This is to certify that the

dissertation entitled

The Relationship Between Selenium and Vitamin E Nutrition and Exercise in Horses

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Animal Science

Major professor
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Date 6/15/84

THE RELATIONSHIP BETWEEN SELENIUM AND VITAMIN E NUTRITION AND EXERCISE IN HORSES

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1984

ABSTRACT

THE RELATIONSHIP BETWEEN SELENIUM AND VITAMIN E NUTRITION AND EXERCISE IN HORSES

By

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The similarities between exercise-induced myopathies in horses and white muscle disease have led to supplementation of both selenium (Se) and vitamin E to heavily exercised horses. Exercise increases O_2 delivery and metabolism in muscle tissue resulting in an increased generation of reactive O_2 byproducts. The Se-containing enzyme glutathione peroxidase (GSHpx) and vitamin E help protect the cell from these reactive O_2 species.

To establish Se status of horses at the MSU Horse Teaching and Research Center (HTRC), plasma and milk Se concentrations of 6 mares and their foals were analyzed and found within normal ranges despite classification of Michigan as a Se-deficient state. Previous studies at the HTRC have offered no explanation for these normal plasma Se concentrations. Analyses of forage, soil and cinder-surfaced

roads support the conclusion that high forage Se was a consequence of soil contamination with Se-rich cinder dust.

Eight Arabian mares were used in a 2x2 double split-plot design to determine the effects of conditioning, exercise and daily supplements of 2.5 mg Se and/or 750 IU vitamin E on blood constituents of the GSHpx system and on muscle and blood enzymes. Three levels of treadmill exercise conditioning were provided: 1) non-conditioned, 2) conditioned for 45 days and 3) conditioned and allowed 2 days of stall rest before sampling. Blood samples were obtained before, during and after exercise. Conditioning mares for 45 days reduced blood malondialdehyde (MDA) and lactate concentrations, indicating improved animal fitness. High blood MDA concentrations in non-conditioned mares, and increased plasma creatine phosphokinase activities after 2 days of rest in conditioned mares, suggest that sudden changes in physical activity should be approached cautiously.

Blood reduced glutathione concentration and erythrocyte glucose-6-phosphate dehydrogenase activity decreased with conditioning. Eighteen weeks of vitamin E supplementation increased plasma alpha-tocopherol concentrations but did not significantly affect any other parameter measured. Erythrocyte GSHpx activities were significantly higher as a result of conditioning and Se supplementation. Mares in this study appeared to have adequate plasma Se levels. However, the observed increases in GSHpx activity associated with

conditioning may indicate a need for supplemental Se when diets of exercising horses are low in this element.

DEDICATION

To my wife Pam, whose faith in me
made this work possible.

ACKNOWLEDGEMENTS

There so many people who helped and supported me during my education it would be impossible to thank them all, so let me start with a blanket thank you to those people whose names are not mentioned here but were instrumental in the completion of this work. Data gathering during this project took many hands. I would like to thank Dr. Joe Rook, Dave Anderson, Chris Johnson, Dave Baer, Dave Cross, Marilyn Loudenslager and especially Mike Yoder, for helping to make things run smoothly. A special thanks to Dr. Wayne Van Huss from the Department of Health and Physical Education for his assistance in monitoring heart rates. I would like to thank Phyllis Whetter and Dr. Pao Ku for their patience and willingness to share their experience with laboratory procedures.

I would like to express a special thank you to Norm Oswald from the University Farms Department for his assistance in the design and fabrication of the treadmill and the many modifications it took to make it operable. His friendship and support will always be remembered.

The willingness of my graduate committee, Dr. Harold Henneman, Dr. Elwyn Miller and Dr. Howard Stowe, to help in giving direction and guidance in my course of study was

greatly appreciated. Most importantly I would like to thank my committee chairman Dr. Duane Ullrey for his constant support and friendship. I feel fortunate to have been able to work with a person whom I so greatly respect and admire.

Lastly I would like to thank my wife, Pam for the many sacrifices she made during our education. Her love and support makes the completion of this work that much more meaningful. I would like to thank my kids Michelle, Kevin and Aaron for understanding that dad couldn't always be there. This degree is indeed a family accomplishment.

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INTRODUCTION

Selenium (Se) and vitamin E have not routinely been added to equine rations as with diets of other species. This may be due primarily to the lack of conclusive evidence of Se-vitamin E deficiency diseases in the horse. Nutritional muscular dystrophy (NMD) does occur in foals and is responsive to vitamin E and Se supplementation (Caple et al., 1978; Gabbedy and Richards, 1970; Schougaard et al., 1972; Wilson et al., 1976). However, the frequency of NMD has been low, even in areas with low soil Se concentrations, and horsemen have not routinely provided supplementation.

Evidence of Se and vitamin E deficiencies in adult horses has been even more elusive. Heimann et al. (1981) demonstrated improved reproductive efficiency in pony mares grazing fescue pasture by providing Se or fertilizing pastures with nitrogen which increased Se content of the forage. Reproductive problems involved perinatal foal losses due to thickening of the placenta or postnatal losses from agalactia. Owen et al. (1977) hypothesized that maxillary myositis and dystrophic myodegeneration in adult horses may be the same disease entity caused by a deficiency of vitamin E and Se. However, conclusive evidence supporting this thesis has not been forthcoming. Dewes (1981) reported

improved athletic performance in horses with parenteral administration of vitamin E. Improvement was seen in attitude and disposition and was thought to be the result of prevention of a mild subclinical myositis, manifested in progressively deteriorating performance and behavior.

There are no less than seven types of myopathies of horses described in the literature. The etiology of most is unknown (Hansen, 1970). Azoturia (Monday morning disease) and tying-up are among the most common and are thought to be the same disease state, varying only in the severity of signs (Farrow et al., 1976). Signs normally appear in well-fit individuals on the first day of exercise after a period of rest; hence, the common name "Monday morning disease". Elevated serum activities of glutamic oxalacetic transaminase and creatine phosphokinase and, at necropsy, lesions of skeletal and cardiac muscle are also described (Cardinet et al., 1967; Lindholm et al., 1974). Affected animals exhibit myoglobinuria and resist further muscular activity. The similarities between NMD in lambs and foals, and exercise-induced myopathies have led veterinarians to prescribe supplementation of Se and vitamin E both prophylactically and therapeutically in the treatment of azoturia and tying-up with favorable results (Farrow et al., 1976; Hill, 1962; Stewart, 1960).

Azoturia and tying-up may occur when conditioning horses for show, race or endurance competition. Because they occur more frequently in some breeds and families than

in others, there is concern that there may be a genetic predisposition in some horses. There is also a tendency for reoccurrence of signs once an animal has had a problem. All of this amounts to losses in both training time and medical expenses which are of concern to a great many horsemen.

The cell has evolved a variety of defense mechanisms to protect against peroxidative membrane damage. Superoxide dismutase, often called the first line of cellular defense, acts on oxygen radicals reducing them to less toxic hydrogen peroxides. The seleno-enzyme, glutathione peroxidase (GSHpx) and vitamin E protect the cell from peroxidative damage as follows. GSHpx, working together with catalase, destroys hydrogen peroxide and organic peroxides. Reducing equivalents for these reactions are provided by the enzymes of the hexose monophosphate pathway (Figure 1). Vitamin E exerts its protective effect by preventing perpetuation of peroxidative chain reactions in unsaturated fatty acids within membranes (Diplock, 1981).

The effects of exercise on this system were first alluded to by Young and Keeler (1962). Immobilizing one limb of Se-deficient lambs significantly reduced muscular lesions in that limb, indicating that exercise exacerbates the effects of Se deficiency. Exercise increases oxidative metabolism at the cellular level which may lead to an increased generation of potentially damaging byproducts. If

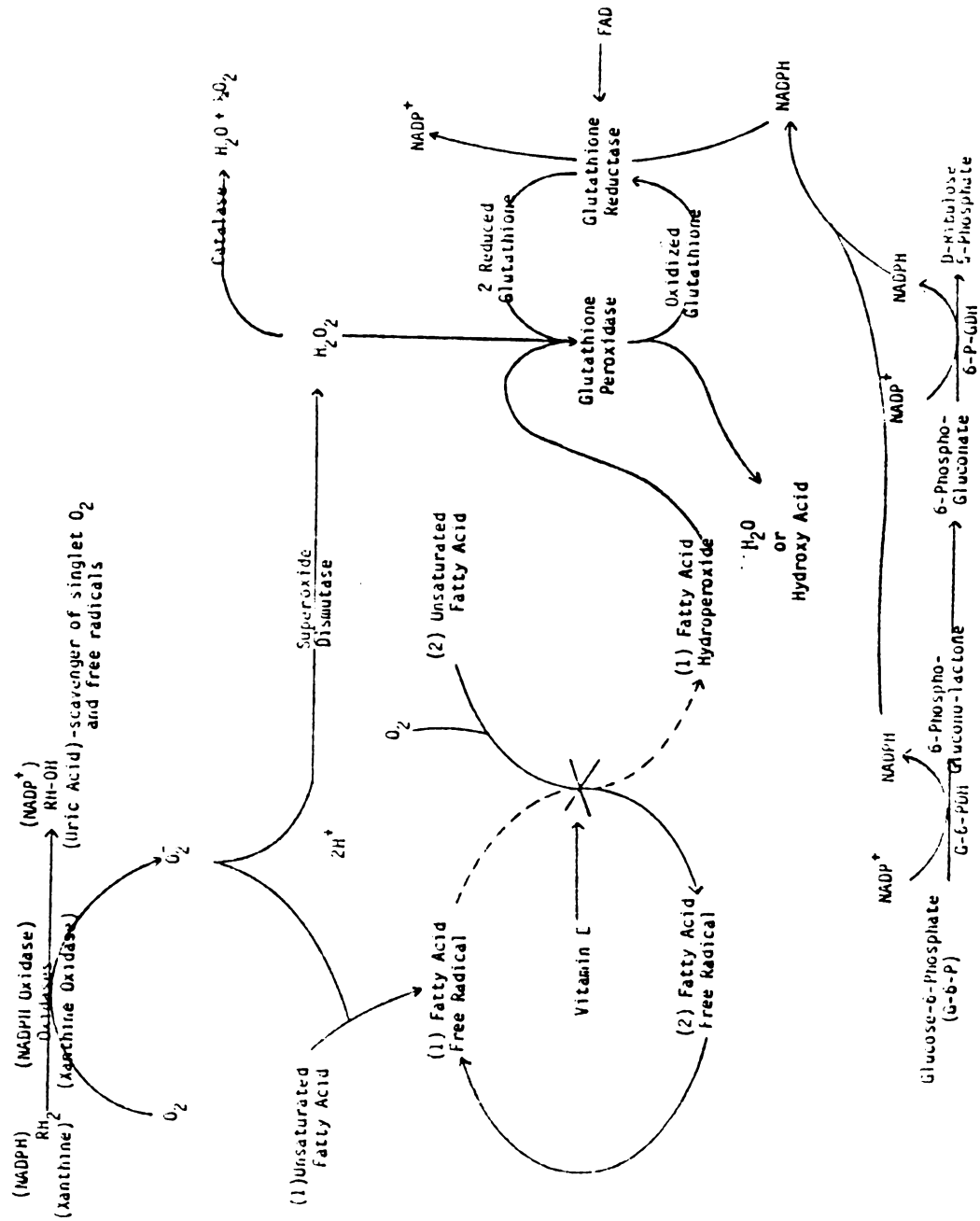


Figure 1. System for cellular protection against O_2 induced damage.

this is indeed the case, then perhaps rigorous exercise programs may increase the need for vitamin E and Se above the minimum requirements.

Thoroughbred horses which had been described as unsatisfactory performers were found to have lower serum Se levels than horses whose performance was as expected (Blackmore et al., 1979). Brady et al. (1978) reported increased peroxidative damage in exercised horses but could not show an increase in GSHpx activity with Se supplementation. However, this study was conducted using non-conditioned horses during a single exercise bout. The need for Se supplementation may increase with conditioning. A constant challenge to the GSHpx system through frequent exercise may stimulate synthesis of GSHpx and hence increase the Se need.

The effect of mega-doses of vitamin E on performance of human and equine athletes has been tested by Sharmon et al. (1971) and Lawrence and Slade (1979), respectively. In both cases, athletes were on conditioning programs and performance was not significantly altered by supplementation. However, the effect of supplementation on limiting peroxidative damage was not assessed.

This study was undertaken to: 1) reproduce as nearly as possible the factors which result in equine exercise myopathies, by varying days of rest and exercise, 2) determine the effect of Se and vitamin E supplementation on changes in serum enzyme activities resulting from exercise-

induced stress, 3) evaluate changes in the erythrocyte GSHpx system due to supplementation and conditioning, 4) design and build an equine exercise treadmill, with adjustable speed and slope of incline, to accurately produce controlled exercise stress for use in studies of this nature, and 5) investigate further the unusually high plasma Se levels found in horses at the MSU Horse Teaching and Research Center.

REVIEW OF THE LITERATURE

Selenium and Vitamin E in Nutrition of the Horse

Selenium Toxicity

Toxicity signs were first reported in horses in the United States by Madison (1860). In the 1930s the causative agent was identified as Se, occurring in high levels in plants that concentrate the element naturally (Franke, 1934). The acute form of toxicity is called blind staggers with the chronic form known as alkali disease. Chronic signs include hair loss (especially of the mane and tail), laminitis and hoof sloughing. Lack of pathologic lesions of the visceral organs has been a feature of poisoned horses at necropsy (Knott and McCray, 1959). Crinion and O'Connor (1978) saw elevated sorbitol dehydrogenase activity, indicative of liver damage, in the serum of horses consuming plants high in Se. However, death in most instances has at least partially been attributed to an inability to forage for food due to lameness. The acute form of toxicity is evidenced by elevated temperature, labored respiration, a bloody froth from the mouth and nose, respiratory failure and death. Acute toxicity normally follows consumption of very high levels of Se (Gerken, 1982).

Selenium Deficiency

The essentiality of Se has over-shadowed its toxic properties in recent years. The occurrence of nutritional muscular dystrophy (NMD) has been correlated to areas of the United State where Se content or availability in soils is low (Muth and Allaway, 1963). It has further been shown that the signs of NMD can be eliminated with the addition of Se to livestock diets (Ewan et al., 1968). NMD is also seen in horses and is responsive to Se and vitamin E supplementation (Caple et al., 1978; Gabbedy and Richards, 1970; Jones & Reed, 1948; Schougaard et al.,1972; Wilson et al.,1976). Foals with NMD show increased serum activities of creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) which would indicate muscle damage and the subsequent release of these enzymes into the circulation. Lesions of cardiac and skeletal muscle, typical of NMD in livestock, are seen at necropsy. Affected foals commonly are nursing mares that are consuming diets low in Se or low in both Se and vitamin E and are seen to improve with supplementation.

Perinatal foal losses in mares grazing fescue pasture were found to respond to Se supplementation (Heimann et al., 1981a). Death of the foals was attributed to suffocation due to a thickened placenta which failed to rupture at parturition or to agalactia. All foals fromSe-supplemented mares survived while 50% of the foals from the non-supplemented group died at birth. In a concurrent study, the

Se status of mares grazing fescue was shown to improve when pastures were fertilized with nitrogen, which increased the Se concentration in the forage (Heimann et al., 1981b).

Selenium Requirement

Serum Se levels in surveys of normal horses conducted by Stowe (1967) and Maylin et al.(1970) ranged from .070 to .124 ppm and .077 to .156 ppm, respectively. Se levels were found to be dependent on whether feed was home grown or commercially obtained, and on quantity and type of bedding used. Stowe (1967) and Bergsten et al. (1970) found lower serum Se levels in suckling foals than in their dams. In a depletion-repletion study, Stowe used orphaned foals on a Torula yeast diet to determine the Se requirement. Minumum serum Se levels reached .037 ppm, and foals on the deficient diet showed a tendency for decreased weight gain when compared to their normally reared contemporaries. Optimal physiological effect from parenteral supplementation was reached at approximately 45 days and gave an estimated requirement of 2.4 ug Se/kg body weight per day.

Bergsten et al. (1970) orally supplemented adult horses with low serum Se levels with sodium selenite at doses of 1.5, 3, 6, 18, or 30 mg Se per week. Serum Se levels responded linearly from 1.5 to 6 mg of supplementation with only a slight increase with higher dose levels. Bergsten et al. (1970) concluded that 6 mg Se per week was the requirement of the horse.

These values can be used to calculate dietary Se intake, approximating a minimum requirement of .09 ppm and .10 ppm daily for the studies of Bergsten et al. and Stowe, respectively. These studies have led the National Research Council to suggest that Se is required at the .10 ppm level in equine diets (NRC, 1978).

Vitamin E

Rations for horses normally contain sun-cured green hays which are high in vitamin E. This fact, in combination with the interrelationship of Se and vitamin E function in maintenance of cellular integrity, has made it difficult to produce substantial evidence of a vitamin E deficiency in horses. Nutritional muscular dystrophy in foals has been shown to respond to Se and vitamin E supplementation (Dodd et al., 1960). However, when supplementing vitamin E only to dystrophic foals, Dodd saw no response and concludedthat Se supplementation alone was of prophylactic benefit.

In a survey of New York breeding farms, plasma vitamin E concentrations varied by horse and season of the year (Maylin et al., 1980). The highest concentrations were seen during spring and summer sampling periods, when intake of fresh rather than stored forage was greatest. It was suggested that during the months of peak reproductive performance, vitamin E content of feeds was increased and this may be useful in maintenance of normal reproductive function. Vitamin E deficiency has been implicated as a possible cause of male sterility and in failure to maintain pregnancies in females of other species. However, Rich et al. (1983) could not improve semen quality in stallions with supplementation of 5,000 or 10,000 IU of vitamin E daily, and concluded that the current NRC recommended minimum requirement was adequate to maintain reproductive performance.

Stowe (1968) fed orphaned foals a vitamin E-deficient diet. Erythrocyte stability, as determined by layering hemolysis, was used to evaluate vitamin E status. Foals were fed the deficient diet for 200 days. Rations were then supplemented with alpha-tocopherol, and the return of erythrocyte stability was monitored. A value of 233 ug alpha-tocopherol per kg of body weight produced normal erythrocyte stability and is currently the recommended minium daily requirement (NRC, 1978).

Vitamin E and the Immune System

Recent developments in immunology have spurred new interest in vitamin E supplementation. Polymorphonuclear leukocytes (PMN) engulf and destroy invading bacteria. The destructive mechanism is closely related to an increased oxygen uptake. The reduced byproducts of this oxygen surge, especially H_2O_2, account for much of the microbicidal activity of the PMN (Baehner et al., 1980; Iyer et al., 1961). The ability of vitamin E to protect erythrocytes from oxidative cellular damage, and because erythrocytes and lymphocytes are of similar cellular origin, interest has been stimulated in the effect of vitamin E supplementation on the immune system (Sheffy and Shultz, 1979). Megadoses of vitamin E have been shown to enhance immune response by increasing phagocytic activity as well as T-cell lymphocyte mitogenesis (Linn et al., 1981; Watson and Petro, 1982; Nockels,1979).

Vitamin E and Exercise

Most of the effects of vitamin E on cellular mechanisms can be directly attributed to its antioxidant properties. When oxygen metabolism is increased, the potential need for additional supplementation is apparent. It was this logic which led to the widespread use of megadoses of vitamin E to enhance performance of human athletes (Cooper, 1972; Shepard, 1980). However, Sharman et al. (1971, 1975) in two separate studies with untrained and highly trained swimmers could show no benefit from vitamin E supplementation when athletic performance was evaluated. Lawrence and Slade (1979) did see increased packed cell volumes (PCV) in exercising horses when supplemented with vitamin E. A decrease in red cell lysis due to supplementation was considered a possible explanation for the increase in PCV.

Vitamin E supplementation of exercising horses is thought to be of benefit in preventing muscle disorders which result from strenuous exercise (McLean ,1973; Geiser, 1975; Hill ,1962). Improvement in performance has not been a consideration except in terms of decreased incidence of muscle damage and the resulting decrease in periods of lay-off for recovery from these disorders.

Myopathies

Disorders which result in muscle cell necrosis are numerous and occur in many species, including man. Most cases in humans are idiopathic and are accompanied by varying degrees of myoglobinuria and elevated serum enzymes (Afifi et al., 1968; Savage et al., 1971; Schutta et al., 1969; Scarpelli et al., 1963). Occurrence of signs is normally associated with some insult, physical exertion, drugs, trauma or mild infections. Kontos et al. (1963) described one such exertional related case study. Myoglobinuria accompanied by muscle soreness and atrophy were reported. Administration of glucose or glycogen produced myoglobinuria in this subject, and it was suggested that an uncoupling of oxidative phosphorylation in skeletal muscle during activity was the cause.

Exertional Myopathy

In most domestic species, myopathies have been shown to be of nutritional origin rather than activity related. White muscle disease in sheep and cattle has been shown to respond to Se and vitamin E supplementation. However, Young and Keeler (1962) demonstrated that muscular activity exacerbates the effect of Se deficiency.

Exertional myopathies in wild animals resulting from the stress of chase and capture occur in various species of ungulates. A profound acidemia has been described and favorable results have been obtained with bicarbonate

infusion immediately post-capture (Harthoorn and Young, 1974; Harthoorn, 1976). Serum creatine phosphokinase (CPK) and SGOT activities as well as plasma lactate concentrations were elevated (Hofmeyr, 1973).

Harthoorn (1976) expressed concern that lactating animals exhibited the lowest plasma Se levels of the age groups studied and were most frequently lost as a result of the stress of chase and capture. However, injecting the animals with a commercially available Se and vitamin E preparation (Bo-Se; 2.19 mg sodium selenite, 68 IU vitamin E) did not significantly change blood chemistry at capture. Similarly, Robbins and Parish (personal communication) could not alter the effects of capture by supplementing the diet of mountain goats with both Se and vitamin E. Harthoorn (1976) concluded that conditioning animals to the capture procedure could reduce the stress on the animal and, subsequently, the severity of capture myopathy.

Muscle Disorders in Horses

Hansen (1970) studied 35 cases of myopathy in horses and classified them into 9 categories. Dark colored urine and elevated activity of SGOT and serum glutamic pyruvate transaminase (SGPT)were commonly seen in many of these syndromes. The relative increase in serum enzymes and the rate of decline during treatment were shown to be useful indicators of prognosis.

Muscle degeneration (Gabbedy and Richards, 1970; Jones

and Reed, 1948; Schougaard et al., 1972; Wilson et al., 1976) or yellow fat disease (Dodd et al., 1960; Platt and Whitwell, 1971) in foals have, at least in part, been attributable to diets low in Se and vitamin E. Lesions of skeletal and cardiac muscle, typical of white muscle disease in lambs were evident at necropsy. In some cases,a generalized fat necrosis and fat discoloration were also present. Owen et al. (1977) reported similar histological muscle findings in adult horses. Low dietary vitamin E intakes were associated with ataxia, muscular incoordination and skeletal muscle lesions in adult and juvenile Mongolian horses (Liu et al., 1983). However, skeletal muscle lesions are not pathognomonic of Se and vitamin E deficiency, and direct evidence supporting this thesis has been limited.

Bowen (1942) and Pope and Heslop (1960) reported myoglobinuria in adult horses. Muscles were flaccid. Horses were extremely weak and resisted movement. In both cases, animals were in outside drylots and fed poor quality diets. Se and vitamin E levels of plasma or feeds were not determined.

Azoturia and tying-up are the most common muscular disorders in horses. Azoturia occurs during the early stages of exercise and is characterized by extreme muscle tenseness and myoglobinuria. Muscles which are most drastically affected are those of the loin and croup. Horses are normally in good physical condition and on high concentrate rations. Azoturia most frequently occurs on the

first day of exercise following a 1 to 2 day period of rest. Tying-up has been associated with excessively nervous animals and is similar to muscle cramps experienced by human athletes after a post-exercise rest period when movement is resumed (Farrow et al., 1976).

Cardinet et al. (1963), in an effort to associate high carbohydrate consumption with the occurence of azoturia and tying-up, evaluated SGOT activity in exercise and at rest in horses fed high grain rations. Activities were elevated in exercise. When exercise was restricted and feed intake was maintained, SGOT activities also rose.

In a later study, Cardinet et al. (1967) compared SGOT and CPK activities in exercising horses and horses with azoturia. SGOT activities rose appreciably in both exercise and azoturia. However, CPK activities were 10-100 times greater in azoturia than in exercise and was preferable to SGOT in diagnostic use. The disappearance rates of both enzymes were useful in predicting rate of animal recovery.

Lindholm et al. (1974) evaluated serum enzyme and muscular changes in 59 Standardbred trotters showing clinical signs of tying-up. SGOT and CPK levels were elevated in all horses. Muscle biopsies showed myofibrillar degeneration and necrosis with invasion of inflammatory cells. Fast twitch fibers were most frequently affected. Muscle samples were low in glycogen, ATP and CPK and had high concentrations of glucose and lactate. It was

suggested that muscular alterations may be caused by a derangement of carbohydrate metabolism resulting from a local hypoxia.

Historically, treatment has consisted of restricting concentrate intake, administration of muscle relaxants and rest. Prophylactic and therapeutic administration of vitamin E and Se has met with widespread acceptance by equine practicioners and appear to produce favorable results (Geiser,1975; Hill, 1962; McLean, 1973; Stewart, 1960).

MATERIALS AND METHODS

Selenium in Plasma and Milk

Four Arabian and two Quarter Horse mares weighing approximately 500 kg and their foals were used to determine plasma and milk Se levels for horses at the MSU Horse Teaching and Research Center. Mares were fed a corn-oats concentrate and alfalfa-bromegrass hay which was grown on the same farm. No supplemental Se was provided. First cutting hay was fed during gestation, and second cutting hay was fed post-parturition. During gestation a 1:1 mix of oats and corn was fed at approximately 25% of total dry matter intake. Concentrate intake was doubled after foaling. Mares were allowed access to grass pastures during the daylight hours, both pre- and post-partum.

Blood samples were taken at approximately 7 and 14 days prior to foaling, at foaling and 1, 4, 7, 14 and 21 days after foaling. Milk samples were taken by allowing the foal to suckle one teat while a 50 ml sample was collected from the other. Colostrum and milk samples were taken on blood sampling days post-partum. Blood samples were centrifuged at 4°C and 2000 x g for 15 minutes to separate plasma. The plasma, colostrum and milk samples were stored in plastic

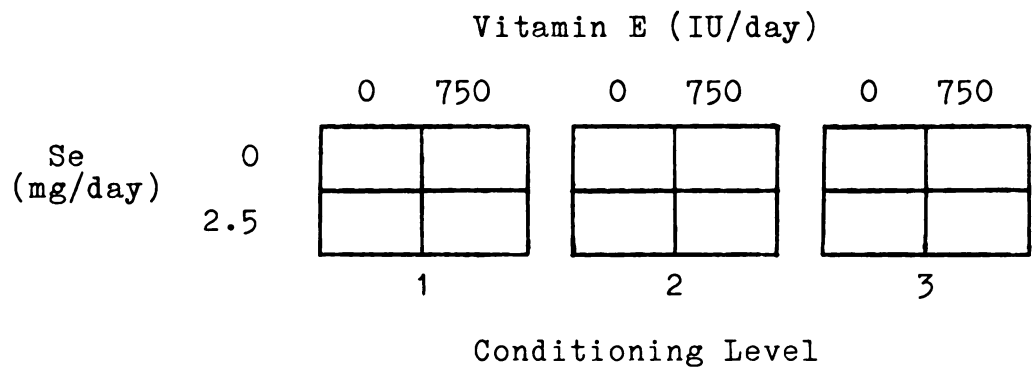
containers and frozen until analyzed. Feedstuffs were analyzed for Se content as were additional potential Se sources such as soil and cinders which are a major portion of the roadways at the MSU horse unit.

Exercise Trial

Animals

Eight Arabian mares ranging in age from 5 to 14 years were used in a 2x2 double split-plot design with repeated measures. Two levels of Se and of vitamin E supplementation were provided at 3 levels of conditioning, with repeated samples taken during each collection period, illustrated as follows:

DOUBLE SPLIT-PLOT DESIGN



The three conditioning levels were 1) non-conditioned (prior to sampling, horses were acclimated to the treadmill then housed in 3.0m x 3.6m box stalls and allowed one hour of free exercise daily); 2) conditioned (horses were conditioned 6 days per week for 45 days); 3) conditioned with rest (horses were conditioned for 3 days beyond conditioning level 2 and allowed 2 days of stall rest immediately prior to sampling).

Diets

Two horses were assigned to each of 4 experimental diets with the concentrate portion of the ration consisting of crimped oats with supplemental energy provided in the form of cracked corn to some horses. Second-cutting alfalfa-bromegrass hay was also fed. Vitamin E and Se concentrations for each of the major components of the ration are given in Table 1. All diets were fed for 2 months prior to blood samples being taken at conditioning level 1.

Mares were weighed at the beginning of the conditioning period and at 2-week intervals during conditioning. A final weight was taken at the completion of the trial. All horses were fed over the entire trial to achieve a desired weight change or to maintain weight, depending upon the animal's physical appearance at the initiation of the feeding period. Mares were in good physical condition at the beginning of the feeding trial. However, limiting exercise caused increased fattening in some mares; after exercise began, other mares lost weight rapidly. An effort was made to minimize fat differences. Therefore, animal weights and feed intakes differed in some cases during the conditioning stage of the trial (Table 2).

Supplemental vitamin E and Se were provided in pelleted form (Table 3) and fed twice daily with the concentrate portion of the ration. Mares were bedded on wood shavings and supplied fresh water and trace mineralized salt ad libitum. Mean daily Se and vitamin E intakes, respectively,

Table 1. Vitamin E and selenium concentrations in air dry matter of feeds.

Feed	Se(ppm)[a]	Vitamin E (mg alpha-tocopherol/kg)[b]
Crimped oats	.022	8.4
Cracked corn	.033	10.4
Alfalfa-bromegrass hay	.408	60.8

a Analyzed values.
b Hoffman-LaRoche Inc.,Nutley,NJ (personal communication).

Table 2. Horse weights and air dry feed intake.

Animal number	Trt.	Weight (kg) Initial	Final	Ave. daily intake(kg)	Intake (% BW/day)
DF	Control	465	477	8.12	1.72
51	diet	365	387	7.49	1.99
32	+Se	414	416	7.17	1.73
37		387	398	7.59	1.93
21	+Vit E	448	448	6.75	1.51
25		488	477	6.75	1.40
18	+Se +Vit E	475	482	6.75	1.41
27		450	446	6.75	1.51

Table 3. Supplement pellet formulation (%).

Ingredient	Supplement pellet		
	Placebo	+Se	+Vit E
Ground corn	65.6	64.5	65.14
Wheat middlings	30.0	30.0	30.00
Se premix[a]	--	5.5	--
Vit E premix[b]	--	--	.66
Ca carbonate	4.2	--	4.20

a 200 mg/kg. Sodium selenite in calcium carbonate carrier.
b 500 IU vitamin E/g. Vitamin E activity in the form of dl-alpha tocopheryl acetate.

for each treatment group were: control, 1.99 mg, 327 IU; plus Se, 4.47 mg, 321 IU; plus vitamin E, 1.93 mg, 1057 IU; plus Se plus vitamin E, 4.43 mg, 1057 IU.

Conditioning

Horses were conditioned on an equine exercise treadmill. During each exercise bout, animals were started slowly, and speed was then increased in two stages. Daily distance traveled by each horse was recorded as well as environmental temperature and total time on the treadmill (Table 4). After each bout, horses were rinsed with warm water and tied until cool. The 22% grade of the treadmill was held constant during the conditioning and sampling periods. One day of rest was provided after every 6 days of conditioning. Time on the treadmill was increased as animals showed visual improvement in signs of fatigue and time required to recover from exercise, normally after 5 to 6 days of conditioning at the preceding level.

Blood Sampling

Blood samples were taken before, during and after exercise (Table 5). Mares were fitted with indwelling jugular venous catheters at 8:00 a.m. on sampling days. These remained in place until the 1 hr post-exercise sample was taken. The 24 hr post-exercise sample was taken by venous puncture. Thirty ml of blood were obtained at each sampling, placed in 2 heparinized 15 ml test tubes and mixed by repeated inversion. Subsamples were taken immediately

Table 4. Conditioning routines[a].

Week	Exercise (min/day)	Distance(m)	Speed (m/min)	Environ. temp. (degree C)
1	6.3	1211.4	192.9	6.8
2	6.7	1437.4	213.9	6.2
3	7.6	1512.4	199.0	8.3
4	8.7	1798.3	206.7	8.3
5	9.6	1946.9	202.8	3.3
6	10.0	1980.0	198.0	1.7

a Daily, except Sunday.

Table 5. Blood sampling times.

Sample no.	Time of sampling
I	Pre-exercise
1	1 minute of exercise
F	Exercise heart rate of 200 bpm
1 hr	1 hr post-exercise
24 hr	24 hr post-exercise

for glutathione and malondialdehyde determinations. A 20 ul sample of whole, non-heparinized blood was taken for lactate analysis.

Heart Rate

Areas on either side of the sternum, approximately 7.5 cm behind the elbow, and in the center of the back, 5.0 cm behind the withers, were clipped and shaven to expose the skin surface. The skin was cleaned with 70% ethanol and surface electrodes were attached. Lead wires were connected to the electrodes with alligator clips and wrapped with an elastic bandage to maintain good contact during the exercise bout. Horses were placed on the treadmill, and an initial heart rate was taken with a model 30-30 Cambridge Electro-Cardiogram recorder (Cambridge Co., Inc., Ossaning, NY). Exercise did not begin until a steady resting heart rate was achieved. Heart rates were monitored during the entire exercise period and for approximately 1 min into the recovery phase.

Blood Analyses

Blood samples were taken to the laboratory immediately after the 1 hr post-exercise samples were obtained. Heparinized whole blood samples were analyzed for packed cell volume (PCV) and hemoglobin concentration as soon as possible. The blood was then centrifuged at 2000 x g for 15 min at 4°C, the resultant plasma was pipetted into plastic containers, residual air was displaced with nitrogen and the

plasma was stored at -20°C until analyzed. The remaining cells were washed three times with physiological saline and refrigerated at 4°C until analyzed for GSHpx and G-6-PDH activity.

Fluorometric Se analyses were conducted on all plasma samples and diet components as described by Whetter and Ullrey (1978). Plasma vitamin E levels were determined by high pressure liquid chromatography (HPLC) using the procedure of Bieri et al. (1979). Glutathione peroxidase activity of erythrocytes and plasma was determined by a modification (Lawrence et al., 1974) of the procedure of Paglia and Valentine (1967). Plasma glutathione-S-transferase activities were measured as described by Habig et al. (1974).

Whole blood was precipitated in 5% metaphosphoric acid, centrifuged at 39,100 x g for 15 min at 4°C, and the resulting supernatant was analyzed for reduced glutathione levels (Beutler et al., 1963). Similarly, 1 ml of whole blood was precipitated in 10% trichloroacetic acid, centrifuged at 1500 x g for 20 minutes at 4°C, and the supernatant analyzed for malonyldialdehyde concentration (Mengel 1967; Placer 1966). Plasma creatine phosphokinase and erythrocyte G-6-PDH activities were evaluated using commercially prepared diagnostic kits (Sigma, 1983a,b).

Lactate levels were determined in the Laboratory for the Study of Human Performance of the Department of Health

and Physical Education. A Roche Lactate Analyzer 640 (Hoffmann-LaRoche Ltd., Bio-Electronics Div., Basle, Switzerland) was used. In this procedure, lactate is oxidized to pyruvate by hexocyanoferrate in the presence of cytochrome b_2; hexocyanoferrate then liberates two electrons to the reaction medium, and they are collected on a platinum electrode. The resulting change in current is measured and used to determine lactate concentration based on lactate standards.

Statistical Analyses

Selenium concentrations in plasma and milk from mares and foals were analyzed as a completely randomized block design with repeated measures. Horses were used as blocks and time as treatment. Statistical analyses were performed on the negative log y scale for all values to minimize heterogeneity of variance. Missing cells were generated by least squares estimation and used to calculate sums of squares. This procedure produces values which, by definition, minimize variance and bias the test statistic upward. Therefore, treatment sums of squares, for marginally significant omnibus test statistics, were adjusted to eliminate biases. Comparisons between selected means were made using Scheffe's test (Gill, 1978).

A multivariate analysis of variance for a double split-plot design with repeated measures was conducted for all exercise values. Similarly,a split-plot analysis was

performed for samples collected over the entire feeding period to evaluate changes due to time on experimental diets (Statistical Packages for the Social Sciences). Scheffe's test was applied to selected comparisons of means as described by Gill (1978).

Treadmill Design and Construction

Providing a constant and repeatable work load for exercise research is difficult, if not impossible, when traditional training and exercising methods are used. Sampling subjects during exercise poses additional problems, as exercise must be interrupted to facilitate blood sampling. The introduction of exercise treadmills in both human and animal exercise studies has greatly increased the accuracy and ease with which these studies may be conducted.

The cost of commercially-constructed equine exercise treadmills, which are variable in both speed and incline of walking surface, has made them difficult to purchase on limited research budgets. Less expensive mills lack much of the versatility needed to conduct extensive exercise research. Most commercially-built treadmills are not designed to take the type of abuse they would be subjected to in a research situation. For these reasons, an equine exercise treadmill was designed and built for use in this and subsequent animal studies.

To produce maximum animal stress, the belt, which provides a non-slip moving walking surface, must travel in excess of 200 m/min. This speed results in a fast trot for pleasure horses. Commercial mills which are designed to travel at this speed use a system of conveyor-type cylindrical rollers beneath the walking surface to decrease friction and facilitate the use of smaller, less expensive

motors to drive the belt.

Traveling at high speed, these rollers are subject to a great deal of stress and, consequently, wear out quickly. Providing an even walking surface with this type of system is also difficult. Rollers must be placed as close together as possible, and a thick, less pliable belting is used to help lessen the vibration which occurs as the horse's hooves are carried over these rollers. The large number of rollers required results in considerable noise and presents some difficulty in acclimating horses to exercise on the treadmill.

Because of the problems resulting from the use of rollers, the treadmill designed for this study used a flat, stainless steel skid sheet as the surface over which the belt was driven. This provided a level walking surface and greatly decreased operating noise. However, eliminating the rollers increased both friction and heat generation, and means of overcoming these problems will be discussed later.

Detailed drawings of all elevations are given in Figures 2 through 4. Measurements are given in the English units currently used for structural materials. The framework was constructed with 4 inch channel iron, and 1 1/2 by 1/4 inch angle iron provided the support for the walking surface. Side panels were 3/4 inch plywood attached to 1 1/2 inch black pipe. Plywood provided a durable, rigid sidewall, which was also helpful in decreasing operating noise.

Table 6. Key for figures 2-6.

Figure 2. Treadmill, side elevation, with motor and walkway removed.

A Adjustable front support.
B Conveyor belt.
C Rear pillow block bearing.
D Loading ramp.
E Loading gate.
F Safety latch, rump rope.

Figure 3. Treadmill, front elevation.

A Curved chest gate.
B Speed adjustment, to variable speed sheave.
C Walkway, handler.
D Right angle gear box, ratio 1:1.
E Adjustable front support.

Figure 4. Treadmill, rear perspective.

A Rump rope.
B Conveyor belt.
C Walkway, handler.
D Motor.
E Loading ramp.

Figure 5. Treadmill, safety latch.

A Chain, to rump rope.
B Mounting bracket.

Figure 6. Treadmill, motor and drive, top view.

A Motor.
B Chain drive, 60 chain, 6.9:1 sprocket ratio.
C Driver conveyor pulley.
D Variable speed adjustment, leading to operator.
E Variable speed sheaves, 10 in., 6:1 ratio.
F Conveyor belt.

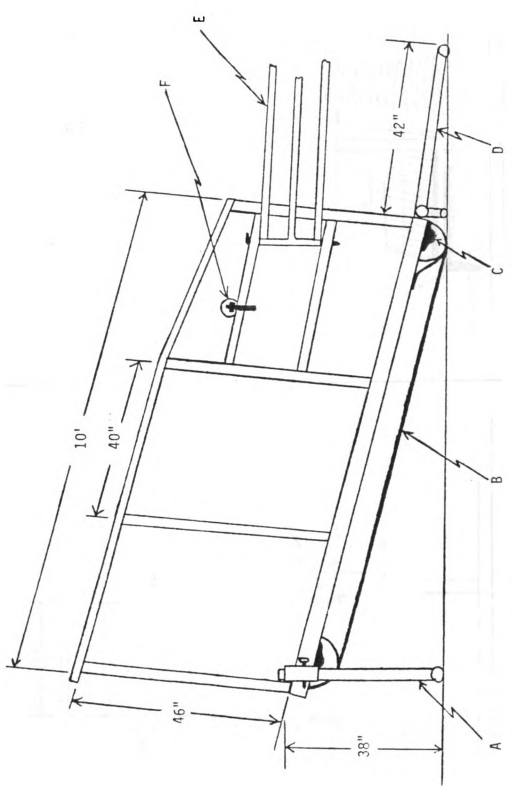


Figure 2. Treadmill, side elevation, with motor and walkway removed.

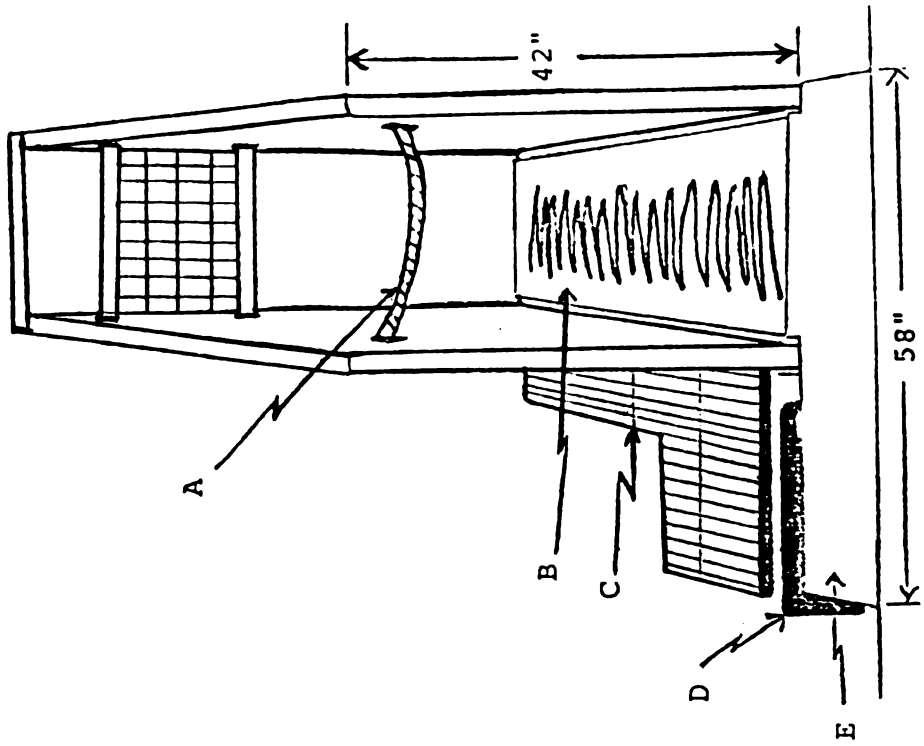


Figure 4. Treadmill, rear perspective.

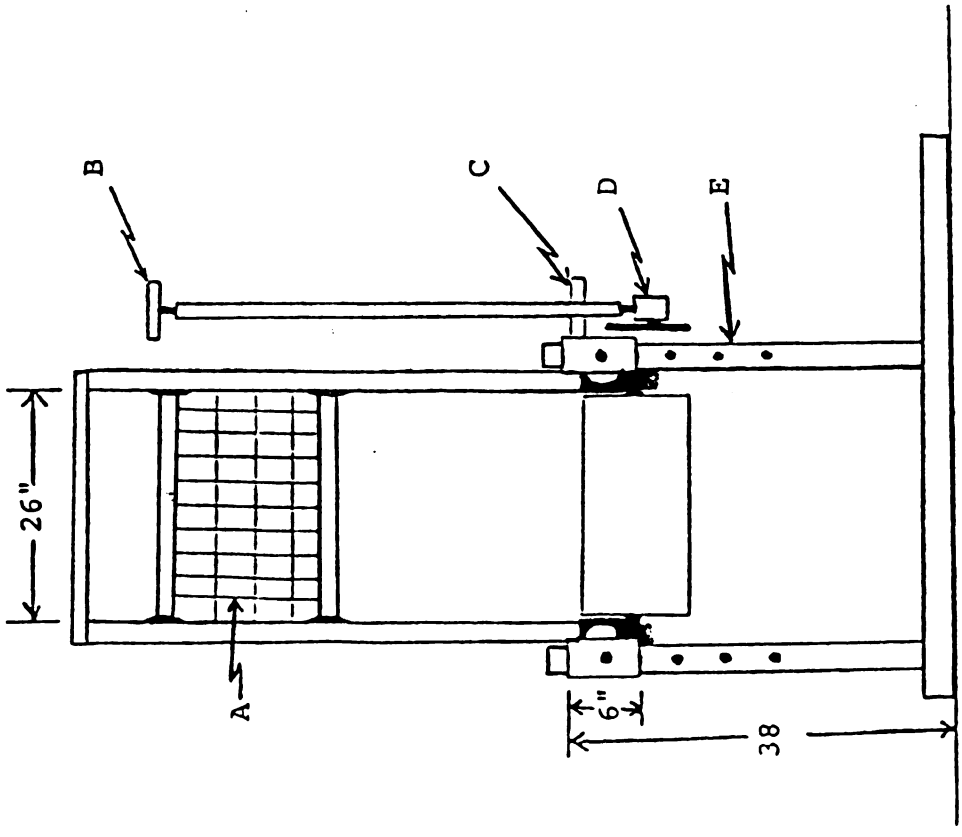


Figure 3. Treadmill, front elevation.

Front supports were constructed of 2 inch black pipe and were adjusted by sliding through a 2 1/2 inch sleeve, 6 inches long. A 1/2 inch steel pin was used to secure the sliding support at any of 4 desired heights. Rear supports were 1/2 by 2 inch flat iron curved to maintain a constant 1 1/2 inch clearance for the rear drive conveyor drum, regardless of how the incline was changed.

The loading ramp was made from 1 1/2 inch pipe and supported with 2 by 1/4 inch angle iron. It was covered with 3/4 inch plywood and carpeted to provide good footing. The ramp was wide enough to accomodate both horse and handler and made loading animals the first time easier and safer. A loading gate also was designed to simplify loading young or balky horses. It was constructed from 1 1/2 inch pipe and hinged to swing away from the mill and then back to provide a narrow walkway to help force animals to load. The angle at which the gate was hung could be adjusted as the incline of the treadmill was changed.

Once horses were on the mill they were restrained in front by a chest gate and in the rear by a cotton rump rope. The chest gate was curved forward from top to bottom so that the stride of the animal was not obstructed when the mill was in operation. The rump rope was secured, after the animal was loaded, with a safety latch (Figure 5) which could be released quickly and easily in all situations. This was particularly useful if a horse should fall or resist exercise.

A walkway for the handler was constructed of metal grating supported by 1 1/2 by 1/4 inch angle iron. This also served as a protective housing for the motor and drive train assembly which were mounted on 4 inch channel iron.

A 5 H.P., 220 volt single phase motor was used. Treadmill speed was adjusted with variable speed sheaves which drove a jackshaft. The conveyor drum was driven from this jackshaft with size 60 chain. The driver sprocket had thirteen teeth while the driven sprocket had 90 teeth, giving a 6.9 to 1 speed reduction (Figure 6). The speed range of the mill was from 10 meters per minute (mpm) to in excess of 500 mpm. Normal operating speed was from 100 to 250 mpm, producing a slow walk to a very fast trot for most horses.

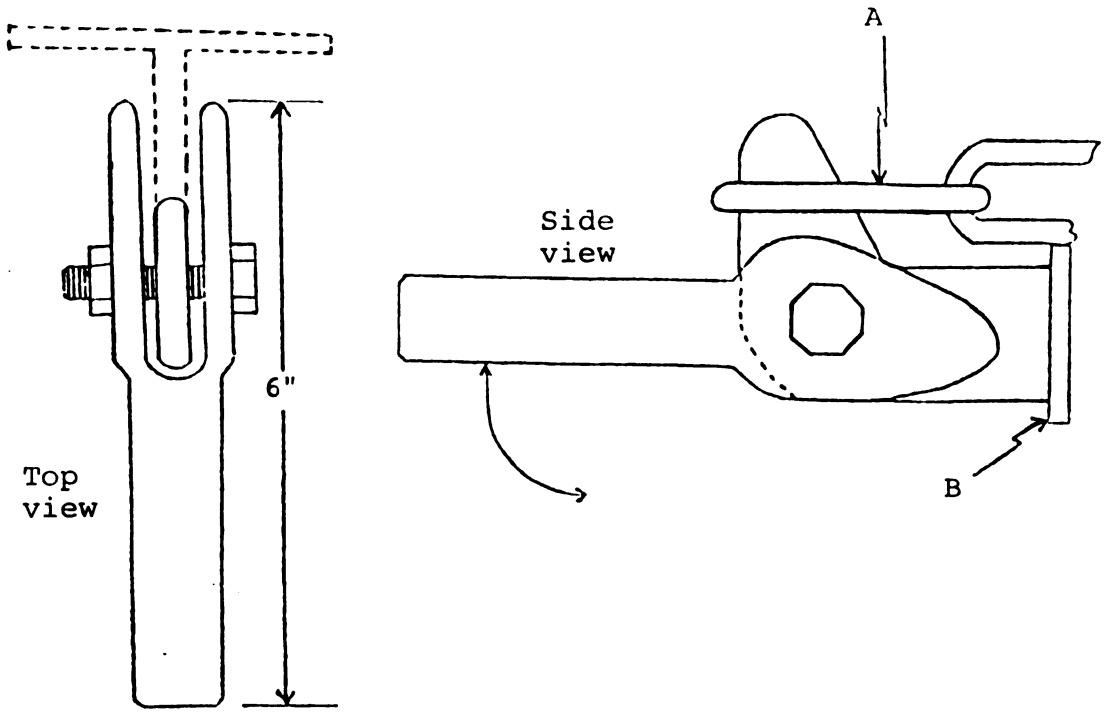


Figure 5. Treadmill, safety latch.

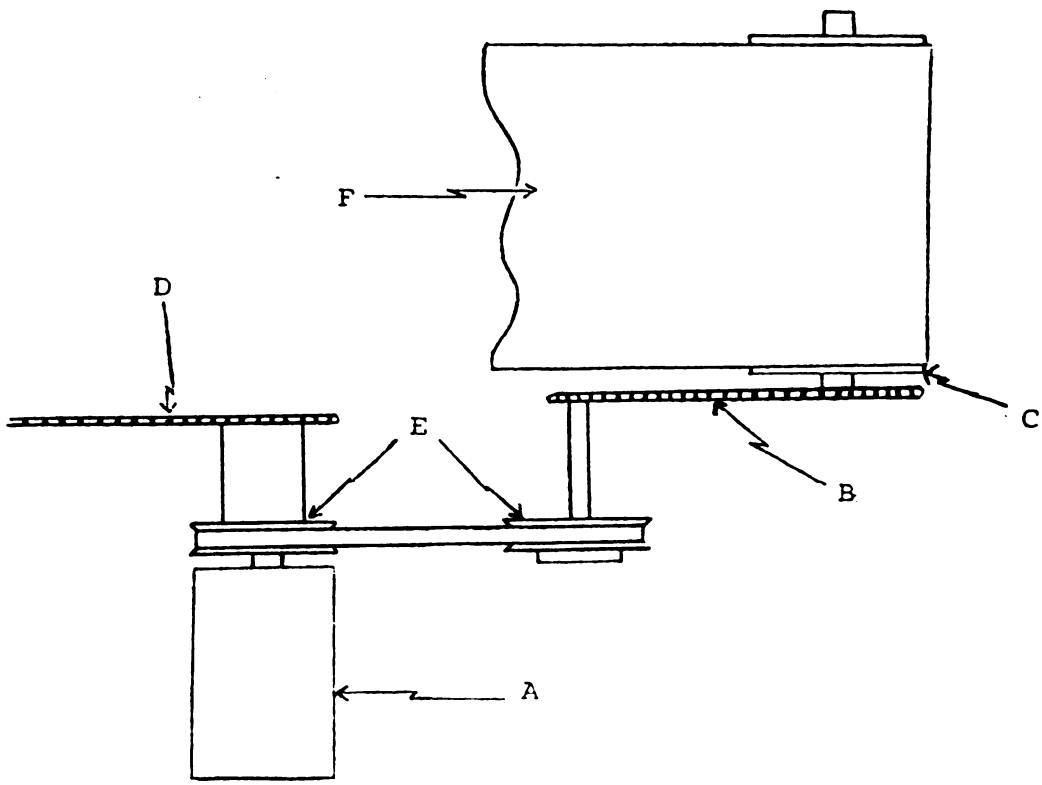


Figure 6. Treadmill, motor and drive, top view.

RESULTS AND DISCUSSION

The Testing, Use and Modification of an Equine Exercise Treadmill

Plates 1 through 4 show the completed treadmill. During the exercise trial, the treadmill was used intensely six days per week. Minor breakdowns did occur, and were corrected as quickly as possible, so that no exercise days were missed. However, some major changes still need to be made to improve its durability and ease of operation.

Most of the early changes dealt with the motor and drive train. To reduce cost, a 3 H.P., 110 volt electric motor was installed when the treadmill was constructed. Treadmills with rollers under the walking surface are equipped with 1 1/2 to 2 H.P. motors. Replacing the rollers with a slide sheet increased the drag and the power needed to drive the belt. The 5 H.P., 220 volt motor (Plate 5), which was installed later, had sufficient power to operate the mill without difficulty.

Finding a durable conveyor belt, which provided good footing for the horses and also helped to reduce friction, was difficult. Grip-Top rubber belting (B. F. Goodrich, Columbus, OH), fulfilled these criteria better than any

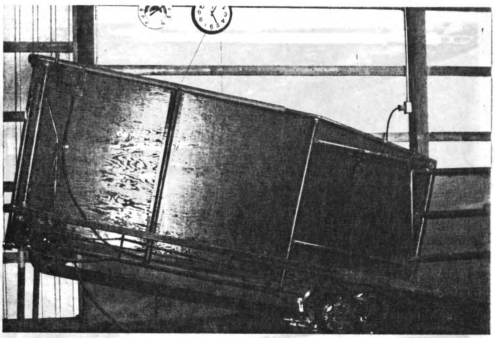


Plate 1. Treadmill, side view.

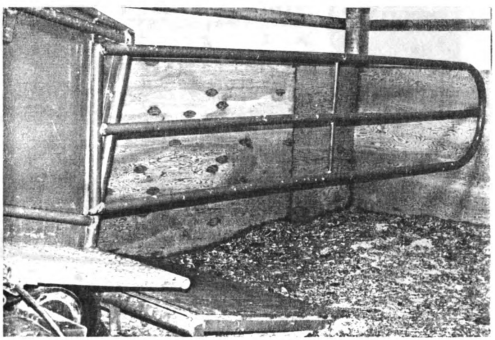


Plate 2. Treadmill, loading gate and ramp.

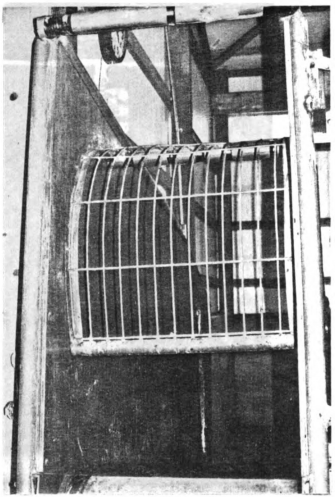


Plate 3. Treadmill, front view.

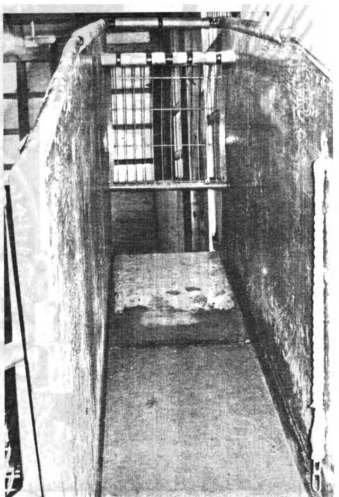


Plate 4. Treadmill, rear view.

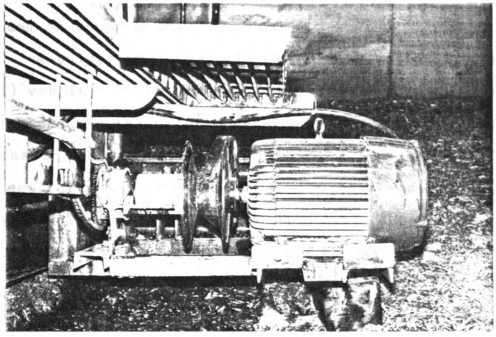


Plate 5. Treadmill, motor and drive.

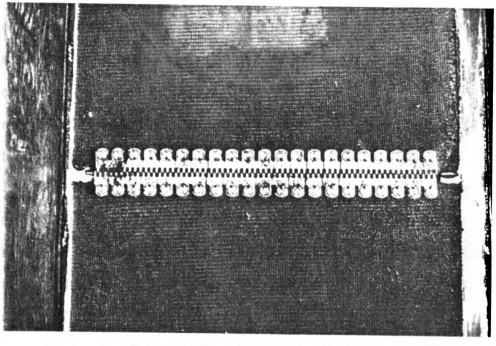


Plate 6. Treadmill, belt, lacings and slide.

other observed commercial conveyor belting (Plate 6). The underside was nylon fiber, as compared to rubber backing found on other belts.

A polypropylene sheet (Cadillac Plastics Co., Detroit, MI) was initially used as the slide. This posed several problems. As the nylon underside of the belt moved over the plastic sheet, static electricity was generated. The electricity was transferred to the horse on the treadmill, and resulted in static shock when contact was made with the pipe supports on the sides of the treadmill. Covering the pipes with rubber pipe insulation solved this problem. However, if the operator touched the horse, both would recieve a shock. An antistatic spray was applied to the underside of the belt and helped to reduce the generation of static electricity.

Belt lacings (Lovejoy Co., Downers Grove, IL) were required to allow for easy conveyor belt installation. Lacings were bolted to both end of the belt, and were connected by sliding a flexible metal cable through the lacings (Plate 6). These lacings cut into the polypropylene sheet and wore it out quickly. Polypropylene is a soft plastic with a low melting point (110^{o} C). When the mill was in operation, the heat generated from friction between the belt and slide warmed the plastic. This caused it to warp and become softer, which made it more susceptible to damage from the belt lacings.

The plastic slide was replaced with a stainless-steel

sheet (Plate 6), which was more durable. Stainless-steel increased friction between the belt and the slide, and also warped when heated. One inch holes were drilled in the plywood, under the slide, to help dissipate some of the heat. This did reduce heat build up. However, when the treadmill was used for an extended period of time, the steel could not cool sufficiently, and would warp and become noisy. Liquid soap was applied to lubricate the underside of the belt. This was effective; however, it was messy and required an additional person for its application.

Tetrafluoroethylene (Teflon; Dupont, Midland, Michigan) is material which fulfills many of the necessary requirements for use as a slide. It is a smooth, heat resistant material (operating temperature, 230° C) that is very durable. Teflon will be used in future tests to replace the stainless-steel slide.

When the incline of the treadmill was maximized, and friction reduced as much as possible, horses could move the belt without the motor running. Some horses, when exercising, were difficult to stop after the motor was turned off. A temporary brake was applied to the driver variable speed pulley. A brake which can be operated by the handler, and applied to the driven conveyor pulley will be installed in the future.

Conclusion

Constructing the treadmill provided a less expensive, reproducible method to stress horses through exercise. Cost of construction was about one-half that of commercial treadmills made similarly. Speeds which were used to exercise horses during this trial were comparable to those attained with other treadmills. However, the maximum speed possible greatly exceeds that of treadmills which can be purchased currently. Having the ability to change the slope of the incline, and exercise horses at various speeds (including the canter), allows for more flexibility when designing future exercise research.

Plasma and Milk Selenium Levels from Mares and Foals

Selenium concentrations of feeds are shown in Table 7. Using these feed values, and assuming a daily intake of 8.75 kg of dry matter (1.75% of body weight), an estimate of dietary Se intake was made. Mares were fed approximately 4.7 and 1.9 mg Se daily from natural feed sources during gestation and lactation, respectively.

The Se concentrations of cinders and soil are also given in Table 7. Cinders from the Michigan State University Power Plant were used to establish and maintain driveways at the Horse Teaching and Research Center for many years. Many of the pastures contain one of these driveways, or large areas where cinders were used as fill to eliminate moisture problems. As a result, pastures are bathed in cinder dust during the summer months. This may explain the unusually high Se concentrations found in hays grown on these fields, and the resulting high plasma Se values observed in horses grazing them (Brady, 1978). Furr et al. (1978) and Mandisodza et al. (1979) found high Se levels in sweet clover grown on fly ash, which is a residue from burning soft coal in electrical power-generating plants. When this sweet clover was fed to livestock, Se concentrations were increased in all tissues studied.

Changes in Se concentration of plasma from mares and foals and mare milk are compared graphically in Figure 7.

Table 7. Se concentration of feeds, bedding, soils, and driveway surfaces.

Item	Se (ppm dry basis)
Crimped oats	.022
Cracked corn	.033
Alfalfa-bromegrass hay	
1st cutting	.717
2nd cutting	.408
Wood shavings (bedding)	.048
Soil (pasture)	.242
Driveway surfaces	
Cinders	2.418
Gravel	.040

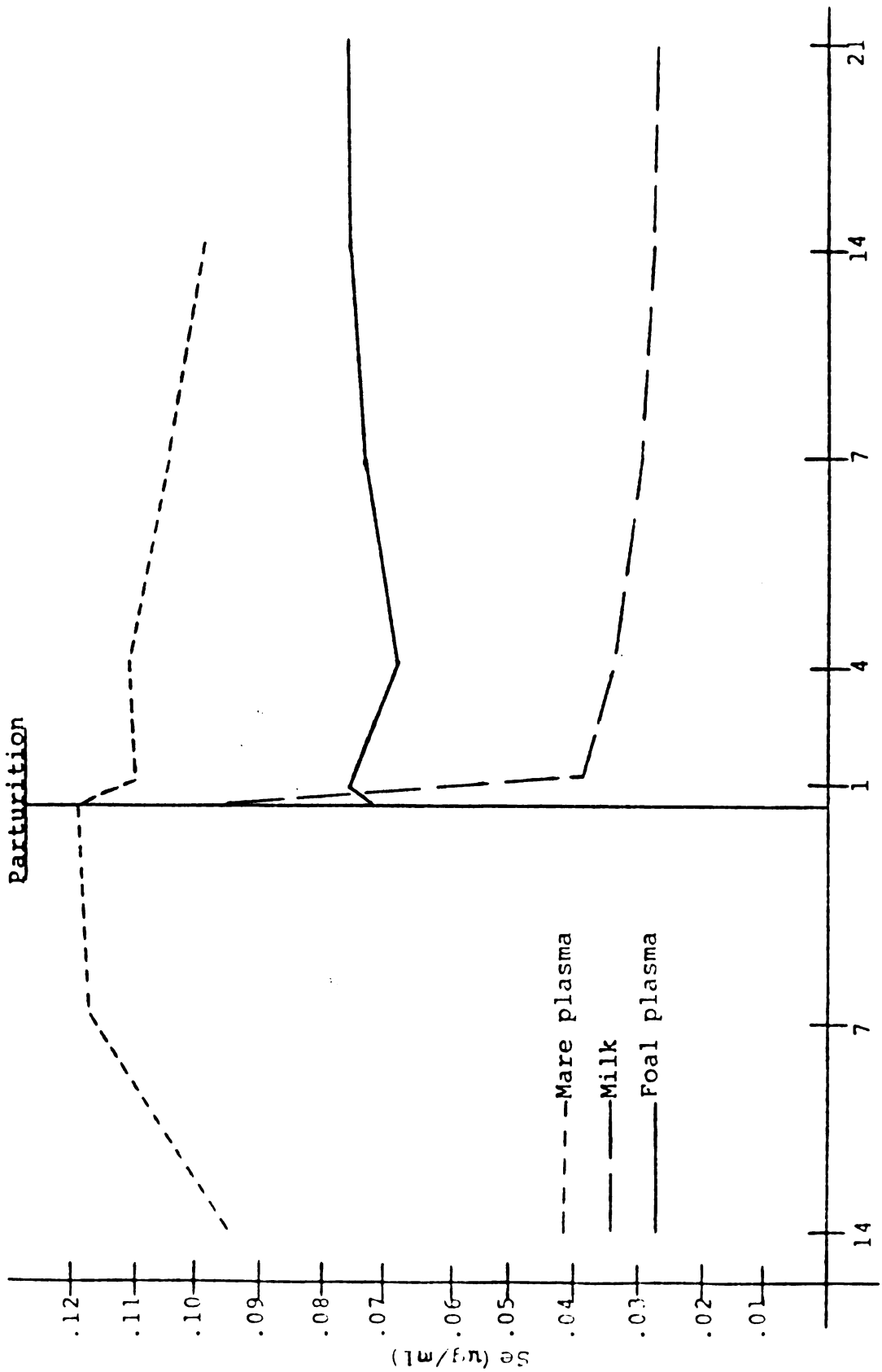


Figure 7. Changes in Se concentration in mare's milk and mare and foal plasma over time.

Foal plasma Se levels remained constant throughout the collection period. Mare plasma Se levels tended to rise prepartum and fall off after parturition; however, these means were not significantly different. Mean Se concentrations of milk and plasma from individual mares and their respective foals are given in Table 8. In general, mares had higher plasma Se levels than their respective foals.

Colostrum Se concentrations were higher than those found in milk ($P<.001$). McConnell and Roth (1964) demonstrated that nearly all of the Se found in milk is protein bound. The decline seen in Se levels, between colostrum and milk, is coincident with the rapid decline in immunoproteins during the first 24 hr of lactation. The average Se concentration of milk samples (.029 ug/ml) compares favorably with Se concentrations found in milk from Hereford cows fed diets with similar Se levels (Perry et al., 1977).

Table 8. Mean Se concentration of mare's milk and plasma and foal plasma.

	Se concentration (ug/ml)			
Mare	Plasma[a]	Colostrum[b]	Milk[c]	Foal plasma[d]
DC	.098±.02[e]	.053	.026±.003	.051±.03
EB	.097±.01	.075	.028±.003	.055±.01
FR	.093±.02	.119	.018±.006	.080±.03
FS	.125±.02	.084	.039±.002	.093±.02
R	.097±.01	.070	.037±.011	.071±.01
Z	.129±.01	.131	.030±.006	.088±.02

a Values represent the mean of 8 samples per mare, obtained 7 and 14 days prepartum, at parturition, and 1, 4, 7, 14 and 21 days after foaling.
b Values represent 1 colostrum sample per mare, obtained at the time of foaling.
c Values represent the mean of 5 samples per mare, obtained at 1, 4, 7, 14 and 21 days after foaling.
d Values represent the mean of 6 samples per foal, obtained at the time of foaling and 1, 4, 7, 14 and 21 days after foaling.
e ±SEM

Conclusions

Plasma Se concentrations of mares, at the MSU Horse Teaching and Research Center, were found to be within normal ranges in an area known to have low soil Se levels. Apparently adequate Se intakes could be provided at this facility by feeding hays which were grown on soils contaminated with Se-rich cinder dust. Plasma Se concentrations of foals in this study were low-normal and may warrant further investigation to ensure that prophylactic administration of Se, to nursing foals, is not needed.

Changes in Blood Parameters During Conditioning and Exercise in Horses

The exercise time required to reach a maximum heart rate of 200 bpm increased, while blood lactate concentrations decreased, with conditioning (Table 9). These changes, due to conditioning, have been used as indicators of improved physical performance in horses (Milne et al., 1976; Milne et al., 1977; Rodiek et al., 1983; Sigler et al., 1979). The two days of rest prior to exercise, provided in conditioning level 3, resulted in a decrease in the time required to reach maximum heart rate, when compared to conditioning level 2. Whether this is a result of an increase in excitability of the horses or an actual decrease in animal fitness is not known. However, Foreman et al. (1983) reported that a one month rest period did not significantly change cardiopulmonary fitness in well-trained thoroughbred horses.

Changes in blood lactate concentrations as a result of conditioning and exercise are given in Table 10. Significantly higher lactate concentrations were seen for all conditioning periods at one minute of exercise and when a heart rate of 200 bpm was reached as compared to initial values. Blood lactate concentrations remained elevated 1 hr after exercise for non-conditioned horses, again indicating improved animal fitness for conditioning levels 2 and 3.

Rodiek et al. (1983) demonstrated the increased

Table 9. The effects of conditioning on heart rate and blood lactate levels.

Item	Conditioning level			$\bar{X}$
	1	2	3	
Speed (m/min)	220	210	219	216
Time (min:sec)[a]	1:50	4:15	2:30	2:52
Lactate (mmoles/l)[b]	9.7	5.3	5.5	6.9

a Exercise time required to reach a heart rate of 200 bpm.
b Lactate concentration when heart rate was 200 bpm.

Table 10. Changes in blood lactate (mmoles/l) as a result of conditioning and exercise.

Time of sampling	Conditioning level 1	2	3	P value
I	1.6[a]	1.0[a]	1.3[a]	NS[e]
1 min	5.2[bc]	3.1[bd]	3.5[bd]	.05
F	9.8[bc]	5.4[bd]	5.5[bd]	.001
1 hr	3.8[ac]	1.5[ad]	1.5[ad]	.01
24 hr	1.3[a]	1.0[a]	1.5[a]	NS
P value	.001	.001	.001	

a,b Values in the same column with different superscripts differ significantly.
c,d Values in the same row with different superscripts differ significantly.
e Values are not significantly different (P>.10).

physical stress associated with increasing the incline of the treadmill. Increases in both heart rate and blood lactate levels were seen when the grade was changed from 3% to 9%. Webb et al. (1979) indicated the same type of response when the incline (9% grade) was held constant and treadmill speed was increased from 123 m/min to 172 m/min. In both studies, maximum heart rate achieved, after 30 minutes of exercise, was approximately 150 bpm.

The treadmill, used in the present study, was operated at a 22% grade and an average speed of 216 m/min. Maximizing physical stress, as measured by blood lactate and heart rate, was accomplished in a minimum amount of exercise time. These values exceed those obtained on conventional treadmills and are similar to values seen in horses under simulated racing conditions (Lindholm and Saltin, 1974).

Blood hemoglobin concentration and hematocrit increased with exercise (Table 11), consistent with the results of Torten and Schalm (1964) who reported splenic release of erythrocytes in horses during exercise. This phenomenon is well documented (Boucher et al., 1981; Kitchen et al., 1965; Parks and Monohar, 1983; Rose et al., 1983) and allows for rapid changes in the oxygen carrying capacity of the blood of horses under stress. It is interesting to note that initial samples had significantly higher hematocrit levels when compared with the 1 and 24 hr post-exercise samples. This may be attributable to splenic release of red cells as

Table 11. Changes in blood hemoglobin and hematocrit and plasma hemoglobin as a result of exercise.

Time of sampling	Hematocrit (%)	Hemoglobin (g/dl)	
		Blood	Plasma
I	42.3[a]	15.2[ab]	.156[de]
1 min	47.8[b]	18.8[a]	.192[d]
F	51.9[b]	20.4[a]	.226[d]
1 hr	34.7[c]	13.5[b]	.119[e]
24 hr	38.9[c]	15.6[ab]	.146[de]

a,b,c Values in the same column with different superscripts differ significantly ($P<.001$).
d,e Values in the same column with different superscripts differ significantly ($P<.01$).

a result of nervous anticipation of exercise.

Centrifugation of blood samples, which were mixed by inversion and used to determine hematocrit and hemoglobin values, produced plasma with noticeably more hemolysis than blood centrifuged immediately after collection. Hemoglobin values for these plasma samples are shown in Table 11. Hemolysis was significantly higher in samples obtained during exercise, when compared to the 1 hr post-exercise samples. The extensive reticulo-endothelial system of the spleen functions to remove damaged erythrocytes from blood (Wintrobe, 1980). Strenuous physical activity increases red blood cell hemolysis (Buskirk, 1980). The increased hemolysis seen in exercise samples may be a result of two factors, the release of these damaged cells back into the circulation and exercise-induced hemolysis.

Summary

Exercise bouts conducted in this study produced hematological and blood lactate changes similar to those reported elsewhere in the literature. The ability to increase per cent grade and treadmill speed provided an effective method of producing maximum stress in a minimum amount of time. Hematocrit values were shown to increase with exercise and level of excitement. This would indicate that care should be taken to minimize animal excitement when blood samples are obtained for baseline hematocrit or hemoglobin determinations in the horse.

Selenium and Vitamin E Supplementation During Exercise and Conditioning in Horses

Changes in plasma Se levels over the 18-week feeding period are shown graphically in Figure 8. Plasma Se concentration increased with Se supplementation above pre-feeding levels($P<.001$). The basal ration would appear to provide dietary Se intakes high enough to maintain plasma Se concentration. However, feeding this ration produced a significant decline in plasma Se over the length of the trial ($P<.001$). Before mares were fed the experimental diets, they were maintained in outside lots and fed only first cutting alfalfa-grass hay. This hay had an analyzed Se content of .711 mg/kg dry matter (DM). Therefore, feeding the basal ration reduced daily dietary Se intake 3 fold and resulted in the observed decrease in plasma Se levels for non-supplemented mares.

Plasma Se concentrations were elevated ($P<.05$) during exercise, when compared to the 1 hr post-exercise sample (Figure 9). These differences are thought to have resulted from changes in plasma volume rather than mobilization of Se from some body store. During severe exercise in humans, hemoconcentration and reduction in plasma volume have been described (Kaltreider and Meneely, 1940; Johnson and Buskirk, 1980). This decrease is thought to result, at least in part, from increased circulatory hydrostatic pressure and loss of water from the vascular compartment. Astrand and

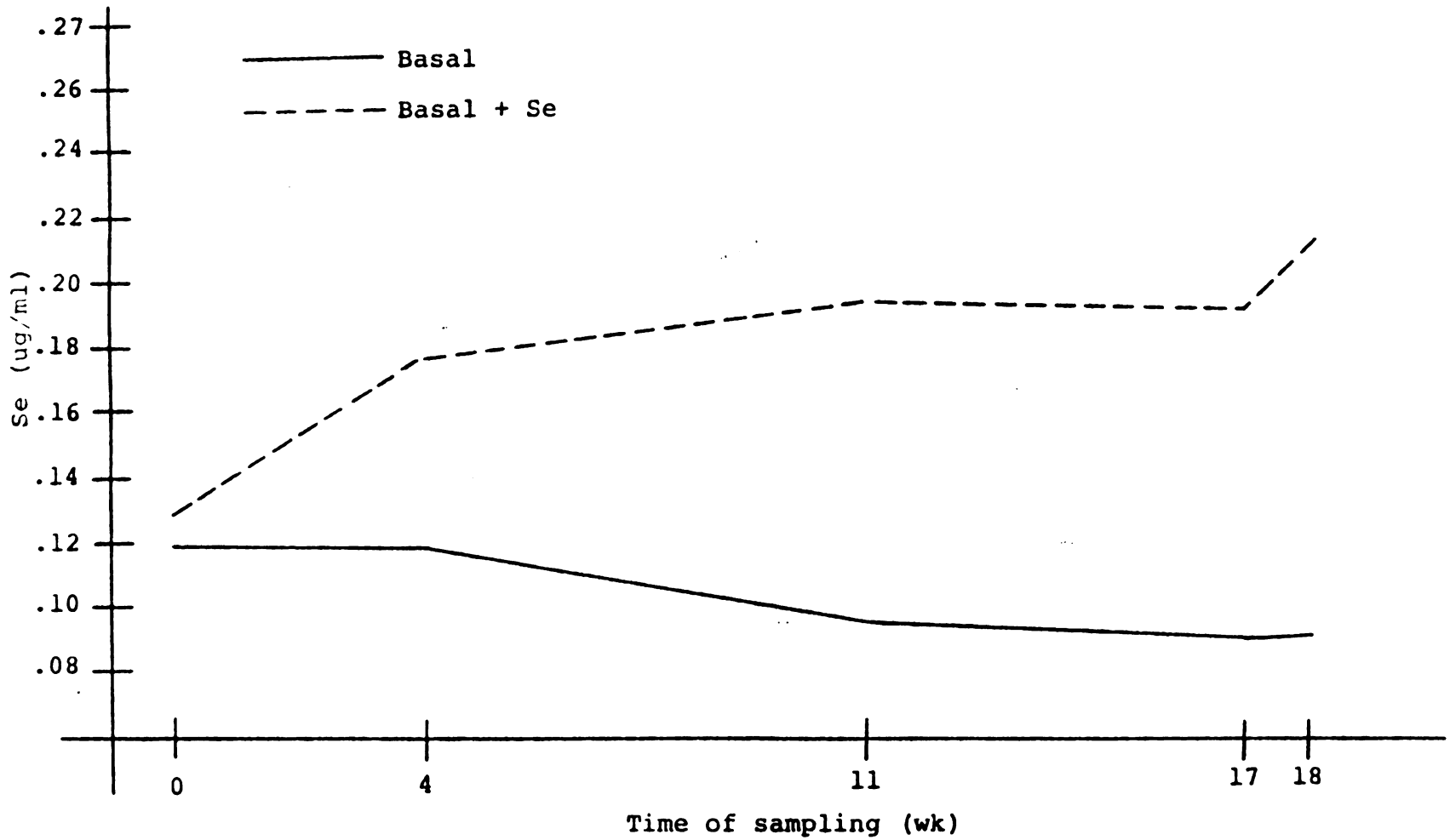


Figure 8. The effects of Se treatment on plasma Se levels.

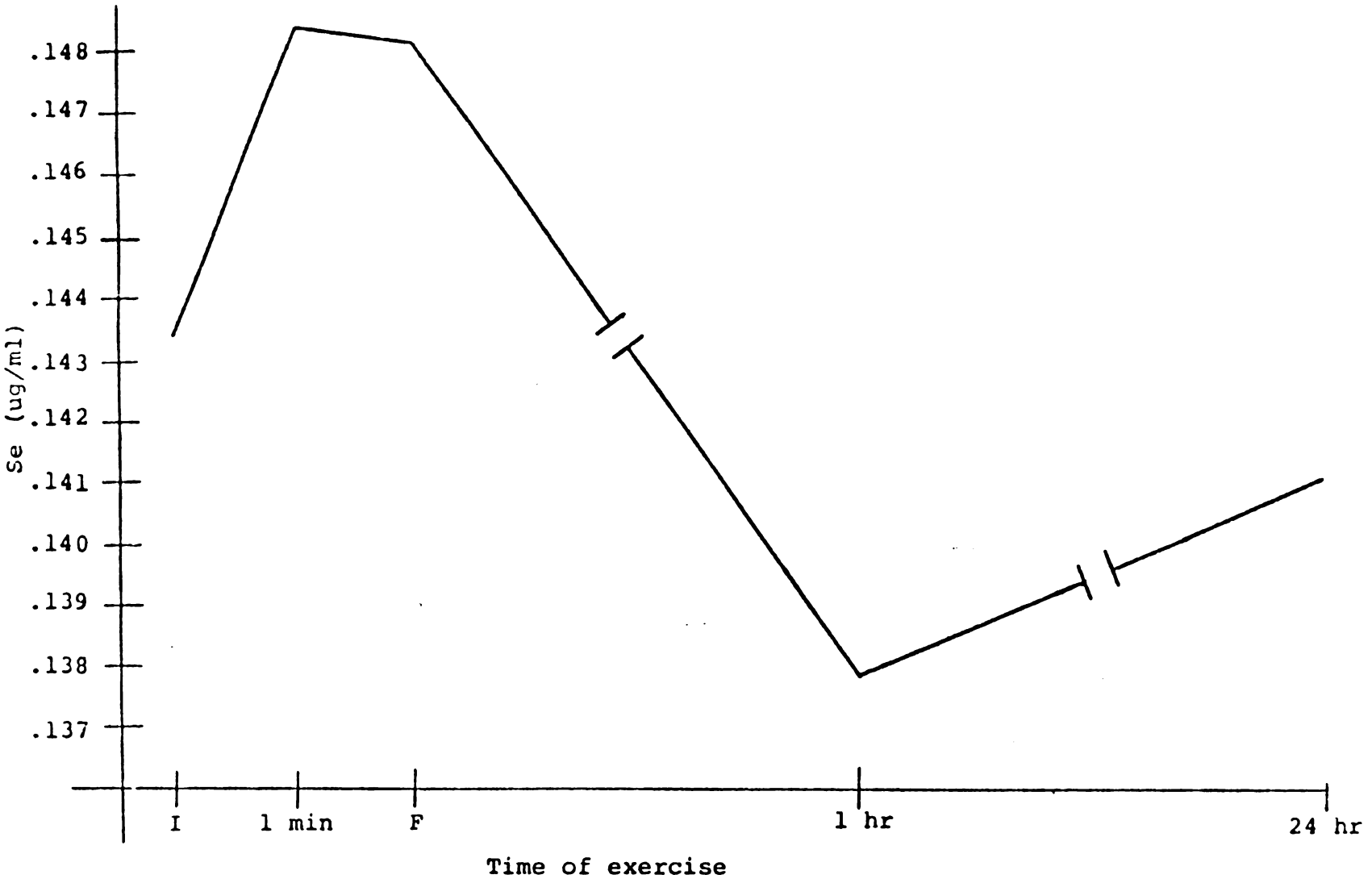


Figure 9. Changes in plasma Se during exercise.

Saltin (1964) found an increase in plasma volume, above pre-exercise levels, 1 hr after a strenuous exercise bout. This initial hemoconcentration and subsequent hemodilution, may explain observed changes in plasma Se concentration during, and 1 hr after strenuous exercise.

Changes in plasma alpha-tocopherol concentrations resulting from vitamin E supplementation are shown in Table 12. Concentrations tended to be higher with supplementation; however, these differences were not significant. Exercise and conditioning did not affect plasma alpha-tocopherol levels.

Mean plasma GSHpx activities were 5.3 and 7.7 EU/g of plasma protein ($P<.01$), for non-supplemented and supplemented mares, respectively. Erythrocyte GSHpx activities increased as a result of conditioning (Table 13). Selenium supplementation augmented the effect of conditioning and resulted in a significant treatment/conditioning interaction ($P<.01$).

Brady et al. (1978) and Shellow et al. (1983) saw no effect of Se supplementation on GSHpx activities in exercised and non-exercised horses, respectively. However, plasma Se levels were within normal ranges in both studies prior to supplementation, and in neither study were horses conditioned. Exercise has been shown to increase peroxidative damage, as determined by changes in lipid peroxide levels, in horses and rats (Brady et al., 1978; Brady et al., 1979). This increase in peroxidative damage

Table 12. The effect of vitamin E supplementation on plasma alpha-tocopherol concentrations (ug/ml).

	Weeks on feed					
Diet	0	4	11	17	18	P value
Basal	3.6	3.1	3.5	2.5	3.3	NS[a]
Basal+vit E	3.4[b]	4.5[bc]	4.6[bc]	4.9[bc]	5.8[c]	.01

a Values are not significantly different (P>.10).
b,c Values with different superscripts in the same row differ significantly.

Table 13 The effects of conditioning and Se treatment on erythrocyte GSHpx activity (EU/g Hb)[a].

Selenium treatment	Conditioning level		
	1	2	3
No added Se	42^{bd}	108^{cd}	104^{cd}
2.5 mg added Se/day	54^{bd}	149^{ce}	154^{ce}

a 1 EU (enzyme unit)= 1 umole NADPH oxidized x min^{-1}.
b,c Values in the same row with different superscripts differ significantly ($P<.001$).
d,e Values in the same column with different superscripts differ significantly ($P<.05$).

is thought to be a result of an increase in oxygen delivery and metabolism which occurs during exercise. Therefore, exercise may increase the need for mechanisms to protect against cellular oxidative damage, and in so doing, stimulates GSHpx synthesis. Godwin (1972) did demonstrate a moderating effect, due to conditioning, on plasma CPK activities in lambs fed a Se-deficient diet.

The lifespan of the mature red blood cell in horses is 151 days (Carter et al. 1974), and, being a non-nucleated cell, it lacks the genetic machinery to alter its protein make-up. The magnitude of change in erythrocyte GSHpx activity, seen in this study, and the length of time required for this change to occur, are difficult to explain within these restrictions. However, extensive destruction of red cells occurs with the onset of intensive conditioning (Davidson, 1964; Johnson and Buskirk, 1980). As conditioning continues, erythropoiesis increases to restore red cell count to normal levels and then decreases to a new level of production commensurate with the needs of conditioning (Johnson and Buskirk, 1980). In effect, the onset of intensive physical conditioning reduces the lifespan of the red cell and may account for the observed rapid changes in erythrocyte GSHpx activity.

Blood malondialdehyde (MDA) levels at 24 hr after exercise were significantly lower than levels during exercise, for non-conditioned mares (Table 14). Conditioning lowered MDA levels, eliminated the effect of

Table 14. The effects of conditioning and time of sampling on blood malondialdehyde concentration (U/ml)[a].

Sampling time	Conditioning level 1	2	3	P value
I	13.7[bd]	3.6[c]	3.2[c]	.001
1 min	8.7[bd]	3.6[c]	3.2[c]	.001
F	11.5[bd]	3.9[c]	3.8[c]	.001
1 hr	9.2[bd]	3.0[c]	3.1[c]	.001
24 hr	5.6[be]	2.2[c]	2.9[c]	.05
P value	.01	NS[f]	NS	

a U (unit) is defined as 10 x absorbance at 535 nm.
b,c Values in the same row with different superscripts differ significantly.
d,e Values in the same column with different superscripts differ significantly.
f Values are not significantly different (P>.10).

exercise, and resulted in a significant conditioning/time of sampling treatment interaction ($P<.05$). Blood MDA levels are a measure of cellular lipid peroxidative damage and, as has been discussed earlier, increase during exercise in non-conditioned horses (Brady et al, 1978). Daily conditioning of rats by swimming for several weeks has been shown to decrease lipid peroxides in both blood and liver (Tani and Aoki, 1981). This is in agreement with the present study and may indicate the importance of conditioning in stimulating cellular protective mechanisms.

Changes in total plasma protein resulting from exercise and conditioning are given in Table 15. Increased plasma protein concentrations during exercise have been described elsewhere (Poso et al., 1983; Rose et al., 1983). Strenuous exercise causes a transient hemoconcentration and a subsequent hemodilution after exercise has stopped. These changes in plasma volume are thought to be responsible for the observed fluctuations in plasma protein concentrations. Plasma glutathione-S-transferase activity tended to rise after exercise (Figure 10). However, there were no significant differences.

Plasma CPK activity was significantly lower at all sample times in non-conditioned horses (Table 16). Cardinet et al. (1967) and Poso et al. (1983) saw elevated plasma CPK activities with exercise in strenuously exercised horses. Increases in CPK activities are thought to indicate muscle damage as a result of extreme exertion. The length of time

Table 15. The effects of level of conditioning and time of sampling on plasma protein (mg protein/ml plasma).

Time of sampling	Conditioning level 1	2	3	P value
I	64[c]	66[cd]	64	NS[e]
1 min	65[ac]	69[bc]	69[b]	.10
F	65[ac]	69[bc]	69[b]	.10
1 hr	57[ad]	63[bd]	66[b]	.01
24 hr	68[ac]	64[bcd]	64[b]	.10
P value	.05	.05	NS	

a,b Values with different superscripts in the same row differ significantly.
c,d Values with different superscripts in the same column differ significantly.
e Values are not significantly different (P>.10).

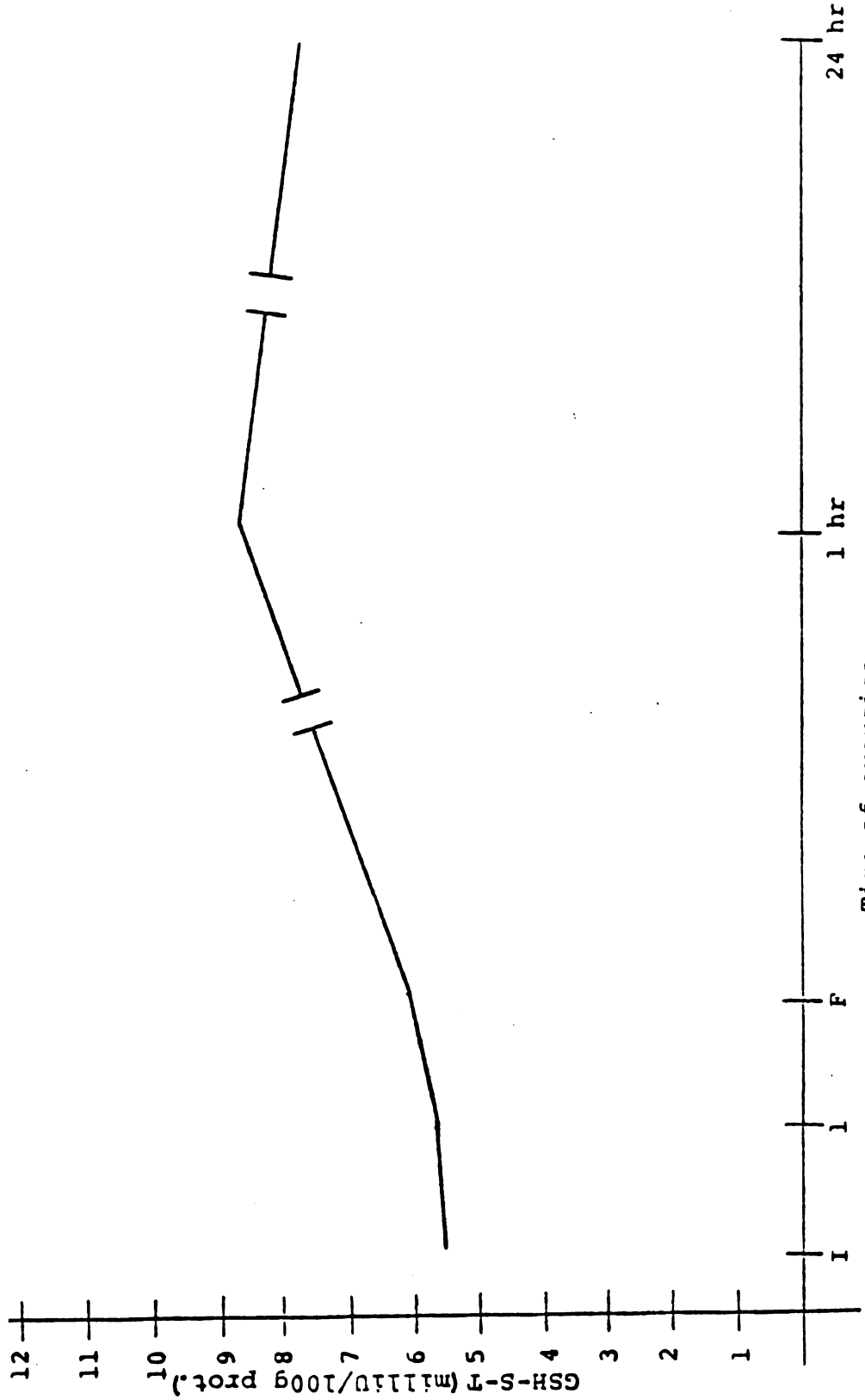


Figure 10. Changes in plasma glutathione-S-transferase activity as a result of exercise (1 milli U = 1000 X EU, EU = umoles CNB conjugated X min^{-1}).

Table 16. The effect of conditioning and time of sampling on plasma creatine phosphokinase activity (IU/100 g protein).

Time of sampling	Conditioning level 1	2	3	P value
I	2.3[a]	4.8[b]	4.6[bd]	.01
1 min	2.4[a]	4.7[b]	5.5[bd]	.001
F	2.7[a]	5.2[b]	4.6[bd]	.01
1 hr	3.0[a]	6.3[b]	5.7[bd]	.001
24 hr	2.5[a]	4.8[b]	9.0[ce]	.001
P value	NS[f]	NS	.001	

a,b,c Values in the same row with different superscripts differ significantly.
d,e Values in the same column with different superscripts differ significantly.
f Values are not significantly different (P>.10).

mares were on the treadmill at conditioning level 1 was shorter than for conditioning levels 2 and 3 (refer to Table 9), and may not have been of sufficient length to produce a level of muscular activity resulting in the leakage of muscle enzymes into the circulation.

The highest CPK activities were seen in conditioning level 3, 24 hr after exercise. This mean was calculated on seven observations. Mare number 37 had a 24 hr CPK activity of 191.5 IU/100 g plasma protein. This value was calculated as an outlyer and removed for statistical analysis. Mare 37 showed no visible signs of lameness or plysical fatigue after this exercise bout. She was, however, an excitable animal and always appeared to be under more emotional stress than other mares. The elevated CPK activities after exercise in conditioning level 3 may indicate the importance of gradually increasing work intensity after days of rest to minimize muscle damage.

Reduced glutathione (GSH) levels in whole blood were elevated ($P<.001$) during exercise (Figure 11). The highest concentration of GSH in blood is within the red cell. As discussed earlier, red cell numbers are increased with exercise in the horse, as a result of splenic contraction. Changes in GSH concentrations may be the result of this rise and subsequent decline in erythrocyte numbers during exercise. The effects of conditioning on blood GSH concentrations are given in Table 17. Concentrations were significantly higher at all sample times for conditioning

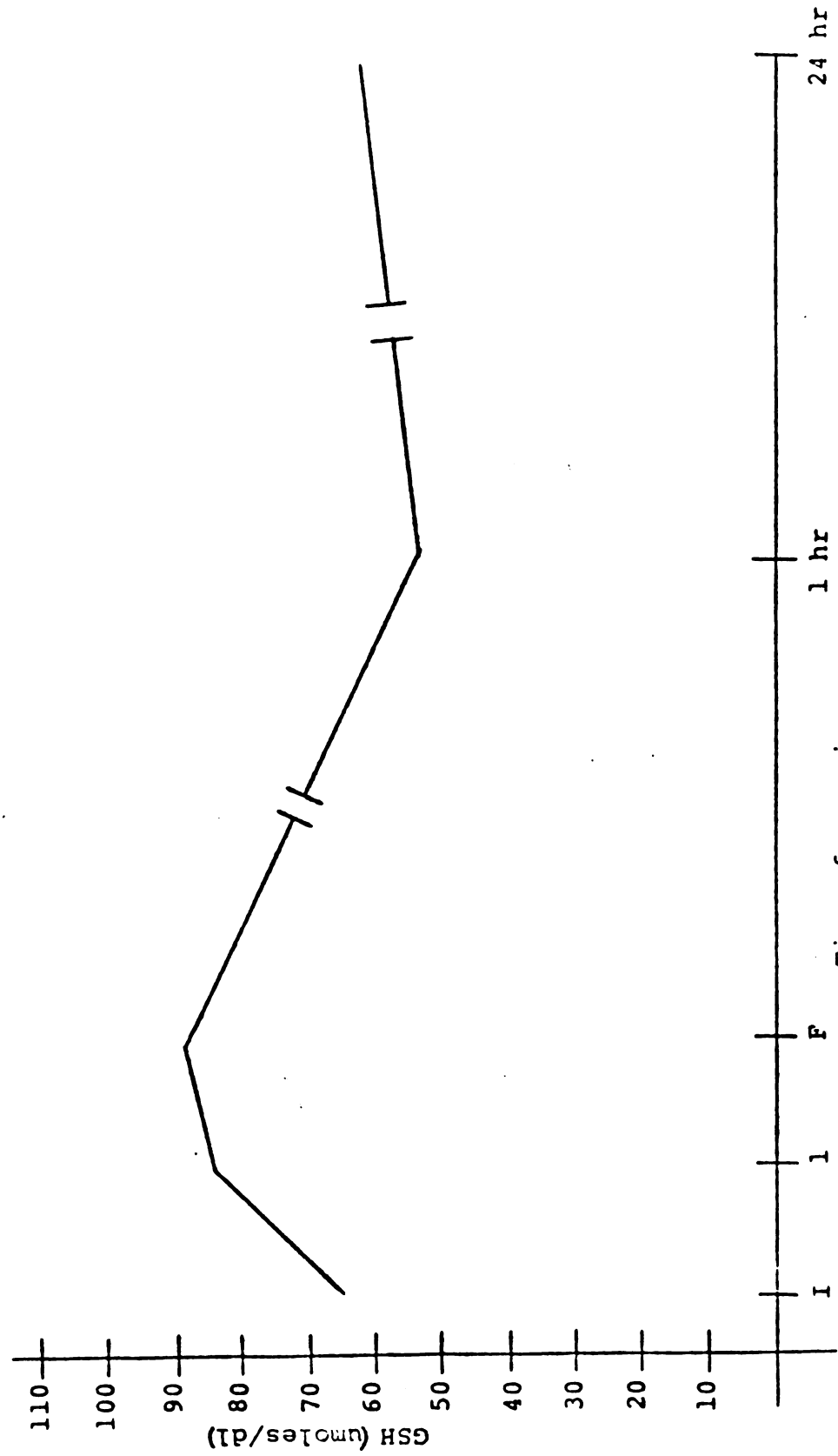


Figure 11. Changes in blood reduced glutathione levels with exercise.

Table 17. The effects of conditioning and time of sampling on blood reduced glutathione levels (umoles/dl).

Time of sampling	Conditioning level 1	2	3	P value
I	76^{ac}	62^{bc}	56^{bc}	.01
1 min	93^{ad}	77^{bd}	80^{bd}	.01
F	100^{ad}	83^{bd}	82^{bd}	.01
1 hr	65^{ac}	52^{bc}	49^{bc}	.01
24 hr	75^{ac}	52^{bc}	58^{bc}	.001
P value	.001	.001	.001	

a,b Values in the same row with different superscripts differ significantly.
c,d Values in the same column with different superscripts differ significantly.

level 1. A significant interaction between vitamin E supplementation and level of conditioning occurred for erythrocyte glucose-6-phosphate dehydrogenase (G-6-PDH) activity (Table 18).

Erythrocyte G-6-PDH activities and GSH levels were affected similarly by conditioning. This result is reasonable, as the reducing equivalents required to maintain GSH levels in the erythrocyte are supplied exclusively from the hexose monophosphate pathway. G-6-PDH is the regulatory enzyme in this pathway, and is responsive to cellular NADPH levels. Glutathione reductase, in the presence of riboflavin, uses NADPH to reduce oxidized glutathione to GSH. Heavy exercise in the rat has been shown to increase liver G-6-PDH activity (Mayanskaya,1982). Maximum activities were reached at 6 and 16 hr after exercise. However, the effects of conditioning were not studied.

The increase in erythrocyte GSHpx activity seen in this study would indicate an increased demand for reducing equivalents provided by the hexose monophosphate pathway. However, the reverse may be true. A reduction in G-6-PDH activity and the resultant assumed rise in oxidized glutathione levels, may stimulate synthesis of GSHpx. This may, in part, account for the magnitude of change seen in red cell GSHpx activity as a result of conditioning. Additional work is needed to determine the effects of exercise and conditioning on these enzymes and further elucidate the reasons for these changes in enzyme activity.

Table 18. The effects of conditioning and vitamin E supplementation on erythrocyte glucose-6-phosphate dehydrogenase activity (IU/g hemoglobin).

Dietary treatment	Conditioning level 1	2	3	P value
Basal (B)	9.2[a]	7.3[b]	7.1[bc]	.001
B + vitamin E	9.1[a]	7.2[b]	7.9[bd]	.001
P value	NS[e]	NS	.01	

a,b Values in the same row with different superscripts differ significantly.
c,d Values in the same column with different superscripts differ significantly.
e Values are not significantly different (P>.10).

Summary

Strenuous conditioning programs appear to increase GSHpx activities in equine erythrocytes. This may indicate an increased need for mechanisms to protect against peroxidative cellular damage resulting from the increased production of oxygen byproducts during exercise. Decreases in G-6-PDH activities seen as a result of conditioning were not expected. The reason for this decline is not understood, and additional research is needed to define the mechanisms responsible. The observations of horsemen indicating an increase in the incidence of muscular disorders as a result of exercise, after periods of rest, is supported by the prolonged increase in CPK activity seen in conditioning level 3.

CONCLUSION

Supplementation of vitamin E, above the levels found in the basal ration, increased plasma alpha-tocopherol concentrations, However, the addition of vitamin E did not significantly affect any other parameter measured. When good quality hays were provided, supplementation with vitamin E appeared to be of no additional benefit to exercising horses.

Hematocrit, hemoglobin and blood lactate levels increased with exercise. These changes were similar to published values for heavily exercised horses. Conditioning mares for 45 days reduced the magnitude of blood MDA and lactate concentration increases resulting from exercise, indicating an improvement in animal fitness.

Erythrocyte GSHpx activity increased as a result of conditioning and Se supplementation. Conditioning had the most profound effect, increasing baseline activities two-fold. Selenium supplementation did not improve animal performance or reduce the potentially damaging effects of exercise, as measured by blood MDA levels and plasma CPK activities. The effects of Se supplementation upon Se-deficient horses during exercise were not studied. However, the conditioning-induced increase in erythrocyte GSHpx

activity may indicate that providing adequate dietary Se intake becomes increasingly more important as the level of physical activity increases.

Blood GSH levels decreased with conditioning. This may have been a result of an increase in oxygen metabolism with exercise, and a decrease in reducing equivalents provided from the hexose monophosphate pathway. Conditioning decreased erythrocyte G-6-PDH activity. The reason for this decrease is unknown and warrants further investigation.

Plasma CPK activities, during and after exercise, were highest in conditioned mares after 2 days of rest. Blood malondialdehyde levels, during exercise, were highest in non-conditioned mares. These results indicate that care should be taken when exercise intensity is increased or when exercise is resumed after periods of rest. Rapid changes in the level of physical activity may cause increased lipid peroxidation and muscle damage and may result in impaired animal performance.

BIBLIOGRAPHY

LITERATURE CITED

Afifi, A.K., R.A. Bergman and J.C. Hawey. 1968. Steroid myopathy: clinical histologic and mytologic observations. Johns Hopk. Med. J. 123:158.

Astrand, P.O. and B. Saltin. 1964. Plasma and red cell volume after prolonged severe exercise. J. App. Phys. 19:829.

Baehner, R.L., L.A. Boser, L.M. Ingraham, C. Butterick and R.A. Haak. 1982. The influence of vitamin E on human polymorphonuclear cell metabolism and function. N.Y. Acad. Sci. 393:250.

Bergsten, G., R. Holmback and P. Lindberg. 1970. Blood selenium in naturally fed horses and the effect of selenium administration. Acta Vet. Scand. 11:571.

Beutler, E.,O. Duron and B.M. Kelly. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61:882.

Bieri, J.G., T.J. Tolliver and G.L. Catignani. 1979. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am. J. Clin. Nutr. 32:2143.

Blackmore, D.J., K. Willett and D. Agnes. 1979. Selenium and gamma-glutamyl transferase activity in serum of Thoroughbreds. Res. Vet. Sci 26:76.

Boucher, J.H., E.W. Ferguson, C.L. Wilhelmsen, N. Statham and R. McMeekin. 1981. Erythrocyte alterations during endurance exercise in horses. J. Appl. Phys. 51:131.

Bowen, J.N. 1942. Myoglobinuria in horses. Vet. Rec. 54:354.

Brady, P.S. 1978. The relationship of the glutathione peroxidase system to physical stress. PhD Thesis. Michigan State Univ.

Brady, P.S., P.K. Ku and D.E. Ullrey. 1978. Lack of effect of Se supplementation on the response of equine erythrocyte glutathione system and plasma enzymes to exercise. J. Anim. Sci. 47:492.

Brady, P.S., L.J. Brady and D.E. Ullrey. 1979. Selenium, vitamin E and the response to swimming stress in the rat. J. Nutr. 109:1103.

Caple, I.W., S.J.A. Edwards, W.M. Forsyth, P. Whiteley, R.H. Selths and L.J. Fulton. 1978. Blood glutathione peroxidase activity in horses in relation to muscular dystrophy and selenium in nutrition. Aust. Vet. J. 54:57.

Cardinet, G.H., M.E. Fowler and W.S. Tyler. 1963. The effects of training, exercise and tying-up on serum transaminase activities in the horse. Am. J. Vet. Res. 24:980.

Cardinet, G.H., J.F. Littrell and R.A. Freedland. 1967. Comparative investigations of serum creatine phosphokinase and glutamic-oxalacetic transaminase activities in equine paralytic myoglobinuria. Res. Vet. Sci. 8:219.

Carter, E.I., V.E. Valli, B.J. McSherry, F.J. Milne, G.A. Robinson and J.H. Lumsden. 1974. The kinetics of hematopoiesis in the light horse. 1. The lifespan of peripheral blood cells in the normal horse. Can. J. Comp. Med. 38:303.

Cooper, D.L. 1972. Drugs and the athlete. J. Am. Med. Assoc. 221:1007.

Crinion, R.A.P. and J.P. O'Connor.1978. Selenium intoxication in horses. Irish Vet. J. 32:81.

Davidson, R.J.L. 1964. Exertional haemoglobinuria: a report on three cases with studies on the haemolytic mechanism. 17:536.

Dewes, H.F. 1981. A possible vitamin E responsive condition in adult horses. N.Z. Vet. J. 25:83.

Diplock, A.T. 1981. The role of vitamin E and selenium in the prevention of oxygen-induced tissue damage. In: Selenium in Biology and Medicine. AVI Publishing Co., Westport, Conn.

Dodd, D.C. and A.A. Blakely, R.S. Thornbury and H.F. Dewes. 1960. Muscle degeneration and yellow fat disease in foals. N. Zealand Vet. J. 8:45.

Ewan, R.C., C.A. Baumann and A.L. Pope. 1968. Effects of selenium and vitamin E on nutritional muscular dystrophy in lambs. J. Anim. Sci. 27:751.

Farrow, W.A., D.H. Roloff, C.W. Westman, J.P. McCay, R.E. O'Neil, W.R. Linfoot, J. Hoagland, J.A. Robberson, H. Hopsin, E.A. Todd and P.O. Backett. 1976. Treatment for azoturia and tying-up. Mod. Vet. Prac. 57:413.

Foreman, J.H., W.M. Bayly, B.D. Grant and P.D. Gollnick. 1983. Cardiopulmonary responses to a traditional method of training the Thoroughbred racehorse. Proc. 8th Eq. Nutr. Phys. Sym. 8:193.

Franke, K.W. 1934. A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. J. Nutr. 8:597.

Furr, A.K., T.F. Parkinson, C.L. Heffron, J.T. Reid, W.M. Haschek, W.H. Gutenmann, C.A. Bache, L.E. St.John and D.J. Lisk. 1978. Elemental content of tissues and excreta of lambs, goats, and kids fed white sweet clover growing on fly ash. J. Agric. Food Chem. 26:847.

Gabbedy, B.J. and R.B. Richards. 1970. White muscle disease in foals. Aust. Vet. J. 46:111.

Geiser, D.R. 1975. The azoturia tying-up syndrome. Vet. Med. Small Anim. Clin. 70:710.

Gerken, H.J. 1982. Consider selenium needs when feeding horses. Feedstuffs. 11/8/82, 29.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. The Iowa State Univ. Press. Ames, Iowa.

Godwin, K.O. 1972. The moderating effect of regular exercise on the development of nutritional muscular dystrophy in lambs. Aus. J. Exp. Agr. Anim. Husb. 12:473.

Habig, W.H., M.J. Pabst and W.B. Jakoby. 1974. Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130.

Hansen, M.A. 1970. Plasma transaminase activity in myopathies of horses. Nord. Ver. Med. 22:617.

Harthoorn, A.M. and E. Young. 1974. A relationship between acid base balance and capture myopathy in zebra and an apparent therapy. Vet. Rec. 95:337.

Harthoorn, A.M. 1976. Physiology of capture myopathy: Study of capture myopathy (overstraining syndrome) in wild ungulates in Southern Africa. Dept. of Zoo. Univ. of Pretoria, Pretoria.

Heimann, E.D., L.W. Garrett, W.E. Loch, J.S. Morris and W.H. Pfander. 1981a. Selenium and reprocuctive abnormalities in pony mares grazing fescue pastures. Proc. 7th Eq. Nutr. Phys. Sym. 7:62.

Heimann, E.D., L.W. Garrett, W.E. Loch, J.S. Morris and W.H. Pfander. 1981b. Selenium status in pony mares grazing fescue pastures. Proc. 7th Eq. Nutr. Phys. Sym. 7:56.

Hill, H.E. 1962. Selenium-vitamin E treatment of tying-up in horses. Mod. Vet. Prac. 43:66.

Hofmeyer, J.M., G.M. Louw and J.S. duPreez. 1973. Incipient capture myopathy as revealed by blood chemistry of chased Zebra. Madoqua. Ser. I. 7:45.

Iyer, G.Y.N., D.M.F. Islam and J.H. Quanstel. 1961. Biochemical aspects of phagocytosis. Nature. 192:535.

Johnson, W.R. and E.R. Buskirk. 1980. Structural and Physiological Aspects of Exercise and Sport. Princeton Book Co. Princeton, NJ.

Jones, T.C. and W.O. Reed. 1948. Muscular dystrophy in a foal. J. Am. Vet. Med. Assoc. 112:170.

Kaltreider, N.L. and G.R. Meneely. 1940. The effects of exercise on the volume of blood. J. Clin. Invest. 19:627.

Kitchen, H., W.F. Jackson and W.J. Taylor. 1965. Hemoglobin and hemodynamics in the horse during physical training. Am. Assoc. Eq. Pract. 11th Conv. 11:97.

Knott, S.G. and C.W.R. McCray. 1959. Two naturally occuring outbreaks of selenosis in Queensland. Aust. Vet. J. 35:161.

Kontos, H.A., E.L. Harley, A.J. Wasserman, J.J. Kelly and J.H. Magee. 1963. Exertional idiopathic paroxysmal myoglobinuria: Evidence for a defect in skeletal muscle metabolism. Am. J. Med. 35:283.

Lawrence, R.A., R.A. Sunde, G.L. Schwartz and W.G. Hoekstra. 1974. Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake. Exp. Eye Res. 18:563.

Lawrence, L.M. and L.M. Slade. 1979. Physiological effects of vitamin E supplementation on exercised horses. Proc. 6th Eq. Nutr. Phys. Sym. 6:56.

Lindholm, A. and B. Saltin. 1974. The physiological and biochemical response of Standardbred horses to exercise of varying speed and duration. Acta Vet. Scand. 15:310.

Lindholm, A., H. Johanssan and P. Kjaersgaard. 1974. Acute rhabdomyolysis (tying-up) in Standardbred horses. Acta Vet. Scan. 15:325.

Linn, T.S., N. Putt, D. Safranski, C. Chung and R.R. Watson. 1981. Effects of vitamin E on cell-mediated immune responses and serum corticosterone in young and maturing mice. Immunology. 44:289.

Liu, S., E.P. Dolensek, C.R. Adams and J.P. Tappe. 1983. Myelopathy and vitamin E deficiency in six Mongolian wild horses. J. Am. Vet. Med. Assoc. 183:1266.

Madison, T.C., 1860. Sanitary Report-Fort Randall. In: Statistical Report on the Sickness and Mortality in Army of the United States, January 1855 to January 1860, R.H. Coolidge, ed. U.S. Congress 36th, 1st Session. Senate Exch. Doc. 52:37.

Mandisodza, K.T., W.G. Pond, D.J. Lisk, D.E. Hogue, L. Krook, E.E. Cary and W.H. Gutenmann. 1979. Tissue retention of Se in growing pigs fed fly ash or white sweet clover grown on fly ash. J. Anim. Sci. 49:535.

Mayanskaya, N.N., L.E. Panin and T.G. Filatava. 1982. Role of lysosomes in changes of G-6-P dehydrogenase activity in the rat liver during exercise. Inst. Clin. Exp. Med. Novosibirsk, USSR. 44:33.

Maylin, G.A., D.S. Rubin and D.H. Lein. 1980. Selenium and vitamin E in horses. Cornell Vet. 70:272.

McConnell, K.P. and D.M. Roth. 1964. Passage of selenium across the placenta and also into the milk of the dog. J. Nutr. 84:340.

McLean, J.G. 1973. Equine paralytic myoglobinuria (azoturia): A review. 49:156.

Mengel, C.E., H.E. Kann and W.E. Merriwether. 1967. Studies of paroxysmal nocturnal myoglobinuria erythrocytes: Increased lysis and lipid peroxide formation by hydrogen peroxide. J. Clin. Invest. 46:1715.

Milne, D.W., R.T. Skarda, A.A. Gabel, L.G. Smith and K. Ault. 1976. Effects of training on BCH values in Standardbred horses. Am. J. Vet. Res. 31:285.

Milne, D.W., A.A. Gabel, W.W. Muir and R.T. Skarda. 1977. Effects of training on heart rate, cardiac output and lactic acid in Standardbred horses using a standard exercise test. J. Eq. Med. Surg. 1:131.

Muth, O.H. and W.H. Allaway. 1963. The relationship of white muscle disease to the distribution of naturally occurring selenium. J. Am. Vet. Med. Assoc. 142:1379.

National Research Council. 1978. Nutrient Requirements of Horses. National Academy of Sciences, Washington, D.C.

Nockels, C.F. 1979. Protective effects of supplemental vitamin E against infection. Fed. Proc. 38:2134.

Owen, R.R., J.N. Moore, H.B. Jopkins and D. Arthur. 1977. Dystrophic myodegeneration in adult horses. J. Am. Vet. Med. Assoc. 171:343.

Paglia, D.E. and W.N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158.

Parks, C.M. and M. Monohar. 1983. Distribution of blood flow during moderate and strenuous exercise in ponies (Equus caballus). J. Am. Vet. Med. Assoc. 44:1861.

Perry, T.W., R.C. Peterson and W.M. Beeson. 1977. Selenium in milk from feeding small supplements. J. Dairy Sci. 60:1698.

Placer, Z.A., L.L. Cushman and B.C. Johnson. 1966. Estimation of products of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analyt. Biochem. 16:359.

Platt, H. and K. E. Whitwell. 1971. Clinical and pathological observations and generalized steatitis in foals. J. Com. Path. 81:499.

Pope, D.C. and C.H. Heslop. 1960. An outbreak of myoglobinuria in light horses. Can. Vet. J. 1:171.

Poso, A.R., T. Soveri and H. Oksanen. 1983. The effect of exercise on blood parameters in Standardbred and Finnish-bred horses. Acta Vet. Scand. 24:170.

Rich, G.A., D.E. McGlothlin, L.D. Lewis, E.L. Squires and B.W. Pickett. 1983. Effect of vitamin E supplementation on stallion seminal characteristics and sexual behavior. Proc. 8th Eq. Nutr. Phys. Sym. 8:85.

Rodiek, A.V., M.A. Russell and L.M. Lawrence. 1983. Cardiovascular fitness of horses conditioned by intermittent or continuous treadmill work. Proc .8th Eq. Nutr. Phys. Sym. 8:199.

Rose R.J., J.R. Allen, D.R. Hodgson, H.J. Stewart and W. Chan. 1983. Responses to submaximal treadmill exercise and training in the horse: Changes in haematology, arterial blood gas and acid base measurements, plasma biochemical values and heart rate. Vet. Rec. 113:612.

Savage, D.L.C., M. Forbes and G.W. Pearce. 1971. Idiopathic rhabdomyolysis. Arch. Dis. Childh. 46:594.

Scarpelli, D.G., M.H. Greider and W.J. Frajola. 1963. Idiopathic recurrent rhabdomyolysis: A clinical, chemical and morphological study. Am. J. Med. 34:426.

Schougaard, H., A. Basse, G. Gissel-Nielson and M.G. Simensen. 1972. Nutritional muscular dystrophy in foals. Nord. Vet. Med. 24:67.

Schutta, H.S., A.M. Kelly and S.I. Zocks. 1969. Necrosis and regeneration of muscle in paroxysmal idiopathic myoglobinuria: electron microscopic observations. Brain. 92:191.

Sharman, I.M., M.G. Down and R.N. Sen. 1971. The effects of vitamin E and training on physiological function and athletic performance in adolescent swimmers. Br. J. Nutr. 26:265.

Sharman, I.M., M.G. Down and N.G. Norgan. 1975. Alleged argogenic properties of vitamin E: In Proceedings of 20th World Congress of Sports Medicine. ed. A.H. Toyne. Melbourne. Aust. Sports Med. Assoc.

Sheffy, B.E. and R.D. Schultz. 1979. Influence of vitamin E and selenium in immune response mechanisms. Fed. Proc. 38:2139.

Shellow, J.S., S.G. Jackson, J.P. Baker and A.H. Cantor. 1983. The influence of dietary selenium levels on the plasma levels of selenium and glutathione peroxidase activity in the horse. Proc. 8th Eq. Nutr. Phys. Sym. 8:21.

Shepard, R.J. 1980. Vitamin E and physical performance. Eur. J. App. Phys. 53:299.

Sigler, D.H., S.P. Webb and G.D. Potter. 1979. Physiological response of horses in different conditioning regimes to a standard exercise tolerance test on the treadmill. Proc. 6th Eq. Nutr. Phys. Sym. 8:193.

Sigma Chemical Company. 1983a. Determination of glucose-6-phosphate dehydroginase in blood at 340 mn. Sigma Technical Bulletin No. 345-UV. St. Louis, MO.

Sigma Chemical company. 1983b. Determination of creatine phosphokinase in serum or plasma at 340 nm. Sigma Technical Bulletin No. 45-UV. St. Louis, MO.

Statistical Packages for the Social Sciences: Update 7-9. 1981. ed. C.H. Hull and N. H. Nie. McGraw-Hill Book Co. New York.

Stewart, J.M. 1960. Selenium therapy in horses. N.Z. Vet. J. 8:82.

Stowe, H. D. 1967. Serum selenium and related parameters of naturally and experimentally fed horses. J. Nutr. 93:60.

Tani, Y. and M. Aoki. 1981. Effects of dietary protein levels and exercise on liver and serum lipid composition of rats. J. Jpn. Soc. Food Nutr. Sci. 34:59.

Torten, M. and O.W. Schalm. 1963. Influence of the equine spleen on rapid changes in the concentration of erythrocytes in peripheral blood. Am. J. Vet. Res. 25:500.

Watson, R.R. and T.M. Petro. 1982. Cellular immune responses, corticosteroid levels and resistance to Listeria monocytogenes and murine leukemia in mice fed a high vitamin E diet. N.Y. Acad. Sci. 393:205.

Webb, S.P., G.D. Potter and D.H. Sigler. 1979. Development of a repeatable standard exercise tolerance test in the horse. Proc. 6th Eq. Nutr. Phys. Sym. 8:193.

Whetter, P.A. and D.E. Ullrey. 1978. Improved flourometric method for determining selenium. J. Assoc. Off. Anal. Chem. 61:927.

Wilson, T.M., H.A. Morrison, N. G. Palmer, G.G. Finley and A.A. Drummel. 1976. Myodegeneration and suspected selenium/vitamin E deficiency in horses. J. Am. Vet. Med. Assoc. 169:213.

Wintrobe, M.M. 1980. Blood Pure and Eloquent. Nat. Acad. Sci. McGraw-Hill Book Co., New York.

Young, S. and R.F. Keeler. 1962. Nutritional muscular dystrophy in lambs-the effect of muscular activity on the symmetrical distribution of lesions. Am. J. Vet. Res. 23:966.