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Creatine Phosphokinase in Detection of Visceral Muscle Injury* (33619)

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Reactions of the Embden-Meyerhof pathway and transamination occur in the majority of mammalian tissues. As a consequence, aldolase, lactic dehydrogenase (LDH) and glutamic-oxalacetic transaminase (GOT) have a ubiquitious distribution. Activity of these enzymes is high in striated muscle, liver, and other tissues limiting their value in specific detection of muscle cellular injury.

Significant creatine phosphokinase (CPK) activity is found only in muscle, brain, thyroid, and kidney (1). Very low CPK activity is found in serum, red cells, and liver. Therefore CPK activity determination is a better indicator of injury to the cellular integrity of those organs in which it is concentrated. Discussion of the advantages and limitations of the enzyme in the diagnosis of skeletal muscle disease employing CPK as an indicator of cellular injury have been published (2). Serum CPK alterations were useful in the diagnosis of myocardial infarction but to the authors' knowledge have not been employed in the detection of injury to visceral muscle.

Urinary bladder muscle was used as a model for visceral muscle in these studies. Since bladder muscle bulk is small and serum dilution effects were anticipated, an *in vivo* method of monitoring arteriovenous CPK differences was employed. The method is unique and permits inferences concerning specific organ injury. Muscle biopsy for CPK estimation during physiologic studies prevents reproducible electrophysiologic experimentation and is limited in patient application. Development of techniques of visceral muscle electronic reflex stimulation and diagnosis of visceral muscle disease demand more sophisticated means of evaluating detrusor muscle cellular changes and energy mechanisms. Accompanying studies of bladder muscle glycogen concentration were employed to assess changes in substrate reserves for energy metabolism. Vesical vein lactate concentration was assessed to determine the extent of anaerobic metabolism during the course of these experiments.

Materials and Methods. Adult female dogs were anesthetized by intravenous pentobarbital (25 mg/kg). The bladder was exposed by suprapubic incision. Ureteral catheters were employed for bladder filling and monitoring of intravesical pressure. Bipolar electrodes were affixed to the bladder by means of sutures (3). Blood was withdrawn from a vesical vein. Stimulation of the bladder muscle every 10 min over a period of 1 hr with 20 V, 1/msec pulses in a pulse train of 40–60-sec duration induced massive bladder contractions to the point of rigor. Vesical vein blood was collected again after the 1-hr period elapsed. The internal iliac arteries were then ligated. This procedure required about 20 min. Collateral circulation was such that ischemia rather than infarction occurred. After 60 min, blood was once again drawn from the vesical vein. The bladder fundus was

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TABLE I. CPK in Vesical Vein Blood before and after Stimulation (IU; $n = 9$).

Dog no.	Before	After
1	13	22
2	6	16
3	18	18
4	29	57
5	22	43
6	20	28
7	33	43
8	31	37
9	19	27
	$p \sim .03$	

then removed and frozen in dry ice and stored at −60° until glycogen studies could be carried out.

Glycogen concentration was determined by the anthrone method (4). Blood samples were centrifuged immediately and the serum was refrigerated. The CPK activity was determined by the method described by Rosalki (5). Lactate concentration was determined by spectrophotometric methods (6). The CPK and lactate studies were carried out within 2 hr after blood collection.

Results. The CPK activity in the vesical vein increased significantly after a period of supramaximal direct electrical stimulation productive of muscle rigor (Table I). Ligation of the internal iliac artery also evoked significant increase in CPK activity in the blood of the vesical vein (Table II). For technical reasons, only 7 dogs were employed for the ligation procedure, although 9 dogs underwent bladder stimulation. The student t test for small samples was utilized for calculation of data significance.

TABLE II. CPK in Vesical Vein Blood before and after Iliac Artery Ligation (IU; $n = 7$).

Dog no.	Before	After
1	22	50
2	16	21
3	18	27
5	43	54
6	27	46
8	19	16
9	22	50
	$p \sim .02$	

Lactate concentration in the vesical vein was not affected by bladder muscle rigor or ischemia. Average serum lactate concentration before stimulation was 10.9 mg/100 ml and immediately after stimulation 10.1 mg/100 ml. Average serum concentration before ligation was 12.6 mg/100 ml and after ligation was 11.6 mg/100 ml. Bladder muscle glycogen concentration was unchanged by rigor and ischemia as compared to normal bladder control values (Table III).

TABLE III. Glycogen Concentration (g/100 g wet wt.).

Normal dog bladder	
	0.07
	0.14
	0.13
	0.10
	0.06
Experimental dog bladder	
Dog 1	0.06
2	0.12
3	0.12
4	0.10[a]
5	0.12
6	0.23
7	0.16[a]
8	0.19
9	0.18
Normal dog gastrocnemius muscle	
	0.78
	0.77
	0.70

[a] Underwent stimulation only.

Discussion. The CPK is an enzyme responsible for the transfer of the high energy phosphate moiety from creatine phosphate to adenosine diphosphate (ADP) forming adenosine triphosphate (ATP). ATP is the energy-yielding compound associated with muscular contraction. Therefore CPK is a pivotal enzyme in energy availability during skeletal muscle contraction. The presence of actin, myosin, CPK, and creatin phosphate in smooth muscle indicate some similarities with energy metabolism of contraction in skeletal muscle.

The isozyme of CPK found in bladder smooth muscle is different than that in

skeletal muscle (1). This finding may reflect an unknown difference in the metabolic requirements of smooth muscle.

Analyses of CPK activity of the smooth muscle of the rabbit urinary bladder showed significant ontogenetic changes which may be of importance and impose limitations in future clinical application of these techniques (7).

Changes in cell membrane integrity have been implicated as the basis for CPK efflux from skeletal muscle in progressive muscular dystrophy (8). Serum CPK activity also increases with anoxia and crush injuries of skeletal muscles (9).

The elevation of CPK activity in the effluent blood from bladder muscle after rigor induced by electrical stimulation and experimental ischemia is therefore likely due to an escape of the enzyme from the muscle cells secondary to membrane injury. The study of changes in CPK activity in effluent venous blood to detect injury to visceral muscle has not been previously utilized to our knowledge.

The failure of lactate concentration to rise under these conditions suggests that the rate of tricarboxylic acid cycle metabolism is sufficient to keep pace with pyruvate formation. An alternative explanation is that other substrates such as fatty acids may be used preferentially during times of stress.

The concentration of glycogen in bladder muscle is approximately one-tenth of the concentration present in skeletal muscle. This may indicate a greater dependence of bladder muscle on circulating carbon sources to be used as substrate for energy metabolism or it may reflect a more efficient system of glucose utilization necessitating smaller glycogen stores. The apparent lack of changes of glycogen concentration in bladder muscle after electrically induced rigor and exposure to ischemia suggests that there are adequate stores of glycogen present to fulfill tissue needs during stress. Future studies employing these techniques will include the effects of reflex induction and denervation on single cell excitability and production of these metabolites.

Summary. Stimulation of dog urinary bladder muscle to the point of rigor and subjection of dog urinary bladder muscle to ischemia caused an increase in the effluent venous CPK activity. The technique of monitoring CPK activity employed in this study allows the detection of specific visceral muscle cellular compromise without the necessity of biopsy during ongoing physiologic experimentation. Lactate concentration in the venous blood of the vesical vein was unchanged by supramaximal stimulation or ischemia of the urinary bladder and suggests that anaerobic metabolism did not play a prominent role in energy metabolism during these experimental stresses. Bladder muscle glycogen was found to be about one-tenth the concentration found in skeletal muscle and was not depleted during the course of these experiments.

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