AN ABSTRACT OF THE THESIS OF

Charles Chase Hoffman, Jr. for the degree of Master of Science

in Animal Science (Physiology) presented on 9 August 1977

Title: Effects of Intramuscular Administration of Selenium in Dairy Cattle on Levels of Erythrocyte Glutathione Peroxidase and Blood Selenium

Abstract approved: Redacted for Privacy

Lloyd Swanson

Three experiments were designed to examine the response of red blood cell (RBC) glutathione peroxidase (GSH-Px) to a single injection of selenium and vitamin E and to determine the value of this enzyme as an indicator of both pre- and post-treatment selenium status in dairy cattle. In Experiment 1, five Holstein heifers were injected intramuscularly with 5 mg selenium equivalent from sodium selenite plus 68 IU vitamin E per 60 kg of body weight and five heifers were left untreated. Blood samples, taken at two week intervals for 14 weeks, were assayed for RBC GSH-Px activity to determine if a single injection of selenium and vitamin E would alter the levels of GSH-Px activity as enzyme units (e.u.)/mg hemoglobin (Hb). In Experiment 2, ten heifers were divided into two groups and received the same treatments as in Experiment 1. Blood samples, drawn at ten day intervals for 30 days, were assayed for both RBC GSH-Px activity and whole blood selenium. Heifers in both experiments had been maintained on an all-forage, low-selenium

(<.02 ppm Se) ration and had relatively low initial levels of RBC GSH-Px activity. In Experiment 1, GSH-Px activity of the treated heifers increased 16% (P<.05), reaching 23.0 ± 2.6 e.u./mg Hb four weeks post-treatment. Meanwhile, enzyme levels for the untreated heifers had decreased 10%. The decline in activity of the untreated heifers was linear (P<.01) over the 14 week observation period. Enzyme activity had returned to a level equivalent to that of the untreated heifers by 12 to 14 weeks. In Experiment 2, RBC GSH-Px activity of treated heifers increased (P<.01) more dramatically, reaching 167% (24.4 ± 3.0 e.u./mg Hb) of initial levels by 30 days post-treatment while whole blood selenium concentrations rose (P<.05) from .016 µg/ml to a plateau of .043 µg/ml from day 10 through 30.

In Experiment 3, 17 parous, nonlactating cows were randomly assigned to one of three treatments as they reached 24 days prepartum (day 259 of gestation). Treatments consisted of an intramuscular injection of 5 mg selenium equivalent from sodium selenite and 68 IU vitamin E per 60 kg body weight, 5 mg selenium and 68 IU vitamin E per 91 kg body weight, or 4 ml vitamins AD&E (2,000,000 IU vitamin A, 300,000 IU vitamin $D_3$, and 200 IU vitamin E). Prior to treatment these cows had been on the same all-forage, low-selenium ration as the heifers for 36.7 ± 6.0 days. Blood samples, taken over a 40 day interval beginning on day 259 ± 1 of gestation, were assayed for RBC GSH-Px activity and for whole blood and plasma selenium levels. No change was observed in any of these characteristics (which averaged 43.9 ± 2.0 e.u./mg Hb, .068 ± .006 µg Se/ml, and .018 ± .003 µg Se/ml, respectively, on day of treatment) during the 40 days post-treatment.

It appears that the difference in response to selenium and vitamin E administration between the heifers of Experiments 1 and 2 and the cows in Experiment 3 was related to pre-treatment levels of RBC GSH-Px activity and blood selenium. While lactating, the cows had been fed a ration containing 5 to 10 kg mixed grain concentrate daily. Relative to the forage (<.02 ppm), this concentrate was high in selenium (.3 ppm). This probably accounts for the higher pre-treatment levels ($P<.01$) of RBC GSH-Px and whole blood selenium for the cows as compared to the heifers (43.9 ± 2.0 vs 14.6 ± 2.3 e.u./mg Hb and .068 ± .006 vs .016 ± .003 µg/ml, respectively).

A regression of pre-treatment levels of RBC GSH-Px activity on whole blood selenium for the cows and heifers in this study was highly significant ($R^2 = .75$). Thus, even though the cows had been on the low-selenium diet for variable periods of time (20 to 108 days), enzyme activity was still a reliable index of the relative pre-treatment selenium status of all the cattle in this experiment. For the heifers, it was also useful as a means of measuring the response to a selenium injection.

# Effects of Intramuscular Administration of Selenium in Dairy Cattle on Levels of Erythrocyte Glutathione Peroxidase and Blood Selenium

by

Charles Chase Hoffman, Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed 9 August 1977

Commencement June, 1978

APPROVED:

Redacted for Privacy

Associate Professor of Animal Science

Redacted for Privacy

Head of Department of Animal Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 9 August 1977

Typed by Kristyanne Sprinker for Charles Chase Hoffman, Jr.

# ACKNOWLEDGMENTS

I wish to acknowledge the Oregon Dairy Farmers (through the Oregon Dairy Products Commission) for funding my assistantship and the Department of Animal Science and the Oregon Agricultural Experiment Station for providing the cattle, the facilities, and funds necessary for my research. In addition, I wish to thank the personnel of the OSU Department of Agricultural Chemistry for their assistance and for the use of their laboratory facilities. My special thanks are extended to Ray Morris and his crew at the OSU Dairy for their assistance and cooperation.

To my advisor, Dr. Lloyd Swanson, for his support and guidance over the course of these studies, to my colleagues, Lindsay, Bill, and KBK, for their contributions to my sanity, to Kristy for her time and help, and to Ingrid and Heidi for their love I offer my sincere gratitude.

TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

# Effects of Intramuscular Administration of Selenium in Dairy Cattle on Levels of Erythrocyte Glutathione Peroxidase and Blood Selenium

## I. INTRODUCTION

In cattle, selenium (Se) deficiencies have been associated with various selenium-responsive diseases including nutritional muscular dystrophy (NMD, also known as "white muscle disease"), depressed growth rates, and possibly diarrhea (Oldfield, 1974). Lowered reproductive performance in sheep has been related to selenium deficiencies (Oldfield, 1974), and many veterinary practitioners also consider this to be the case in cattle.

Recently, selenium has also been reported to be a useful prophylaxis against retained placentas in dairy cows. A placenta which has not been expelled within 12 hr of parturition is considered to be retained. In a dairy herd located in an area with a history of NMD, Trinder (1969) observed that prepartum intramuscular (im) injection of 15 mg selenium from sodium selenate and 680 IU vitamin E reduced the incidence of retained placentas from 42% to 0%. Julian et al. (1976a) reported similar success in preventing retained placentas in dairy cows. Prepartum administration of selenium and vitamin E eliminated the incidence of retained placentas compared to an incidence of 50% and 20% in untreated cows fed rations containing 8.5% crude protein (CP) and .02 ppm SE or 15% CP and .06 ppm Se, respectively. They found intramuscular injection (50 mg selenium from sodium selenite and 680 IU vitamin E) 20 days prepartum or oral administration of selenium (12.5 mg sodium selenite daily for five consecutive days beginning 60 days

prepartum and then once weekly until parturition) to be equally effective in preventing retained placentas. Julian et al. observed that injections on day 20 prepartum raised plasma selenium from .025 to .065 µg/ml on day 10 prepartum and to .085 µg/ml on the day of parturition. Possibly, a portion of this increase in plasma selenium may be attributable to the higher selenium content of the 15% CP rations received by one-half of the injected cows. In a field study on four dairy farms, Julian et al. (1976b) found that prepartum injection of 50 mg selenium and 680 IU vitamin E reduced the incidence of retained placenta from an average of 51% to 9%. They reported that a single injection on day 20 prepartum was as effective as injections on days 40 and 20 prepartum. Further study by Trinder et al. (1973) indicated that injections of selenium and vitamin E must be given within four weeks prepartum to be an effective prophylaxis against retained placenta. Trinder et al. also observed that selenium and vitamin E together were more effective as a prophylactic than selenium alone. These two observations may be a consequence of the low dosage of selenium used by Trinder relative to that of Julian et al. (1976) and the dosage (5 mg Se and 681 IU vitamin E) recommended by Burns-Biotech, sole U.S. manufacturers of injectable selenium products. Thus, in situations where supplemental selenium is to be administered parenterally, features of selenium uptake and metabolism become important to determine the timing of injections necessary for obtaining optimal results.

As described by McConnell (1963), the disappearance of selenium from whole blood, plasma, and red blood cells (RBC's) is a component rate function. Following parenteral administration in rats, $^{75}Se$

initially appears in greatest amounts in the plasma (McConnell, 1941). However, it is removed from the plasma much faster than from the RBC's leaving the concentration of selenium in RBC's elevated above basal levels 24 hr post-treatment. In dogs, RBC's account for most of the increased activity of whole blood after $^{75}Se$ administration (McConnell and Cooper, 1950). They found selenium to be associated with hemoglobin--six to eight times as much with the heme as with the globin. They also found that $^{75}Se$ activity in the bone marrow does not increase following injection, indicating that selenium fixation does not occur there. The magnitude of *in vitro* $^{75}Se$ uptake by ovine RBC's was found by Wright and Bell (1963) to be inversely proportional to the level of dietary selenium intake. In the absence of oxygen, uptake of $^{75}Se$ as selenite was depressed. Thus, they asserted that the selenium transport mechanism is active and requires active respiratory processes. Weswig *et al.* (1965) observed that significant differences of *in vitro* uptake of $^{75}Se$ in ovine whole blood could be detected by 30 days after the sheep had been on a low-selenium dietary regime. This suggests that the patterns of metabolism and excretion of selenium are not like those of iron, in which iron is re-utilized by re-incorporation into new RBC's. McConnell (1963) found that, following a single injection of $^{75}Se$, the rate of $^{75}Se$ disappearance from RBC's in dogs was greatest at 100-120 days. This would indicate that selenium, once incorporated into the RBC, remains throughout the life span of the cell.

In a review, Ammerman and Miller (1975) consider organically bound Se to be the superior source of supplemental selenium for ruminants. It is the most biologically available form and also the least toxic.

Water soluble selenium, both as selenate and selenite, has also been effective in preventing Se deficiency symptoms. However, selenite may be less stable in supplementary feed than selenate. Selenium in plants is mostly organically bound and water soluble and is not present as either selenate or selenite. Jenkins and Hidiroglou (1972) observed that Se-deficient animals retain more Se from feed than those previously fed normal or excessive amounts of selenium in their diets. There is a rapid urinary excretion of inorganic selenium by Se-sufficient animals.

From studies in rats, Rotruck et al. (1973) found that selenium functions as an integral part of the enzyme glutathione peroxidase (GSH-Px). This was substantiated in bovine blood by Flohe et al. (1973), who determined that GSH-Px contains four gram atoms of selenium per mole. Oh et al. (1974) observed that this enzyme accounts for 75% of the selenium found in ovine erythrocytes.

According to a review by Tappel (1974) GSH-Px is present in the cytosol and functions as an antioxidant which serves to spare vitamin E by reducing peroxides. This action decreases the potential number of free radicals which can be created from the polyunsaturated fatty acids of the phospholipid cell membrane. In contrast, vitamin E is an integral part of the plasmalemma and "intercepts" free radicals, preventing further damage to cell walls. This interrelationship makes it difficult to accurately determine the requirements for selenium and vitamin E. Neither vitamin E nor selenium alone have completely prevented NMD but small deficiencies in one or the other may be compensated for by additional selenium or vitamin E (Ammerman and Miller, 1975; Jenkins et al. 1974).

Using data from a four month feeding trial with dairy cattle, Allen et al. (1975) found RBC GSH-Px to be significantly correlated with whole blood selenium ($R^2$ = .92). Allen also found increases in both the selenium concentration of whole blood and RBC GSH-Px activity following four monthly injections of selenium and vitamin E (the dose was not reported). Boyd (1975), Thompson et al. (1976), and Wilson and Judson (1976) also found a significant relationship between whole blood selenium and RBC GSH-Px in cattle. Thus, measurement of glutathione peroxidase appears to be a useful means of monitoring the selenium status of the bovine.

Three experiments were designed to examine the response of RBC GSH-Px to a single injection of selenium and vitamin E and to determine the value of this enzyme as an indicator of both pre- and post-treatment selenium status in heifers and cows. Experiment 1 was undertaken using dairy heifers with relatively low initial levels of RBC GSH-Px to determine if a single intramuscular injection of selenium and vitamin E would cause a measurable change in activity levels of this enzyme, and the time course any such change would follow. In Experiment 2, levels of both whole blood selenium and RBC GSH-Px activity were quantified following administration of selenium and vitamin E to define the value of the enzyme as an indicator of both the initial and the post-injection selenium status of the heifers. Finally, Experiment 3 was designed to determine if selenium injections (three to four weeks prepartum) of non-lactating cows with adequate blood levels of selenium (>.05 μg/ml whole blood) would cause a detectable change in RBC GSH-Px activity or blood selenium levels. In addition to whole blood, plasma was also assayed

for selenium content to determine if plasma selenium levels, uninfluenced by the compound effect of RBC life span, could be a more sensitive index of an animal's selenium status. In conjunction with a three year study of the incidence of retained placentas, these experiments were undertaken to examine the value of these blood characteristics as indices of the potential prophylactic effect of prepartum selenium and vitamin E administration on the incidence of retained placentas.

# II. EXPERIMENTS 1 AND 2

## Materials and Methods

For the first experiment, ten pregnant Holstein heifers (466 ± 17 kg) were selected from a group which had been on a ration consisting entirely of forages from areas known to produce feed low in selenium (<.02 ppm). Through week 5 of the experiment this ration consisted of improved pasture grasses supplemented with grass hay from the Klamath Falls area. For the duration of Experiment 1 and Experiment 2 the ration was made up of ca. 60% corn silage harvested from O.S.U. lands and 40% grass hay. The heifers remained on a similar diet throughout the experimental period. Five heifers were assigned randomly to each of two groups. Treatments consisted of an injection of 1 ml MU-SE[1] per 60 kg body weight[2] for the experimental group and a sham injection for the untreated group. Intramuscular injections were given in the gluteal region. Blood samples were collected in heparinized tubes by tail vein puncture prior to treatment and every two weeks thereafter for 14 weeks. The blood samples were placed on ice immediately after collection and remained on ice during preparation for storage.

Red blood cells were prepared for storage using the method of Shull (1976). Briefly, .5 ml whole blood was washed in 5 ml .9%

---

[1]MU-SE, Burns-Biotech Laboratories, Oakland, CA 94621. (Each ml contains 5 mg selenium equivalent from sodium selenite and 68 IU vitamin E.)

[2]This dose was the higher of two dosages suggested by Dr. George McConnell, Burns-Biotech.

saline and centrifuged at 495 X g for 5 min. The supernatant was aspirated and discarded; cells were lysed in 2.5 ml .003M phosphate buffer (pH 7) containing .02M dithiothreitol. This hemolyzed RBC preparation was frozen for storage in .5 ml aliquots. Stored RBC preparations were assayed for GSH-Px activity after every second sampling period using a modified method of Paglia and Valentine (1967). To reduce inter-assay and intra-assay variation it was found necessary to maintain sample hemolysates below $10^{o}$C (on ice or in an ice-water bath) throughout the pre-reaction period. The photometric reaction took place in cuvettes (in 1 ml volume) at $27^{o}$C after a four minute equilibration period. The cuvette concentration of hydrogen peroxide was quadrupled to 28.3 X $10^{-5}$M and the concentration of glutathione reductase was doubled to 0.67 enzyme units (e.u.) per ml. Two pools of RBC preparations, obtained from dairy cows and stored in .5 ml aliquots, were analyzed in each assay as internal standards. Hemoglobin concentration of the assay hemolysates was determined by the method of Shenk et al. (1934). GSH-Px activity was expressed in e.u. per mg hemoglobin (Hb).

In the second experiment, ten Holstein heifers (529 ± 12 kg), maintained on the same low-selenium forage diet, were assigned to two treatments balanced according to pre-treatment blood GSH-Px activity. Treatments were the same as in Experiment 1, except that blood samples were taken at ten day intervals for 30 days post-treatment. Blood samples were assayed for RBC GSH-Px activity and whole blood levels of selenium. Forage samples were also analyzed for selenium content.

For the Se assay, 2 ml whole blood was stored in 10 ml nitric acid at $5^{o}$C for 2 to 12 days before digestion began. Samples were then digested

and assayed for selenium by the procedure of Olson (1969) with modifications by Whanger et al. (1975).

Data were analyzed statistically by split plot analysis of variance and regression analysis (Steel and Torrie, 1960). The LSD method was used to compare consecutive means within treatments.

## Results and Discussion

In the first experiment, the inter-assay coefficient of variation for the standard red blood cell preparations was 30.4%. Thus, for statistical analyses, all GSH-Px activity values were adjusted via the red blood cell internal standards to a common point of reference. Enzyme activities for these internal standards were 14.0 and 36.2 e.u./mg Hb. The coefficient of variation for the internal standards within each assay was only 9.1%. RBC GSH-Px activity of heifers in this experiment ranged from 7.9 to 37 e.u./mg Hb. These values are similar to those reported by Thompson et al. (1976) and Wilson and Judson (1976), which ranged from 6.6 to 41 and 2 to 30 e.u. mg/Hb, respectively. Storage time in the freezer (one to three weeks) seemed to increase the relative GSH-Px activity slightly, in that the most recent set of samples which were run in an assay always fell below the regression line, except where values were increasing. Wilson and Judson (1976) observed that storage time up to six days had no effect on GSH-Px activities of bovine blood.

Experiment 1. As determined by regression analysis, RBC GSH-Px activity of the untreated heifers declined linearly from 13.8 to 9.3

e.u./mg Hb during the sampling period ($R^2$= .62; P<.01) (Figure 1). This suggests that, either dietary intake of selenium had been decreased prior to the beginning of the experiment and body reserves of selenium were becoming depleted, or that the standard RBC preparations were increasing in relative enzyme activity over time. We are at a loss to explain a possible increase in relative activity, as Hb concentrations of the assay hemolysates remained unchanged during this time. In contrast, GSH-Px activity for the selenium-injected group increased through week 4 and then declined rapidly ($\hat{Y}$ = 20.28 + .86X - .097$X^2$; $R^2$ = .34, P<.01) to pre-injection levels by week 10 and approached the activity levels of the untreated heifers by week 12 to 14 (Figure 1). At week 4, GSH-Px activity was significantly greater than at either 0 or 10 weeks. The difference between group means was greatest on week 6 (10.6 ± 1.2 vs 21.6 ± 1.6 e.u./mg Hb), indicating that the maximum GSH-Px response to treatment was achieved between four and six weeks post-treatment. The initial difference between mean GSH-Px activity of the two groups (14.6 ± .9 vs 19.8 ± 2.5 e.u./mg Hb for treated and untreated heifers, respectively) was due to random selection. Pre-treatment enzyme activity levels of treated heifers were higher than the mean GSH-Px activity of the untreated heifers. Enzyme activity of one heifer in particular was much higher (29.5 e.u./mg Hb) than the others and showed no response to treatment. Deletion of this heifer decreased the mean enzyme activity of the treated heifers to 17.3 ± .8 e.u./mg Hb and actually increased the relative response of GSH-Px to treatment. Values for RBC GSH-Px activity for individual heifers are given in Appendix A. Using split plot analysis, the interaction of time vs treatment was significant,

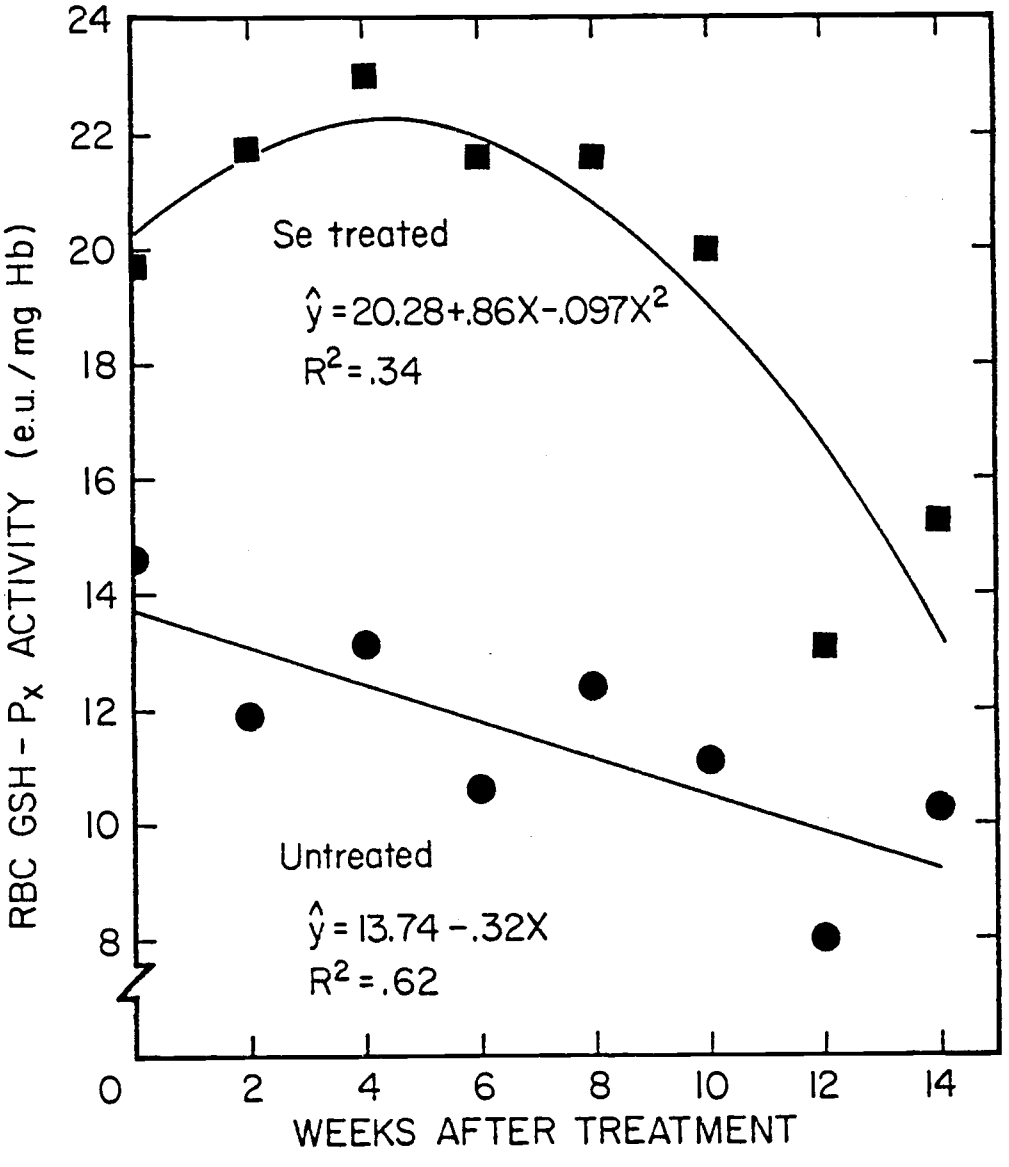


Figure 1. Regression of red blood cell (RBC) glutathione peroxidase (GSH-Px) activity on time in ten dairy heifers, half of which had been injected (im) with 5 mg selenium per 60 kg BW. (Experiment 1).

indicating that the change in the difference between the means of the treated vs the controls was also significant, i.e., selenium administration had produced a significant effect in changing levels of GSH-Px activity.

Experiment 2. RBC GSH-Px activity increased even more dramatically than in Experiment 1. Within ten days of Se-injection, GSH-Px activity had increased significantly (Table 1; Appendix B1). Activity increased linearly ($\hat{Y} = 11.76 + 3.24X$; $R^2 = .23$, $P<.01$), attaining 167% (24.4 ± 3.0 e.u./mg Hb) of the initial activity (14.6 ± 2.3 e.u./mg Hb) by day 30. This agrees with our results in Experiment 1, in which peak activity was attained between 28 and 42 days post-treatment. Response of erythrocyte GSH-Px activity in lambs, following a change in selenium intake has been shown to accompany the turnover of RBC's (Oh et al., 1976). Similarly, McConnell (1973) found the rate of $^{75}Se$ disappearance from RBC's in dogs to be greatest at 100 to 120 days. As in the first experiment, both sampling time and the interaction of treatments vs time were significant.

The life span of bovine RBC has been estimated to be 70 to 126 days in calves and 144 to 160 days in mature cows (as reviewed by Johnson and Schwartz, 1970). Assuming that the life span of a RBC is 120 days in these heifers and that GSH-Px is incorporated into the RBC during erythropoeisis, a 67% increase in total RBC GSH-Px activity at day 30 indicates that the 25% of the RBC's synthesized during this time period (30 days) contained 368% of the enzyme activity of RBC's synthesized prior to treatment. Synthesis of GSH-Px in new RBC's would be

TABLE 1. Red blood cell (RBC) glutathione peroxidase (GSH-Px) activity and whole blood selenium (Se) concentrations of ten dairy heifers, half of which were treated with selenium[1] and vitamin E (Experiment 2).

| Treatment | Days after treatment | | | |
|---|---|---|---|---|
| | day 0 | day 10 | day 20 | day 30 |
| | RBC GSH-Px activity (e.u./mg Hb) | | | |
| Se treated | $14.6^{a} \pm 2.3$ | $18.7^{b} \pm 3.4$ | $21.7^{b} \pm 4.5$ | $24.4^{c} \pm 3.0$ |
| Untreated | $13.3^{d} \pm 1.7$ | $14.5^{d} \pm 1.9$ | $14.7^{d} \pm 2.0$ | $14.9^{d} \pm 1.0$ |
| Difference | $.3^{NS}$ | $4.2^{NS}$ | $7.0^{NS}$ | 9.5* |

Error a mean square (heifers within groups) = 130.0
Error b mean square (time X within heifer) = 5.0

| | Whole blood Se (μg/ml) | | | |
|---|---|---|---|---|
| Se treated | $.016^{a} \pm .002$ | $.047^{b} \pm .010$ | $.041^{b} \pm .004$ | $.040^{b} \pm .007$ |
| Untreated | $.016^{c} \pm .004$ | $.012^{c} \pm .002$ | $.019^{c} \pm .005$ | $.020^{c} \pm .006$ |
| Difference | 0 | .035* | .022* | .020* |

Error a mean square (heifers within groups) = .00033
Error b mean square (time X within heifer) = .00011

[1]Treatment consisted of an intramuscular injection of 1 ml MU-SE/60 kg BW. MU-SE contains 5 mg selenium from sodium selenite and 68 IU vitamin E/ml.

*Differences between means of treatment groups are significant (P<.05)

$^{a,b,c,d}$Means with difference superscripts within a row are different (P<.05).

expected to continue at this increased level until the supplementary selenium above that which is provided continuously by the diet had been utilized. At this point, synthesis of GSH-Px into new RBC's would revert back to pre-treatment levels. By this logic one would expect RBC GSH-Px activity to plateau after reaching a peak and remain at this higher level of activity until the first of the RBC's synthesized following treatment began to be destroyed. As observed in Experiment 1, this was not the case. GSH-Px activity in the Se-treated heifers rapidly decreased to that of the untreated heifers by 12 to 14 weeks post-treatment. This suggests either a shorter life span than assumed or a decrease in GSH-Px activity of RBC's as they mature. Johnson and Schwartz (1970) suggested that RBC's may be produced in two populations, one of which has a much shorter life span than the other. This concept could partially account for the rapid depletion of GSH-Px activity.

Analysis of the corn silage indicated that the selenium content was less than .013 ppm (the values for duplicate samples were non-dectable and .013 μg Se/g of silage). However, the grass hay was found to contain .19 ppm Se. This was surprising since forages, especially alfalfa hay, grown in Oregon's Klamath Falls Basin, normally contain less than .02 ppm Se (P. H. Weswig, personal communication). Repetition of the assay on the same sample two months later found .14 ppm Se (values of duplicates were .13 and .15 ppm). Thus, it appears that this sample actually contained high amounts of selenium. In consideration of the very low levels of whole blood selenium ( .02 μg/ml) and the history of low-selenium forages from the Klamath area, I feel that this sample must have become contaminated prior to analysis. Unfortunately, by the time

the selenium analysis had been completed all of this grass hay had been fed; thus, it was impossible to resample the hay. There is also a possibility that the grass hay was actually high in selenium, but in a form which was biologically unavailable. In view of these considerations, steps have been taken to obtain samples from grass hay harvested from the same fields during the following season (1977). For the purposes of discussion in this thesis I have assumed the all-forage ration to be selenium-deficient (containing .02 ppm Se).

These dairy heifers had a barely detectable level of 0.016 µg Se/ml whole blood (Table 1; Appendix B2). Jenkins et al. (1974) considered a whole blood selenium level of .018 µg/ml in beef cattle deficient relative to nutritional muscular dystrophy. In dairy cattle, Trinder et al. (1973) reported on a selenium-responsive problem with retained placentas in a dairy herd with mean whole blood selenium levels of .061 to .073 µg/ml. Also in dairy cattle, Perry (1976) considered serum selenium levels less than .02 µg/ml as deficient. Plasma contains only 20-35% of the selenium in whole blood (Jordan and Obst, 1974). These data indicate that our heifers were selenium-deficient. However, selenium-responsive diseases have not been diagnosed in our dairy herd, possibly due to a selenium and vitamin E injection given to newborn calves and to the grain which is fed to calves and breeding-age heifers. In this experiment, administration of selenium and vitamin E (average of 44.5 mg Se and 605 IU vitamin E per heifer) raised whole blood selenium levels significantly by 269% within ten days and blood selenium remained at this level throughout 30 days (Table 1). The untreated heifers remained below .02 µg Se/ml whole blood thoughout the sampling period. Although

whole blood selenium levels of the selenium-injected heifers remained constant after day 10, RBC GSH-Px activity in these same heifers continued to rise during the sampling period. It is probable that this plateau in whole blood selenium represents both an increase of Se incorporated into RBC GSH-Px and a concomitant decrease in unincorporated plasma and RBC selenium which is available to tissues and for RBC synthesis. Van Vleet (1975) found elevated concentrations of selenium in both liver and kidney as long as 23 days post-treatment in calves given a dosage of selenium equivalent to that received by our heifers.

These data demonstrate that the increased blood levels of selenium allow for increased selenium incorporation into GSH-Px. It also indicates that, following injection of selenium, a single determination of GSH-Px activity may not be indicative of the changing Se status of the animal. In selenium deficient cattle, maximum GSH-Px activity and increased whole blood selenium are most likely to be detected following a single Se-injection at 28 to 42 days and 10 to 30 days, respectively. However, we have also observed that in Se-adequate lactating cattle, RBC GSH-Px activity is unchanged following a single selenium injection (unpublished observations).

# III. EXPERIMENT 3

## Materials and Methods

Seventeen parous, nonlactating Holstein cows (having completed 3.8 ± .5 lactations) were randomly assigned to one of three treatments as they reached 24 days prepartum (day 259 ± 1 of gestation). Treatments consisted of a single injection of 1 ml MU-SE per 91 kg body weight, 1 ml MU-SE per 60 kg body weight, or 4 ml vitamins AD&E (Injacom),[3] hereafter referred to as Se 1/91, Se 1/60, or AD&E, respectively. Injections were given (im) in the thigh or gluteal area. Blood samples were drawn prior to treatment, day 10 post-treatment, day of parturition (for Se analysis only) and three days postpartum (for GSH-Px analysis only), and on days 20 and 40 post-treatment. Samples were taken on day 20 to provide a uniform point of reference for cows which did not parturate when expected. Thus, each cow was sampled six times except when day of parturition occurred on a scheduled sample day.

Samples from days 24 and 14 prepartum, day 3 postpartum and day 40 post-injection were prepared for storage and assayed for RBC GSH-Px activity by the methods described in Experiment 1. All blood samples (except day 3 postpartum) were also analyzed for whole blood and plasma selenium concentrations by methods described in Experiment 1.

---

[3]Injacom, Roche Chemical Division, Hoffman-LaRoche Inc., Nutely NJ 07110. (Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin D, and 50 IU vitamin E.)

The cows were fed an all-forage ration throughout the dry period (beginning about 60 days prepartum). These forages were the same as fed in Experiments 1 and 2 (60% corn silage and 40% grass hay). Approximately ten days prepartum, the cows were brought into the barn and given 4-5 kg of mixed grain concentrate (containing .3 ppm Se) daily until parturition.

Data were analyzed statistically by split plot analysis of variance and regression analysis (Steel and Torrie, 1960).

## Results and Discussion

The regression of RBC GSH-Px activity on whole blood Se, using pre-treatment blood samples from ten heifers from Experiment 2 and the 17 nonlactating cows of this experiment was highly significant ($\hat{Y} = 10.12 + 462.47X$; $R^2 = .752$)(Figure 2). Enzyme activity and whole blood selenium in these heifers and cows ranged from 8.0 to 59.0 e.u./mg Hb and .010 to .100 µg Se/ml, respectively. These data agree with Allen et al. (1975), who obtained a highly significant coefficient of determination ($R^2 = .831$) with data from a four month feeding trial, and with Thompson et al. (1976), whose regression ($R^2 = .32$) was based on values from a survey of cows which tended to be low in whole blood Se (<.05 µg/ml). In our experiment, RBC GSH-Px activity was a good estimate of pre-treatment whole blood selenium levels even though cows had been on the low Se ration for variable lengths of time (20 to 108 days).

No significant changes in RBC GSH-Px activity were detected following any of the treatments; initial enzyme activity for all cows

averaged 43.9 ± 2.0 e.u./mg Hb (Table 2; Appendix C1). Surprisingly, enzyme activity had not increased by day 40 post-treatment when cows had been fed a ration high in concentrates (relative to forage the concentrate was high in selenium content--.02 vs .30 ppm Se, respectively) for more than 30 days. This lack of response is similar to a preliminary study (C. Hoffman and L. Swanson, unpublished data), in which we were unable to observe a change in RBC GSH-Px activity following an injection of 1 ml MU-SE per 60 kg body weight in lactating cows fed a ration made up of 5 to 10 kg of concentrate. Thus, in cows with adequate Se blood levels, it is unlikely that measurement of RBC GSH-Px activity can serve as an indicator of the potential effects of Se injections.

Furthermore, changes could not be detected in blood selenium following treatment (Table 3; Appendix C2). Pre-treatment whole blood Se for all cows was .068 ± .006 µg/ml. It appears that 36.7 ± 6.0 days on a Se deficient forage ration was not sufficient to allow whole blood Se to decrease to the point where it would be responsive to injections of Se and vitamin E. Oh et al. (1976) found that the response of RBC GSH-Px activity to a change in the level of dietary selenium was related to the life span of RBC's. The life span of RBC's in the bovine is approximately 100 to 150 days (Johnson and Schwartz, 1970). McConnell (1963) observed that the rate of $^{75}Se$ disappearance from RBC's in dogs was greatest at 100 to 120 days. However, Weswig et al. (1965) could detect increased RBC in vitro uptake of $^{75}Se$ in ovine blood after only 30 days on a low Se diet.

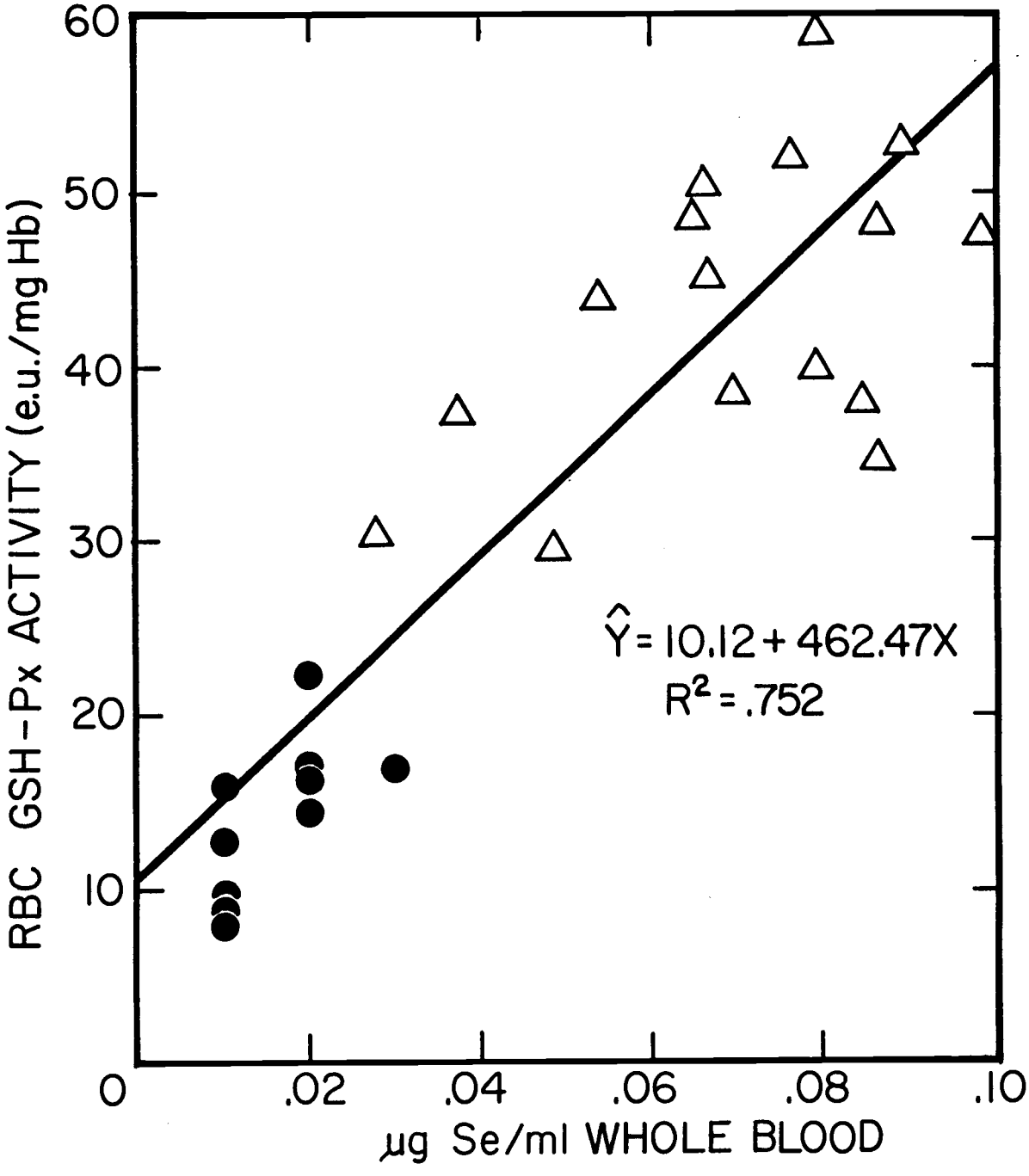


Figure 2. Regression of red blood cell (RBC) glutathione peroxidase (GSH-Px) activity on whole blood selenium in cows (Δ) and heifers ( ● ).

TABLE 2. Red blood cell glutathione peroxidase activity for nonlactating cows injected 24 days prepartum with 1 ml MU-SE[1] per 60 kg BW (Se 1/60), 1 ml MU-SE per 91 kg BW (Se 1/91), or 4 ml Injacom[2] (AD&E). (Experiment 3).

| Treatment | Days post-treatment | | | |
|---|---|---|---|---|
| | 0[3] | 10 | 25 ± 4.9[4] | 39 |
| | e.u./ml Hb | | | |
| Se 1/60 n = 5 | 45.2 ± 2.8[5] | 47.2 ± 5.0 | 43.0 ± 2.2 | 40.7 ± 2.5 |
| Se 1/91 n = 6 | 43.1 ± 4.2 | 41.2 ± 3.2 | 40.7 ± 2.5 | 43.0 ± 2.2 |
| AD&E n = 6 | 43.5 ± 3.5 | 43.0 ± 3.8 | 36.4 ± 3.9 | 37.1 ± 3.2 |

[1]Each ml contains 5 mg selenium as sodium selenite and 68 IU vitamin E.

[2]Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin $D_3$ and 50 IU vitamin E.

[3]Represents pre-treatment sample

[4]Represents sample taken on day 3 postpartum

[5]Mean ± SE.

Plasma, however, contained only .018 ± .003 µg/ml (Table 3; Appendix C3). Perry et al. suggest that serum selenium levels below .020 ppm are borderline to deficiency. However, even at these low initial levels, plasma Se did not differ among treatment groups at ten days post-injection. In contrast, Julian et al. (1976a) observed an increase in plasma selenium from .025 ppm on day 20 prepartum to .065 and .085 ppm on day 10 prepartum and day of parturition, respectively, following administration (im) of 50 mg Se equivalent from sodium selenite and 680 IU vitamin E. This dosage was similar to the average dose of 56 mg Se equivalent and 762 IU vitamin E received by cows in our Se 1/60 group. Julian is not explicit as to which cows were included in this observation. It appears that some portion of the cows referred to above were receiving the 15% CP, adequate selenium (.06 ppm) ration. Thus, some of the observed increase in plasma selenium may be attributable to the increased selenium of the diet. This would explain why plasma levels continued to rise between day 10 prepartum and time of parturition. Oh (1972) was able to increase whole blood Se from .070 to .101 µg/ml on day of parturition with a single selenium injection (6 mg selenium from sodium selenite/60 kg) 30 days prepartum.

As a result of excessively high variability between two of the selenium assays, blood selenium values for the other three sampling periods (day of parturition, days 20 to 40 post-treatment) are not included (Appendix C4). Since no changes in blood Se following injection could be detected ten days post-treatment, it is doubtful that increases in successive samples, if detected, could be attributed to

TABLE 3. Blood selenium levels of nonlactating cows injected 24 days prepartum with 1 ml MU-SE[1] per 60 kg BW (Se 1/60), 1 ml MU-SE per 91 kg BW (Se 1/91), or 4 ml Injacom[2] (AD&E), (Experiment 3).

| Treatment | Whole blood Se | | Plasma Se | |
|---|---|---|---|---|
| | days post-treatment | | | |
| | 0[3] | 10 | 0 | 10 |
| | μg/ml | | | |
| Se 1/60 n = 5 | .077[4] ± .010 | .085 ± .006 | .027 ± .004 | .033 ± .009 |
| Se 1/91 n = 6 | .063 ± .006 | .073 ± .009 | .010 ± .004 | .030 ± .005 |
| AD&E n = 4 | .075 ± .006 | .075 ± .006 | .020 ± .007 | .018 ± .005 |

[1]Each ml contains 5 mg selenium as sodium selenite and 68 IU vitamin E.

[2]Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin $D_3$ and 50 IU vitamin E.

[3]Represents pre-treatment sample

[4]Mean ± SE.

treatment. Such changes would more logically be a consequence of increases in the selenium content of the ration.

These results are contrary to those observed in our previous experiment in heifers (Experiments 1 and 2). In Experiment 2, RBC GSH-Px activity was significantly higher by day 10 post-treatment and had risen 67% by day 30. Whole blood Se had risen 190% by day 10 from .016 to .047 µg/ml and remained above .04 µg/ml through day 30. Those heifers and the cows in this experiment all received the same all-forage ration. However, prior to this dietary regime, the cows had been fed 5 to 10 kg of concentrate daily throughout their previous lactation. Thus, pretreatment whole blood selenium was significantly greater in the cows compared to the heifers, .068 ± .006 vs .016 ± .004 µg Se/ml, respectively. Initial enzyme activity was also higher ($P<.05$) in the cows than in heifers (43.9 ± 2.0 vs 14.6 ± 2.3 e.u./mg Hb). Maximum GSH-Px activity exhibited by the heifers at 30 to 42 days following injection was still significantly lower than that observed in the cows (24 ± 3.0 vs 43.9 ± 2.0 e.u./mg Hb, respectively). Thus, due to the higher pretreatment blood Se status of these cows, we were unable to demonstrate a response in either blood selenium or RBC GSH-Px activity following a single injection of selenium and vitamin E.

# BIBLIOGRAPHY

Allen, W. M., W. H. Parr, P. H. Anderson, S. Berrett, R. Bradley, and D. S. P. Patterson. 1975. Selenium and the activity of glutathione peroxidase in bovine erythrocytes. Vet. Rec. 96(16):360.

Ammerman, C. B., and S. M. Miller. 1975. Selenium in ruminant nutrition: A review. J. Dairy Sci. 58(10):1561.

Boyd, J. W. 1975. Blood selenium and propionic acid. Vet. Rec. 96(20):458.

Flohé, L., W. A. Günzler, and H. H. Schock. 1973. Glutathione peroxidase: a selenoenzyme. Fed. European Biochem. Soc. Letters. 32:132.

Jenkins, K. J., M. Hidiroglou, J. M. Wauthy, and J. E. Proulx. 1974. Prevention of nutritional muscular dystrophy in calves and lambs by selenium and vitamin E additions to the maternal mineral supplement. Can. J. Anim. Sci. 54:49.

Jenkins, K. T., and M. Hidiroglou. 1972. A review of selenium/vitamin E responsive problems in livestock: A case for selenium as a feed additive in Canada. Can. J. Anim. Sci. 52:591.

Johnson, L. W., and S. Schwartz. 1970. Isotopic studies of erythrocyte survival in normal and prophyric cattle: influence of light exposure, blood withdrawal and spleenectomy. Amer. J. Vet. Res. 31(12):2167.

Judson, G. J., and J. M. Obst. 1975. Diagnosis and treatment of selenium inadequacies in the grazing ruminant. In Trace Elements in Soil--Plant--Animal Systems. Ed. D. J. D. Nicholas and A. R. Egan, Academic Press, New York.

Julien, W. E., H. R. Conrad, J. E. Jones, and A. L. Moxon. 1976a. Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. J. Dairy Sci. 59:1954.

Julien, W. E., H. R. Conrad, and A. L. Moxon. 1976b. Selenium and vitamin E and incidence of retained placentas in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. J. Dairy Sci. 59:1960.

McConnell, K. P. 1941. Distribution and excretion studies in the rat after single subtoxic subcutaneous injection of sodium selenate containing radioselenium. J. Biol. Chem. 141:427.

McConnell, K. P., and B. J. Cooper. 1950. Distribution of selenium in serum proteins and red blood cells after subcutaneous injection of sodium selenate containing radioselenium. J. Biol. Chem. 183:459.

McConnell, K. P. 1963. Metabolism of selenium in the mammalian organism. J. Agr. Food Chem. 11(5):385.

Oh, S. H. 1972. Relationship between selenium content of feed, blood and milk, and the problem of retained placenta in dairy cows. M.S. Thesis, University of Wisconsin.

Oh, S. H., H. E. Ganther, and W. G. Hoekstra. 1974. Selenium as a component of glutathione peroxidase isolated from ovine erythrocytes. Biochem. 13:1825.

Oh, S. H., R. A. Sunde, A. L. Pope, and W. G. Hoekstra. 1976. Glutathione peroxidase response to selenium intake in lambs fed a formula yeast-based, artificial milk. J. Anim. Sci. 42:977.

Oldfield, J. E. 1974. The selenium story: some reflections on the "moon-metal." New Zealand Vet. J. 22(6):85.

Olson, O. E. 1969. Fluorometric analysis of selenium in plants. J. Ass. Official Anal. Chem. 52:627.

Paglia, D. E., and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70(1):158.

Perry, T. W., D. M. Caldwell, and R. C. Peterson. 1976. Selenium content of feeds and effect of dietary selenium on hair and blood serum. J. Dairy Sci. 59(4):760.

Rotruck, J. T., A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588.

Shenk, J. H., J. L. Hall, and A. H. King. 1934. Spectrophotometric characteristics of hemoglobin. J. Biol. Chem. 105:741.

Shull, L. R. 1976. Selenium effects and species differences in hepatic metabolism of pyrrolizidine alkaloids and other xenobiotics. PhD Thesis, Oregon State University.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Tappel, A. L. 1974. Selenium glutathione peroxidase and vitamin E. Amer. J. Clin. Nutr. 27:960.

Thompson, R. H., C. H. McMurray, and W. J. Blanchflower. 1976. The levels of selenium and glutathione peroxidase activity in blood of sheep, cows and pigs. Res. Vet. Sci. 20:229.

Trinder, N., C. D. Woodhouse, and C. P. Renton. 1969. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. Vet. Rec. 85:550.

Trinder, N., R. J. Hall, and C. P. Renton. 1973. The relationship between the intake of selenium and vitamin E on the incidence of retained placentae in dairy cows. Vet. Rec. 93:641.

Van Vleet, J. F. 1975. Retention of selenium in tissues of calves, lambs and pigs after parenteral injection of a selenium - vitamin E preparation. Am. J. Vet. Res. 36(9):1335.

Weswig, P. H., S. A. Roffler, M. A. Arnold, O. H. Muth, and J. E. Oldfield. 1965. In vitro uptake of selenium-$^{75}$ by blood from ewes and their lambs on different selenium regimes. Amer. J. Vet. Res. 27(116):128.

Whanger, P. D. 1975. Interaction and methods of analysis of selenium and vitamin E as related to white muscle disease. Laboratory Methods and Nutrition of Animal Feeds. AAFCO-AOAC Workshop Proceedings. Salem, Ore.

Wilson, P. S., and G. J. Judson. 1976. Glutathione peroxidase activity in bovine and ovine erythrocytes in relation to blood selenium concentration. Brit. Vet. J. 132:428.

Wright, P. L., and M. C. Bell. 1963. Selenium and vitamin E influence upon the in vitro uptake of $Se^{75}$ by ovine blood cells. Proc. Soc. Expl. Biol. Med. 114:379.

# APPENDICES

APPENDIX A. Red blood cell glutathione peroxidase activity levels for each of ten dairy heifers, half of which were treated with selenium and vitamin E.[1] (Experiment 1).

| Groups | Weeks post-treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0[2] | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
| | enzyme units/mg Hb | | | | | | | |
| Treated | | | | | | | | |
| 1379 | 17.5 | 17.4 | 20.7 | 19.2 | 18.6 | 19.4 | 10.9 | 12.4 |
| 1382 | 29.5 | 30.3 | 31.6 | 26.8 | 24.9 | 25.3 | 15.8 | 16.3 |
| 1392 | 19.5 | 22.8 | 26.0 | 22.6 | 23.6 | 21.0 | 16.0 | 16.5 |
| 1395 | 16.6 | 22.1 | 20.3 | 21.7 | 19.8 | 18.3 | 9.8 | 14.8 |
| 1399 | 15.7 | 16.0 | 16.5 | 17.8 | 21.3 | 16.1 | 13.1 | 16.5 |
| Mean | 19.8 | 21.7 | 23.0 | 21.6 | 21.6 | 20.0 | 13.1 | 15.3 |
| Untreated | | | | | | | | |
| 1378 | 14.8 | 10.0 | 11.2 | 8.1 | 10.7 | 9.2 | 6.5 | 6.6 |
| 1381 | 12.7 | 8.4 | 12.0 | 11.4 | 11.9 | 13.8 | 7.0 | 9.2 |
| 1383 | 13.4 | 10.5 | 13.5 | 7.6 | 14.6 | 10.7 | 9.7 | 19.9 |
| 1398 | 13.9 | 12.6 | 12.9 | 11.8 | 11.1 | 8.9 | 6.6 | 6.4 |
| 1400 | 18.1 | 17.9 | 15.9 | 13.9 | 13.7 | 12.7 | 10.0 | 9.6 |
| Mean | 14.6 | 11.9 | 13.1 | 10.6 | 12.4 | 11.1 | 8.0 | 10.3 |

[1] Treatment consisted of an intramuscular injection of 1 ml MU-SE (5 mg of selenium as sodium selenite and 68 IU vitamin E) per 60 kg body weight

[2] Represents pre-treatment sample

APPENDIX B1. Red blood cell glutathione peroxidase activity levels for each of ten dairy heifers, half of which were treated with selenium and vitamin E.[1] (Experiment 2).

| Groups | Days post-treatment | | | |
|---|---|---|---|---|
| | 0[2] | 10 | 20 | 30 |
| | enzyme units/mg Hb | | | |
| Treated | | | | |
| 1368 | 7.9 | 10.5 | 12.9 | 19.7 |
| 1381 | 12.5 | 13.9 | 13.4 | 19.5 |
| 1401 | 16.6 | 24.0 | 24.8 | 29.2 |
| 1403 | 22.0 | 29.2 | 37.4 | 33.9 |
| 1410 | 14.0 | 15.9 | 20.1 | 19.7 |
| Mean | 14.6 | 18.7 | 21.7 | 24.4 |
| Untreated | | | | |
| 1378 | 9.4 | 9.9 | 9.2 | 12.2 |
| 1398 | 8.9 | 10.9 | 10.7 | 13.3 |
| 1402 | 16.6 | 18.0 | 16.3 | 15.6 |
| 1406 | 15.6 | 14.0 | 19.0 | 17.6 |
| 1407 | 15.8 | 19.6 | 18.3 | 15.6 |
| Mean | 13.3 | 14.5 | 14.7 | 14.9 |

[1]Treatment consisted of an intramuscular injection of 1 ml MU-SE (5 mg of selenium as sodium selenite and 68 IU vitamin E) per 60 kg body weight

[2]Represents pre-treatment sample

APPENDIX B2. Whole blood selenium levels for each of ten dairy heifers, half of which were treated with selenium and vitamin E.[1] (Experiment 2)

| Groups | Days post-treatment | | | |
|---|---|---|---|---|
| | 0[2] | 10 | 20 | 30 |
| | µg/ml | | | |
| Treated | | | | |
| 1368 | .01 | .045 | .033 (est.) | .025 |
| 1381 | .01 | .010 | .035 | .036 |
| 1401 | .02 | .060 | .050 | .027 |
| 1403 | .02 | .070 | .050 | .063 |
| 1410 | .02 | .050 | .035 | .050 |
| Mean | .016 | .047 | .041 | .040 |
| Untreated | | | | |
| 1378 | .01 | .010 | .010 | .010 |
| 1398 | .01 | .010 | .010 | .010 |
| 1402 | .03 | .020 | .025 | .030 |
| 1406 | .01 | .010 | .015 | .040 |
| 1407 | .02 | .010 | .035 | .010 |
| Mean | .016 | .012 | .019 | .020 |

[1]Treatment consisted of an intramuscular injection of 1 ml MU-SE (5 mg of selenium as sodium selenite and 68 IU vitamin E) per 60 kg body weight

[2]Represents pre-treatment sample

APPENDIX C1. Individual values of red blood glutathione peroxidase activity levels for 17 nonlactating cows injected 24 days prepartum with 1 ml MU-SE[1] per 60 kg BW (Se 1/60), 1 ml MU-SE per 91 kg BW (Se 1/91), or 4 ml Injacom[2] (AD&E). (Experiment 3).

| Cows / Treatment | Days post-treatment | | | | |
|---|---|---|---|---|---|
| | 0[3] | 10 | 25±4.9[4] | 39 | Day of postpartum sample[5] |
| | RBC GSH-Px (e.u./mg Hb) | | | | |
| Se 1/60 | | | | | |
| 1047 | 47.5 | 56.8 | 48.2 | 55.3 | 24 |
| 1247 | 48.2 | 36.2 | (42.2) est | (42.2) est | 16 |
| 1284 | 39.2 | 42.8 | 52.2 | 47.3 | 24 |
| 1186 | 52.7 | 56.1 | 58.8 | 54.1 | 24 |
| 1251 | 37.6 | 33.9 | 34.1 | (35.2) est | 31 |
| Mean | 45.2±2.8 | 47.2±5.0 | 47.1±4.2 | 46.8±3.8 | |
| Se 1/91 | | | | | |
| 1068 | 37.9 | 44.0 | 34.0 | 43.0 | 24 |
| 1067 | 38.4 | 32.2 | 35.8 | 34.5 | 18 |
| 1276 | 29.5 | 33.7 | 40.1 | 40.0 | 34 |
| 962 | 44.4 | 41.7 | 38.5 | 44.4 | 17 |
| 1256 | 59.2 | 53.8 | 49.7 | 50.8 | 21 |
| 1248 | 49.4 | 41.5 | 45.8 | 45.3 | 21 |
| Mean | 43.1±4.2 | 41.2±3.2 | 40.7±2.5 | 43.0±2.2 | |
| AD&E | | | | | |
| 1309 | 34.4 | 39.5 | (37.0) est | (37.0) est | |
| 1339 | 48.5 | 47.8 | 45.2 | 36.2 | 25 |
| 1299 | 50.4 | 53.5 | 41.4 | 48.6 | 32 |
| P-20 | 52.1 | 45.0 | 39.9 | 40.0 | 38 |
| P-28 | 30.5 | 26.3 | 22.2 | 19.4 | 18 |
| 1091 | 45.3 | 46.1 | 37.1 | (37.1) est | 31 |
| Mean | 43.5±3.7 | 43.0±3.8 | 37.1±3.2 | 36.4±3.9 | |

[1]Each ml contains 5 mg selenium as sodium selenite and 68 IU vitamin E

[2]Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin $D_3$, and 50 IU vitamin E

[3]Represents pre-treatment sample

[4]Represents sample taken three to five days postpartum

[5]As days post-treatment

APPENDIX C2. Individual whole blood selenium levels of nonlactating cows injected 24 days prepartum with 1 ml MU-SE[1] per 60 kg BW (Se 1/60), 1 ml MU-SE per 91 kg BW (Se 1/91), or 4 cc Injacom[2] (AD&E). (Experiment 3).

| Cows | Days post-treatment | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 0[3] | 10 | 19.8±1.5[4] | 20 | 39 | Day of parturition[5] |
| | µg/ml | | | | | |
| Se 1/60 | | | | | | |
| 1047 | .094[6] | .099[6] | .081[6] | .081[6] | .160[6] | 20 |
| 1247 | .085[6] | .092[6] | .094[6] | .094[6] | ---- | 20 |
| 1284 | .077[6] | .090[6] | ---- | .069[7] | .073[8] | 18 |
| 1186 | .090[7] | .072[7] | .072[7] | .104[7] | .163[8] | 11 |
| 1251 | .038[7] | .072[7] | .087[8] | .108[8] | .099[8] | 28 |
| Mean | .077 | .085 | | | | |
| Se 1/91 | | | | | | |
| 1068 | .083[6] | .050[6] | .083[6] | .083[6] | .112[6] | 20 |
| 1067 | .063[6] | .081[6] | .112[6] | .055[6] | .133[8] | 13 |
| 1276 | .049[7] | .049[7] | .089[8] | .059[7] | .083[8] | 29 |
| 962 | .055[7] | .090[7] | .090[7] | .076[7] | .086[8] | 13 |
| 1256 | .080[7] | .066[7] | .147[7] | .103[8] | .100[8] | 17 |
| 1248 | .045[7] | .103[7] | .100[8] | .118[8] | .121[8] | 16 |
| Mean | .063 | .073 | | | | |
| AD&E | | | | | | |
| 1309 | .085[6] | .070[6] | .090[6] | ---- | ---- | 17 |
| 1339 | .064[6] | .083[6] | ---- | .036[7] | .125[8] | 18 |
| 1299 | .066[7] | .086[7] | .135[8] | .076[7] | .122[8] | 27 |
| P-20 | .083[7] | .060[7] | .154[8] | .105[8] | .122[8] | 31 |
| P-28 | .028[7] | ---- | .080[8] | .067[8] | .034[8] | 12 |
| 1091 | .066[7] | .109[8] | .125[8] | .128[8] | .167[8] | 26 |
| Mean | .085 | .070 | | | | |

[1]Each ml contains 5 mg selenium as sodium selenite and 68 IU vitamin E

[2]Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin $D_3$, and 50 IU vitamin E

[3]Represents pre-treatment sample

[4]Represents sample taken day of parturition

[5]As days post-treatment

[6]Selenium assay of 13 April 1977

[7]Selenium assay of 21 April 1977

[8]Selenium assay of 14 June 1977

APPENDIX C3. Individual plasma selenium levels of nonlactating cows injected 24 days prepartum with 1 ml MU-SE[1] per 60 kg BW (Se 1/60), 1 ml MU-SE per 91 kg BW (Se 1/91), or 4 cc Injacom[2] (AD&E). (Experiment 3).

| Cows | Days post-treatment | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 0[3] | 10 | 19.8±1.5[4] | 20 | 39 | Day of parturition[5] |
| | µg/ml | | | | | |
| Se 1/60 | | | | | | |
| 1047 | .024[6] | .050[6] | .037[6] | .037[6] | .011[7] | 20 |
| 1247 | .033[6] | .037[6] | .057[6] | .057[6] | ---- | 20 |
| 1284 | .026[6] | .035[6] | .035[7] | .035[7] | .049[8] | 18 |
| 1186 | .038[7] | .041[7] | .035[7] | .007[8] | .035[8] | 11 |
| 1251 | .014[7] | ND[7] | .034[7] | .038[8] | .052[8] | 28 |
| Mean | .027 | .033 | | | | |
| Se 1/91 | | | | | | |
| 1068 | .022[6] | .048[6] | .029[6] | .030[6] | .007[7] | 20 |
| 1067 | .008[6] | .037[6] | .048[6] | .029[6] | .084[8] | 13 |
| 1276 | ND[7] | .017[7] | .035[8] | .021[7] | .035[8] | 29 |
| 962 | ND[7] | .031[7] | .052[7] | .003[7] | .019[8] | 13 |
| 1256 | .014[7] | .021[7] | .043[7] | .052[8] | .060[8] | 17 |
| 1248 | .014[7] | .031[7] | .033[8] | .057[8] | .090[8] | 16 |
| Mean | .027 | .033 | | | | |
| AD&E | | | | | | |
| 1309 | .011[6] | .005[6] | .015[6] | ----[7] | ----[8] | 17 |
| 1339 | .019[6] | .033[6] | .012[7] | .007[7] | .068[8] | 18 |
| 1299 | .044[7] | .017[7] | .030[8] | .011[7] | .033[8] | 27 |
| P-20 | .035[7] | .003[7] | .038[8] | .035[8] | .035[8] | 31 |
| P-28 | .007[7] | .014[8] | .022[8] | .049[8] | .030[8] | 12 |
| 1091 | ND[7] | .034[8] | .030[8] | .005[8] | .027[8] | 26 |
| Mean | .020 | .018 | | | | |

[1] Each ml contains 5 mg selenium as sodium selenite and 68 IU vitamin E

[2] Each ml contains 500,000 IU vitamin A, 75,000 IU vitamin $D_3$, and 50 IU vitamin E

[3] Represents pre-treatment sample

[4] Represents sample taken day of parturition

[5] As days post-treatment

[6] Selenium assay of 13 April 1977

[7] Selenium assay of 21 April 1977

[8] Selenium assay of 14 June 1977

ND = nondetectable

APPENDIX C4

By observation, the blood selenium values obtained from the assay of June 13, 1977 appeared to be excessively high when compared to the values of the previous assays. For example, whole blood values for replicate samples from two cows (1047 and 1068) were 88% and 70% higher in the assay of June 13 vs the assay of April 22 (see following table). Values from the assays of April 13 and 22 are reasonable and compare favorably to values obtained from prior assays. Thus, I consider the values from the June 13 assay to be unrepresentative of the true blood selenium levels of these cows. However, even though inter-assay variation may be intolerably high considering the June 13 assay, I feel confident of the relative values obtained within any given assay. The inter-assay coefficient of variation was 9.1% based on seven observations of replicated samples from two cows in the April 22 assay. Variation of duplicate standards was less than 10% in each assay. Thus, I feel confident about using the blood selenium values from the first two sampling periods (days 0 and 10) of the cows in Experiment 3. Each pair of samples from each cow was analyzed within one of the April assays; therefore, the relative blood levels for days 0 and 10 are accurate and the absolute selenium levels are acceptable.

Replicate Values for Whole Blood Selenium

| Assay | Cow Number | |
|---|---|---|
| | 1047 (12/4)[1] | 1068 (12/4) |
| | µg Se/ml | |
| April 22 | .085±.002[2] (n = 3) | .066±.005 (n = 4) |
| June 13 | .160 (n = 1) | .122 (n = 1) |

[1]Cow number (date of sample)

[2]Mean ± std. deviation