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THE RELATIONSHIP OF THE GLUTATHIONE PEROXIDASE SYSTEM TO PHYSICAL STRESS

By

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ABSTRACT

THE RELATIONSHIP OF THE GLUTATHIONE PEROXIDASE SYSTEM TO PHYSICAL STRESS

By

Paul Scott Brady

There are some data which suggest a relationship between lesions of selenium (Se) and vitamin E (E) deficiency and strenuous exercise. To investigate this phenomenon, a series of four studies was conducted. The first two studies involved exercising horses by running on a soft sand track, and monitoring blood parameters before and subsequent to exercise. In the second of the equine studies, one-half of the horses were provided with supplemental Se in the trace mineral salt. In all of the equine studies, plasma enzymes and erythrocyte malondialdehyde (MDA) rose immediately subsequent to exercise. Both MDA and plasma enzymes have been used as indicators of tissue damage and peroxidation in Se/E deficiency. Parameters of the glutathione peroxidase system were generally unresponsive to exercise, with the exception of glutathione reductase (GR). Total GR activity was consistently elevated subsequent to exercise. The active GR activity also tended to be elevated; however, the response was more variable. Se supplementation had no effect on any of the parameters measured, including gluathione peroxidase (GSH-Px) activity.

The third study was conducted with male weanling Holtzman rats fed torula yeast-based diets supplemented and unsupplemented with Se and E. The rats were killed after four weeks on the diet either prior to exercise, immediately after exercise or 24 hr after exercise. Rats were exercised by swimming to exhaustion. MDA was measured in liver and muscle as an indicator of lipid peroxidation. Enzymes of the glutathione peroxidase system were also determined. Liver and muscle MDA rose subsequent to exercise in liver and muscle among all dietary groups. Liver MDA values returned to baseline within 24 hr subsequent to exercise. Muscle values remained somewhat elevated. In liver, MDA response was reduced by dietary E, but not Se. Muscle values were uneffected by diet. None of the glutathione system enzymes responded to exercise, although Se supplementation resulted in reduced hepatic GR and NADP-linked dehydrogenase activities, and markedly increased GSH-Px activity.

The forth study dealt with the response of white-tailed deer to dietary Se and E. Adult female deer were fed diets supplemented and unsupplemented with Se and E for two years. Mortality of offspring was substantially increased among deer fed diets not supplemented with E. This response related well with in vivo and in vitro indicators of peroxidation among the adults. That is, while E supplementation

reduced mortality among the young, Se supplementation of the adults' diet had no influence on mortality or peroxidation at the levels used.

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My deepest appreciation must go to my wife, Linda, who now undoubtedly holds the record for most GSH-Px assays by Hafeman's modification.

I wish to acknowledge the support of NIH. My tenure as a predoctoral trainee has been both enjoyable and rewarding.

As always, with any piece of work, there are many people who aid in the work in the course of their jobs, out of friendship or just to experience different techniques. I won't try to name these poor fellows...I'm almost certain to forget someone. So, let me take the safe route and offer a blanket "THANK YOU" (So, who can accuse me of forgetting anyone?).

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INTRODUCTION

Lesions of selenium-vitamin E deficiency have been reported in a number of species subsequent to physical exertion (Young & Keeler, 1962; Young et al, 1975; Ullrey, 1973; Harthoorn & Young, 1974; Muth, 1963). Degeneration of the muscle fibers, or "white muscle disease", is the most common lesion, although liver and kidney necrosis may occur (Harthoorn & Young, 1974). Muscle lesions are generally symmetrical; however, Young and Keeler (1962) produced asymmetrical lesions in lambs by restraining one limb. Lesions in the restrained limb were absent or greatly reduced. Thus, it appears that physical exertion may contribute to the development of selenium-vitamin E deficiency lesions at least in muscle.

The metabolic roles of selenium (Se) and vitamin E (E) are now reasonably well defined. This represents recent work. Still, with the growing knowledge of the function of these nutrients, the present series of studies represents an attempt to relate the known metabolic effects of Se and E to the phenomenon of stress-induced deficiency signs. The horse, rat and white-tailed deer were used in these studies. Hartley and Grant (1961) and Hill (1963) have suggested that various exercise myopathies of the horse may be responsive to Se or E. No such suggestions have been made for the rat. Yet, unlike the horse, this species is inexpensive as a laboratory animal and conditions

are more easily controlled. The white-tailed deer like the horse appears more sensitive to Se or E deficiency in the face of physical stress.

REVIEW OF LITERATURE

Early studies demonstrated that dietary Se and E were mutually sparing (Calvert et al, 1962; Schwarz & Foltz, 1957). At least one definable function of E was as an antioxidant (Green, 1972). Primarily on this basis, an antioxidant function was also postulated for Se. It was not until 1972 that Rotruck and coworkers (1972) demonstrated the mode of Se's antioxidant effect. Their finding, that Se is an essential component of glutathione peroxidase (EC 1.11.1.9), served to confirm not only the antioxidant role of Se, but also that the role of E was also as an antioxidant.

The Glutathione Peroxidase System. Glutathione peroxidase and glutathione reductase (EC 1.6.4.2) serve as the basis of an enzyme system for the reduction of various peroxides. The peroxidase (GSH-Px) will reduce a wide range of lipid peroxides (Christopherson, 1968 & 1969; Flohe et al, 1974), hydrogen peroxide (Cohen & Hochstein, 1963) and, perhaps, hydroxyl free radical (McCay et al, 1976). The basic system is presented in figure 1. Electrons are transferred via the GSH-Px from reduced glutathione (GSH) to the peroxide. The enzyme is quite specific in its requirement for GSH (Flohe et al, 1974). Two moles of GSH are conjugated to form one mole of oxidized glutathione (GSSG). The glutathione reductase (GR) then catalyzes the

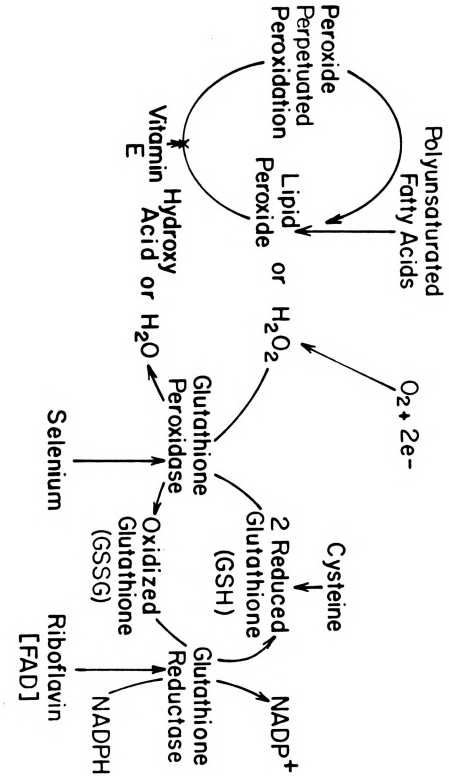

Figure 1. The glutathione peroxidase system.

reduction of GSSG using NADPH for the reducing equivalents (Beutler & Yeh, 1963; Rieber et al, 1968). However, GSH may serve functions other than peroxide reduction. GSH serves as a reservoir for cysteine in rat liver (Tateishi et al, 1977) and functions in amino acid transport (Meister, 1976). Thus, to consider GSH and GR solely committed to the process of peroxide detoxication is an oversimplification.

In the mature erythrocyte, NADPH for the GR is provided solely via the hexose monophosphate shunt. NADPH competitively inhibits glucose-6-phosphate dehydrogenase, and provides a major control of glucose flux through this pathway. However, Jacob and Jandl (1966) working with the erythrocyte model and Eggleston and Krebs (1974) working with rat liver showed that GSSG decreased inhibition of glucose-6-phosphate dehydrogenase (G6PD) by NADPH. Therefore, not only does the ratio of NADPH/NADP modulate flux through the hexose pathway, but also the ratio of GSSG/GSH.

Because the erythrocyte glutathione peroxidase system has been extensively studied, a tendency has developed to assume the hexose shunt to be the major source of reducing equivalents for this system in all tissues (Chow & Tappel, 1974). Stark et al (1975) have suggested that under certain dietary regimens malic enzyme may serve as the primary source of reducing equivalents for GR.

In the erythrocyte, then, G6PD may be thought to be linked directly to the glutathione pathway. A source of

glucose is essential for the erythrocyte to maintain NADPH concentration. Rotruck et al (1971) found that dietary Se protected RBC from in vitro hemolysis only when glucose was included in the incubation medium. Conversely, G6PD deficiency, a genetic anomaly extensively studied in man, results in hemolysis only upon oxidant stress (Beutler, 1972).

GR require an FAD (flavin adenine dinucleotide) cofactor; indeed, GR activity has been used as an indicator of riboflavin status (Glatzle et al, 1970). Riboflavin deficiency may lead to a moderate normocytic anemia in swine (Wintrobe et al, 1944; Brady et al, 1978). This anemia is probably not associated with impairment of the glutathione peroxidase system, however. Beutler (1974) has reported that reduction of GR activity by 50% did not influence GSH levels. In fact, GR activity may fall by 75% in the swine erythrocyte without decrease in the level of GSH (Brady et al, 1978). Both increased activity of GR (Gaetani et al, 1973) and enhanced binding of FAD (Flatz, 1970) has been reported among G6PD-deficient erythrocytes. Erythrocyte GR and G6PD activities may also show a close relationship, even where overt deficiency of one enzyme or the other is not involved (Brady et al, 1978b).

The GSH which GR spends its time trying to keep reduced, is a simple tripeptide (γ-glutamylcysteinylglycine). Its distribution is ubiquitous (Beutler, 1974). GSH is synthesized in two steps:

1) glutamate + cysteine $\xrightarrow[\text{synthetase}]{\gamma\text{-glutamylcysteine}}$ γ-glutamyl-cysteine,

2) γ-glutamylcysteine + glycine + ATP $\xrightarrow{\text{glutathione synthetase}}$ GSH.

This process is caried out in all tissues including erythrocytes (Beutler, 1975). Turnover of GSH is very rapid; in the mature erythrocyte, half-life is about four days (Dimant et al, 1955). The erythrocyte concentration of GSH is also quite high, about 2mM in most species (Beutler et al, 1955). It has been calculated that the erythrocyte could replace its store of GSH within minutes, based on the V_{max} for the synthesizing enzymes (Williams et al, 1975). Why then is GR needed? There are two probable reasons. First, the process of continual synthesis of GSH would prove costly in terms of substrate consumption. Further, GSSG is quite toxic to the cell (Srivastava & Beutler, 1975). It is important for the erythrocyte and other cells to keep intracellular GSSG concentrations low. In addition to reducing GSSG via GR, cells will actively dump GSSG to the extracellular space (Srivastava & Beutler, 1975; Chance et al, 1977).

Vitamin E. Vitamin E, while not directly linked to the glutathione peroxidase system, does serve a parallel function as a free radical quenching agent (Tappel, 1970; Urano et al, 1977). It terminates the free radical perpetuation of peroxidation. However, E is lipid soluble, while GSH-Px and associated enzymes and metabolites are water soluble. This has caused McCay et al (1976) to suggest that the peroxide on which E and GSH-Px act is not the same. This is still a question of active interest.

Exercise and Peroxidation. The evidence linking exercise and the generation of peroxides is minimal. To begin, lesions of Se/E deficiency have been reported subsequent to exercise in a broad range of species (Harthoorn & Young, 1974; Young et al, 1975; Ullrey, 1973; Muth, 1963). A number of these reports have suggested that dietary Se and E were adequate, prior to the production of the stress-induced lesions (Young et al, 1975; Ullrey, 1973; Harthoorn & Young, 1974). The problem with these reports is that the lesions produced by Se/E deficiency are not pathognomonic. The white muscle disease, hepatic necrosis, etc. are suggestive of a deficiency of one or both of these nutrients but do not provide conclusive proof of such a deficiency.

Elevation of various plasma enzymes has been shown to occur with Se/E deficiency (Olson, 1974; VanVleet, 1975; Whanger et al, 1977). Such elevations are used as indicators of preclinical deficiency, and are thought to reflect membrane damage with subsequent leaking of the enzymes into the plasma. Similarly, exercise may result in transient, but very substantial, increases in plasma enzymes (Cardinet et al, 1963; Milne et al, 1976; Thompson, 1962; King et al, 1976). Still, it is unclear whether the apparent change in membrane permeability associated with muscular exertion occurs via the same process as that found with Se/E deficiency.

There are very limited data relating exercise to lesions of nutritional muscular dystrophy or to oxidative

attack. Young and Keeler (1962) were able to produce asymmetrical muscle lesions by restraining one limb of lambs on dystrophogenic diets. These workers did not determine Se or E content of their diets. Stokinger (1963) produced ozone toxicity at reduced ozone levels (subtoxic) in rats by exercising the animals intermittently during exposure. "March hemoglobinuria", a hemolytic disorder brought on by exercise (hence, "march"), has been shown to be the result of a genetic deficiency of erythrocyte GSH-Px (Bernard et al, 1975).

The sum of these findings suggest that Se/E deficiency may be exacerbated by exercise. Two questions remain: what is the effect of Se/E supplementation on the response to exercise and 2) can strenuous exercise induce lesions (biochemical or otherwise) of Se/E deficiency where these nutrients would otherwise be present in the diet in adequate concentration to prevent deficiency?

RAPID CHANGES IN EQUINE ERYTHROCYTE GLUTATHIONE REDUCTASE WITH EXERCISE

INTRODUCTION. The erythrocyte glutathione peroxidase system has been presented (fig.1). Considerable interest has centered on this pathway with the identification of Se as a component of GSH-Px (Rotruck et al, 1972). This system is of interest because lesions of Se/E deficiency, including muscle degeneration (white muscle disease) and hepatic necrosis, have been reported in a wide range of species subsequent to exercise, as discussed previously. Because the only known role for Se is as a component of GSH-Px, it seems likely that lesions in animals subjected to exercise might be related to insufficient GSH relative to the rate of peroxidation.

Work has been done with the production of muscle lesions by running zebra, a relatively close relative of the domestic horse, to exhaustion (Harthoorn & Young, 1974). Further, exercise-induced myopathies of the domestic horse (azoturia, myositis, etc.) have been reported (Siegmund, 1973). In fact, it has been suggested that the equine myopathies respond favorably to Se supplementation (Siegmund, 1973; Hartley & Grant, 1961).

The response of the erythrocyte system was determined

in the hope of defining a metabolic limiting factor to GSH availability in this biochemically simple tissue; however, in view of the ubiquitous distribution of this system, the erythrocyte might in many respects serve as a reasonable model for other tissues.

MATERIALS AND METHODS. Six mature horses (5 Quarter-horses and 1 Arabian) were used. These animals had been occasionally exercised, but had not been regularly trained. Horses were run on a circular sand track until they refused to maintain their pace. An average of 2 km in 7.4 min was run before this point. Heparinized jugular blood samples were taken before (base line), immediately after and 1 hr after exercise.

Blood GSH was determined by the method of Beutler et al (1963), with the modification that blood was precipitated directly, without prior hemolysis, to reduce oxidation. Blood lactate (Hohorst, 1963) and pyruvate (Bucher et al, 1963) were determined enzymatically. The hematocrit (packed cell volume) and hemoglobin (Crosby et al, 1954) were also determined.

Erythrocytes were washed twice with cold isotonic saline (0.9% NaCl) and lysed with cold distilled water. Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.44) (Langdon, 1966), GSH-Px (Hafeman et al, 1974), and active and total GR (Sauberlich et al, 1972) were determined at 37°.

Data were analyzed by analysis of variance using a

randomized complete block design. Where a significant ($P<0.05$) exercise effect was found, data were further analyzed by Tukey's ω-procedure (Steel & Torrie, 1960).

RESULTS. The data are presented in table 1. Hemoglobin (Hb) and hematocrit (PCV) were markedly increased immediately after horses were exercised, but returned to base line within one hr after exercise. The mean corpuscular hemoglobin concentration (MCHC) remained unchanged.

Lactate and pyruvate also increased significantly immediately after exercise with return to near base line values after one hr. The ratio of lactate:pyruvate was increased with exercise. GSH/dl blood was also increased with exercise. However, GSH/dl erythrocyte did not change with exercise. GSH-Px and G6PD activities were not affected by treatment.

GR did exhibit an exercise effect. Total GR increased by over 30% immediately after exercise. Within one hr after exercise total GR had not declined significantly. Active GR also increased immediately after exercise and remained unchanged one hr after exercise. The percent of total GR that was active actually was depressed immediately subsequent to exercise, increasing somewhat by one hr after exercise.

DISCUSSION. Changes in Hb and PCV can be attributed to splenic release of erythrocytes with exercise (Turner & Hodgetts, 1959). The MCHC remained constant, as would be expected were spleen release involved. Splenic storage of erythrocytes is a dynamic process (Turner & Hodgetts, 1959).

Table 1. Blood variables in the exercised horse (n=6).

Variable	Base line (before exercise)	Immediately after exercise	At 1 hour after exercise	SEM
Hemoglobin (g/dl of blood)	11.7^{a}	15.8^{b}	11.2^{a}	0.2
Hematocrit (%)	35.0^{a}	47.1^{b}	33.0^{a}	0.7
Mean corpuscular hemoglobin concentration (%)	33.3	33.7	33.6	0.4
Reduced glutathione (mg/dl of blood)	15.4^{a}	23.6^{b}	17.3^{a}	1.6
(mg/dl of RBC)	43.5	50.2	52.3	3.7
Lactate (mg/dl of blood)	3.0^{a}	26.0^{b}	5.7^{a}	3.6
Pyruvate (mg/dl of blood)	0.8^{a}	1.4^{b}	0.8^{a}	0.1
Lactate/pyruvate (molar ratio)	3.6^{a}	18.2^{b}	6.7^{a}	2.3
Glutathione peroxidase ($-\Delta\log\lvert GSH\rvert \cdot 10^{3} \cdot min^{-1} \cdot mg$ hemoglobin^{-1})	19.8	17.8	16.8	1.4
Glucose-6-phosphate dehydrogenase (IU/g hemoglobin)	6.3	6.1	6.5	0.3
Glutathione reductase				
Total (IU/g of hemoglobin)	1.3^{a}	1.8^{b}	1.6^{ab}	0.1
Active (IU/g of hemoglobin)	1.1^{a}	1.2^{b}	1.2^{b}	0.04
Percent active	81.0^{a}	66.6^{b}	72.6^{c}	1.4

$^{a-c}$Means with different superscripts are significantly ($P<0.05$) different.

SEM = standard error of the mean; this value is generated via the analysis of variance.

Because the stored cells are constantly being exchanged with circulating cells, changes in GR activity should not be due to release of younger cells. While G6PD activity has been shown to decrease with increasing cell age, GR is not influenced by the age of the erythrocyte (Oski, 1970). For these reasons, it is likely that the increase in total GR was due to activation of a pre-existing inactive form of the enzyme.

Increased GR activity has been reported in the G6PD deficient erythrocyte (Gaetani et al, 1973). It has been suggested that synthesis of GR is increased in response to inadequate NADPH. In the present study, the time was too short to allow for protein synthesis, even if it were assumed that a large population of reticulocytes were released into the circulation (for which evidence is also lacking). There are several problems with the assumption that NADPH supplies had become limiting and that latent GR was activated in response. For one, erythrocyte G6PD is over 90% inhibited by NADPH under most conditions (Holten et al, 1976). If NADPH supplies declined, inhibition of G6PD would be removed and the rate of NADP reduction increased. Further, NADPH inhibition of G6PD is removed by GSSG (Jacob & Jandl, 1966).

Increased GR activity in G6PD deficiency has been linked to concentrations of NADPH below the GR K_m for NADPH (Gaetani et al, 1973). Changes in the cell membrane associated with early stages of peroxidation may modulate GR

activity. GR activity has been increased in vitro by incubation of lysate with stroma at 50°. It is possible that GR exists in an inactive, membrane-bound form and in an active soluble form. Such a situation exists for human erythrocyte glyceraldehyde-3-phosphate dehydrogenase (McDaniel & Kirtley, 1974).

The majority of variables associated with the GSH-Px system showed no response to exercise in the erythrocyte. While blood GSH increased, GSH is known to be restricted to the erythrocyte (Ray & Prescott, 1975). GSH/dl erythrocyte did not increase with exercise.

LACK OF EFFECT OF SELENIUM SUPPLEMENTATION ON THE RESPONSE OF THE EQUINE ERYTHROCYTE GLUTATHIONE SYSTEM AND PLASMA ENZYMES TO EXERCISE

INTRODUCTION. As mentioned in the previous study, Hartley and Grant (1961) and Hill (1963) have suggested that various exercise myopathies of the horse may be Se/E responsive. The previous study demonstrated that GR increased rapidly subsequent to exercise, while the other measured components of the glutathione peroxidase system were not altered. The present study was designed to determine 1) if exercise, like Se/E deficiency, might result in increased peroxidation and 2) if Se supplementation could alter this response.

MATERIALS AND METHODS

Experiment 1. Six adult Quarterhorses (ave. wt. 510kg) were run on a soft sand track an average of 2.2 km in 10 min. Heparinized venous blood samples were taken before (base line), immediately after, one hr after and 24 hrs after exercise.

Aliquots were deproteinized immediately for GSH determination (Beutler et al, 1963, as modified previously), lactate (Gutman & Wahlefeld, 1974) and pyruvate (Czok & Lamprecht, 1974). Hb and PCV were determined as before.

Erythrocytes and plasma were separated, washed and lysed as before. In addition an aliquot of erythrocytes was used for determination of malondialdehyde (MDA), an indicator of lipid peroxidation (Placer et al, 1966), after Mengel et al (1967).

The lysed erythrocytes were used for determination of G6PD (Kornberg & Horecker, 1955), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.49)(Horecker & Smyrniotis, 1955), and total and active GR, as described before. All activities were assayed at 37^{o} and expressed per g Hb.

Plasma was assayed for glutamic oxalacetic transaminase activity (GOT; EC 2.6.1.1)(Sigma Tech. Bull. No. 505, Sigma Chemical Co., St.Louis). Plasma Se was determined after Whetter and Ullrey (1978).

Experiment 2. Eight adult horses (4 Arabians and 4 Quarterhorses; ave. wt. 528 kg) were assigned to two dietary treatments. Both groups were fed on pasture, essentially ad libitum, plus 450 g/ day corn:oat concentrate (1:1, by volume). The concentrate was supplemented with 30g trace mineral salt daily. This amount of concentrate plus salt was readily consumed. The salt was either supplemented or unsupplemented with 60ppm Se as sodium selenite, calculated to provide 0.15ppm supplemental Se daily on a whole diet basis. Blood samples were taken before and after exercise, as in the previous experiment, at 0, 2 and 4 weeks on the dietary regimens. PCV, Hb, GSH, MDA and erythrocyte enzymes were determined as in Experiment

1. In addition, erythrocyte GSH-Px was determined (Hafeman et al, 1974).

Plasma GOT, creatine phosphokinase (CPK; EC 2.7.3.2) (Sigma Tech. Bull. No. 520), alkaline phosphotase (AP; EC 3.1.3.1) (Sigma Tech. Bull. No. 104) and lactate dehydrogenase (LDH; EC 1.1.1.28) (Sigma Tech. Bull. No. 500). Reticulocyte counts were made at week 4 (Ham, 1956). Plasma, forage, trace mineral salt, corn and oats were analyzed for Se (Whetter & Ullrey, 1978).

Statistical analysis. Data were analyzed by analysis of variance using a split plot design for Experiment 1 and a split-split plot design for Experiment 2 (Steel & Torrie, 1960).

RESULTS AND DISCUSSION. Data from the first experiment are presented in Table 2. Both Hb and PCV rose immediately after exercise, but returned to base line within 1 hr. This response was similar to the previous study and is consistent with splenic release of erythrocytes (Persson & Bergsten, 1976). MDA and plasma GOT showed a similar response, rising immediately after exercise and returning to base line within 1 hr.

Blood lactate and pyruvate were also highest immediately after exercise, returning to base line within 24 hrs. Similar increases have been reported previously (Milne et al, 1976). Erythrocyte G6PD and 6PGD activities did not exhibit any immediate effect of exercise; however, G6PD activity doubled between 1 and 24 hrs after exercise. Total and active GR rose immediately after exercise and

Table 2. Whole blood, erythrocyte (RBC) and plasma variables in exercised horses (Experiment 1).

Variable	Base line	Hours after exercise			SEM
		0	1	24	
Whole blood					
Hemoglobin (g/dl of blood)	13.4	17.3	13.1	14.0	0.5**
Hematocrit (%)	35.3	48.2	33.1	35.1	1.1**
Mean corpuscular hemoglobin concentration (%)	38.0	35.9	39.6	39.8	1.6^{ns}
Lactate (umol/ml)	0.8	3.4	1.6	0.8	0.3**
Pyruvate (umol/dl)	6.6	11.9	7.8	7.0	1.1**
RBC					
MDA (10A/ml)	7.6	14.9	8.3	7.8	1.5*
GSH (umol/ml)	1.4	1.5	1.9	1.8	0.1*
G6PD (IU/g Hb)	0.9	0.9	0.9	2.1	0.2**
6PGD (IU/g Hb)	0.3	0.3	0.3	0.3	0.04^{ns}
Total GR (IU/g Hb)	3.9	5.2	4.7	4.6	0.3*
Active GR (% of total)	103	100	100	98	2.3^{ns}
Plasma					
GOT (S-F U/ml)	165	184	164	NA	2**

Each value is the mean of 6 animals.
SEM = standard error of the mean; ^{ns} = no significant exercise effect ($P \geq 0.05$; * = $P<0.05$; ** = $P<0.01$).
NA = not available.

fell toward base line within 24 hrs, basically the same response as in the first equine study. However, in the present study, the percent active GR remained constant at about 100%. Plasma Se was found to be 0.16 ± 0.02 ug/ml. Stowe (1967) has reported very similar values for Se-adequate horse in Kentucky.

The data for the second experiment are presented in Table 3. None of the measured parameters showed significant effects of Se, weeks-fed-diet or Se x exercise interaction; therefore, values were pooled to demonstrate the exercise effects. Only GR showed a significant effect of weeks-fed-diet x exercise interaction (fig. 2). While GR activity was always elevated immediately after exercise, subsequent response varied by week of sampling. Active GR was essentially equal to total at all points during this experiment. The reason for the 15 to 30% increase in erythrocyte GR activity immediately after exercise remains unclear. However, when reticulocytes were determined at week 4, no increase was seen with exercise. This would suggest that the erythrocyte age profile, which might alter enzyme activity, was not altered with exercise.

No attempt was made to separate G6PD and 6PGD activities in this experiment. Pooled activity corresponded well with G6PD + 6PGD activities from Experiment 1; however, the increase in G6PD at 24 hrs was not seen.

Notably, erythrocyte GSH-Px, which requires Se for activity, showed no effect of supplemental Se, although a

Table 3. Whole blood, erythrocyte (RBC) and plasma values in exercised horses (Experiment 2).

Variable	Base line	Hours after exercise 0	1	24	SEM
Whole blood					
Hematocrit (%)	36.5	46.8	35.3	36.1	1.0**
Reticulocytes/10^3 RBC	7.9	7.9	10.9	9.3	1.4^{ns}
RBC					
MDA (10A/ml)	7.2	8.4	7.8	7.6	0.2^{ns}
GSH (umol/ml)	2.2	2.1	2.2	2.0	0.1^{ns}
G6PD + 6PGD (IU/g Hb)	1.7	1.4	1.3	1.3	0.2^{ns}
GSH-Px (U/mg Hb)	22.1	20.8	19.5	17.4	0.9**
Plasma					
AP (Bessey-Lowry U/ml)	5.0	5.6	5.1	5.1	0.1**
CPK (Sigma U/ml)	11.2	13.3	11.6	11.8	0.4*
GOT (Sigma-Frankel U/ml)	168	189	173	170	4**
LDH (Berger-Broida U/ml)	725	800	733	717	27**

Each value is the mean of 24 observations with the exception of reticulocytes which are means for 8 animals taken at week 4.
SEM = standard error of the mean; ^{ns} = not significant; * and ** indicate significance at the 0.05 and 0.01 levels, respectively.

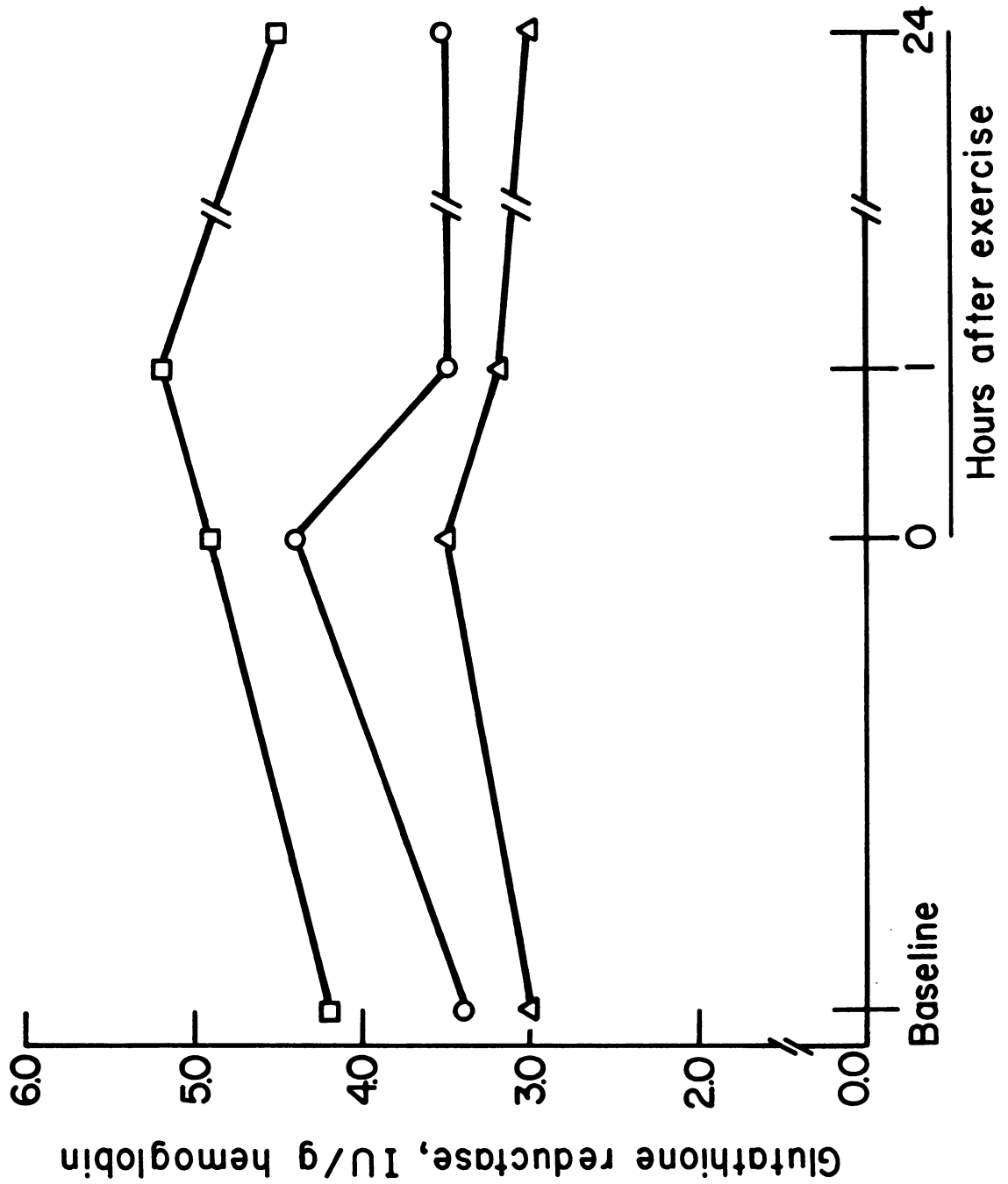

Figure 2. Erythrocyte glutathione reductase (active) as a function of time after exercise. Each point is the mean of 8 observations. o = initial values; ◘ = after 2 weeks fed diets; Δ = after 4 weeks fed diets.

slight decline in activity after exercise was noted. Further, plasma Se was not altered by Se supplementation of the diet. Mean (± SEM) plasma Se was 0.15 ± 0.004 ug/ml. These data again suggest that dietary Se was adequate prior to supplementation (Stowe, 1967). Michigan feeds are reported to be low in Se (Ku et al, 1972) and analysis of the feeds available to these animals would support this (corn, oats and forage contained 0.035, 0.025 and 0.030 ppm Se, respectively). Whether the horses were able to maintain adequate Se stores because of a unique feature of their physiology or because of an unidentified source of Se was available to them is unclear. Horses had been maintained in Michigan on essentially this diet for at least 12 months. While overt Se deficiency has not been unequivocally demonstrated in the horse, Stowe (1967) reduced plasma Se to 0.035 ppm on semi-purified diets on a torula yeast-based diet.

As in the previous study, plasma enzyme activity (AP, CPK, GOT and LDH) as well as erythrocyte MDA increased after exercise. Stowe (1967) has associated increased plasma GOT with subclinical Se deficiency in the horse. Further, Cardinet et al (1963) have shown a relationship between large increases in plasma CPK and the probability that a horse will "tie-up", a syndrome characterized by stiffness, difficulty of movement, swelling of legs and myoglobinuria. Increased plasma LDH, GOT and CPK have been reported in many species after exercise (Milne et al, 1976; Thompson,

1962; King et al, 1976), and with Se/E deficiency (Olson, 1974; VanVleet, 1975; Hayes et al, 1969; Whanger et al, 1977; 1977b). Increased plasma enzymes are associated with increased membrane permeability or destruction (Cardinet et al, 1963; Thompson, 1962).

Increased MDA has been reported with Se deficiency (Whanger et al, 1976; Noguchi et al, 1973; McCay et al, 1976) and is thought to reflect tissue peroxidation (Placer et al, 1966). If the basic process resulting in increased plasma enzymes and erythrocyte MDA with exercise was related to peroxidation of muscle and other tissues, supplementation of the diet with Se did not alter this response. Since Se supplementation did not result in increased plasma Se or erythrocyte GSH-Px, it appears that the horses in these experiments were adequate in Se, even though identified dietary sources were quite low in Se. The possibility remains for a different response to Se and exercise in Se-deficient animals.

SELENIUM, VITAMIN E AND THE RESPONSE TO SWIMMING STRESS IN THE RAT

INTRODUCTION. To this point, data have been presented which suggest that exercise does result in increased peroxidation, at least in the erythrocyte. Increased plasma enzymes might indicate damage to various tissue membranes. In the Se-adequate horse, Se supplementation does little to alter the response to exercise. The present study with the laboratory rat was undertaken to 1) determine if peroxidation could be measured in liver and muscle subsequent to exercise and 2) investigate this response in Se/E deficient- as well as adequate-animals.

MATERIALS AND METHODS

Experiment 1. Eighty male weanling Holtzman rats (The Holtzman Co., Madison, WI), ave. initial wt. 66 ± 1g (Mean ± SEM), were assigned to four torula yeast-based diets (Table 4): 1) unsupplemented with Se or E (-Se-E); 2) supplemented with 0.5 ppm Se as sodium selenite (+Se-E); 3) supplemented with 50 ppm E as DL-α-tocopheryl acetate (-Se+E); or 4) supplemented at the above levels with both Se and E (+Se+E). Diets and water were provided to individually housed rats for four weeks, a point just prior to the time when mortality might be expected on this diet

Table 4. Composition of basal Torula yeast-based diet.

Ingredient	Percent of diet
Torula yeast	30.0
Sucrose	55.7
Lard (Vitamin E-free)	5.0
Cod liver oil	3.0
Salt mix[1]	5.0
Vitamin mix[2]	1.0
DL-methionine	0.3
	100.0

[1]Salt mix (g/kg): $CaCO_3$, 543.0; $MgCO_3$, 25.0; $MgSO_4$, 16.0; NaCl, 69.0; KCl, 112.0; KH_2PO_4, 212.0; ferric ammonium citrate, 20.5; KI, 0.08; $MnSO_4$, 0.35; NaF, 1.0; $AlK(SO_4)_2 \cdot 12H_2O$, 0.17; $CuSO_4$, 0.9.

[2]Vitamin mix (per 100g): glucose monohydrate, 88.58 g; thiamin·HCl, 40 mg; riboflavin, 25 mg; pyridoxine·HCl, 20 mg; calcium-DL-pantothenate, 200 mg; choline chloride, 10 g; niacin, 1 g; menaquinone, 10 mg; folic acid, 20 mg; biotin, 10 mg; vitamin B_{12} triturate (0.1% B_{12} in mannitol), 100 mg; retinyl acetate, to provide 2500 IU/100g diet; ergocalciferol, to provide 200 IU/100g diet.

with this strain of rat (Hafeman et al, 1974). At the end of four weeks, the rats were assigned to three exercise groups: an unexercised control (base line), which was decapitated at the same time as exercised rats; a second group, exercised by swimming to exhaustion and killed immediately by decapitation; and a third group, exercised as before, but killed 24 hrs later. Rats were swum in groups of five to six, with 2% of their body weight in lead weight attached to their tails, until exhausted, a period of 10 to 15 min. All animals were killed within a three hr span of time on three consecutive days. Blood, liver and left hind limb were taken for analysis.

Experiment 2. Forty-eight male weanling Holtzman rats were assigned to diet and exercise groups as in the previous experiment. Only liver was taken in this study.

Blood. Heparinized blood samples were collected on decapitation (Experiment 1 only) and assayed for GSH. PCV was used to correct this value to an erythrocyte basis. Washed erythrocytes were prepared as in previous studies and assayed at 37^{o} for GSH-Px, G6PD, active and total GR activities as before. All activities were expressed per unit Hb.

Liver. Liver was removed, washed and homogenized in 0.15M KCl. Cytosol was prepared by centrifugation at 48,000 x g for 90 min. Hepatic GSH-Px, active GR (which is equal to total in this tissue) and G6PD activities were determined as above. In addition, "malic enzyme" (ME; EC 1.1.1.40) and NADP-isocitrate dehydrogenase (ICDH;

EC 1.1.1.42) activities were assayed (Frenkel et al, 1972). All hepatic enzyme activities were expressed per unit protein (Lowry et al, 1951).

Hepatic fat soluble antioxidant (FSAO) concentration was determined using an α-tocopherol standard (Glavind, 1963). FSAO was expressed as ug α-tocopherol equivalents per g liver, wet weight. Hepatic MDA was determined in the second experiment and expressed as a relative index of absorbance at 532nm per g liver, wet weight (Placer et al, 1966).

Muscle. Left hind limb was assayed for MDA, GSH-Px and active GR as above. As for hepatic GR, muscle GR showed complete saturation with FAD.

Statistical analysis. Data were analysed as a 2 x 2 x 3 factorial design with unequal replications. Linear correlations of the various enzymes were also run (Gill, 1978). The level required for statistical significance was set as $P<0.05$.

RESULTS AND DISCUSSION. Weight gain was not altered by diet. Animals gained approximately 95 g or an average of 3.8 g/day. While a longer period fed Se or E deficient diets might be expected to depress gain, a lack of dietary effect in a period as short as these experiments would be anticipated (Hafeman et al, 1974).

Erythocyte GSH concentration was not altered by diet. However, exercise resulted in a slight but significant decline, from 2.3 ± 0.2mM at base line to 2.0 ± 0.2mM at 0 and 24 hrs after exercise. This observation is in

contrast to the horse, where GSH is unchanged or slightly elevated subsequent to exercise. RBC enzymes are presented (fig. 3). Active and total GR activities fell subsequent to exercise. Since the percent active GR remained constant with exercise, only active GR is presented. G6PD activity also fell following exercise. GR and G6PD activities were directly correlated (table 5). A probable reason for the concomittant decline in activities of both enzymes could be change in the erythrocyte age profile toward older cells (Oski, 1970). G6PD activity is known to decline with increasing cell age. Such an age dependency has not been reported for GR. Neither of these enzymes showed a response to dietary Se or E.

RBC active GR as a percent of total was 95% or greater among -Se-E, +Se-E and -Se+E rats, but only 86% among rats fed the +Se+E diet. Although percent active GR does respond to G6PD activity (Flatz, 1970; Ajmar et al, 1972; Brady et al, 1978), the relationship of Se and E to FAD binding of this enzyme has not been previously investigated. Indeed, the mechanism for alteration in GR affinity for FAD is not known.

Erythrocyte GSH-Px activity did, of course, respond to dietary Se (but not to dietary E). Values were quite comparable to those reported for this strain of rat after four weeks fed this diet (Hafeman et al, 1974). Unlike erythrocyte GR and G6PD, erythrocyte GSH-Px activity was not affected by exercise. Equine GSH-Px may show a slight decline

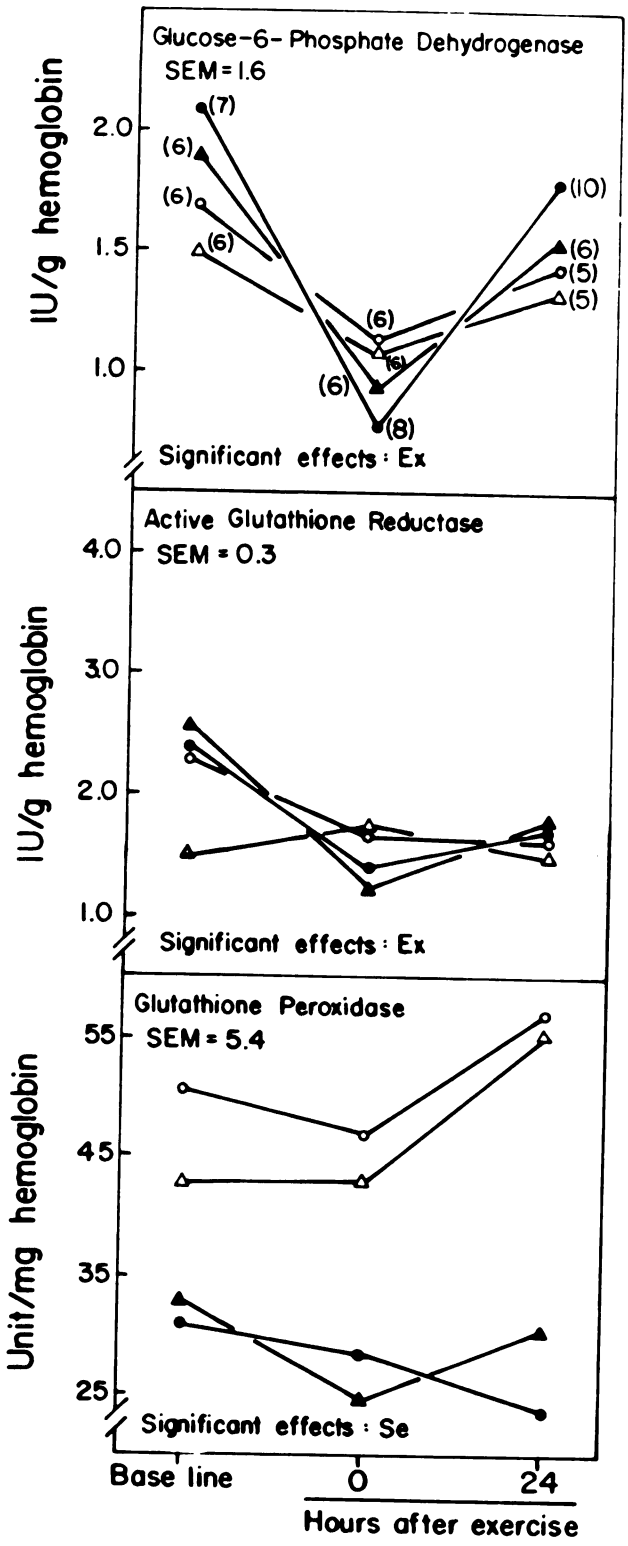

Figure 3. Erythrocyte enzymes as a function of time after exercise. Number per mean is given in parentheses: ● = -Se-E; ▲ = -Se+E; o = +Se-E; Δ = +Se+E.

after exercise.

To facilitate discussion of hepatic enzyme response, only data from the first experiment are presented (fig.4). However, the responses in both experiments were quite comparable. Since none of the enzymes showed dietary E or exercise effects, data were pooled to show the Se effects. Of the hepatic enzymes measured only ICDH did not respond to dietary Se. Although ICDH was slightly lower among Se-supplemented rats in Experiment 1, this effect was reversed in Experiment 2. In neither experiment was the difference statistically significant.

Hepatic GSH-Px activity was markedly depressed when Se was not added to the diet. At the same time, hepatic ME, G6PD and GR activities were reduced by addition of Se to the diet. In fact, GR exhibited a significant negative correlation with GSH-Px in both experiments (table 5). Still, a relatively large number of animals was necessary to demonstrate this relationship. The relationship of the NADP-linked dehydrogenases to GSH-Px or GR was not as easily defined. While G6PD correlated with GSH-Px in the first experiment, this relationship did not appear in the second (table 5). ME did not correlate with GSH-Px, but did show a relationship to GR in the second study. Stark et al (1975) have reported a high correlation of hepatic ME and GR activities among rats on certain dietary regimens. This relationship was variable in the present circumstance. Although not presented, G6PD, ME and ICDH activities were

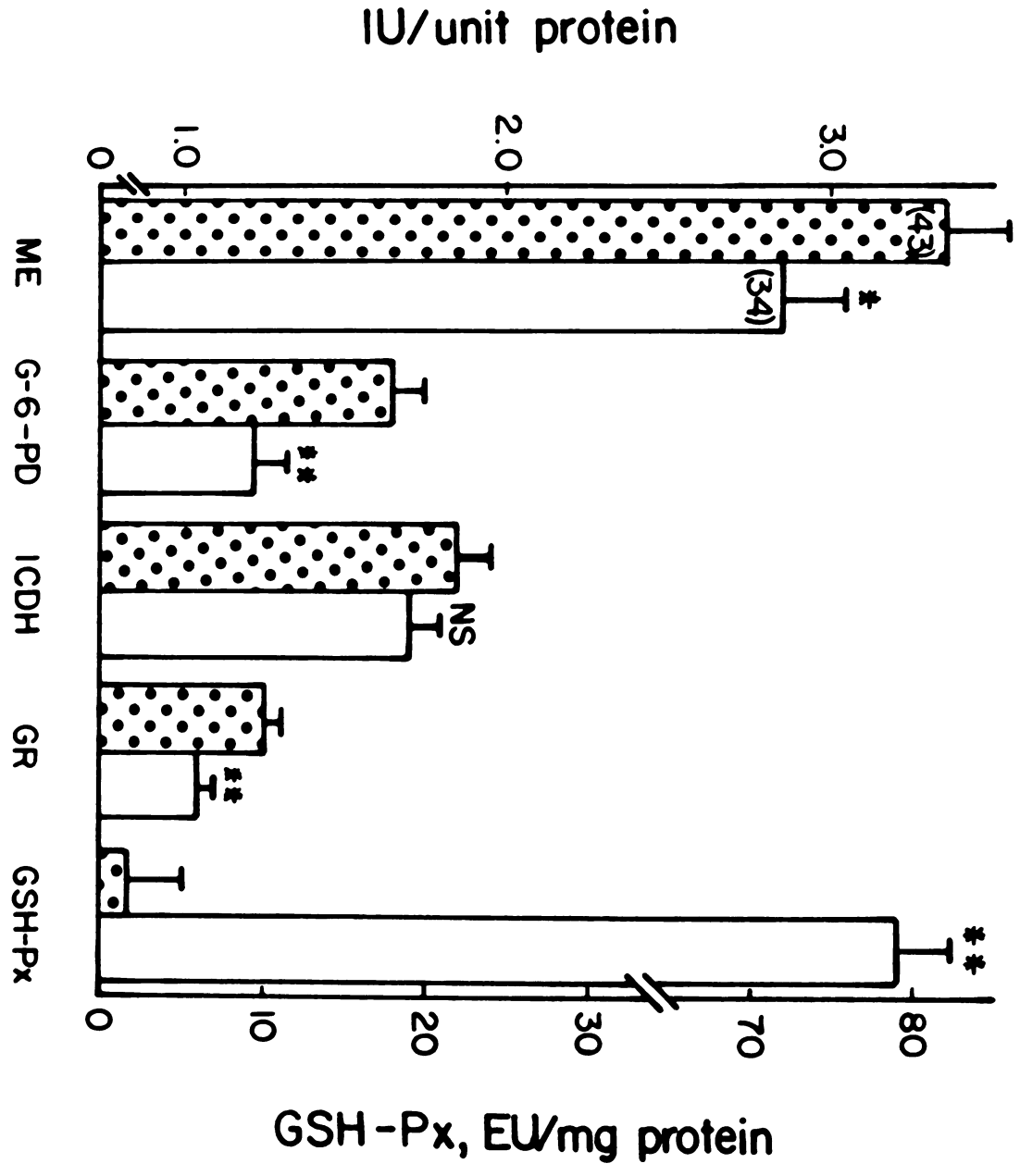

Figure 4. Hepatic enzyme activities. Number per mean is given in parentheses. Stippled bars represent -Se±E diets. The error bars are SEM. NS = no significant effect of dietary Se; * = $P<0.05$; ** = $P<0.01$.

Table 5. Significant linear correlations among rats fed diets supplemented and unsupplemented with Se and E.

Y	=	Slope	(X)	+	Intercept ;	r^1
Erythrocyte (Experiment 1) df=68[2]						
G6PD	=	0.79	GR	+	0.65	; 0.66**
Liver (Experiment 1) df=74						
G6PD	=	-21.0	GSH-Px	+	39.4	;-0.30**
GR	=	- 4.7	GSH-Px	+	77.0	;-0.34**
GR	=	-27.2	ME	+	314.5	;-0.02
Liver (Experiment 2) df=44						
G6PD	=	-1425	GSH-Px	+	2934	;-0.07
GR	=	-82.6	GSH-Px	+	1094	;-0.30*
GR	=	0.28	ME	-	0.42	; 0.43**

[1] r is the linear correlation coefficient; * and ** indicate significance at 0.05 and 0.01 levels, respectively.

[2] df indicates the degrees of freedom for the linear correlation.

all interrelated. While the response of GR, ME and G6PD to Se deficiency suggests an interrelation with GSH-Px activity, a simple linear correlation does not clarify this relationship. Hepatic FSAO concentration increased with dietary E supplementation (fig.5), but did not respond to dietary E or exercise. FSAO has been shown previously to reflect dietary E intake (Trostler et al, 1978).

Muscle GSH-Px activity was quite similar to the hepatic enzyme in its response to dietary Se, and its lack of response to dietary E and exercise. Activity was increased roughly 25-fold in response to Se supplementation (4.2 ± 5.0 versus 91.2 ± 5.0 U/100mg protein). GR in this tissue did not respond to diet or exercise. Mean (± SEM) activity was 1.7 ± 0.3 IU/100mg protein.

The MDA concentration or index has been used as a measure of lipid peroxidation (Placer et al, 1966). Hepatic (fig.6) and muscle (fig.7) MDA increased immediately after exercise. Hepatic values returned to base line within 24 hrs while muscle values did not. Dietary E reduced the response in liver but not in muscle. Dietary E also reduced the base line level of peroxidation. Dietary E had no effect on the base line level of MDA nor on the subsequent response to exercise. While Se supplementation would be expected to result in reduced levels of MDA (Noguchi et al, 1973; McCay et al, 1976; Whanger et al, 1976), the duration of the present studies may have been too short for this response to manifest itself. I had expected to induce mortality among

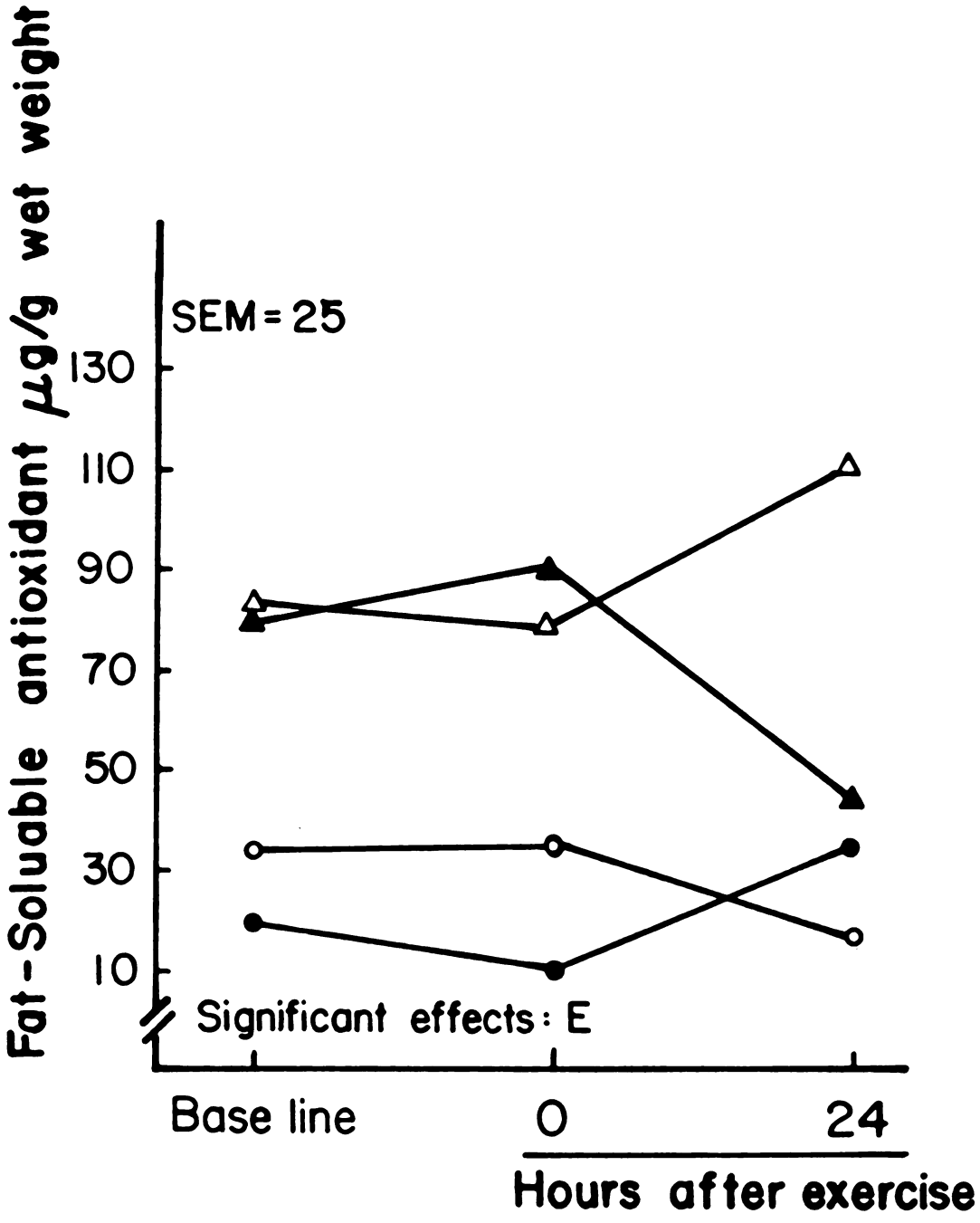

Figure 5. Hepatic fat soluble antioxidant concentration as a function of time after exercise. Numbers per mean are per Fig.3. ● = -Se-E; ▲ = -Se+E; o = +Se-E; Δ = +Se+E.

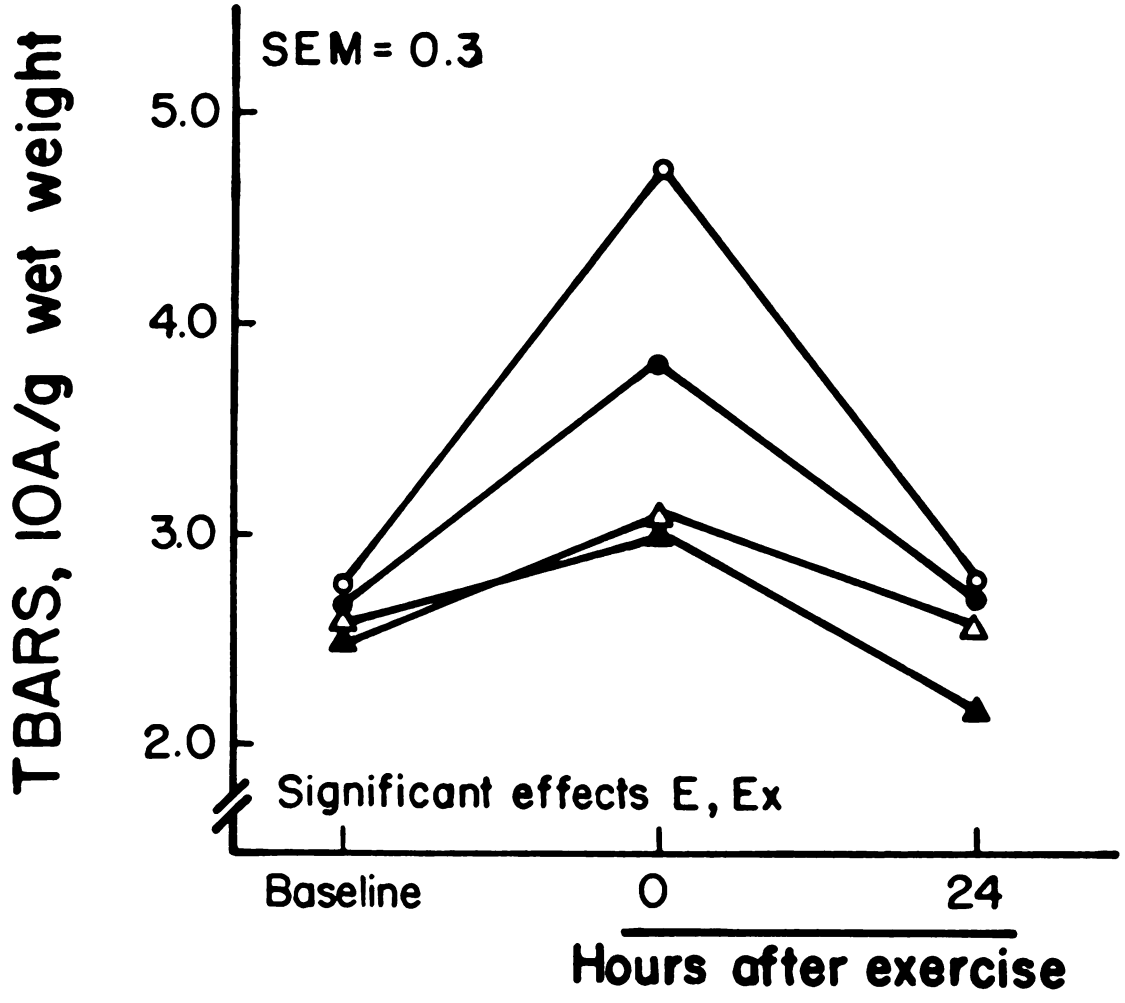

Figure 6. Hepatic TBARS (MDA) concentration as a function of time after exercise. Each value is the mean of 4 observations. ● = -Se-E; ▲ = -Se+E; o = +Se-E; Δ = +Se+E.

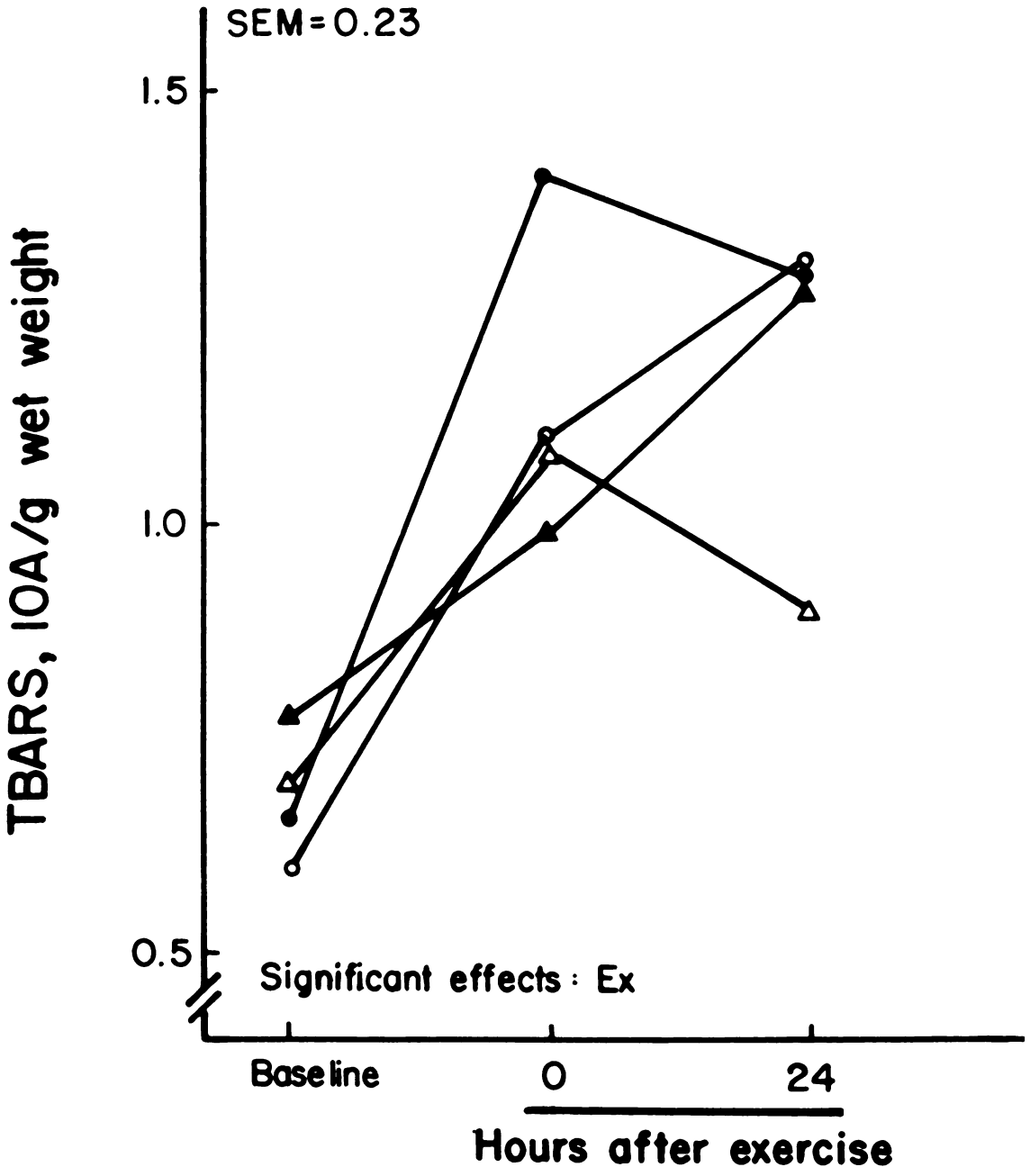

Figure 7. Muscle TBARS (MDA) concentration as a function time after exercise. Numbers per mean are per Fig.3. ● = -Se-E; ▲ = -Se+E; o = +Se-E; Δ = +Se+E.

Se/E deficient animals subsequent to exercise. However, no deaths occurred before, during or within 24 hrs of exercise among any of the dietary treatments in either experiment. At the same time, marked reduction in muscle, liver and erythrocyte GSH-Px activity on the Se deficient diets was observed; and depressed FSAO concentration in liver of E deficient animals was found. The animals of these experiments showed responses to Se deficiency similar to reported values for GSH-Px. The report of Hafeman et al (1974) also indicates no mortality with four weeks on the Se deficient diet. Clearly, there is more to the development of lesions and death from Se/E deficiency than the reduction of GSH-Px activity and E stores.

From the data collected in these experiments, it is unlikely that even extreme physical exertion will induce Se/E deficiency signs in a Se or E-supplemented animal. Even with a combined deficiency of Se and E lesions of deficiency were not produced with exercise. At the same time, peroxidation was apparently increased as a result of exercise. Thus, a relationship to Se and E nutriture is suggested, but exercise may induce lesions only when lesions are already an imminent concern. Then, the possibility exists for a different response with a more prolonged deficiency. It may also be of interest to determine the response to training of the exercise-induced peroxidation.

THE EFFECT OF DIETARY SELENIUM AND VITAMIN E ON BIOCHEMICAL PARAMETERS AND SURVIVAL OF YOUNG AMONG WHITE-TAILED DEER (Odoccileus virginianus)

INTRODUCTION. Up to this point the various studies have considered the effect of exercise on the response of rats and horse glutathione system parameters and peroxidation. While this study does not deal directly with the problem of exercise, the white-tailed deer is of some interest because deer have been reported to respond to physical stress with lesions of Se/E deficiency. This is particularly true of the young. Stuht et al (1971) have reported mortality among young fed a diet containing 0.2 ppm Se but not supplemented with E. The present study addresses the question of Se and E effects on biochemical parameters and reproduction in this species.

MATERIALS AND METHODS. Thirty-two adult female white-tailed deer were assigned to a complete pelleted diet (table 6), supplemented with 0 or 45 IU vitamin E (as DL-α-tocopheryl acetate), and 0 or 0.15 mg Se (as sodium selenite) per kg diet, for two years. The basal diet contained 0.04 ppm Se and 5.5 ppm E. Animals had been maintained

Table 6. Composition of basal (low Se/E) diet for white-tailed deer (Brady & Ullrey, 1975)[1].

Ingredient	Percent (%)
Corn cob product[2]	35.0
Corn grain	18.7
Soybean meal (49% crude protein)	23.95
Alfalfa meal (17% crude protein)	5.0
Cane molasses	5.0
Wheat, soft winter	10.0
Soybean oil	1.0
Limestone (38% calcium)	0.4
Trace mineral salt[3]	0.5
Vitamin A & D premix[4]	0.25
Calcium propionate	0.2
	100.0

[1]Contained 0.04 ppm Se and 5.5 ppm α-tocopherol (Horwitz, 1975) by analysis.

[2]Material remaining after hard cylinder of cob is removed. Cell wall constituents comprised 81.2%; acid detergent fiber, 37.5%; lignin, 6.5%, by weight. A product of The Anderson's, Maumee, OH.

[3]A product of Diamond Crystal Salt Co., Akron, OH.

[4]Supplied 3300 IU vitamin A as retinyl acetate and 220 IU ergocalciferol per kg of mixed diet.

in captivity throughout their lives. Prior to this study, deer were fed a pelleted diet identical to the +Se+E diet. Mean age of the female deer was 3.1 ± 0.5 years (mean ± SEM) at the start of the study. Deer averaged 59.3 ± 2.2 kg. The animals were individually housed in outdoor pens. Pen floors were of packed earth and individual shelters were provided. Diet and water were available ad libitum.

Animals were weighed, restrained using CI-744 (Parke-Davis Co., Ann Arbor, MI) and blood samples taken bimonthly for the first year and quarterly for the second. Deer were mated in the fall of both years and survival of the offspring followed to weaning at about 4 months of age. All deaths were evaluated clinically and histologically for evidence of white muscle disease (WMD), the primary lesion of Se/E deficiency reported for this species (Stuht et al, 1971). At weaning in the second year, twelve male young (three per treatment) and the surviving adult females were killed. Blood, liver and muscle were taken for analysis.

Blood. Blood samples were taken by jugular venipuncture using heparin as an anticoagulant. Hemoglobin (Crosby et al, 1954) and hematocrit were determined. Aliquots of whole blood were deproteinized immediately and transported on ice to the laboratory (3 to 5 hrs) for lactate (first year only)(Gutman & Wahlefeld, 1974), MDA (Hunter et al, 1963) and GSH (Beutler et al, 1963) determination. GSH was once again expressed per ml erythrocyte.

Erythrocytes were separated from plasma by

centrifugation. Washed erythrocytes were lysed and assayed for G6PD, total and active GR and GSH-Px activities as previously described. All activities were assayed at 37° and expressed per unit hemoglobin. Serum was frozen (-20°) and susequently analyzed for Se (Whetter & Ullrey, 1978) and α-tocopherol (Desai, 1968).

Tissues. Muscle and liver samples were taken for ME, ICDH, G6PD, GR and GSH-Px assay as described for the rat. MDA and FSAO were also determined.

In vitro hemolysis. Erythrocytes from the 12 month sample were used for time-dependent (Draper & Csallany, 1969) and hydrogen peroxide-dependent (Rotruck et al, 1971) hemolysis tests. Time-dependent hemolysis was carried out for 24 hrs, using erythrocytes from 3 deer per diet at 37°. Peroxide-dependent hemolysis was also performed at 37° using erythrocytes from 5 deer per treatment. Both tests were run with and without exogenous glucose (150 mg/dl, final concentration) to provide reducing equivalents needed for the function of the glutathione peroxidase system (Rotruck et al, 1972; Rotruck et al, 1971).

Statistical analysis. Data were analyzed as a 2 x 2 factorial design with unequal replication (Gill, 1978). Mortality data for the two years were pooled and analyzed by chi-square analysis (Steel & Torrie, 1960). Simple linear correlations for the relationship of Se and GSH-Px were determined (Steel & Torrie, 1960).

RESULTS. Both plasma Se (fig.8) and E (fig.9) had essentially stabilized within 10 to 12 months fed the respective diets. To facilitate discussion, only 0 and 12 month values are presented (table 7). Plasma Se, erythrocyte GSH-Px and blood lactate showed significant effects of dietary Se, while plasma E was the only measured blood variable to respond to dietary E. Neither GR nor G6PD in the erythrocyte showed a significant response to diet. Percent active GR was also unresponsive to diet.

Although blood MDA did not respond to diet, in vitro hemolysis was altered by dietary E (table 8). In the absence of glucose, dietary E reduced hemolysis 30 to 90%. In the presence of glucose, the E effect was lost for the time-dependent hemolysis. Glucose addition did not significantly reduce hemolysis. The hemolysis data are generally consistent with mortality data for the young (table 9). Mortality, and mortality with WMD lesions was reduced when the parental diet contained E, but did not vary in response to dietary Se. Even when adults were fed the +Se+E diet there was some mortality with WMD.

Blood variables for the offspring are presented (table 10) and are quite comparable to the adult values, showing significant effects of dietary Se on plasma Se and erythrocyte GSH-Px. Dietary Se did, however, result in reduced erythrocyte MDA among the young, unlike the adults. MDA and plasma E were responsive to dietary E in the young. Again,

Figure 8. Plasma selenium among white-tailed deer as a function of months fed diets. ○ = -Se±E; △ = +Se±E.

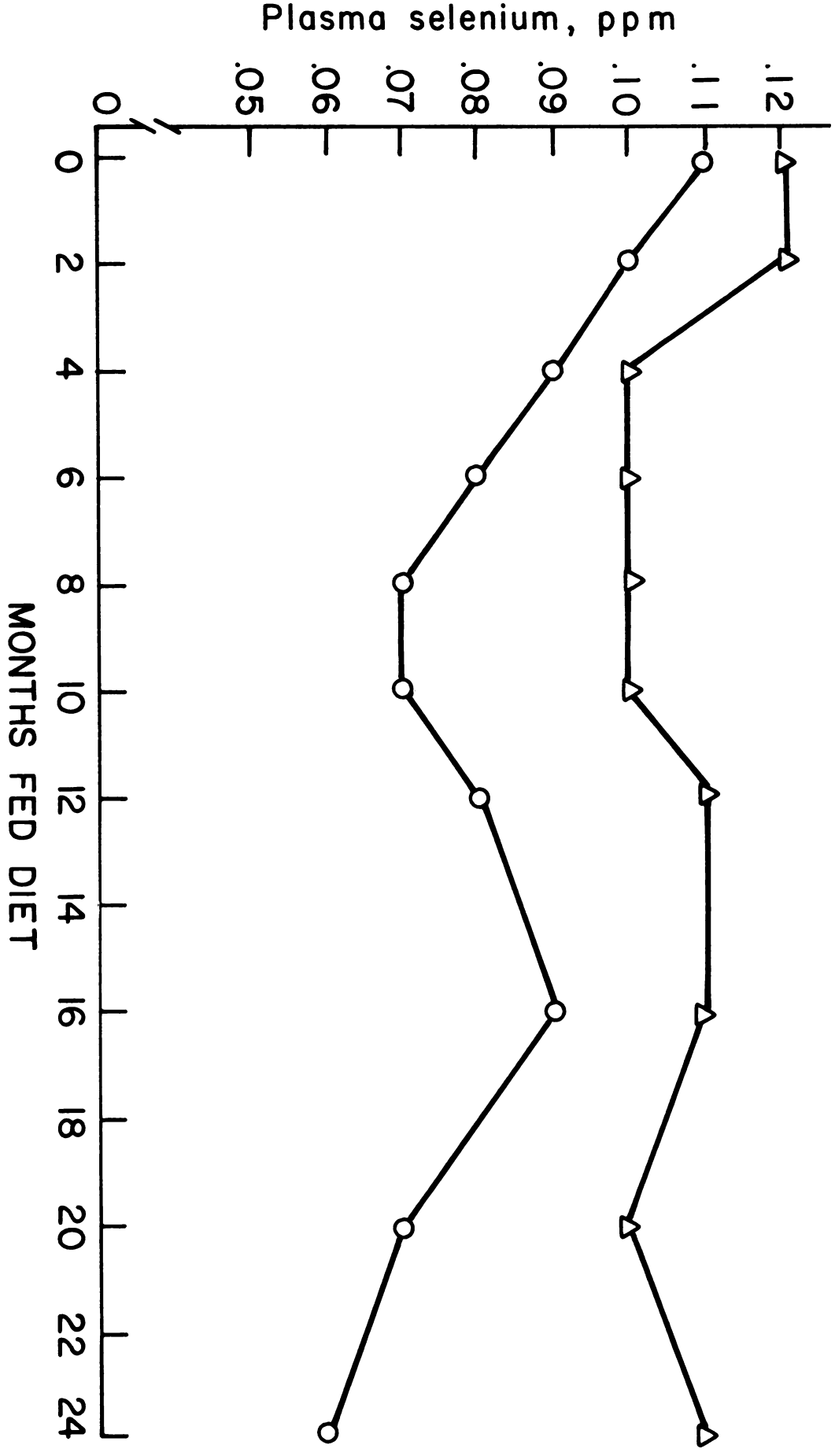
Plasma selenium, ppm
0
.05
.06
.07
.08
.09
.10
.11
.12
0
2
4
6
8
10
12
14
16
18
20
22
24
MONTHS FED DIET

Figure 9. Plasma α-tocopherol among white-tailed deer as a function of months fed diets. o = ±Se-E; Δ = ±Se+E.

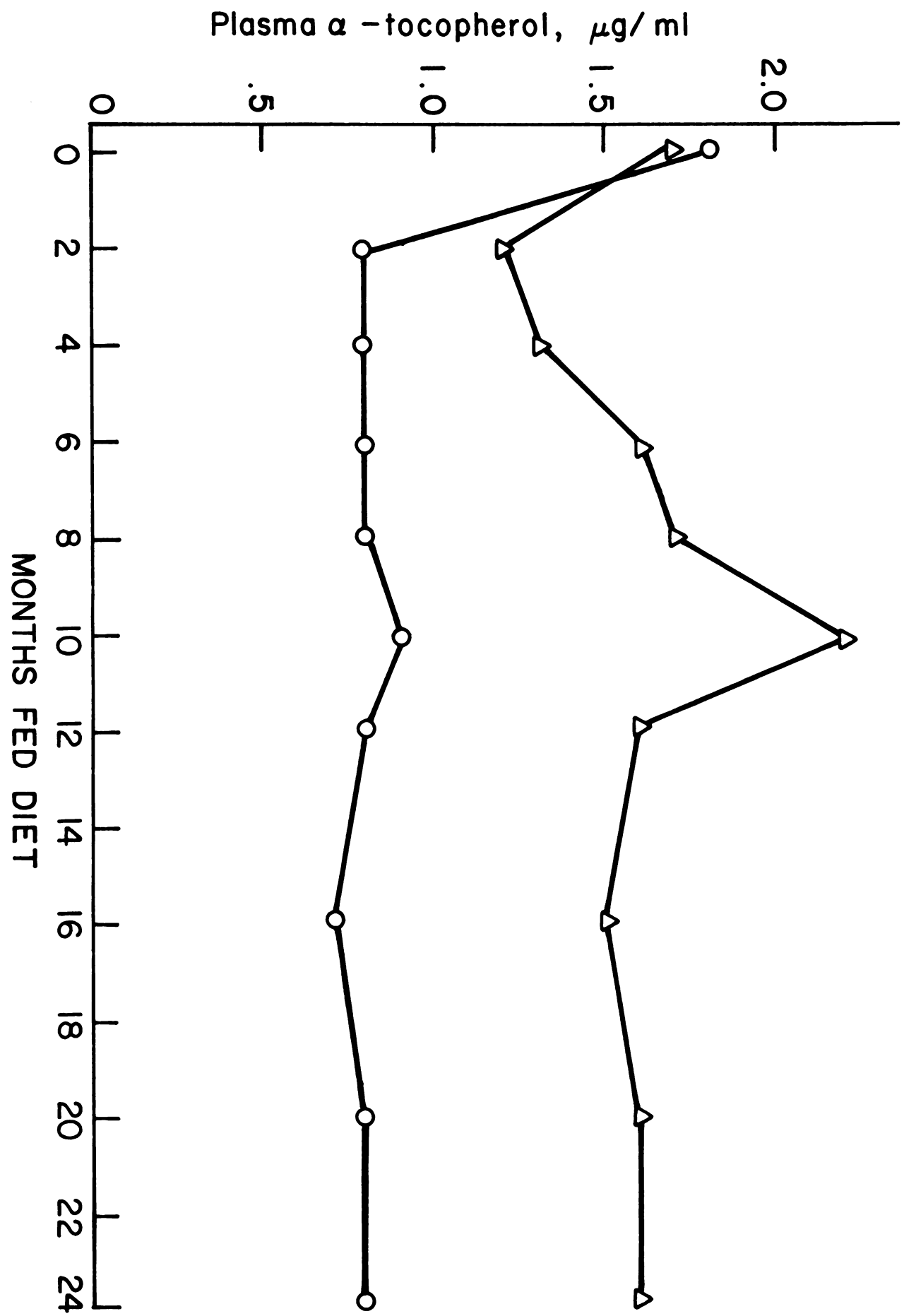
Plasma α -tocopherol, μg/ ml
0
.5
1.0
1.5
2.0
0
2
4
6
8
10
12
14
16
18
20
22
24
MONTHS FED DIET

Table 7. Initial and 12 month body weight and blood variables among adult female white-tailed deer fed diets supplemented and unsupplemented with Se and E.

Variable	Months fed diet	Diet				SEM[1]	Significant effects[2]
		-Se-E	+Se-E	-Se+E	+Se+E		
Body weight, kg	0	57.3[3]	60.4	59.0	60.0	2.2	ns
	12	60.1	61.9	60.6	61.5	5.1	ns
Plasma							
Selenium, ug/ml	0	0.11	0.12	0.12	0.12	0.005	ns
	12	0.07	0.10	0.07	0.11	0.005	Se
Vitamin E, ug/ml							
	0	1.81	1.83	1.58	1.80	0.12	ns
	12	1.03	0.90	2.10	2.20	0.13	E
Whole blood							
Lactate, mM	0	7.2	8.4	7.3	6.9	1.8	ns
	12	10.6	4.9	7.2	4.1	1.5	Se
MDA, 10A/ml	0	2.8	2.6	3.3	3.1	1.5	ns
	12	4.2	4.0	3.3	2.9	2.0	ns
Erythrocyte							
GSH, mM	0	0.24	0.21	0.22	0.25	0.08	ns
	12	0.21	0.15	0.16	0.14	0.03	ns
G6PD, IU/g Hb	0	2.4	2.3	2.0	2.4	0.2	ns
	12	2.2	2.2	2.1	2.4	0.2	ns
Active GR, IU/g Hb							
	0	2.1	1.8	2.0	2.4	0.3	ns
	12	2.8	2.2	2.2	3.0	0.2	ns
Percent active GR, %							
	0	92	87	88	94	4	ns
	12	84	86	84	83	3	ns
GSH-Px, U/mg Hb	0	42.0	38.4	36.5	35.6	3.1	ns
	12	15.3	30.4	15.6	30.2	2.3	Se

[1]Standard error of the mean.
[2]ns = no significant effect of diet (P>0.05); Se = significant effect of dietary Se; E = significant effect of dietary E.
[3]Each value is the mean of 8 deer at 0 months. At 12 months, -Se-E represents 8 deer; +Se-E, 7 deer; -Se+E, 7 deer; and +Se+E, 5 deer.

Table 8. In vitro hemolysis among Se and E supplemented and unsupplemented white-tailed deer after 12 months fed the respective diets.

Test	Diet				SEM[1]	Significant effects[2]
	-Se-E	+Se-E	-Se+E	+Se+E		
	% hemolysis					
Time-dependent (24 hr)[3]						
-Glucose	28	32	4	10	7	E
+Glucose[4]	20	23	6	4	8	ns
Hydrogen peroxide-dependent[5]						
-Glucose	32	33	3	3	7	E
+Glucose[4]	23	13	3	3	6	E

[1]Standard error of the mean.

[2]ns = no significant effect of diet (P>0.05); E = significant effect of dietary E.

[3]Each value is the mean of three deer.

[4]Glucose added to provide final concentration of 150 mg/dl.

[5]Each value is the mean of five deer.

Table 9. Pooled (first and second year) mortality data for offspring of Se and E supplemented and unsupplemented white-tailed deer.

Variable	Diet				Significant effects[1]
	-Se-E	+Se-E	-Se+E	+Se+E	
Young born	27	30	19	20	ns
Young surviving to weaning	9	10	9	11	ns
Young dying prior to weaning	18	20	10	9	E
Young dying with WMD lesions	16	16	5	4	E

[1]ns = no significant dietary effects (P>0.05); E = significant effect of dietary E by chi-square analysis.

Table 10. Body weight and blood variables among male offspring of female white-tailed deer fed diets supplemented and unsupplemented with Se and E, at weaning.

Variable	Parent's diet				SEM[1]	Significant effects[2]
	-Se-E	+Se-E	-Se+E	+Se+E		
Body weight, kg	21.2[3]	24.9	27.4	22.6	2.7	ns
Plasma						
Se, ug/ml	0.04	0.08	0.05	0.07	0.006	Se
E, ug/ml	0.34	0.34	0.88	0.83	0.12	E
Whole blood						
MDA, 10A/ml	3.1	1.2	0.9	1.3	0.3	Se, E, Se x E
Erythrocyte						
GSH, mM	0.56	0.70	0.62	0.45	0.07	ns
G6PD, IU/g Hb	2.3	2.3	2.2	2.5	0.5	ns
Active GR, IU/g Hb	2.1	2.4	2.2	2.3	0.4	ns
Percent active GR, %	90	89	86	94	4	ns
GSH-Px, U/mg Hb	17.8	35.7	27.7	38.3	3.5	Se

[1]Standard error of the mean.

[2]ns = no significant effect (P>0.05); E = significant effect of dietary E; Se = significant effect of dietary Se; Se x E = significant interaction.

[3]Each value is the mean of three deer.

GR, percent active GR and G6PD activities were not altered by diet.

Hepatic and muscle variables for adults (table 11) and young (table 12) are presented. The adults showed elevated hepatic FSAO concentrations with dietary E supplementation. This effect was lacking among the young. Dietary Se significantly affected hepatic GSH-Px activity, and hepatic and muscle Se concentration among adults and young. Muscle GSH-Px activity was significantly increased among the young of Se-supplemented adults. Se supplementation also resulted in reduced hepatic MDA levels among the young.

Hepatic and muscle G6PD, GR, ME and ICDH activities were not affected by diet. Measurable ME activity was found only in liver of young. ICDH activity was also too low to measure in muscle of both young and adults.

The relationship of tissue Se concentration and GSH-Px activity is presented for erythrocytes, liver and muscle (table 13). GSH-Px activity in all tissues showed a significant linear relationship to tissue Se concentration.

DISCUSSION. Initially, an interesting question is how wild species in general, and white-tailed deer, in particular, survive in a Se-deficient environment such as that of Michigan (Ku et al, 1972; Kubota et al, 1967). Clearly, on the basis of plasma, muscle and liver Se and erythrocyte, muscle and liver GSH-Px activity, white-tailed

Table 11. Hepatic and muscle variables among adult female white-tailed deer fed diets supplemented and unsupplemented with Se and E.

Variable	Parent's diet				SEM[1]	Significant effects[2]
	-Se-E	+Se-E	-Se+E	+Se+E		
Number of animals	8	7	7	5		
Liver						
Weight, kg	1.2	1.1	1.1	1.4	0.2	ns
MDA, U/g	4.0	3.6	3.5	3.4	0.3	ns
FSAO, ug/g	2.8	3.0	6.4	4.9	1.1	E
G6PD, IU/g protein	7.3	6.8	5.4	6.8	2.0	ns
ICDH, IU/g protein	111	115	130	131	2	ns
ME, IU/g protein	<0.5					
GR, IU/g protein	46	44	46	45	3	ns
GSH-Px, U/mg protein	2.8	10.7	2.9	16.1	1.8	Se
Se, ug/g	0.24	0.36	0.24	0.34	0.02	Se
Muscle						
G6PD, IU/g protein	1.8	1.8	1.6	1.3	0.5	ns
ICDH, IU/g protein	<0.5					
ME, IU/g protein	<0.5					
GR, IU/100mg protein	1.4	1.0	1.1	1.0	0.2	ns
GSH-Px, U/mg protein	6.1	6.4	4.4	5.9	0.8	ns
Se, ug/g	0.07	0.09	0.06	0.08	0.01	Se

[1]Standard error of the mean.
[2]ns = no significant effect of diet (P>0.05); Se and E = significant dietary effects of Se and E, respectively.

Table 12. Hepatic and muscle variables among male offspring of white-tailed deer fed diets supplemented and unsupplemented with Se and E.

Variable	Parent's diet				SEM[1]	Significant effects[2]
	-Se-E	+Se-E	-Se+E	+Se+E		
Liver						
Weight, g	539[3]	536	505	529	51	ns
MDA, U/g	1.7	0.7	2.1	0.6	0.5	Se
FSAO, ug/g	1.8	2.9	2.0	1.6	0.8	ns
G6PD, IU/g protein	0.5	1.1	0.5	1.2	0.5	ns
ICDH, IU/g protein	65	95	75	75	21	ns
ME, IU/g protein	0.8	2.4	2.3	1.4	0.2	ns
GR, IU/g protein	51	43	97	70	29	ns
GSH-Px, U/mg protein	2.0	11.2	7.2	13.0	0.6	Se
Se, ug/g	0.21	0.31	0.19	0.29	0.02	Se
Muscle						
G6PD, IU/g protein	2.5	1.8	2.0	2.0	1.3	ns
ICDH, IU/g protein	<0.5					
ME, IU/g protein	<0.5					
GR, IU/g protein	44	22	28	21	10	ns
GSH-Px, U/mg protein	1.4	6.4	2.0	6.1	1.7	Se
Se, ug/g	0.05	0.07	0.05	0.07	0.004	Se

[1]Standard error of the mean.
[2]ns = no significant diet effect (P>0.05); Se = significant effect of dietary Se.
[3]Each value is the mean of three deer.

Table 13. Linear relationships of Se concentration (ug/ml plasma or ug/g tissue) and GSH-Px activity (U/mg Hb or U/mg protein), where GSH-Px = bSe + a, among white-tailed deer adults and offspring.

	a	b	r[1]
Adult			
Erythrocyte	-1.8	287.2	0.71**
Liver	-13.5	71.0	0.72**
Muscle	1.1	63.0	0.51**
Young			
Erythrocyte	2.0	464.0	0.92**
Liver	-8.2	66.4	0.82**
Muscle	-9.7	227.5	0.95**

[1]r is the linear correlation; ** designates a highly significant ($P<0.01$) linear correlation.

deer fed on a defined diet were susceptible to Se depletion. Equally clearly, Se depletion, per se, did not result in any untoward effects. There was no evidence of increased lipid peroxidation, as reflected by MDA and in vitro hemolysis, nor was there increased preweaning mortality of young.

At the same time, others have reported that GSH-Px activity does reflect Se status (Hafeman et al, 1974; Oh et al, 1976; Reddy & Tappel, 1974; Chow & Tappel, 1974), that Se deficiency will result in increased erythrocyte hemolysis (Rotruck et al, 1972b), and that increased lipid peroxidation, as reflected by increased MDA, is a sequel to Se deficiency (Noguchi et el, 1973).

Although GSH-Px activity in liver, muscle and erythrocytes did parallel Se concentration, other gross and metabolic responses were lacking among the adult deer fed the deficient diets for 2 years. Although little is known of the specific requirement of deer for Se, the requirement for domestic ruminants is now thought to be in excess of 0.1 ppm (Oh et al, 1976; 1976b; Whanger et al, 1977; Ullrey et al, 1977). The 0.04 ppm provided by the -Se diets is typical of values for forage and grains from most areas of Michigan (Oldfield et al, 1971; Muth & Allaway, 1963; Ullrey, 1974).

Blood and liver MDA was also unresponsive to dietary E. Again, others have shown this (Noguchi et al, 1973) and other parameters of lipid peroxidation (Dillard et al, 1977) to increase with E deficiency. The prolonged period (3 to 5 hrs) before analysis may have led to increased variability

of MDA values with subsequent loss of sensitivity for this indicator of peroxidation. In vitro hemolysis, hepatic FSAO and plasma E among adults, and plasma E and mortality among the young were responsive to dietary E. Hemolysis (Draper & Csallany, 1969), hepatic FSAO (Trostler et al, 1977) and plasma E (Hayes et al, 1969; Machlin et al, 1977) responses to dietary E have been reported previously for other species.

Although evidence of dietary influence on tissue Se and E was demonstrated among adults, the only mortality resulted from trauma during and after handling. This was not true of the young. Mortality was E-related among the young. However, even when Se and E were included in the parents' diet, some mortality with lesions of WMD was found. It is uncertain why this is so. Others have reported mortality in zebra from acidosis with lesions identical to WMD (Harthoorn & Young, 1974). The WMD lesions are, then, not pathognomonic for Se/E deficiency but represent the end product of several means of muscle destruction. But blood lactate was high among the adults when compared to other species (Beutler, 1975; Harthoorn & Young, 1974; Brady et al, 1978; Harvey & Kaneko, 1975). Lactate was further elevated among deer fed the low Se diets after 12 months. Thus, there apparently is some relationship between acidosis and Se.

Mortality, even from +Se+E parents ran as high as 50%, rising to 67% when E was not present in the diet. Other

sources of mortality included parasitism, congenital defect, nephritis, heat stress, and unknown causes. While mortality was significantly lower when E was included in the parents' diet, tissue variables among the young contribute little toward an explanation of the observed effect. Plasma E and blood MDA responded to the parents' diet, hepatic FSAO and MDA did not. It is certainly possible that the surviving fawns were those least susceptible to E (and Se) deficiency. Biochemical parameters were not determined for young dying prior to weaning.

From these data, it appears that dietary E is important for survival of young, at least to weaning. E did not appear important to the survival of adults. Dietary Se had no measurable influence on survival of young or adults during the course of this study. A "non-Se glutathione peroxidase" has been described (Lawrence & Burk, 1976; Prohaska & Ganther, 1976) and identified as glutathione-S-transferase (EC 2.5.1.18)(Prohaska & Ganther, 1977) in the rat. Non-Se GSH-Px has also been described in other species (Lawrence & Burk, 1977). While it is possible that Se-dependent GSH-Px is not critical to survival of deer, it remains uncertain if any GSH-Px activity is needed, since non-Se dependent activity was not measured.

A number of other comments may be of comparative interest. While G6PD and the hexose monophosphate shunt is the only source of NADPH for GR in the erythrocyte (Beutler, 1975), a number of NADP-dependent dehydrogenases

are available in liver and other tissues. These would include ME and ICDH, as well as G6PD. ME activity was essentially absent from muscle of adults and young, and liver of adults. Hanson and Ballard (1968) have also reported low hepatic ME activity in the adult bovine. Hepatic ICDH is also quite high in the bovine, much as observed for the deer. G6PD was the only measurable dehydrogenase in muscle of adults and young.

There were some differences between the young and adults. Young tended to have lower hepatic G6PD and ICDH activities than the adults, but measurable ME. Otherwise hepatic, muscle and erythrocyte enzyme activities were comparable.

Erythrocyte GSH was low among young and adult deer when compared to the 1 to 2 mM reported for other species (Beutler, 1975; Harvey & Kaneko, 1975; Reid et al, 1948; Agar & Stephens, 1975). The young had higher GSH concentrations than the adults. Also, erythrocyte percent active GR was on the order of 85% among adults at 12 months. Less than 83% would be considered indicative of a riboflavin deficiency in man and rat (Sauberlich et al, 1972; Glatzle et al, 1970). We do not have percent active GR values for other ruminants. Thus, these low values could be characteristic of ruminants in general or a transient effect of handling stress in white-tailed deer, much like the shift observed in the first horse study.

CONCLUSIONS

In the initial studies with the horse, it was demonstrated the erythrocyte MDA, an indicator of lipid peroxidation, increases subsequent to exercise. This increase is accompanied by elevations of a number of plasma enzymes. These data suggest that exercise results in 1) an increased level of in vivo lipid peroxidation and 2) an increase in permeability of muscle membrane. Addition of Se to a diet apparently adequate in Se did nothing to alter these responses. The effects of dietary E were not investigated.

The subsequent work with rats indicated that exercise also yields increased lipid peroxidation in liver and muscle, a response which again was independent of dietary Se (even where Se deficiency was pronounced) and only slightly altered by dietary E.

On the basis of these studies, it seems likely that exercise could exacerbate Se/E deficiency, if an animal were on the borderline of deficiency to begin with. No support is lent to the suggestion that Se- or E-adequate animals could spontaneously develop lesions of deficiency subsequent to strenuous exercise. The species employed may not have provided adequate models for this problem, however.

Exercise-induced myopathies have not been reported in the rat. Such myopathies have been reported for the horse, and, indeed, linked to Se deficiency. However, controlled data are lacking.

The final study with white-tailed deer, a "sensitive species", was not in any sense an exercise study. It is a difficult problem to obtain resting samples from deer. Still, as a first step, a knowledge of Se/E contributions to the survival of this species would be of interest.

Its GSH-Px activity (erythrocyte, liver or muscle; young or adult) is quite low, even on Se-supplemented diets. However, dietary Se had no effect on subsequent mortality of the young. E, on the other hand, did alter the mortality of the young. The one clearly documented instance of stress-induced mortality in this species followed accidental omission of E from the diet (Stuht et al, 1971).

This suggests a possible relationship to stress-induced lesions in swine, a species not investigated in this series of studies. E is a relatively expensive ingredient and it is generally kept to a minimum in commercial diets. Further, with the legalization of Se supplementation of swine diets and the known sparing effect of Se on E, there may be a tendency to reduce the dietary E concentration. It is possible that the reports of stress-induced lesions result when swine, particularly young swine, are fed diets low in E and subjected to some physical stress, such as

comingling of litters.

Certainly, a great deal of work is yet to be done to confirm this possibility. The data presented herein merely strengthen the likelihood of a relationship of exercise to increased lipid peroxidation, a property shared with Se/E deficiency.

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