THE EFFECTS OF EXERCISE AND CONDITIONING ON SERUM CONSTITUENTS IN THE HORSE

by

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ABSTRACT

The Effects of Exercise and Conditioning on Serum Constituents in the Horse. (May 1979) Janice Rae Ballard, B. S., Texas A&M University Undergraduate Fellows Advisor: Dr. G. D. Potter

Nine previously sedentary Quarter Horse mares were randomly assigned to three treatment groups of 3 mares each. Horses in treatment group I served as controls. Horses in treatment groups II and III were conditioned by daily exercise for 2.25 miles at a trot and gallop, respectively. On days 0, 14 and 28 of a 28 day conditioning period the horses in all three treatment groups were subjected to a submaximal exercise tolerance test on an equine treadmill. Blood samples were taken at rest, 10 and 30 min. of exercise and 10 and 30 min. of recovery during the exercise test. From these blood samples serum was extracted and analyzed for calcium, glucose, albumin, inorganic phosphorus, blood urea nitrogen, creatine, total bilirubin, alkaline phosphitase, CKP, LDH, SGOT, SGPT, and total serum protein. Serum levels of these parameters were monitored in order to relate changes in response to exercise and conditioning to an objective means of measuring fitness levels in horses.

The serum levels of calcium, albumin, inorganic phosphorus, blood urea nitrogen, creatine, total serum protein, total bilirubin, alkaline phosphitase and SGPT were within clinically normal ranges and did not vary greatly with exercise or conditioning. Serum glucose, CPK, LDH and SGOT levels increased in response to the exercise tolerance test. The magnitude of this increase remained consistent throughout the conditioning period for SGOT. On the other hand, serum CPK, LDH and glucose levels were lower on day 28 of conditioning than on day zero for all three treatment groups at 30 minutes of exercise during the exercise test. Because the control group responed in similar manner to the trot and gallop groups, it is difficult to conclude from this study that measuring serum CPK, LDH and glucose levels in response to and exercise test can be useful in measuring fitness levels in horses.

DEDICATION

This paper is dedicated to those horses participating in the 1978 World Three Day Event who were eliminated during the endurance phase of the event because of inadequate physical conditioning.

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INTRODUCTION

The horses utilized for today's equine performance events are required to perform at a high level of physical exertion. The three-day event and endurance trail rides, for example, are designed primarily to test a horse's physical fitness and stamina. Success in such events depends not only on behavioral training but also on proper physical conditioning. Thus, the trainer, in addition to employing effective training techniques, must also develop efficient methods of conditioning a horse and be able to evaluate when a horse is adequately fit.

Limited research has been conducted in the area of equine exercise physiology. Consequently, the horse industry relies more upon art than science for determinations of levels of conditioning. Research is needed to determine the most efficient methods of conditioning and to develop an objective test of a horse's fitness level.

The primary purpose of this research is to study changes in serum enzymes and other constituents which may aid in developing an objective test to measure levels of fitness in horses.

Objectives

 Monitor change in serum constituents over a 28-day conditioning period, as indicated by response to a standard submaximal exercise tolerance test on an equine treadmill.

Citations follow the style and form of the <u>Journal of Animal</u> <u>Science</u>.

- Relate change to objective methods and techniques of measuring physical fitness in horses.
- 3. Compare effectiveness and efficiency of two conditioning schedules.

LITERATURE REVIEW

Strenuous physical exercise in the horse is accompanied by changes in several biochemical parameters. The magnitude and duration of such changes may indicate an animal's ability to produce and utilize energy to sustain muscular activity.

The serum enzymes creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and serum glutamic oxalacetic transaminase (SGOT) have been studied as to their response to exercise, stress and conditioning. These enzymes are related by their role in producing the energy used in sustaining muscular activity. These enzymes function in the muscle tissue and are believed to be released into the bloodstream due to an increased cell membrane permeability (Highman and Altland, 1963; Anderson, 1975).

Anderson (1975) has shown that in horses maintained under strictly controlled conditions of diet and exercise, normal serum CPK, LDH and GOT levels vary within narrow limits. Thus, changes in these enzymes in reponse to exercise are easily detectable.

Creatine phosphokinase is the enzyme that catalyzes the reaction between creatine phosphate and ADP to form available energy in the form of ATP. A measurement of CPK levels in the serum, therefore, indicates the demand of muscle cells for energy from stored reserves. Anderson (1975); Cardinet <u>et al</u>. (1967); Codazza <u>et al</u>. (1974) and Aitken <u>et al</u>. (1974) have observed that strenuous exercise causes increased serum CPK levels. In addition, Aderson (1975) and Aitken <u>et al</u>. (1974) have shown that the increase in serum CPK levels due to exercise becomes less pronounced as horses are subjected to regular moderate to hard exercise over several weeks. It seems likely that as a horse becomes more "fit" he relies less on stored energy for muscular activity and thus, less CPK is generated in the muscle tissue. It has not been shown whether repeated exercise results in decreased serum CPK values because of the cell membrane's decreased sensitivity to the stimulus that increases permeability or a decrease in the stimulus itself. But, since SCPK levels decrease with repeated exercise, Anderson (1975) and Aitken <u>et al</u>. (1974) have suggested that monitoring the elevation in SCPK levels due to strenuous exercise may be a useful means of assessing a horse's fitness level. Milne (1976), on the other hand, found no basis for using SCPK values as a guide to measuring fitness.

Lactic dehydrogenase is the enzyme needed to produce lactic acid from pyruvic acid to yield energy. This reaction takes place when the animal is unable to perform the work aerobically and is forced to meet his energy demands via less efficient anaerobic respiration. LDH levels in the serum may therefore reflect an animal's ability or lack thereof to maintain muscular activity aerobically. Anderson (1975); Codazza <u>et al</u>. (1974) and Aitken <u>et al</u>. (1974) observed increased serum LDH levels in horses in response to exercise. In addition, Anderson (1975) has shown that repeated exercise lessens the SLDH increase due to exercise and thus measures of SLDH levels may reflect a horse's fitness. Milne (1976), however, did not observe an increase in SLDH in response to exercise or a decrease in SLDH activity over a period of repeated exercise.

Serum glutamic oxalacetic transaminase, like creatine phosphokinase and lactic dehydrogenase, is related to the production of energy since it is used to transfer amine groups from protein structures and in doing

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so creates α Keto glutaric acid, an intermediate metabolyte of the Krebs Cycle. In addition to reflecting the use of protein for energy, serum GOT levels reflect the synthesis and breakdown of muscle tissue.

Cornelius <u>et al</u>. (1963) observed that horses undergoing strenuous daily exercise had average SGOT levels twice as high as horses not in training. Although they did not observe an increase in SGOT resting levels, Codazza <u>et al</u>. (1974) and Cardinet <u>et al</u>. (1963) found an increase in SGOT values following exercise. They suggested that increased SGOT activity at the onset of the conditioning period represents damage to muscle fibers. Anderson (1975) and Cardinet <u>et al</u>., however, observed that SGOT levels are inconsistently affected by exercise. Similarly, Milne (1976) found exercise to have little effect on SGOT levels.

In addition to the work done with respect to the serum enzymes, limited research has been done concerning the response of total serum protein, glucose, creatine, and calcium to exercise. Milne (1974) found a significant increase in serum calcium after heavy exercise that most likely represents a shift of calcium out of muscle cells as a result of acidosis. Soliman and Nadim (1967), however, found serum calcium levels to drop slightly after prolonged strenuous exercise which most likely reflects Ca losses in sweat.

Linholm and Saltin (1974) observed eight to ten fold increases in serum glucose levels following exercise. Englehardt <u>et al</u>. (1973) and Codazza <u>et al</u>. (1974), however, observed slight or no increase in serum glucose levels in response to exercise. Codazza <u>et al</u>. (1974) observed a significant increase in both total serum protein and creatine following exercise.

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The research that has been conducted to monitor the response of various serum constituents in response to exercise and conditioning has produced a wide variety of results. Further study of the serum enzymes CPK, LDH and GOT may yield more accurate information regarding the response of these enzyme to exercise and conditioning. This information may then be applied to assessing the feasibility of monitoring serum enzyme levels in response to an exercise test to evaluate an animal's level of fitness.

EXPERIMENTAL PROCEDURE

A group of nine previously sedentary Quarter Horse mares were randomly assigned to three treatment groups of 3 mares each. Treatment group I was a control treatment and horses in this group received no forced exercise. Horses in treatment group II were exercised daily at a trot of approximately 200 meters/minute for 3.6 km (2.25 miles). Horses in treatment group III were galloped daily at a pace of approximately 400 meters/minute for 3.6 km (2.25 miles). The horses were kept in semi-confinement and fed a commercially available pelleted feed and bermudagrass hay twice daily to meet NRC (1978) requirements. The horses were weighed daily and fed to maintain constant body weight throughtout the experiment.

The horses were conditioned according to their respective treatments for a period of 28 days. Before, during and immediately after the conditioning period, at days 0, 14 and 28, respectively, the mares were subjected to a standardized submaximal exercise tolerance test on an equine treadmill (Webb, 1978). The test was administered so that the effects of the treatments could be measured physiologically.

The exercise test was designed to stress the horse to a level just short of his maximum capability. This involved attaining heart rates of at least 180 beats per minute and required horses to ultimately resort to anaerobic respiration for the supply of energy. A treadmill, preset at a 9° incline, was used for the test because it facilitated a repeatable standard work load. The exercise tolerance test consisted of 30 minutes of exercise at a trot; the first 20 minutes of work were done at approximately 8.9 km/hr and the remaining 10 minutes at approximately 9.7 km/hr. In order to monitor serum constituent levels in response to exercise, 20 ml blood samples were drawn during the 30 minute exercise test and also during a 30 minute recovery period. Blood samples were obtained prior to the exercise test while the horses were at rest. Subsequent samples were taken at 10 minutes of exercise, 30 minutes of exercise, 10 minutes of recovery and 30 minutes of recovery. A catheter placed in the left jugular vein facilitated the collection of blood samples.

In order to obtain serum samples the blood was allowed to clot in test tubes and then centrifuged to separate the cell and serum fractions. The serum was then extracted and stored in plastic vials at -5° C for later analysis.

Upon completion of the experiment the serum samples were taken to the Texas Veterinary Diognostic Laboratory for analyses. The samples were analyzed using a Technicon SMA 12/60 Multichannel Biochemical Analyzer (Technicon Instruments Corporation, 1974), which automatically and simultaneously analyzes individual serum samples for 12 biochemical parameters. These parameters include: calcium, albumin, inorganic phosphorus, glucose, blood urea nitrogen, creatine, total bilirubin, alkaline phosphitase, creatine phosphokinase (CPK), lactic dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). In addition, total serum protein content was determined by refractometric determinations of specific gravity (American Optical, 1976).

The data were statistically analyzed by analysis of variance techniques. Differences between means were tested for significance by Duncan's New Multiple Range Means Test. In addition, relationships between the serum enzymes at the various intervals during the exercise tolerance test

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were measured by standard correlation analyses. (Steel and Torrie, 1960) Because one mare, due to emotional problems, would not work on the treadmill, observations in the trot treatment group were based on 2 horses rather than 3.

RESULTS AND DISCUSSION

Response of Creatine Phosphokinase to Exercise and Conditioning

Correlations between serum CPK values at the various sampling points during the exercise tolerance test were positive and very high. (Table 1). This suggests that for the purposes of monitoring changes in serum CPK levels over a conditioning period, one measurement during the exercise tolerance test may indicate overall CPK response to conditioning as good as another. It is necessary, however, to take additional measurements during the exercise test to observe the effects of stenuous exercise on serum CPK levels.

	Exer	Exercise 10min 30min		very
	10min			30min
Rest	0.99	0.96	0.97	0.92
10min exercise		0.98	0.98	0.91
30min exercise			0.97	0.93
lOmin recovery				0.93

TABLE 1. CREATINE PHOSPHOKINASE CORRELATION COEFFICIENTS DURING A SUBMAXIMAL EXERCISE TEST

Mean CPK values were calculated by treatment groups for the various sampling points during the exercise tolerance test. These mean values for the three exercise tests are shown in table 2.

The mean resting CPK value was 157.5 IU/ℓ , with a range of 121 IU/ℓ

Tuestment	Days of		Exercise		Recovery	
group	tioning	Rest	10min	30min	lOmin	30min
Control	0	147.3	172.7	209.7	171.7	180.3
	14	152.0	170.0	173.3	156.7	166.7
	28	121.0	145.7	147.3	140.7	141.0
Trot	0	191.0	226.0	265.0	237.0	253.5
	14	138.0	139.0	150.5	141.5	205.5
	28	141.5	164.5	163.0	159.0	130.5
Gallop	0	189.6	215.7	229.7	219.7	235.0
	14	148.0	167.3	166.3	162.0	162.0
	28	189.3	215.7	217.0	219.0	217.7

TABLE 2. MEAN SERUM CREATINE PHOSPHOKINASE VALUES DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)

to 191 IU/ℓ . These values fall within clinically normal ranges and are consistent with those found by Anderson (1975) and Milne (1976).

Serum CPK levels increased in response to the exercise tolerance test. In the first test, before any of the animals were conditioned, serum CPK levels increased from an overall mean resting value of 175.6 IU/ℓ to a post exercise value of 234.5 IU/ℓ . The CPK levels remained elevated or decreased only slightly during the 30 minute recovery period. This is consistent with findings by Anderson (1975), but contrasts findings of Milne (1976).

Over the 28 day conditioning period the magnitude of the increase in

serum CPK levels due to the exercise tolerance test decreased in horses in all of the three treatment groups. Differences between the treatments were not statistically significant. In addition, within each treatment, differences over the four week conditioning period were not significant.

Because the control group responded in similar manner as did the trot and gallop groups in this study, it is difficult to conclude whether differences in serum CPK levels in response to exercise are useful in objective assessment of individual horses' levels of fitness.

Response of Lactic Dehydrogenase to Exercise and Conditioning

Relationships of serum LDH values at the various sampling points during the exercise tolerance test were measured (Table 3). Resulting correlation coefficients were positive and high suggesting that any one measurement during the exercise test may reflect changes in serum LDH levels over the conditioning period.

	Exer	cise	Reco	overy
	lOmin	30min	10min	30min
Rest	0.89	0.64	0.81	0.76
10min exercise		0.66	0.85	0.83
30min exercise			0.74	0.83
lOmin recovery				0.90

TABLE 3. LACTIC DEHYDROGENASE CORRELATION COEFFICIENTS DURING A SUBMAXIMAL EXERCISE TEST

The mean resting serum LDH values, by treatment groups for the three consecutive exercise tolerance tests, are shown in Table 4. Serum LDH resting levels ranged from 218.3 IU/ ℓ to 374.3 IU/ ℓ , with an overall mean of 275.0 IU/ ℓ . These resting values fall within clinically normal ranges, but are considerably lower than those observed by Anderson (1975) and Milne (1976) who found values of 695 IU/ ℓ and 550 IU/ ℓ , respectively. Horses in the gallop treatment group had significantly higher (P<.05) resting serum LDH levels than horses in the trot and control treatment groups (Table 4).

Turaturat	Days o	Maana hu		
group	0	14	28	mean by trt.
Control	302.3	242.3	218.3	254.3 ^a
Trot	191.0	216.0	274.0	227.0 ^a
Gallop	374.3	353.7	311.7	346.5 ^b
Mean by week	289.2	270.7	268.0	

TABLE 4. MEAN RESTING SERUM LACTIC DEHYDROGENASE VALUES OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)

a,^b Values within a column having different superscripts differ significantly (P<.05).

Tables 5 and 6 show the mean serum LDH values during the exercise tolerance test at 10 minutes of exercise and 30 minutes of exercise, repectively. Comparing data in tables 4, 5 and 6, it appears that serum LDH levels increased in response to the exercise test. This is similar to the observations by Anderson (1975) and contrasts findings by Milne (1976).

At 30 minutes of exercise during the exercise test on day zero of conditioning, serum LDH levels increased to a mean post-exercise level of 378.0 IU/ℓ from a mean resting level of 289.2 IU/ℓ (Tables 4 and 6). While there were treatment differences in resting serum LDH values, these differences were not observed at 10 and 30 minutes of exercise during the exercise tolerance test.

At 30 minutes of exercise, mean LDH values were significantly lower (P<.05) on day 28 of conditioning than on day zero. This decreasing level of serum LDH measured in response to the 28 day conditioning period was observed in the control group as well as in the trot and gallop treatment groups. Anderson (1975) found similar decreasing trends for the conditioned horses, those subject to daily strenuous exercise, but control horses did not demonstrate this training effect.

Treatment group	Days o	Moan by		
	0	14	28	trt.
Control	383.3	276.0	272.3	295.6
Trot	318.5	258.5	294.0	290.3
Gallop	415.3	446.7	256.0	372.7
Mean by week	372.7	327.1	274.1	

TABLE 5. MEAN SERUM LACTIC DEHYDROGENASE VALUES AT 10 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/L)

Twootmont	Days o	ioning	Moon hu	
group	0	14	28	mean by trt.
Control	399.3	320.7	254.3	324.8
Trot	306.0	284.0	210.0	266.7
Gallop	428.7	307.7	267.7	334.7
Mean by week	378.0 ^a	304.7 ^a ,	^b 244.0	

TABLE 6. MEAN SERUM LACTIC DEHYDROGENASE VALUES AT 30 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)

TABLE 7. MEAN SERUM LACTIC DEHYDROGENASE VALUES AT 10 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)

Treatment group	Days o	Maan hu		
	0	14	28	mean by trt.
Control	347.0	267.3	235.3	283.2
Trot	293.5	279.0	188.0	253.5
Gallop	348.3	422.7	273.7	348.2
Mean by week	329.6	323.0	232.3	

Tables 7 and 8 show mean serum LDH values during the recovery phase of the 3 exercise tests at 10 minutes and 30 minutes of recovery, respectively. At 10 minutes of recovery there were no significant differences in mean LDH values by treatment groups over the 28 day conditioning period. At 30 minutes of recovery mean LDH values by treatment were not significantly different, however, values on day 28 of conditioning were significantly lower (P<.05) than those on days 0 and 14.

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Tuestment	Days o	Maan by		
group	0	14	28	trt.
Control	318.3	283.7	187.3	263.1
Trot	262.5	291.0	183.5	245.7
Gallop	376.7	405.3	220.7	334.2
Mean by week	319.2 ^a	326.7 ^a	197.2 ^b	

TABLE 8. MEAN SERUM LACTIC DEHYDROGENASE VALUES AT 30 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/L)

a,b Values within a row having different superscripts differ significantly (P<.05).

Response of Serum Glutamic Oxalacetic Transaminase to Exercise and Conditioning

Table 9 shows correlation coefficients for SGOT values at various intervals during the exercise tolerance test. The values are high and positive; thus, any measurement during the exercise test may represent response of SGOT to conditioning.

Mean resting levels of SGOT for the three exercise tests during the conditioning period are shown in Table 10. The values, averaged by treatment groups, ranged from 148.5 IU/ℓ to 326.3 IU/ℓ , with an overall mean of 259.6 IU/ℓ . These values are within normal clinical ranges and are

	Exerc	cise	Reco	Recovery		
	10min	30min	10min	30min		
Rest	0.85	0.71	0.81	0.80		
10min exercise		0.66	0.85	0.79		
30min exercise			0.74	0.79		
10min recovery				0.90		

TABLE 9. SERUM GLUTAMIC OXALACETIC TRANSAMINASE CORRELATION COEFFICIENTS DURING A SUBMAXIMAL EXERCISE TEST

TABLE 10. MEAN RESTING SERUM GLUTAMIC OXALACETIC TRANSAMINASE VALUES OVER A 28 DAY CONDITIONING PERIOD (IU/*ℓ*)

Theatment	Days o	ioning	Moan	
group	0	14	28	trt.
Control	253.7	275.3	248.3	259.1 ^{a,b}
Trot	148.5	264.0	265.0	225.8 ^b
Gallop	260.7	326.3	294.3	293.8 ^a
Mean by week	221.0 ^b	288.5 ^a	269.2 ^{a,b}	

a,^b Values within a row or column having different superscripts differ significantly (P<.05)

similar to those found by Anderson (1975) and Milne (1976). Mean resting SGOT levels were significantly higher (P<.05) on day 14 of conditioning

than on day zero. In addition, mean resting SGOT levels in horses in the gallop treatment group were significantly higher (P<.05) than horses in the trot treatment but were not significantly different than values for horses in the control group. Cornelius <u>et al</u>. (1963) observed that horses in training (regular strenuous exercise) had resting SGOT levels twice as high as horses not in training. Observations in this study confirm his findings, although not to the same degree, in that horses in the gallop treatment group had higher resting SGOT levels than horses in the trot and control groups on day 28 of conditioning.

TABLE 11. MEAN SERUM GLUTAMIC OXALACETIC TRANSAMINASE VALUES AT 10 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/L)

Treatment group	Days (Maan hu		
	0	14	28	trt.
Control	290.3	315.3	276.7	294.1 ^a
Trot	270.5	290.0	302.0	287.5 ^a
Gallop	299.0	374.3	324.7	332.7 ^b
Mean by week	286.6	326.5	301.2	

a,^b Values within a column having different superscripts differ significantly (P<.05)

Mean SGOT levels at 10 and 30 minutes of exercise during the exercise tolerance tests on days 0, 14 and 28 of conditioning are shown in Tables 11 and 12, respectively. Serum GOT levels increased in response to the exercise tolerance test (Tables 10, 11 and 12). These trends were observed

by Cardinet <u>et al</u>. (1963) but not by Anderson (1975) and MIlne (1976). At 10 minutes of exercise, horses in the gallop treatment group had significantly higher (P<.05) SGOT levels than horses in the other two groups. This is not surprising, however, since their resting SGOT levels were also significantly higher (P<.05).

Treatment group	Days o	Moon by		
	0	14	28	trt.
Control	299.7	383.3	276.0	386.3
Trot	290.0	301.5	294.5	295.3
Gallop	332.0	372.7	298.3	334.3
Mean by week	307.2	352.5	289.6	

TABLE 12. MEAN SERUM GLUTAMIC OXALACETIC TRANSAMINASE VALUES AT 30 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)

At both 10 and 30 minutes of exercise, the increase in SGOT levels in response to the exercise test appeared similar in all three treatments over the conditioning period. There were no apparent treatment trends in SGOT levels at the end of the exercise tolerance test. This is similar to results shown by Anderson (1975) and Milne (1976), but contrasts findings by Cardinet <u>et al.</u> (1963).

Tables 13 and 14 show mean SGOT levels at 10 and 30 minutes of recovery during the exercise tolerance tests, respectively. No significant differences were observed between treatment groups during recovery at the three testing points during the conditioning period. However, at 30 min.

TABLE 13. MEAN SERUM GLUTAMIC OXALACETIC TRANSAMINASE VALUES AT 10 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/L)

Treatment group	Days o	of condit	Mean by	
	0	14	28	trt.
Control	275.7	284.0	258.7	272.8
Trot	307.0	276.5	262.5	282.0
Gallop	286.3	355.0	300.0	313.8
Mean by week	289.7	305.2	273.7	

TABLE 14. MEAN SERUM GLUTAMIC OXALACETIC TRANSAMINASE VALUES AT 30 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/*L*)

Treatment group	Days o	of condit	Maara	
	0	14	28	mean by trt.
Control	260.0	267.0	243.0	258.8 ^a
Trot	272.5	288.0	266.0	275.5 ^{a,b}
Gallop	282.0	260.0	304.0	315.3 ^b
Mean by week	271.5	305.0	271.0	

 a,b Values within a column having different superscripts differ significantly (P<.05)

of recovery horses in the gallop treatment group had significantly higher (P<.05) mean SGOT levels than horses in the control and trot groups, consistent with higher levels at rest and 10 minutes of exercise during the exercise test.

Response of Glucose to Exercise and Conditioning

Resting serum glucose levels are shown in Table 15. The resting values ranged from 74.3 mg% to 94.0 mg%. These values are within normal clinical ranges. Horses in the trot and gallop treatment groups had mean serum glucose levels higher than those in the control group, with significant differences (P<.05) between the trot and control groups. Although there were no treatment by week interactions it appears that resting serum glucose levels of horses in the gallop group consistently increased over the conditioning period.

			· · · · · · · · · · · · · · · · · · ·			
Thostmont	Days o	Days of conditioning				
group	0	14	28	trt.		
Control	74.3	80.7	77.3	77.4 ^b		
Trot	88.0	84.5	94.0	88.8 ^a		
Gallop	78.0	88.3	89.0	85.1 ^{a,b}		
Mean by week	85.8	84.5	79.1			

TABLE 15. MEAN RESTING SERUM GLUCOSE VALUES OVER A 28 DAY CONDITIONING PERIOD (mg%)

^{a,b} Values within a column having different superscripts differ sinificantly (P<.05)

Tables 16 and 17 show mean serum glucose levels at 10 and 30 minutes of exercise, respectively. Glucose levels increased in response to the exercise tolerance test (Tables 15, 16 and 17) consistent with responses found by Lindlolm and Saltin (1974) but inconsistent with findings of Englehardt et al. (1973). At 10 minutes of exercise, horses in the control

Treatment group	Days o	Days of conditioning				
	0	14	28	trt.		
Control	79.9	93.0	81.7	84.8 ^b		
Trot	120.0	95.0	102.5	105.8 ^a		
Gallop	89.0	84.0	81.3	84.8 ^b		
Mean by week	93.3	90.1	86.8			

TABLE 16. MEAN SERUM GLUCOSE VALUES AT 10 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

^{a,b} Values within a column having different superscripts differ significantly (P<.05).

TABLE 17. MEAN SERUM GLUCOSE VALUES AT 10 MINUTES OF EXERCISE DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Treatment group	Days o	Maria		
	0	14	28	trt.
Control	117.7	121.7	108.0	115.7
Trot	129.0	108.5	113.0	116.8
Gallop	108.7	109.0	90.3	102.7
Mean by week	117.1 ^a	113.6 ^a ,	^b 102.6 ^b	

 $^{\rm a,b}$ Values within a row having different superscipts differ significantly (P<.05).

and gallop groups had significantly lower (P<.05) glucose levels than horses in the trot group. In addition, at 30 minutes of exercise during the exercise test mean glucose values on day 28 of conditioning were significantly lower (P<.05) than values on day 0. Although there were no treatment by week interactions, horses in the gallop group had consistently decreasing glucose levels produced in response to the exercise test over the conditioning period.

The mean serum glucose values at 10 and 30 minutes of recovery during the exercise tolerance test are shown in Tables 18 and 19, respectively. At both sampling points, horses in the gallop group had significantly lower (P<.05) mean glucose levels than horses in the control group. Since no prior research has been done dealing with the response of glucose to conditioning, these results cannot be compared to others.

	L					
Theatment	Days o	Days of conditioning				
group	0	14	28	trt.		
Control	110.7	111.7	107.0	109.8 ^a		
Trot	117.0	104.5	111.0	110.8 ^a		
Gallop	104.3	97.7	94.0	98.7 ^b		
Mean by week	109.9	104.6	103.1			

TABLE 18. MEAN SERUM GLUCOSE VALUES AT 10 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

a,b Values within a column having different superscipts differ significantly (P<.05).

Treatment group	Days o	Days of conditioning			
	0	14	28	trt.	
Control	106.3	96.0	88.0	96.8 ^{a,b}	
Trot	105.5	96.0	102.0	101.2 ^a	
Gallop	89.3	89.3	88.3	89.0 ^b	
Mean by week	99.8	93.5	91.6		

TABLE 19. MEAN SERUM GLUCOSE VALUES AT 30 MINUTES OF RECOVERY DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

^{a,b} Values within a column having different superscripts differ significantly (P<.05).

Response of Additional Serum Constituents to Exercise and Conditioning

Several additional serum constituents were measured along with the four previously discussed. Appendix Tables 1 thru 9 show the mean levels of serum creatine, total bilirubin, inorganic phosphorus, total serum protein, calcium, blood urea nitrogen, albumine, alkaline phosphitase, and serum glutamic pyruvic transaminase, in that order. Values for these various parameters were all within normal clinical ranges and exercise neither increased or decreased levels. In addition, conditioning caused no response in these various parameters.

GENERAL DISCUSSION

The modern horse, much like a human athlete, is called upon to perform various strenuous activities which he would not encounter in the wild. The cutting horse, for example, is asked to stop and turn much harder than natural circumstances would dictate. Similarly, horses in a steeplechase are asked to jump very difficult obstacles, perform at a high speed and have stamina to last the entire race. Thus, demands placed upon horses today make it necessary for trainers to gain a better understanding of how to adequately condition horses in order to compete sucessfully in such activities.

In conditioning horses for strenuous activities the main objective is developing and expanding the capabilities of the muscular system. Thus, research has been directed at gaining knowledge of the functioning of muscle tissue and factors that affect it. With respect to this, several enzymes have been studied as to their response to exercise and conditioning. The enzymes creatine phosphokinase, lactic dehydrogenase and glutamic oxaloacetic transaminase function within the muscle cells to produce energy. These enzymes are believed to be spilled into the bloodstream because of an increase in cell membrane permeability; thus, monitoring serum levels of these enzymes may reflect needs of the muscle cells for energy.

Research dealing with these enzymes has produced a wide variety of results leaving many questions unanswered. On one hand these serum enzymes have been shown to increase in response to strenuous exercise with the magnitude of this increase diminishing with conditioning. A logical explaination of this phenomenon would be that a more physiologically fit horse is more efficient in using energy to perform a task, and thus a lower level of enzymes related to energy production would be needed resulting in lower levels of these enzymes being found in the serum. Supporters of this theory suggest that monitoring serum levels of these enzymes following a strenuous exercise test may reflect a horse's level of fitness. On the other hand, other workers have not observed similar changes in these serum enzymes in response to exercise and conditioning. It is obvious, then, that further research in the field is needed.

Discrepancies in prior research may be explained by studying several factors inherent to research of this type. For example, the initial level of physical fitness of horses used in these studies may vary, contributing to apparent contradictions in published results. Similarly, differences in the duration and intensity of exercise used to condition horses and the length of the conditioning period itself may result in varied findings. In addition, the variations in exercise tolerance tests with respect to the demand they place on the muscular systems of horses may relate to conflicting data in the literature. And finally, differences in environmental factors, especially extremes in temperature and humidity, may offer explaination as to varied research results. Thus, controlling or limiting variation in these factors is necessary if researchers are to produce uniform results and conclusions.

In this study serum levels of creatine phosphokinase, lactic dehydrogenase and glutamic oxaloacetic transaminase increased in response to the exercise tolerance test. The magnitude of these elevated levels remained consistent throughout the conditioning period for SGOT. Thus, monitoring SGOT levels in horses during an exercise test may not reflect physiological fitness. On the other hand, over the conditioning period, decreasing amounts of serum CPK and serum LDH were measured in response to the exercise test for all three treatment groups, (control, trot and gallop). It could be suggested then, that horses in the trot and gallop groups displayed a training effect, meaning that the conditioning program developed the muscular system such that on day 28 of conditioning these horses were more efficient in their energy use, as evidenced by lower serum CPK and LDH levels post exercise. This suggestion, however, ignores the existence of a similar "training effect" in the control horses. Obviously, post exercise levels of serum CPK and LDH should have remained constant over the conditioning period instead of decreasing. One explaination of this behavior might be linked to the exercise test itself. The use of an equine treadmill adds an additional stress factor in addition to the work load produced during the test. Thus, over the 3 consecutive tests, horses in the control group may have displayed a training effect that more nearly represents emotional adaptation to the testing apparatus. Another explaination could be that the amount of exercise received from the exercise test produced a gradual change in physical fitness in control horses. If these suggestions are true for the control group, then it is possible that similar observations in horses in the trot and gallop groups did not reflect a true training effect or conditioning of the muscular system. On the other hand, a true training effect may have been present but was masked by these other factors.

Research efforts in the future should attempt to eliminate the emotional response of horses to the treadmill before the actual experiment

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begins. In addition, an experiment to study the possibility that three strenuous exercise periods over 28 days may cause an increase in fitness is in order. Another possible experiment would be to use the same horses for the control and exercise groups, allowing a long enough sedentary period in between conditioning periods to erase prior training effects. In a test such as this each horse would serve as his own control, and variations in results due to inherent differences between horses would be eliminated.

Research studying the effects of exercise and conditioning is difficult because of the many variables present. But, because conclusions regarding this field of study are desparately needed in the horse industry, which expends large amounts of time and money for conditioning horses, the search for objective means of monitoring fitness and comparing conditioning programs for horses should be continued.

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SUMMARY AND CONCLUSIONS

In this study serum levels of calcium, albumin, inorganic phosphorus, glucose, blood urea nitrogen, creatine, total bilirubin, alkaline phosphitase, creatine phosphokinase (CPK), lactic dehydrogenase (LDH), serum glutamic oxalactic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and total serum protein were studied as to their response to exercise and conditioning for the purpose of relating changes in levels of these parameters to an objective means of measuring fitness levels in horses. Serum levels of calcium, albumin, inorganic phosphorus, blood urea nitrogen, creatine, total bilirubin, alkaline phosphitase, serum glutamic pyruvic transaminase and total serum protein fell within normal clinical ranges and did not change greatly in response to the standardized submaximal exercise tolerance test. In addition, serum levels in horses in all three treatments (control, trot and gallop) did not change over the 28 day conditioning period. Thus, from this study it cannot be concluded that monitoring serum levels of these constituents can be useful in objective assessment of horses' fitness levels.

Serum levels of CPK, LDH, SGOT and glucose increased in response to the exercise tolerance test. Over the 28 day conditioning period, the magnitude of this increase remained constant for SGOT. Serum CPK, LDH and glucose levels, however, increased less in response the exercise test on day 28 of conditioning than on day 0. These decreasing levels due to conditioning were observed in all three treatment groups. Control values should not have indicated a training effect and reasons for this are not fully understood. But because control horses responded to exercise and conditioning in a similar manner to the horses in the trot and gallop treatment groups, it is difficult to conclude that monitoring serum CPK, LDH and glucose in response to an exercise tolerance test can be useful in measuring an individual horse's level of fitness.

There were no significant differences between the responses of horses in the trot treatment group versus horses in the gallop treatment group to exercise and conditioning. Thus, from this study, evaluations of the efficiency of these two conditioning schedules cannot be made.

LITERATURE CITED

- Aitken, M. M., Anderson, M. G., Mackensie, G. and Sanford, J. 1974. Correlations between physiological and biochemical parameters used to assess fitness in the horse. J. S. Afr. Vet. Assoc. 45:361.
- American Optical Scientific Instrument Division. 1976. Publication No. 10400A-101. Buffalo, N.Y.
- Anderson, M. G. 1975. The influence of exercise on serum enzyme levels in the horse. Equine Veterinary Journal. 7:3.
- Cardinet, G. H., Fowler, M. E. and Tyler, W. S. 1963. The effects of training, exercise and tying-up on serum transaminase activities in the horse. Am. Journal of Vet Research. 24:980.
- Cardinet, G. H., Littrell, J. F., and Freedland, R. A. 1967. Comparative investigations of serum creatine phosphokinase and glutamicoxaloacetic transaminase activities in equine paralytic myoglobinuria. Research in Veterinary Science. 8:219.
- Codazza, D., Maffeo, G. and Redaelli, G. 1974. Serum enzyme changes and haemato-chemical levels in thoroughbreds after transport and exercise. J. S. Afr. Vet. Assoc. 45:331.
- Cornelius, C. E., Burnham, L. G. and Hill, H. E. 1963. Serum transaminase activities of thoroughbred horses in training. J. Am. Vet. Med. Assoc. 142:639.
- Engelhardt, W. V., Hörnicke, H., Ehrlein, H. and Schimidt, E. 1973. Lactat, pyruvat, glucose und wasserstoffionen im vernösen blut bei reitpferden in unterschiedlichem trainingzustand. Zbl. Vet. Med. A-20:173.
- Highman, B. and Altland, P. D. 1960. Serum evzyme rise after hypoxia and effect of autonomic blockade. Am. J. Physiol. 199:981.
- Lindholm, A. and Saltin, B. 1974. The physiological and biochemical response of standardbred horses to exercise of varying speed and duration. Act. Vet. Scand. 15:310.
- Milne, D. W. Gabel, A. A., Muir, W. W. and Skarda, R. T. 1976. Effects of exercise and training on cardiovascular and biochemical values in the horse. Proc. Am. Assoc. of Equine Pract. 22:211.
- N. R. C. 1973. Nutrient requirements of horses. National Academy of Science, Wash. D. C.

- Soliman, M. K. and Nadim, M. A. 1967. Calcium, sodium and potassium levels in the serum and sweat of healthy horses after strenuous exercise. Zbl. Vet. Med. A-14:53.
- Steel, R. G. D. and Torrie, J. H. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. Inc., New York, N. Y.
- Technicon Instruments corporation. 1974. Technical Publication No. U A 4-0160-00. Tarrytown, New York.
- Webb, S. P. 1978. Unpublished research. Texas A&M University, College Station, Texas.

APPENDIX

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Turaturat	Days of		Exer	Exercise		very
group	tioning	Rest	10min	30min	10min	30min
Control	0	1.7	1.9	2.4	2.3	2.2
	14	1.9	2.2	2.5	2.4	2.3
	28	2.0	2.2	2.4	2.5	2.4
Trot	0	1.8	2.0	2.3 /	2.2	2.2
	14	1.8	2.0	2.3	1.9	2.2
	28	2.0	2.2	2.4	2.5	2.4
Gallop	0	1.8	2.0	2.2	2.2	2.2
	14	1.8	2.0	2.2	2.1	2.2
	28	1.8	2.1	2.2	2.2	2.2

APPENDIX TABLE 1. MEAN SERUM CREATINE LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Tuestment	Days of		Exercise		Recovery	
group	tioning	Rest	lOmin	30min	lOmin	30min
Control	0	0.69	0.71	0.70	0.60	0.52
	14	0.74	0.90	0.88	0.60	0.67
	28	0.64	0.82	0.79	0.60	0.46
Trot	0	0.61	0.61	0.75	0.43	0.67
	14	0.54	0.65	0.67	0.45	0.68
	28	0.57	0.67	0.49	0.87	0.34
Gallop	0	0.38	0.47	0.43	0.38	0.41
	14	0.51	0.55	0.41	0.59	0.66
	28	0.63	0.56	0.48	0.65	0.39

APPENDIX TABLE 2. MEAN TOTAL BILIRUBIN LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Treatment group	Days of		Exercise		Recovery	
	condi- tioning	Rest	lOmin	30min	10min	30min
Control	0	3.2	3.6	4 1	34	3 1
	14	3.1	3.6	3.9	3.3	3.0
	28	2.5	2.9	3.0	2.7	2.5
Trot	0	3.9	4.3	4.6	4.0	3.9
	14	2.6	2.8	3.1	2.5	2.4
	28	2.9	3.2	3.4	2.8	2.3
Gallop	0	3.7	4.3	4.4	3.9	3.5
	14	2.8	3.5	3.3	3.2	3.0
	28	2.8	3.0	3.3	2.8	2.4

APPENDIX TABLE 3. MEAN INORGANIC PHOSPHORUS LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Twootmont	Days of		Exercise		Recovery	
group	tioning	Rest	10min	30min	10min	30min
Control	0	7.4	8.6	8.7	8.0	7.3
	14	7.5	8.5	8.6	7.7	7.3
	28	7.1	7.9	7.8	7.3	6.9
Trot	0	7.5	8.1	8.6	8.2	7.7
	14	7.5	7.7	7.9	7.5	7.4
	28	7.0	7.8	7.8	7.2	6.9
Gallop	0	7.0	7.9	7.8	7.7	7.1
	14	6.8	7.7	7.3	7.1	7.3
	28	6.7	7.2	7.3	6.6	6.9

APPENDIX TABLE 4. MEAN TOTAL SERUM PROTEIN LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Two atmost	Days of		Exercise		Recovery	
group	tioning	Rest	10min	30min	10min	30min
Control	0	12.4	13.3	12.6	11.9	12.2
	14	12.6	13.2	12.6	11.9	12.1
	28	11.7	11.8	11.7	11.3	12.1
Trot	0	12.4	12.2	12.0	11.1	12.1
	14	12.4	12.5	12.4	12.0	12.6
	28	12.6	13.3	12.7	12.2	12.3
Gallop	0	12.6	12.8	11.8	11.9	12.3
	14	11.3	11.9	11.1	11.1	12.0
	28	11.6	11.2	11.1	11.3	11.7

APPENDIX TABLE 5. MEAN SERUM CALCIUM LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Tuestment	Days of		Exercise		Recovery	
group	tioning	Rest	10min	30min	10min	30min
Control	0	14.7	14.6	15.2	15.3	15.2
	14	14.7	15.2	15.4	15.5	15.2
	28	13.3	13.2	13.6	13.9	13.9
Trot	0	16.9	16.1	16.5	16.7	17.2
	14	16.3	16.3	16.7	17.1	17.2
	28	17.0	17.2	17.0	17.3	17.3
Gallop	0	15.4	15.2	15.4	16.2	16.0
	14	16.7	17.2	17.2	17.3	18.0
	28	15.3	15.7	15.5	15.8	16.0

APPENDIX TABLE 6. MEAN BLOODUREA NITROGEN LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

Two atmost	Days of		Exercise		Recovery	
group	tioning	Rest	10min	30min	lOmin	30min
Control	0	3.2	3.4	3.6	3.3	3.5
	14	3.2	3.5	3.5	3.3	3.1
	28	3.0	3.2	3.2	3.1	2.9
Trot	0	3.2	3.3	3.4	3.2	3.2
	14	3.0	3.2	3.3	3.2	3.2
	28	3.1	3.3	3.3	3.3	3.1
Gallop	0	3.0	3.4	3.4	3.2	3.2
	14	3.0	3.4	3.3	3.2	3.2
	28	3.0	3.2	3.1	3.0	3.1

APPENDIX TABLE 7. MEAN SERUM ALBUMIN LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (mg%)

T	Days of	Exercise			Recovery	
lreatment group	condi- tioning	Rest	10min	30min	10min	30min
Control	0	267.3	309.3	325.3	301.3	272.3
	14	274.0	311.7	333.0	297.0	273.7
	28	229.3	260.7	253.7	246.7	237.3
Trot	0	270.5	294.0	313.5	277.5	288.0
	14	264.0	291.5	310.5	294.5	291.5
	28	262.5	307.5	302.5	206.5	266.5
Gallop	0	339.0	383.7	379.0	358.3	355.7
	14	360.6	403.7	365.0	396.3	384.7
	28	332.7	372.0	353.7	347.3	342.0

APPENDIX TABLE 8. MEAN SERUM ALKALINE PHOSPHITASE LEVELS DURING SUBMAX-IMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ \mathcal{L}).

Treatment group	Days of		Exercise		Recovery	
	condi- tioning	Rest	10min	30min	10min	30min
Control	0	2.3	0.0	0.0	0.0	0.0
	14	1.3	0.0	0.0	0.0	0.3
	28	1.7	0.0	0.0	0.0	0.0
Trot	0	2.0	0.0	0.0	0.0	0.0
	14	2.0	1.0	3.0	0.0	2.5
	28	0.5	1.0	0.0	0.0	0.0
Gallop	0	1.0	0.0	0.0	1.0	0.0
	14	5.7	3.3	1.3	3.3	4.3
	28	2.0	1.7	1.3	0.3	0.3

APPENDIX TABLE 9. MEAN SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS DURING SUBMAXIMAL EXERCISE TESTS OVER A 28 DAY CONDITIONING PERIOD (IU/ℓ)