

# Plasma Creatine Phosphokinase Activity in the Cold-Exposed and Cold-Acclimated Chicken<sup>1</sup> (35755)

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(Introduced by R. F. Krause)

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Serum creatine phosphokinase (CPK) activity is probably the most specific and sensitive enzyme test available for confirmation of disease or injury to skeletal muscle and myocardium (1). Elevations of serum CPK have been reported in muscular dystrophy (1, 2), myocardial infarction (1), and after strenuous physical exercise (3).

More recently elevations in plasma CPK activity have been reported in rats subjected to cold environments (4, 5) and freezing injury (5).

In this study the effects of cold exposure and cold acclimation on plasma CPK activity in the chicken are reported.

*Materials and methods.* Male chickens (White Rock  $\times$  Cornish) fed a commercial diet *ad libitum* were used for all studies.

I. *The effect of acute cold exposure on plasma CPK activity.* Sixteen 9-week-old chickens maintained at 17–19° were divided into two groups. One group was exposed to 2–3° for 4 hr, while the control group remained at 17–19°. Blood samples were taken at zero time and after 4 hr of temperature treatment.

II. *The effect of cold acclimation on plasma CPK activity.* Thirty-six 1-day-old chicks were divided into three groups and housed six per cage in three environmental chambers. At 5 weeks of age each group was exposed to 4–7, 8–11, or 19–21° (control) for 4 weeks. Blood samples were taken weekly from zero through 4 weeks of cold exposure.

In both experiments, 3-ml blood samples were drawn via the brachial vein with syringes rinsed with heparin (75 units/ml). Samples were centrifuged at 800g for 25 min and plasma was stored at –18°.

Plasma CPK activity was measured utilizing a fluorescent Ninhydrin method (6).

This method was reported to be at least three times more sensitive than the colorimetric methods. Enzyme activity was expressed in units per milliliter of plasma, 1 unit being the amount of CPK that will liberate 1  $\mu$ mole of creatine/min/ml  $\times 10^3$ . The data were evaluated by analysis of variance (7).

*Results and Discussion.* When chickens were exposed to 2–3° for 4 hr, plasma CPK activity increased from 1138 to 3176 units/ml compared to control values of 1321 before and 1540 units/ml after 4 hr at 17–19°.

The difference in CPK levels between the cold-exposed group before and after treatment for 4 hr was significantly greater ( $p < 0.05$ ) than the difference between the two times for the control group.

An increase of 3000% in plasma CPK activity in rats following freezing injury has been demonstrated (5). Conclusions were drawn by these authors that the increased activity was caused by destruction of the muscle sarcolemma and escape of CPK into the vascular system. It seems unlikely that extensive muscle damage occurred in the chickens cold exposed to 2–3°. Muscular activity could account for the increased CPK levels since this enzyme has been reported to increase in the serum following exercise (3). It is reasonable to speculate that the higher CPK levels during cold exposure may result from increased muscular activity associated with shivering thermogenesis.

Plasma CPK activity of chickens acclimated to various environmental temperatures is reported in Table II. After exposure for 1 week to 8–11 or 4–7°, chickens had significantly ( $p < 0.05$ ) elevated levels of CPK when compared with chickens at 19–21°. CPK activity was increased approximately 40% above

TABLE I. Effect of 4-hr Exposure to 2-3° on Plasma CPK Activity<sup>a</sup> of 9-Week-Old Chickens (means ± standard errors).

Temp (°)	Exposure	
	Before	After
17-19 (control)	1321 ± 86 (4) <sup>b</sup>	1540 ± 188 (4)
2-3	1138 ± 88 (12)	3176 ± 461 (12)

<sup>a</sup> Expressed in units per millimeter of plasma, 1 unit being the amount of CPK that will liberate 1 μmole of creatine/min/ml × 10<sup>3</sup>.

<sup>b</sup> Number of observations per mean.

<sup>c</sup> Difference from control values significant,  $p < 0.05$ .

ens exposed to 8-11 or 4-7° for 1 week was probably due to muscular shivering in the birds' attempt to maintain body temperature. After 2 weeks of temperature treatment as the chickens became cold acclimated and nonshivering thermogenesis became operative, muscular activity in the form of shivering was virtually absent, thus no elevated CPK levels were seen.

*Summary.* Chickens cold exposed to 2-3° had significantly elevated levels of plasma CPK compared with controls. After an initial increase during the first week, no significant difference was seen in plasma CPK activity of chickens cold acclimated at either 8-11 or

TABLE II. Effect of Cold Acclimation on Plasma CPK Activity<sup>a</sup> of Chickens 5-9 Weeks of Age (means ± standard errors).

Temp (°)	Age (weeks)				
	5	6	7	8	9
19-21 (control)	435 <sup>b</sup> ± 47	411 ± 33	628 ± 77	605 ± 56	1074 ± 83
8-11	460 ± 59	575 <sup>c</sup> ± 69	595 ± 79	592 ± 68	942 ± 121
4-7	440 ± 42	678 <sup>c</sup> ± 58	616 ± 79	707 ± 112	822 ± 80

<sup>a</sup> Expressed in units per millimeter of plasma, 1 unit being the amount of CPK that will liberate 1 μmole of creatine/min/ml × 10<sup>3</sup>.

<sup>b</sup> Each value is the mean of 12 observations.

<sup>c</sup> Difference from control values significant,  $p < 0.05$ .

control values upon exposure to 8-11° and 70% above control values upon exposure to 4-7°. After 2-4 weeks of cold treatment, no significant differences were seen in plasma CPK activity of the cold-acclimated chickens compared to chickens at 19-21°.

CPK activity gradually increased in the control chickens with age (Table II). This would be expected if serum CPK level is taken to be an indication of total muscle mass. A similar trend was shown in data reported by Holliday *et al.* (8).

A previous report (5) described an increased CPK activity in plasma from rats exposed 6 weeks at 2°. In contrast, the present findings are in partial agreement with the study by Dieter *et al.* (4), who reported no difference in serum CPK activity in rats maintained at 25 or 1.7° for 4-6 weeks.

The increased CPK activity seen in chick-

4-7° for 2-4 weeks when compared with controls. It is concluded that muscle activity (shivering thermogenesis) in cold-exposed chickens resulted in elevated plasma CPK, while the absence of muscle involvement (nonshivering thermogenesis) in cold-acclimated chickens resulted in no alterations in plasma CPK activity.

Published with the approval of the director of the West Virginia Agricultural Experiment Station as scientific series paper No. 1155. Supported by National Institute of Health grant No. 5R01GM-12884-05.

The authors are grateful to Mrs. Beverly Barnett, M. T. (ASCP) and Mrs. Gerda Carney, M. T. for excellent technical assistance.

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Received Feb. 9, 1971. P.S.E.B.M., 1971, Vol. 137.