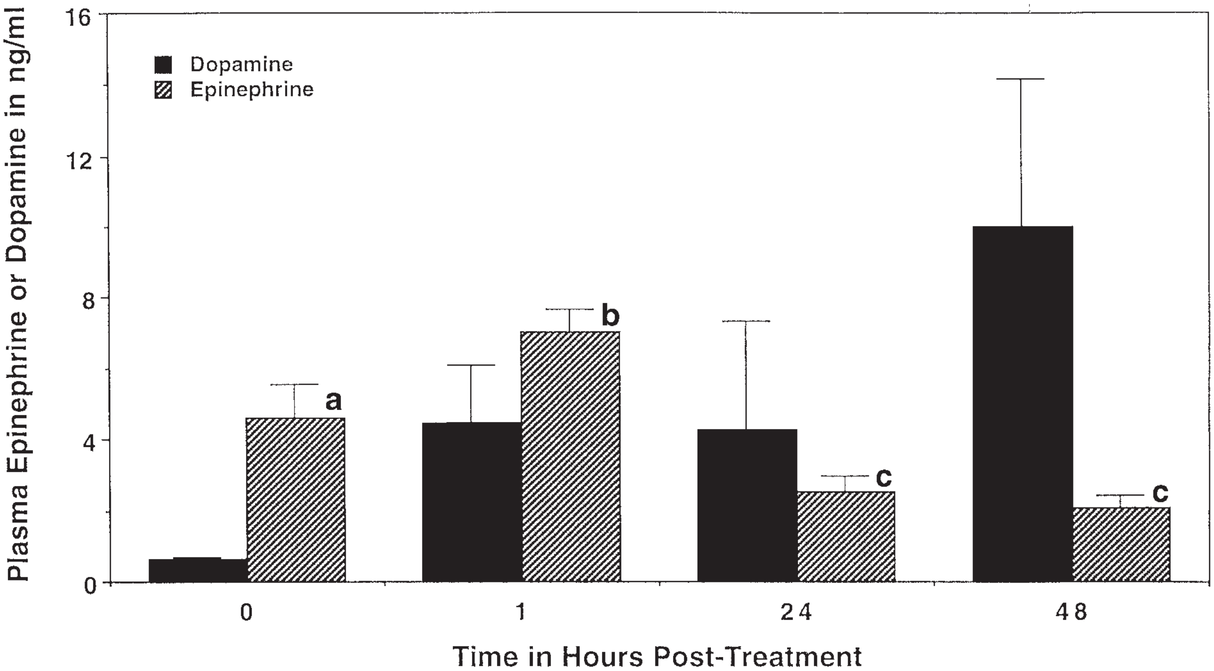

Fig. 1. Plasma dopamine and epinephrine values in juvenile alligators prior to and following submersion in ice water. The bar represents the mean value and the line above, the standard error of the mean (SEM), n = 9. Significant differences between sampling times are indicated by letters. Columns that share a letter are not significantly different from one another.

the initial value at 24 and 48 hr. Plasma triglycerides, however, increased more than threefold from initial values at 24 hr and remained significantly elevated at 48 hr ($P < 0.01$) (Fig. 3). Hematocrit declined significantly at 24 hr and 48 hr ($P < 0.01$).

Differential white blood cell counts are presented in Table 2. Total WBC increased significantly by 48 hr ($P < 0.05$). Lymphocytes and heterophils increased at 48 hr whereas basophils decreased by 48 hr. Azurophils decreased at 1 hr and returned to initial values by 48 hr. Eosinophils were basically unchanged. The percentage of different white cell types during the experiment is presented in Table 3. Heterophils increased from about 36 to 52% at 48 hr. Azurophils declined by about 50% and basophils by about 70% at 48 hr.

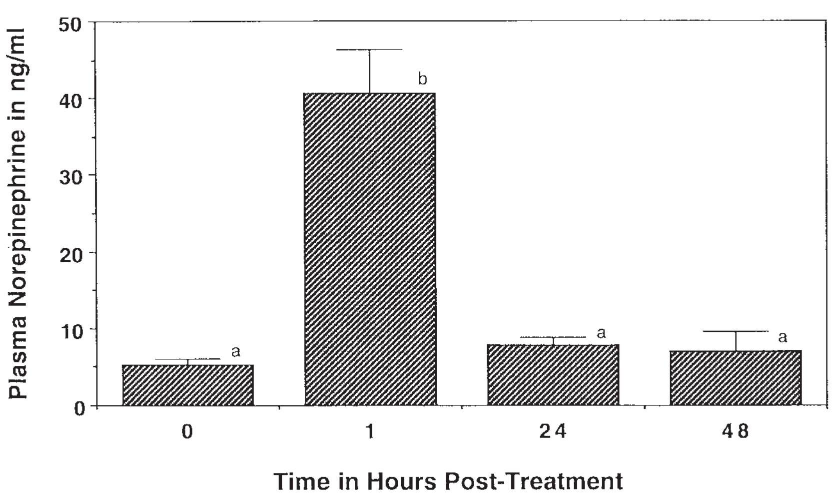

Fig. 2. Plasma norepinephrine in juvenile alligators prior to and following submersion in ice water. Mean and SEM as in Fig. 1, n = 9. Significant differences are noted as in Fig. 1.

TABLE 1. Plasma chemistry of alligators following cold shock

	0 hr	1 hr	24 hr	48 hr
Sodium mM/liter	146 ± 3.6	141 ± 1.5	152 ± 2.4	146 ± 2.2
Potassium mM/liter	3.96 ± 0.17	5.37 ± 0.15*	4.64 ± 0.18	4.14 ± 0.15
Chloride mM/liter	95.8 ± 5.9	90.6 ± 3.2	103.7 ± 3.6	112.1 ± 2.1*
Glucose mM/liter	9.97 ± 0.9	10.89 ± 0.69	10.57 ± 0.94	11.69 ± 0.99
Calcium mM/liter	2.53 ± 0.25	2.29 ± 0.15	2.46 ± 0.21	2.62 ± 0.09
Magnesium mM/liter	0.62 ± 0.03	0.80 ± 0.05*	0.63 ± 0.02	0.77 ± 0.03
Phosphorus mM/liter	1.86 ± 0.09	3.23 ± 0.16*	1.59 ± 0.08	1.44 ± 0.06
Cholesterol mg/dL	87.4 ± 5.7	76 ± 3	59 ± 2*	58 ± 2.2*
Hematocrit	17.5 ± 1	17.4 ± 0.6	13.4 ± 0.5*	12.9 ± 0.5*
Corticosterone ng/ml	2.79 ± 0.79	12.45 ± 2.48*	6.76 ± 1.40	8.74 ± 2.90

*Indicates significantly different from 0 hr, $P < 0.05$.

DISCUSSION

The rapidity with which the alligators lost all reflexes when immersed in ice water was surprising. The relatively small body mass (~700 g) and the permeability of the skin of alligators this size may have been a factor. Presumably the central nervous system was rapidly cooled to close to 0°C and thus nervous conduction ceased (Rosenberg, ’77, ’78). Complete immobilization was maintained for at least 30 min after the alligators were removed from the ice bath, but recovery was rapid upon immersion in tepid water. Large alligators have been observed moving slowly when body temperatures were as low as 2–5°C (Hagan et al., ’83), temperatures that would probably be lethal to caimans. Colbert et al. (’46) concluded from their studies that “alligators are affected much less severely by adverse conditions of cold for brief periods than they are by adverse conditions of heat.” In our study we can also conclude that brief periods of extreme cold are not unduly stressful to alligators. While the sudden cold shock delivered to the alligators in this study is unlikely to occur in nature, the fact that these animals are able to tolerate the gradually decreasing seasonal temperatures that are occasionally below freezing suggests that they are physiologically able to tolerate such a shock. Large alligators can apparently respond to some stimuli at temperatures as low as 5°C (Hagen et al., ’83), but in the closely related *Caiman crocodilus* auditory fibers cease firing below 11°C (Smolders and Klinke, ’84). Similarly,

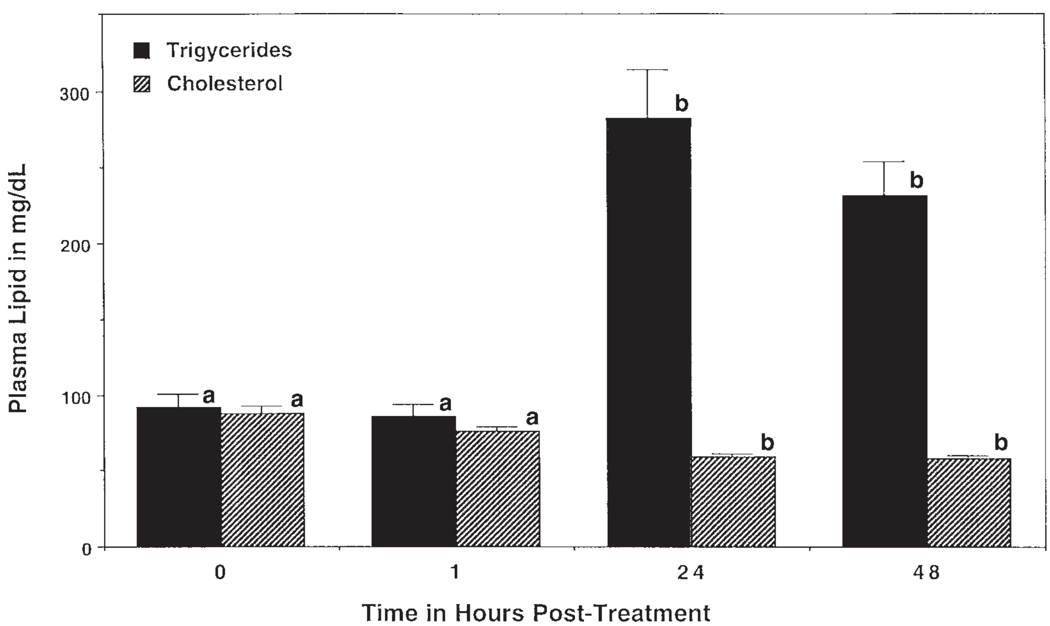

Fig. 3. Plasma triglycerides in juvenile alligators prior to and following submersion in ice water. The bar represents the mean value and the line above the standard error of the mean (SEM), n = 7. Significant differences are indicated as in Fig. 1.

TABLE 2. Differential white cell counts following cold shock in juvenile alligators

	0 hr	1 hr	24 hr	48 hr
Total WBC	4405 ± 1557	4661 ± 729	9058 ± 1025*	14344* ± 2487
Total heterophils	2515 ± 231	1494 ± 280	5103 ± 676*	7681 ± 1439*
Total azurophils	2160 ± 620	895 ± 221	1338 ± 216	1846 ± 411
Total lymphocytes	1022 ± 417	1461 ± 380	1674 ± 423	4125 ± 1447*
Total basophils	995 ± 276	623 ± 270	590 ± 109	422 ± 55*
Total eosinophils	580 ± 150	450 ± 95	352 ± 68	723 ± 256

*Indicates different from time 0, $P < 0.05$.

hatchling *Caiman crocodilus* are unable to produce distress call at temperatures below 10°C (Garrick and Garrick, '78). It is not known if alligators are similarly affected at these temperatures, but the behavioral observations cited above suggest that this is not the case.

The large increase in norepinephrine and the relatively minor increase in epinephrine at 1 hr post-treatment are in agreement with the suggestion of deRoos et al. ('89) that the peripheral nervous system is more important in the immediate response to stress in alligators than the adrenal medullary tissue. It has been argued, however, that in ducks subjected to forced dives, up to 80% of the circulating norepinephrine came from the adrenal medulla and not the peripheral sympathetic nerve endings (Lacombe and Jones, '90). Catecholamine responses are generally extremely rapid and it is possible that the immediate response to the cold shock could have been missed, but as the alligators were completely immobile and heart rate and circulation were greatly reduced and did not recover until the animal was warmed, the 1-hr sample is probably representative of the response to the shock. The large post-cold-shock increase in norepinephrine, however, could be an artefact due to the result of a shut down in the plasma clearance of the hormone and thus an abnormally high concentration at the 1-hr sample. Alligators held under restraint also show an increase in norepinephrine, but less than one fourth the values seen in this study (Lance and Elsey, '99). The results suggest that cold shock elicits a much greater peripheral sympathetic discharge in the alligator than handling stress, but the source of the increase in circulating norepinephrine is at present uncertain. Both epinephrine and norepinephrine were close to 4 ng/ml at the initial bleed indicating an extremely rapid sympathetic response to the disturbance in the minute prior to getting a blood sample. A similar but even greater rise in catecholamines in response to handling was reported in tree lizards, *Urosaurus ornatus* (Matt et al., '97).

There was a significant rise in plasma corticosterone, but not as great as that seen when simple restraint alone is used (Lance, '92; Lance and Elsey, '99). Elevated corticosterone is seen in turtles following prolonged submergence in anoxic water at 22°C but not during the time they are submerged (Keiver et al., '92a). In turtles submerged in anoxic water at 5°C plasma corticosterone declined during the treatment, but returned to control within one day of recovery (Keiver et al., '92b). Decreased temperature appears to decrease the secretion of corticosterone in reptiles (Dauphin-Villemant et al., '90). Surprisingly, there was no significant increase in plasma glucose in the alligators in this study following immersion in the ice bath. In contrast, both corticosterone and glucose show highly significant increases following restraint stress in alligators (Lance, '94; Lance and Elsey, '99). Glucose is released into the blood in response to catecholamines, and both epinephrine and norepinephrine have been shown to cause a marked rise in plasma glucose when injected into alligators (Coulson and Hernandez, '83; Lance and Elsey, unpublished). In the present

TABLE 3. White cell percentages following cold shock in juvenile alligators

	0 hr	1 hr	24 hr	48 hr
% Heterophils	35.6 ± 5.0	38.3 ± 5.9	56.4* ± 4.4	52.0* ± 4.6
% Azurophils	26.3 ± 3.3	14.6* ± 2.8	15.1* ± 1.7	13.6* ± 1.5
% Lymphocytes	18.6 ± 3.6	36.0* ± 4.9	21.8 ± 4.1	26.0 ± 5.1
% Basophils	10.2 ± 1.7	6.9 ± 1.1	6.2 ± 0.8	3.4* ± 0.6
% Eosinophils	7.1 ± 1.0	10.1 ± 1.4	4.0* ± 0.7	4.8 ± 1.0

*Indicates significantly different from initial $P < 0.05$.

study there was a significant rise in plasma epinephrine and a large increase in norepinephrine at 1 hr post-treatment, but no increase in glucose. The lack of any increase in glucose in the presence of such high levels of catecholamines could be due to failure of catecholamine receptors to respond at low temperature. Turtles subjected to anoxia at 22°C show increased levels of catecholamines and an increase in plasma glucose (Keiver et al., ’92a), however, in the same species of turtle subjected to anoxia at 5°C plasma catecholamines increased and plasma glucose remained unchanged (Keiver et al., ’92b). The authors showed that at 5°C catecholamines were unable to stimulate hepatic glycogenolysis in the turtles, but were able to do so at 22°C. Mammals and birds subjected to cold stress undergo a number of well-known physiological adjustments to increase heat production that include shivering thermogenesis, increased thyroid hormone secretion, and increased lipid metabolism. Poikilotherms subjected to cold are unable to compensate for heat loss and generally go into metabolic arrest (Hochachka, ’86). It is possible that epinephrine and norepinephrine failed to stimulate glucose secretion in the cold-shocked alligators because the receptor system was unresponsive. A somewhat analogous situation has been reported in an amphibian in which epinephrine stimulated prostaglandin synthesis in the lungs of warm-acclimated American bullfrogs, but failed to stimulate synthesis in cold-acclimated frogs (Herman and Martinez, ’88).

It has been known since Metchnikoff demonstrated that alligators were unable to produce antitoxins when held at 20°C, but were able to mount a vigorous response when held at 30°C (Metchnikoff, ’01) that temperature plays an important role in reptile immunity. Low temperatures have been reported to result in immunosuppression in frogs (Maniero and Carey, ’97), but the effect of sudden and short cold shock on the immune system does not appear to have been studied. There were a number of changes in white blood cells in the alligators following immersion in melted ice. Total white cells more than tripled by 48 hr after the cold shock (Table 2), but individual cell types were affected differently. Heterophils and lymphocytes increased at 24 and 48 hr, whereas basophils decreased by 48 hr only. Eosinophils were relatively unchanged and azurophils declined at 1 hr but returned to baseline at 24 and 48 hr. Exactly what causes these changes is not clear. Sympathetic discharge is known to cause contraction of splenic smooth muscle and thus increase the number of circulating white cells in mammals, and a similar mechanism may have caused the rise in leukocytes in the alligators. Despite these major shifts in white cell numbers the overall changes do not indicate a suppression of immune function as was seen in restraint stress (Lance and Elsey, ’99).

Mammals that are exposed to cold undergo a series of metabolic and physiological adjustments to increase heat production and prevent heat loss. Among these is an increase in lipid metabolism and an increase in circulating free fatty acids, but a decrease in plasma triglycerides (Himms-Hagen, ’72). Teleost fish that are acclimated to cold show a general increase in serum lipids (Hazel and Prosser, ’74). The unusual increase in plasma triglycerides, but the simultaneous decrease in plasma cholesterol at 48 hr after the cold shock in the alligators is at present impossible to explain. If, as is the case in mammals, the increase in catecholamines increased lipid metabolism then a decrease in plasma triglycerides should have occurred. This mechanism, however, has not been demonstrated in poikilotherms. It is possible that the cold shock initiated a change in lipid metabolism that resulted in the increased plasma lipids similar to what is seen in cold-acclimated fish, but the mechanism for this increase in triglycerides in the alligators is unknown.

It has been suggested that cold stress causes shifts in tissue fluids and shifts in plasma ions (Munday and Blane, ’61). In alligators cooled from 30°C to 20°C only minor changes in potassium were noted (Douse and Mitchell, ’91). Given that these temperatures are similar to what an alligator could experience in a single day, such a result is not unexpected. Plasma sodium was reported to increase in rats and pigeons subjected to cold stress and to remain unchanged in snakes. But in lizards subjected to a similar zero-degree cold stress both sodium and potassium declined significantly (Munday and Blane, ’61). Such data are difficult to interpret. Jena and Patnaik (’95) suggest that such changes in fluid electrolytes in cold-stressed reptiles are due to a suppression of sodium and potassium ATPase in various organs and thus a suppression of sodium and potassium transport. How this can cause a decrease in one species and no change in another is not clear. The alligators in this study showed a slight increase in sodium at 24 hr and an increase in potassium, magnesium, and phosphate at 1 hr. Chloride showed a significant increase at 48 hr. Whether these changes are due to membrane transport changes brought on by the cold or by the massive discharge of norepinephrine needs further investigation.

In summary, alligators exposed to a brief cold shock undergo a series of complex physiological and hormonal changes, many of which need further investigation, but recovery is rapid and long-term effects appear negligible.

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LITERATURE CITED

Barton AJ. 1955. Prolonged survival of a released alligator in Pennsylvania. Herpetologica 11:210.

Boyer TH. 1992. Clinical anesthsia of reptiles. Bull Assoc Reptiles Amphib Vet 2:10–13.

Brandt LA, Mazzotti FJ. 1990. The behavior of juvenile *Alligator mississippiensis* and *Caiman crocodilus* exposed to low temperature. Copeia 3:867–871.

Brisbin IL Jr., Standora EA, Vargo MJ. 1982. Body temperatures and behavior of American alligators during cold winter weather. Am Mid Nat 107:209–218.

Colbert EH, Cowles RB, Bogert CM. 1946. Temperature tolerances in the American alligator and their bearing on the habits, evolution, and extinction of the dinosaurs. Bull Am Mus Nat Hist 86:329–373.

Coulson RA, Hernandez T. 1983. Alligator metabolism: studies on chemical reactions in vivo. Comp Biochem Physiol 74:1–182.

Dauphin-Villemant C, Leboulenger F, Vaudry H. 1990. Adrenal activity in the female lizard *Lacerta vivipara* Jacquin during artificial hibernation. Gen Comp Endocrinol 79:210–214.

deRoos, R, Rumpf RP, Berry MW. 1989. Evidence that somatic and sympathetic nervous system neurotransmitters, not circulating epinephrine, mediate the immediate responses to a sudden threat in the American alligator (*Alligator mississippiensis*). J Exp Zool 250:1–10.

Douse MA, Mitchell GS. 1991. Time course of temperature effects on arterial acid-base status in *Alligator mississippiensis*. Respir Physiol 83:87–102.

Garrick LD, Garrick RA. 1978. Temperature influences on hatchling *Caiman crocodilus* distress calls. Physiol Zool 51:105–113.

Hagan JM, Smithson PC, Doerr PD. 1983. Behavioral response of the American alligator to freezing weather. J Herpetol 17:402–404.

Hazel JR, Prosser CL. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol Rev 54:620–677.

Herman CA, Martinez JM. 1988. Epinephrine stimulates prostaglandin synthesis by bullfrog lung from warm-acclimated, but not cold-acclimated animals. J Exp Zool 248:101–108.

Himms-Hagan J. 1972. Lipid metabolism during cold-exposure and during cold-acclimation. Lipids 7:310–323.

Hochachka PW. 1986. Defense strategies against hypoxia and hypothermia. Science 231:234–241.

Jena BS, Patnaik BK. 1995. Age-related responses of hepatic ATPases to starvation and cold stress in male garden lizards. Comp Biochem Physiol 111B:545–552.

Joanen T, McNease L. 1987. Alligator farming research in Louisiana. In: Webb GJW, Manolis SC, Whitehead PJ, editors. Wildlife management: crocodiles and alligators. Chipping Norton, Australia: Surrey Beatty & Sons Pty Ltd. p 329–340.

Joanen T, McNease L. 1990. The effects of a severe winter freeze on wild alligators in Louisiana. Lae, Papua New Guinea: Proc. 9th Working Meeting of the IUCN/SSC Crocodile Specialist Group. p 21–32.

Keiver KM, Weinberg J, Hochachka PW. 1992a. The effect of anoxic submergence and recovery on circulating levels of catecholamines and corticosterone in the turtle, *Chrysemys picta*. Gen Comp Endocrinol 85:308–315.

Keiver KM, Weinberg J, Hochachka PW. 1992b. Roles of catecholamines and corticosterone during anoxia and recovery at 5°C in turtles. Am J Physiol 263:R770–R774.

Lacombe AMA, Jones DR. 1990. The source of circulating catecholamines in forced dived ducks. Gen Comp Endocrinol 80:41–47.

Lance VA. 1992. Evaluating pain and stress in reptiles. In: Schaeffer DO, Kleinow KM, Krulisch L, editors. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW. p 101–106.

Lance VA. 1994. Life in the slow lane: Hormones, stress, and the immune system in reptiles. In: Davey KG, Peter RE, Tobe SS, editors. Perspectives in comparative endocrinology. Ottawa: National Research Council of Canada. p 529–534.

Lance VA, Lauren D. 1984. Circadian variation in plasma corticosterone in the American alligator, *Alligator mississippiensis* and the effects of ACTH injections. Gen Comp Endocrinol 54:1–7.

Lance VA, Elsey RM. 1999. Plasma catecholamines and plasma corticosterone following restraint stress in juvenile alligators. J Exp Zool 283:559–565.

Lee JR, Burke VJ, Gibbons JW. 1997. Behavior of hatchling *Alligator mississippiensis* exposed to ice. Copeia 1:224–226.

Maniero GD, Carey C. 1997. Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to cold. J Comp Physiol B 167:256–263.

Matt KS, Moore MC, Knapp R, Moore IT. 1997. Sympathetic mediation of stress and aggressive competition: plasma catecholamines in free-living male tree lizards. Physiol Behav 61:639–647.

Metchnikoff E. 1901. L'immunité dans les maladies infectieuses. Paris: Masson and Cie.

Munday KA, Blane GF. 1961. Cold stress of the mammal, bird and reptile. Comp Biochem Physiol 2:8–21.

Reid IR. 1997. Glucocorticoid osteoporosis—mechanisms and management. Eur J Endocrinol 137:209–217.

Rosenberg ME. 1977. Temperature and nervous conduction in the tortoise. J Physiol 289:50P–51P.

Rosenberg ME. 1978. Thermal relations of nervous conduction in the tortoise. Comp Biochem Physiol 60A:57–63.

Smolders JWT, Klinke R. 1984. Effects of temperature on the properties of primary auditory fibres of the spectacled caiman, *Caiman crocodilus* (L.) J Comp Physiol A 155:19–30.

Steger R, Matt K, Bartke A. 1985. Neuroendocrine regulation of seasonal reproductive activity in the male golden hamster. Neurosci Biobeh Rev 9:191–201.