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Changes in Plasma Enzyme Activity Elicited by Running Exercise in the Dog[1] (38013)

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It has frequently been observed that, in mammals, both pathological and sustained physiological changes in various organ systems bring about alterations in the blood plasma levels of enzymes normally located within the intracellular compartment (1, 2). These enzymes are usually present in low activity in the blood plasma of the resting healthy mammal. It is assumed that in pathological situations tissue necrosis allows the escape of cellular contents into the interstitial fluid (1). In physiological situations, however, an increase in the permeability of the cell-limiting membrane may occur (3, 4).

Physical activity is an example of a physiological situation which is often accompanied by an increase in plasma enzyme activity (3, 5). The mechanisms responsible for the exercise-induced increase in plasma enzyme activity have not been elucidated despite several studies related to this topic (6, 7). In particular, data obtained from experiments on the anaesthetized dog are difficult to assess since there is little information concerning the plasma enzyme response to exercise in the conscious dog (8, 9).

The experiments reported here were performed to establish the typical plasma enzyme response to running exercise in the dog.

Material and Methods. Six adult dogs were used in this study (10–14 kg, of either sex). The exercise was performed by running on a treadmill (Quinton Model 24-72). All dogs used in these experiments had been previously accustomed to short (5-min) bouts of treadmill running and ran without visible apprehension. Three dogs made a total of 4 runs each, with a recovery period of at least 1 week between each run. These runs varied in duration, speed, and grade. A further 3 dogs were tested at the workload that induced the largest increases in plasma enzyme activity in the first 3 animals.

All runs were performed between 10:00 AM and 3:00 PM. Each animal was brought into the experimental area 30 min prior to a run and allowed to rest quietly. During this time, the dog was placed in a harness and a control rectal temperature determined. The exercise electrocardiogram (ECG) was obtained from a bipolar sternal lead attached during this period.

Two minutes before the run, a control sample of blood (4–5 ml) was collected from a cephalic vein. One milliliter of blood was immediately added to ice-cold 3% perchloric acid for blood lactate determination. The remainder was used in the determination of haematocrit and for plasma enzyme analysis. The blood was then heparinized and centrifuged at 1600*g* for 5 min and the supernatant plasma separated and stored at 4°.

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The exercise started at an initial speed and elevation of 1.5 mph and 0% grade, and the treadmill was then rapidly adjusted (within 15 sec) to the desired speed and elevation. ECG and heart rate were continuously recorded on a Grass Model 7 Polygraph. A second venous blood sample was taken within 2 min of the end of the run and treated in the same manner as the control sample. Rectal temperature was measured immediately following collection of the blood sample.

Plasma creatine phosphokinase (PCPK) and plasma glutamicoxalacetic transaminase (PGOT) were assayed spectrophotometrically within 12 and 24 hr, respectively, of obtaining the sample (10, 11). Reagents were obtained from Calbiochem, Los Angeles, CA. Plasma lactic dehydrogenase (PLDH) was assayed colorimetrically, within 48 hr of obtaining the sample, by the method of Babson and Phillips (12). An estimation of the relative activities of the "H" and "M" subunits of LDH was made according to the procedure of Babson (13). Reagents for PLDH assays were obtained from Warner–Chilcott Laboratories, Morris Plains, NJ. Activities of all enzymes were expressed in international units (IU) per liter of plasma.

Lactate determinations were performed on whole blood, deproteinized with 3% perchloric acid, according to the enzymatic method of Pfleiderer and Dose (14).

Results. The data obtained from 3 dogs (J, S, and C) each performing 4 separate runs are presented in Table I. Elevated plasma enzyme levels were seen following 10 of the 12 runs. Following a run, PLDH was elevated more frequently than PGOT or PCPK. The changes in plasma enzyme activity did not correlate with the changes in haematocrit, elevation in rectal temperature, increase in blood lactate, or the steady-state exercise heart rate. However, there did appear to be a relationship between the intensity of the exercise and the magnitude of the plasma enzyme response. In general, it appeared that the greater the speed and grade of the run, the larger the increase in PLDH and PGOT. Furthermore, with one exception, a postexercise eleva-

TABLE I. Plasma Enzyme Changes and Heart Rate During Running Exercise of Varied Intensity and Duration in the Dog.

Dog	J	J	J	J	S	S	S	S	C	C	C	C
Speed (mph)	4	6	6	10	4	6	6	10	4	6	10	10
Grade (%)	0	10	10	10	0	10	10	10	0	10	10	10
Duration (min)	30	15	30	15	30	15	30	15	30	15	15	30
Heart rate (beats/min)	207	236	235	252	—	—	225	225	156	—	213	207
Increase in PCPK (IU/liter)	0	0	0	12	7	0	0	11	0	0	0	8
(%)	0	0	0	86	17	0	0	33	0	0	0	42
Increase in PGOT (IU/liter)	0.0	0.0	1.8	7.2	2.2	0.0	2.1	1.8	0.0	0.0	6.7	9.0
(%)	0	0	21	46	14	0	10	11	0	0	56	60
Increase in PLDH (IU/liter)	0.0	3.5	0.0	5.8	2.1	13.1	2.1	8.1	0.0	3.5	6.7	14.7
(%)	0	39	0	72	11	111	12	86	0	31	68	171

TABLE II. Heart Rate, Rectal Temperature, Venous Haematocrit, Blood Lactate, and Plasma Enzyme Responses to Running Exercise in the Dog ($n = 6$) (15 min, 10 mph, 10% grade).

	Mean ± SEM
Heart rate (beats/min)	229 ± 10
Change in rectal temperature (°)	+1.3 ± 0.3
Final rectal temperature (°)	40.6 ± 0.3
Change in haematocrit (%)	+0.2 ± 2.2
Increase in blood lactate (mg%)	57.8 ± 10.5
Increase in PCPK	
(IU/liter)	13 ± 4
(%)	72 ± 35
Increase in PGOT	
(IU/liter)	6.7 ± 1.2
(%)	59 ± 14
Increase in PLDH	
(IU/liter)	9.5 ± 2.1
(%)	151 ± 61
PLDH ratio before	2.6 ± 0.1
after	2.7 ± 0.1

tion of PCPK was seen only after the most intense exercise.

Table II summarizes the data obtained from 6 dogs performing a standard run (15 min, 10 mph, 10% grade). At the end of the run, PLDH and PGOT were elevated in all dogs while PCPK was increased in all but one animal. The dogs in which the largest changes in PLDH occurred also demonstrated the largest alterations in PGOT and PCPK. There was no change in the PLDH isoenzyme ratio following exercise although there was about a twofold rise in total PLDH activity. This meant that there were proportionally similar increases in the "H" and "M" subunit activity of LDH in the plasma during exercise.

It was noted that the mean increase in activity of a particular plasma enzyme, during exercise, showed less variance when expressed as the absolute value than when calculated as the percent change from the resting value. From this we concluded that the magnitude of an exercise-induced change in plasma enzyme activity was probably independent of the resting plasma enzyme activity.

Discussion. It is difficult to compare our results with those previously reported. Wagner and Critz (8) found that running dogs at 10 mph and 10° (17%) elevation for 1 hr elicited an exercise heart rate of less than 180 beats/min and resulted in an approximately fivefold increase in serum CPK. In contrast, in the current study heart rates of over 200 beats/min were achieved in 4 runs lasting for 30 min, with a detectable increase in PCPK occurring in only one of these.

In another study, Bedrak (9) measured the serum activities of 8 different enzymes before, during, and after light exercise of long duration (2-hr walk, 4 km/hr, 8% grade). Serum GOT (assayed colorimetrically) increased throughout the exercise (33%) and was further elevated at the end of a 2-hr recovery period (84%). There was a substantial increase in serum LDH after the first 30 min of exercise (43%), with no further change during the remainder of the exercise or recovery periods. Serum CPK was not assayed. Taking into account the duration and intensity of the exercise used in this study, the magnitude of the exercise-induced changes in serum GOT and serum LDH concur with the observations of the present study.

We have previously reported that simulation of the cardiovascular response to exercise in the chloralose-anaesthetized dog by diencephalic stimulation consistently evoked small increases in PCPK and PGOT (15). When compared to the data then available on the plasma enzyme changes occurring in spontaneous exercise, the changes in PCPK seemed insignificant (8). However, from the data obtained in the present study, it now seems that an important contribution to the exercise-evoked increases in plasma enzyme activity may derive in some way from the marked cardiovascular adjustments to exercise.

The quantitative and qualitative similarities between the plasma enzyme increases observed in the dog during running exercise (current study) and during simulated exercise (7) (electrical stimulation of skele-

tal muscle) are interesting. They suggest that skeletal muscle and the process of skeletal muscle contraction may be the primary site and mechanism of loss of intracellular enzymes during exercise. Furthermore, the electrical stimulation of skeletal muscle may be helpful for studying, in the anaesthetized animal, the mechanism of the plasma enzyme response to exercise.

Summary. In the conscious dog, running exercise at a sufficient speed and gradient elicited increases in PCPK, PGOT, and PLDH. The duration of the exercise did not appear to be as important as the speed or grade in determining the plasma enzyme response. Following exercise, PLDH was elevated more frequently than PGOT. PCPK was elevated only after exercise at relatively high workloads. The changes in plasma enzyme activity did not appear to be directly related to the exercise heart rate or to the increases in rectal temperature or blood lactate concentration.

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