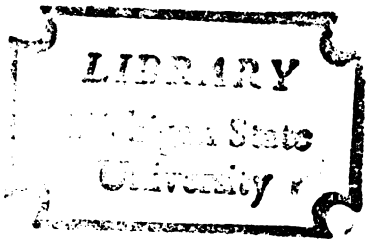

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presented by

Matthew J. Parsons

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of the requirements for

Ph.D. degree in Animal Science

E. R. Miller

Major professor

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SOME ASPECTS OF SELENIUM METABOLISM IN THE YOUNG PIG

By

Matthew James Parsons

A DISSERTATION

Submitted to
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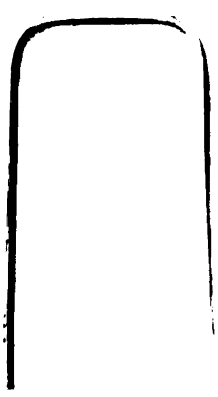

ABSTRACT

SOME ASPECTS OF SELENIUM METABOLISM

IN THE YOUNG PIG

By

Matthew James Parsons

The effects of dietary riboflavin supplementation, selenium source and level of supplementation on the performance and selenium metabolism of weanling pigs were studied. Pigs fed riboflavin-supplemented (10 mg/kg) casein based diets for 18 days gained faster than pigs fed the riboflavin-unsupplemented diets. Percent active erythrocyte glutathione reductase declined rapidly when pigs were placed on riboflavin-unsupplemented diets and was obviously lower after 12 days of consuming the riboflavin-unsupplemented diets than the erythrocyte percent active glutathione reductase of pigs fed the riboflavin-supplemented diets. Percent active erythrocyte glutathione reductase values of as low as 50% were needed before other riboflavin deficiency signs became evident.

Supplementation of diets with riboflavin (10 mg/kg) resulted in increased liver and erythrocyte glutathione peroxidase activity and decreased kidney and muscle glutathione peroxidase activity in trials one and two. The selenium concentration of liver and heart were increased, while plasma selenium levels were decreased by riboflavin-supplementation.

Riboflavin-supplementation and selenium source did not affect apparent selenium absorption. However, riboflavin supplementation did

decrease urinary selenium and concomitantly increased the apparent retention of selenium. Urinary selenium excretion was decreased when selenomethionine was used as the selenium source rather than sodium selenite. As a result more selenium was apparently retained when selenomethionine was fed.

In trial four riboflavin supplementation increased kidney, muscle, heart and brain glutathione peroxidase activity when sodium selenite was fed as the selenium source, but not when selenomethionine was fed as the selenium source. Tissue selenium concentrations were not affected by riboflavin supplementation of diets. Feeding selenomethionine as the supplemental selenium source at 67% of the level of selenium from sodium selenite resulted in higher muscle, heart and brain selenium concentrations than feeding the higher level of selenium as sodium selenite.

Serum glutathione peroxidase activity was more responsive to dietary selenium intake than erythrocyte glutathione peroxidase activity. When pigs were reared under practical conditions, serum glutathione peroxidase activity plateaued at 0.35 ppm selenium in a corn-soybean meal diet at two weeks postweaning. Gains, feed intake and feed efficiency were not affected by dietary selenium level.

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SECTION I

Introduction

The glutathione peroxidase system requires the mineral selenium, the sulfur amino acid cysteine, and the two water soluble vitamins riboflavin and niacin. Glutathione peroxidase (EC 1.11.1.9) is a selenium containing enzyme that has been isolated from erythrocytes (Rotruck et al., 1972a and 1973), plasma (Burk and Gregory, 1980), liver (Sunde et al., 1978 and Burk and Gregory, 1980) and muscle (Sunde et al., 1978). Glutathione peroxidase normally exists as a tetramer (Awasthi et al., 1975; Sunde et al., 1978; and Ladenstein et al., 1979) which contains four atoms of selenium per molecule of enzyme. The active site of the enzyme appears to involve the selenocysteine moities present in the molecule (Tappel et al., 1978a, and Ladenstein et al., 1979).

Glutathione is a cysteine containing tripeptide (γ-glutamylcysteinylglycine) that provides reducing equivalents for the GSH-Px catalyzed reduction of lipid peroxides or hydrogen peroxide to hydroxy acids or water to protect tissues from oxidative damage (Mills, 1957; and Mills and Randall, 1958). The sulfhydryl group of glutathione is the active site (Styer, 1975). Niacin as a component of nicotinamide adenine dinucleotide phosphate (NADPH) is essential to provide reducing equivalents to reduce oxidized glutathione (Jacob and Jundl, 1966; Eggleston and Krebs, 1974).

Glutathione reductase (EC 1.6.4.2) is a flavoprotein (Buzard and Kopko, 1963) that contains two flavin adenine dinucleotide (FAD) molecules per molecule of enzyme (Staal et al., 1969). It catalyzes the reduction of oxidized glutathione using reduced nicotinamide adenine

dinucleotide phosphate as a source of reducing equivalents. The erythrocyte glutathione reductase activity coefficient has been used as an index of riboflavin status in rats (Glatzle et al., 1970 and Tillotsen et al., 1972), man (Glatzle, 1968 and Sauberlich et al., 1972) and pigs (Brady et al., 1979).

Since riboflavin is a relatively unstable vitamin, and since unsupplemented practical swine diets are generally deficient in riboflavin (Pond and Maner, 1974), it is possible that a riboflavin deficiency may exist. A riboflavin deficiency could cause an apparent selenium deficiency by decreasing the glutathione reductase activity and consequently lowering the supply of reduced glutathione available to the glutathione peroxidase enzyme. In an attempt to test this hypothesis, Brady et al. (1979) reported decreased in vitro hepatic glutathione peroxidase activity and a decreased hepatic selenium content in young pigs on riboflavin-deficient diets. They did find the expected erythrocyte glutathione reductase response to dietary riboflavin, but hepatic and muscle in vitro glutathione reductase activity was not affected by riboflavin supplementation.

Studies by Groce et al. (1973a) have shown that a lower proportion of absorbed selenium from seleniferous corn was excreted in urine than from sodium selenite. Increased tissue selenium levels have been reported by Mahan and Moxon (1978b) and Mandisodza et al. (1979) when organic selenium sources were fed rather than sodium selenite.

Four trials were conducted to further study the effects of riboflavin supplementation and selenium source on the absorption and metabolism of selenium. Also the effects of riboflavin supplementation and

selenium source on the glutathione peroxidase system were examined.

Materials and Methods

Eight one-week old pigs weighing 2.84 ± .19 kg (mean ± standard error) were randomly allotted to either the riboflavin-deficient diet shown in table 1 or a riboflavin-supplemented diet. The riboflavin-supplemented diet (10 mg/kg of diet) was mixed by adding riboflavin to the selenium chromium premix. In trial one sodium selenite was used as the selenium source. The riboflavin-unsupplemented diet contained .17 ppm selenium, and the riboflavin-supplemented diet contained .20 ppm selenium. Pigs fed the riboflavin-supplemented diet were pair fed to pigs fed the riboflavin-deficient diet. Feeding was done twice daily and pigs were trained to rapidly consume a mixture of one part diet and one part water.

Pigs were weighed and blood samples taken every three days for twelve days. Erythrocytes were obtained by centrifugation and washed three times in ice cold saline (.9% NaCl). Erythrocytes were used for determination of percent active glutathione reductase. After eighteen days on the diets all pigs were killed. Liver, kidney, heart, muscle, brain and erythrocyte samples were frozen for glutathione peroxidase and glutathione reductase activity assays. Liver, muscle, heart, brain and plasma samples were taken for selenium analysis.

Liver, kidney, heart, muscle and brain samples were homogenized in isotonic saline and centrifuged to obtain the cytosolic fraction for enzyme activity determinations. Erythrocytes were hemolyzed in double strength Drabkins Solution for enzyme assays.

Glutathione peroxidase activity was determined by the method of Paglia and Valentine (1967) using hydrogen peroxide as the substrate.

TABLE 1. COMPOSITION OF THE RIBOFLAVIN-DEFICIENT DIET

Ingredient	Internat'l. ref. no.	g/kg diet
Casein, vitamin free[1]	5 01 162	300
Glucose[2]	4 02 125	471
α-Cellulose[3]		50
Mineral premix[4]		60
Lard	4 04 790	50
Vitamin premix[5]		30
Corn oil	4 07 882	10
Selenium/chromium premix[6]		29
		1000

[1]"Vitafree", United States Biochemical Corporation, Cleveland, OH.

[2]"Dextrose 2001", CPC International, Englewood Cliffs, NJ.

[3]"Solka-Floc", Brown Co., Berlin, NH.

[4]Provided per kg diet: 6.0 g KCl, 1.2 mg KI, 630 mg $FeSO_4.7H_2O$, 30 mg $CuSO_4$, 60 mg $CoCO_3$, 60 mg $MnSO_4.H_2O$, 240 mg $ZnSO_4.H_2O$, 1.2 g $MgCO_3$, 15.0 g $NaHCO_3$, 21.6 g $CaHPO_4.2H_2O$, 7.5 g $CaCO_3$, 7.7 g cerelose.

[5]Provided per kg diet: 3 mg thiamin mononitrate, 40 mg nicotinamide, 30 mg D-calcium pantothenate, 2 mg pyridoxine hydrochloride, 13 mg p-aminobenzoate, 80 mg ascorbic acid, 130 mg myo-inositol, 1.3 g choline chloride, 260 µg folate, 50 µg biotin, 100 µg cyanocobalamin (0.1% triturate in mannitol), 20 mg D, L-α-tocopheryl acetate, 40 µg menadione, 1.5 mg retinyl palmitate, 12.5 µg ergocalciferol.

[6]Provided per kg diet: 2 mg Cr.

Glutathione reductase activity was determined by the method described by Glatzle et al. (1970). Total glutathione reductase activity was determined by saturating the reaction mixture with FAD and incubating at 37°C for seven minutes, prior to the start of the reaction. Selenium concentrations were determined by a modification of the Olsen method (Whetter and Ullrey, 1978).

In trial two, eight one-week old pigs weighing 2.75 ± .15 kg (mean ± standard error) were randomly allotted to a riboflavin-deficient or a riboflavin-supplemented diet containing 10 mg of supplemental riboflavin per kg of diet. The diets used in trial two were the same as the diets used in trial one, except the selenium source was DL-selenomethionine (Calbiochem-Behring Corp., LaJolla, CA). The riboflavin-deficient diet contained .50 ppm selenium and the riboflavin-supplemented diet contained .54 ppm selenium. All other procedures in trial two were the same as in trial one.

Statistical analysis of data from trials one and two was done as a two by two factorial design. The main effects were trial and riboflavin supplementation. Selenium source and trial were confounded. Significance was determined using the F-test (Gill, 1978a). Erythrocyte total, active and percent active glutathione reductase activities were analyzed as a split-plot design (Gill, 1978b).

In a third trial twelve three-week old pigs weighing 5.6 ± .13 kg (mean ± standard error) were randomly allotted to a selenium balance study. The main effects of the study were riboflavin supplementation and selenium source. The pigs were fed three times per day in individual feeding cages and were returned to individual collection cages as soon as they completed the meal. Seventy-five grams of a basal diet,

which was similar to the diet in table 1, but contained no supplemental selenium or riboflavin, were fed each morning. A 25 g meal containing 0.11 mg of selenium as sodium selenite or selenomethione and 0 or 2 mg of supplemental riboflavin was fed each day at noon. Treatment diets were mixed by adding riboflavin and selenium to the chromium premix. A third meal of 50 g of the basal diet was fed in the evening. This approach was taken to minimize the problems associated with the decreased feed intake of pigs on the riboflavin-deficient diets that was experienced in trials one and two. Each meal was mixed with an equal part of water and pigs were trained to rapidly consume the slurry. After a nine day adaptation period a three day total urine and fecal collection was made on each pig. Feces were dried in a drying oven, weighed and ground for selenium analysis. Urine was weighed and an aliquot was taken for selenium analysis. All urine, feces and feed selenium concentrations were determined by a modification of the Olson method (Whetter and Ullrey, 1978).

In trial four, sixteen pigs weighing 4.74 ± .50 kg (mean ± standard error of treatment means) that had been weaned and started on a corn-soybean meal diet for one week, were randomly allotted to a 2 x 2 factorial design. The main effects were riboflavin supplementation (0 or 2 mg/day) and supplemental selenium source. Since selenium retention results of pigs fed riboflavin supplemented diets in trial three indicated the selenium from the sodium selenite supplemented diet was only 67% as available as selenium from the selenomethionine supplemented diet, the supplemental selenium intake as selenomethionine was 67% of the supplemental selenium intake from sodium selenite in trial four (.12 mg/day as selenomethionine or .18 mg/day as sodium selenite).

Total daily selenium intakes were .13 mg and .19 mg respectively for pigs fed the selenomethione supplemented diet and the sodium selenite supplemented diet, respectively. Pigs were fed for fourteen days by the system described in trial three. On the fifteenth day all pigs were bled, and serum samples were obtained for selenium analysis and erythrocytes were obtained to determine the percent active glutathione reductase. Pigs were then killed and liver, muscle, kidney, heart and brain samples were frozen for selenium, glutathione peroxidase activity and glutathione reductase activity determinations. The methods of determination were the same as those described in trial one.

Data from trials three and four were analyzed as 2 x 2 factorial designs (Gill, 1978a). The main effects were selenium source and riboflavin supplementation.

Results and Discussion

Trials one and two. Pigs fed diets supplemented with 10 mg of riboflavin per kg of diet gained significantly faster ($P<.01$) and were heavier than pigs fed the riboflavin-unsupplemented diets after eighteen days on the trial ($P<.08$). Pigs fed the riboflavin-unsupplemented diets gained 74.3 g/day, while pigs fed the riboflavin-supplemented diets gained 115.5 g/day (see table 2). Krider et al. (1949) and Forbes and Haines (1952) reported an increase in gains of starter pigs with riboflavin supplementation of diets. Miller et al. (1954) reported an increase in gain with increasing riboflavin supplementation up to 3 mg/kg. Feed efficiency was improved by riboflavin supplementation in studies of Krider et al. (1949) and Miller et al. (1954). Terrill et al. (1953), however, found a more variable response in feed efficiency.

TABLE 2. THE EFFECT OF SUPPLEMENTAL RIBOFLAVIN ON BODY WEIGHT AND GAINS OF PIGS

	Trial I		Trial II				
	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect		
Item	0(mg/kg)	10	0	10	0	10	S.E.M.[a]
Initial weight, kg	2.99	2.70	2.67	2.84	2.83	2.77	.27
Final weight[b], kg	4.26	4.66	4.07	5.03	4.17	4.85	.37
Average daily gain[c], g/day	70.4	109.0	78.1	122.0	74.3	115.5	7.2

[a]Standard error of treatment means.

[b]Supplemental riboflavin effect, $P<.08$.

[c]Supplemental riboflavin effect, $P<.01$.

Erythrocyte active glutathione reductase values (table 3) and percent active glutathione reductase values (table 5) were lower ($P<.01$) for pigs fed the riboflavin-unsupplemented diets. This depression in activity has been described in rats (Glatzle et al., 1968), humans (Glatzel et al., 1970) and pigs (Brady et al., 1979). However, Brady et al. (1979) reported that erythrocyte percent active glutathione reductase plateaued at 75% when pigs were fed 4 mg of riboflavin per kilogram of diet. The values reported here for pigs fed the supplemented diet appear to be lower than that value and are considerably lower than the 80% value suggested by Glatzle et al. (1970) as a minimum percent active erythrocyte glutathione reductase for human subjects on riboflavin-sufficient diets.

There was a decline in total ($P<.01$) (table 4), active ($P<.01$) (table 3) and percent active ($P<.02$) (table 5) erythrocyte glutathione reductase activity, however, more importantly there was an interaction of time and riboflavin supplementation for active ($P<.01$) and percent active ($P<.09$) erythrocyte glutathione reductase activity. During the course of the study active erythrocyte glutathione reductase activity was maintained close to the initial activity for pigs fed riboflavin-supplemented diets, but active erythrocyte glutathione reductase activity was lowered by feeding unsupplemented diets. The percent active erythrocyte glutathione reductase generally declined throughout the study for pigs fed the riboflavin-unsupplemented diets, while values for pigs fed riboflavin-supplemented diets did not change or increased slightly. These results were expected since erythrocyte active glutathione reductase activity in the pig has been shown to be responsive to

TABLE 3. THE EFFECTS OF SUPPLEMENTAL RIBOFLAVIN AND DAYS ON TRIAL ON ACTIVE ERYTHROCYTE GLUTATHIONE REDUCTASE ACTIVITY[a] OF PIGS

Days on trial[bce]	Trial I Supplemental riboflavin		Trial II Supplemental riboflavin		Mean supplemental riboflavin effect[b,e]		
	0(mg/kg)	10	0	10	0	10	Mean
0	16.4	16.6	13.7	12.4	15.1	14.5	14.8
3	7.3	11.8	7.8	12.7	7.6	12.3	9.9
6	7.7	13.1	7.4	10.6	7.6	11.9	9.7
9	6.9	16.2	6.9	14.3	6.9	15.3	11.1
12	3.7	12.4	4.7	10.7	4.2	11.6	7.9
Mean	8.4	14.0	8.1	12.1	8.3	13.1	

[a]Expressed as micromoles of glutathione reduced/minute/mg of hemoglobin.

[b]Standard error of treatment means = .67; standard error of period means = .66.

[c]Time on trial effect, $P<.01$.

[d]Supplemental riboflavin effect, $P<.01$.

[e]Supplemental riboflavin x time interaction, $P<.01$.

TABLE 4. THE EFFECTS OF SUPPLEMENTAL RIBOFLAVIN AND DAYS ON TRIAL ON TOTAL ERYTHROCYTE GLUTATHIONE REDUCTASE ACTIVITY[a] OF PIGS

Days on trial[b,c]	Trial I Supplemental riboflavin 0(mg/kg)	10	Trial II Supplemental riboflavin 0	10	Mean supplemental riboflavin effect[b] 0	10	Mean
0	26.2	26.3	25.3	21.6	25.8	24.0	24.9
3	14.8	19.5	17.3	17.5	16.1	18.5	17.3
6	19.6	18.9	17.6	14.0	18.6	16.5	17.5
9	17.6	28.6	16.8	21.4	17.2	25.0	21.1
12	20.0	21.9	25.1	19.5	22.6	20.7	21.6
Mean	19.6	23.0	20.4	18.8	20.1	20.9	

[a]Expressed as micromoles of glutathione reduced/minute/mg of hemoglobin.

[b]Standard error of treatment means = 1.4; standard error of period means = 1.3.

[c]Time on trial effect, $P<.01$.

TABLE 5. THE EFFECTS OF SUPPLEMENTAL RIBOFLAVIN AND DAYS ON TRIAL ON ERYTHROCYTE PERCENT ACTIVE GLUTATHIONE REDUCTASE[a] OF PIGS

	Trial I		Trial II				
Days on	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect[b,d,e]		
trial[b,c,e]	0(mg/kg)	10	0	10	0	10	Mean
0	62.9	65.0	54.9	57.7	58.9	61.4	60.2
3	50.9	62.3	46.7	72.9	48.8	67.6	58.2
6	41.9	69.5	42.2	75.3	42.1	72.4	52.3
9	39.9	68.2	45.3	67.7	42.6	68.0	55.3
12	19.2	57.2	18.0	57.0	18.6	57.1	37.9
Mean	43.0	64.4	41.4	66.1	42.2	65.3	

[a]All values expressed as percent.

[b]Standard error of treatment means = 2.4; standard error of period means = 4.2.

[c]Time on trial effect, $P<.02$.

[d]Supplemental riboflavin effect, $P<.01$.

[e]Time on trial x supplemental riboflavin interaction, $P<.09$.

dietary riboflavin (Brady et al., 1979). Furthermore, it is interesting to note the speed with which the erythrocyte active glutathione reductase activity responds to the lack of dietary riboflavin. Baker et al. (1966) reported that the clearance of circulating riboflavin was very rapid. In subjects judged to have adequate circulating riboflavin, the half-time for clearance of a 5 mg dose of riboflavin was fourteen minutes, but subjects with low circulating riboflavin cleared half of the dose in six minutes. They indicated that the increased clearance in deficient patients was due to greater avidity of tissues for administered riboflavin.

Riboflavin supplementation significantly decreased actual liver weight ($P<.07$) and liver weight as a percent of body weight ($P<.01$) (table 6). Also, pituitary weight was decreased ($P<.08$) by riboflavin supplementation. Heart ($P<.03$) and brain ($P<.02$) weights were increased by supplemental dietary riboflavin, but when expressed as a percent of body weight there was no consistent trend.

Supplementation of diets with riboflavin resulted in a 22% increase ($P<.12$) in liver glutathione peroxidase activity (table 7) and an increase in erythrocyte glutathione peroxidase activity ($P<.02$). However, riboflavin supplementation lowered kidney ($P<.04$) and muscle ($P<.08$) glutathione peroxidase activity. Glutathione peroxidase activity of heart and brain were not affected by level of riboflavin in the diet. Plasma selenium concentration (table 8) was decreased ($P<.05$) by riboflavin supplementation, while liver ($P<.11$) and heart ($P<.01$) selenium concentrations were increased. The selenium concentration of muscle and brain were not affected by riboflavin supplementation. It is interesting to note the interaction ($P<.08$) of trials and dietary riboflavin on heart selenium concentration. Pigs in trial two which

TABLE 6. THE EFFECTS OF SUPPLEMENTAL RIBOFLAVIN ON THE ACTUAL WEIGHT AND PERCENT OF BODY WEIGHT OF SELECTED ORGANS

	Trial I		Trial II					
	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect		Significance of riboflavin	
Item	0(mg/kg)	10	0	10	0	10	effect	S.E.M.[a]
Liver								
weight,g	112.0	88.2	117.0	101.0	114.5	94.6	.07	10.1
% of body weight	2.72	1.90	2.92	2.03	2.82	1.97	.01	.32
Kidney								
weight,g	31.3	30.6	28.5	35.2	29.9	32.9	.18	2.2
% of body weight	.75	.66	.70	.71	.73	.69	—	.05
Heart								
weight,g	20.7	22.5	19.6	26.4	20.2	24.5	.03	1.8
% of body weight	.49	.48	.48	.53	.49	.51	.11	.01
Brain								
weight,g	43.6	46.1	42.2	47.2	42.9	46.7	.02	1.5
% of body weight	1.05	1.00	1.05	.95	1.05	.98	.28	.06
Pituitary								
weight,mg	55.8	38.8	51.3	50.5	53.6	44.7	.08	4.8

[a]Standard error of treatment means.

TABLE 7. THE EFFECT OF SUPPLEMENTAL RIBOFLAVIN ON LIVER, KIDNEY, MUSCLE, HEART, BRAIN AND ERYTHROCYTE GLUTATHIONE PEROXIDASE ACTIVITY

	Trial I		Trial II					
	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect		Significance of riboflavin	
Item	0(mg/kg)	10	0	10	0	10	effect	S.E.M.[a]
Liver[b]	1.68	2.06	1.71	2.07	1.70	2.07	.12	.23
Kidney[b]	1.53	1.26	2.39	1.19	1.96	1.23	.04	.34
Muscle[c]	179.0	76.2	161.0	74.3	170.0	75.3	.08	16.4
Heart[c]	257.0	264.0	453.0	373.0	355.0	318.5	—	84.3
Brain[c]	497.0	285.0	567.0	611.0	532.0	448.0	—	62.8
Erythrocyte[d]	11.4	16.8	13.3	27.4	12.4	22.1	.02	3.7

[a]Standard error of treatment means.

[b]Values expressed as millimoles of glutathione oxidized/minute/mg of protein.

[c]Values expressed as micromoles of glutathione oxidized/minute/mg of protein.

[d]Values expressed as micromoles of glutathione oxidized/minute/g of hemoglobin.

TABLE 8. THE EFFECT OF SUPPLEMENTAL RIBOFLAVIN ON LIVER, MUSCLE, HEART, BRAIN AND PLASMA SELENIUM CONCENTRATION OF PIGS

	Trial I		Trial II					
	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect		Significance of riboflavin	
Item	0(mg/kg)	10	0	10	0	10	effect	S.E.M.[a]
Liver, μg/g[b]	1.05	1.75	.77	1.18	.91	1.47	.11	.46
Muscle, μg/g[b]	.06	.06	.08	.08	.07	.07	—	.07
Heart, μg/g[b]	.23	.27	.25	.36	.24	.32	.01	.02
Brain, μg/g[b]	.21	.22	.24	.24	.23	.23	—	.09
Plasma, μg/ml	.18	.15	.17	.15	.18	.15	.05	.01

[a]Standard error of treatment means.

[b]Wet tissue basis.

were fed selenomethionine as a supplemental selenium source tended to accumulate selenium in heart tissue when the diet was supplemented with riboflavin.

Erythrocyte percent active glutathione reductase was increased ($P<.01$) by feeding the riboflavin-supplemented diets for eighteen days. Values at the end of the trial for supplemented pigs are similar to values reported by Brady et al. (1979) for pigs on an adequately supplemented diet (table 9). Also, brain glutathione reductase activity was increased ($P<.04$) by dietary supplementation with riboflavin. In trial one muscle glutathione reductase was not affected by riboflavin, but in trial two when selenomethionine was fed, muscle glutathione reductase activity was decreased ($P<.05$) by riboflavin supplementation. Heart glutathione reductase activity was elevated when diets were supplemented with riboflavin in trial one, but activity was depressed by supplementation in trial two ($P<.01$).

The effects of riboflavin supplementation on liver selenium content, glutathione peroxidase activity and glutathione reductase activity are generally in good agreement with the trends reported by Brady et al. (1979). They reported more variable results with these parameters in muscle but these current data appear to give trends similar to those which they reported.

Trial three. During the three day collection period selenium intakes ranged from 110 µg/day to 116 µg/day (table 10). There was no effect of either dietary treatment (supplemental riboflavin or selenium source) on fecal selenium concentration and fecal excretion, and consequently apparent selenium absorption was not affected. Urinary selenium concentration ($P<.03$) and urinary excretion ($P<.01$) were

TABLE 9. THE EFFECT OF SUPPLEMENTAL DIETARY RIBOFLAVIN FOR EIGHTEEN DAYS ON LIVER, KIDNEY, MUSCLE, HEART AND BRAIN GLUTATHIONE REDUCTASE ACTIVITY AND PERCENT ACTIVE ERYTHROCYTE GLUTATHIONE REDUCTASE

	Trial I		Trial II					
	Supplemental riboflavin		Supplemental riboflavin		Mean supplemental riboflavin effect		Significance of riboflavin	
Item	0(mg/kg)	10	0	10	0	10	effect	S.E.M.[a]
Glutathione reductase activity, micromoles of glutathione reduced/mg protein/minute								
Liver	307	323	265	305	286	314	.32	27
Kidney	300	342	316	275	308	309	—	28
Muscle	6.10	6.12	7.42	5.31	6.76	5.72	.06	.51
Heart	98	134	131	101	115	118	—	11
Brain	42.1	50.2	38.8	48.4	40.5	49.3	.04	4
Percent active glutathione reducatase, %								
Erythrocyte	41.6	70.4	23.7	71.8	32.7	71.1	.01	6.9

[a]Standard error of treatment means.

TABLE 10. THE EFFECT OF DIETARY RIBOFLAVIN SUPPLEMENTATION AND SELENIUM SOURCE ON SELENIUM BALANCE

Selenium source	Sodium selenite		Seleno-methionine		Mean supplemental riboflavin effect		Level of significance	Mean source effect: Sodium selenite	Mean source effect: Seleno-methionine	Level of significance	S.E.M.[a]
Supplemental riboflavin (mg/day)	0	2	0	2	0	2					
Parameter											
Basal diet consumed, g	375	375	375	375							
Treatment diet consumed, g	75	75	75	75							
Total selenium intake, µg	346.95	337.63	328.88	337.33							
Feces, g	28.3	22.7	24.7	25.3	26.5	24.0	--	25.5	25.0	--	4.8
Fecal selenium concentration, µg/g	2.07	1.90	1.92	1.45	2.00	1.68	0.20	1.99	2.69	0.24	.23
Fecal selenium, µg	58.18	42.06	45.15	49.41	51.66	39.65	0.18	50.12	41.19	0.31	8.20
Apparent absorbed selenium, µg	288.77	295.56	283.73	300.09	286.25	297.82	0.20	292.17	291.91	--	8.20
Urine, g	2075.7	1224.7	1990.0	1565.3	2033.8	1395.0	0.17	1650.2	1777.7	--	425.3
Urine selenium concentration, µg/ml	.103	.125	.036	.066	.082	.095	--	.114	.064	0.03	.018
Urinary selenium, µg	203.18	148.04	105.47	84.09	157.19	116.06	0.09	178.47	94.78	0.01	21.54
Apparent retained selenium, µg	79.86	147.53	178.26	216.00	129.06	181.76	0.06	113.70	197.13	0.01	24.37
Apparent % absorbed	83.2	87.5	86.3	89.0	84.7	88.3	0.19	85.4	87.6	0.38	2.4
Apparent % retained	23.0	43.7	54.2	64.8	38.6	53.7	0.07	33.4	59.1	0.01	7.2
% of absorbed selenium retained	32.0	49.6	62.6	71.6	47.3	60.6	0.08	40.8	67.1	0.01	6.6

[a]Standard error of treatment means.

generally decreased when selenomethionine was used as the selenium source, instead of sodium selenite. As a result more selenium was retained ($P<.01$) when selenomethionine was used. It appears from these data that more of the absorbed selenium from selenomethionine is incorporated into tissues than from sodium selenite.

Groce et al. (1973a) reported that a smaller proportion of ingested selenium was excreted in urine when seleniferous corn was fed as a selenium source rather than sodium selenite. In both studies higher fecal selenium losses were reported when seleniferous corn was fed rather than sodium selenite. However, Groce et al. (1973a) reported a decrease in fecal selenium loss when the seleniferous corn diet was supplemented with vitamin E.

The selenium content of muscular tissues was higher in pigs fed diets in which the selenium sources were distillers grains or fish meal than in pigs fed corn-soybean meal diets containing sodium selenite (Mahan and Moxon, 1978b). Mandisodza et al. (1979) reported higher kidney, liver and skeletal muscle selenium concentrations when seleniferous white clover was fed rather than sodium selenite. The metabolic significance of the higher retention of selenium from organic sources is not clear, since Wang and Spallholz (1980) reported that selenomethionine restored rat liver glutathione peroxidase activity much more slowly than selenite or high selenium yeast in selenium depleted rats. Yet, Schwartz and Foltz (1958) reported that sodium selenite and D-L selenomethione had similar factor 3 activity in rats. Recently, Sunde and Hoekstra (1980a) have shown with perfused rat liver that selenite and selenide are metabolically closer than selenocysteine to the immediate selenium precursor used for glutathione peroxidase synthesis.

In another study Sunde et al. (1981) reported that low dietary methionine levels decreased the biopotency of selenomethionine in rapidly growing rats.

Riboflavin supplementation did not significantly affect the apparent absorption of selenium. However, it did decrease the urinary selenium loss ($P<.09$) and concomitantly increased the apparent retention of selenium ($P<.06$) (table 10). Riboflavin supplementation appeared to improve the efficiency of selenium incorporation into body tissues. Tappel (1978b) has proposed that glutathione reductase re- duces selenite to hydrogen selenide, which is then incorporated into protein to form glutathione peroxidase. Since FAD is a cofactor for glutathione reductase, this mechanism may be responsible for the increase in selenium retention when diets were supplemented with riboflavin. However, since Tappel (1978b) has suggested that selenomethionine is converted to selenocysteine, before being incorporated into glutathione peroxidase and since there was no significant interaction of riboflavin supplementation and selenium source on selenium retention, it seems unlikely the increased selenium retention is due strictly to riboflavin stimulated glutathione peroxidase synthesis.

Trial four. In trial four the pigs fed the riboflavin-unsupplemented diets did not show the expected growth depression (table 11) as was reported by Krider et al. (1949), Forbes and Haines (1952) and Miller et al. (1954). However, Terrill et al. (1953) did report an inconsistant growth response to riboflavin supplementation. Although the erythrocyte percent active glutathione reductase of pigs fed the riboflavin-unsupplemented diets was lower ($P<.03$) than that of pigs fed the riboflavin-supplemented diets (table 12), the values for the

TABLE II. THE EFFECT OF SUPPLEMENTING WITH EQUAL AVAILABLE SELENIUM INTAKES FROM SODIUM SELENITE OR SELENOMETHIONINE AND RIBOFLAVIN SUPPLEMENTATION ON BODY WEIGHT, GAINS AND SELECTED ORGAN WEIGHTS OF PIGS

	Selenium source and intake				Level of significance			
	Sodium selenite (.18 mg/day)		Selenomethionine (.12 mg/day)					
	Supplemental riboflavin		Supplemental riboflavin		Riboflavin	source	inter-	
Item	0 (mg/day)	2	0	2	effect	effect	action	S.E.M.[a]
Initial body weight, kg	4.65	4.87	4.71	4.75				.50
Final body weight, kg	5.69	6.13	6.16	6.06				.57
Average daily gain, g	69.2	84.0	96.7	87.7			.26	13.9
Liver								
weight, g	114	112	121	120		.25		6.6
% of body weight	2.05	1.84	2.00	2.03				13.84
Kidney								
weight, g	27.2	29.1	34.9	31.5		.08		2.8
% of body weight	.49	.48	.57	.52	.31	.06		.03
Heart								
weight, g	22.6	28.4	27.6	27.8	.23		.26	2.5
% of body weight	.41	.46	.45	.46	.06	.32	.14	.02
Brain								
weight, g	5.46	5.39	5.08	5.51	.32		.18	1.78
% of body weight	.99	.89	.84	.95			.27	.09

[a]Standard error of treatment means.

unsupplemented pigs were higher in this trial than in trials one and two, thus, indicating that the pigs on the unsupplemented diet in this study may not have been as depleted of riboflavin at the end of the trial as pigs in trials one and two.

Liver and brain actual weight and as a percent of body weight were not significantly affected by either dietary treatment (table 11). Kidney weight ($P<.08$) and as percent of body weight ($P<.06$) was increased when selenomethionine was fed rather than sodium selenite. Heart weight was not affected by treatments, but as a percent of body weight heart was heavier for pigs fed the riboflavin-supplemented diets ($P<.06$). These tissue weight results show a less severe effect of feeding the riboflavin-unsupplemented diet than was reported in trials one and two.

Erythrocyte percent active glutathione reductase was increased by riboflavin supplementation ($P<.03$). Heart glutathione reductase activity tended to be increased by riboflavin supplementation ($P<.14$), but liver, kidney, muscle and brain activities were not affected (table 12). This is similar to the results of Brady et al. (1979) for erythrocytes, liver and muscle. The selenium source fed did not affect glutathione reductase activities in any of the tissues that were assayed.

There was no significant effect of either dietary treatment on tissue glutathione peroxidase activity. However, there were interactions of selenium source and riboflavin supplementation for kidney ($P<.03$), muscle ($P<.03$), heart ($P<.08$) and brain ($P<.01$) indicating that riboflavin supplementation increases incorporation of selenium

TABLE 12. THE EFFECTS OF SUPPLEMENTING WITH EQUAL AVAILABLE SELENIUM INTAKE FROM SODIUM SELENITE OR SELENOMETHIONINE AND RIBOFLAVIN SUPPLEMENTATION ON LIVER, KIDNEY, MUSCLE, HEART AND BRAIN GLUTATHIONE REDUCTASE ACTIVITY, AND ERYTHROCYTE PERCENT ACTIVE GLUTATHIONE REDUCTASE OF PIGS

	Selenium source and intake							
	Sodium selenite (.18 mg/day)		Selenomethionine (.12 mg/day)		Level of significance			
	Supplemental riboflavin		Supplemental riboflavin					
Item	0 (mg/day)	2	0	2	Riboflavin effect	Source effect	Interaction	S.E.M.[a]
Liver, EU/g protein[b]	412	401	359	402		.28	.26	23
Kidney, EU/g protein[b]	437	385	380	378				40
Muscle, EU/g protein[b]	6.35	6.63	7.13	6.35				.88
Heart, EU/g protein[b]	33.8	34.8	34.3	39.3	.14	.22	.32	2
Brain, EU/g protein[b]	14.5	15.0	14.0	13.0		.30		1.2
Erythrocyte								
% active, %	60.1	77.3	62.1	73.6	.03			5.6

[a]Standard error of treatment means.

[b]EU = 1 micromole of NADPH oxidized/minute.

from sodium selenite into glutathione peroxidase (table 13). As discussed earlier, Tappel (1978b) has suggested that the flavoprotein glutathione reductase is involved in glutathione peroxidase synthesis from sodium selenite. The interaction observed here of selenium source and riboflavin supplementation for kidney, muscle, heart and brain tends to support the hypothesis that glutathione reductase is involved in the incorporation of selenium from sodium selenite into glutathione peroxidase, since riboflavin supplementation increased tissue *in vitro* glutathione peroxidase activity when sodium selenite was used as the supplemental selenium source, but did not increase *in vitro* glutathione peroxidase activity when selenomethionine was fed as the supplemental selenium source. *In vitro* glutathione reductase activity of these tissues was not greatly affected by riboflavin supplementation, however, Baker *et al.* (1966) reported that tissues avidly take up circulating riboflavin when tissue levels are low and that tissues are very slowly depleted of riboflavin when a deficient diet is fed. Consequently, tissue riboflavin levels would be expected to decline more slowly than circulating riboflavin levels when a riboflavin-deficient diet is fed. This was observed in this study as the percent active glutathione reductase was depressed ($P<.03$) when the riboflavin-unsupplemented diet was fed to the pigs, however, other tissue (liver, kidney, muscle, heart and brain) glutathione reductase activities were not affected by riboflavin supplementation. From these results it appears that glutathione reductase may be involved in the incorporation of selenium from sodium selenite into glutathione peroxidase.

Tissue selenium concentrations were not affected by riboflavin supplementation (table 14). Muscle ($P<.01$), heart ($P<.05$) and brain

($P<.01$) selenium levels were higher for pigs fed diets with selenomethionine rather than sodium selenite as the selenium source. In trial three the selenomethionine-supplemented diet with supplemental riboflavin was 1.5 times more effective as a selenium source, based on selenium retention, than the sodium selenite supplemented diet with supplemental riboflavin. In this trial the selenium levels of the sodium selenite-supplemented diets were 1.5 times the selenium levels of the selenomethionine supplemented diets. The selenium levels in muscle ($P<.05$), heart ($P<.01$) and brain ($P<.01$) for pigs fed selenomethionine supplemented diets are higher than those fed sodium selenite supplemented diets. Mahan and Moxon (1978b) and Mandisodza et al. (1980) have also reported higher tissue selenium concentrations when pigs were fed organic selenium sources rather than sodium selenite.

Since glutathione peroxidase activities for muscle, heart and brain were not increased when selenomethionine was fed as the supplemental selenium source rather than sodium selenite, it appears that selenomethionine must be incorporated into some other protein in these tissues. Black et al. (1978) have identified two selenium containing proteins in ovine heart, muscle, kidney and liver, but have reported that glutathione peroxidase activity was only associated with one of these peaks. Beilstein et al. (1980) have separated three selenium containing proteins from lamb heart and muscle. Based on chromatographic results the selenium in these proteins appears to be selenocysteine. Excess selenomethionine may be converted to selenocysteine and incorporated into proteins other than glutathione peroxidase in some tissues. This could account for the increased tissue selenium

TABLE 13. THE EFFECTS OF SUPPLEMENTING WITH EQUAL AVAILABLE SELENIUM INTAKE FROM SODIUM SELENITE OR SELENOMETHIONINE AND RIBOFLAVIN SUPPLEMENTATION ON LIVER, KIDNEY, MUSCLE, HEART, BRAIN AND SERUM GLUTATHIONE PEROXIDASE ACTIVITY OF PIGS

	Selenium source and intake				Level of significance			
	Sodium selenite (.18 mg/day)		Selenomethionine (.12 mg/day)					
	Supplemental riboflavin		Supplemental riboflavin		Riboflavin	Source	Inter-	
Item	0 (mg/day)	2	0	2	effect	effect	action	S.E.M.[a]
Liver, EU/g protein[b]	900	1140	967	1070				196
Kidney, EU/g protein[b]	495	723	1040	564		.20	.03	150
Muscle, EU/g protein[b]	51.3	70.5	90.0	59.5		.21	.03	11
Heart, EU/g protein[b]	55.3	100.0	152.0	69.3			.08	34.6
Brain, EU/g protein[b]	31.0	69.5	103.0	27.5			.01	20.5
Serum, EU/g protein[a]b	28.2	26.3	29.2	26.7		.32		2.3

[a]Standard error of treatment means.

[b]EU = 1 micromole of NADPH oxidized/minute.

TABLE 14. THE EFFECTS OF SUPPLEMENTING WITH EQUAL AVAILABLE SELENIUM INTAKE FROM SODIUM SELENITE OR SELENOMETHIONE AND RIBOFLAVIN SUPPLEMENTATION ON LIVER, KIDNEY, MUSCLE, HEART, BRAIN AND PLASMA SELENIUM CONCENTRATIONS OF PIGS

	Selenium source and intake							
	Sodium selenite (.18 mg/day)		Selenomethionine (.12 mg/day)		Level of significance			
	Supplemental riboflavin		Supplemental riboflavin		Riboflavin	Source	Inter-	
Item	0 (mg/day)	2	0	2	effect	effect	action	S.E.M.[a]
Liver[b]	.79	.90	.88	.92				.09
Kidney[b]	2.03	2.00	1.91	1.97				.08
Muscle[b]	.10	.12	.15	.16	.23	.01		.01
Heart[b]	.24	.27	.30	.29		.05		.02
Brain[b]	.17	.19	.23	.21		.01	.01	.01
Plasma[c]	.16	.16	.17	.17				.01

[a]Standard error of treatment means.

[b]Concentrations in µg/g of wet tissue.

[c]Concentrations in µg/ml.

concentration without increased glutathionine peroxidase activity observed in heart, muscle and brain of pigs receiving selenomethionine in this study.

The lack of clear cut riboflavin deficiency signs in trial four may be due in part to the fact that these pigs were fed a starter diet containing 10% dried whey product for one week after weaning. The calculated riboflavin content of this diet is 7.5 mg/kg, which is considerably higher than the 3 mg/kg recommendation of NRC (1979). Furthermore, the loss of riboflavin from the body once the animal is placed on a riboflavin-deficient diet appears to be quite slow (Baker et al., 1966).

In conclusion, erythrocyte percent active glutathione reductase appears to be a sensitive indicator of riboflavin status of the pig. Values below 50% are needed before other riboflavin deficiency signs become evident. Riboflavin supplementation of diets can increase the retention of absorbed selenium and it may aid in the incorporation of selenium from sodium selenite into glutathione peroxidase.

A higher percent of the selenium consumed as selenomethione was retained and a larger proportion of the absorbed selenium was incorporated into tissues than selenium from sodium selenite. Feeding selenomethionine as the supplemental selenium source at 67% of the level of selenium from sodium selenite resulted in higher muscle, heart and brain selenium concentrations than feeding the higher level of selenium as sodium selenite.

SECTION II

Introduction

The metabolic role of selenium as a component of glutathione peroxidase was elucidated in 1972 by Rotruck et al. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide and lipid peroxides to water (Mills, 1959) and hydroxy acids (O'Brien and Little, 1967), respectively. The active center of the molecule contains four selenocysteine moieties (Ludenstein et al., 1979). The major portion of erythrocyte selenium has been reported to be associated with the glutathione peroxidase molecule (Rotruck et al., 1973; Oh et al., 1974; and Sunde et al., 1978). Sunde et al. (1978) have shown that only 10% of ovine liver selenium was associated with glutathione peroxidase. Wegger et al. (1980) noted that there was a highly significant positive correlation between selenium concentration and glutathione peroxidase activity in pig liver, but not in pig kidney. Pig liver (Young et al., 1977b; McDowel et al., 1977; Mahan et al., 1977; and Mahan and Moxon, 1978a), muscle (Groce et al., 1971; Ku et al., 1972; Mahan et al., 1977; Young et al., 1977a; and Mahan and Moxon, 1978a), kidney (Mahan et al., 1977; McDowell et al., 1977; and Mahan and Moxon, 1978a) and blood (Mahan et al., 1977; McDowell et al., 1977; and Meyer et al., 1981) selenium concententrations have been shown to respond to dietary selenium intake. Using rat repletion assays Hoekstra et al. (1973) reported that liver glutathione peroxidase activity responds to supplemental dietary selenium, but that enzyme activity plateaued at 0.1 µg/g of selenium of diet. Also, they found that erythrocyte glutathione peroxidase activity was restored at a rate consistent with the life span of the erythrocyte. Using similar techniques Wang and Spullholz

(1980) reported that rat liver glutathione peroxidase was responsive to dietary selenium intake. Scott and Naguchi (1973) and Bunk et al. (1980) found that plasma glutathione peroxidase activity of chickens responded to dietary selenium concentration. Chow and Tappel (1974) and Smith et al. (1974) have shown plasma glutathione peroxidase to be the most sensitive measure of selenium intake in rats. Gabrielsen and Opstvedt (1980) have developed a selenium bioavailability assay in chicks using plasma glutathione peroxidase as a measure of selenium availability. This assay requires only nine days to complete because of sensitivity of plasma glutathione peroxidase activity to dietary selenium intake. However, little work has been done to study the responsiveness of pig serum and erythrocyte glutathione peroxidase to supplemental selenium intake. The trials reported here were designed to study the responsiveness of serum glutathione peroxidase to dietary selenium levels. In addition, the effects of environmental temperature and weaning stress on performance and serum glutathione peroxidase activity were evaluated.

Materials and Methods

Trial V. Fifteen four-week old pigs which weighed 7.20 ± .46 kg (mean ± standard error) were randomly allotted to three dietary treatments. The pigs used in this study were farrowed and raised by gilts which had received no supplemental selenium or vitamin E since the gilts were weaned. The basal (B) selenium-unsupplemented diet is shown in table 15. Additions of 0.1 (B+.1) or 0.2 (B+.2) µg of selenium as sodium selenite per g of diet were made in place of cerelose. The selenium levels of the diets are shown in table 16. The pigs were housed in raised stainless steel decks in groups of five pigs per pen.

TABLE 15. BASAL DIET FOR TRIALS V AND VI

Ingredient	Amount
Ground shelled corn (IFN 4-02-931)	736.0
Soybean meal, dehulled (IFN 5-04-612)	200.0
Synthetic lysine (78.4% L-lysine)	2.0
Mono-dicalcium phosphate (18.5% Ca, 21% P)	14.0
Calcium carbonate (38% Ca) (IFN 6-01-069)	12.5
Vitamin trace-mineral premix[a]	5.0
Salt	3.0
Antibotic premix[b]	2.5
Vitamin E premix[c]	5.0
Glucose[d] (IFN 4-02-125)	20.0
	1000.0
Calculated analysis	%
Lysine	.96
Methionine-cystine	.58
Tryptophan	.17
Calcium	.81
Phosphorus	.60

[a]Supplies 3,300 IU vitamin A; 660 IU vitamin D; 3.3 mg riboflavin; 13.2 mg d-pantothenic acid; 17.6 mg niacin; 19.8 µg vitamin B_{12}; 126.7 mg choline chloride; 2.2 mg menadione sodium bisulfate; 74.8 mg zinc; 59.4 mg iron; 37.4 mg manganese; 9.9 mg copper; and 2.75 mg iodine per kg of diet.

[b]Provides 110 mg chlortetracycline, 110 mg sulfamethazine and 55 mg penicillin per kg of diet.

[c]Supplies 11 IU vitamin E per kg of diet.

[d]"Dextrose 2001," CPC International, Englewood Cliffs, NJ.

Feed was available free-choice from a self-feeder and water was available at all times.

The pigs were weighed and blood samples taken at 0, 14 and 28 days of the study. Erythrocytes were washed three times in ice cold saline (.9% NaCl). Erythrocyte and serum glutathione peroxidase activity was determined by the method of Paglia and Valentine (1967). Plasma selenium levels were assayed by a modification of the Olson method (Whetter and Ullrey, 1978). Serum tocopherol and tocopherol acetate were determined by the high pressure liquid chromatographic method of Widicus and Kirk (1979). On the twenty-eighth day of the trial hematocrit and hemoglobin values were also determined. Using these values, the body weight, enzyme activity values and blood volume values from Kornegay et al. (1964) serum pool and erythrocyte pool glutathione peroxidae values were calculated.

The statistical analyses of these data were done as a split-plot design when time effects were considered. All other analyses were done as a completely randomized design (Gill, 1978a and 1978b).

Trial VI. Nine pigs weighing 7.62 ± .36 kg (mean ± standard error) were randomly allotted to the three dietary treatments described in trial V. The selenium levels of the feeds are shown in table 16. The pigs used in this study were farrowed and raised by sows that had been fed corn-soybean meal diets that were supplemented with vitamin E (11 IU/kg) and selenium (0.1 ppm). Blood samples were taken at 0, 14 and 28 days of the study for serum and erythrocyte glutathione peroxidase activity and plasma selenium level determinations. The methods used for laboratory and statistical analysis were described in trial V.

TABLE 16. SELENIUM LEVELS OF DIETS FED IN TRIALS V AND VI

Diet	Trial V	Trial VI
Basal (B)	.06[a]	.06
B+0.1 ppm selenium	.16	.19
B+0.2 ppm selenium	.29	.25

[a] µg/g.

Trial VII. One hundred and two five-week old pigs were allotted to two replicates of a 2 x 3 factorial design. The main effects were environmental temperature and level of selenium supplementation. The basal corn-soybean meal diet (table 17) contained 0.1 ppm of supplemental selenium. The basal diet (B) was supplemented with an additional 0.2 µg/g and 0.4 µg/g of selenium as sodium selenite to formulate the B+.2 and B+.4 diets, respectively. The selenium levels of the diets are shown in table 18. The daily high and low temperatures for each environmental treatment were recorded. The average daily high temperature was 22.1 ± 0.57°C (mean ± standard error) and the average daily low temperature was 20.5 ± 1.01°C (mean ± standard error) in the cool room. The average daily high and low temperatures in the warm room were 28.6 ± .69 (mean ± standard error) and 25.3 ± 1.64 (mean ± standard error), respectively. In the cool room there were nine pigs per pen housed in 2.0 x 2.4 m pens on a totally slatted floor of aluminum slats. For the first two-weeks a 1.2 x 1.2 m solid plywood panel was provided for the pigs to lay on. In the warm room eight pigs were allotted per pen. The pens were 1.2 x 2.4 m with a 1.2 x 0.9 m solid cement hovered area and the remainder of the pen being aluminum slats. Feed and water were available ad libitum.

All pigs were weighed initially and at 14 and 28 days after the start of the trial. Feed intakes were determined at 14 and 28 days. Blood samples were taken from the pigs in one replicate initially and at 14 days. Serum glutathione peroxidase activity was determined by the method of Paglia and Valentine (1967). A modification of the Olson method (Whetter and Ullrey, 1978) was used for analysis of serum for

TABLE 17. BASAL DIET FOR TRIAL VII

Ingredient	Amount
Ground shelled corn (IFN 4-02-931)	756.0
Soybean meal, dehulled (IFN 5-04-612)	200.0
Mono-dicalcium phosphate (18.5% Ca, 21% P)	14.0
Calcium carbonate (38% Ca) (IFN 6-01-069)	12.5
Vitamin trace-mineral premix[a]	5.0
Selenium vitamin E premix[b]	5.0
Salt	3.0
Antibiotic premix[c]	2.5
Synthetic lysine (78% L-lysine)	2.0
	1000.0

[a]Supplies 3,300 IU vitamin A; 660 IU vitamin D; 3.3 mg riboflavin; 13.2 mg d-pantothenic acid; 17.6 mg niacin; 19.8 µg vitamin B_{12}; 126.7 mg choline chloride; 2.2 mg menadione sodium bisulfite; 74.8 mg zinc; 59.4 mg iron; 37.4 mg manganese; 9.9 mg copper, and 2.75 mg iodine per kg of diet.

[b]Supplies 16.5 IU of vitamin E and 0.1 mg of selenium per kg of diet.

[c]Provides 110 mg chlortetracycline, 110 mg sulfamethazine and 55 mg penicillin per kg of diet.

TABLE 18. SELENIUM LEVELS OF DIETS IN TRIAL VII

Diet	Selenium, µg/g
Basal	.17
B+.2 ppm selenium	.35
B+.4 ppm selenium	.64

selenium. Blood reduced glutathione levels were determined using the method described by Beutler et al. (1963).

Blood samples were also taken on days 3 and 7 of the study from eight pigs in the warm room allotted to the basal diet and eight pigs in the same room allotted to the B+.2 diet. Serum glutathione peroxidase activity and blood reduced glutathione levels were determined by methods already described.

Statistical analysis of the main trial was done as a 2 x 3 factorial design (Gill, 1978a). The initial, 3 and 7 day serum glutathione peroxidase activity and blood reduced glutathione levels, were analyzed as a split-plot design (Gill, 1978b).

Results and Discussion

The average daily gains of the pigs in trials five and six were not affected by the dietary treatments (table 19). Wastell et al. (1972) and Glienke and Ewan (1977) reported a growth response to supplementary selenium when added to vitamin E supplemented torula yeast diets. However, Groce et al. (1971), Ku et al. (1973), Mahan et al. (1978a), Mahan et al. (1978b) and Mandisodza et al. (1979) have observed no growth response to selenium supplementation of corn-soybean meal diets for pigs.

There was an interaction ($P<.10$) of time and supplementary selenium level on serum glutathione peroxidase activity in trial five. Pigs fed the unsupplemented diet exhibited a decline in serum glutathione peroxidase activity during the trial. The decline was rapid and reached a low point by 14 days of the trial. Pigs fed the B+.2 diet exhibited an increase in serum glutathione peroxidase activity, while there was a much larger increase in serum glutathione peroxidase

TABLE 19. THE EFFECT OF SELENIUM SUPPLEMENTATION ON AVERAGE DAILY GAIN IN TRIALS V AND VI

	Supplemental selenium (µg/g)			
Item	0	0.1	0.2	S.E.M.[a]
Trial V, average daily gain, g	428	412	450	43
Trial VI, average daily gain, g	352	367	517	77

[a]Standard error of treatment means.

TABLE 20. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON SERUM GLUTATHIONE PEROXIDASE ACTIVITY[a] IN TRIAL V

Day of trial[b,c,e]	Supplemental dietary selenium[d,e] (µg/g) 0	0.1	0.2	Mean
0	19.8	13.6	6.3	13.2
14	3.4	11.6	10.6	8.5
28	3.6	70.1	23.6	32.4
Mean	8.9	31.8	13.5	

[a]Expressed as µmoles of glutathione oxidized/minute/g of protein.

[b]Standard error of treatment means = 6.4; standard error of period means = 6.6.

[c]Time effect (P<.05).

[d]Diet effect (P<.10).

[e]Interaction of time and diet (P<.10).

activity of pigs fed the B+.1 diet during the study (table 20). Scott and Naguchi (1973) reported plasma glutathione peroxidase activity of chickens fed selenium deficient diets fell to less than 10% of the normal activity within 5 days after being placed on the deficient diet. Gabrielsen and Opstvedt (1980) observed a response in plasma glutathione peroxidase of chicks within seven days after being placed on a repletion or a depletion diet.

In trial six an interaction ($P<.10$) of time and supplementary selenium level on serum glutathione peroxidase was also observed (table 21). The trends in this trial were similar to those in trial five. There was a significant diet effect in trial six with pigs fed supplemented diets having higher serum glutathione peroxidase activity values than those fed the unsupplemented diet. Chavez (1979) has reported higher plasma glutathione peroxidase activity in weaning pigs, fed selenium-supplemented (0.1 ppm selenium as sodium selenite) torula yeast diets than in pigs fed the unsupplemented diet. Meyer et al. (1980) observed greater plasma glutathione peroxidase activity at two weeks post-weaning when corn-soybean meal diets were supplemented with 0.1 ppm of selenium as sodium selenite.

In trial five total serum pool glutathione peroxidase activity was calculated using blood volume estimates from Kornegay et al. (1964). Pigs fed the basal diet had lower serum pool glutathione peroxidase activity ($P<.01$) than pigs fed the supplemented diets (table 22).

Total serum pool glutathione proxidase acitivity in trial five and serum glutathione peroxidase activity in trial six appeared to plateau at 0.1 ppm supplemental selenium. In both trials pigs fed 0.1 ppm supplemental selenium were able to maintain serum glutathione

TABLE 21. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON SERUM GLUTATHIONE PEROXIDASE ACTIVITY[a] IN TRIAL VI

Day of trial[b,c]	Supplemental dietary selenium[b,c,d] (µg/g) 0	0.1	0.2	Mean
0	12.10	11.60	9.97	11.2
14	7.35	17.00	10.80	11.7
28	6.33	13.60	15.00	11.6
Mean	8.59	14.07	11.92	

[a]Expressed as µmoles of glutathione oxidized/minute/g of protein.

[b]Standard error of treatment means = .94; standard error of period means = 1.07.

[c]Significant dietary effect ($P<.05$).

[d]Significant interaction of time and diet ($P<.10$).

TABLE 22. THE EFFECT OF SELENIUM SUPPLEMENTATION FOR FOUR WEEKS ON SERUM AND ERYTHROCYTE POOL OF GLUTATHIONE PEROXIDASE ACTIVITY IN TRIAL V

	Supplemental dietary selenium (µg/g)			
Item	0	0.1	0.2	S.E.M.[a]
Erythrocyte pool glutathione peroxidase activity, µmoles of glutathione oxidized/minute/pig	854.2	978.9	737.0	161.0
Serum pool glutathione peroxidase activity, µmoles of glutathione oxidized/minute/pig	66.3[b]	291.7[c]	346.8[c]	50.5

[a]Standard error of treatment means.

[b,c]Means with different superscripts are significantly different ($P<.01$).

peroxidase activity throughout the study. Meyer et al. (1981) reported that plasma glutathione peroxidase plateaued at 0.35 ppm of supplemental selenium at 5 weeks postweaning. However, Chavez (1979) has shown more variable results. In a trial with two week old pigs the plasma glutathione peroxidase activity was maintained by supplementing a torula yeast diet with 0.1 ppm selenium as sodium selenite, but in another trial 0.1 ppm supplemental selenium did not maintain plasma glutathione peroxidase activity of two week old pigs.

Erythrocyte glutathione peroxidase activity was not affected by dietary selenium supplementation in trials five (table 23) and six (table 24), however there was a decline in activity with increasing time in trial five ($P<.01$). The erythrocyte pool glutathione peroxidase activity at 28 days was not affected by dietary selenium in trial five (table 22). Hoekstra et al. (1973) noted that rat erythrocyte glutathione peroxidase activity was restored (after depletion) by dietary selenium at a rate consistent with the life span of the erythrocyte. The life span of the swine erythrocyte has been estimated to be from 72 days (Withrow and Bell, 1969) to 86 days (Bush et al., 1955) and Talbor and Swensen (1963) have suggested the swine erythrocyte has a half-life of 28 days. Using these values approximately 30% to 50% of the erythrocyte pool should have been turned over during a 28 day trial. However, erythrocyte destruction in swine is due to both an age dependent process and a random process. Bush et al. (1955) and Withrow and Bell (1969) concluded that random destruction accounts for the larger portion of the cells which are destroyed. Tao (1973) observed some random destruction of erythrocytes, but suggested that age-dependent destruction was the primary destructive mechanism in pigs. It

TABLE 23. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON ERYTHROCYTE GLUTATHIONE PEROXIDASE ACTIVITY[a] IN TRIAL V

Day of trial[b,c]	Supplemental dietary selenium[b] (µg/g) 0	0.1	0.2	Mean
0	8.33	8.71	13.90	10.31
14	7.26	8.99	7.70	7.98
28	5.11	6.23	4.08	5.14
Mean	6.90	7.98	8.56	

[a]Expressed as µmoles of glutathione oxidized/minute/g of hemoglobin.

[b]Standard error of treatment means = .49; standard error of period means = .88.

[c]Significant time effect (P<.01).

TABLE 24. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON ERYTHROCYTE GLUTATHIONE PEROXIDASE ACTIVITY[a] IN TRIAL VI

Day of trial	Supplemental dietary selenium[b] (µg/g) 0	0.1	0.2	Mean
0	9.74	7.86	7.72	8.44
14	2.92	3.15	1.54	2.54
28	4.70	4.28	5.37	4.78
Mean	5.79	5.10	4.88	

[a]Expressed as µmoles of glutathione oxidized/minute/g of hemoglobin.

[b]Standard error of treatment means = 2.61; standard error of period emans = 2.38.

appears that newly formed erythrocytes that would reflect recent dietary selenium intakes may be destroyed by the random destruction process. Therefore, a longer period of time may be needed in swine before dietary induced changes in erythrocyte glutathione peroxidase activity could be observed than in species such as sheep which exhibit primary age dependent erythrocyte destruction (Withrow and Bell, 1969).

Serum glutathione peroxidase activity appears to be a more sensitive indicator of selenium intake than erythrocyte glutathione peroxidase activity in pigs. Smith et al. (1974) reported that plasma glutathione peroxidase activity was the most responsive to dietary selenium followed in order by heart, kidney and erythrocyte glutathione peroxidase activity in rats. Chow and Tappel (1974) have also shown similar results with both depletion and repletion studies.

In trial five there was a significant ($P<.01$) effect of supplemental selenium on plasma selenium level. More importantly, there was an interaction ($P<.01$) of supplemental dietary selenium and days on treatment for plasma selenium concentrations in both trials five (table 25) and six (table 26). In both trials pigs fed the basal diet showed a rapid decline in plasma selenium levels during the first fourteen days of the study. In trial five, the plasma selenium level of pigs fed the B+.1 diet increased slightly throughout the study, while plasma selenium values increased greatly when pigs were fed the B+.2 diet. Pigs fed the unsupplemented diets in trial six exhibited only a slight rise in plasma selenium levels during the study.

Groce et al. (1973b) reported that serum selenium levels were increased by selenium supplementation up to 0.1 μg/g. Young et al. (1977b) observed that serum selenium concentration of six-week old

TABLE 25. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON PLASMA SELENIUM CONCENTRATIONS[a] IN TRIAL V

Day of trial[b,d]	Supplemental dietary selenium[b,c,d] (µg/g) 0	0.1	0.2	Mean
0	.05	.05	.05	.05
14	.02	.07	.10	.06
28	.02	.07	.12	.07
Mean	.03	.06	.09	

[a]Expressed as µg/ml.

[b]Standard error of treatment means = .006; standard error of period means = .021.

[c]Significant diet effect (P<.01).

[d]Significant interaction of diet and time (P<.01).

TABLE 26. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON PLASMA SELENIUM CONCENTRATIONS[a] IN TRIAL VI

Day of trial[b]	Supplemental dietary selenium[b,c] (µg/g) 0	0.1	0.2	Mean
0	.11	.12	.10	.11
14	.06	.14	.14	.11
28	.05	.15	.13	.11
Mean	.07	.14	.12	

[a]Expressed as µg/ml.

[b]Standard error of treatment means = .008; standard error of period means = .004.

[c]Significant interaction of time and diet ($P<.01$).

boars plateaued at 0.56 ppm dietary selenium, when a high moisture corn-soybean meal diet was fed. The serum selenium values reported by Young et al. (1977b) tended to be higher (.14 to .18 µg/ml) than the plasma selenium concentrations in trials five and six. Mahan and Moxon (1978a) observed that serum selenium concentrations declined rapidly in the first seven days postweaning when pigs were fed unsupplemented corn-soybean meal diets. Furthermore, they reported that pigs raised by sows fed selenium-supplemented diets (0.1 ppm supplemental selenium) had higher serum selenium levels at weaning than pigs raised by sows fed unsupplemented corn-soybean meal diets. This trend is evident in trials five and six. Pigs used in trial five were raised by gilts fed selenium-unsupplemented diets, while pigs in trial six were raised by sows fed selenium-supplemented diets. The initial serum selenium values were .05 and .11 µg/ml, respectively, for trials five and six (tables 25 and 26). Mahan and Moxon (1978a) reported that supplementing starter diets with 0.1 ppm of selenium as sodium selenite resulted in an increase in serum selenium levels of pigs raised by sows fed selenium-unsupplemented diets, but did not affect serum selenium levels of pigs raised by sows fed selenium-supplemented diets. Similar trends were noted in trials five and six.

Initial serum tocopherol values were lower in trial five (table 27) than in trial six (table 28) (.32 and .50 µg/ml, respectively). Pigs used in trial five were raised by gilts fed corn-soybean meal diets that were not supplemented with vitamin E or selenium, while pigs used in trial six were raised by sows fed vitamin E (11 IU/kg) and selenium (0.1 ppm) supplemented corn-soybean meal diets. In trial five there was an interaction ($P<.10$) of supplemental selenium and days on

TABLE 27. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON SERUM TOCOPHEROL LEVELS[a] IN TRIAL V

Day of trial[b,c]	Supplemental dietary selenium[b,c] (µg/g) 0	0.1	0.2	Mean
0	.48	.17	.31	.32
14	.19	.36	.30	.28
28	.22	.19	.38	.26
Mean	.30	.24	.33	

[a]Expressed as µg of tocopherol and tocopheryl acetate/ml.

[b]Standard error of treatment means = .05; standard error of period means = .05.

[c]Significant interaction of time and diet ($P<.10$).

TABLE 28. THE EFFECT OF SELENIUM SUPPLEMENTATION AND DAYS CONSUMING DIETS ON SERUM TOCOPHEROL LEVELS[a] IN TRIAL VI

Day of trial	Supplemental dietary selenium[b] (µg/g) 0	0.1	0.2	Mean[c]
0	.53	.42	.54	.50
14	.48	.56	.46	.50
28	.81	.67	.59	.69
Mean	.61	.55	.53	

[a]Expressed as µg of tocopherol and tocopheryl acetate/ml.

[b]Standard error of treatment means = .09; standard error of period means = .04.

[c]Time effect ($P<.01$).

treatment on serum tocopherol level. Serum tocopherol levels of pigs fed the basal diet declined during the first two weeks of the study and remained low throughout the study. Pigs fed the B+.2 diet exhibited a slight rise in serum tocopherol levels during the study. During the first 14 days the serum tocopherol concentration of pigs fed the B+.1 diet rose, but then declined to a level similar to the pig fed the unsupplemented diet. In trial six there was no significant effect of selenium supplementation on serum tocopherol concentration. Both Meyer et al. (1981) and McDowell et al. (1977) have reported no effect of supplemental dietary selenium on circulating tocopherol levels.

In trials five and six, serum glutathione peroxidase activity was more responsive postweaning than erythrocyte glutathione peroxidase activity to dietary selenium levels during the four weeks. At least 0.1 ppm of supplemental selenium was needed to maintain serum glutathione peroxidase activity and plasma selenium levels throughout the four-week post weaning period. It appears that the intake of selenium and vitamin E by the dam influences the weaning plasma selenium, and serum tocopherol levels, as well as the response of pigs to supplemental selenium.

Supplemental dietary selenium did not affect rate of gain, average daily feed intake or feed efficiency in trial seven (table 29). Similar results have been reported by Groce et al. (1971), Ku et al. (1973) Mahan et al. (1978a), Mahan et al. (1978b), Mandisodza et al. (1979) and Meyer et al. (1981). Average daily feed intake was not significantly affected by the environmental temperature. Pigs in the cool room gained faster ($P<.04$) than pigs in the warm room (.52 vs .46 kg/day) during the second two-week period of the trial. During the

TABLE 29. THE EFFECT OF SELENIUM SUPPLEMENTATION AND ENVIRONMENTAL TEMPERATURE ON FEED INTAKE, GAINS AND FEED EFFICIENCY IN TRIAL VII

	Cool room				Warm room				Level of significance		Error
	Diet				Diet						
Item	B	B+.2	B+.4	Mean	B	B+.2	B+.4	Mean	Diet effect	Environment effect	mean square
Initial weight, kg	8.53	8.44	8.44	8.47	8.75	8.80	8.48	8.68			
Average daily gain, kg											
0-14 days	.11	.17	.13	.14	.14	.12	.14	.13			.046
14-28 days	.51	.51	.50	.51	.47	.46	.45	.46		.04	.062
0-28 days	.31	.33	.30	.31	.30	.30	.29	.30			.045
Average daily feed intake, kg											
0-14 days	.31	.39	.35	.35	.35	.33	.36	.35			.025
14-28 days	.95	1.01	.95	.97	.84	.83	.84	.84			.154
0-28 days	.64	.70	.64	.66	.59	.58	.60	.59			.078
Feed/gain ratio											
0-14 days	2.78	2.39	2.66	2.61	2.62	2.81	2.56	2.66			.155
14-28days	1.87	1.97	1.90	1.91	1.79	1.81	1.88	1.83		.05	.005
0-28 days	2.04	2.11	2.10	2.08	1.99	1.93	2.03	1.98		.02	.004

first two-week period, however, the gains were not affected by the environmental treatment. Also, overall gains for the trial were not affected by the environmental treatment. Feed required per unit of gain was lower for pigs in the warm room for the second two-week period (1.83 vs 1.91, P<.05) resulting in an improved overall feed efficiency (1.98 vs 2.08, P<.02). Improved caloric efficiency with increasing temperature has been reported (Nichols et al., 1980) in growing pigs in the same range of temperatures used in this study. Similar trends have been observed in heavier pigs, also (Jensen et al., 1969 and Sugahara et al., 1970).

The serum glutathione peroxidase activity (table 30) was higher for pigs fed the B+.2 and B+.4 than for pigs fed the basal diet (P<.05). The serum glutathione peroxidase activity appeared to be maximum at 0.35 ppm selenium (B+.2 diet). Meyer et al. (1981) has suggested that the point at which plasma glutathione peroxidase activity peaks declines from one to five weeks post-weaning. At 14 days postweaning, Meyer et al. (1981) reported that plasma glutathione peroxidase activity plateaued at 0.4 ppm dietary selenium.

Plasma selenium level increased (P<.01) with each increment of supplemental dietary selenium at two weeks postweaning (table 30). The plasma selenium concentration did not plateau over a range of dietary selenium used in this study (0.17 to 0.64 ppm). Meyer et al. (1981) observed that plasma selenium levels plateaued at 0.45 ppm dietary selenium at two weeks post weaning. However, Young et al. (1977b) reported that serum selenium values plateaued at 0.56 ppm dietary selenium in six-week old boars fed high moisture corn diets. Mahan and

TABLE 30. THE EFFECT OF SELENIUM SUPPLEMENTATION FOR FOURTEEN DAYS AND ENVIRONMENTAL TEMPERATURE ON BLOOD PARAMETERS

	Cool room			Warm room			Level of significance		Error
	Diet			Diet					
Item	B	B+.2	B+.4	B	B+.2	B+.4	Diet effect	Environment effect	mean square
Serum glutathione peroxidase activity, EU/mg protein[a,b]	26.6	32.9	37.0	27.0	34.5	35.6	.01		49.8
Circulating reduced glutathione, µmoles/ml	677	651	647	668	609	751			3010
Plasma selenium, µg/ml[c]	.09	.15	.18	.08	.15	.17	.01		.001

[a] 1 EU = 1 µmole of NADPH oxidized/minute.

[b] Means for .2 and .4 ppm supplemented diets are greater than mean of basal diet ($P<.05$).

[c] Significant effect of each increment of supplemental selenium ($P<.01$).

Moxon (1978) observed that serum selenium values did not plateau in weanling pigs over a range of 0 to 0.3 ppm supplemental selenium.

Circulating reduced glutathione levels of pigs were not affected by their dietary or environmental treatment (table 30). Since the reduction of lipid peroxides by glutathione peroxidase is a first order reaction with respect to reduced glutathione (O'Brien and Little, 1967), one could conclude that the reduced glutathione supply could limit *in vivo* glutathione peroxidase activity, since there are differences in *in vitro* glutathione peroxidase activity, or one must conclude that reduced glutathione is available in excess of the needs for *in vivo* activity. Since glutathione is rapidly synthesized (Anderson and Mosher, 1951) and since oxidized glutathione is a positive effector of glucose-6-phosphate dehydrogenase, (Jacob and Jandl, 1966) it seems likely that after fourteen days of the study ample reduced glutathione would be available to support reduction of lipid peroxides.

During the second two-week period, one pig in the cool room being fed the B+.4 diet died. Liver and muscle samples contained 2.24 and 0.51 µg of selenium/g of dry tissue, respectively. The liver selenium concentration is clearly above the level of 0.52 µg/g reported by Young *et al.* (1977b) for pigs that died of vitamin E-selenium deficiency, and is similar to the value of 2.05 µg/g reported by Ku *et al.* (1973) for pigs fed selenium supplemented diets (0.4 ppm supplemental selenium). Survey work done by Young *et al.* (1977a) would indicate that the liver selenium concentration is consistant with adequate supplemental dietary selenium. The muscle selenium level of this pig was greater than that of pigs fed deficient diets (0.24 µg/g) reported by Young *et al.*

(1977b) and also higher than the level for pigs fed selenium supplemented diets (0.4 ppm supplemental selenium) of 0.46 µg/g reported by Ku et al. (1973). It appears that the cause of death was not related to the dietary treatment.

During the first week of the trial (first week postweaning), pigs fed the B+.2 diet had higher ($P<.01$) serum glutathione peroxidase activity than pigs fed the basal diet (table 31). Glutathione peroxidase activity was also affected by time on the diets ($P<.01$), as the activity rapidly increased during the first three days after weaning. More importantly, there was a significant interaction of days on treatment and supplemental selenium ($P<.05$) for serum glutathione perixodase activity. Pigs fed the B+.2 diet exhibited a greater rise in serum glutathione peroxidase activity by three days and maintained serum glutathione peroxidase activity higher throughout the remainder of the week, than pigs fed the basal diet. These results are consistent with the results reported by Meyer et al. (1981). Mahan et al. (1977) has suggested that there is a very short carryover of selenium from the dam to the progeny.

Circulating reduced glutathione levels (table 31) were higher in pigs fed the B+.2 diet than the B diet ($P<.06$). Reduced glutathione concentrations declined throughout the study in pigs fed the basal diet and increased during the study in pigs fed the B+.2 diet ($P<.04$). Since reduced glutathione is the rate limiting substrate for glutathione peroxidase (O'Brien and Little, 1967) and since glutathione has been shown to be synthesized more rapidly in rat liver than most proteins (Anderson and Mosher, 1951) the decline in reduced glutathione in pigs fed the basal diet may reflect a lower in vivo glutathione peroxidase activity than for pigs fed the B+.2 diet. It is unlikely

TABLE 31. THE EFFECT OF LEVEL OF SELENIUM SUPPLEMENTATION DURING THE FIRST WEEK POSTWEANING ON SERUM GLUTATHIONE PEROXIDASE ACTIVITY AND BLOOD REDUCED GLUTATHIONE LEVELS

	Diet and selenium concentration								Level of significance		
	B (.17 µg/g)				B+.2 (.35 µg/g)						
	Days postweaning				Days postweaning				Diet	Day of trial	Interaction
Item	0	3	7	Mean	0	3	7	Mean	effect	effect	
Serum glutathione peroxidase activity, EU/g protein[b]	15.7	24.6	19.6	20.0	16.8	36.1	33.1	28.7	.01	.01	.05
Circulating reduced glutathione, µmoles/ml[c]	613	547	506	555	569	616	676	620	.06		.04

[a] 1 EU = 1 µmole of NADPH oxidized/minute.

[b] Standard error of treatment means = 1.6; standard error of period means = 1.6.

[c] Standard error of treatment means = 27; standard error of period means = 40.

that changes in the reduced glutathione supply are due to slow regeneration of reduced glutathione from oxidized glutathione if all other essential dietary nutrient requirements are met, since oxidized glutathione releases the inhibition of glucose-6-phosphate dehydrogenase by reduced nicotine adenine dinucleotide phosphate (Jacob and Jandl, 1966 and Eggleston and Krebs, 1974). This release would allow the hexose monophosphate shunt to produce more reducing equivalents which could be used to reduce glutathione.

The data from this study indicate that weanling pigs require at least 0.35 ppm selenium to maximize serum glutathione peroxidase activity at two weeks postweaning. Serum glutathione peroxidase activity responds to dietary selenium levels up to at least 0.35 ppm during the first week post weaning.

CONCLUSIONS

1. Erythrocyte percent active glutathione reductase is a sensitive indicator of the riboflavin status of pigs. Values below 50% are needed before other riboflavin deficiency signs become evident.

2. Riboflavin supplementation of swine diets increased selenium retention and appeared to increase the incorporation of selenium from sodium selenite into glutathione peroxidase.

3. A higher percent of the selenium from selenomethionine was retained and incorporated into body tissues than selenium from sodium selenite. Muscle, heart and brain selenium concentrations indicated that the retention of selenium from selenomethionine was 1.5 times greater than that from sodium selenite.

4. Serum glutathione peroxidase activity was more responsive to dietary selenium level than was erythrocyte glutathione peroxidase activity over a four week period. Serum glutathione peroxidase activity appeared to peak at lower dietary selenium concentrations than did plasma selenium levels.

5. Under routine rearing conditions, feeding a diet containing 0.35 ppm selenium resulted in a higher serum glutathione peroxidase activity by three days postweaning than feeding a diet containing 0.17 ppm selenium. This advantage was maintained throughout the remainder of the first week after weaning.

6. At least 0.35 ppm dietary selenium was required for serum glutathione peroxidase activity to become maximum at two weeks postweaning when pigs were reared under routine management conditions.

LITERATURE CITED

Literature Cited

Anderson, E.I. and W.A. Mosher. 1951. Incorporation of S^{35} from DL-cystine into glutathione and protein in the rat. J. Biol. Chem. 188:51.

Awasthi, Y.C., E. Beutler and S.K. Srivastava. 1975. Purification and properties of human erythrocyte glutathione peroxidase. J. Biol. Chem. 250:5144.

Baker, H., O. Frank, S. Feingold, R.A. Gellene, C.M. Leevy and S.H. Hunter. 1966. A riboflavin assay suitable for clinical use and nutritional surveys. Am. J. Clin. Nutr. 19:17.

Beilstein, M.A., M.J. Tripp and P.D. Whanger. 1980. Evidence of selenocysteine in low molecular weight selenium containing proteins in ovine muscle. Fed. Proc. 39:338. (Abstr.).

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

Black, R.S., M.J. Tripp, P.D. Whanger and P.H. Weswig. 1978. Selenium proteins in ovine tissues: III Distribution of selenium and glutathione peroxidase in tissue cytosol. Bioinorganic Chem. 8:161.

Brady, P.S., L.J. Brady, M.J. Parsons, D.E. Ullrey and E.R. Miller. 1979. Effects of riboflavin deficiency on growth and glutathione peroxidase system enzymes in the baby pig. J. Nutr. 109:1615.

Bunk, M.J., M.S. Cupp and G.F. Combs, Jr. 1980. Relationship of selenium dependent glutathione peroxidase activity to nutritional pancreatic atrophy in selenium deficient chicks. Fed. Proc. 39:556.

Burk, R.F. and P.E. Gregory. 1980. ^{75}Se incorporation into liver and plasma fractions of Se-deficient (0 Se) and control (c). Fed. Proc. 39:1678 (Abstr.).

Bush, J.A., N.I. Berlin, W.N. Jensen, A.B. Brill, G.E. Cartwright and M.M. Wintrobe. 1955. Erythrocyte life span in growing swine as determined by glycine-2-C^{14}. J. Exp. Med. 101:451.

Buzard, J.A. and F. Kopko. 1963. The flavin requirement and some inhibition characteristics of rat tissue glutathione reductase. J. Biol. Chem. 238:464.

Chavez, E.R. 1979. Effect of dietary selenium on glutathione peroxidase activity in piglets. Can. J. Anim. Sci. 59:67.

Chow, C.K. and A.L. Tappel. 1974. Response of glutathione peroxidase to dietary selenium in rats. J. Nutr. 104:444.

Eggleston, L.V. and H.A. Krebs. 1974. Regulation of the pentose phosphate cycle. Biochem. J. 138:425.

Ewan, R.C., M.E. Wastell, E.J. Bicknell and V.C. Speer. 1969. Performance and deficiency symptoms of young pig fed diets low in vitamin E and selenium. J. Anim. Sci. 29:912.

Forbes, R.M. and W.T. Haines. 1952. The riboflavin requirement of the baby pig I. At an environmental temperature of 85°F and 70% relative humidity. J. Nutr. 47:411.

Gabrielsen, Bjørn O. and J. Opstvedt. 1980. A biological assay for determination of availability of selenium for restoring blood plasma glutathione peroxidase activity in selenium-depleted chicks. J. Nutr. 110:1089.

Gill, J.L. 1978a. Design and Analysis of Experiments in the Animal and Medical Sciences, Volume I (1st Ed.). Iowa State University Press, Ames.

Gill, J.L. 1978b. Design and Analysis of Experiments in the Animal and Medical Sciences, Volume 2 (1st Ed.). Iowa State University Press, Ames.

Glatzle, D., F. Weber and O. Wiss. 1968. Enzymatic test for the detection of a riboflavin deficiency. NADPH-dependant glutathione reductase of red blood cells and its activation by FAD in vitro. Experientia (Basel) 24:1122.

Glatzle, D., W.F. Korner, S. Christeller and O. Wiss. 1970. Method for the detection of a biochemical riboflavin deficiency stimulation of $NADPH_2$-depentent glutathione reductase from human erythrocytes by FAD in vitro. Investigations on the vitamin B_2 status in healthy people and geriatric patients. Internat. J. Vit. Res. 40:166.

Glienke, L.R. and R.C. Ewan. 1977. Selenium deficiency in the young pig. J. Anim. Sci. 45:1334.

Groce, A.W., E.R. Miller, K.K. Keahey, D.E. Ullrey and D.J. Ellis. 1971. Selenium supplementation of practical diets for growing-finishing swine. J. Anim. Sci. 32:905.

Groce, A.W., E.R. Miller, J.P. Hitchcock, D.E. Ullrey and W.T. Magee. 1973a. Selenium balance in the pig as affected by selenium source and vitamin E. J. Anim. Sci. 37:942.

Groce, A.W., E.R. Miller, D.E. Ullrey, P.K. Ku, K.K. Keahey and D.J. Ellis. 1973b. Selenium requirements in corn-soy diets for growing-finishing swine. J. Anim. Sci. 37:948.

Hitchcock, J.P., E.R. Miller, K.K. Keahey and D.E. Ullrey. 1978. Effects of arsanilic acid and vitamin E upon utilization of natural or supplemental selenium by swine. J. Anim. Sci. 46:425.

Hoekstra, W.G., D. Hafeman, S.H. Oh, R.A. Sunde and H.E. Ganther. 1973. Effect of dietary selenium on liver and erythrocyte glutathione peroxidase in the rat. Fed. Proc. 32:885 (Abstr.).

Jacobs, H.S. and J.H. Jandl. 1966. Effects of sulfhydryl inhibition on red blood cells. III Glutathione in the regulation of the hexose monophosphate shunt. J. Biol. Chem. 24:4243.

Jensen, A.H., D.E. Kuhlman, D.E. Becker and B.G. Harmon. 1969. Response of growing-finishing swine to different housing environments during winter seasons. J. Anim. Sci. 29:451.

Kornegay, E.T., E.R. Miller, B.E. Brent, C.H. Long, D.E. Ullrey and J.A. Hoeffer. 1964. Effect of fasting and refeeding on body weight, rectal temperature, blood volume and various blood constituents in growing swine. J. Nutr. 84:295.

Krider, J.L., S.W. Terrill and R.F. VanPoucke. 1949. Response of weanling pigs to various levels of riboflavin. J. Anim. Sci. 8:121.

Ku, P.K., W.T. Ely, A.W. Groce and D.E. Ullrey. 1972. Natural dietary selenium, α-tocopherol and effect on tissue selenium. J. Anim. Sci. 34:208.

Ku, P.K., E.R. Miller, R.C. Wahlstrom, A.W. Groce, J.P. Hitchcock and D.E. Ullrey. 1973. Selenium supplementation of naturally high selenium diets for swine. J. Anim. Sci. 37:501.

Ladenstein, R., O. Epp, K. Bartels, A. Jones and R. Huber. 1979. Structure analysis and molecular model of the selenoenzyme glutathione peroxidase at 2.8 Å resolution. J. Mol. Biol. 134:199.

Mahan, D.C., A.L. Moxon and Mariana Hubbard. 1977. Efficacy of inorganic selenium supplementation to sow diets on resulting carry-over to their progeny. J. Anim. Sci. 46:738.

Mahan, D.C. and A.L. Moxon. 1978a. Effect of increasing the level of inorganic selenium supplementation in the post-weaning diets of swine. J. Anim. Sci. 46:384.

Mahan, D.C. and A.L. Moxon. 1978b. Effects of adding inorganic or organic selenium sources to the diets of young swine. J. Anim. Sci. 47:456.

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

McDowell, L.R., J.A. Froseth, R.C. Piper, I.A. Dyer and G.H. Kroening. 1977. Tissue selenium and serum tocopherol concentrations in selenium-vitamin E deficient pigs fed peas (Pisum sativum). J. Anim. Sci. 45:1326.

Meyer, W.R., D.C. Mahan and A.L. Moxon. 1981. Value of dietary selenium and vitamin E for weanling swine as measured by performance and tissue selenium and glutathione peroxidase activities. J. Anim. Sci. 52:302.

Miller, E.R., R.L. Johnston, J.A. Hoefer and R.W. Luecke. 1954. The riboflavin requirement of the baby pig. J. Nutr. 52:405.

Mills, G.C. 1957. Hemoglobin catabolism I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. 229:189.

Mills, G.C. and H.P. Randall. 1958. Hemoglobin catabolism II. The protection of hemoglobin from oxidative breakdown in the intact erythrocyte. J. Biol. Chem. 232:589.

Mills, G.C. 1959. The purification and properties of glutathione peroxidase of erythrocytes. J. Biol. Chem. 234:502.

Morris, V.C. and O.A. Levander. 1980. Metabolism of selenium (Se) fed to rats as selenite or high selenium yeast. Fed. Proc. 39:338. (Abstr.).

Nichols, D.A., D.R. Ames and R.H. Hines. 1980. Effect of temperature on performance and efficiency of finishing swine. J. Anim. Sci. 51:(Suppl. 1) 63 (Abstr.).

NRC. 1979. Nutrient Requirements of Domestic Animals, No. 2. Nutrient Requirements of Swine. Eighth Revised Ed. National Academy of Science-National Research Council. Washington, DC.

O'Brien, P.J. and C. Little. 1967. An intracellular glutathione peroxidase with a lipid peroxide as substrate. Biochem. J. 103:31P.

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

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

Pond, W.G. and J.H. Maner. 1974. Swine Production in Temperate and Tropical Environments. W.H. Freeman and Company, San Francisco.

Rotruck, J.T., W.G. Hoekstra, A.L. Pope, H. Ganther, A. Swanson and D. Hafeman. 1972. Relationship of selenium to glutathione peroxidase. Fed. Proc. 31:691 (Abstr.).

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.

Sauberlick, H.E., J.H. Judd, Jr., G.E. Nichoalds, H.P. Broquist and W.J. Darby. 1972. Application of the erythrocyte glutathione reductase assay in evaluating riboflavin nutritional status in a high school student population. Amer. J. Clin. Nutr. 25:756.

Schwarz, K. and C.M. Foltz. 1958. Factor 3 activity of selenium compounds. J. Nutr. 233:245.

Scott, M.L. and T. Noguchi. 1973. Metabolic function of selenium in prevention of exudative diathesis in chicks. Fed. Proc. 32:885. (Abstr.).

Smith, P.J., A.L. Tappel and C.K. Chow. 1974. Glutathione peroxidase activity as a function of dietary selenomethionine. Nature (London). 247:392.

Staal, G.E.J., J. Visser and C. Veeger. 1969. Purification and properties of glutathione reductase of human erythrocytes. Biochem. Biophys. Acta, 185:39.

Stryer, L. 1975. Biochemistry. W.H. Freeman and Company, San Francisco.

Sugahara, M., D.H. Baker, B.G. Harmon and A.H. Jensen. 1970. Effect of ambient temperature on performance and carcass development in young swine. J. Anim. Sci. 31:59.

Sunde, R.A., H.E. Ganther, and W.G. Hoekstra. 1978. A comparison of ovine liver and erythrocyte glutathione peroxidase. Fed. Proc. 37:757. (Abstr.).

Sunde, R.A. and W.G. Hoekstra. 1980a. Selenium incorporation into glutathione peroxidase in the isolated perfused rat liver. Fed. Proc. 39:555 (Abstr.).

Sunde, R.A. and W.G. Hoekstra. 1980b. Incorporation of selenium into liver glutathione peroxidase in the Se-adequate and Se-deficient rat. Proc. Soc. Exp. Biol. Med. 165:291.

Sunde, R.A., G.E. Gutzke and W.G. Hoekstra. 1981. Effect of dietary methionine on the biopotency of selenite and selenomethionine in the rat. J. Nutr. 111:76.

Talbot, R.B. and M.J. Swenson. 1963. Survival of CR^{51} labeled erythrocytes in swine. Proc. Soc. Exp. Biol. Med. 112:573.

Tao, R.V.P. 1973. Biochemistry and metabolism of mammalian blood glycosphingolipids. Ph.D. Thesis. Michigan State University, East Lansing, MI.

Tappel, A.L., J.W. Forstrom, J.J. Zabowski, D.E. Lyons and W.C. Hawkes 1978a. The catalytic site of rat liver glutathione peroxidase as selenocysteine and selenocysteine in rat liver. Fed. Proc. 37:706 (Abstr.).

Tappel, A.L. 1978b. Measurement of and protection from *in vivo* lipid peroxidation. Proc. of "Conference on Biochemical and Clinical Aspects of Oxygen".

Terrill, S.W., C.B. Ammerman, D.E. Walker, R.M. Edwards, H.W. Norton and D.E. Becker. 1955. Riboflavin studies with pigs. J. Anim. Sci. 14:593.

Tillotson, J.A., and E.M. Baker. 1972. An enzymatic measurement of the riboflavin status in man. Am. J. Clin. Nutr. 25;425.

Wang, C.S. and J. Spallholz. 1980. Biological availability of selenium from sodium selenite, selenium yeast, monoselenodiactic acid and selenomethionine for glutathione peroxidase. Fed. Proc. 39:555 (Abstr.).

Wastell, M.E., R.C. Ewan, M.W. Vorhies and V.C. Speer. 1972. Vitamin E and selenium for growing and finishing pigs. J. Anim. Sci. 34:969.

Wegger, I., K. Rasmussen and P. F. Jørgensen. 1980. Glutathione peroxidase activity in liver and kidney as indicator of selenium status in swine. Livestock Prod. Sci. 7:175.

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

Widicus, W.A. and J.R. Kirk. 1979. High pressure liquid chromatographic determination of vitamins A and E in cereal products. J. Assoc. Off. Anal. Chem. 62:637.

Withrow, G. and M.C. Bell. 1969. Erythrocytic life span estimations in growing sheep and swine using ^{75}Se. J. Anim. Sci. 28:240.

Young, L.G., A.G. Castell and D.E. Edmeades. 1977a. Influence of dietary levels of selenium on tissue selenium of growing pigs in Canada. J. Anim. Sci. 44:590.

Young, L.G., R.B. Miller, D.E. Edmeades, A. Lun, G.C. Smith and G.J. King. 1977b. Selenium and vitamin E supplementation of high moisture corn diets for swine reproduction. J. Anim. Sci. 45:1051.