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thesis entitled

INFLUENCE OF MONENSIN ON SELENIUM STATUS

AND RELATED FACTORS IN GROWING SWINE

presented by

Christopher James Horvath

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Large Animal Clinical Sciences

Major professor
Howard D. Stowe

Date September 11, 1989

INFLUENCE OF MONENSIN ON SELENIUM STATUS AND RELATED FACTORS IN GROWING SWINE

By

Christopher James Horvath

AN ABSTRACT OF A THESIS

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

MASTER OF SCIENCE

Department of Large Animal Clinical Science

1989

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ABSTRACT

INFLUENCE OF MONENSIN ON SELENIUM STATUS
AND RELATED FACTORS IN GROWING SWINE

By

Christopher James Horvath

Monensin is an ionophoretic antibiotic which selectively transports alkali metal cations across biological membranes and affects the absorption, retention and excretion of minerals, including trace elements. Monensin toxicosis in growing swine produces acute, degenerative cardiac and skeletal myopathy resembling vitamin E-selenium (Se) deficiency. Selenium is an essential trace element incorporated in glutathione peroxidase (GSH-Px), an antioxidant enzyme that protects cellular membranes. These experiments examined the effects of monensin on growth, Se balance, antioxidant status, and serum and tissue concentrations of selected elements in growing swine, including pigs which were genetically hypo- or hyperselenemic. Monensin consumption did not affect serum Se values or Se balance but increased serum GSH-Px activities. Monensin had no effect on body weight or serum leakage enzymes and inconsistent effects on serum and tissue mineral values. No interactions occurred between monensin and genetic Se status. Hyper-Se pigs grew faster and had higher serum Se and GSH-Px values than hypo-Se pigs. Hypo-Se and hyper-Se pigs were hypo- and hypercupremic, respectively, suggesting genetic regulation of Cu status. We conclude that monensin toxicosis in swine is not the result of direct nutritional antagonism of Se metabolism but it may be more prevalent in pigs with inadequate antioxidant status. Genetic control of Se and Cu status occurs in swine.

This work is dedicated to
my wife, Donna, for her love;
my mentor, Howard, for taking a chance;
my friend, Paul, for his encouragement;
my dog, Nicki, for her companionship;
my mother, Jackie, for her blessings;
my father, Donald, for his example.

This Master's

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illustrations; Ms. S

Jones, John Baker,

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ACKNOWLEDGEMENTS

This Master's Thesis would not have been possible without the assistance and support of a great number of people. I would like to thank the Veterinary Clinical Center Resach Grant and the Biomedical Research Support Grant programs for funding; Dr. Maynard Hogberg for use of the facilities at the Michigan State University Swine Research Center; Drs. Elwin R. Miller and Glen Waxler for their participation and guidance as committee members; Anne House and Tonie Theil for excellent technical assistance; Drs. Harold Tveden, Emmett Braselton, and Mike Slanker for disscusion and assistance with sample assays; Drs. John Gill, Thomas Adams, Thomas Herdt, and Kent Refsal for their advice on statistical analysis and computer graphics; Ms. Maggie Hoffman for assistance with the illustrations; Ms. Shirley Eisenhauer for typing the manuscript; Drs. Kent Ames, John Baker, Charles Coy, and Michelle Kopcha for their understanding; Dr. Dan Ciszewski for discussion, empathy and laughter; and, most importantly, Dr. Howard D. Stowe for his patience, expertise and compassionate guidance. I thank you all.

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INTRODUCTION

Selenium (Se) is an essential trace element which is incorporated in the metalloenzyme, glutathione peroxidase (GSH-Px), that provides protection against *in vivo* peroxidation of cellular membranes. In swine, Se deficiency disease is characterized by acute severe degenerative disease of liver and cardiac and skeletal muscle.

At Michigan State University, H. D. Stowe and E. R. Miller were intrigued by the apparently diverse susceptibility of commercially raised swine to naturally occurring vitamin E-selenium deficiency disease (VESD). Following reports by Atroshi *et al.* (1981) of the genetic determination of erythrocyte GSH-Px activity in Finn sheep, Stowe and Miller (1982, 1985) were successful in selecting for populations of crossbred pigs which were born relatively hypo- or hyperselenemic and remained so for life.

At the same time, other investigators were finding that monensin significantly affected absorption, retention and excretion of many monovalent and divalent minerals, including trace elements (Starnes *et al.*, 1984; Elsasser, 1984; Kirk *et al.*, 1985a, 1985b; Greene *et al.*, 1986). In particular, Costa *et al.* (1984, 1985, 1987) had documented increased Se absorption and retention in monensin-fed cattle. Ionophores were suggested to have potential benefit as modifiers of trace element diseases (Kirk *et al.*, 1985b). However, the magnitude and direction of ionophore-mediated shifts in mineral balance were unpredictable (Elsasser,

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To decrease coccidia oocyst shedding after farrowing, Roberts and Walker (1981, 1982) had recommended feeding monensin to sows. Several investigators then reported monensin toxicosis, presumably due to delayed monensin metabolism (Meingassner et al., 1979), in growing swine simultaneously fed the antibiotic tiamulin (Drake, 1981; Pott and Skov, 1981; Stansfield and Lamont, 1981). The clinical and pathologic appearance in these cases resembled VESD disease, but attempts to experimentally reproduce monensin-tiamulin interactive myopathy in swine were not always successful (D.J.S. Miller, 1981). This suggested that susceptibility to monensin toxicosis was not uniform among animals.

Van Vleet and co-workers at Purdue University soon completed several studies in pigs that confirmed that the lesions of acute monensin toxicosis were similar to those of VESD and that pretreatment with vitamin E/Se partially ameliorated experimental monensin toxicosis (Van Vleet et al., 1983a, 1983b; Van Vleet and Ferrans, 1984a, 1984b). Circumstantial evidence also incriminated monensin consumption as the precipitating event in several cases of naturally occurring nutritional myopathy in cattle (Hosie and Rollo, 1985; Smith et al., 1985). These observations were compatable with antagonistic effect of monensin on Se metabolism.

In contrast, Anderson et al. (1983) reported that monensin and Se supplementation produced additive increases in the blood GSH-Px concentrations of pregnant ewes and that these effects were subsequently carried over to their lambs. This finding suggested that monensin might have some beneficial effects on Se metabolism.

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thereby influence the antioxidant status of growing pigs. Conversely, if monensin toxicosis were mediated in part by a peroxidative mechanism, as Van Vleet and Ferrans (1984a) had proposed, then individual susceptibility to monensin toxicosis might depend on the Se status of the pig, which is genetically regulated. Innate differences in antioxidant status might explain the sporadic occurrence of monensin toxicosis within a group of animals.

Accordingly, the objectives of this thesis were to determine:

1. The effects of continual oral monensin intake on growth, antioxidant status, serum leakage enzyme activity and serum mineral concentrations in growing pigs fed corn-soybean diets, with or without supplemental Se (Experiment 1);

2. The effects of continual intake of monensin without supplemental Se on growth, antioxidant status, serum leakage enzyme activities, and serum and tissue mineral concentrations in pigs from genetically hypo-selenemic or hyperselenemic populations (Experiment 2);

3. The influence of monensin on Se balance in weanling pigs (Experiment 3).

1. Introduction

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I. Introduction

Selenium (Se) has been recognized as an essential trace element since 1957 when Schwarz and Foltz demonstrated that it prevented liver necrosis in rats. Previously, selenium had been studied primarily for its toxic effects in livestock. Soon, a wide variety of degenerative diseases in many species was associated with Se-deficient conditions.

Rotruck et al. (1973) discovered that Se had a biological role as a component of glutathione peroxidase (GSH-Px; EC 1.11.1.9) an enzyme which had been shown to protect erythrocyte hemoglobin from oxidative damage (Mills, 1957). It is now understood that the selenoenzyme GSH-Px is responsible, in concert with vitamin E and the sulfur-containing amino acids, for preventing peroxidative damage to biological membranes.

This review will examine the function of Se in the antioxidant protection system and the consequences of Se-deficiency disease.

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II. Selenium Distribution in Soil and Plants.

Geographic distribution of selenium is variable depending on soil type, but most soils contain .1-2.0 ppm Se (Mayland, 1985). The ratio of chemical forms of selenium present is a function of the type of soil. Acidic, poorly aerated soils contain predominantly selenides (Se^{-2} and elemental Se (Se^{0}), which are less bioavailable to plants than the selenites (SeO_3^{-2}) and selenates (SeO_4^{-2}) contained in aerated, alkaline soils (Mayland, 1985).

The Se concentration within plants depends on its availability in soil and the capacity of the plant for Se uptake. Plants can be considered Se accumulators if they contain more than several thousand parts per million (ppm) of Se (primary Se indicator plants) or several hundred ppm Se (secondary Se absorbing plants) (Rosenfeld and Beath, 1964). Fortunately, most of the grains and grasses that are fed to livestock contain less than 50 ppm Se when grown on seleniferous soils.

Several investigators have mapped plant and soil Se levels throughout North America and related them to the occurrence of Se toxicosis or deficiency (Muth and Allaway, 1963; Allaway and Hodgson, 1964; Kubota et al., 1967). In the United States, large areas of the Atlantic, Great Lakes, Midwest, Southeastern, Pacific and Northwestern regions have soils or plants containing marginal or inadequate concentrations of Se. The North Central, Great Plains and Southwestern areas typically have adequate or potentially toxic concentrations of Se in plants or soils.

III. Vitamin E-Se

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III. Vitamin E-Selenium Deficiency Disease (VESD).

A. Overview.

Vitamin E and Se deficiency diseases (VESD) have been recognized in a number of domestic livestock and laboratory species. Rosenfeld and Beath (1964) offer a valuable discussion, and other comprehensive reviews are available (Andrews et al., 1968; Jenkins and Hidiroglou, 1972; Ammerman and Miller, 1975; Lannek and Lindberg, 1975).

It is generally accepted that vitamin E exerts its antioxidant activity as a lipid-soluble component of bipolar cellular membranes where it acts to interrupt the chain of lipid peroxidation initiated by some oxidant stressor or resulting from normal cellular activities (Andrews et al., 1968; Combs and Scott, 1977). Excess dietary unsaturated fatty acids may increase lipid peroxidation and hasten the onset of nutritional myopathy (Jenkins and Hidiroglou, 1972). The sulfur-containing amino acids, cystine, cysteine, and methionine, may exert some sparing effects because they can supply selenocysteine for the synthesis of glutathione (GSH), a necessary substrate for GSH-Px (Ammerman and Miller, 1975). Vitamin E, Se, and the sulfur amino acids have complementary, overlapping, protective effects, but they are separately necessary dietary components.

Vitamin E/Se deficiency is associated with hepatic necrosis and "suckling paralysis" in rats (Schwarz and Foltz, 1957); skeletal myopathy, exudative diathesis and encephalomalacia in chicks (Lannek and Lindberg, 1975); steatitis, skeletal myopathy or "tying-up syndrome" in horses (Owen et al., 1977); "stiff lamb disease" or "white muscle disease" in sheep and calves (Maas, 1983; Maas et al., 1984, 1985); and "hepatosis dietetica", "mulberry heart disease", exudative diathesis and nutritional myopathy in

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In man, Se-deficiency (Keshan disease) is endemic in certain regions of China (Yang et al., 1983). Young women and children with Keshan disease have abnormal electrocardiograms, gallop rhythms, and cardiomyopathy which result in heart failure. Koller and Exon (1986) made the point that Se deficiency across species is characterized by nutritionally induced skeletal and cardiac myopathies, more prevalent in young, growing individuals and often precipitated by stress. For the most part, this discussion will focus on the role of Se in nutritionally related diseases of swine.

B. VESD in Swine.

1. Review.

Vitamin E-Selenium deficiency disease became increasingly prevalent in the 1960's as a result of management changes in swine production. These included confinement rearing, a switch to corn-soybean meal diets, use of high-moisture grains low in vitamin E and ensiled with propionic acid, earlier weaning, and the increased "stress" inherent in intensified production systems (Mortimer, 1983; Whitehair and Miller, 1985).

Testicular degeneration in boars and mastitis-metritis-agalactia complex in sows were reported as part of this syndrome (Trapp et al., 1970; Whitehair et al., 1982a, 1982b). However, weaned, rapidly growing pigs were observed to develop gastric ulcers, skeletal and cardiac myopathies, microangiopathies, exudative diathesis, and hepatic necrosis (Obel, 1953; Michel et al., 1967, 1969; Trapp et al., 1970). Clinical disease entities were occasionally seen alone but usually occurred in combinations (Tollersrud and Nafstad, 1970; Moir and Masters, 1979).

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Numerous workers have investigated the use of vitamin E and Se for prevention of these nutritional syndromes (Obel, 1953; Michel et al., 1969; Mahan et al., 1971; Sharp et al., 1972; Van Vleet et al., 1973; Niyo et al., 1977). The combined results indicate that adequate protection requires both vitamin E and Se supplementation. Currently, it is legal (and recommended) to add .3 mg supplemental Se/kg to diets for pigs of all ages (Food and Drug Administration, 1987).

2. Lesions in Disease.

a. Liver Tissue.

"Hepatosis dietetica" was described by Obel (1953) as affecting post-weaning pigs of less than 3-4 months of age. Michel et al. (1967, 1969) and Trapp et al. (1970) later reported the condition in Michigan pigs. Affected animals were occasionally noted to be depressed or inappetent but were often found dead. Findings on postmortem examination included extensive, patchy hepatic necrosis and enlargement (Andrews et al., 1968; Trapp et al., 1970). Focal, multilobular areas of noninflammatory, coagulative necrosis of hepatocytes and interlobular fibrosis were seen microscopically (Moir and Masters, 1979).

b. Cardiac Tissue.

Death due to "mulberry heart disease" or "dietetic microangiopathy" also occurred in post-weaning pigs with few premonitory signs. Skin was occassionally marked by irregularly shaped "bruises" (Lannek and Lindberg, 1975). Lesions of circulatory failure predominated, with fluid accumulation in the pericardial, thoracic, and abdominal cavities, and pulmonary and mesenteric edema (Trapp et al., 1970; Jenkins and Hidiroglou, 1972; Lannek and Lindberg, 1975; Moir and Masters, 1979).

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Lesions usually involved the atria and ventricles bilaterally, although the right side was more severely affected (Van Vleet et al., 1970).

c. Skeletal Muscle Tissue.

"White muscle disease" or nutritional muscular dystrophy (NMD) often had a sudden onset but rarely caused mortality by itself. Affected pigs appeared listless and had an incoordinated gait or were unable to stand. Occasionally, myoglobinuria has been observed (Andrews et al., 1968). Skeletal muscle lesions appeared as chalky, white areas within heavy muscle groups (Whitehair and Miller, 1985), but macroscopic lesions were often absent (Michel et al., 1969).

Histologically, skeletal muscle underwent hyaline necrosis, with loss of striations, vacuolization, fragmentation, and mineral deposition (Michel et al., 1969; Trapp et al., 1970; Niyo et al., 1971; Moir and Masters, 1979). Varying degrees of skeletal myofiber regeneration and macrophage infiltration have been seen (Van Vleet et al., 1976) and selective destruction of type I (aerobic) skeletal muscle fibers has been reported (Ruth and Van Vleet, 1974; Van Vleet et al., 1976).

C. Serum Leakage Enzymes.

1. Review.

Development of cardiac or skeletal muscle degeneration and liver necrosis causes cellular enzymes to leak from damaged tissue; these enzymes can be measured as an index of the extent of vitamin E-Se deficiency lesions.

Creatine kinase (EC 2.7.3.2) or creatine phosphokinase (CPK) is found in skeletal and cardiac muscle and brain tissue, where it catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP)

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(Rosalki, 1967). The resulting high energy phosphate, creatine phosphate, is stored by muscles for contraction (Kramer, 1980). Although three CPK isoenzymes exist and many cells contain CPK, elevations in serum CPK are highly suggestive of cardiac or skeletal muscle damage (Kramer, 1980). Plasma half-life of CPK is only 6 hours, and values reportedly return to normal by 48 hours once muscle damage ceases (Duncan and Prasse, 1977).

Alanine amino transferase (ALT) (EC 2.6.1.2) or glutamic-pyruvate transaminase (GPT) is found in liver and other cells, where it catalyzes the reversible transamination of L-alanine and α-oxoglutarate to pyruvate and glutamate (Kramer, 1980). Hepatic plasma membrane damage allows ALT leakage into serum, but only cats and dogs have sufficient hepatic ALT activity for it to serve as a liver-specific indicator (Duncan and Prasse, 1977).

Aspartate aminotransferase (AST) (EC 2.6.1.1) or glutamic-oxaloacetic transaminase (GOT), catalyzes the transamination of L-aspartate and α-oxoglutarate to oxaloacetate and glutamate (Kramer, 1980). The presence of AST in most tissues, including liver, muscle, and erythrocytes, prevents its use as an organ-specific enzyme. Plasma half-life of AST is greater than that of CPK, and plasma AST values are therefore not as useful for determining the temporal aspect of disease.

Lactate dehydrogenase (LDH) (EC 1.1.1.27) catalyzes the reversible oxidation of L-lactate to pyruvate and is found in all tissues in a variety of isoenzymes (Kramer, 1980). Serum isoenzyme profiles can be used reliably to determine whether liver, heart, or skeletal muscle damage predominates (Prasse, 1969).

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2. Enzyme Leakage in Disease.

a. Cattle, Sheep and Horses.

Nutritional myopathies associated with the loss of cellular membrane integrity result in significant enzyme leakage into plasma, which usually reflect muscle damage since muscle tissue has the greatest mass (Kramer, 1980). In a review of VESD, Lannek and Lindberg (1975) cited numerous studies in which sheep and cattle had increased serum ALT, AST, LDH, or CPK activities. Horses (Owen et al., 1977) and foals (Dill and Rebhun, 1985) with VESD-related dystrophic myodegeneration had elevated serum CPK and AST values. Nutritional myopathy was diagnosed in yearling beef cattle (Smith et al., 1985) and young lambs (Maas et al., 1984) in part on the basis of greatly elevated AST or CPK concentrations in serum. Experimental models of NMD in cattle showed consistent elevations in CPK and AST values (McMurray and McEldowney, 1977; McMurray and Rice, 1982; McMurray et al., 1983). Tollersrud (1971) found significant increases in serum AST, ALT, and LDH values in experimentally induced nutritional muscular dystrophy in lambs prior to onset of illness. Therefore, elevated serum activities of AST, CPK and LDH are considered useful aids for the diagnosis of Se-responsive diseases of sheep, cattle, and horses (Maas, 1983; Maas and Koller, 1985; Dill and Rehbun, 1985).

b. Swine.

Wretlind et al. (1959) established normal values for serum ALT and AST in swine plasma. Pigs with naturally occurring VESD had increased activities of ALT and AST (Wretlind et al., 1959; Orstadius et al., 1959; Grant, 1961). Pigs with experimentally induced VESD had significantly greater serum AST activities than pigs treated with Se and/or vitamin E

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(Niyo et al., 1977). Simesen et al. (1982) found higher mean serum ALT, but not AST, activities in pigs fed a Se-deficient diet compared to pigs supplemented with either Se, vitamin E, or the antioxidant ethoxyquin. Fontaine et al. (1977) demonstrated increased serum CPK, but not AST, activities in pigs fed Se- or vitamin E-deficient diets. They suggested that subclinical skeletal muscle damage occurred without liver necrosis.

Increased AST activities and muscular stiffness were thought to be evidence of subclinical disease in Se-deficient pigs (Van Vleet et al., 1975) but similar increases were seen in healthy pigs subjected to physical exertion and heat stress. Bengtsson et al. (1978) fed Se-deficient diets and found increased serum AST values in growing pigs that subsequently developed VESD, but elevations were intermittent and not consistent. Other workers showed that serum ALT, AST and LDH activities were good indicators of subclinical VESD in pigs, but were not necessarily good predictors of the organ systems found to be involved at necropsy (Tollersrud and Nafstad, 1970; Tollersrud, 1973). Some reviewers (Lannek and Lindberg, 1975; Glienke and Ewan, 1977) have concluded that serum enzymes are of great value in the diagnosis of liver, cardiac or skeletal muscle degeneration in VESD of swine. However, Simesen et al. (1979) failed to find an association between hyposelenemia and serum ALT or AST values, even in pigs dying of VESD. Apparently, serum leakage enzymes may not be as reliable in swine as in cattle or sheep for revealing VESD tissue damage.

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IV. Selenium and Antioxidation.

A. Se-Dependent Glutathione Peroxidase.

Mills (1957) discovered an enzyme, glutathione peroxidase (GSH-Px) (EC 11.11.1.9), that used reduced glutathione (GSH) as a hydrogen donor and catalyzed the breakdown of hydrogen peroxide (H_2O_2), thus protecting bovine erythrocytes from hemoglobin oxidation. The same year, Schwarz and Foltz (1957) demonstrated that Se was an essential trace element in rats and could prevent liver necrosis, which was also prevented by vitamin E and delayed by sulfur-containing amino acids. However, it was not until 1973 that Rotruck et al. discovered Se to be a component of GSH-Px, the enzyme which prevented oxidative damage to rat erythrocytes and hemoglobin.

The structure of GSH-Px has been described and its function reviewed (Ganther et al., 1976; Stadtman, 1980; Flohe, 1982). Glutathione peroxidase is a soluble protein with a molecular weight of about 85,000 daltons and composed of four identical 21,000 molecular weight subunits (Flohe, 1982). Each subunit contains about 180 amino acids of identical sequence and a single Se atom, which resides in a selenocysteine residue at position 35 (Flohe, 1982). Forstrom et al. (1978) demonstrated that the reduced form of GSH-Px in rat liver contains selenocysteine in the form of selenol (-SeH) at the catalytic site. The Se content of GSH-Px is four gram atoms per mole.

Using reduced glutathione (GSH) as substrate and hydrogen donor, GSH-Px catalyzes the reduction of hydrogen peroxide (H_2O_2) (Eq. 1) or an array of organic peroxides (ROOH) (Eq. 2) to yield oxidized glutathione (GS:SG), water (H_2O) and an organic hydroxy acid (ROH) (Ganther et al.,

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1976; Flohe, 1982). This can be represented as follows:

$$\text{Eq. 1:} \quad 2GSH + H_2O_2 \xrightarrow{\text{GSH-Px}} GS{:}SG + 2H_2O$$

$$\text{Eq. 2:} \quad 2GSH + ROOH \xrightarrow{\text{GSH-Px}} GS{:}SG + ROH + H_2O$$

Glutathione peroxidase is highly specific for the donor substrate, GSH, but has relatively low specificity for the peroxide substrate and will use either organic (lipid) hydroperoxides or H_2O_2 (Ganther et al., 1976).

B. Se-Independent Glutathione-S-Transferase

Lawrence and Burk (1978) identified a non-Se-dependent glutathione peroxidase, more properly called glutathione-S-transferase-B (GSH-S-Tr) (EC 2.5.1.18), that has a function similar to GSH-Px. Glutathione-S-transferase was isolated from a wide variety of species, but tissue distribution and activities of GSH-Px and GSH-S-Tr were highly variable. This would have made determination of Se-dependent antioxidant status difficult if the enzymes shared common substrate specificities, but GSH-S-Tr was found to have an extremely low specificity for H_2O_2 and a high specificity for organic hydroperoxides (Lawrence and Burk, 1978). For accurate determination of Se-dependent GSH-Px activities, assays must be performed using H_2O_2 as a substrate rather than organic hydroperoxides, such as cumene hydroperoxide or tertiary butyl hydroperoxide.

The role of non-Se-dependent GSH-S-Tr in protecting biological membranes from oxidative damage is unclear, but may be important in Se-deficient tissues or animals (Stadtman, 1980; Van Vleet, 1980). GSH-S-Tr activity is probably induced in an antioxidant crisis since Arthur

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C. Other Selenoproteins.

Several bacterial enzymes, including formate dehydrogenases, clostridial glycine reductase, xanthine dehydrogenase and nicotinic acid hydroxylase, are known to be Se-dependent enzymes (Stadtman, 1980). To date, the major requirement of mammalian and avian species for Se is believed to be as a component of GSH-Px.

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V. Nutritional Muscular Dystrophy (NMD) and Lipid Peroxidation.

A. Vitamin E and NMD.

1. Overview.

A relationship among dietary polyunsaturated fatty acids (PUFAs), primarily arachidonic acid (C 20:4), linolenic acid (C 18:3), and linoleic acid (C 18:2), vitamin E and the development of NMD has been recognized. Since PUFAs reside within biological membranes, in vivo lipid peroxidation could explain observed membrane damage in myopathic disease (Machlin, 1984). Vitamin E, or α-tocopherol, is also located within cell membranes, where it is able to "quench" free radicals produced by lipoperoxidation (Noguchi et al., 1973). The molar ratio of PUFAs to vitamin E within membranes is approximately 1000:1 (Tappel, 1973).

Of more than eight different tocopherol compounds, d-α-tocopherol is the most abundant and active (Combs and Scott, 1977), and is associated with PUFAs in plant oils, particularly linoleic acid (Linder, 1985a). Indirect evidence for the role of vitamin E as a membrane antioxidant was provided, in part, by the sparing effects of synthetic antioxidants, such as ethoxyquin, on vitamin E-deficiency disease syndromes (Machlin, 1984). Tappel (1962) first proposed that vitamin E served in vivo antioxidant functions and protected tissue lipids from free radical attack. Direct evidence of in vivo lipid peroxidation was not easily found, however.

2. Methods for Detecting Peroxidation.

Results of attempts to quantify peroxides within diseased muscle tissue have been inconsistent (Machlin, 1984). Most workers measured the reaction of malonyldialdehyde, which is produced by lipid peroxidation, with thiobarbituric acid (TBA) as an index of free radical-induced lipid

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peroxidation (McMurray and Dormandy, 1974). Tissues with decreased antioxidant capacity or increased free radical production *in vivo* would be expected to have high concentrations of TBA reactants, even *in vitro*.

Chow *et al*. (1973) estimated *in vitro* lipid peroxidation by the reaction of malonyldialdehyde with phosphatidyl ethanolamine to yield fluorescent products that were measured spectrophotometrically. Measurement of lipoperoxide-derived pentane and ethane production in breath has been used to quantify *in vivo* lipid peroxidation (Tappel, 1980). More recently, Arthur *et al*. (1987a) used a "spin trapping" proceedure to show that the amount and rate of formation of free radicals was elevated in homogenates of heart from rats with combined vitamin E and Se deficiency.

3. Lipid Peroxidation in Disease.

McMurray and McEldowney (1977) reported that young cattle put out to graze spring pasture developed classical nutritional myopathy of skeletal and cardiac muscle, with 50- to 100-fold increases in serum CPK and AST values. Histologic examination revealed mitochondrial swelling, hyalin degeneration, cell lysis and necrosis, and calcific mineralization (McMurray and Rice, 1982). Whole blood GSH-Px activity was not detectable, and blood Se levels were very low. Treatment with vitamin E, Se, or both prevented serum enzyme increases and clinical disease. These investigators theorized that dietary concentrations of Se and vitamin E were naturally low and that high concentrations of dietary PUFAs in the young grass resulted in overwhelming hydroperoxide production, membrane damage and enzyme leakage (McMurray and McEldowney, 1977). Serum PUFA concentrations were measured in grass-fed calves and a 230% increase

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Subsequently, an experimental model for NMD was developed using vitamin E- and Se-deficient calves fed linolenic acid (McMurray and Rice, 1982; McMurray et al., 1983). These calves exhibited 100-fold increases in CPK activity and clinical and histologic signs of NMD, which were prevented by pretreatment with vitamin E and/or Se. Increased plasma linolenic acid concentrations, ECG changes, and myoglobinuria were seen in calves in this model; severe skeletal muscle degeneration was most pronounced in Type I fibers and myocardial lesions were greatest in the left ventricle (Kennedy and Rice, 1987). This seemed to substantiate the idea that PUFAs underwent in vivo peroxidation, with hydroperoxide and free radical production and ensuing tissue damage (McMurray et al., 1983; Rice et al., 1985). Later, Rice et al. (1987) showed that the phospholipid membranes of muscle cells from vitamin E- and Se-deficient cattle contained increased amounts of arachidonic acid, and that the toxicity of unsaturated fatty acids was due to their incorporation in the muscle cell membranes.

Allen et al. (1975a) described an outbreak of degenerative myopathy with myoglobinuria and 50- to 100-fold elevations in serum CPK and LDH concentrations in yearling cattle. They estimated muscle peroxide content and found increased TBA-reactant compounds, presumably malonyldialdehyde. Desai et al. (1964) reported increased TBA-reactants prior to onset of membrane damage in vitamin E- and methionine-deficient chicks. Patterson et al. (1969) showed increased muscle peroxides in pigs with acute myopathy induced by iron injection. Iron is a potent oxidant and can precipitate acute VESD-like lesions in Type I, aerobic muscle fibers of

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pigs with marginal antioxidant status (Cook et al., 1982).

Several other reports support lipid peroxidation as the mechanism responsible for membrane damage in VESD. Vitamin E-deficient rabbits had myopathic lesions that were felt to have been initiated by lipid peroxidation (Van Vleet et al., 1968). Jackson et al. (1983) showed that muscle from vitamin E-depleted rats had reduced antioxidant capacity and increased TBA-reactant compounds when stressed with oxidants. However, these investigators were unable to demonstrate that exercise-induced muscle damage was mediated by free radical reactions. Resistance of erythrocytes to in vitro lipid peroxidation, based on malonyldialdehyde detection, was described by Fontaine and Valli (1977) as a potential method of evaluating vitamin E status in pigs.

B. Mechanism of Lipid Peroxidation.

Lipid peroxidation occurs when a polyunsaturated fatty acid (LH) reacts with an oxidant stressor to form an unstable lipid free radical intermediate ($L^{\cdot}$)(Eq. 3). The lipid free radical can react with oxygen (O_2) to form a lipoperoxy free radical ($LOO^{\cdot}$)(Eq. 4), which further reacts with lipids to form lipid hydroperoxides (LOOH) and lipid free radicals (Eq. 5)(Tappel, 1980; Machlin, 1984). This self-propagating reaction can be represented as follows:

$$\text{Eq. 3} \qquad LH \xrightarrow{\text{oxidant stressor}} L^{\cdot}$$

$$\text{Eq. 4} \qquad L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$

$$\text{Eq. 5} \qquad LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$$

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Oxidant initiating processes may be endogenous or exogenous. Membrane-bound enzymes, such as xanthine oxidase (EC 1.2.3.2), a molybdenum-dependent enzyme (Ammerman and Miller, 1975), and the cytochrome P_{450} oxidase system catalyze reactions of oxidizable substrates and oxygen to produce hydroxyl free radicals ($OH^{\cdot}$) and superoxide anions ($^{\cdot}O_2$) (King et al., 1975; Linder, 1985a). Superoxide can react with H_2O_2 to produce hydroxyl ($OH^{\cdot}$) or peroxide free radicals ($HOO^{\cdot}$). These free radicals can attack PUFAs in phospholipid mitochondrial, microsomal and plasma membranes, producing organic ($ROO^{\cdot}$) or lipid free radicals ($LOO^{\cdot}$).

Without antioxidant protection, cell membranes would rapidly destabilize and tissue integrity would be lost. Unchecked free radical propagation would result in damage to cellular proteins, producing a chaotic sequence of polymerization and cross-linking, especially among membrane-bound enzymes (Tappel, 1973). This peroxidative cascade can be interrupted at many different points. The copper and zinc-dependent enzyme superoxide dismutase (SOD) (EC 1.15.1.1), can convert $^{\cdot}O_2$ to H_2O_2, and the iron-dependent enzyme catalase (EC 1.11.1.6) can convert H_2O_2 to H_2O (Linder, 1985b). Singlet oxygen can be "quenched" by carotene (McMurray and Rice, 1982). It is no surprise, then, that additional antioxidant capacity is also nutritionally dependent on vitamin E, Se, and the sulfur-containing amino acids.

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VI. Glutathione Peroxidase Antioxidant Functions in Erythrocytes.

A. Erythrocyte Integrity and Hexose Monophosphate (HMP) Shunt.

Glutathione peroxidase activity has been found in virtually all tissues studied so far (Van Vleet, 1980) but most early research focused on protection of red blood cell membranes. Membranes containing high levels of PUFAs in their phospholipids, such as erythrocyte plasma membranes and mitochondrial and microsomal membranes, are prone to lipid peroxidation (Combs et al., 1975). In fact, controlled lipoperoxidation is probably a metabolic process common to all tissues (King et al., 1975; Combs and Scott, 1977). Erythrocyte plasma membranes, in direct contact with molecular oxygen, may be even more susceptible to PUFA peroxidation and consequent hemolysis (Tappel, 1973; Combs et al., 1975).

Erythrocytes are heavily dependent on the hexose monophosphate (HMP) or pentose phosphate shunt to supply the reducing equivalents (H^+) necessary to resist oxidation. Ganther et al. (1976) and Flohe (1982) have reviewed this system.

Cellular glucose is phosphorylated by hexokinase (EC 2.7.1.1) to yield glucose-6-phosphate (G-6-P) (Eq. 6), which can be metabolized in the glycolytic (Embden-Meyerhoff) pathway or diverted to the HMP shunt. In the latter case, the enzyme glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49) oxidizes G-6-P to 6-phosphogluconate (6-PG) while reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH (Eq. 7). Additional reducing equivalents are generated (Eq. 8) when 6-PG is converted to ribulose-5-phosphate (R-5-P) by 6-phosphogluconate dehydrogenase (6-PGD; EC 1.1.1.44).

The enzyme glutathione reductase (GS:SG-Red; EC 1.6.4.2) then

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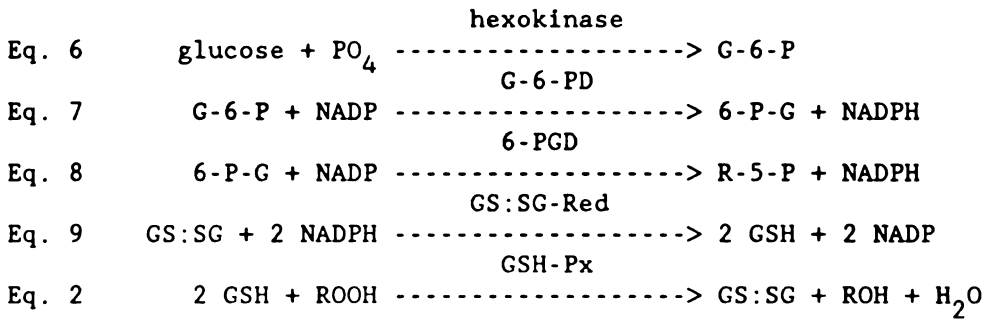
utilizes NADPH as a hydrogen donor to convert oxidized glutathione (GS:SG) to its reduced form (GSH) (Eq. 9). Reduced glutathione is then available as substrate for reduction of toxic organic hydroperoxides (ROOH) by GSH-Px (EC 1.11.1.9) as seen before (Eq. 2). This process can be summarized as:



Oxidation of glucose via the HMP shunt is the only method of maintaining reduced NADP in the mature red blood cell (Ganther et al., 1982). Protective effects of glucose against hemoglobin oxidation and erythrocyte hemolysis were once thought to be due to maintenance of adequate supplies of GSH (Rotruck et al., 1973), which has some direct antioxidant ability (Hoekstra, 1975). However, Rotruck et al, (1973) showed that GSH-Px was ultimately responsible for the HMP-derived reducing equivalents necessary to limit oxidative damage.

B. Sulfur-Containing Amino Acids.

Selenium, with an atomic weight of 78.96, is closely related to sulfur (AW 32.06) in chemical properties and organic forms. Consequently, the sulfur amino acids, methionine, cystine and cysteine frequently contain Se (Stadtman, 1980). These amino acids are known to have sparing effects against lipid peroxidation and the development of VESD lesions (Bieri, 1959; Witting and Horwitt, 1964).

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Once the role of reduced glutathione in antioxidation was elucidated, Chow and Tappel (1974) proposed that the sulfur amino acids were incorporated into GSH. This scheme is now widely accepted (Lawrence et al., 1974) and can be depicted as follows:

$$\text{Eq. 10} \qquad \text{Glu} + \text{Cys} \xrightarrow{\gamma\text{-GC-synthetase}} \gamma\text{-Glu-Cys}$$

$$\text{Eq. 11} \qquad \gamma\text{-Glu-Cys} + \text{Gly} \xrightarrow{\text{GSH-synthetase}} \gamma\text{-L-Glu-L-Cys-Gly (GSH)}$$

Glutamate (Glu) and cysteine (Cys) are combined via γ-glutamylcysteine synthetase (γ-GC-synthetase; EC 6.3.2.2) to form a dipeptide, γ-glutamylcysteine (γ-Glu-Cys)(Eq. 10). Glutathione synthetase (EC 6.3.2.3) then adds glycine (Gly) to yield the tripeptide reduced glutathione, or γ-L-glutamyl-L-cysteinylglycine (γ-L-Glu-L-Cys-Gly) (Eq. 11) (Flohe, 1982; Cooper and Bunn, 1987). Therefore, both the metalloenzyme, GSH-Px, and its substrate, GSH, contain a selenocysteine residue.

C. Hemolytic Anemias.

Mature mammalian erythrocytes have no nucleus, ribosomes, or mitochondria and are therefore incapable of protein synthesis or oxidative phosphorylation. Most glucose is metabolized via the glycolytic pathway, and only 10% via the HMP shunt (Cooper and Bunn, 1987). As mentioned, erythrocytes depend on the GSH-Px and HMP shunt systems to protect hemoglobin and plasma membranes from oxidative damage. Defects in this cooperative system have been associated with several nutritional or congenital anemias in man.

Congenital deficiencies in several enzymes of the glycolytic pathway, including pyruvate kinase and glucose-phosphate isomerase, have

been reported, but the most common defect is in the HMP shunt enzyme, G-6-PD (Ganther et al., 1976). Glucose-6-phosphate dehydrogenase deficiency is known as "favism" because it is characterized by an exceptional sensitivity to fava beans, which produce a fulminating hemolytic crisis if consumed (Cooper and Bunn, 1987). Regardless of cause, affected individuals are usually young, have a nonspherocytic hemolytic anemia, and display no clinical problems unless exposed to some type of oxidant initiator.

The source of oxidant stress may be viral or bacterial infection, but is typically an oxidizing drug, such as the antimalarials (primaquine, chloroquine), sulfonamides, nitrofurantoins, or some analgesics (phenacetin, acetanilid) (Cooper and Bunn, 1987). These drugs, or xenobiotics, generate superoxide ($\cdot O_2$), H_2O_2, or lipoperoxides (LOOH) within red cells (Flohe, 1982) and greatly increase the amount of glucose routed through the HMP shunt, thereby supplying reducing equivalents.

Individuals with defective HMP shunt enzyme activity are unable to meet the increased demand for NADPH and GSH production. Hemoglobin sulfhydryl groups are oxidized and hemoglobin precipitates out as Heinz bodies (Cooper and Bunn, 1987). Acute hemolysis is uncommon, but drug-induced hemolytic episodes promote increased clearance of damaged erythrocytes by the reticuloendothelial system (Flohe, 1982).

Defects in the GSH, GS:SG-Red, and GSH-Px system are associated with hemolytic anemias also. Genetic impairment of glutathione synthesis exists in deficiencies of γ-glutamylcysteine synthetase and glutathione synthetase (Flohe, 1982). Deficiencies in GS:SG-Red were once thought to be congenital, but are now known to be due to a deficiency of riboflavin,

a coenzyme precursor (Ganther et al., 1976; Flohe, 1982).

A congenital impairment of GSH-Px activity, resulting in hemolysis, has been suggested, but nutritional Se deficiency is more likely involved (Ganther et al., 1976). Once again, it was Rotruck et al. (1973) who found that Se-deficient rat erythrocytes were more susceptible to H_2O_2-dependent hemolysis, and thereby discovered that GSH-Px was a selenoenzyme. The idea that Se deficiency may affect GSH-Px activity was supported by a report of Se-deficient cattle with Heinz body formation and anemia which responded to Se supplementation (Morris et al., 1984).

D. Glutathione Peroxidase and HMP Shunt Enzymes in VESD.

Chow and Tappel (1972) investigated the effect of ozone on lipid peroxidation, GSH-Px and the HMP shunt enzymes in lung tissue of vitamin E-deficient rats. They found significantly increased concentrations of TBA-reactants and increased GSH-Px, GS:SG-Red, and G-6-PD activities in ozone-treated rats. Vitamin E pretreatment partially ameliorated these increases, suggesting in vivo inhibition of lipid peroxidation. Further, they concluded that oxidant stress induced GSH-Px, GS:SG-Red and G-6-PD activities, thereby providing the necessary reducing equivalents for NADPH and GSH. Consequently, they suggested that GSH-Px activities might serve as an index for monitoring the extent of oxidant damage.

Additional studies (Chow and Tappel, 1973; Chow et al., 1973) confirmed that the activity of GSH-Px and GS:SG-Red, as well as several HMP shunt and glycolytic pathway enzymes, was induced by oxidant stressors capable of initiating lipid peroxidation (corn oil, cod liver oil, ozone). In vitamin E-deficient rats fed corn oil, there was a highly significant correlation between tissue GSH-Px, GS:SG-Red and G-6-PD activities and the

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quantity of malonyldialdehyde-related fluorescent products (Chow and Tappel, 1973). This was strong evidence that GSH-Px and associated enzymes were adaptive enzymes induced by tissue peroxidative challenge.

It remained to be shown that GSH-Px activity was dependent on dietary Se levels. Following the discovery (Rotruck et al., 1973) that Se was incorporated into GSH-Px, Chow and Tappel (1974) showed that Se-depleted rats supplemented with selenomethionine had significant, linear increases in GSH-Px activity in all tissues. Selenium was determined to be an integral part of GSH-Px, rather than a cofactor, because administration of an RNA synthesis inhibitor (actinomycin D) and a protein synthesis inhibitor (puromycin) failed to suppress the increase in GSH-Px activity (Chow and Tappel, 1974).

Omaye and Tappel (1974b) subsequently discovered a linear relationship between the GSH-Px activity in chick tissues and the logarithm of the dietary Se concentration. They suggested that plasma GSH-Px was probably synthesized in the liver, was highly correlated with dietary Se levels, and that analysis of plasma GSH-Px activity might indicate abnormal Se nutrition.

VII. Integrated Mechanism of Antioxidation.

A. General Concept.

The currently accepted model for membrane antioxidation is complex and involves vitamin E, the selenoenzyme GSH-Px and the sulfur-containing amino acids. A schematic representation of these interrelationships is presented in Figure 1 [after Omaye and Tappel, 1974a; Chow and Tappel, 1973, 1974].

Oxidant initiators begin the process and can include a wide variety of exogenous and endogenous metabolic compounds. Environmental oxidants could be toxic metals (Ag, Cd, Cu, Fe, Hg, Pb), ozone (O_3), nitrous oxide (NO_2), oxidizing drugs, dietary PUFAs, or irradiation with x-rays or UV light (Demopoulos, 1973; Tappel, 1980). Toxic metabolites like H_2O_2, $^{\cdot}O_2$, $OH^{\cdot}$, and singlet oxygen are produced routinely by most cells (Tappel, 1980; McMurray and Rice, 1982), especially phagocytic cells (Ganther et al., 1976). Xanthine oxidase and cytochrome P_{450} oxidize xenobiotic compounds with formation of $^{\cdot}O_2$ and H_2O_2 (Linder, 1985a).

Once started, the production of free radicals is self-renewing and propagates through the PUFAs contained within the phospholipid membrane, producing lipoperoxides (LOOH) and other organic hydroperoxides (ROOH). The membranes of subcellular organelles, such as mitochondria and endoplasmic reticulum, are rich in PUFAs and highly vulnerable to oxidative processes (Tappel, 1973, 1980). Damage to these organelles may interfere with cellular respiration, electron transport, protein synthesis or electrolyte homeostasis.

The structure of lipid-soluble vitamin E allows it to locate within intracellular and extracellular phospholipid membranes (Machlin, 1984; Combs et al., 1985), where it resides close to PUFAs and the membrane-

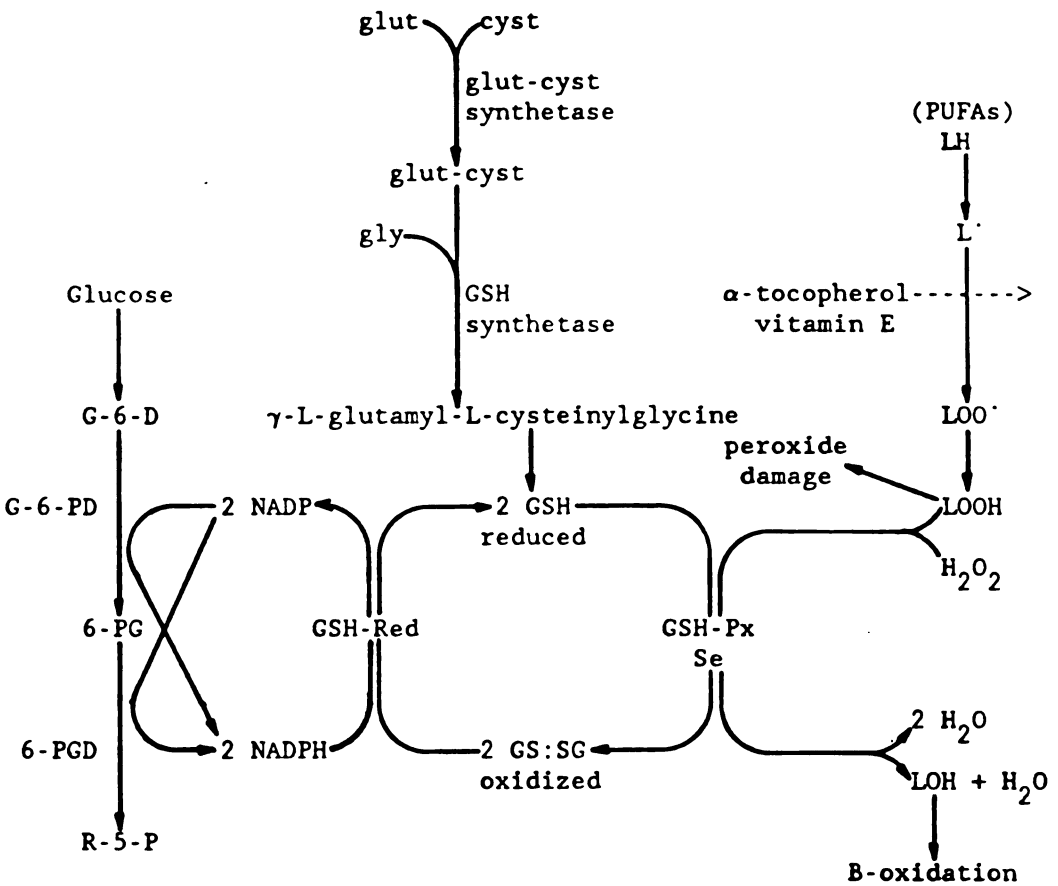

Figure 1. Proposed relationship of the hexose monophosphate shunt to the <u>in vivo</u> lipid peroxidation protection system.

bound enzymes that generate free radicals (Molenaar et al., 1973). Here, vitamin E serves as a free radical chain-breaker (Tappel, 1962), interrupting this cascade with its own oxidation and thereby preventing fatty acid hydroperoxide formation (Noguchi et al., 1973; Rotruck et al., 1973). A reactive hydroxyl group on the phenyl ring of α-tocopherol is oxidized, forming a relatively stable free radical that is then oxidized to a quinone compound and excreted in the urine (Linder, 1985a). Other peroxides may be formed in the process, but they can be reduced by GSH-Px (Chow and Tappel, 1974).

Glutathione peroxidase is associated with the aqueous phase of the cytosol (Noguchi et al., 1973). Here it bathes the cellular interior in reducing power. Superoxide anions, whether from random exogenous or controlled endogenous peroxidation, are reduced to H_2O_2 by superoxide dismutase (Combs and Scott, 1977). Catalase, contained within peroxisomes, further reduces H_2O_2, but the bulk of the extraperoxisomal antioxidant work is done by GSH-Px (Ganther et al., 1976; Flohe, 1982).

Organic or lipid hydroperoxides are likewise reduced to hydroxyacids by GSH-Px and/or the related enzyme, GSH-S-transferase (GSH-S-Tr), depending on the species and tissue (Lawrence and Burk, 1978). Adequate cytosolic antioxidant function requires glucose and the HMP shunt enzymes for reducing equivalents (NADPH), Se for GSH-Px activity, and the sulfur- (and Se-) containing amino acids as precursors for GSH (Chow and Tappel, 1973, 1974; Hoekstra, 1975).

B. Implications.

Tissues, cells or membranes which are rich in PUFAs, are in direct contact with molecular oxygen, or have high rates of endogenous

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peroxidation, will be prone to autoxidation if exposed to oxidant stress during states of vitamin E or Se deficiency. Accordingly, VESDs are characterized by hepatic necrosis, myopathic degeneration, hemolytic anemias, and decreased phagocytic/immune function. Incomplete sparing effects of vitamin E and Se may be a reflection of their different cellular locations and specific activities or the extent to which catalase and GSH-S-Tr are responsible for antioxidation within a given tissue or species.

Mitochondrial membranes, usually studied in liver tissue, contain high levels of unsaturated fatty acids, vitamin E, and hemoproteins which are lipoperoxidation catalysts (Combs et al., 1975; Machlin, 1984). They are intimately associated with oxygen since their function is oxidative phosphorylation. While the mitochondrial matrix probably contains SOD, GSH-Px, GS:SG-Red, and GSH (Flohe, 1982), excessive intramitochondrial peroxide production might overwhelm this defense system.

It would appear that mitochondrial damage is one of the most critical effects of membrane peroxidation in VESD. Linolenate hydroperoxide inhibited hepatic mitochondrial oxidative phosphorylation in rats (Tappel, 1965), and certain xenobiotics caused mitochondrial H_2O_2 formation (Flohe, 1982). Mitochondrial peroxidation resulted in swelling and lysis (Tappel, 1962; Noguchi et al., 1973) and mitochondrial disruption was one of the earliest lesions observed in muscle of animals with nutritional myopathy (Van Vleet, et al., 1976; Machlin, 1984). Abnormal ultrastructural characteristics of jejunal epithelial mitochondrial membranes were a prominent feature in vitamin E-deficient children and ducklings (Molenaar et al.,1973).

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VIII. Dietary Selenium and Biological Antioxidant Status.

A. Overview.

Rotruck et al. (1973) theorized that measurement of GSH-Px activity might provide a means of determining Se requirements and of identifying Se deficiency in humans or other animals. Chow and Tappel (1974) and Omay and Tappel (1974) soon found that supplemental dietary Se increased plasma and tissue GSH-Px activities in Se-deficient rats and chicks, respectively, and it was proposed that plasma GSH-Px activity could be an indicator of tissue Se status. Other researchers were quick to adopt this tool to survey livestock for Se status.

It must be noted that it is not possible to compare these reports directly because of differences in species, source of GSH-Px activity sampled, laboratory procedures, and expression of enzyme activity units. The assay techniques employed may have measured enzyme activity directly by the disappearance of GSH, indirectly by the enzymatically-coupled oxidation of NADPH or by some other method. Use of different peroxide substrates (organic hydroperoxides or H_2O_2), particularly in older work, may have affected the proportion of measured activity due to Se-dependent GSH-Px and/or non-Se-dependent GSH-S-Tr. Only values within one study can be directly compared (Ganther et al., 1976), but significant trends can be followed across different reports.

As an estimate of the biological Se status of livestock, glutathione peroxidase activity was surveyed in sheep erythrocytes in Britain (Anderson et al., 1979); in whole blood of cattle in Sweden (Carlstrom et al., 1979); in cattle erythrocytes in Devon (Bloxham et al., 1979); in bovine and ovine erythrocytes in Australia (Wilson and Judson, 1976;

Jelinek et al., 1975) or whole blood in Northern Ireland (Thompson et al., 1976); in cattle serum in the Great Lakes area of the American Midwest (Stevens et al., 1985); in erythrocytes of horses in Australia (Caple et al., 1978); and in whole blood of pigs in Denmark (Jensen, 1977). The global consensus of these reports is that whole blood, erythrocyte, plasma or serum GSH-Px assays offer useful estimations of biological Se status of livestock within a given area, if consuming locally grown feed.

The effect of supplemental dietary Se on blood and tissue GSH-Px activities has been studied in cattle (Allen et al., 1975b; Scholz et al., 1981) and sheep (Oh et al., 1974; Moksnes and Norheim, 1983; Jelinek et al., 1985). Plasma or tissue GHS-Px activity and Se concentrations are highly correlated with dietary Se concentrations, although plasma or erythrocyte GSH-Px values increase to a certain range, above which additional Se supplementation does not increase activity. This plateau is generally considered to represent the physiologic requirement for Se, as a component of GSH-Px, although plasma and tissue Se concentrations will continue to increase when Se is supplemented above this point.

B. Studies in Swine.

1. Source of Dietary Se.

The source of dietary Se and its relationship to Se bioavailability and GSH-Px-associated antioxidant status has been much studied. Ku et al., (1972) found a significant linear relationship (r = .95) between naturally occurring (organic) dietary Se concentrations of less than .5 mg Se/kg diet and the Se content of longissimus dorsi muscle, while Groce et al., (1973b) found that longissimus dorsi muscle Se content and serum Se levels reached a plateau when diets were supplemented with .1 mg Se/kg

as (inorganic) sodium selenite. Compared to sodium selenite, proportionately more Se from seleniferous corn was excreted in the feces and less in the urine (Groce et al., 1973a).

Dietary vitamin E content has been shown to affect Se availability also, as supplemental vitamin E was shown to increase urinary Se excretion and lower Se retention in pigs (Groce et al., 1973a, 1973b). Meyer et al., (1981) found that increasing levels of supplemental vitamin E decreased Se concentrations of many tissues, probably by lowering Se retention.

Sankari (1985) showed that, at a supplemental level of .3 mg Se/kg, sodium selenite was superior to selenomethionine in increasing plasma Se concentration and GSH-Px activity but that both parameters plateaued rapidly, after which there was no apparent difference. On the other hand, selenomethionine was not superior for increasing muscle Se concentration (Sankari, 1985).

Together, these studies suggest that pigs may have a physiologic threshold for Se as a component of GSH-Px (Groce et al., 1973; Sankari, 1985) but that the source of dietary Se, as well as the dietary vitamin E content, affect the availability of Se for GSH-Px synthesis.

2. Amount of Dietary Se.

The concept of a physiologic threshold led many investigators to examine the effect of dietary Se concentrations on body Se and GSH-Px stores. Moksnes et al. (1982) found a linear response, without a threshold effect, in liver and muscle Se concentrations of pigs fed 0-2.2 mg Se/kg as sodium selenite. Mahan and Moxon (1978) reported a linear increase in serum Se values over time with .1 mg supplemental Se/kg as

Na_2SeO_3, but .3 mg/kg produced a rapid plateau.

Plasma Se concentrations were found to increase linearly, reach a plateau (at .45 mg Se/kg) and then increase cubically (upward curvilinear response) with supplemental Se concentrations from .1-2.0 mg/kg (Meyer et al., 1981). In the same study plasma GSH-Px activity increased, then plateaued with concentrations of dietary Se up to .35 mg/kg. Parsons et al. (1982a) found that at least .1 mg supplemental Se/kg was required to maintain serum GSH-Px activity and plasma Se values in weanling pigs. Over the range of dietary Se levels of .02-.12 mg/kg, weanling pigs were found to have linear increases in serum Se and GSH-Px values and a quadratic increase in serum GSH-Px activity over time (Adkins and Ewan, 1984).

While feeding pigs concentrations of up to .15 mg Se/kg, Groce et al. (1973b) observed that tissue and serum Se concentrations plateaued. In another study, serum GSH-Px activity was seen to plateau in response to .35 mg dietary Se/kg, while plasma Se concentrations increased with dietary Se concentrations (up to .64 mg Se/kg) but did not plateau (Parsons et al., 1982b). Hakkarainen et al. (1978) suggested that blood Se concentrations and serum GSH-Px activity tended to plateau at about .14 mg dietary Se/kg.

Sankari (1985) reported that plasma Se concentrations and GSH-Px activity had curvilinear relationships with dietary Se during early supplementation. Later, dietary Se and plasma Se levels had a linear relationship but the difference in response of serum GSH-Px activity depended on whether Se was supplemented in nutritionally adequate or excessive concentrations. In growing pigs, plasma GSH-Px activity

plateaued at the physiological requirement for the selenoenzyme with diets containing .1 mg supplemental Se/kg; additional supplemental Se produced an earlier plateau (Sankari, 1985).

3. Correlation of GSH-Px and Se.

Levander (1983) made the point that functional tests of Se status, such as GSH-Px enzyme activity, would be more accurate for assessing Se bioavailability than would measures of total tissue or blood Se content. This premise required that there be a highly significant correlation between measured GSH-Px activity and *in vivo* Se status.

Jensen (1977) measured whole blood GSH-Px activity in pigs of different Se backgrounds and concluded that it was useful for evaluating Se status. Ewan (1976) described high correlations ($r = .78$-$.90$) between tissue GSH-Px activity and Se content in young pigs injected with Se. However, in a survey of slaughter-weight pigs, Thompson *et al*. (1976) reported a poor correlation ($r = .27$) between whole blood Se values, which ranged from 93-193 ng Se/ml, and GSH-Px activity. They concluded that blood GSH-Px activity was not a good indicator of biological Se status in pigs.

In contrast, Siversten *et al*. (1977) found a highly significant correlation ($r = .90$) between erythrocyte GSH-Px activity and whole blood Se in growing pigs with blood Se concentrations from 10-200 ng Se/ml. Using graded Se supplementation in Se-deficient diets, Hakkarainen *et al*. (1978) reported a significant correlation ($r = .96$) between serum GSH-Px activity and whole blood Se concentrations (range 10-185 ng Se/ml) in growing pigs. This correlation was more pronounced for concentrations of less than 75 ng Se/ml ($r = .94$), which is in the Se-deficient range for

pigs of this age, than for concentrations from 75-200 ng Se/ml (r = .74). In pigs with serum Se concentrations of 5-88 ng/ml, Adkins and Ewan (1984) found significant correlation with serum GSH-Px activity (r = .81). The correlation between plasma GSH-Px and blood Se, which ranged from undetectable amounts to almost 200 ng Se/ml, was lower in another study (r = .65) (Chavez, 1979a).

Plasma GSH-Px activity has been shown to increase as a function of age or time in pigs on a Se-adequate diet. The correlation between plasma Se concentration and GSH-Px activity was more significant for young pigs (45-125 ng Se/ml; r = .81) than for older pigs (88-275 ng Se/ml; r = .55) (Sankari, 1985). An almost linear, age-dependent increase in erythrocytes and plasma GSH-Px was also reported by Jorgensen et al. (1977).

4. GSH-Px and Se as Indicators of Antioxidant Status.

Several investigators concluded that whole blood, plasma or serum GSH-Px activities were reliable indicators of innate Se status of pigs (Jensen, 1977; Jensen et al., 1979; Chavez 1979a, 1979b) or suggested that GSH-Px activity might be of diagnostic use in Se-deficient syndromes (Jorgensen et al., 1977). However, animal antioxidant status would also be affected by dietary vitamin E levels and plasma or tissue GSH-S-Tr activity.

Jensen et al. (1979) used in vitro erythrocyte lipid peroxidation (ELP) and whole blood GSH-Px activity to demonstrate subclinical vitamin E-Se deficiency in growing pigs. In this study, GSH-Px activity was related to dietary Se supplementation but was not affected by vitamin E or the antioxidant, ethoxyquin. The formation of TBA reactants in ELP was inhibited by vitamin E supplementation, as Fontaine and Valli (1977)

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reported, but not influenced by Se or ethoxyquin consumption. Chavez (1979a, 1979b) confirmed that dietary Se, but not vitamin E, significantly affected plasma or tissue GSH-Px activity in weanling pigs.

Earlier, Ewan (1976) had reported a high correlation (r = .86) between liver Se content and GSH-Px activity in pigs and Lawrence and Burk (1978) had shown that pig liver achieved up to 67% of its total GSH-Px activity from Se-independent GSH-S-Tr. Meyer et al. (1981) subsequently showed that liver GSH-S-Tr activity remained similar, although total and Se-dependent GSH-Px activities increased with increasing dietary Se level.

While vitamin E and GSH-S-Tr undoubtedly contribute to maintenance of normal antioxidant capacity, Se-dependent functions have been better studied. Researchers have shown that GSH-Px activity is a more reliable indicator of Se status at low or adequate dietary Se intake (Siversten et al., 1977; Hakkarainen et al., 1977; Sankari, 1985) than at levels above the nutritional requirement for Se (Thompson et al., 1976; Chavez, 1979a; Sankari, 1985). Sankari (1985) concluded that animals on Se adequate diets have a biological GSH-Px plateau effect; blood and tissue Se concentrations include functional and nonfunctional Se pools; and plasma Se level is not a valid estimate of tissue Se levels since tissue Se stores continue to accumulate past the point of physiological benefit.

About the same time, Peter et al. (1985a) demonstrated that liver, plasma and whole blood Se and GSH-Px values did not accurately reflect the muscle or whole body Se retention in sheep fed Se as Na_2SeO_3 or selenomethionine. Furthermore, tissue Se concentrations were found to be affected by feed intake, as well as dietary Se concentrations, suggesting that discrepancies in tissue Se concentrations in natural cases of NMD may

be due to differences in intake levels (Peter et al., 1985b). These workers were careful to point out that the Se "status" of an animal cannot be predicted from the Se concentration of any particular tissue (Peter et al., 1985a, 1985b).

Therefore, simultaneous measurement of Se concentration and GSH-Px activity will give the most accurate assessment of Se-dependent antioxidant status.

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IX. Selenium, Glutathione Peroxidase and Genotype.

A. Cattle and Sheep.

Cawley and Bradley (1978) reported a syndrome of acute myocardial degeneration in dairy calves associated with low blood Se concentrations and erythrocyte GSH-Px activity. Affected calves (n = 40) were almost exclusively the progeny of Friesian bulls and cows; only one calf born to Friesian heifers serviced by Angus bulls died. Although this appeared to suggest that calves born to cows were more susceptible to disease than those of heifers, Rogers and Poole (1978) were interested in the apparent breed difference. They described a similar outbreak in Friesian calves in which 25 of 120 calves sired by the same Friesian bull died over a three year period. Blood Se and GSH-Px assays were not determined, but disease incidence declined rapidly after the bull was culled.

Garden and Sproat (1978) soon after recorded an outbreak of nutritional myopathy in 40 beef calves in which serum AST and CPK values were raised and erythrocyte GSH-Px activity was low. In this herd, 10 calves from an Angus bull were affected, 5 of which died, yet none of 20 calves sired by a Shorthorn bull developed NMD. Collectively, these cases raised the possibility of an inherited predisposition in cattle to nutritional myopathy.

Although Thompson *et al*. (1976) found a high correlation (r = .92) between whole blood Se concentrations and GSH-Px activity in sheep, they also noted that sheep fell into two distinct groups, one with low blood Se (21-67 ng/ml) and GSH-Px (2-20 IU/g Hb) values, and the other with high blood Se (133-249 ng/ml) and GSH-Px (77-179 IU/g Hb) values. They did not discuss whether Se values varied as a function of location or management

conditions, but genotype may have had an effect.

Wilson and Judson (1976) and Anderson et al. (1978) reported significant correlations between blood Se and erythrocyte GSH-Px values in cattle and sheep, but regression analysis suggested that there were species differences in Se requirements and GSH-Px properties. Langlands et al. (1980) speculated that breed, as well as species, differences in Se and GSH-Px relationships might exist. They were then able to show that genotype was a significant source of variation in blood Se and GSH-Px values between breeds of Bos taurus and Bos indicus cattle. Bos indicus breeds had higher values and there was a tendency towards heterosis in crossbred cattle. In sheep, significant differences in Se and GSH-Px levels were associated with breeds and sire groups within breed. Dorset-Merino cross lambs displayed a heterosis effect on both blood parameters (Langlands et al., 1980).

Shortly thereafter, Atroshi et al. (1981) discovered that erythrocyte GSH-Px activity was genetically determined in Finn sheep and apparently controlled by a single pair of autosomal alleles, with the gene for high GSH-Px dominant over that for low GSH-Px. Further work led to the development of genetically selected Finn sheep with high or low erythrocyte GSH-Px activity (Sankari and Atroshi, 1983). These sheep had a significant correlation ($r = .91$) between blood Se content and erythrocyte GSH-Px activity. Relative differences in GSH-Px activity were maintained when Se was supplemented, but blood Se and GSH-Px levels appeared to increase more readily in high GSH-Px sheep than low GSH-Px sheep. It was not known if this was due to enhanced Se absorption.

B. Swine.

While studying blood Se and GSH-Px interactions in swine, a number of investigators described significant differences among litters of pigs (Jensen et al., 1979; Adkins and Ewan, 1984; Friendship and Wilson, 1985). Jorgensen et al. (1977) analyzed erythrocyte GSH-Px activity and found that there were greater variations among pigs than in a single animal over time, and that variation among litters was greater than within litters. They concluded that there was strong evidence for genetic control of porcine erythrocyte GSH-Px and that cellular Se levels might be similarly controlled.

Stowe and Miller (1982, 1985) were successful in developing populations of pigs that were relativley hyposelenemic (hypo-Se) or hyperselenemic (hyper-Se) and that maintained their genetic Se status while commonly reared. On the basis of mean 10- and 30-day serum Se concentrations, first-generation (F1) pigs could be assigned to hypo-Se (mean = 75 ng Se/ml) or hyper-Se (mean = 108 ng Se/ml) groups which differed by 33 ng Se/ml. When supplemented with either .1 or .3 mg Se/kg, hypo-Se pigs had a greater serum Se response than hyper-Se pigs, although relative selenemic status persisted. Plasma GSH-Px activity had an inconsistent relationship with serum Se levels or Se supplementation rates, but was better correlated with serum Se levels than was erythrocyte GSH-Px activity (Stowe and Miller, 1982, 1985).

The difference in relative genetic Se status was shown to intensify in second-generation pigs, as F2 populations differed by an average of 42 ng Se/ml serum (Stowe, 1986). Compared to hyper-Se pigs, hypo-Se pigs absorbed less dietary Se and, even though excreting less Se, had lower overall Se retention rates (Stowe, 1986).

X. Nutritional and Genetic Muscular Dystrophies.

Discovery of the genetic regulation of antioxidant protection in animals (Jorgensen et al., 1977; Langlands et al., 1978; Atroshi et al., 1981; Sankari and Atroshi, 1983; Stowe and Miller, 1982, 1985) was exciting news for it validated the concept of an inherited predisposition to nutritional muscular dystrophy. It remained to be shown that diminished antioxidant status was present in cases of genetic muscular dystrophy (MD) in man and other species.

Several forms of genetic muscular dystrophy occur in man and many have similar symptomatology as nutritional muscular dystrophy. Horwitt (1965) suggested that human tocopherol deficiency might be associated with creatinuria since vitamin E-deficient rats with NMD developed creatinuria proportional to the level of dietary unsaturated fats. However, evidence for a relationship between antioxidant deficiency and genetic muscular dystrophy in man was lacking. Since then, human MD has been associated with low or normal plasma vitamin E concentrations, increased Se excretion, greatly elevated serum CKP activity, creatinuria and muscle weakness (Pennington, 1980; Jackson et al., 1983; Linder, 1985a, 1985b), but the disease is not reversed by vitamin E, Se or antioxidant administration (Omaye and Tappel, 1974; Linder, 1985a).

Omaye and Tappel (1974) found that in vitro muscle tissue GSH-Px and GS:SG-Red activities and TBA-reactant product concentrations were higher in genetically dystrophic strains of chickens and mice than in control animals and were highest in strains of dystrophic chickens bred for high muscle lipid content. They theorized that the degree of lipid peroxidation was dependent on muscle tissue fat content and that

peroxidation induced GSH-Px and GS:SG-Red enzyme activity. These results correlated well with those that showed increased activities of the HMP shunt enzymes (G-6-PD and 6-P-GD) and a tendency for increased tissue Se content, presumably in GSH-Px, in dystrophic mice compared to normal mice (Omaye and Tappel, 1974). Thus, animals with genetic MD do not have defective GSH-Px antioxidant capacity but may have increased *in vivo* lipid peroxidation, with concommitant muscle membrane damage and enzyme leakage.

Accelerated lipid peroxidation in human muscular dystrophies was documented by Kar and Pearson (1979). Although they did not measure GSH-Px activity, significantly higher quantities of TBA-reactive products and increased catalase and GS:SG-Red, but not SOD, activities occurred in muscles of patients with MD. Once again, activation of the protective GSH-Px system in response to lipoperoxidation was inferred.

Several investigators have suggested that genetic MD may be associated with an underlying cellular membrane defect (Kar and Pearson, 1979; Pennington, 1980), perhaps due to lipid peroxidation of membranes and/or altered calcium regulation (Omage and Tappel, 1974). Lucy (1980) reviewed the evidence for primary muscle cell membrane defect in genetic MD and concluded that the observed membrane abnormalities may well be secondary to some unrecognized biochemical lesion.

REVIEW OF MONENSIN

I. Introduction.

Monensin ($C_{36}H_{62}O_{11}$-H_2O) is an ionophoretic antibiotic isolated from culture filtrates of Streptomyces cinnamonensis grown in fermentation broths (Haney and Hoehn, 1968; Stark et al., 1968). Monensin (MW 688) is a pentacyclic, monocarboxylic acid which has no UV absorption, is acid-labile and poorly soluble in water; the sodium salt of monensin (MW 710) is readily soluble in lipids or organic solvents (Agtarap et al., 1967; Agtarap and Chamberlin, 1968).

Monensin is approved for and widely used as a growth promotant in cattle and coccidiostat in cattle and poultry. As an ionophore, monensin has the ability to selectively transport alkali metal cations, particularly sodium (Na^+), across biological membranes (Pressman, 1976). This property interferes with normal ion exchange mechanisms and is responsible for the effects of monensin in prokaryotic cells. In ruminants, monensin has antibiotic activity against Gram-positive organisms of the ruminal microflora and causes a shift toward propionate-producing Gram-negative organisms that allows more efficient feed utilization (Bergen and Bates, 1984). The coccidiostatic properties of monensin are probably due to changes in transmembrane ion transport also.

In eukaryotic cells, the ionophoretic properties of monensin have significant effects on cardiovascular tissue (Pressman and Fahim, 1983),

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sometimes with disastrous consequences. Monensin toxicosis has been reported in rats, rabbits, mice, poultry, dogs, sheep, cattle, horses and pigs, and there is a great deal of difference in species susceptibility to toxicosis (Langston et al., 1985). While toxicity is apparently dose-dependent, the lesions of monensin toxicosis are remarkably constant across species.

This review will discuss the structure and mechanism of action of monensin, its effect on prokaryotic and eukaryotic cells, as well as signs of toxicity and the underlying pathophysiologic process.

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II. Ionophore Antibiotics.

A. Overview.

Early research showed that monensin existed in several forms which were closely related to other antibiotic compounds (nigerisin, dianemycin, lasalocid) (Gorman et al., 1968). Collectively, the monensins and other monocarboxylic antibiotics shared the ability to bind alkali metal cations and transport them across mitochondrial membranes of rat liver in vitro (Estrada-O. et al., 1968). It was proposed that these antibiotics chelated metal cations, forming a complex of neutral charge and hydrophobic perimeter which then allowed the facilitated transport of cations down their concentration gradients across mitochondrial membranes (Estrada-O. et al., 1968). Because ion selectivity is peculiar to each ionophore, they are widely used in studies of ion metabolism and transport. Most biological membranes consist of an ordered arrangement of lipids and proteins about 100 angstroms thick which represents an effective barrier against the passage of alkali ions from the aqueous phase, where they are in solution, across the membrane (Langer, 1972). The fact that ions do move across membranes, however, implies that there must be methods of ion transport, i.e.: "natural ionophores". Several different classes of artificial ionophore compounds exist which have been used to study cellular ion transport: the channel-forming quasi-ionophores, neutral ionophores, and monocarboxylic acid ionophores (Pressman, 1976).

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B. Channel-Forming Quasi-Ionophores.

This group of compounds, including gramicidin A and monazomycin, is the least understood, but potentially most important, since it is recognized that ions are transported via natural cellular "channels" (Ovchinnikov, 1979). Most of these ionophores are believed to exist as dimers and to form helical "tunnels" within membranes through which ions can travel. Gramicidin A has a known ion selectivity scheme ($H^+ > Cs^+ - Rb^+ > K^+ > Na^+ > Li^+$), but its affinity for K^+ is only four times that for Na^+ ions (Kinsky, 1970).

C. Neutral Ionophores.

The neutral ionophores, including valinomycin, enniatin A, B and C, nonactin and monactin, are unique in that they all exist as "macrocyclic" structures which have a hydrophobic exterior and hydrophilic interior (Kinsky, 1970; Pressman, 1976). Valinomycin (MW 110) is the most studied neutral ionophore and consists of a cyclic molecular backbone of alternating α-aminoacids and α-hydroxy acids which is able to "wrap around" cations and transport them across membranes, behaving almost like a "shuttle bus" (Kinsky, 1970). The cation selectivity of valinomycin ($Rb^+ > K^+ > Cs^+ > Ag^+ > Na^+ > Li^+$) is determined by the stability of the antibiotic-cation complex and is such that there is a 10,000:1 preference for K^+ over Na^+ ions (Kinsky, 1970).

Ovchinnikov (1979) has labeled ionophores as "complexones" and pointed out that valinomycin behavior resembles an enzyme because it has activity at very low concentrations; is regenerated after each transport to repeat the process; "catalyzes" ion transport across an energy barrier; has high selectivity for substrate (K^+); and "shapes" its active center

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to fit the substrate. Lauger (1972) has calculated that a single valinomycin molecule can transport 1,000 K^+ ions per second across a membrane.

Neutral ionophores can behave as "electrogenic" or "electrophoretic" carriers depending on whether they alter pre-existing membrane potential or carry ions as a result of an imposed potential (Pressman, 1976). They form charged cation complexes and transport cations down their electrochemical gradients (Pressman and Fahim, 1982). Because the macrocyclic ionophores transfer ions across membranes along with their net charge, they can severely disrupt normal electrophysiologic membrane processes (Pressman and Fahim, 1983). This makes them highly toxic to eukaryotic cells (Bassett et al., 1978).

D. Monocarboxylic Acid Ionophores.

The family of monocarboxylic ionophores includes salinomycin, nigericin, lasalocid, A23187, and monensin, most of which have been used in animal production, either for their growth promotant or coccidiostatic properties. As a class, these antibiotics have the distinction of existing in two forms, depending on whether they are complexed to a metal cation or not, and of transporting ions across membranes as electrically neutral zwitterions (Pressman and Fahim, 1983). Consequently, these molecules function as ion carriers in a type of facilitated "exchange-diffusion" (Pressman, 1976; Pressman and Fahim, 1983).

The structure of monensin was worked out by Agtarap et al., (1968) and Pinkerton and Steinrauf (1970), and is shown in Figure 2 [after Agtarap et al., 1967; Agtarap and Chamberlin, 1967; Pinkerton and Steinrauf, 1970; Whitlock et al., 1978; Elsasser, 1984]. When

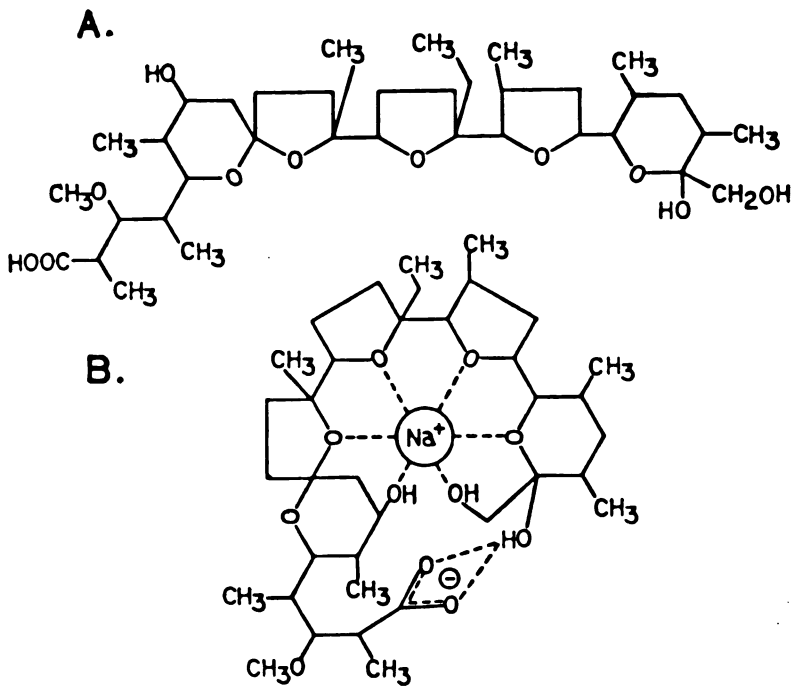

Figure 2. Structure of the protonated, acyclic form of monensin (A) and the cyclic, lipophilic ionophore molecule complexed with a metal cation (B).

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uncomplexed, monensin exists as a protonated, acyclic molecule with a single terminal carboxyl group (Figure 2A); but when complexed with a metal cation, monensin assumes a cyclic, lipophilic zwitterion form (Figure 2B) (Pressman, 1976; Bergen and Bates, 1984). Monensin attains the structure of crystalline sodium monensin by enveloping the Na^+ ion and hydrogen bonding between the deprotonated carboxyl group and hydroxyl groups on the opposite end (Pressman, 1968).

In the complexed form, the molecular "backbone" of monensin serves to form a "pocket" for cations by focusing the oxygen atoms into the interior where they can replace the oxygen atoms usually present in the water of solvation that surrounds a cation in an aqueous solution (Pinkerton and Steinrauf, 1970; Pressman and Fahim, 1983). The resulting ionophore-cation complex is strongly hydrophobic and therefore quite soluble in organic solvents or phospholipid membranes.

The flexible backbone structure permits complexation-decomplexation kinetics, which characterize the carboxylic ionophores (Pressman, 1976). This process has been diagramatically illustrated in Figure 3 [after Pressman, 1976] and has been described (Pressman 1976; Painter et al., 1982; Bergen and Bates, 1984). The protonated, acyclic form of monensin (HM) diffuses to the polar membrane interface where it loses a proton (H^+) from the carboxyl group and is trapped in the anionic form (M^-). An alkali metal cation, such as sodium (Na^+) in aqueous solution ($H_2O{\cdot}Na^+$) encounters the ionophore anion and is complexed by it, displacing the water of solvation. The cyclic zwitterion complex (Na^+M^-) can then free itself from the interface and diffuse across the lipid membrane interior to the opposite interface where the cation is released in an analogous,

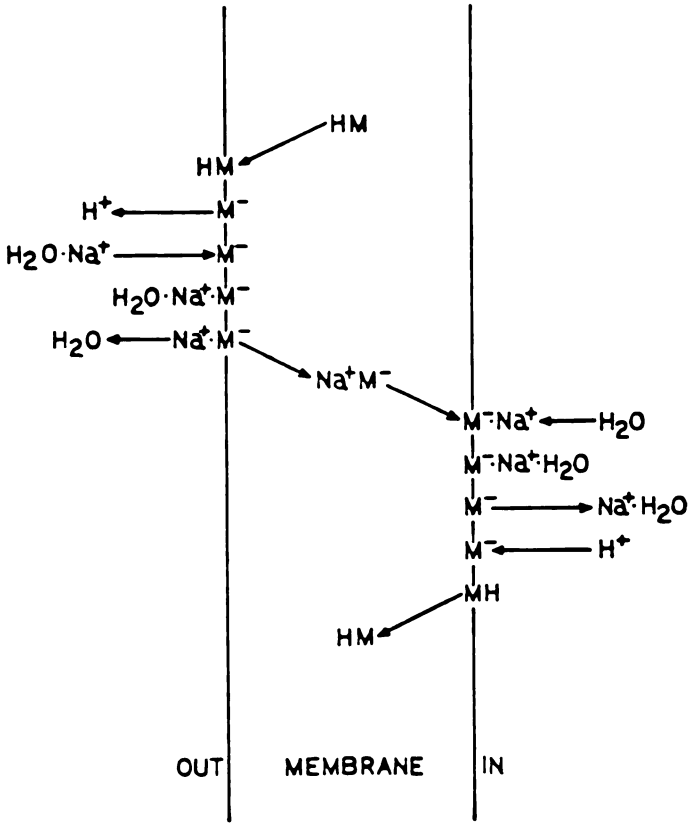

Figure 3. Proposed method for transportation of sodium across a phospholipid membrane by monensin.

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but opposite, process. Combination with a proton restores monensin to its low energy, HM form again, which is less polar and able to traverse the membrane once more. This process can be repeated up to several hundred times per second (Pressman and Fahim, 1983).

A similar mechanism has been proposed for all of the "open" carboxylic ionophores, most of which carry only monovalent cations (Pressman, 1976). Monovalent cation transporters accommodate different metal cations by virtue of their flexible molecular backbone, which results in different degrees of conformational "best fit." The ion selectivity peculiar to each species of ionophore is determined by the radius and valence of the cation, the antibiotic molecular structure, as well as the pH and hydration state (Kinsky, 1970; Elsasser, 1984). The ion affinity of monensin is $Na^+ > K^+ > Li^+ > Rb^+ > Cs^+$ and the preference for Na^+ over K^+ is about 10:1 (Pressman, 1976; Pressman and Fahim, 1983). However, transport rate is not necessarily synonymous with ion affinity, so that one ion may be carried more efficiently than another even though bound with less affinity (Elsasser, 1984).

Lasalocid is unique in that it can apparently transport either monovalent or divalent cations by assuming either monomeric or dimeric forms (Pressman and Fahim, 1982; Elsasser, 1984). Lasalocid can transport Ba^{+2}, $Ca^{+}2$, Cu^{+2}, Fe^{+2}, Mg^{+2}, Ni^{+2} and Zn^{+2} ions (Pressman and Fahim, 1982) and the affinity for Ca^{+2} is about equal to that of Na^+ (Pressman, 1976). A related compound, A23187, has an even greater affinity for divalent cations, especially Ca^{+2} (Pressman, 1976).

The ability of ionophores to shuttle cations across membranes is responsible for their biological properties. At neutral pH the

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monocarboxylic antibiotics are negatively charged and can exchange protons for monovalent cations across membranes (Kinsky, 1980). This behavior may yield an ionophore-impregnated membrane freely permeable to H^+ ions.

Sandeaux et al. (1982) studied monensin transport across artificial bimolecular lipid membranes under different conditions of pH and Na^+ ion concentration (e.g., $[Na^+]$). They determined that if the pH is equal on both sides of a membrane, but $[Na^+]$ is greater on one side, monensin will carry Na^+ down its concentration gradient, while increasing $[H^+]$ on the opposite side. However, if the $[Na^+]$ is the same on both sides, and $[H^+]$ is higher (pH lower) on one side, monensin will transport H^+ ions down their concentration gradient (Sandeaux et al., 1982).

In summary, monensin will carry cations in either direction across membranes, and the direction of ion flux is determined by the electrochemical gradient between metal cations and protons (Bergen and Bates, 1984). If the potential of the Na^+ ion gradient exceeds the H^+ ion gradient, monensin will drive H^+ against its gradient and vice versa. The exchange-diffusion process is electrically neutral since Na^+ ions are traded 1:1 for H^+ ions, and therefore it serves as a metal cation antiport (Harold, 1972; Bergen and Bates, 1984).

The monovalent ion transporters are not alone in these capabilities. Workers have shown that lasalocid and A23187 are able to transport Ca^{+2} ions against their concentration gradient, provided there exists a Na^+, Li^+, or H^+ gradient (Malaisse and Couturier, 1978; Malaisse et al., 1980).

It is known that a number of "natural ionophores" exist within membranes of prokaryotic and eukaryotic cells (Malaisse et al., 1980; Elsasser, 1984). These ion counter-transport systems are responsible for

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maintaining ionic homeostasis or for generating ionic gradients necessary for normal cell function. Insertion of synthetic ionophores into membranes of these cells might be expected to have significant, even adverse, effects.

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III. Ionophore Effects in Prokaryotic and Eukaryotic Cells.

A. Mitchell's Chemiosmotic Theory.

In 1961, Mitchell proposed that oxidative and photosynthetic phosphorylation may be coupled to electron and proton transfer across membranes by a chemiosmotic mechanism. Harold (1972) has written an excellent review of this theory and its implications for energy conservation and ion transport in mitochondrial and bacterial membranes.

In essence, Mitchell's chemiosmotic theory states that enzyme systems are physically oriented within a membrane in such a way that, with the use of a reversible ATPase enzyme, electron and proton translocation can be driven across a membrane which is impermeable to ions, thereby maintaining a gradient of pH and electrical potential (Mitchell, 1961; Harold, 1972). In principle, oxidation of NADH would not result in the formation of H_2O, but rather in the translocation of H^+ and OH^- ions in opposite directions across the membrane, with proton accumulation outside the membrane (Harold, 1972). As substrate oxidation pumps protons out of the mitochondria, energy from ATP is used and a negative charge is left in the interior. The combination of chemiosmotic (pH) and electrical potential "pulls" at H^+ ions, creating a "proton motive force" (PMF) which can be used to drive ATPase "backwards," resulting in oxidative phosphorylation of ADP to yield ATP (Lehninger *et al.*, 1967; Harold, 1972). It is the PMF and diffusion of H^+ ions down their gradient that makes possible other energy-linked mitochondrial functions, such as metabolism and ion transport.

Agents, such as the carboxylic ionophores, which transport metal cations and protons in a 1:1 ratio could severely tax normal cellular ion

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and energy kinetics by promoting chemiosmotic neutrality. Several different types of alkali metal cations can be transported across the membrane by molecules of one type of ionophore at the same time, thereby achieving multi-ionic equilibrium. Regardless of the individual polyether antibiotic ion selectivity schemes, the final transmembrane ionic equilibrium produced by different compounds is the same (Pressman, 1976; Pressman and Fahim, 1983).

B. Monensin and Prokaryotic Cells.

Harold's review (1972) of bacterial membrane energy conservation and transformation makes strong use of Mitchell's chemiosmotic theory. Like mitochondria, bacteria generate electrochemical gradients across their membranes that are essential for active transport of ions and oxidative phosphorylation (Harold, 1972). Ionophores might be expected to interfere with these gradients, much as monensin transported Na^+ and H^+ ions across artificial membranes until electrochemical neutrality was reached (Sandeaux et al., 1976).

Monensin's antibacterial activity is primarily directed against Gram-positive organisms in the rumen of cattle (Haney and Hoehn, 1968). Bergen and Bates (1984) have reviewed the effects of ionophores on rumen microflora and proposed an explanation for their antibiotic spectra.

Prokaryotic cells accomplish active transport of ions either directly by coupling it to the proton gradient or indirectly through a Na^+ gradient established by a membrane-bound Na^+-H^+ antiporter (Bergen and Bates, 1984). Most rumen bacteria, being obligate anaerobes, synthesize ATP by coupling fermentation to substrate level phosphorylation and consume ATP for Na^+ and H^+ transport. Gram-positive organisms tend to

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rely on substrate level phosphorylation for ATP synthesis, while Gram-negative organisms couple ATP synthesis to electron transfer and proton extrusion (Bergen and Bates, 1984).

Ordinarily the $[Na^+]$ within rumen bacteria is greater than in the surrounding environment, and $[H^+]$ is less. Therefore, the net effect of dietary monensin on the ruminal bacteria is to decrease intracellular $[Na^+]$ and increase internal $[H^+]$. Collapse of the proton gradient (PMF) requires expenditure of metabolic energy to actively pump H^+ ions out of the cell. Gram-positive organisms, which rely on the use of ATP generated by substrate level phosphorylation for H^+ extrusion, must devote much of their cellular energy to that task and may be overwhelmed, resulting in cell death. On the other hand, Gram-negative organisms, which couple H^+ extrusion to electron transport to generate a transmembrane potential, may be stressed but will survive (Bergen and Bates, 1984). The net result is a shift of ruminal microflora toward the Gram-negative spectrum.

Presumably, the coccidiostatic effects of the carboxylic antibiotics are due to similar actions as in the Gram-positive bacteria. Monensin, salinomycin, and narasin produce morphologic damage to coccidia (Pressman and Fahim, 1982), and monensin-treated coccidia sporozoites swell and lyse (Bergen and Bates, 1984).

C. Monensin and Eukaryotic Cells.

1. Ion Transport Across Plasma Membranes.

a. Overview.

Eukaryotic cells are certainly no less dependent on ion transport for energy metabolism than are prokaryotic cells, hence they should be at least as susceptible to ionophore-induced cation disturbances. It is

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erhaps ironic that our understanding of energy-linked ion transport in ukaryotic cells would not have been possible without the extensive use of ionophores as uncoupling agents. Artificial manipulation of ion fluxes with ionophores made possible the study of the ion transport systems of cellular membranes, especially in mitochondria.

The relative concentrations of Na^+, K^+ and Ca^{+2} ions in the extracellular, cytosolic, and intramitochondrial compartments are not equal. Maintaining these electrochemical gradients across membranes impermeable to ions is the job of "natural ionophores" (Malaisse and Couturier, 1978; Malaisse et al., 1980). Technically, these naturally occurring ion transporters should not be considered true ionophores since they are often energy-dependent, are not mobile within the membrane, and maintain, rather than dissipate, ionic gradients across a membrane (Pressman and Fahim, 1982). Nevertheless, much is now known about coupled ion transport in mammalian cells.

b. Na^+-K^+-ATPase Pump.

Relative to the extracellular fluid, intracellular fluid (cytosol) has a higher $[K^+]$ but lower $[Na^+]$. Establishment of this difference in $[Na^+]$ and $[K^+]$ requires that Na^+ ions be moved against concentration and electrical gradients. Energy for this work is supplied by the Na^+-K^+-ATPase enzyme incorporated into virtually all cell membranes (Sweadner and Golden, 1980). Because three Na^+ ions are extruded for every two K^+ ions admitted (at the cost of one ATP molecule), the Na^+-K^+-ATPase pump is electrogenic, creating a current across the membrane. Smith and Rozengurtz (1978) used monensin in cultured fibroblasts to show that the internal $[Na^+]$ limited the Na^+-K^+-ATPase

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pump. The energy generated by Na^+ extrusion can be used to drive a variety of secondary ion transport systems within cell membranes.

c. Na^+-Ca^{+2} Counter Transport.

Extracellular [Ca^{+2}] is much greater than cytosolic [Ca^{+2}]. Extrusion of Ca^{+2} from a cell is against an electrochemical gradient as well. Lasalocid was used to develop a model for Na^+-Ca^{+2} counter transport (Malaisse and Couturier, 1978). It was shown that maintenance of a primary Na^+ gradient is sufficient to create a secondary Ca^{+2} gradient and drive Ca^{+2} ions against this gradient. An "electrically-silent" Na^+-Ca^{+2} exchange protein (antiporter) couples influx of two Na^+ ions with extrusion of one Ca^{+2} ion (Sweaden and Goldin, 1980).

2. Ion Transport Across Mitochondrial Membranes.

a. Overview.

Cation transport in mitochondria of various mammalian tissues has been extensively studied and reviewed by Lehninger et al. (1967) and Harold (1972). Mitchell's chemiosmotic hypothesis (1961) is generally accepted as uniting energy production with ion transport. Within the cristae (inner membrane) of mitochondria is an enzyme chain, consisting of proton and electron carriers, which catalyzes the oxidation of NADH or other substrates (respiration) and which is coupled to the synthesis of ATP (oxidative phosphorylation) (Harold, 1972). Proton extrusion generates a membrane potential which is harnessed by the cell to do useful "work", such as the energy-linked uptake of metal cations (Ca^{+2}, K^+, Mn^+, Mg^{+2}, Na^+) into mitochondria (Lehninger et al., 1967).

b. Na^+-H^+ Antiporter.

Relative to the cytosol, mitochondria have a lower [Na^+]. In 1969, Mitchell and Moyle discovered that liver mitochondria employed a Na^+-H^+ antiport system to extrude Na^+. The pH gradient established across the mitochondrial membrane by respiration forced H^+ ions out of the matrix; proton re-entry was coupled to Na^+ efflux.

c. Na^+-Ca^{+2} Exchange System.

Mitochondria have a greater [Ca^{+2}] than is present in the cytosol. An energy-dependent process of accumulating Ca^{+2} ions was shown to reduce the extra-mitochondrial (cytosolic) [Ca^{+2}] *in vitro* (Lehninger *et al.*, 1967). However, Na^+ ions were shown to displace Ca^{+2} from heart mitochondria *in vitro* (Carafoli *et al.*, 1974). In 1976, Crompton *et al.* determined the existence of a transmembrane Na^+-Ca^{+2} antiporter which coupled uptake of three Na^+ ions with efflux of two Ca^{+2} ions. This counter transport system was located on the inner membrane of heart, but not liver, mitochondria. It promoted the extrusion of Ca^{+2} against its electrochemical gradient, but was separate from the energy-dependent Ca^{+2} influx system. Both liver and heart mitochondria had Na^+-H^+ antiporters which were distinct from the Na^+-Ca^{+2} system (Crompton *et al.*, 1977).

Further work by Crompton *et al.* (1978) in rats, rabbits and cattle, identified a Na^+-Ca^{+2} antiporter in mitochondria of skeletal muscle, brain and some secretory tissues, but not liver, kidney or smooth muscle tissue. Extrusion of Ca^{+2} was mediated by Na^+, but not K^+, influx.

3. Role of "Natural Ionophores" in Cardiac Contractility.

a. Overview.

Contraction of cardiac cells is a highly sophisticated event requiring the cooperation of many different ion exchange systems in order to modify myoplasmic [Ca^{+2}]. Braunwald (1982) offers an excellent review of the integrated process as it is affected by Ca^{+2} blocking agents. Interaction of the various "natural ionophores" can perhaps be best understood by examining their function in heart tissue.

Extracellular [Ca^{+2}] is much greater than that within the cytoplasm, but mitochondria, and especially the sarcoplasmic reticulum, contain significant amounts of Ca^{+2}. The higher extracellular [Ca^{+2}] surrounding cardiac sarcolemma is conveyed to the interior of the cell by invaginations known as transverse (T) tubules which are in close approximation to the Ca^{+2}-rich sarcoplasmic reticulum. Running the length of a myofiber are interdigitated actin and myosin filaments whose contractile behavior is activated by an increase in myoplasmic [Ca^{+2}] (Braunwald, 1982).

When an action potential arrives at the myocyte, it initiates membrane depolarization by allowing Na^{+} ions to leak down their concentration gradient through "fast" Na^{+} channels. A second inward current then develops due to entry of Ca^{+2} ions through "slow" Ca^{+2} channels (Sweadner and Goldin, 1980). In some way, extracellular Ca^{+2} entry triggers release of Ca^{+2} ions from the sarcoplasmic reticulum, elevating myoplasmic [Ca^{+2}] and activating the myofilament contractile system. Repolarization is due to an outward K^{+} current which restores resting membrane potential. Relaxation results when Ca^{+2} influx stops

and sarcoplasmic reticulum actively sequesters myoplasmic Ca^{+2} against a concentration gradient. The process of muscle contraction-relaxation is entirely dependent on the regulation of myoplasmic [Ca^{+2}] (Braunwald, 1982).

b. Regulation of Myoplasmic Ca^{+2} Concentration.

In the resting cardiac sarcolemma, Na^+ extrusion by Na^+-K^+-ATPase generates a Na^+ ion gradient; a coupled Na^+-Ca^{+2} antiport extrudes Ca^{+2}. Depolarization facilitates Na^+ and Ca^{+2} entry down their concentration gradients via "fast" and "slow" channels, respectively (Braunwald, 1982). Sarcoplasmic reticulum then releases additional Ca^{+2} and contraction results.

The elevated [Ca^{+2}] is reduced in several ways. The sarcolemmal Na^+-Ca^{+2} antiport, coupled to the Na^+ gradient created by Na^+-K^+-ATPase, extrudes Ca^{+2}. An energy-dependent Ca^{+2}-ATPase in the sarcolemma also extrudes Ca^{+2} (Braunwald, 1982). Additional myoplasmic Ca^{+2} is sequestered in sarcoplasmic reticulum by a Ca^{+2}-Mg^{+2}-ATPase. Mitocondria accumulate Ca^{+2} through an energy-dependent Na^+-Ca^{+2} antiport, coupled to a Na^+-H^+ antiport (Crompton et al., 1977, 1978). The Na^+-H^+ antiport is probably driven by proton extrusion linked to mitochondrial respiration (Crompton, 1976).

c. Alteration of Normal Contractility.

Clearly, myocardial contractility is complex, and any compounds that interfere with the normal ion gradients, transport mechanisms or respiration will disrupt the system. The cardiac glycosides, digitalis and ouabain, are known to bind to Na^+-K^+-ATPase, thereby inhibiting Na^+ extrusion (Langer, 1977). As a result, intracellular [Na^+] increases; at

the same time, intracellular [Ca^{+2}] increases (Crompton et al., 1976). The increase in myoplasmic [Ca^{+2}] is due to Na^+-driven release of intramitochondrial Ca^{+2} via the Na^+-Ca^{+2} antiporter (Langer, 1977) and to reversal of the sarcolemmal Na^+-Ca^{+2} exchange system in an attempt to extrude Na^+ (Braunwald, 1982). The net effects of digitalis in cardiac tissue are increased myoplasmic [Ca^{+2}], enhanced contractility, and positive inotropy (Sweadner and Goldin, 1980).

Monocarboxylic ionophores, especially monensin, transport Na^+ ions down their concentration gradient across phospolipid membranes directly. Therefore, these compounds may have significant effects on any membrane process reliant on an electrochemical gradient maintained by osmoregulation. Tissues highly dependent on Na^+-Ca^{+2} ion fluxes for normal function, such as heart and skeletal muscle, might be most severely affected by monensin.

4. Cardiovascular Effects of Monensin.

Pressman (1976) reasoned that, like the cardiac glycosides, ionophores may also have inotropic properties, and found that lasalocid did produce positive chronotropic and inotropic effects in isolated, perfused rabbit hearts (Pressman, 1973). However, while lasalocid transports Ca^{+2} ions directly, it also accepts a fairly wide range of monovalent cations and, therefore, has limited inotropic applications. Monensin, with a strong Na^+ preference, was found to have more potent cardiovascular effects (Pressman, 1976).

Sutko et al. (1977) showed that monensin ($Na^+ > K^+$ 10:1) and nigericin ($K^+ > Na^+$ 50:1) produced biphasic contractility responses in guinea pig and cat cardiac muscle in vitro; initial positive inotropy at

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low concentrations was followed by a negative inotropic effect at higher concentrations. Positive inotropy partially due to the indirect effect of ionophores on catecholamine release. Shlafer et al. (1978) confirmed a significant biphasic inotropic effect for monensin in isolated, driven rabbit and cat left atria.

Saini et al. (1979) then studied the effects of monensin on coronary blood flow in anesthetized dogs. At low doses (<25 ug/kg), monensin produced vasodilation, resulting in increased coronary blood flow and decreased total peripheral resistance. At high doses (>50 ug/kg), monensin caused dose-dependent increases in myocardial contractility and aortic blood pressure.

The common mechanism for myocardial contracture and endogenous catecholamine release was suggested to be an increase in intracellular $[Na^+]$ with secondary increase in cytoplasmic $[Ca^{+2}]$ as a result of Na^+-Ca^{+2} exchange (Shlafer et al., 1978; Saini et al., 1979). Basset et al. (1978) were able to confirm this theory by showing that monensin augmented K^+-dependent contracture in isolated cat ventricular myocardium only if Na^+ ions were present in solution. Monensin transported Na^+ ions down their concentration gradient from outside to inside across the sarcolemma; intracellular Na^+ ions were then exchanged for extracellular Ca^{+2} ions, enhancing cardiotonicity (Basset et al., 1978).

Most early reports of the cardiovascular effects of monensin suggest that practical applications might be found for ionophores in medicine (Pressman, 1976; Saini et al., 1979). However, severe adverse cardiac effects were soon observed with monensin. Shlafer et al. (1979) noted that, in the presence of only 50 nM ouabain, monensin produced arrhythmias

and irreversible atrial contracture in isolated rabbit left atria. Burt and Berns (1980) produced identical effects in monensin- and ouabain-treated cardiac cell cultures. Ouabain is known to increase intracellular Na^+ concentrations by inhibiting the Na^+-K^+-ATPase enzyme.

Furthermore, at even lower concentrations than those producing positive inotropy in atrial muscle, monensin decreased respiratory control of isolated cardiac mitochondria by 85% (Shlafer and Kane, 1980). Because the same concentration of monensin that poisoned mitochondria produced positive inotropic effects in intact myocytes, rather than negative effects or contracture, Shlafer and Kane (1980) suggested that sarcolemmal membrane was the site of ionophore insertion and inotropic activity. These researchers predicted that if monensin were administered to patients with cardiac ischemia and the attendant increase in sarcolemmal permeability, monensin might have direct access to subcellular organelles.

Shortly thereafter, Kabell et al. (1981) used anesthetized dogs with experimentally induced myocardial infarction to show that monensin increased blood flow in normal tissue, but accelerated the development of conduction delays and the onset of myocardial tachycardia in ischemic tissue.

In anesthetized dogs given 100 ug monensin/kg intravenously, or in conscious dogs given 2 mg monensin/kg orally, strong vasopressive and positive chronotropic effects were observed (Fahim and Pressman, 1981). Conscious rabbits given monensin at 200 ug/kg intravenously or 10 mg/kg orally had less pronounced cardiovascular effects. Interestingly, one hour after consuming monensin, dogs developed dyspnea and restlessness and were unable to stand; rabbits behaved similarly 4-5 hours after oral treatment and within one minute after monensin injection.

IV. Monensin Pharmacokinetics.

The fate of ingested monensin is unclear but has been studied with various assay methods. Initially, a "bioautographic" assay was used to measure monensin content of chick tissues (Donoho and Kline, 1968). Monensin concentration was estimated from the zone of inhibition of Bacillus subtilis growth on agar poured over thin-layer chromatography plates: an indirect measure of antibiotic activity. The test sensitivity claimed was 25 ppb (.025 ug/g) but it was advised that this test be used qualitatively, rather than quantitatively (Donoho and Kline, 1968). These researchers reported that monensin was not detected at withdrawal time in muscle, liver or kidney tissue of chicks fed 134 g monensin/ton for 56 days. Fat contained .05-.10 ppb monensin at 0 hours but no detectable amounts after 24 hours of withdrawal.

Herberg et al. (1978) administered ^{14}C-labeled monensin to steers and found that radioactive metabolites were excreted in feces, but not urine, and that about 95% of the ^{14}C was recovered in feces. Twelve hours after oral treatment, monensin content was estimated at .59 ppm in liver tissue on the basis of radioactivity but was not detected in other edible tissues. In a second trial, liver tissue contained radioactivity comparable to up to .425 ppm monensin, but bioautographic assay suggested monensin content of less than .015 ppm (Herberg et al., 1978).

Donoho et al. (1978) identified varying amounts of six different metabolites of monensin in feces of steers and rats fed ^{14}C-labeled monensin. The total concentration of metabolites, based on radioactivity, in steer liver was .59 ppm, but monensin only accounted for 3% of the total radioactivity, according to bioautographic assay. Davison (1984)

then fed ^{14}C-monensin to bile duct-cannulated chickens and calves and found that 11 to 31% and 36 to 40% of the ^{14}C-monensin was absorbed, respectively. Calf bile contained one metabolite but no monensin. At least 50% of the fecal ^{14}C was present as monensin, although three metabolites were also detected. Chicken bile contained four metabolites, as well as monensin. It was concluded that monensin was absorbed and extensively metabolized but that most of the absorbed ^{14}C-monensin was secreted in the bile and passed in the feces.

Collectively, these studies confirmed a loss of antibiotic activity after metabolization of monensin but failed to determine whether ionophoretic activity remained.

Donoho (1984) reviewed a large number of monensin pharmacokinetic studies and concluded that monensin concentration in blood was low even in intoxicated animals; biliary excretion was the major route of elimination; and even intoxicating doses of monensin did not result in high tissue monensin concentrations. When fed to cattle and chickens at the recommended levels (30 and 120 mg/kg, respectively), monensin was not detected (<.05 ppm) in edible tissues by the bioautographic method. (Donoho, 1984). Currently, the recommended withdrawal time for monensin is 0 and 3 days in cattle and poultry, respectively (Heitzman et al., 1986).

In 1981, Fahim and Pressman reported the development of a sensitive (.005 ppm) radiochemical assay which measured monensin content of homogenized tissue or plasma samples by the extent of $^{22}Na^{+}$ uptake, an ionophoretic activity. They employed this test in studies designed to correlate the pharmacokinetic or pharmacologic effects of monensin. Dogs

were shown to have faster oral absorption, faster plasma clearance (shorter $t_{1/2}$), and more significant cardiovascular and toxic effects than rabbits. They suggested that carnivores and herbivores may differ in their metabolism of monensin. Tissue residues in rabbits were about .5 ug/g (.5 ppm), which was 10 times the maximal residue allowed by the FDA in cattle and chickens (Fahim and Pressman, 1981). Heitzman et al. (1986) have developed an enzyme-linked immunosorbent assay sensitive to 5 ppb of monensin in bovine plasma.

Because ionophore function depends on insertion in a lipid membrane, it seemed logical that ingested monensin should penetrate gastrointestinal cells and be absorbed; Pressman and Fahim (1983) confirmed this in dogs, rabbits and sheep. It is therefore unlikely that monensin should be undetectable in tissues of animals fed significant amounts without withdrawal, as Donoho and Kline (1968) had reported using their bioautographic method. Perhaps monensin metabolites lose their antibiotic capabilities but maintain their ionophoretic properties. If so, the radiochemical assay (based on $^{22}Na^{+}$ transport) would detect significantly smaller residues than the bioautographic method (Pressman and Fahim, 1982, 1983).

Pressman and Fahim (1982) pointed out that daily monensin consumption at the approved levels could amount to about 600 ug/kg BW in cattle and 7,000 ug/kg BW in chickens. They proposed that poultry fed 120 ng monensin/kg diet for 7 days would have about .7 ppm monensin in the liver and .6 ppm in the muscle, which might decline to .1 ppm if a one-day withdrawal period were observed. This was greater than the allowable residue of .05 ppm monensin. At this rate, a 70 kg man consuming 250 g

of poultry would ingest 150-175 ug monensin, or 2 ug/kg BW (Pressman and Fahim, 1983).

The toxic dose of oral monensin in humans is not known, but workers exposed during manufacture have developed headache, nausea, epistaxis, and skin rash (Pressman and Fahim, 1982). It is therefore possible that contamination of the food chain with monensin could have adverse effects in individuals with pre-existing heart disease or receiving cardiac glycoside medication.

V. Monensin Toxicosis in Animals Other Than Swine.

A. Overview.

Accidental or experimental monensin toxicosis has been reported in mice, rats, poultry, dogs, horses, sheep, goats, cattle, and pigs (Langston et al., 1985; Osweiler et al., 1985). Most outbreaks of monensin poisoning are related to accidental mixing errors or inclusion of monensin in feed for a species which has low monensin tolerance. Reports of monensin toxicosis suggest that exposure may be sporadic within a group of animals due to differences in amount of contaminated feed consumed or to uneven distribution of monensin within the feed.

Species susceptibility to monensin toxicity is widely variable and is often reported as the dose at which 50% of the animals consuming monensin would be likely to die (LD_{50}). The monensin LD_{50} values for a number of species have been established (Table 1).

For the most part, signs of toxicosis are similar between species, but there are some exceptions, based largely on amount and duration of intake. Acute, high dose toxicosis often produces death with few premonitory signs or pathologic lesions, while chronic or low-dose exposure results in significant clinical and pathologic derangements.

B. Poultry.

Monensin is approved for use as a coccidiostat in broilers at 90-100 /ton (≈120 mg/kg diet) (Lanston et al., 1985) and the LD_{50} for monensin chickens is generally considered to be 200 mg/kg BW (Hanrahan et al., 81). Monensin poisoning has been reported in broiler chickens (Beck and ries, 1979; Chalmers, 1981; Hanrahan et al., 1981) and turkeys (Stuart, 8). The related monocarboxylic ionophores, salinomycin and narasin,

Table 1. Monensin lethal dose-50 (LD_{50}) values reported for different species.

Species	LD_{50} (mg/kg BW)	Reference
Mouse	44	Haney and Hoehn, 1968
	70(male), 96(female)	Todd et al., 1984
	125	Anon., 1978; Beck and Harries, 1979
Rat	35	Anon., 1978; Beck and Harries, 1979
	40(male), 29(female)	Todd et al., 1984
Rabbit	42	Todd et al., 1984
Poultry	200	Anon., 1976
	284	Haney and Hoehn, 1968
Dog	>20(male), >10(female)	Todd et al., 1984
Horse	2-3	Matsuoka, 1976
Sheep	12	Anon., 1978; Confer et al., 1983; Potter et al., 1984
Goat	24	Anon., 1978; Raisbeck and Miller, 1981
Cattle	22	Anon., 1978; Raisbeck and Miller, 1981
	27	Potter and Miller, 1979 in Janzen et al., 1981
	50-80	Whitlock et al., 1978
Pig	17	Anon., 1980; Potter et al., 1984
	50	Van Vleet et al., 1983a
Monkey	>160	Todd et al., 1984
Trout	>1000	Todd et al., 1984

have been incriminated in outbreaks of poisoning in turkeys (Davis, 1983; Stuart, 1983; Horrox, 1984).

Clinical signs in chickens accidentally exposed to feed containing 197 (Chalmers, 1981) or 325 mg monensin/kg (Beck and Harries, 1979), included anorexia, depression, infertility, decreased egg production, paralysis, and death. Many birds were found dead in a characteristic sternal position, with head and neck outstretched. In one report of accidental toxicosis in birds and a subsequent trial feeding, congestion of head, neck, lung and liver tissue was apparent, suggestive of circulatory failure, but histologic changes were minimal (Beck and Harries, 1979). In another report few gross lesions were observed but, histologically, cardiac and skeletal muscle had extensive degenerative myopathy, with fragmentation, mineralization and loss of cross-striations (Chalmers, 1981). Attempts to reproduce the disease were unsuccessful because remaining feed contained less than 5 mg monensin/kg.

Hanrahan et al. (1981) fed 150, 200, or 250 mg monensin/kg BW to chickens which developed toxicosis. Pericardial fluid accumulation, myocardial streaking, pallor and ventricular enlargement were present, with mitochondrial degeneration histologically. Mild to severe degenerative myopathy of skeletal muscle was present and most pronounced in Type I (red) fibers which are aerobic in nature.

In adult turkeys, but not young ones, 218-300 mg monensin/kg caused thirst, dyspnea, prostration, paralysis and death, with only mild myocardial pallor evident on gross postmortem examination (Stuart, 1978). Results of histologic examination were not presented, but an experimental trial with 200 mg monensin/kg reproduced disease. Halvorson et al. (1982)

reported that concentrations of monensin and salinomycin less than those normally fed to chickens produced extensive myodegeneration and mortality in adult, but not juvenile, turkeys. Salinomycin at 15-30 mg/kg diet produced a similar clinical course and degenerative myopathy with more acute mortality (Stuart, 1983). Davis (1983) produced ionophore toxicosis and mortality in young turkeys with 70 mg narasin/kg but not with 100 mg monensin/kg.

Collectively, these cases showed that there were differences in susceptibility of poultry to ionophore toxicosis depending on the type and amount of ionophore consumed and the age of the animal.

C. Dogs.

One report of monensin toxicosis in dogs that ate feed containing 150 mg monensin/kg described clinical signs of anorexia, weakness, myoglobinuria, ataxia and paresis (Wilson, 1980). In one animal, serum AST and LDH activities were moderately elevated and CPK values greatly increased. Todd et al. (1984) related information from toxicity trials in which dogs fed monensin at doses from 0-50 mg/kg BW developed mild, transient to severe, fatal myodegenerative disease of skeletal and cardiac muscle. Dogs fed up to 7.5 mg monensin/kg BW monensin initially had anorexia, lethargy, weight loss, and increased ALT and CPK values but no pathologic lesions. Fahim and Pressman (1981) reported similar clinical signs in dogs fed 2 mg/kg BW.

D. Horses.

As early as 1975, it was recognized that monensin-treated broiler feed might be toxic or fatal to horses (Stoker, 1975). Matsuoka (1976) first produced experimental monensin toxicosis in a feeding study where

horses were fed 0, 31, 125, or 279 mg monensin/kg diet. Toxicity was dose-dependent and characterized by anorexia, sweating, weakness, reluctance to move, ataxia, recumbency, and death. No gross lesions were seen, but "toxic tubular nephritis" and "toxic hepatitis" were evident histologically. Serum AST activity and blood urea nitrogen (BUN) values were elevated, apparently due to the liver and kidney damage.

In a second trial, single doses of 1, 2, 3, 4, or 20 mg monensin/kg BW were administered and all horses receiving at least 3 mg/kg BW died within 36 hours without changes in serum enzyme values or gross lesions. Histologic diagnosis was "toxic tubular nephritis" and "toxic hepatitis", and the LD_{50} was stated to be 2-3 mg/kg BW (Matsuoka, 1976).

Soon after, Nava (1978) described a natural outbreak of monensin toxicosis in which 26 horses died after developing posterior weakness, reluctance to move and ataxia. Serum AST and BUN values were elevated, and acute cardiovascular collapse and myocardial degeneration were diagnosed. Horses surviving beyond two weeks developed cardiac arrhythmias, increased heart rates and edema. Monensin was found at concentrations of 0, 215 and 274 mg/kg in feed samples and at 50 and 100 mg/kg in stomach contents of two dead horses.

Beck and Harries (1979) reported two cases of equine monensin toxicosis marked by sweating, depression, ataxia and cardiac myopathy with circulatory failure. Monensin was detected at 9.5, 19, and 110 mg/kg in the feed. Whitlock et al. (1979) found monensin at 70-150 mg/kg in horse feed which had been contaminated with a poultry premix and caused toxicosis and/or death in several horses and two ponies. Feed refusal, common after first exposure to monensin, prevented disease in many horses.

Cardiac failure occurred in two horses consuming cattle feed containing 7500 mg monensin/kg (about 18-20 mg/kg BW) (Ordidge et al., 1979). A third horse recovered and was found to have no cardiac abnormalities. However, Muylle et al. (1981) found electrocardiographic (ECG) evidence of cardiac disease in 8 of 32 horses examined for poor performance 3-5 months after accidental exposure to monensin. Four affected horses had elevated LDH-5 (skeletal muscle isoenzyme) values. Six of these animals were necropsied and had pale myocardial and skeletal muscles and signs of circulatory failure. Renal tubular nephrosis and marked granular degeneration and fibrous replacement of myocytes were seen histologically (Muylle et al., 1981).

Mollenhauer et al. (1981) studied the ultrastructural lesions of monensin toxicosis in three ponies given a single oral dose of 4 mg/kg BW. Myocardial tissue had severe mitochondrial swelling and lipidosis but apparently normal sarcoplasmic reticulum. Hepatic tissue had increased sarcoplasmic reticulum (usually associated with increase microsomal mixed-function oxidase activity) and increased numbers of peroxisomes (site of catalase activity). These authors concluded that heart mitochondria were preferentially damaged by monensin (Mollenhauer et al., 1981).

Amend et al. (1980) investigated the pathophysiology of experimental monensin toxicosis in horses. In ponies treated with 2-3 mg monensin/kg BW, death occurred in 24 hours. Hemoconcentration, polyuria, hypovolemic shock and elevations in serum BUN, creatinine and osmolality were manifestations of toxic tubular nephrosis. Analysis of serum zymograms indicated early elevations in LDH due to erythrocyte hemolysis, followed by increases in LDH from cardiac muscle, CPK from skeletal muscle and AST

from skeletal muscle, cardiac muscle and erythrocytes. Serum Na^{+} content did not vary much, but transient decreases were seen in serum concentrations of Ca^{+2} (1-2 mg/dL during first 12 hours) and K^{+} (1-2 mEq/L first 12-16 hours). Initial abnormalities in ECGs were associated with hypokalemia (loss of T waves), but subsequent S-T segment depression was indicative of myocardial injury. Amend et al. (1980) also reported dose-related destruction of myocardial mitochondria.

E. Sheep.

Monensin is not approved for use in sheep but is often fed as a coccidiostat at 11-33 mg/kg of feed (Langston et al., 1985; Bourque et al., 1986). The LD_{50} for monensin in sheep is reported to be 11.9 mg/kg BW (Anon., 1978). While testing the therapeutic value of monensin against ovine coccidiosis, Bergstrom and Jolley (1977) produced toxicity or death in sheep treated with 4 or 8 mg/kg BW but found no lesions at necropsy. Nation et al. (1982) then reported accidental toxicosis in two flocks of sheep exposed to 152-550 mg monensin/kg of feed. Diarrhea, muscle pain, hindquarter muscle atrophy, posterior paresis and death occurred. Subacute and chronic poisoning cases were marked by hemorrhagic enteritis and skeletal muscle damage but minimal myocardial degeneration. Hind limb muscles were pale with white, linear streaks which histologically were the result of hyaline degeneration of myofibers and calcification of the sarcoplasm and fibrosis.

Bourque et al. (1986) describe feed refusal, stiff gaits, hind limb weakness, "humped up" posture, "tip-toe" walking, but few deaths in lambs consuming feed containing 110 mg monensin/kg. Serum Ca^{+2} values were low and CPK and AST concentrations were 50-100 times normal. Two lambs

necropsied had myocardial and skeletal muscle pallor, with evidence of regeneration in skeletal muscle. Serum CPK and AST activities were increased in 2 of 3 sheep receiving either 12, 16, or 24 mg monensin/kg BW as a single oral dose (Anderson et al., 1984). These sheep had dose-dependent myocardial and skeletal muscle degeneration and vacuolation which was shown by electron microscopy to be due to severe mitochondrial disruption and swelling. Hepatocytes had dose-related increases in smooth endoplasmic reticulum, a nonspecific finding attributed to attempts by the mixed-function oxidase system to detoxify foreign substances (Anderson et al., 1984).

Confer et al. (1983) described progressive, high amplitude mitochondrial swelling and vacuolation and myofibrillar necrosis over six days in sheep receiving one oral dose of 12 mg/kg BW of monensin. Myonecrosis was more severe in skeletal than cardiac muscle and most pronounced in sheep treated with 8 mg monensin/kg BW daily for three days.

F. Cattle.

In cattle, monensin is approved for use as a growth promotant at 50-200 mg/hd/day in pastured cattle or 50-360 mg/hd/day (about 1 mg/kg BW) in feedlot cattle (Anon., 1978). This can be fed as 5-30 g monensin/ton of complete feed (5.5-33 ppm) or in the form of supplements containing no more than 1200 g monensin/ton (Anon., 1978). However, monensin is sometimes fed at 200 mg/hd/day for prevention of acute bovine pulmonary emphysema (Hammond et al., 1982) or at 16-33 g/ton of feed for prevention of coccidiosis (Langston et al., 1985).

Accidental toxicity due to monensin is more prevalent in cattle than any other species. Collins and McCrea (1978) described feed refusal,

hemorrhagic gastroenteritis and heart failure, with right ventricular hemorrhage and cardiac enlargement in 6 of 44 pastured yearling bull calves consuming a grain supplement containing 2000 mg monensin/kg rather than 200 mg/kg. In this case and another (Malone, 1978), monensin was thought to predispose heifers grazing turnips to nitrite toxicity.

Beck and Harries (1979) described two outbreaks of monensin toxicosis; in the first, 2 of 6 feeder calves exposed to 56-160 mg monensin/kg in a grain mixture died, and in the second case, 74 of 500 feedlot steers died after consuming up to 4 g monensin/hd/day (17 mg/kg BW). Anorexia, dark, watery feces, and dyspnea were common clinical signs, and postmortem examination revealed cardiac enlargement and hemorrhage and lobular hepatic necrosis. Mitochondria of both liver and heart were destroyed and vacuolated. Similar incidents of accidental poisoning and circulatory failure, in which the amount of monensin consumption was unknown, were reported in a group of bulls at a performance test station (Janzen et al., 1981), and in 117 of 1,994 feedlot cattle where monensin was not properly mixed in a liquid dispensing system (Kimberling and Schweitzer, 1983; Schweitzer et al., 1984).

Raisbeck and Miller (1981) identified monensin toxicosis in a herd of pastured calves allowed access to a mix of corn and pellets which contained 1200 g monensin/ton. Sudden death and exercise intolerance were associated with a globular myocardium and congestive heart failure, the characteristic sequelae of myocardial necrosis. Serum CPK and AST activities were not significantly elevated, and skeletal muscle was not affected. In another outbreak of toxicosis in yearling calves, the

skeletal muscle of one animal with cardiac myopathy was not affected, although serum CPK values were mildly to markedly increased in six other animals (Collery, 1983). Wardrope et al. (1983) found gastroenteritis, globular cardiomyopathy and heart failure, with or without hepatic swelling, in an outbreak in which 9 of 40 dairy calves died after consuming a concentrate mixture containing up to 200 mg monensin/kg.

Geor and Robinson (1985) described death in 20 of 110 feedlot cattle exposed to 400 g monensin/ton. The clinical picture was diarrhea, dyspnea, reluctance to move, and recumbency before death. In one animal sampled, serum CPK, AST and LDH activities were greatly elevated and hypocalcemia, hyponatremia, and hyperkalemia were present. Postmortem examination revealed pulmonary edema, hydrothorax, ascites, and ventricular dilation compatable with right-sided heart failure. However, myocardial necrosis and gastroenteritis could not be demonstrated, although focal skeletal muscle necrosis was observed.

Potter et al. (1984) reviewed outbreaks of monensin toxicosis and feeding studies in cattle. They concluded that toxicosis was almost exclusively the result of mixing errors or improper usage and that mortality was predictable, based on exposure studies. However, it would seem that sub-lethal exposure to monensin results in a variable clinical presentation, depending upon the amount and duration of consumption. Animals dying acutely may have no postmortem lesions (Raisbeck and Miller, 1981), while those affected subacutely or chronically may have involvement of different organ systems. Antemortem clinicopathologic changes may, likewise, be variable.

Experimentally induced monensin toxicosis has been studied in cattle

to clarify the clincopathologic course. Van Vleet et al. (1983c) gave monensin as a single oral dose of 25 mg/kg BW (group A) or as two doses of 40 mg/kg BW at a 7-day interval (group B) to beef cattle and monitored the effects. Calves in groups A and B were euthanatized after 4 or 11 days on trial, respectively. Anorexia, lethargy, and diarrhea developed within 24 hours and were most severe in group B calves, one of which died on day 7. Mild to moderate decreases were seen in mean serum Na^+, K^+ and Ca^{+2} concentrations in both groups and were attributed to diarrhea. Mean serum CPK and AST activities were increased three- to four-fold in group B, but not group A, calves and corresponded with the chemical, but not visual, detection of urinary myoglobin. Only group B calves developed ECG abnormalities which included prolongation of Q-T and QRS intervals and first degree heart block.

Significant lesions were found in the heart, skeletal muscle and rumen on necropsy examination of the treated calves (Van Vleet et al., 1983c). Hearts were not dilated but had yellow-brown areas of necrosis in the ventricles, most prominent in transverse section. The myocardial tissue was affected by a delayed onset of necrosis which was characterized by contraction bands and sarcoplasmic vacuolation due to mitochondrial swelling and lipid droplet accumulation. Lesions of congestive heart failure were noted only in the calf that died. Skeletal muscle was not grossly abnormal, but sections of triceps brachii, vastus lateralis, diaphragm, and, especially, tongue had similar dose-related lesions as the myocardium. The gastro-intestinal tract was full of watery contents and appeared normal, but rumenitis was diagnosed microscopically (Van Vleet et al., 1983c).

Galitzer et al. (1983) gave a single oral dose of 25 mg monensin/kg BW to six steers and measured increases in serum CPK and LDH activity and decreases in serum Ca^{+2}, K^{+} and Cl^{-} concentrations, but did not report the magnitude of the changes. Five of these cattle died within 12 days and had typical lesions of monensin toxicosis, as did six steers given 50 or 100 mg/kg BW lasalocid (Galitzer et al., 1986).

Collectively, this work supported the idea that the pathophysiology of monensin toxicosis in cattle was dependent on dose and duration of exposure. Serum electrolyte leakage, enzyme concentrations, ECG recordings and urinalysis may be useful aids in antemortem diagnosis, especially in subacute or chronic monensin poisoning.

VI. Monensin Toxicosis in Swine.

A. Overview.

Unlike monensin toxicosis in poultry, dogs, horses, sheep or cattle, poisoning in swine is not usually associated with consumption of accidentally contaminated feed or with mixing errors. Rather, it results from the intentional use of monensin as a coccidiostat in feed for growing pigs. It is important to review porcine coccidiosis and its discussion in the literature before describing cases of natural or experimental monensin toxicosis in swine.

B. Porcine Coccidiosis.

Diarrhea in neonatal pigs is caused by a variety of etiologic agents, all of which can be life-threatening. One of the most prevalent sources of disease is coccidoisis, caused by Isosopora suis (Biehl and Hoefling, 1986). The disease was first recognized in the late 1970's in the United States and subsequently reported world-wide by the early 1980's (Current, 1987). Pigs of 6-10 days of age develop pasty, then fluid, diarrhea, weight loss, and dehydration, and they may die within several days. Surviving pigs are often unthrifty. Morbidity may reach 100% and mortality 20% (Biehl and Hoefling, 1986).

Upon necropsy examination, portions of ileum and jejunum may be found to contain yellow fluid, have a fibrinonecrotic pseudomembrane (without intraluminal hemorrhage), and histologic evidence of villous atrophy and necrotic enteritis (Current, 1987). Developmental stages of the parasite may or may not be present within the tissues inspected. Affected pigs are more susceptible to other enteric infections and are unresponsive to antibiotic therapy (Tubbs, 1986, 1987).

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Harleman and Meyer (1983) have reviewed the life cycle of Isospora suis. Sporozoites released from an ingested, sporulated oocyst invade an intestinal epithelial cell. Here, they become meronts and, through asexual reproduction (merogony), produce merozoites which are released from the cell to invade other host cells. Second-generation meronts undergo merogony, and this process is repeated several times.

After 1-3 asexual generations, merozoites enter a sexual reproduction phase (gametogony). Some merozoites become microgamonts, which divide to form microgametes, and some become macrogamonts, which become macrogametes without dividing. One microgamete and one macrogamete unite to produce a zygote, which is encapsulated and then released as an oocyst by excystation. The unsporulated oocyst is passed in the feces and, under proper environmental conditions, sporulates (sporogony) to become infective and begin the process in another susceptible piglet. Extraintestinal cycles of I. suis in piglets have been reported (Harleman and Meyer, 1983) but not confirmed (Stuart et al., 1982).

Neonatal coccidiosis has been experimentally reproduced with I. suis oocysts and found to be virtually identical to natural outbreaks (Lindsay et al., 1985). The prepatent period of I. suis is 4-5 days (Harleman and Myers, 1983; Current, 1987), and affected pigs develop diarrhea by 3-5 days postinoculation (Lindsay et al., 1985). This suggests that day-old piglets exposed to oocysts could be shedding oocysts, infective for other pigs, at 5-6 days of age (Current, 1987).

Swine can also be infected with 8-10 species of Eimeria, but this genus is usually isolated from adult swine (Lindsay et al., 1984) and is not considered to be of enteropathogenic significance in young pigs (Biehl

and Hoefling, 1986; Tubbs, 1986; Current, 1987). A study of the prevalence of I. suis and Eimeria spp. oocysts revealed that I. suis was not detected in feces of sows on farms with neonatal coccidiosis and in only .4% of fecal samples from sows on farms without neonatal coccidiosis (Lindsay et al., 1984). Eimeria spp. oocysts were found in 81.8% of sows from farms with neonatal coccidiosis and in 94.8% of sows from farms without. Therefore, it was concluded by these investigators (Lindsay et al., 1984) and others (Biehl and Hoefling, 1986; Tubbs, 1986; Current, 1987) that I. suis oocysts most likely are not shed by sows.

If I. suis oocysts are not shed by sows, then the source of infection is probably a carry-over from previous farrowings in contaminated farrowing houses (Current, 1987). The high temperatures common to farrowing houses (35-38^{o}C) favor the sporulation of I. suis, which can become infective in 12 hours at 37^{o}C, while inhibiting the sporulation of Eimeria spp. oocysts (Lindsay et al., 1982; Tubbs, 1986; Current, 1987).

Assuming the farrowing house to be the source of infection, control of neonatal coccidiosis would best be accomplished by farrowing house sanitation (Tubbs, 1987). Ernst et al. (1985) credited improved sanitation with reducing the proportion of sows positive for Eimeria spp. from 9.9% to .4%, and for I. suis from 1.2% to 0%. In this study the feces of piglets contained no Eimeria spp. oocysts, but prevalence of I. suis oocysts in litter composite fecal samples decreased from 100% to 19.8%. No association between sow shedding and pig infestation with I. suis was seen.

The role of sows in oocyst shedding in neonatal coccidiosis is

controversial. Harleman and Meyer (1983) state that colostral immunity to I. suis is poor, that "stress" may precipitate oocyst shedding in older pigs, and that pregnant gilts or sows stressed by farrowing do shed I. suis oocysts. In a review article, Tubbs (1986) mentioned that other investigators believe I. suis oocysts may be carried on the sow's body, shed in feces in numbers below detection levels, or shed at farrowing due to a periparturient relaxation of immunity.

It is interesting that the practice of introducing sows to the farrowing house up to 7 days prior to farrowing, coupled with the very short prepatent period of I. suis (4-5 days), and a periparturient decline in immune function might allow sows to become reinfected in the farrowing house, much like their piglets. Oocyst shedding by sows might then reflect I. suis-contaminated housing and function as a source of oocyst amplification, rather than as the primary source of infection in piglets.

C. Use of Coccidiostatic Agents in Swine.

In 1980, Roberts et al. reported outbreaks of diarrhea in piglets associated with Isospora suis oocysts and rotavirus. They stated that a coccidiostat, amprolium (a thiamine analogue), fed to sows at 1 kg/ton of feed before they entered the farrowing house appeared to decrease sow oocyst shedding (genus not reported) and the incidence of neonatal diarrhea. Later reports (Roberts and Walker, 1981, 1982) correlated infection in pigs with I. suis oocyst shedding of sows from 4-5 days before to 2-3 days after farrowing.

Control was attempted in several herds by thorough cleaning between farrowing periods and use of several coccidiostatic regimens (Roberts and Walker, 1982). Amprolium was fed either to piglets for the first 3-4 days

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of life, or to sows for one week prior to farrowing and while in the farrowing house. The latter method was ineffective in controlling diarrhea in one herd, so monensin was fed to sows of that herd at 100 g/ton prior to and after farrowing, with reported success (Roberts and Walker, 1982).

Many coccidiostatic agents have been used in the field, but these have not been consistently effective (Harleman and Meyer, 1983; Biehl and Hoefling, 1986; Tubbs, 1986) and are often not recommended (Lindsey et al., 1984; Biehl and Hoefling, 1986; Current, 1987). In fact, many authors feel that the reduced incidence of neonatal coccidiosis is due more to improved sanitation and awareness than to coccidiostat usage (Lindsay et al., 1984; Ernst et al., 1985; Tubbs, 1986, 1987; Current, 1987).

Nevertheless, shortly after Roberts and Walker (1981, 1982) recommended the nonapproved use of monensin as a coccidiostat in prepartum sows, cases of monensin toxicosis began to appear in growing pigs.

D. Monensin-Tiamulin Interactions in Poultry and Swine.

Early cases of monensin toxicosis in swine involved growing pigs simultaneously medicated with monensin and tiamulin. Tiamulin hydrogen fumarate is a semisynthetic, diterpene compound derived from the antibiotic pleuromutilin which was isolated from Pleurotis mutilus (Schultz et al., 1983). Tiamulin is approved for water-additive use against swine dysentery (Treponema hyodysenteriae) in pigs at 60 mg/L (8.8 mg/kg BW) (Anon., 1983c; Miller et al., 1986; Olson, 1986) and against mycoplasmosis (Mycoplasma spp.) in poultry at 250 mg/L (Umemura et al., 1984).

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Meingassner et al. (1979) first reported on the potential interaction of tiamulin and polyether antibiotics (monensin and lasalocid) in chickens. Although tiamulin alone had no anticoccidial activity, its simultaneous use with monensin or lasalocid enhanced the coccidiostatic efficacy of both compounds. These researchers suggested that host metabolism of polyether antibiotics may be altered by tiamulin and were able to show that tiamulin reduced monensin elimination by 60% in isolated, perfused rat liver, perhaps by competing for the same degradative enzymes. Slower elimination was responsible for an "overdosing" effect which depressed weight gains in chicks at high levels of monensin but not lasalocid (Meingassner et al., 1979).

Naturally occurring cases of interactive toxicity in poultry and swine soon followed. Horrox (1980) reported a 40% mortality rate over about ten days in turkey poults medicated with tiamulin and consuming feed accidentally containing monensin. Drake (1981) then described necropsy lesions similar to nitrite poisoning in 2 of 20 pigs weighing 15 kg which had been exposed to monensin in the feed (1 kg/ton) and tiamulin in the water. Affected animals had pink urine, a serosanguinous nasal discharge, and discoloration of the ventral abdomen. Pigs receiving only monensin were not affected, and withdrawal of tiamulin stopped the outbreak.

Several groups then attempted to experimentally reproduce these interactive death losses, with mixed results. Miller (1981) was unable to cause death or clinical disease in pigs three months of age consuming feed containing 170 mg monensin/kg, with or without 200 mg tiamulin/kg. Pott and Skov (1981) offered feed containing 100 mg monensin/kg, with or without 200 mg tiamulin/kg, to pigs weighing 17 kg. Over a ten-day

period, pigs fed both compounds became anorectic, depressed, lethargic, recumbent and ataxic, and died. Necropsy revealed pink urine (possibly myoglobinuria), but histopathologic lesions were not seen in the heart, liver or kidneys. They theorized that illness may be the result of neurotoxicity, a direct effect on myocytes, or generalized electrolyte imbalances (Pott and Skov, 1981).

Stansfield and Lamont (1981) used the standard doses of 100 mg monensin/kg feed and 60 mg tiamulin/L water, as Drake (1981) had reported, to study interactive effects in pigs weighing 24 kg. Anorexia, pyrexia, dyspnea, ataxia, quadriplegia, and coma developed within three days. Mean serum CPK values rose from 236 IU/L prior to treatment to 18,405 IU/L on day 2 and 99,350 IU/L on day 3. Necropsy revealed normal hearts but pale skeletal muscle which was characterized by myopathy of Type I (aerobic) fibers. They concluded that the unapproved use of monensin, advocated by Roberts and Walker (1981, 1982) for control of coccidiosis (100 ppm), was indeed able to produce toxicosis in pigs also medicated with tiamulin (Stansfield and Lamont, 1981).

Umemura et al. (1984) further described the histopathology of lesions of monensin-tiamulin toxicosis in broiler chicks. One-week-old chicks, fed 80 mg monensin/kg and drinking 250 mg tiamulin/L, developed anorexia, depression and drowsiness and often assumed a position of sternal recumbency with dropped wings. Necropsy revealed no gross lesions but, microscopically, skeletal muscles of the neck and legs were found to have severe, non-necrotic, degenerative changes with evidence of regeneration. Because cardiac and pectoral muscles were usually involved in myopathic disease, but were spared in this study, the findings were

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considered distinctive for monensin-tiamulin interaction.

Subsequently, Umemura et al. (1985) studied the myotoxicity of monensin and tiamulin in pigs weighing 4.5-6.0 kg. Pigs offered 500 mg tiamulin/L alone in water had no signs of disease and no histologic lesions. Pigs offered 200 mg monensin/kg feed and 250 mg tiamulin/L water were anorectic and lost weight. Macroscopic lesions were absent, but severe myonecrosis and degeneration of both Type I and II myofibers were seen, microscopically, to preferentially affect skeletal muscle of the tongue, diaphragm and legs. Cardiac muscle was not affected.

Another monovalent, monocarboxylic ionophore, salinomycin, has also been shown to cause interactive toxicity with monensin in pigs (Miller et al., 1986). Twelve-week-old pigs, given 80 mg salinomycin/kg diet and various concentrations of tiamulin in the feed or as an injection, developed dose-related signs of toxicity which included anorexia, recumbency, hind limb ataxia, muscle tremors, dyspnea and cyanosis. Serum AST, ALT and CPK values were elevated in affected pigs but, once again, no gross lesions were seen at necropsy, and histologic examination was apprarently not performed (Miller et al., 1986).

E. Experimental Monensin Toxicosis in Swine.

Several experimental studies have been carried out to determine the toxicity of monensin alone in pigs. Acute single-dose oral administration after a 16-18 hour fasting period produced an estimated LD_{50} of 16.8 mg monensin/kg BW in pigs weighing 8-25 kg (Anon., 1980). Signs of toxicosis included dyspnea, blueish discoloration of skin, ataxia, diarrhea, and death. Ten 21-33 kg pigs, consuming 500 mg monensin/kg total diet, had increased serum AST values; four died within ten days, with histologic

evidence of a severe degenerative myopathy in two pigs (Anon., 1980). However, monensin at 100 mg/kg diet, was found to have no toxic effects in 26 litters of nursing pigs, in 18 gilts from 35 kg BW through lactation, or in 8 gilts and 6 sows from ten days prior to breeding through 28 days of lactation (Anon., 1980).

Van Vleet et al. (1983a) performed a graded-dose trial on five groups of four, non-fasted, 20 kg, weanling pigs by administering monensin at 10, 20, 30, 40, or 50 mg/kg BW. Dose-dependent clinical signs included anorexia, lethargy, diarrhea, dyspnea, ataxia, myoglobinuria and death. One pig receiving 30 mg monensin/kg BW and three pigs receiving 50 mg/kg BW died within 24 hours. Remaining pigs were euthanatized at four days.

Bilaterally symmetrical areas of chalky white discoloration were observed in the thigh, shoulder, and loin muscles. These areas were marked by dose-related hyaline necrosis and macrophage infiltration, with some myocyte regeneration. Cardiac myonecrosis was infrequently detected, but most commonly involved the left atrium. These investigators concluded that the LD_{50} in pigs was probably close to 50 mg monensin/kg BW (Van Vleet et al., 1983a).

Van Vleet et al. (1983b) then dosed ten weanling, 20 kg pigs with 40 mg monensin/kg BW orally following a 16 hour fast to study clinicopathologic changes. No pigs died but by 24 hours post-treatment all pigs were anorectic, lethargic and had diarrhea. Two pigs were dyspneic, ataxic, and reluctant to stand. Electrocardiographic abnormalities included wide or notched P waves, prolonged Q-T intervals and first degree heart block. By 24 hours, mean serum AST values were increased to 9,000 IU/L and CPK values were over 200,000 IU/L, 80-95% of

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which was due to elevations in the CPK isoenzyme of skeletal muscle origin. Significant changes were not observed in serum Ca^{+2}, Cl^{-}, K^{+}, or Na^{+} concentrations.

In this study as well, bilateral areas of pallor were apparent in skeletal muscles of the thigh on transverse section. Histologically, the findings consisted of hyaline necrosis, mitochondrial mineralization, and macrophage infiltration, with regeneration in many muscle groups. Damage was more pronounced in muscles ordinarily containing a high proportion of Type I fibers, although specific identification of skeletal muscle fiber types was not done. Myocardial pallor was also evident grossly, and again confined to the left atrium. Microscopically, myocardial necrosis with hypercontraction bands, macrophage infiltration and fibrosis was evident (Van Vleet et al., 1983b).

Van Vleet and Ferrans also performed ultrastructural examinations of the cardiac (1984a) and skeletal (1984b) muscles of pigs with acute monensin toxicosis after administration of 40 mg monensin/kg BW. In skeletal muscle, primarily Type I fibers, necrosis with disruption of contractile material and persistence of empty sarcolemmal "tubes" were evident. Sarcoplasmic reticulum and mitochondria were swollen and disrupted and contained flocculent matrical densities. Macrophage infiltration accompanied attempts by myoblasts to regenerate myofibers. Myonecrosis was categorized as polyfocal and monophasic (Van Vleet and Ferrans, 1984b). Cardiac muscle, predominantly left atrium, had extensive necrosis with contraction bands, myocyte degeneration and myofibrillar lysis (Van Vleet and Ferrans, 1984a). Mitochondria were initially swollen and then accumulated matrical densities. These were subsequently

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released by myofiber lysis and were ingested by infiltrating macrophages. The external laminae of necrotic cardiocytes remained as empty "tubes", but myocardial regeneration was not observed.

Thus, it is clear that in swine, as in other species, monensin toxicosis appears to be directed at skeletal and cardiac muscle where the earliest lesions are of mitochondrial disruption. Severe degenerative myopathy occurs in these striated muscle tissues, which are highly dependent on Na^{+} and Ca^{+2} ion regulation for contractility and normal function. Disruption of cation homeostasis in these tissues by the monocarboxylic ionophores has harmful or fatal consequences.

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VII. Proposed Mechanism of Monensin Toxicosis.

Small oral doses of monensin have a positive inotropic effect but only in the presence of Na^+ ions (Basset et al., 1978). Monensin-induced Na^+ influx results in a secondary increase in intracellular [Ca^{+2}], which facilitates contraction (Pressman and Fahim, 1981). This effect is augmented by endogenous catecholamine release (Fahim and Pressman, 1981). At higher concentrations, monensin has negative inotropic effects and can produce hypercontraction of atrial cells in vitro (Sutko et al., 1977). Hypercontracture is probably due to uncompensated myocardial Ca^{+2} overload (Shlafer and Kane, 1980). In combination with inhibitors of the Na^+-K^+-ATPase pump, monensin produces irreversible atrial contracture (Shlafer et al., 1979) and cell death (Burt and Berns, 1980).

The action of monensin is to uncouple oxidative phosphorylation in mitochondria (Estrada-O., 1968). Mitochondrial swelling is one of the earliest lesions seen in skeletal or cardiac muscle of many species with monensin toxicosis (Van Vleet and Ferrans, 1983, 1984a, 1984b). Elevations in intracellular [Ca^{+2}] prompt subcellular organelles, primarily mitochondria and sarcoplasmic reticulum, to sequester Ca^{+2} ions (Braunwald, 1982). Wrogeman and Pena (1976) have proposed that mitochondrial Ca^{+2} overload may be the common mechanism of a wide variety of muscle diseases characterized by hypercontraction and cell necrosis.

Based on their findings in skeletal and cardiac muscle of calves and pigs with monesin toxicosis, Van Vleet and co-workers proposed that intracellular Ca^{+2} overloading, secondary to elevated intracellular Na^+ content, was the underlying pathophysiologic process of monensin toxicosis (Van Vleet et al., 1983; Van Vleet and Ferrans, 1983, 1984a, 1984b).

REVIEW OF MONENSIN-MINERAL INTERACTIONS

I. Introduction.

W. J. Miller (1975) authored a paper on mineral metabolism and homeostasis which made several important points. For normal physiologic functions, animals must maintain tissue concentrations of the biologically active forms of minerals, particularly trace elements, within a narrow critical range, despite wide fluctuations in dietary mineral content. Tissue mineral concentrations outside the range governed by homeostasis become liabilities; mineral deficiencies or toxicities may follow.

Differences of several orders of magnitude exist between macromineral and trace element requirements, suggesting that different methods of homeostatic control must have evolved. Unlike the macrominerals, trace elements are rarely available as free ions; usually they are found in protein complexes. Interspecies differences in mineral requirements and tolerances are considerable. However, mineral homeostasis is uniformly accomplished by regulation of mineral absorption, retention, tissue distribution and by control of excretion by urinary, fecal or mammary routes (W. J. Miller, 1975).

E. R. Miller and Kornegay (1983) discussed the numerous interactions among trace elements and other minerals or nutrients in swine. Absorption, transport and distribution of different minerals within a living system are inseparably related. Dietary excess of one mineral may

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precipitate deficiency states of another mineral (Ashmead and Christy, 1985; E. R. Miller and Kornegay, 1983). While significant diseases of macromineral deficiency or toxicity are recognized (i.e., rickets, grass tetany, parakeratosis, white muscle disease), increasing evidence suggests that disruptions in trace element homeostasis may be just as important (E. R. Miller, 1985). In the latter case, mineral-disease interactions may be dependent on the role of trace elements in various enzyme systems (E. R. Miller and Kornegay, 1983). Perturbations in availability of minerals for incorporation in metalloenzymes may alter enzyme activity (Ashmead and Christy, 1985). Enzymatic involvement in disease resistance is recognized (i.e., GSH-Px and NMD) and implies that genotype may also be a significant determinant of disease susceptibility.

Participation of macrominerals (Ca^{+2}, K^{+}, Mg^{+2}, Na^{+}) in so-called "natural ionophore" systems is vital to cellular energetics, metabolism and function. Alterations in normal macromineral or trace element homeostatic mechanisms (absorption, distribution, excretion) may produce overt disease or subtle changes in disease resistance or susceptibility. The ionophoretic antibiotics used as feed additives could therefore have either adverse or beneficial effects on the mineral metabolism and/or nutritional status of livestock. For that reason, mineral-ionophore interactions deserve study.

This review will focus on the known interactions of ionophores, particularly monensin, with minerals, including selenium, and the influence of these interactions on disease processes.

II. Ionophore Interactions With Minerals Other Than Selenium.

A. Ionophore Effects on Mineral Balance.

Ionophores have the ability to transport monovalent or divalent cations across membranes and can directly influence the transport of some minerals. Indirectly, they may alter the metabolism of other minerals, particularly if transport of one mineral is coupled to that of another which is affected by an ionophore. Elsasser (1984) outlined five areas of potential ionophore-mineral interaction: 1) presence and bioavailability of ions in feed and water; 2) uptake and transport of ions; 3) distribution and storage of ions; 4) element-element interactions; and 5) homeostatic and regulatory mechanisms of ions. Additionally, Elsasser (1984) reviewed ionophore-induced alterations in normal tissue function which were ascribed to interference with natural osmoregulatory systems.

Several studies have examined the interactive effects of ionophores and monovalent cations on production parameters. Bartov and Jensen (1980) reported a significant growth depression in broiler chicks fed 100 or 120 mg monensin/kg of an animal protein diet, but not when included in a corn-soybean meal diet. Lower K^+ concentrations in the animal protein diet suggested that K^+ may have been limiting when monensin was fed. In broiler chicks fed 100 mg monensin/kg diet, significant growth rate depression occurred unless birds were also fed .2% magnesium potassium sulfate ($MgKSO_4$) (Charles and Duke, 1981). Cervantes et al. (1982) subsequently found that .3% supplemental K^+ alleviated the growth depression observed in broiler chicks fed diets containing fish meal and 140 or 160 mg monensin/kg. The same effect was obtained in chicks fed 160

mg monensin/kg of a corn-soy diet with K^+, but not Na^+, supplementation. Ferrell et al. (1983) described increases in rate of gain with K^+ supplement in feedlot steers fed 33 mg monensin or lasalocid/kg diet.

Other work has focused on the effects of ionophores on mineral availability, absorption, retention and excretion. Dvorak et al. (1980) found no difference between bulls fed monensin at 125 mg/hd/day and control animals in ruminal fluid concentrations of Ca^{+2}, Cu^{+2}, Fe^{+2}, Mg^{+2}, P, and Zn^{+2}, or in plasma Ca^{+2}, Cu^{+2}, Fe^{+2}, K^+, and Zn^{+2} concentrations. Starnes et al. (1984) fed 33 mg monensin or lasalocid/kg diet to steers and measured apparent absorption and retention of macrominerals. Relative to controls, ionophore-treated animals had decreased ruminal fluid concentrations of Ca^{+2}, K^+, and Mg^{+2}; increased apparent absorption of Mg^{+2}, Na^+, and P; and increased apparent retention of Mg^{+2} and P. There was a tendency towards increased apparent absorption of Ca^{+2} and increased apparent retention of Ca^{+2}, K^+, and Na^+. In treated animals, plasma Ca^{+2}, K^+, Mg^{+2}, Na^+, and P concentrations were not different, but plasma Cu^{+2} and Zn^{+2} values were increased, even though apparent absorptions and retentions were not (Starnes et al., 1984). Although they have different ion selectivity patterns, comparable results were found for lasalocid ($K^+ > Na^+ = Ca^{+2} > Mg^{+2}$) and monensin ($Na^+ > K^+$).

Elsasser (1984) designed several trials to investigate divalent mineral transport in ionophore-treated animals. He noted that, compared to controls, sheep fed 10 or 30 ppm monensin had significantly lower serum Ca^{+2}, higher serum P, but similar serum Mg^{+2} concentrations. Chickens had altered intestinal transport of ^{45}Ca, ^{64}Cu and ^{59}Fe in the presence of monensin or lasalocid, whether from the diet or acutely infused into

isolated loops of duodenum. Hepatic storage of Cu^{+2}, Fe^{+2}, and Zn^{+2} in chickens and Cu^{+2} and Zn^{+2} in sheep were altered by monensin and lasalocid. It was concluded that ionophores affected bioavailability, absorption and tissue distribution of divalent minerals but that the direction in which metabolism was altered was unpredictable (Elsasser, 1984).

At Texas A & M University, several balance trials were conducted in sheep fed 20 mg monensin/kg diet. Kirk et al. (1985a, 1985b) reported that, compared to controls, monensin-treated sheep had no difference in mineral intake; no difference in rumen fluid mineral concentrations, except for lowered Zn^{+2} values; increased apparent absorption of K^{+}, P, and Zn^{+2}; increased apparent retention of K^{+}, Mg^{+2}, P, and Zn^{+2} but decreased apparent retention of Na^{+}; decreased total excretion of all minerals, except for Na^{+}; no differences in serum mineral concentrations; and no differences in tissue mineral concentrations, except lowered Na^{+} in ileum and lowered Ca^{+2} in liver. Greene et al. (1986) found that, compared to controls, sheep consuming monensin had increased intake, apparent absorption and apparent retention of Ca^{+2} and Mg^{+2}, and decreased excretion of Mg^{+2}; increased apparent absorption and decreased excretion of K^{+}; no difference in Na^{+} or P balance; and no difference in serum mineral concentrations, except for increased Na^{+}.

Monensin at 125 mg/hd/day was reported to increase absorption of orally administered ^{65}Zn in steers fed hay (Costa et al., 1985). In pastured heifers implanted with a ruminal monensin delivery device (100 mg/day), plasma concentrations of Zn^{+2} were increased, but those of Cu^{+2} and Fe^{+2} were not (Costa, 1987).

Clearly, monensin (and lasalocid) consumption affected the bioavailability, uptake, retention, distribution and excretion of many monvalent or divalent cations,as well as one related anion (P). Since the direction and extent of alteration were not predictable (Van Vleet, 1986), it was not clear whether these effects would be beneficial or harmful to the animal consuming an ionophore.

B. Monensin-Mineral Interactions and Disease.

The possibility that ionophores might affect mineral metabolism in a beneficial manner was intriguing, for it might have practical applications. Perhaps ionophore-mediated uptake of some metal ion, particularly a trace element, would affect resistance to deficiency or toxicity states (Kirk et al., 1985). This concept has been most explored in ruminant Mg^{+2} homeostasis.

In 1978, Martens et al. reported that Mg^{+2} absorption across isolated rumen epithelium in sheep was directed against an electrical gradient and was dependent on the transmural potential difference. Active transport of Mg^{+2} was thought to utilize the Na^{+}-K^{+}-ATPase pump, because it was blocked by ouabain, a known inhibiter of the Na^{+}-K^{+}-ATPase pump (Martens et al., 1978). Meanwhile, Green et al. (1983a, 1983b) were studying the effect of dietary K^{+} concentrations on development of hypomagnesemic ("grass") tetany in beef cattle. They reported that increased dietary K^{+} resulted in a linear decrease in Mg^{+2} absorption and a linear increase in Mg^{+2} excretion (Greene et al., 1983a, 1983b).

Working with Greene, Kirk et al. (1985b) soon reported that monensin increased the apparent retention of Mg^{+2} in lambs, as Starnes et al. (1984) had shown in cattle. Smith and Rozengurtz (1978) had already

demonstrated that monensin stimulated the Na^+-K^+-ATPase pump in cultured fibroblasts, so Kirk et al. (1985b) proposed that monensin facilitated Mg^{+2} absorption by driving the Na^+-K^+-ATPase pump. Further, they suggested that monensin might be of practical use in the prevention of hypomagnesemia.

Greene et al. (1986) pursued this idea by investigating the effects of monensin and various concentrations of ruminal K^+ on absorption of Mg^{+2} in lambs. They observed no significant interaction effect between monensin and K^+ on mineral balance. However, the addition of monensin increased apparent absorption and retention of Mg^{+2} and decreased fecal Mg^{+2} excretion. Increasing concentrations of K^+ decreased apparent absorption of Mg^{+2} and increased fecal Mg^{+2} excretion. They concluded that monensin might prove useful for preventing the decreased Mg^{+2} absorption seen in ruminants consuming diets high in K^+, but did not go so far as to suggest that monensin might prevent grass tetany (Greene et al., 1986).

Other workers studying the influence of ionophore feeding on mineral metabolism anticipated advantageous effects, forcasting the use of ionophores for prevention of trace element deficiencies (Costa et al., 1984). Kirk et al. (1985b) had even suggested that monensin might prevent Zn^{+2} deficiency in ruminants, although this condition was uncommonly encountered. The likelihood of precipitating a toxic state of some trace element was not given much consideration, and ionophore-induced trace element deficiencies seemed even less likely.

III. Ionophore-Selenium Interactions.

A. Monensin-Selenium Interaction.

1. Overview.

Both monensin toxicosis and vitamin E-Se deficiency diseases produce severe degenerative myopathy of skeletal and cardiac muscles in a wide variety of species. The clinical signs, clinicopathogic findings, and gross, microscopic and ultrastructural lesions are strikingly similar. It is tempting to entertain the possibility of an antagonistic relationship between monensin and selenium, especially since other divalent cations are directly or indirectly affected by monensin.

2. Monensin and Nutritional Myopathy.

Two reported cases of nutritional myopathy (NMD) may have involved monensin toxicosis. In the first, Maas et al. (1984) described NMD in a flock of lambs in which affected animals were 45-60 days old and had been determined to have adequate blood Se status 30 days prior to onset of disease. They had been consuming a creep mixture containing 43.8 g monensin/ton since ten days of age. A diagnosis of vitamin E deficiency was made based on inadequate plasma concentrations and no more lambs were affected after supplementation of vitamin E. There is some chance that the disease described was related to the monensin feeding.

In the second case, Smith et al. (1985) reported "enzootic NMD" in a group of yearling bulls fed an experimental, high-energy ration consisting of high-moisture corn and corn silage (shown to be deficient in vitamin E and Se by assay) without supplemental vitamin E or Se. The sentinel case involved a bull that developed diarrhea, lethargy, dyspnea, myoglobinuria, teeth grinding, and reluctance to bear weight on the hind

limbs. It was treated with oral fluids and an injection of vitamin E/Se. Although recumbent for six days the bull recovered over 17 days, during which time serum AST values declined from 6,909 to 238 IU/L (normal = 39-72 IU/L) and serum CPK values from 114,444 to 260 IU/L (normal = 40-200 IU/L). Muscle biopsy indicated acute degeneration with repair.

In this group of bulls, serum vitamin E concentrations were very low, serum GSH-Px activities were inadequate, and serum CPK and AST values were moderately elevated (Smith et al., 1985). At slaughter, evidence of acute myodegenerative disease with subsequent repair was found in skeletal muscle; heart was not examined. In contrast, animals from an adjacent pen, fed haylage (with adequate vitamin E and Se content), corn silage and 70 mg monensin/kg diet, had low serum GSH-Px activity, but serum vitamin E concentrations were six times higher than in the other group. At slaughter these animals had no remarkable lesions.

These authors (Smith et al., 1985) stated that the spontaneous nature of the disease and clinical signs of diarrhea and anorexia were misleading for an outbreak of NMD. However, considering the clinical signs in the sentinel animal, the return of serum leakage enzymes to baseline values, and the postmortem findings of acute myodegeneration with repair in animals not treated with vitamin E/Se, it seems possible that accidental monensin exposure occurred. Assays of feed or tissues for monensin were not performed.

Inadequate vitamin E and selenium status was implicated in another report of monensin toxicosis. Hosie and Rollo (1985) described NMD associated with monensin toxicosis in twelve 3-5 month old bulls fed straw ad libitum and barley top-dressed in a trough with a balancer containing

300 mg monensin/kg. Competition for bunk space allowed larger bulls to preferentially select barley over the balancer. On day one, the smallest bull was found recumbent, kicking and opisthotonic, and it was euthanatized. Five other animals were depressed, dyspneic and tachycardic. Signs were more pronounced in one smaller bull from which a blood sample was taken. The balancer was withdrawn from the trough. On day three, blood samples were taken from the five affected animals, and they were treated with vitamin E/Se injection.

Gross and histologic examination of tissues from the sentinal animal confirmed severe, acute degeneration of skeletal muscle, kidney and liver tissue. Concentrations of vitamin E and Se were less than 50% of expected values. The most affected animal had a greatly increased blood CPK value on day one (9,580 IU/L) which was lower on day three (4,920 IU/L). This bull had very low serum vitamin E concentration and mildly depressed blood GSH-Px activity. The four other bulls had adequate serum AST, CPK and GSH-Px values, but depressed serum vitamin E levels (< 50% of normal). These animals reportedly recovered over ten days, but the most affected bull was found dead four weeks later; no necropsy was performed. The four remaining bulls had adequate serum vitamin E and blood GSH-Px values at ten weeks.

Because the lesions in one animal were of skeletal, but not cardiac, myodegeneration, and because the myopathy apparently responded to vitamin E/Se injection, Hosie and Rollo (1985) concluded that monensin had induced nutritional myopathy. This may have been an invalid speculation, based on the assumption of cause and effect. Clinical recovery may have been as much due to limited ionophore insult and subsequent monensin withdrawal

as to treatment with vitamin E and Se. Although serum vitamin E concentrations of the four affected bulls that survived were inadequate, blood GSH-Px values were within adequate limits. Vitamin E and GSH-Px values were not determined on any of six unaffected pen-mates, but it is implied that they were protected by adequate antioxidant status. It is perhaps more likely that vitamin E, Se or GSH-Px values in the bulls that died were depressed for the same reason that monensin consumption was increased: limited access to appropriate feed. In that sense, this was an incidence of nutritional myopathy associated with monensin toxicosis.

Very few other reports of monensin toxicosis simultaneously evaluated the antioxidant status of affected animals. Raisbeck and Miller (1981) considered NMD as a differential diagnosis for calves with monensin toxicosis, but blood Se values were within normal limits. Nation et al. (1982) diagnosed monensin toxicosis in a flock of lambs, but blood and liver Se concentrations were within expected ranges. Another case of monensin toxicosis in lambs with adequate blood GSH-Px activity was reported (Bourque et al., 1986). Thus, monensin toxicosis is apparently not limited to animals with marginal vitamin E/Se status.

B. Monensin Toxicosis and Vitamin E/Selenium.

1. Similarities Between Monensin Toxicosis and VESD.

Van Vleet and co-workers at Purdue University investigated vitamin E/Se-deficiency disease in rabbits (Van Vleet et al., 1968), chicks (Van Vleet and Ferrans, 1976) and pigs (Van Vleet et al., 1970, 1973, 1975, 1976; Ruth and Van Vleet, 1974). They have studied experimental monensin toxicosis in cattle (Van Vleet et al., 1983c, 1985; Van Vleet and Ferrans, 1983) and pigs (Van Vleet et al., 1983a, 1983b; Van Vleet and Ferrans,

1984a, 1984b). Both acute monensin toxicosis and VESD in pigs were characterized by severe degenerative myopathy of cardiac and skeletal muscle (Van Vleet et al., 1983a). Disruption of mitochondria, sarcoplasmic reticulum and plasma membranes preceded hypercontraction and myofibrillar lysis in skeletal muscle (Van Vleet et al., 1976; Van Vleet and Ferrans, 1984b) or cardiac muscle (Van Vleet and Ferrans, 1984a, 1984b) of swine in both diseases. Mitochondrial disruption and swelling was due to fluid influx (Mollenhauer et al., 1981; Anderson et al., 1984).

In swine, monensin toxicosis had a predeliction for left atrial tissue (Van Vleet and Ferrans, 1984a). Primarily type I (aerobic) fibers of skeletal muscle were affected in both disease syndromes (Ruth and Van Vleet, 1974; Van Vleet et al., 1976, 1983b; Van Vleet and Ferrans, 1984b). Type I (red, fast) fibers contain more mitochondria and have a higher lipid content than type II (white, slow) fibers (Ruth and Van Vleet, 1974) and may be more susceptible to peroxidation due to their aerobic nature (Van Vleet and Ferrans, 1984b).

Consequently, Van Vleet and Ferrans (1984a) theorized that three mechanisms might be involved in monensin toxicosis: first, was a direct Ca^{+2} overload of myocytes, as Shlafer and Kane (1980) had proposed; second, was the possibility of catecholamine toxicity as had been implied (Saini et al., 1979; Fahim and Pressman, 1981; Kabell et al., 1981); and third, was the possibility of peroxidative damage, although monensin was not known to induce peroxidation. This latter idea was of interest because of the obvious relationship to vitamin E/Se status and because it could be tested.

2. Vitamin E/Se Pretreatment and Monensin Toxicosis.

In 1983, Van Vleet et al. (1983a, 1983b) examined the effect of vitamin E/Se pretreatment on development of monensin toxicosis in swine. One day before 12 pigs weighing 20 kg were treated orally with 50 mg monensin/kg BW, six of them were injected intramuscularly with a combined vitamin E/selenium preparation (.25 mg selenium as Na_2SeO_3; 17 IU α-tocopherol acetate/kg BW). Vitamin E and Se status prior to and after treatment was not recorded. Pretreated pigs remained relatively healthy in appearance, but the control group developed transient anorexia, lethargy, diarrhea and a stiff gait; three died within 24 hours. Mean serum AST and CPK activities were greatly increased in both groups one day after monensin treatment but less so for pigs receiving vitamin E/Se (1,100 and 25,000 IU/L, respectively) than for pigs not pretreated (9,000 and 245,000 IU/L, respectively). The CPK zymogram was of skeletal muscle origin and skeletal, rather than cardiac, myopathy was more prevalent postmortem. The frequency and severity of myonecrosis were greater in pigs not pretreated and lesions were absent in three of six pigs receiving vitamin E/Se. It was concluded that the partial protection afforded by vitamin E/Se may indicate the involvement of peroxidation-induced damage in monensin toxicosis of swine (Van Vleet et al., 1983a). It should be noted that the monensin dose (50 mg/kg BW) was nearly three times the reported LD_{50} for pigs (16.8 mg/kg BW) (Anon., 1980).

A similar trial was subsequently performed in cattle (Van Vleet et al., 1985). At 72 and 24 hours before oral administration of 50 mg monensin/kg BW, five of ten calves weighing 180 kg were injected with a combined vitamin E/Se product (.25 mg selenium; 17 IU α-tocopherol/kg BW).

Compared to pretreated calves, those not receiving vitamin E/Se had lower mean serum selenium (.034 vs .338 mg/L) and α-tocopherol concentrations (.270 vs .798 mg/dL). Control animals showed signs of toxicosis by 24 hours, and all calves died by day three. Pretreated calves were not clinically ill until 48 hours, and mortality was slightly delayed, with four calves dying by day four and one dying on day ten. No difference in frequency or severity of postmortem lesions occurred and myonecrosis was more pronounced in cardiac than skeletal muscle. Cattle were considered to be more susceptible to monensin toxicosis than weanling pigs (Van Vleet et al., 1985). The monensin dose used in this study was twice that reported as the LD_{50} for cattle (22 mg/kg BW) (Anon., 1978).

C. Effect of Monensin on Selenium Balance.

Ionophore consumption was known to alter mineral metabolism, either directly or indirectly. For example, monensin had been shown to enhance ruminant Mg^{+2} absorption (Starnes et al., 1984; Greene et al., 1986) and Kirk et al. (1985b) had speculated that monensin feeding might prevent nutritional diseases resulting from mineral (Mg^{+2}, Zn^{+2}) deficiencies. On the other hand, the relationship between monensin toxicosis and nutritional myopathy seemed to imply that the ionophore adversely affected Se metabolism. It was possible that monensin precipitated nutritional disease (VESD/NMD) by interfering with trace element (Se^{+2}) metabolism.

Perhaps the most interesting development in monensin-Se interaction research was a serendipitous one. In 1983, Anderson et al. were studying the effect of monensin treatment and selenium status on coccidiosis in sheep. Four groups of pregnant ewes were fed a Se-deficient diet and allotted to a 2x2 factorial design using 0 or 10 mg/monensin/hd/day with

or without two oral treatments of Se at about six and four weeks prior to lambing. Blood GSH-Px activity was measured in ewes at parturition and in lambs at birth and 4, 8 and 12 weeks of age.

Quite unexpectedly, they found that ewes treated with monensin, but not Se, had significantly higher mean blood GSH-Px activity than those receiving no Se or Se alone; ewes treated with both Se and monensin had an even greater, apparently additive, increase in blood GSH-Px values. Furthermore, these differences carried over to lambs born to ewes of the respective groups and were maintained until 12 weeks of age. Blood GSH-Px activity increased and plateaued between four and eight weeks of age in all groups, except the controls, in which it decreased. At eight and 12 weeks of age, blood Se concentrations in lambs maintained the treatment effects and were well correlated with blood GSH-Px activity ($r = .94$). Consequently, the increase in blood GSH-Px activity was believed to be due to a true increase in available Se (Anderson et al., 1983).

Soon after, Costa et al., (1985) confirmed that ionophores affected Se balance. They showed that dietary monensin (and narasin) enhanced whole-body retention of ^{75}Se given orally to steers. Because the ionophores had no influence on whole-body retention of intravenous ^{75}Se, it was concluded that ionophores increased absorption of dietary Se. These workers echoed the sentiments of others (Starnes et al., 1984; Kirk et al., 1985b) when they suggested that ionophores might act as useful modifiers of trace element metabolism (Costa et al., 1984, 1985).

Further research did not substantiate this claim. Costa et al. (1987) used a ruminal monensin delivery device (100 mg monensin/day) in heifers at pasture to demonstrate that, although serum Se concentrations

were low in all animals (<20 ng Se/ml), monensin-treated heifers had consistently higher whole blood and plasma Se values. Blood GSH-Px activity was not increased by monensin treatment, and blood Se values did not increase enough to make Se supplementation unnecessary.

In contrast to Costa et al. (1984, 1985), Wheeler (1984) expressed concern that many swine rations were already supplemented at Se concentrations above those recommended and that monensin-enhanced Se absorption might upset the delicate balance between adequate and toxic concentrations of this trace element in pigs.

Jensen (1986) alleviated concerns of Se toxicity, at least in poultry. He showed that, in broiler chicks fed Se-toxic diets and 120 mg monensin/kg diet, an interaction between monensin and Se reduced hepatic Se concentrations in chicks fed the highest supplemental Se (5 mg Se/kg diet). However, there were no interactions between monensin or Se and the plasma or liver Se content of chicks fed Se-deficient diets. It was also suggested that ruminant and monogastric animals differed with respect to monensin influence on Se balance (Jensen, 1986).

It is evident from the limited number and conflicting results of reports on ionophore-mineral interactions that much remains to be learned. Ionophoretic manipulation of trace element nutrition may well prove to be a two-edged sword.

IV. Selenium-Mineral Interactions.

Ionophore-mediated alterations in mineral metabolism may occur indirectly. Selenium is known to have interrelationships with other trace elements, as have been reviewed by Hill (1975). In rats, Se toxicity (growth depression) has been alleviated by tungsten (W), germanium (Ge), and arsenic (As), but not fluorine (F), molybdenum (Mo), zinc (Zn), cobalt (Co), nickel (Ni), gallium (Ga), or uranium (U). The As-Se interaction occurred in chicks, dogs, cows and pigs (Hill, 1975). There were also "sparing" interactions of Se against sulfate (SO_4^-), mercury (Hg), cadmium (Cd), or copper (Cu) toxicity, so as to render the most toxic of the interaction pair less harmful. Initially, these interactions were believed to be due to the formation of less noxious compounds or altered metabolism (Hill, 1975), but the discovery of the role of Se in GSH-Px suggested a protective effect of Se against membrane peroxidation (Van Vleet et al., 1977).

Some of the manifestations of mineral toxicity are oxidative or peroxidative in nature. Iron (Fe) injection toxicosis in vitamin E-/Se-deficient pigs is possibly due to membrane lipid peroxidation of Type I skeletal muscle fibers (Cook et al., 1982). Cobalt (Co) toxicity in swine caused cardiomyopathy, with elevations in serum CPK and AST values, myocardial pallor and hypercontraction bands, mitochondrial swelling and membrane disruption, and myofibrillar lysis (Van Vleet et al., 1977). Pretreatment with vitamin E/Se prevented not only clinical signs of Co toxicity but the increase in serum leakage enzymes and gross and histopathologic lesions as well (Van Vleet et al., 1977). Later, Van Vleet et al. (1981) induced VESD lesions in weanling pigs fed silver (Ag),

Co, tellurium (Te), Zn, Cd, and vanadium (V). The extent of lesions evident at necropsy ranged from mild, subclinical (Co, Te, Zn, Cd or V) to severe, fatal cardiac and skeletal muscle necrosis (Ag). Serum GSH-Px values were markedly decreased over time by Te, and hepatic Se concentrations were increased by Ag (Van Vleet et al., 1981).

Thus, it would seem that mineral interactions with Se may have direct or indirect effects on the development of peroxidative-like disease in swine. Ionophoretic alterations in homeostasis of other minerals may likewise affect Se balance.

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EXPERIMENTAL PROCEDURES AND PROTOCOL

I. Experiment 1.

A. Materials and Methods.

Twenty-four pigs (13 males, 11 females) were selected from eight litters of crossbred pigs born within a 10-day period at the Michigan State University Swine Research Center (MSU-SRC). Sows had been housed in a common gestation barn and fed a corn-soybean meal based gestation diet containing 0.1 mg supplemental Se/kg. All sows farrowed in crates in the same farrowing house and were fed a lactation diet containing 0.1 mg supplemental Se/kg. The lactation diet served as creep feed for the piglets.

At weaning, pigs were separated into four groups so as to have similar numbers of males and females, no more than 2 pigs from any one litter, and similar mean body weights (range 9.9 to 10.3 kg). Pigs were housed in four adjacent pens in a common nursery barn and fed ad libitum a standard starter diet (Appendix A) without supplemental Se.

After a 7-day acclimation period, a 42-day feeding trial was begun (from 46 to 88 days of age). Groups were fed ad libitum the basal corn-soybean diet alone (control;C) or containing 0.3 mg supplemental Se/kg (C+Se), 150 mg monensin/kg (Rumensin, Eli Lilly Company, Greenfield, IN) (C+M) or both (C+M+Se). On days 0, 14, 28, and 42 of Experiment 1, pigs were weighed and blood samples were collected from the anterior vena cava

in evacuated blood collection tubes (Vacutainer, Becton Dickinson, Rutherford, NJ), which were refrigerated and allowed to clot. After centrifugation, serum was pipetted off, divided into three 1-2 ml aliquots, and stored frozen at -20°C in graduated polystyrene vials (Walter Sarstedt, Inc., Princeton, NJ). Serum was then assayed to determine Se content, glutathione peroxidase (GSH-Px), aspartate amino transferase (AST) and creatine phosphokinase (CPK) activities, and serum vitamin E and multielement (Ca, Cu, Fe, K, Mg, Na, P, Zn) values.

All samples collected on a given day were handled, stored and processed identically to minimize variation between groups. Serum Se concentrations were determined by the fluorometric procedure of Whetter and Ulrey (1978). Serum GSH-Px activity (EU = uM GSH-Px oxidized/min/ml serum) was determined after storage for 8-12 months by an indirect spectrophotometric procedure (Paglia and Valentine, 1967) which measured the enzymatically coupled oxidation of NADPH. Vitamin E assays were made by a high performance liquid chromatography method (Bieri et al., 1979) as modified by Stowe and Miller (1985). The method of Karmen et al. (1955), as modified by Rodgerson et al. (1974), was used to determine serum AST activity; serum CPK activity was determined by Rosalki's method (1967). An inductively-coupled argon plasma emission spectroscopy procedure (Braselton et al., 1981; Stowe et al., 1985) was used for multielement (Ca, Cu, Fe, K, Mg, Na, P, Zn) analysis.

B. Statistical Analysis.

Data were subjected to a computer-generated two-way analysis of variance (ANOVA) procedure for a split-plot, 4x2 factorial design corrected for repeated measurements by using the general linear models

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(GLM) method of Barr, Goodnight, Sall and Helwig (Statistical Analysis Systems [SAS] Institute, Carey, NC). Fisher's variance ratio test (F test) was used to determine significant differences between the main effects of treatment group and gender. No significant main effects or interactions were observed for gender so this factor was subsequently omitted from the ANOVA procedure.

Conditional comparisons of treatment means within periods were then made according to a Bonferroni t-test procedure described by Gill (1986) for split-plot designs with repeated measurements. If split-plot structure had been ignored, analysis would have been performed as if each observation were on a different subject and random error would not have been separated into variation among and variation within subjects. The result would be an exaggeration of the apparent significance of difference among treatment means and reduced sensitivity of tests for trends or interaction effects (Gill and Hafs, 1971; Gill, 1978).

The correlation (CORR) procedure of the SAS program was used to perform Pearson's correlation analysis on measured variables and determine their correlation coefficients. Regression analysis of serum GSH-Px activity on serum Se concentration was calculated using the HP-15049 Math/Statistics Module curve-fitting program designed for use with the HP-41CX hand-held microcomputer (Hewlett-Packard, Portable Computer Division, Corvallis, OR). Plots of mean values for selected variables over time were then constructed using the Sigmaplot Software Package graphics program (Jandel Corporation, Sausalito, CA) with an IBM-PC XT computer (International Business Machines Corporation, Boca Raton, FL) and HP-7470A graphics plotter (Hewlett-Packard, Corvallis, OR). All values are expressed as mean $\pm$ standard deviation (SD).

II. Experiment 2.

A. Materials and Methods.

Twenty-four 8-week-old, Yorkshire/Landrace crossbred pigs were selected from litters of second-generation (F2), genetically hypo- and hyperselenemic pigs (hypo-Se and hyper-Se, respectively) (Stowe and Miller, 1985) born within a 5-day period at the MSU-SRC. On the basis of mean serum Se concentrations at 10 and 30 days of age, twelve of the most hypo-Se (6 males, 6 females) pigs were selected from a total of 30 pigs from three litters. The mean ($\pm$ standard deviation [SD]) of group 10- and 30-day serum Se concentrations for the hypo-Se pigs was 76.4 $\pm$ 3.0 ng Se/ml (range 71.0-80.5 ng Se/ml). Similarly, twelve of the most hyper-Se pigs (6 males, 6 females) were selected from 22 pigs from two litters. The mean ($\pm$ SD) of group 10- and 30-day serum Se concentrations for the hyper-Se pigs was 106.3 $\pm$ 10.3 ng Se/ml (range 97.5-125.5 ng Se/ml).

Four pigs (2 males, 2 females) from both the hypo-Se and hyper-Se groups were assigned to each of three groups of eight pigs. They were housed in three adjacent group pens in a nursery barn for an acclimation period of 14 days, where they were fed ad libitum a standard starter diet (Appendix A) with only 0.1 mg supplemental Se/kg. On day 0 of Experiment 2 (76 days of age), pigs were moved to the grower portion of the same barn and fed ad libitum a grower ration (Appendix A) containing 0.1 mg supplemental Se/kg and either 0, 200, or 400 mg monensin/kg. A similarly-treated finishing ration was fed from days 35-77, followed by a withdrawal diet, without monensin, from days 77-105 of Experiment 2.

On days 0, 7, 28, 56, 70 and 98 on experiment, all pigs were weighed and blood (20 ml) was collected from the anterior vena cava for processing

as described for Experiment 1. Pigs were slaughtered on day 105 (181 days of age), and samples of liver and kidney tissue from each pig were collected and frozen for determination of tissue Se and multielement analysis, according to the methods of Whetter and Ulrey (1978) and Stowe and Miller (1985), respectively.

B. Statistical Analysis.

Data were subjected to ANOVA for a 2x3x2 factorial, split-plot design with repeated measurements by the same methods as for Experiment 1. The main effects of genetic Se status, monensin treatment level and gender, as well as their interaction effects, were examined for significance. Gender had no effect on any variable (except body weight) and so was omitted from the ANOVA procedure, leaving a 2x3 factorial design. Values for day 98 were not included in this statistical analysis because treatment ended at 77 days. Likewise, data from one pig withdrawn from each of the monensin treatment groups in this trial for reasons unrelated to the experiment were not included.

Loss of two pigs left unequal numbers of observations between control (n = 8) and monensin-treated (n = 7) groups and necessitated modification of the Bonferroni t-test procedure used to make conditional comparisons of treatment means (J. L. Gill, Personal Communication). The formula for variance of the contrast (Eq. 6 in Gill, 1986) was now:

$$V_{(\bar{q}k)} = (\sum_{i=1}^{t} c^2)(\hat{\sigma}^2[1/n_1 - 1/n_2])$$

and the formula for approximate number of degrees of freedom (Eq. 8 in Gill, 1986) became:

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$$\hat{\nu} = p^2(\sigma^2)^2/([MS_{E_{(1)}}/(t-1)] + [(p-1)MS_{E_{(2)}}{}^2/(n_1-1)(n_2-1)]).$$

Mean kidney and liver tissue values were subjected to an ANOVA procedure to test for significance of monensin treatment and genetic Se status, followed by a Tukey's Studentized range test on mean values using the SAS program. All values are expressed as mean $\pm$ standard deviation (SD).

III. Experiment 3.

A. Materials and Methods.

Eleven weight-matched, weanling pigs were randomly selected from two litters and acclimated over 9 days to individual stainless steel metabolism cages. They were fed twice daily a gruel mixture containing finely ground feed (Appendix A) and water at 1.75 and 3.50% of body weight, respectively, in separate individual holding pens. Five control and six treated pigs were fed either 0 or 300 mg monensin/kg and 0.1 mg supplemental Se/kg initially. Monensin level was reduced to 150 mg/kg after 6 days of acclimation due to palatability problems.

A 6-day metabolism cage balance trial was then performed (Stowe and Miller, 1986; Stowe, 1986). Blood samples were collected on days -9, 0 and 6 of Experiment 3 (31 to 46 days of age) for assay as in Experiment 1. Average daily Se intake was calculated and all urine and feces collected from day 0 to 6 for determination of individual percent urinary, fecal and total Se excretion and percent Se retention (Stowe, 1986). Additionally, creatinine and Se concentrations were determined on 6-day individual pooled samples of serum and urine to allow calculation of renal creatinine:Se clearance ratios (Traver et al., 1977; Coffman, 1980; Neiger and Hagemoser, 1985).

B. Statistical Analysis.

Analysis of variance for the main effect of treatment group on all serial samples was performed as in Experiment 1. Gender effect was not considered. A Tukey's Studentized range test was used to compare group means of the Se metabolism parameters. All values are expressed as mean ± standard deviation (SD).

RESULTS

I. Experiment 1.

All pigs continued to eat well, and no clinical abnormalities were observed during this trial. Gender had no effect on any variable but females tended to have higher GSH-Px activities than males ($p<.10$). Body weight was not affected by gender (Table 2) or treatment (Table 3).

On day 0 of Experiment 1 (46 days of age) mean serum Se concentrations were not different between groups (Table 3) but were much lower than the expected values of 100-160 ng Se/ml for pigs of this age (Appendix B). Despite Se supplementation mean serum Se concentrations remained at inadequate or marginally adequate levels throughout the study. Pigs not supplemented with Se had inadequate serum Se concentrations at all times (Table 3).

There was a marked effect of treatment on serum Se concentrations ($p<.0001$) since supplemental Se increased serum Se values over time ($p<.001$) (Figure 4 and Table 3). Monensin treatment had no effect on serum Se concentrations (Figure 4).

All GSH-Px values were very low on day 14, even when re-assayed, suggesting that samples were handled improperly at some point. However, treatment affected serum GSH-Px activity ($p<.005$) since serum GSH-Px activity increased over time in pigs receiving supplemental Se, regardless of monensin treatment ($p<.0001$) (Figure 5 and Table 3). Monensin

treatment did not affect serum GSH-Px activity. Correlation between serum Se concentrations and serum GSH-Px activity over all days was fair ($r = .57$, $p<.0001$) (Figure 6) but increased sustantially if day 14 values were excluded ($r = .83$, $p<.0001$) (Figure 7).

Mean serum vitamin E concentrations were not different among groups at any time (Table 3). Although mean serum vitamin E values increased over time ($p<.0001$), they were considered to be inadequate for pigs of this age (Appendix B) on all days except day 28 (Table 3).

Mean serum CPK (Figure 8) and AST (Figure 9) activities were not affected by either Se or monensin treatment and were highly variable. Correlation between serum CPK and AST values was poor ($r = .27$, $p<.01$). Likewise, there was poor correlation between serum GSH-Px and CPK or AST activities over all days ($r = -.00$, $p<.98$ and $r = -.07$, $p<.49$, respectively) and when day 14 values were omitted ($r = .30$, $p<.01$ and $r = .02$, $p<.90$, respectively).

All other mean serum mineral values (Table 4) were within the expected ranges for pigs of this age (Appendix C) and no obvious trends over time were observed. Treatment had no effect on serum Ca, Cu, Fe, K, Mg, Na or Zn values. There was a moderate difference in serum phosphorus values between groups ($p<.05$), due primarily to low initial and final P concentrations in the group receiving monensin and Se.

Error terms for the variables in Experiment 1 are summarized in Appendix D.

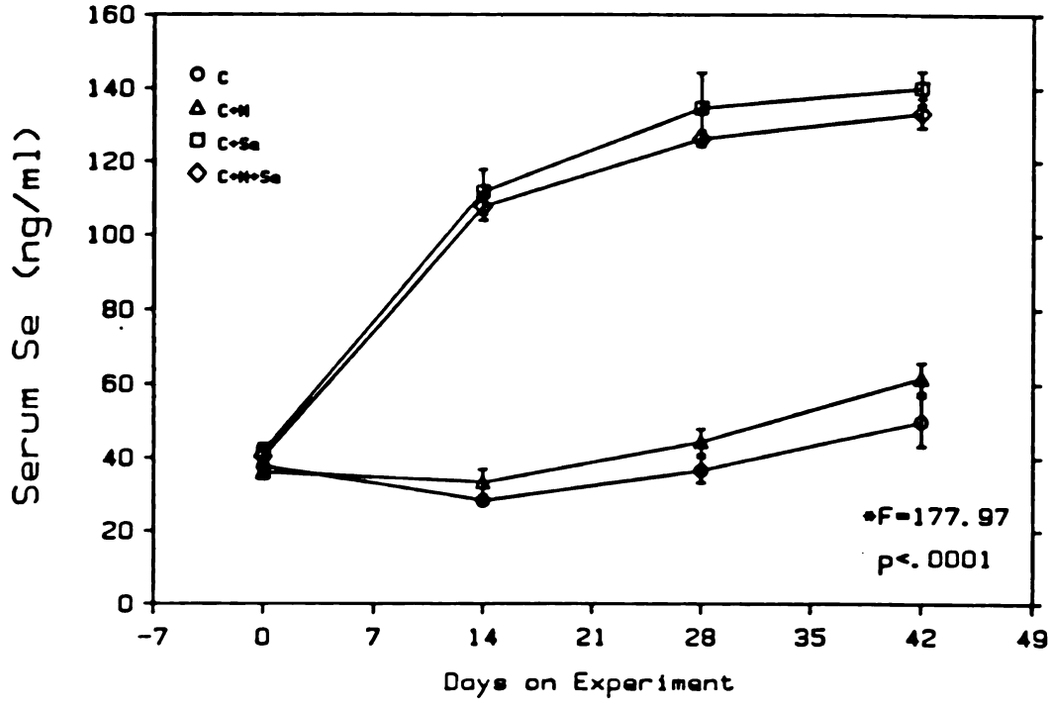

Figure 4. Effect of treatment on serum Se concentrations in Experiment 1.

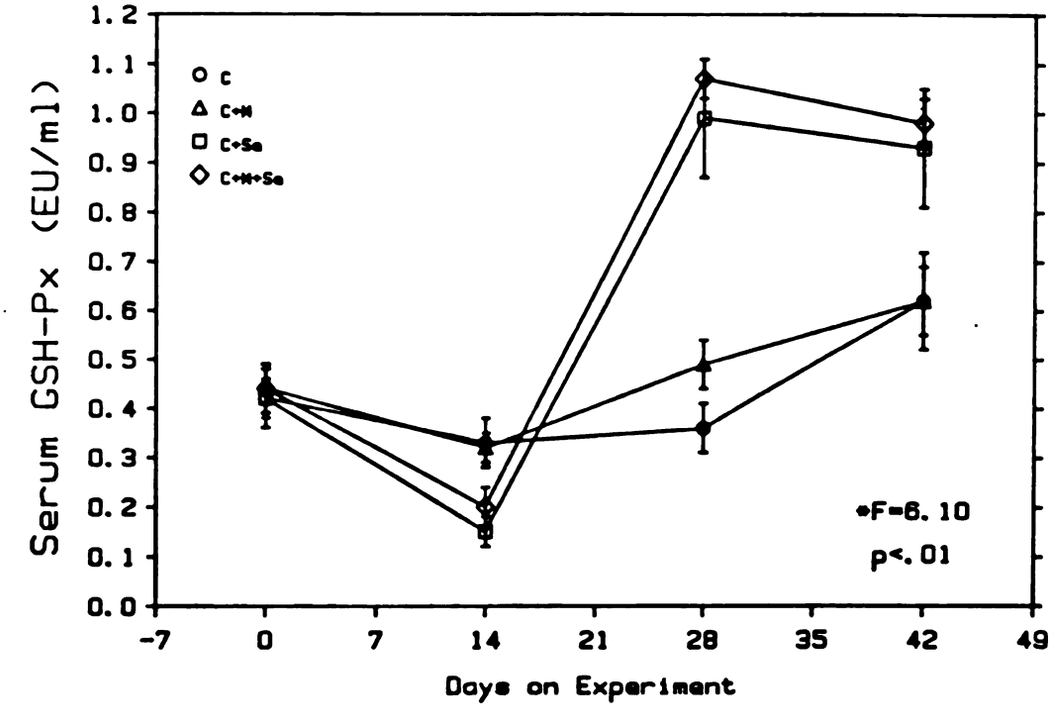

Figure 5. Effect of treatment on serum GSH-Px activity in Experiment 1.

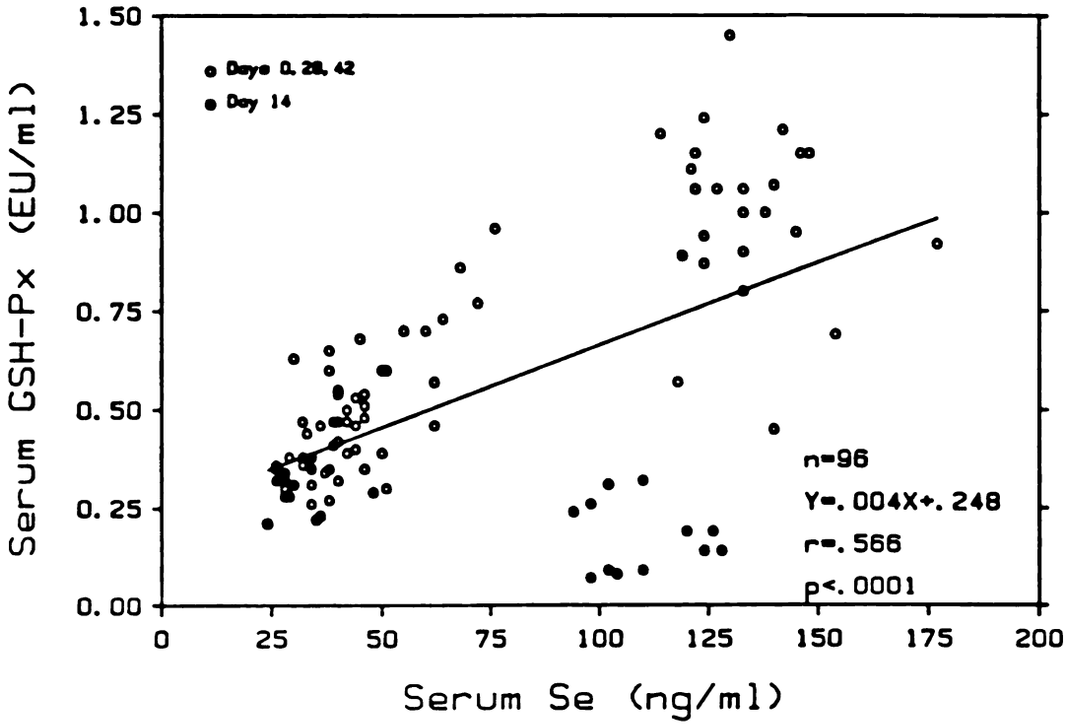

Figure 6. Regression analysis of serum GSH-Px activity on serum Se concentration in Experiment 1, including values from all days.

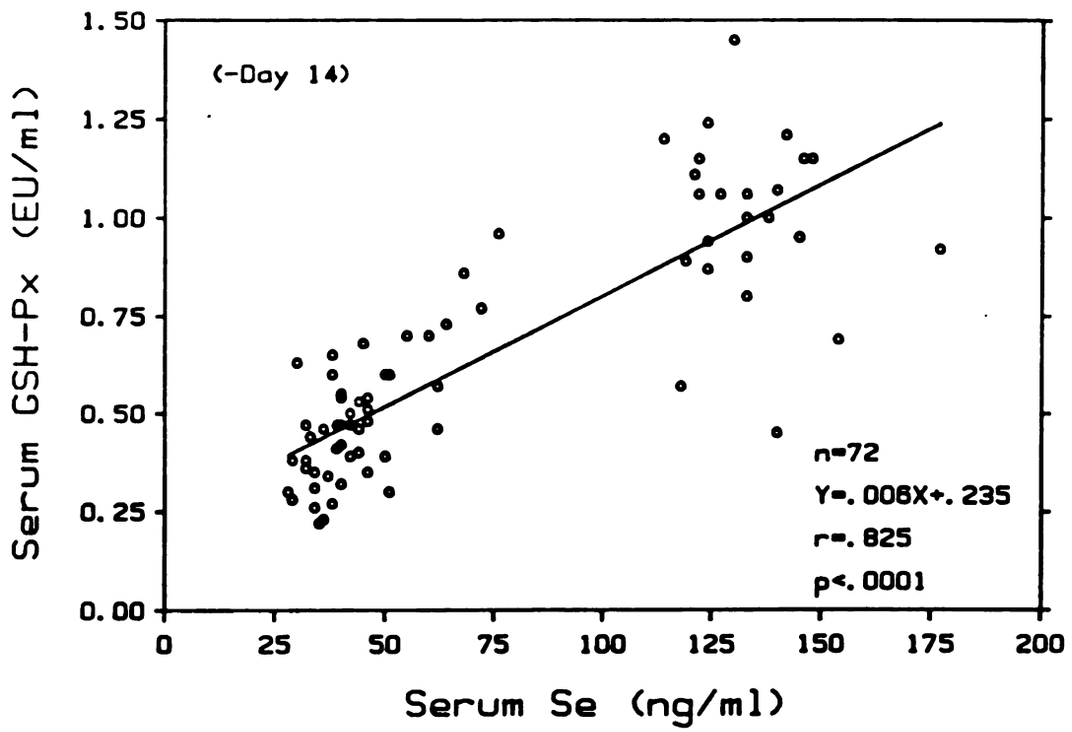

Figure 7. Regression analysis of serum GSH-Px activity on serum Se concentration in Experiment 1, excluding values from day 14.

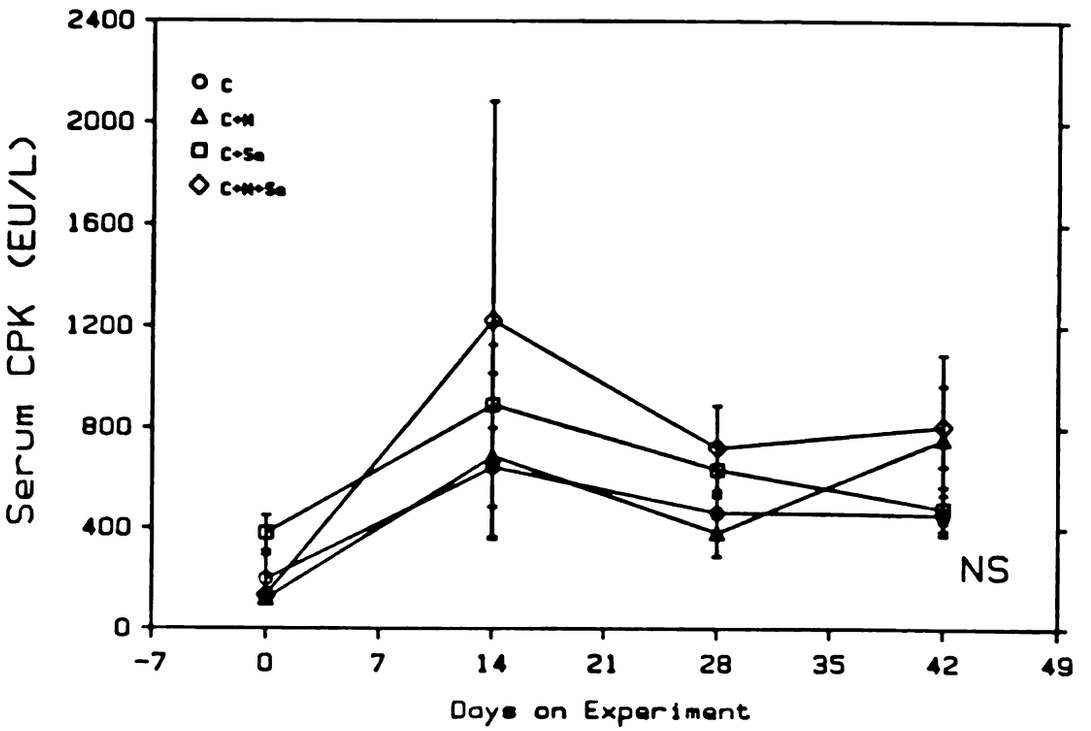

Figure 8. Effect of treatment on serum CPK activity in Experiment 1.

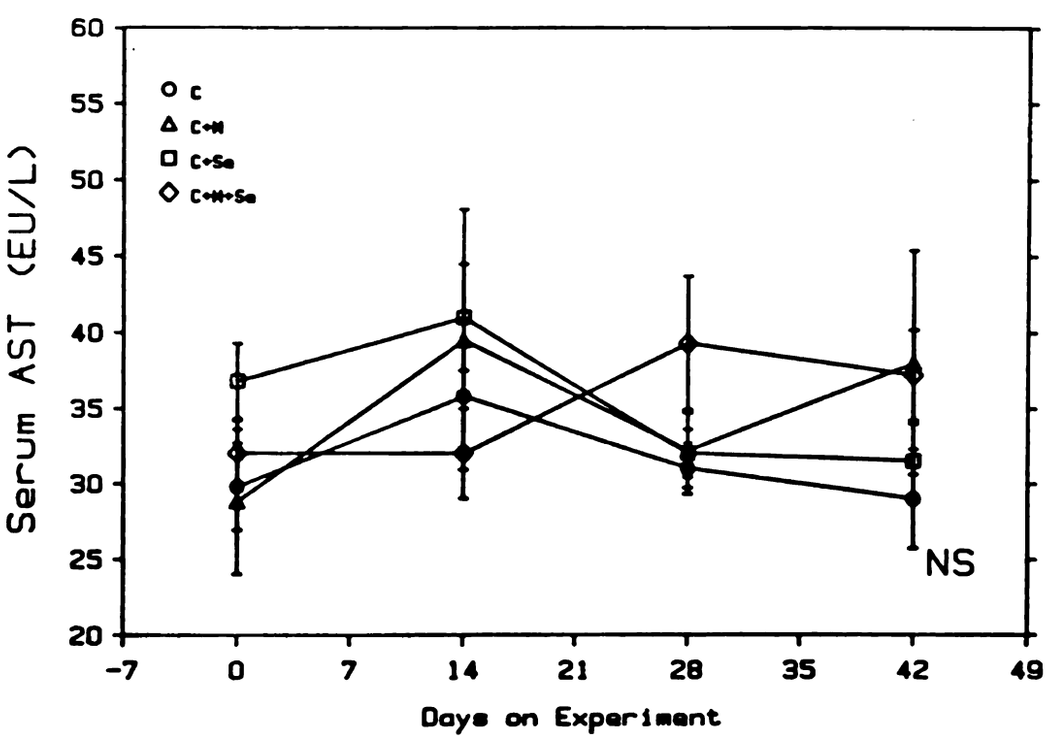

Figure 9. Effect of treatment on serum AST activity in Experiment 1.

Table 2. Effect of gender on body weight and serum Se, GSH-Px, CPK, and AST concentrations in Experiment 1.

Variable	Day	Gender: Male	Gender: Female	F Value	PR>F
Body Weight (kg)	0[+]	14.0 ± 2.6[#]	13.4 ± 2.9	.35	.5617
	14	24.8 ± 4.3	24.1 ± 4.5		
	28	31.4 ± 4.4	31.0 ± 5.1		
	42	42.8 ± 5.2	41.1 ± 6.3		
Serum Se (ng/ml)	0	39.4 ± 3.7[*]	38.4 ± 6.9[*]	.69	.4180
	14	70.3 ± 44.0[*]	70.2 ± 40.2[*]		
	28	79.2 ± 46.3[*]	93.0 ± 50.7[*]		
	42	96.0 ± 45.0[*]	96.6 ± 43.1[*]		
Serum GSH-Px (EU/ml)	0	.39 ± .10	.48 ± .12	3.64	.0746
	14	.24 ± .12	.27 ± .08		
	28	.65 ± .33	.84 ± .36		
	42	.77 ± .21	.82 ± .34		
Serum CPK (EU/ml)	0	178 ± 151	240 ± 205	.40	.5353
	14	794 ± 675	939 ± 1536		
	28	544 ± 251	554 ± 363		
	42	530 ± 343	731 ± 593		
Serum AST (EU/ml)	0	30.5 ± 9.7	33.5 ± 5.5	2.33	.1462
	14	39.9 ± 10.8	42.0 ± 12.7		
	28	32.6 ± 7.1	34.8 ± 7.6		
	42	32.5 ± 8.3	35.6 ± 13.7		

[#]Mean ± SD.

[+]Pigs were 46 days of age on day 0.

[*]Mean values were below the expected range for pigs of this age (see Appendix B).

Table 3. Effect of treatment on body weight and serum Se, GSH-Px, CPK, AST, and vitamin E concentrations in Experiment 1.

Variable	Day	Treatment Group: C	C + MO	C + Se	C + Se + MO	F Value	PR>F
Body Weight (kg)	0[+]	13.3 ± 2.9[#]	14.4 ± 2.9	14.5 ± 3.0	12.5 ± 2.2	.21	.8911
	14	24.6 ± 5.2	25.4 ± 4.0	24.6 ± 4.7	23.5 ± 4.3		
	28	29.7 ± 5.9	31.7 ± 4.3	31.7 ± 5.2	31.8 ± 3.7		
	42	40.4 ± 6.7	43.1 ± 5.0	43.0 ± 7.0	41.5 ± 4.8		
Serum Se (ng/ml)	0	37.7 ± 5.3[*]	36.0 ± 4.6[*]	41.7 ± 5.3[*]	40.3 ± 5.4[*]	177.97	.0001
	14	28.3 ± 2.9[a*]	33.3 ± 8.8[a*]	111.7 ± 14.8[b]	107.7 ± 9.3[b]		
	28	36.7 ± 8.6[a*]	44.5 ± 8.5[a*]	134.7 ± 23.4[b*]	126.3 ± 5.2[b*]		
	42	50.0 ± 16.6[a*]	61.7 ± 9.7[a*]	140.2 ± 11.0[b]	133.3 ± 10.1[b]		
Serum GSH-Px (Eu/ml)	0	.42 ± .16	.44 ± .11	.42 ± .10	.44 ± .13	6.10	.0041
	14	.33 ± .03	.32 ± .06	.15 ± .07	.20 ± .11		
	28	.36 ± .13[a]	.49 ± .12[a]	.99 ± .30[b]	1.07 ± .11[b]		
	42	.62 ± .16[a]	.62 ± .25[a]	.93 ± .30[b]	.98 ± .13[b]		

Table 3 (cont'd.).

Serum CPK (EU/L)	0	197 ± 236	118 ± 56	379 ± 174	131 ± 48	.98	.4213
	14	642 ± 387	685 ± 808	892 ± 580	1224 ± 2108		
	28	461 ± 185	381 ± 230	632 ± 265	720 ± 414		
	42	452 ± 197	754 ± 820	477 ± 216	806 ± 394		
Serum AST (EU/L)	0	29.8 ± 7.1	28.8 ± 11.7	36.8 ± 6.1	32.0 ± 5.3	.36	.7802
	14	35.8 ± 8.3	39.5 ± 21.1	41.0 ± 8.5	32.0 ± 7.5		
	28	31.0 ± 4.2	32.2 ± 6.1	32.0 ± 4.0	39.3 ± 10.8		
	42	29.0 ± 8.1	38.0 ± 18.0	31.5 ± 6.0	37.2 ± 7.4		
Serum Vit.E (ug/ml)	0	.16 ± .06*	.14 ± .04*	.16 ± .05*	.09 ± .07*	1.41	.2685
	14	.25 ± .13*	.58 ± .19*	.46 ± .30*	.51 ± .50*		
	28	.98 ± .28*	1.22 ± .50*	1.07 ± .19*	1.22 ± .45*		
	42	.78 ± .24*	.97 ± .30*	.68 ± .21*	.80 ± .25*		

#Mean ± SD.

+Pigs were 46 days of age on day 0.

a,bMeans within periods (days) without common superscript letters differ ($p<.01$).

*Mean values are below the expected range for pigs of this age (see Appendix B).

Table 4. Effect of treatment on serum mineral concentrations in Experiment 1.

Variable	Day	Treatment Group: C	C + MO	C + Se	C + Se + MO	F Value	PR>F
Serum Ca (ug/ml)	0	113 ± 6[#]	117 ± 7	119 ± 6	114 ± 9	2.05	.1385
	14	105 ± 4	111 ± 11	106 ± 15	103 ± 4		
	28	107 ± 5	109 ± 2	109 ± 7	106 ± 6		
	42	100 ± 4	109 ± 4	111 ± 7	107 ± 7		
Serum Cu (ug/ml)	0	1.68 ± .31	1.67 ± .28	1.77 ± .20	1.70 ± .30	1.19	.3373
	14	1.65 ± .12	2.13 ± .42	2.12 ± .49	1.95 ± .37		
	28	1.93 ± .12	2.12 ± .33	2.02 ± .18	2.00 ± .21		
	42	2.10 ± .17	2.27 ± .33	2.40 ± .33	2.40 ± .33		
Serum Fe (ug/ml)	0	2.40 ± .66	3.30 ± 1.21	3.95 ± 1.19	3.18 ± 1.09	2.00	.1466
	14	2.60 ± .56	2.03 ± .64	2.18 ± .76	1.90 ± .37		
	28	2.55 ± .93	1.90 ± .77	3.00 ± .87	2.95 ± .42		
	42	2.28 ± .59	3.12 ± 1.13	4.47 ± 3.75	3.22 ± 1.96		
Serum K (ug/ml)	0	218 ± 20	212 ± 38	228 ± 26	196 ± 23	.48	.6997
	14	214 ± 23	218 ± 19	196 ± 29	217 ± 24		
	28	182 ± 21	172 ± 33	190 ± 14	164 ± 12		
	42	227 ± 19	261 ± 43	234 ± 33	237 ± 12		

Table 4 (cont'd.).

Serum Mg (ug/ml)	0	23.3 ± 2.9	25.0 ± 1.3	25.0 ± 2.4	24.2 ± 2.4	1.21	.3315
	14	23.5 ± 5.6	28.5 ± 1.6	25.0 ± 4.7	25.3 ± 2.9		
	28	31.0 ± 7.1	30.5 ± 2.7	31.2 ± 4.8	32.5 ± 4.4		
	42	19.7 ± 1.9	22.5 ± 2.2	23.5 ± 3.2	21.7 ± 2.5		
Serum Na (ug/ml)	0	3533 ± 82	3500 ± 63	3500 ± 63	3400 ± 110	1.94	.1557
	14	3350 ± 105	3467 ± 808	3467 ± 258	3350 ± 105		
	28	3033 ± 103	3117 ± 98	3067 ± 103	3117 ± 147		
	42	3200 ± 110	3317 ± 75	3333 ± 82	3235 ± 81		
Serum P (ug/ml)	0	162 ± 11	167 ± 16	169 ± 10	146 ± 12	3.64	.0304
	14	169 ± 11	172 ± 14	165 ± 21	159 ± 3		
	28	176 ± 22	170 ± 8	185 ± 17	178 ± 11		
	42	147 ± 7	150 ± 7	162 ± 10	135 ± 7		
Serum Zn (ug/ml)	0	1.10 ± .15	1.18 ± .21	1.28 ± .15	1.25 ± .31	1.90	.1620
	14	.92 ± .15	1.22 ± .15	1.15 ± .15	1.07 ± .12		
	28	1.15 ± .22	1.22 ± .15	1.25 ± .23	1.13 ± .08		
	42	1.10 ± .15	1.05 ± .10	1.23 ± .25	1.18 ± .20		

#Mean ± SD.

+Pigs were 46 days of age on day 0.

II. Experiment 2.

No signs of monensin toxicosis were observed in treated pigs during this study. One hypo-Se male consuming 400 mg monensin/kg died of respiratory arrest after a blood sample was obtained on day 28. Necropsy examination revealed no significant cardiac or skeletal muscle lesions, but an extensive suppurative bronchopneumonia was present.

Another hypo-Se male from the group consuming 200 mg monensin/kg developed a large umbilical hernia/abscess and was culled on day 42. Gross postmortem examination was unremarkable. Upon histologic examination of the heart, myocardial fibers appeared swollen, fragmented, and granular with vacuolization, loss of cross-striation and mild mineralization. Moderate to severe nuclear rowing with many fragmented, pyknotic nuclei was present. These changes occurred in all chambers of the heart but were most pronounced in the right atrium. Sections of skeletal muscle had enlarged, hyalinized, eosinophilic fibers with signs of early mineralization.

Gender did not affect the measured variables, except that male pigs were heavier than females ($p<.05$) (Table 5). There were no interactions between the main effects of monensin treatment and genetic Se status for any variable. Body weight was not affected by monensin treatment (Figure 10 and Table 6) but hyper-Se pigs were heavier than hypo-Se pigs ($p<.05$) (Figure 11 and Table 7).

On days 0 and 7 of Experiment 2 (76 and 83 days of age, respectively) all groups had mean serum Se concentrations below the range of expected values (140-190 ng Se/ml) for pigs of this age (Appendix B). Mean serum Se concentrations reached adequate levels by day 28.

Pigs consuming monensin had serum Se values no different than controls (Figure 12) but genetic Se status affected serum Se values ($p<.001$) (Figure 13). Serum Se concentrations were greater in hyper-Se pigs than in hypo-Se pigs on days 28, 56 and 70 ($p<.05$). Serum Se values seemed to plateau at day 56 for both hypo-Se (156.7 $\pm$ 15.7 ng Se/ml) and hyper-Se (209.1 $\pm$ 21.7 ng Se/ml) pigs (Table 9).

Mean serum GSH-Px activity was affected by treatment ($p<.01$), with monensin-treated pigs tending to have greater values than controls on all days except day 0 ($p<.05$) (Figure 14). GSH-Px activity peaked at day 56 for all groups, but values in monensin-treated pigs fell almost to those of controls after monensin withdrawal (Table 6).

Genetic Se status affected serum GSH-Px activity ($p<.001$), with greater mean values for hyper-Se pigs on days 28, 56 and 70 ($p<.05$) (Figure 15). Peak GSH-Px activity occurred on day 56 for both hypo-Se (1.35 $\pm$ .39 EU/ml) and hyper-Se (2.04 $\pm$ .32 EU/ml) groups, although values in hypo-Se pigs appeared to plateau at 28 days (Table 7). Serum Se concentrations and serum GSH-Px activity were well correlated ($r = .77$, $p<.0001$) (Figure 16).

Compared to the expected ranges for animals of a given age (Appendix B), mean serum vitamin E concentrations for all groups of pigs were inadequate on almost all days, although they tended to increase with time ($p<.10$) (Tables 6 and 7).

Serum CPK values were quite variable and were not affected by monensin treatment, although mean serum CPK activity tended to increase over time for all groups ($p<.05$) (Figure 17 and Table 6). Genetically hypo-Se pigs had consistently higher serum CPK values than hyper-Se pigs

on all days except 0 ($p<.01$) with the difference greatest on day 70 ($p<.05$) (Figure 18 and Table 7).

Monensin treatment approached significance for serum AST activity ($p<.10$) because mean values for the 400 mg/kg group were greater than those of either the 200 mg/kg or control groups on all days except 0 and 98 (Figure 20 and Table 6). Genetic Se status had no effect on serum AST concentrations (Figure 21 and Table 7). There was poor correlation between CPK and AST values ($r = -.05$, $p<.59$) and between GSH-Px and CPK ($r = -.07$, $p<.43$) or AST activities ($r = .29$, $p<.001$).

Main effects of monensin treatment (Table 8) and genetic Se status (Table 9) were not significant for Ca, Fe, Mg, Na, P or Zn values. However, monensin tended to increase serum Fe concentrations ($p>.05$); overall mean values were 2.81, 3.47, and 3.99 ug Fe/ml for pigs consuming 0, 200, and 400 mg monensin/kg diet, respectively.

Serum Cu concentrations were not affected by monensin treatment (Figure 21 and Table 8) but were affected by genetic Se status ($p<.01$) (Figure 22 and Table 9). Hyper-Se pigs had higher mean Cu values than hypo-Se pigs on all days and were significantly higher on days 0 and 56 ($p<.05$). Overall mean values for serum Cu in hypo-Se and hyper-Se pigs were 1.77 and 1.99 ug Cu/ml, respectively.

Serum K concentrations were affected by monensin treatment and were lower on all days in groups consuming monensin ($p<.0001$), although only significant on day 7 ($p<.05$) (Figure 23 and Table 8). The overall mean serum K values were 250, 213 and 198 ug K/ml in groups consuming 0, 200 and 400 mg monensin/kg, respectively. Genetic Se status also affected serum K values ($p<.005$), since hypo-Se pigs (overall mean = 235 ug K/ml)

had higher concentrations than hyper-Se pigs (overall mean = 211 ug K/ml) on all days but were significantly higher only on day 7 ($p<.05$) (Figure 24 and Table 9).

Monensin treatment did not affect liver or kidney tissue Se content but hyper-Se pigs had greater kidney Se concentrations than hypo-Se pigs ($p<.05$) (Table 10). On average, kidney tissue Se content was about 5.5 times that of liver tissue, but correlation between kidney and liver tissue Se concentrations was poor ($r = .35$).

Mean liver tissue mineral values were within expected ranges (Appendix C) with the exception of Na values, which were lower, and P values, which were higher, than expected (Tables 10 and 11). All mean kidney tissue mineral values (Tables 10 and 11) were within expected ranges (Appendix C).

Monensin treatment at 200, but not 400 mg/kg, increased liver tissue concentrations of Mg and Na and decreased liver P content compared to control pigs ($p<.05$). No differences occurred in kidney mineral concentrations as a result of monensin treatment (Table 10). Genetic Se status affected liver Cu and Zn concentrations, with higher Cu values and lower Zn values in hypo-Se pigs ($p<.05$) (Table 11).

Error terms for the variables in Experiment 2 are summarized in Appendix E.

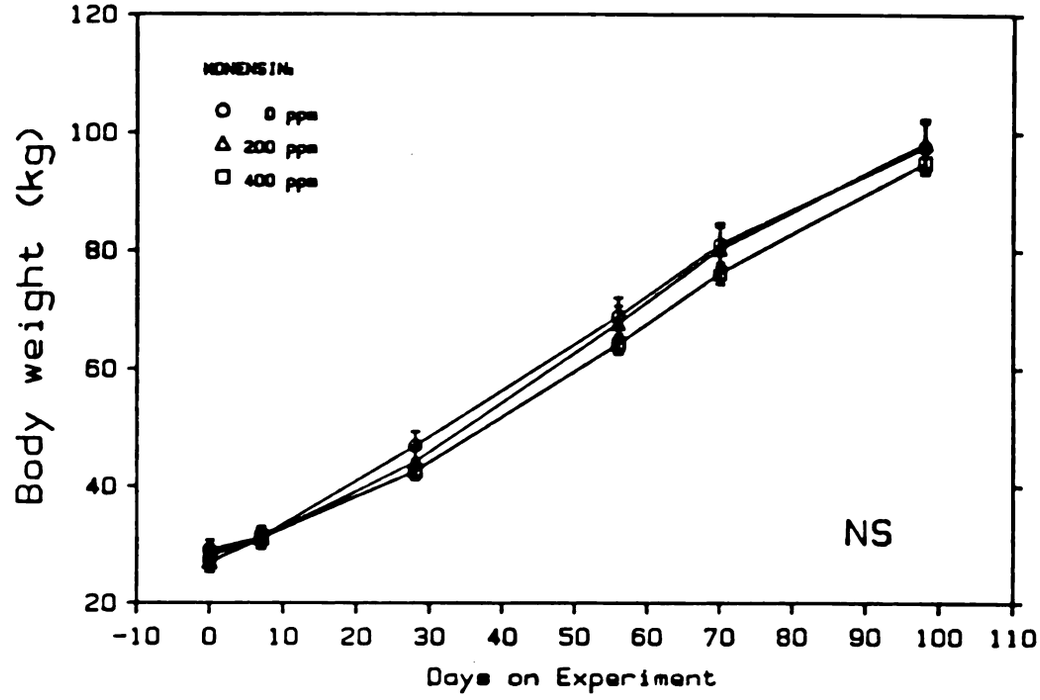

Figure 10. Effect of monensin on body weight in Experiment 2.

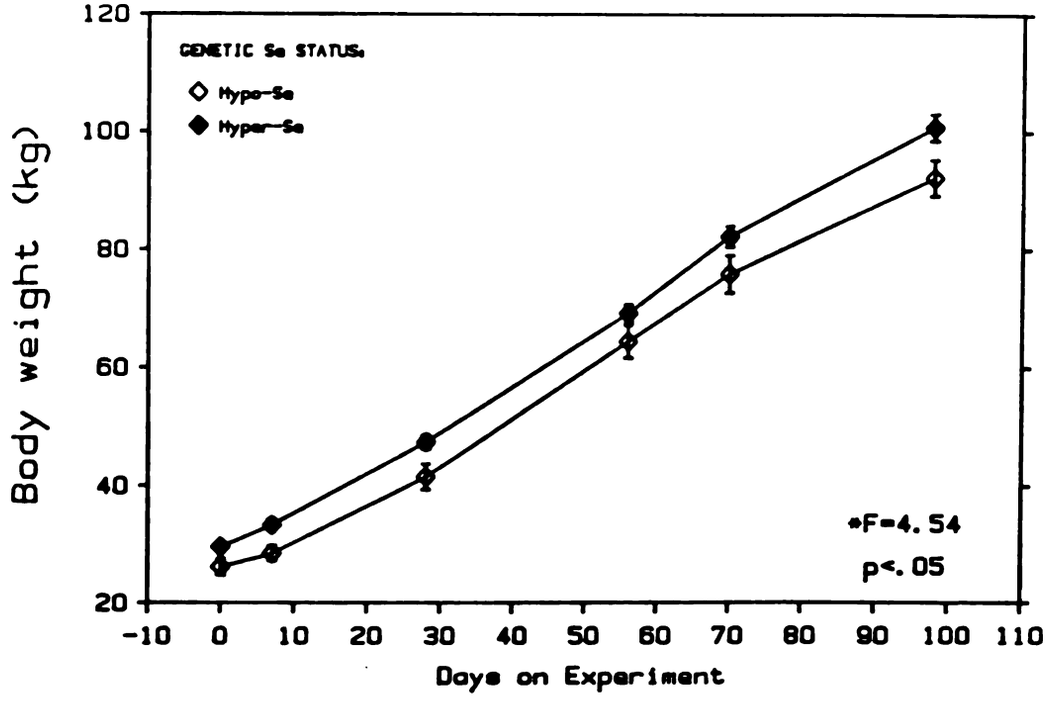

Figure 11. Effect of genetic Se status on body weight in Experiment 2.

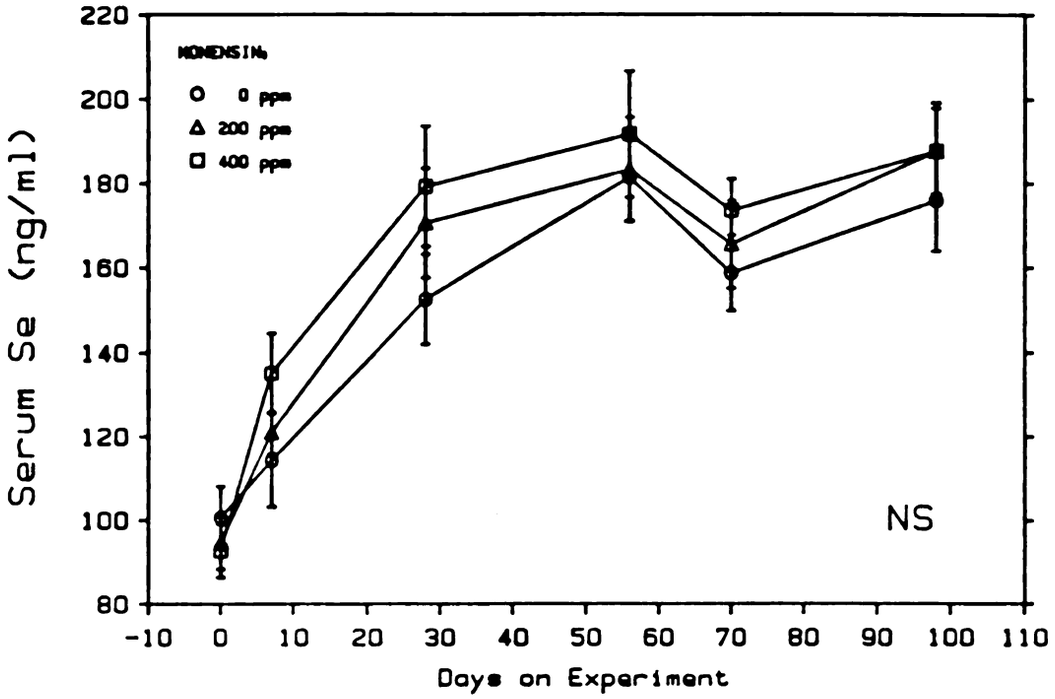

Figure 12. Effect of monensin on serum Se concentrations in Experiment 2.

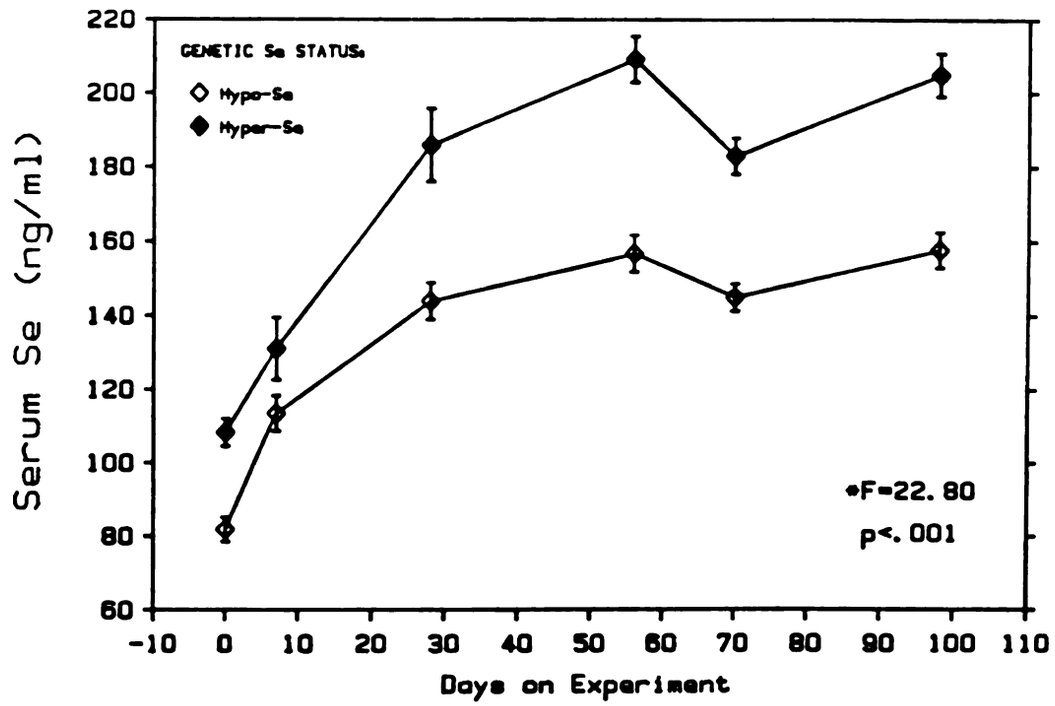

Figure 13. Effect of genetic Se status on serum Se concentrations in Experiment 2.

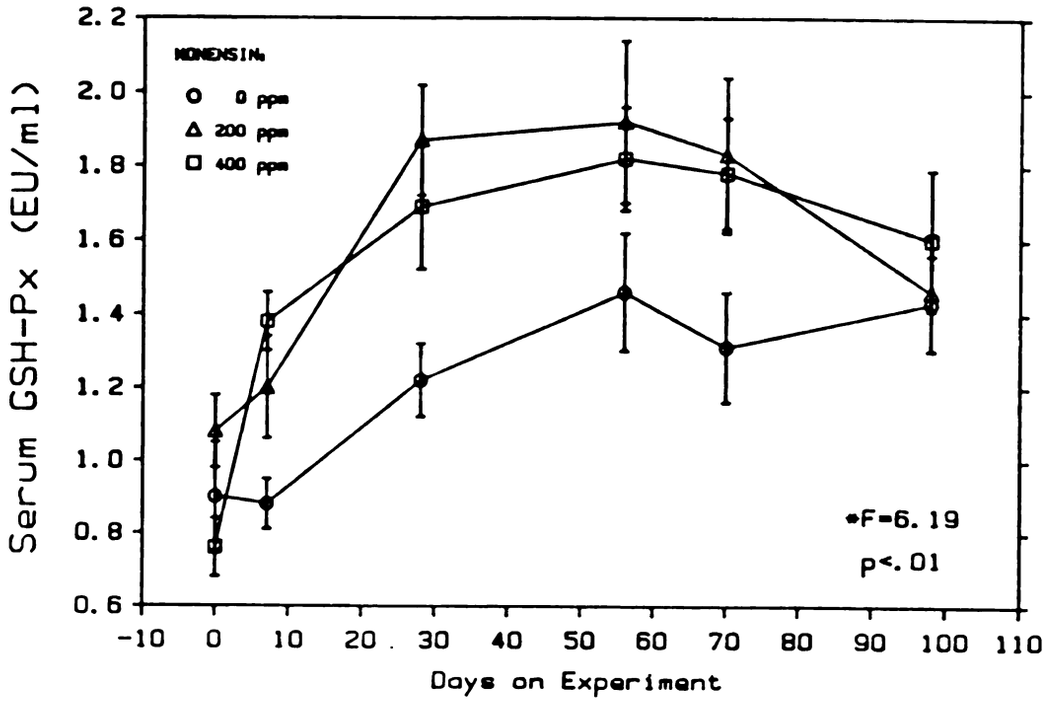

Figure 14. Effect of monensin on serum GSH-Px activity in Experiment 2.

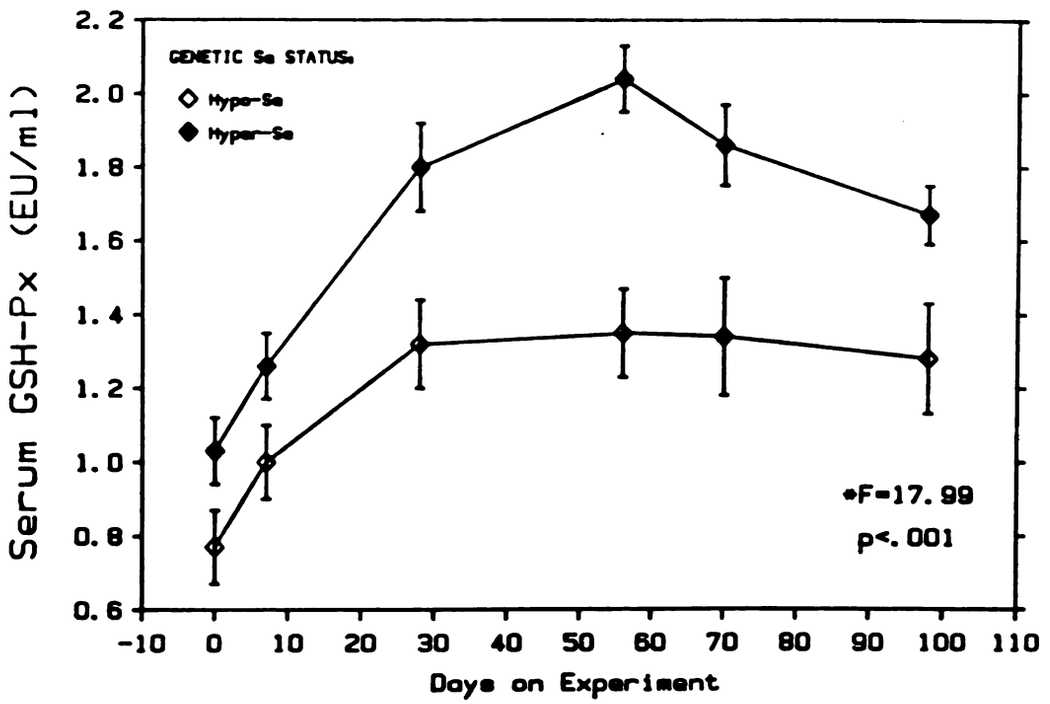

Figure 15. Effect of genetic Se status on serum GSH-Px activity in Experiment 2.

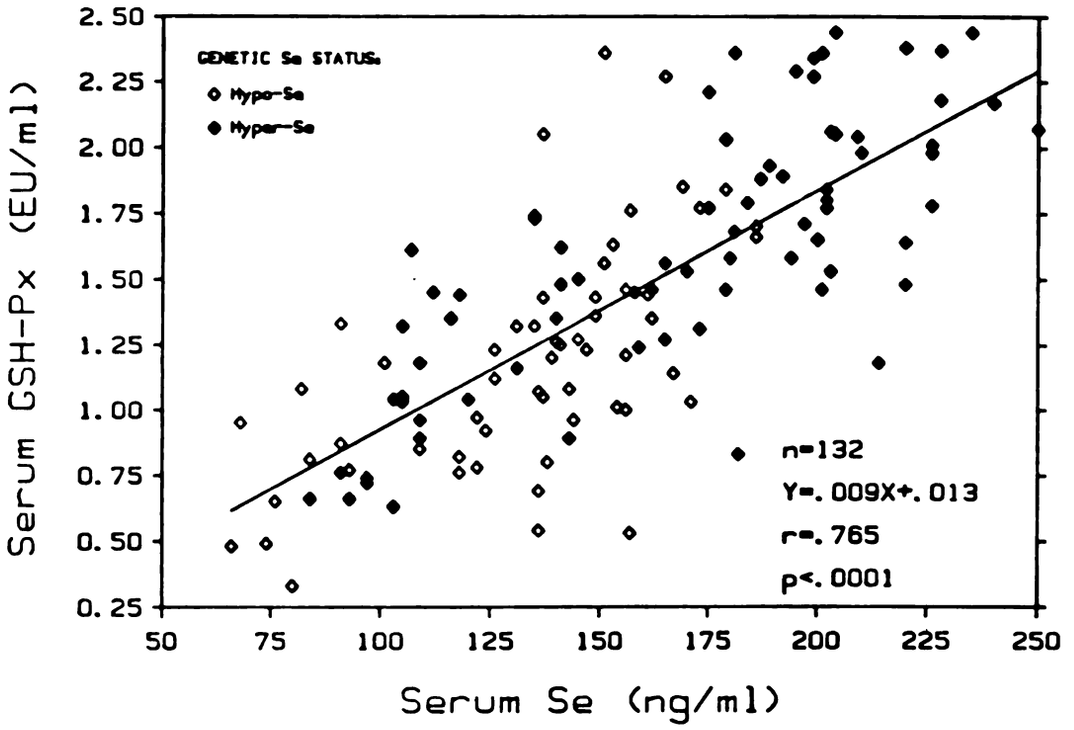

Figure 16. Regression analysis of serum GSH-Px activity on serum Se concentration in Experiment 2.

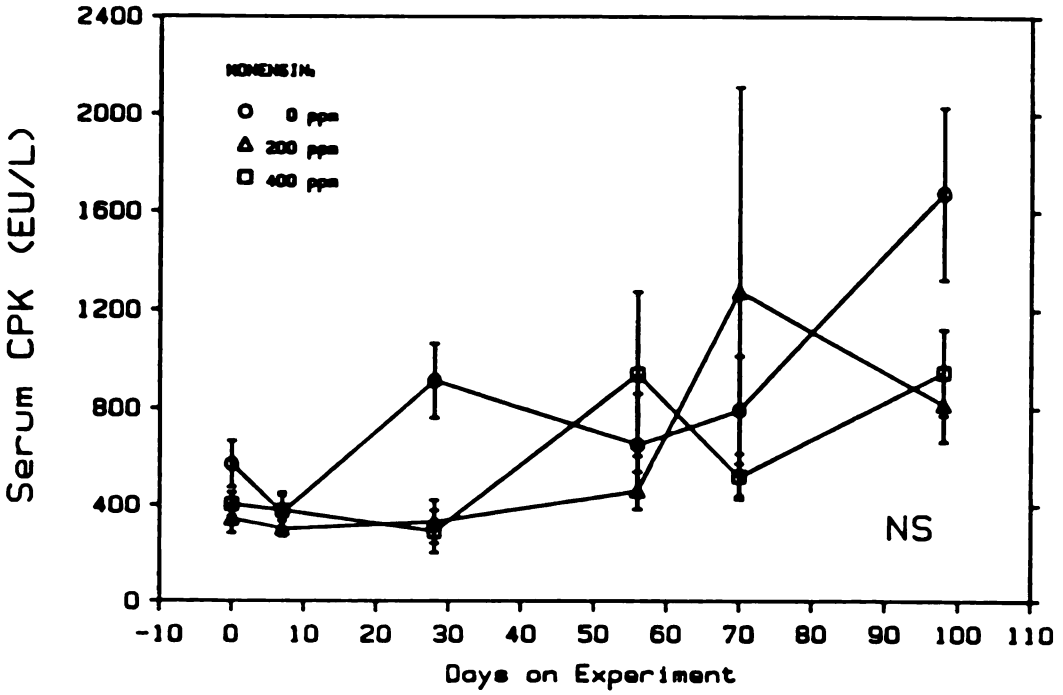

Figure 17. Effect of monensin on serum CPK activity in Experiment 2.

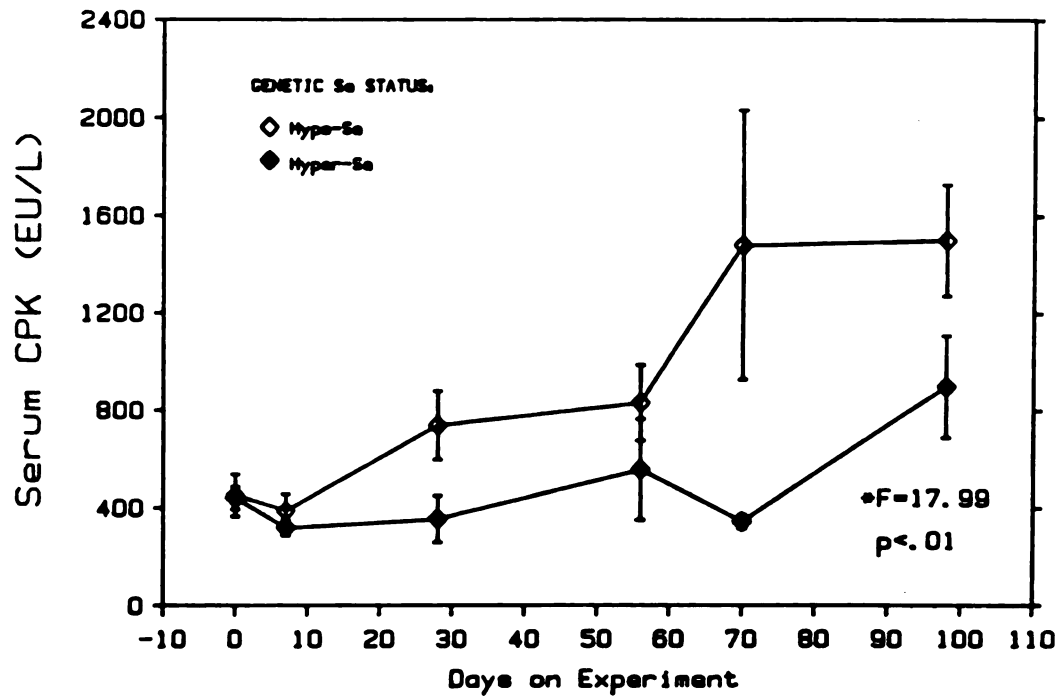

Figure 18. Effect of genetic Se status on serum CPK activity in Experiment 2.

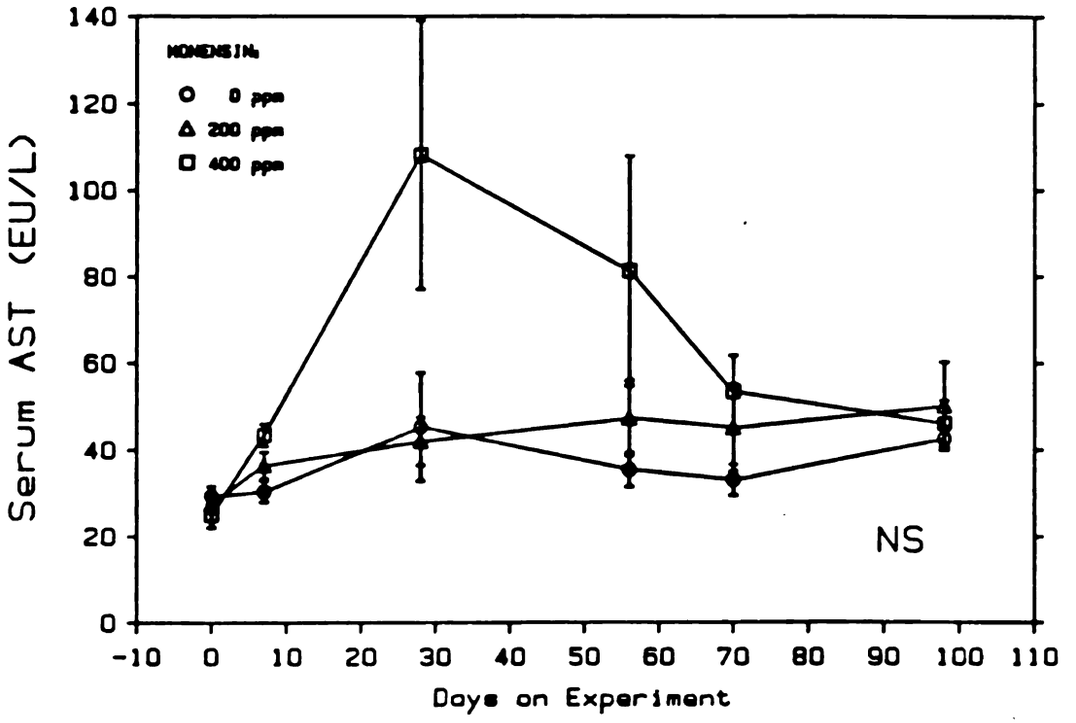

Figure 19. Effect of monensin on serum AST activity in Experiment 2.

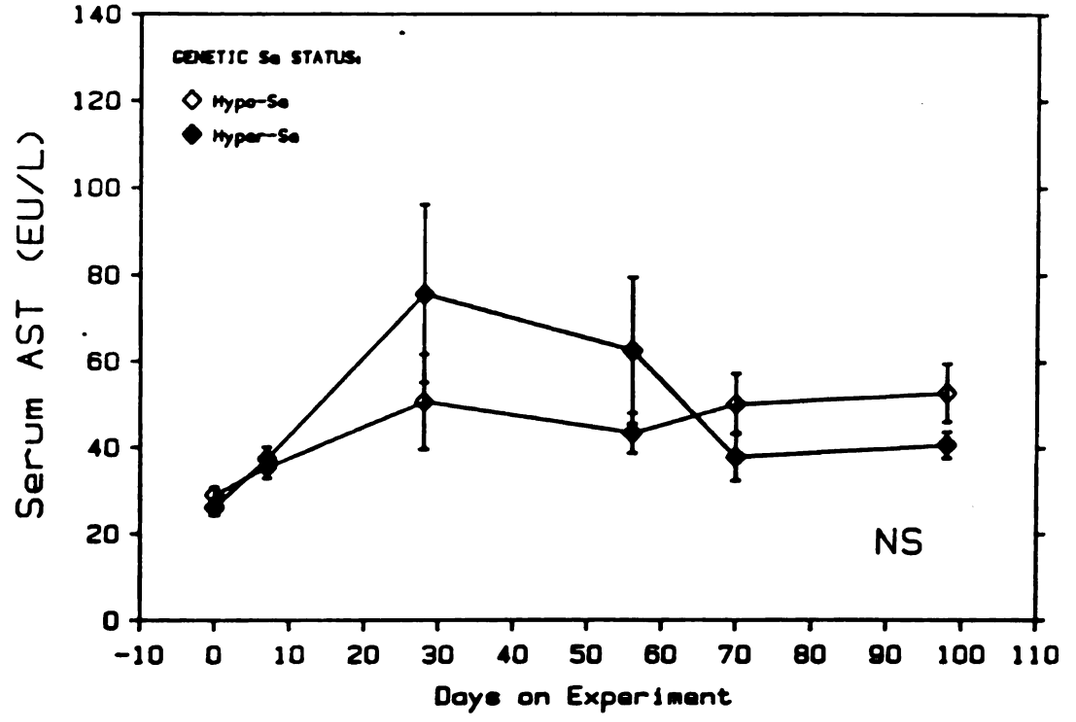

Figure 20. Effect of genetic Se status on serum AST activity in Experiment 2.

Table 5. Effect of gender on body weight and serum Se, GSH-Px, CPK, and AST concentrations in Experiment 2.

Variable	Day	Gender: Male	Gender: Female	F Value	PR>F
Body weight (kg)	0[+]	29.5 ± 3.1[#]	26.6 ± 4.3	5.10	.0433
	7	32.6 ± 3.3	29.8 ± 4.9		
	28	46.0 ± 5.4	42.4 ± 6.3		
	56	70.1 ± 6.1	64.2 ± 7.5		
	70	83.3 ± 6.7	75.6 ± 8.8		
	98	101.7 ± 6.2	92.6 ± 10.3		
Serum Se (ng/ml)	0	92.1 ± 16.7[*]	96.6 ± 20.2[*]	.88	.3667
	7	114.1 ± 24.3[*]	127.5 ± 25.6[*]		
	28	155.7 ± 31.9	171.8 ± 37.6		
	56	187.8 ± 31.9	183.2 ± 34.5		
	70	169.4 ± 21.3	162.5 ± 26.8		
	98	192.2 ± 28.6	176.2 ± 30.2		
Serum GSH-Px (EU/ml)	0	.93 ± .36	.87 ± .30	.10	.7614
	7	1.06 ± .39	1.14 ± .33		
	28	1.56 ± .52	1.52 ± .41		
	52	1.77 ± .53	1.69 ± .48		
	70	1.68 ± .44	1.58 ± .57		
	98	1.70 ± .24	1.32 ± .46		
Serum CPK (EU/ml)	0	473 ± 243	387 ± 176	.17	.6904
	7	304 ± 156	374 ± 174		
	28	526 ± 422	566 ± 471		
	56	975 ± 804	437 ± 289		
	70	687 ± 607	1003 ± 1674		
	98	1341 ± 855	1028 ± 704		
Serum AST (EU/ml)	0	27.3 ± 8.0	26.7 ± 6.0	.00	.9463
	7	34.6 ± 7.7	38.1 ± 9.8		
	28	64.3 ± 36.5	65.3 ± 72.5		
	56	50.2 ± 25.1	56.8 ± 57.3		
	70	41.8 ± 23.4	44.7 ± 20.4		
	98	47.9 ± 21.7	44.6 ± 13.2		

[#]Mean ± SD.

[+]Pigs were 76 days of age on day 0.

[*]Mean values are below the expected range for pigs of this age (see Appendix B).

Table 6. Effect of monensin on body weight and serum Se, GSH-Px, CPK, AST, and vitamin E concentrations in Experiment 2.

Variable	Day	Monensin (mg/kg diet) 0	200	400	F Value	PR>F
Body weight (kg)	0[+]	29.1 ± 4.6[#]	27.0 ± 4.4	28.1 ± 3.2	.63	.5463
	7	31.2 ± 5.4	31.0 ± 4.7	31.2 ± 3.5		
	28	46.9 ± 6.9	44.2 ± 7.1	42.6 ± 4.0		
	56	68.9 ± 8.9	67.7 ± 8.0	64.2 ± 4.4		
	70	81.1 ± 10.2	80.3 ± 10.0	76.2 ± 4.9		
	98	97.6 ± 12.3	98.2 ± 11.0	94.9 ± 4.9		
Serum Se (ng/ml)	0	100.6 ± 21.2[*]	94.6 ± 16.6[*]	92.7 ± 16.9[*]	.72	.5004
	7	114.3 ± 31.5[*]	120.9 ± 13.1[*]	135.1 ± 25.2[*]		
	28	152.6 ± 29.9	170.6 ± 34.4	179.3 ± 37.7		
	56	181.4 ± 29.3	183.3 ± 32.8	191.7 ± 39.6		
	70	158.8 ± 25.1	165.6 ± 27.5	173.6 ± 20.2		
	98	176.0 ± 34.3	187.7 ± 30.3	187.7 ± 27.0		
Serum GSH-Px (EU/ml)	0	.90 ± .43	1.08 ± .25	.76 ± .22	6.19	.0102
	7	.88 ± .21	1.20 ± .37	1.38 ± .22		
	28	1.22 ± .29	1.87 ± .39	1.69 ± .45		
	56	1.46 ± .45	1.92 ± .57	1.82 ± .37		
	70	1.31 ± .43	1.83 ± .56	1.78 ± .39		
	98	1.43 ± .35	1.46 ± .43	1.60 ± .50		

Table 6 (cont'd.).

Serum CPK (EU/L)	0	570 ± 272	341 ± 156	402 ± 133	.49	.6223
	7	368 ± 203	301 ± 86	376 ± 202		
	28	912 ± 430	331 ± 242	289 ± 235		
	56	650 ± 595	460 ± 210	939 ± 890		
	70	793 ± 626	1275 ± 2218	519 ± 250		
	98	1676 ± 1000	816 ± 407	947 ± 465		
Serum AST (EU/L)	0	29.3 ± 6.5	27.6 ± 5.3	25.0 ± .2	2.88	.0852
	7	30.4 ± 7.1	36.4 ± 8.3	43.4 ± 6.8		
	28	45.3 ± 35.5	42.0 ± 14.8	108.1 ± 82.1		
	56	35.4 ± 11.4	47.3 ± 22.9	81.3 ± 70.4		
	70	33.0 ± 10.0	45.1 ± 26.8	53.4 ± 22.1		
	98	42.6 ± 7.8	50.1 ± 26.9	46.0 ± 14.4		
Serum Vit. E (ug/ml)	0	.60 ± .21*	.67 ± .26*	.61 ± .13*	.36	.7008
	7	.39 ± .12*	.45 ± .08*	.46 ± .22		
	28	.57 ± .27	.79 ± .18*	.98 ± .26*		
	56	.90 ± .21*	.76 ± .28	.78 ± .41*		
	70	.86 ± .43*	.97 ± .34*	.70 ± .41*		
	98	.68 ± .22	.54 ± .34	.45 ± .24		

#Mean ± SD.

+Pigs were 76 days of age on day 0.

*Mean values are below the expected range for pigs of this age (see Appendix B).

Table 7. Effect of genetic Se status on body weight and serum Se, GSH-Px, CPK, AST, and vitamin E concentrations in Experiment 2.

Variable	Day	Genetic Se Status: Hypo Se	Genetic Se Status: Hyper Se	F Value	PR>F
Body wt. (kg)	0[+]	26.2 ± 4.3[#]	29.6 ± 3.2	4.54	.0489
	7	28.5 ± 4.2	33.3 ± 3.3		
	28	41.5 ± 6.9	47.4 ± 4.1		
	56	64.4 ± 8.9	69.2 ± 5.3		
	70	75.8 ± 10.1	82.2 ± 6.2		
	98	92.2 ± 9.9	100.8 ± 7.9		
Serum Se (ng/ml)	0	81.8 ± 10.9*	108.2 ± 13.2*	22.80	.0002
	7	113.4 ± 15.5*	131.0 ± 29.6*		
	28	143.9 ± 15.7[a]	185.9 ± 34.2[b]		
	56	156.7 ± 15.7[a]	209.1 ± 21.7[b]		
	70	144.9 ± 11.6[a]	182.9 ± 16.9[b]		
	98	157.7 ± 15.2	204.9 ± 20.4		
Serum GSH-Px (EU/ml)	0	.77 ± .30	1.03 ± .32	17.00	.0006
	7	1.00 ± .31[a]	1.26 ± .33[b]		
	28	1.32 ± .38[a]	1.80 ± .41[b]		
	56	1.35 ± .39[a]	2.04 ± .32[b]		
	70	1.34 ± .50[a]	1.86 ± .38[b]		
	98	1.28 ± .46	1.67 ± .28		
Serum CPK (EU/L)	0	450 ± 277	439 ± 164	9.52	.0071
	7	387 ± 219	318 ± 114		
	28	738 ± 447	354 ± 333		
	56	831 ± 493	557 ± 720[b]		
	70	1479 ± 1749[a]	343 ± 100[b]		
	98	1499 ± 724	897 ± 731		
Serum AST (EU/L)	0	28.9 ± 6.4	26.1 ± 6.7	.33	.5719
	7	35.4 ± 8.3	37.3 ± 9.7		
	28	50.6 ± 35.0	75.6 ± 71.3		
	56	43.3 ± 14.7	62.5 ± 58.9		
	70	50.1 ± 22.5	37.8 ± 19.4		
	98	52.7 ± 21.4	40.6 ± 10.8		
Serum Vit. E (ug/ml)	0	.68 ± .18*	.59 ± .21*	.38	.5452
	7	.45 ± .15*	.42 ± .15*		
	28	.75 ± .23*	.79 ± .34*		
	56	.89 ± .21*	.76 ± .35*		
	70	.84 ± .46*	.85 ± .36*		
	98	.62 ± .23*	.51 ± .30*		

[#]Mean ± SD.

[+]Pigs were 76 days of age on day 0.

[a,b]Means within periods (days) without common superscript letters differ ($p<.01$).

*Mean values are below the expected range for pigs of this age (see Appendix B).

Table 8. Effect of monensin on serum mineral concentrations in Experiment 2.

Variable	Day	Monensin (mg/kg diet) 0	200	400	F Value	PR>F
Serum Ca (ug/ml)	0[+]	104 ± 6[#]	104 ± 4	108 ± 8	1.71	.2126
	7	99 ± 7	103 ± 13	111 ± 12		
	28	101 ± 4	107 ± 8	106 ± 7		
	56	99 ± 3	92 ± 7	97 ± 3		
	70	96 ± 9	97 ± 3	94 ± 8		
	98	86 ± 5	94 ± 3	95 ± 7		
Serum Cu (ug/ml)	0	2.11 ± .29	2.26 ± .38	2.09 ± .33	1.68	.2185
	7	1.84 ± .29	1.97 ± .15	1.99 ± .16		
	28	1.60 ± .24	1.93 ± .34	1.67 ± .18		
	56	1.79 ± .19	1.90 ± .36	1.86 ± .22		
	70	1.86 ± .23	1.99 ± .21	1.83 ± .19		
	98	1.79 ± .20	1.94 ± .59	1.70 ± .19		
Serum Fe (ug/ml)	0	2.95 ± 1.00	3.83 ± 1.61	5.37 ± 4.30	3.21	.0671
	7	3.80 ± 1.69	3.09 ± .67	4.99 ± 3.06		
	28	2.35 ± .84	4.10 ± 2.89	2.56 ± .40		
	56	1.94 ± .58	2.47 ± 1.61	2.44 ± .93		
	70	2.42 ± 1.48	2.70 ± 1.45	4.04 ± 2.70		
	98	3.41 ± 1.60	4.64 ± 2.52	4.53 ± 3.54		
Serum K (ug/ml)	0	272 ± 24[a]	277 ± 16[b]	262 ± 15[b]	19.85	.0001
	7	287 ± 36[a]	218 ± 23[b]	178 ± 28[b]		
	28	140 ± 27	125 ± 10	120 ± 5		
	56	254 ± 23	236 ± 27	236 ± 11		
	70	298 ± 27	282 ± 19	276 ± 22		
	98	253 ± 50	138 ± 35	118 ± 4		

Table 8 (cont'd.).

Serum Mg (ug/ml)	0	20.3 ± 2.4	19.3 ± 2.6	18.3 ± 2.1	2.08	.1575
	7	21.8 ± 2.0	23.4 ± 4.5	23.6 ± 1.4		
	28	19.0 ± 2.0	22.4 ± 2.5	20.0 ± 2.1		
	56	22.8 ± 1.3	21.9 ± 2.3	21.1 ± 1.1		
	70	21.5 ± 1.8	22.9 ± 1.6	21.3 ± 2.4		
	98	18.5 ± 2.1	19.7 ± 4.7	17.6 ± 2.0		
Serum Na (ug/ml)	0	3225 ± 89	3329 ± 76	3314 ± 69	.19	.8327
	7	3175 ± 128	3143 ± 151	3157 ± 207		
	28	3038 ± 119	3071 ± 125	3157 ± 181		
	56	3125 ± 46	3014 ± 135	3100 ± 58		
	70	3050 ± 76	3086 ± 69	2986 ± 107		
	98	2788 ± 113	2729 ± 49	2786 ± 157		
Serum P (ug/ml)	0	148 ± 10	165 ± 6	151 ± 8	.85	.4452
	7	142 ± 15	157 ± 23	154 ± 18		
	28	134 ± 8	140 ± 15	137 ± 12		
	56	139 ± 7	123 ± 11	134 ± 9		
	70	134 ± 11	137 ± 5	129 ± 14		
	98	109 ± 10	120 ± 10	123 ± 15		
Serum Zn (ug/ml)	0	1.04 ± .13	.84 ± .15	.89 ± .18	.01	.9929
	7	.87 ± .15	.86 ± .05	.90 ± .20		
	28	.84 ± .07	.93 ± .21	.94 ± .11		
	56	.90 ± .09	.93 ± .19	.93 ± .10		
	70	.90 ± .13	1.01 ± .18	.91 ± .09		
	98	1.29 ± .20	1.19 ± .17	1.07 ± .11		

[#]Mean ± SD.

[+]Pigs were 76 days of age on day 0.

[a,b]Means within periods (days) without common superscript letters differ ($p<.01$).

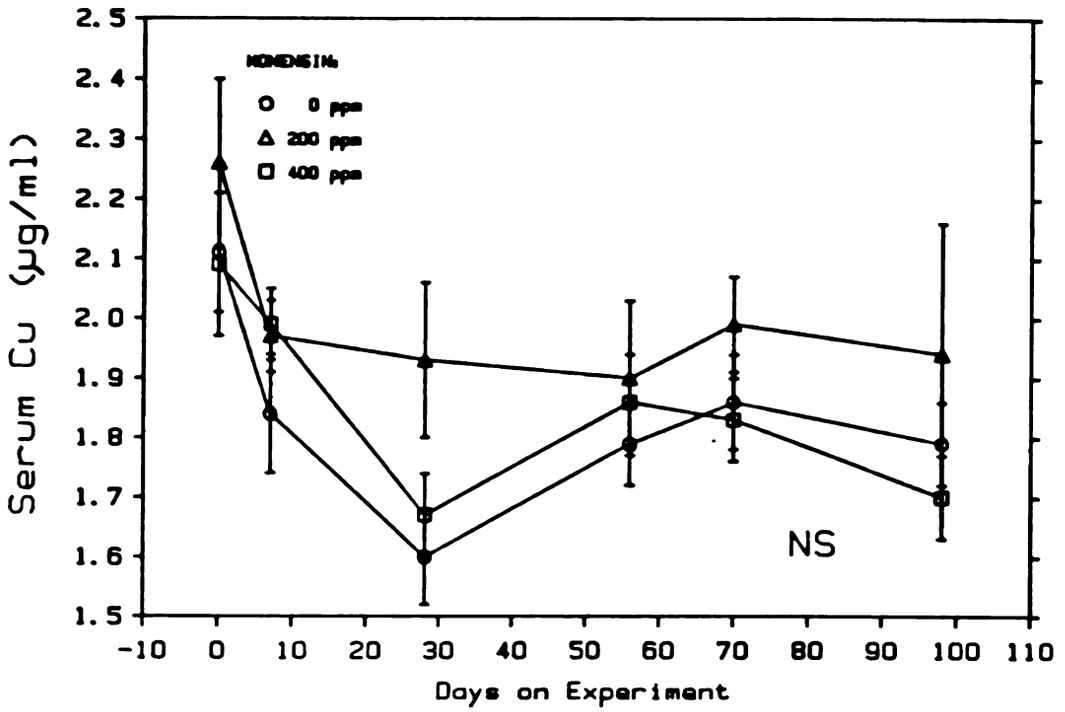

Figure 21. Effect of monensin on serum Cu concentrations in Experiment 2.

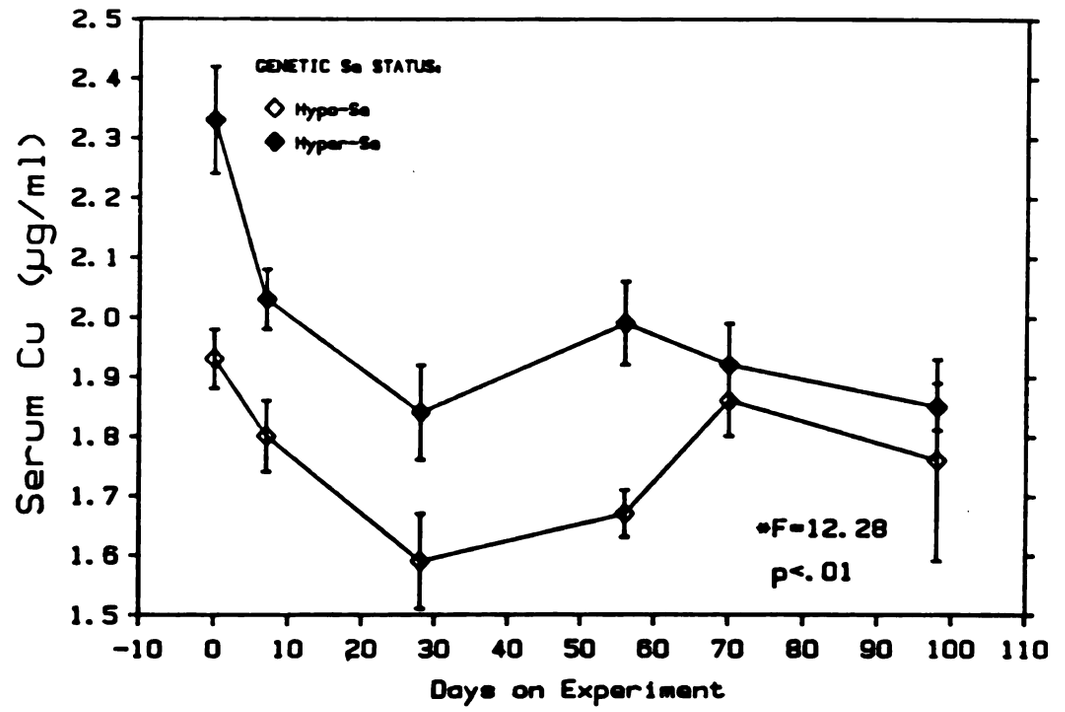

Figure 22. Effect of genetic Se status on serum Cu concentrations in Experiment 2.

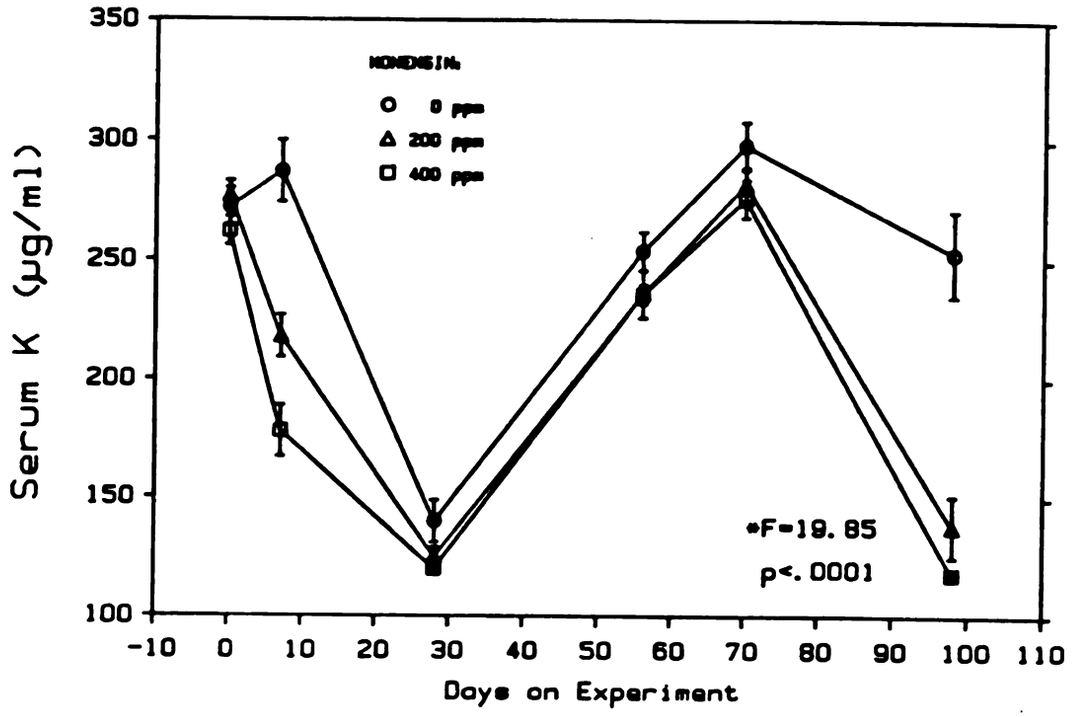

Figure 23. Effect of monensin on serum K concentrations in Experiment 2.

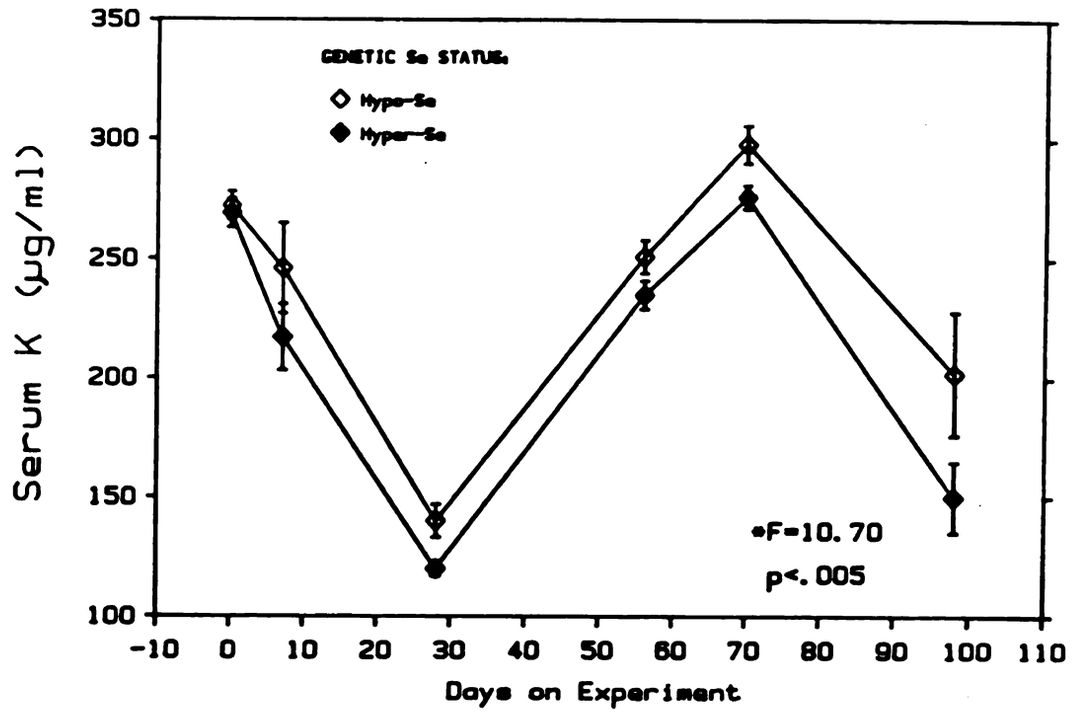

Figure 24. Effect of genetic Se status on serum K concentrations in Experiment 2.

Table 9. Effect of genetic Se status on serum mineral concentrations in Experiment 2.

	Day	Genetic Se Status: Hypo Se	Genetic Se Status: Hyper Se	F Value	PR>F
Serum Ca (ug/ml)	0[+]	102 ± 5.2[#]	108 ± 5.3	1.12	.3065
	7	101 ± 11.2	107 ± 11.5		
	28	103 ± 6.5	106 ± 6.5		
	56	99 ± 3.5	95 ± 6.0		
	70	98 ± 6.6	94 ± 6.5		
	98	92 ± 4.6	91 ± 8.1		
Serum Cu (ug/ml)	0	1.93 ± .17[a]	2.33 ± .31[b]	12.28	.0029
	7	1.80 ± .19	2.03 ± .18		
	28	1.59 ± .26	1.84 ± .26		
	56	1.67 ± .14[a]	1.99 ± .24[b]		
	70	1.86 ± .18	1.92 ± .24		
	98	1.76 ± .53	1.85 ± .14		
Serum Fe (ug/ml)	0	4.35 ± 3.49	3.71 ± 2.00	1.88	.1898
	7	4.27 ± 2.79	3.68 ± 1.34		
	28	3.01 ± 1.43	2.94 ± 2.15		
	56	2.48 ± .81	2.09 ± 1.27		
	70	3.45 ± 2.60	2.67 ± 1.30		
	98	4.83 ± 2.86	3.60 ± 2.26		
Serum K (ug/ml)	0	272 ± 18	269 ± 21	10.70	.0048
	7	246 ± 59[a]	217 ± 50[b]		
	28	140 ± 22	120 ± 9		
	56	251 ± 22	235 ± 20		
	70	298 ± 27	276 ± 18		
	98	202 ± 83	150 ± 52		

Table 9 (cont'd.).

Serum Mg (ug/ml)	0	18.0 ± 2.3	20.4 ± 1.9	.44	.5187
	7	22.2 ± 3.0	23.4 ± 3.3		
	28	20.6 ± 2.2	20.3 ± 2.9		
	56	22.5 ± 1.4	21.5 ± 1.8		
	70	22.2 ± 2.3	21.6 ± 1.7		
	98	19.6 ± 4.0	17.8 ± 1.9		
Serum Na (ug/ml)	0	3270 ± 106	3300 ± 74	.58	.4566
	7	3110 ± 129	3200 ± 171		
	28	3070 ± 149	3100 ± 148		
	56	3100 ± 67	3067 ± 115		
	70	3060 ± 84	3025 ± 97		
	98	2760 ± 126	2775 ± 106		
Serum P (ug/ml)	0	151 ± 11	157 ± 11	4.14	.0587
	7	143 ± 20	157 ± 17		
	28	134 ± 11	139 ± 12		
	56	132 ± 10	133 ± 13		
	70	133 ± 9	134 ± 12		
	98	115 ± 10	119 ± 15		
Serum Zn (ug/ml)	0	.88 ± .18	.97 ± .16	.63	.4400
	7	.85 ± .14	.90 ± .14		
	28	.90 ± .15	.97 ± .10		
	56	.93 ± .14	.91 ± .12		
	70	1.00 ± .16	.89 ± .11		
	98	1.18 ± .19	1.19 ± .19		

[#]Mean ± SD.

[+]Pigs were 76 days of age on day 0.

[a,b]Means within periods (days) without common superscript letters differ ($p<.01$).

Table 10. Effect of monensin on liver and kidney tissue Se and mineral concentrations in Experiment 2.

Variable	Monensin Intake (mg/kg)	Liver Tissue	F Value	PR>F	Kidney Tissue	F Value	PR>F
Se	0	1.50 ± .12[#]	.22	.8048	7.73 ± 1.23	1.37	.2814
	200	1.49 ± .13			8.58 ± 1.01		
	400	1.53 ± .11			8.44 ± 0.80		
Ca	0	62 ± 13	.77	.4784	88 ± 33	1.93	.1775
	200	61 ± 3			126 ± 89		
	400	75 ± 55			72 ± 9		
Cu	0	9.07 ± 2.91	.18	.8347	6.50 ± 1.64	.06	.9444
	200	9.90 ± 4.00			6.26 ± 3.12		
	400	9.86 ± 7.28			6.23 ± 0.96		
Fe	0	208 ± 49	1.22	.3204	61 ± 44	.89	.4308
	200	223 ± 26			47 ± 12		
	400	191 ± 55			42 ± 7		
K	0	3138 ± 226	.19	.8279	2700 ± 355	1.22	.3221
	200	3086 ± 90			2771 ± 315		
	400	3143 ± 276			2971 ± 150		
Mg	0	227 ± 3[a]	3.85	.0433	171 ± 19	.27	.7687
	200	203 ± 8[b]			165 ± 13		
	400	206 ± 6[a,b]			167 ± 7		
Na	0	732 ± 104[a]	4.03	.0382	1363 ± 342	1.37	.2813
	200	894 ± 129[b]			1214 ± 90		
	400	748 ± 94[a,b]			1200 ± 58		
P	0	4863 ± 358[a]	4.35	.0309	2938 ± 277	.16	.8530
	200	4486 ± 247[b]			2886 ± 204		
	400	4514 ± 146[a,b]			2971 ± 160		
Zn	0	88 ± 26	2.17	.1465	28 ± 6	.60	.5623
	200	71 ± 21			24 ± 8		
	400	69 ± 19			27 ± 3		

[#]Values expressed as ug/g dry weight (mean ± SD).

[a,b]Means between monensin treatment groups without common superscript letters differ ($p<.01$).

Table 11. Effect of genetic Se status on liver and kidney tissue Se and mineral concentrations in Experiment 2.

	Genetic Se Status	Liver Tissue	F Value	PR>F	Kidney Tissue	F Value	PR>F
Se	Hypo	1.52 ± .07[#]	.13	.7226	7.72 ± 1.03[a]	4.39	.0524
	Hyper	1.50 ± .14			8.65 ± .92[b]		
Ca	Hypo	72 ± 45	.88	.3611	95 ± 74	.01	.9405
	Hyper	61 ± 10			95 ± 40		
Cu	Hypo	12.14 ± 6.17[a]	6.13	.0248	6.54 ± 2.83	.19	.6679
	Hyper	7.46 ± 1.11[b]			6.17 ± .96		
Fe	Hypo	191 ± 51	3.38	.0848	45 ± 10	.71	.4112
	Hyper	221 ± 35			55 ± 37		
K	Hypo	3130 ± 258	.08	.7803	2640 ± 306	.19	.6701
	Hyper	3117 ± 159			2692 ± 297		
Mg	Hypo	211 ± 14	.00	.9815	169 ± 14	.06	.8106
	Hyper	211 ± 17			167 ± 14		
Na	Hypo	781 ± 126	.01	.9078	1210 ± 57	1.20	.2890
	Hyper	795 ± 135			1308 ± 291		
P	Hypo	4690 ± 307	.51	.4858	2910 ± 213	.23	.6363
	Hyper	4583 ± 324			2950 ± 224		
Zn	Hypo	66 ± 20[a]	5.78	.0287	29 ± 7	2.58	.1279
	Hyper	86 ± 22[b]			24 ± 4		

[#]Values expressed as ug/g dry weight (mean ± SD).

[a,b]Means between genetic Se status groups without common superscript letters differ ($p<.01$).

III. Experiment 3.

Most pigs ate their gruel mixture well once the monensin concentration was decreased from 300 to 150 mg/kg. No clinical differences between controls and monensin-treated pigs were observed.

Serum Se concentrations were below expected ranges (Appendix B) on day -9 (31 days of age) but reached adequate levels by day 0 (Table 12).

Treatment with monensin did not affect body weight or serum Se, GSH-Px, CPK or AST values. Serum Se, and GSH-Px values increased over time in both groups (Table 12).

Serum Se and GSH-Px values ($r = .65$, $p<.0001$) and serum CPK and AST values ($r = .61$, $p<.0002$) were highly correlated, but serum GSH-Px and CPK ($r = .06$, $p<.75$) or AST ($r = .09$, $p<.64$) activities were not.

All mean serum mineral values were within expected ranges (Appendix C). Monensin treatment moderately affected serum Ca ($p<.01$), K ($p<.05$), P ($p<.05$), and Zn ($p<.005$) concentrations, which were lower in pigs receiving monensin than in controls (Table 13).

Selenium balance was not affected by monensin treatment as there were no differences in average daily Se intake, percent Se excretion in urine or feces, percent Se retention, or percent renal Se clearance compared to control animals (Table 14).

Error terms for the variables in Experiment 3 are summarized in Appendix F.

Table 12. Effect of treatment on body weight and serum Se, GSH-Px, CPK, and AST concentrations in Experiment 3.

Variable	Day[+]	----Treatment Group----- Control	Monensin	F Value	PR>F
Body weight (kg)	-9	9.0 ± 1.4[#]	8.7 ± .7	.24	.6335
	0	7.2 ± 1.2	6.9 ± .6		
	6	7.9 ± 1.7	7.7 ± .8		
Serum Se (ng/ml)	-9	85.2 ± 8.1[*]	78.8 ± 9.7[*]	.29	.6034
	0	116.2 ± 13.5	113.8 ± 14.3		
	6	133.8 ± 14.8	128.3 ± 12.1		
Serum GSH-Px (EU/ml)	-9	.71 ± .13	.69 ± .11	.27	.6156
	0	.87 ± .14	.83 ± .25		
	6	.97 ± .21	.90 ± .23		
Serum CPK (EU/L)	-9	180 ± 89	118 ± 55	.05	.8265
	0	107 ± 44	99 ± 29		
	6	170 ± 94	198 ± 163		
Serum AST (EU/L)	-9	25.4 ± 8.1	24.3 ± 6.4	.00	.9613
	0	23.0 ± 8.3	21.3 ± 5.2		
	6	31.8 ± 9.4	33.0 ± 11.1		

[#]Mean ± SD.

[+]Pigs were 31 days of age on day -9.

[*]Mean values are below the expected range for pigs of this age (see Appendix B).

Table 13. Effect of treatment on serum mineral concentrations in Experiment 3.

Variable	Day	----Treatment Group----- Control	Monensin	F Value	PR>F
Serum Ca (ug/ml)	-9[+]	95 ± 14[#]	97 ± 8	12.41	.0065
	0	113 ± 4[a]	104 ± 2[b]		
	6	113 ± 9	106 ± 5		
Serum Cu (ug/ml)	-9	1.56 ± .05	1.53 ± .14	.02	.8961
	0	1.98 ± .08	2.00 ± .19		
	6	2.06 ± .23	2.02 ± .23		
Serum Fe (ug/ml)	-9	2.72 ± .99	2.55 ± .81	.00	.9762
	0	2.70 ± 1.02	3.62 ± .67		
	6	3.50 ± 1.47	2.62 ± .52		
Serum K (ug/ml)	-9	208 ± 20	192 ± 13	5.69	.0409
	0	283 ± 23	256 ± 30		
	6	336 ± 23	299 ± 25		
Serum Mg (ug/ml)	-9	18.8 ± 3.4	23.5 ± 2.1	.65	.4423
	0	19.0 ± 3.2	19.0 ± 2.5		
	6	21.0 ± 6.2	18.0 ± 3.6		
Serum Na (ug/ml)	-9	3120 ± 110	3083 ± 41	2.48	.1495
	0	3780 ± 228	3467 ± 197		
	6	3640 ± 321	3483 ± 271		
Serum P (ug/ml)	-9	120 ± 11	119 ± 13	5.73	.0403
	0	162 ± 20	135 ± 14		
	6	147 ± 26	129 ± 18		
Serum Zn (ug/ml)	-9	.96 ± .38	.83 ± .14	19.09	.0018
	0	1.00 ± .07[a]	.68 ± .10[b]		
	6	.96 ± .19	.82 ± .12		

[#]Mean ± SD.

[+]Pigs were 31 days of age on day -9.

[a,b]Means between treatment groups without common superscript letters differ ($p<.01$).

Table 14. Effect of monensin on Se balance in Experiment 3.

	Treatment Groups			
	Control		Monensin	
	mean ± SD	range	mean ± SD	range
Mean daily Se intake (ug/kg BW/day)	6.65 ± .68	5.45-7.11	6.81 ± .51	6.05-7.36
Se excretion (% of intake)				
Total	50.9 ± 6.9	43.6-60.8	44.7 ± 11.9	29.3-59.3
Feces	39.1 ± 5.8	32.1-46.4	30.5 ± 15.0	9.9-52.4
Urine	11.9 ± 3.8	7.4-16.8	14.2 ± 4.5	6.9-19.4
Se retention (% of intake)	49.1 ± 6.9	40.7-70.7	55.3 ± 11.7	39.3-56.4
Se:creatinine clearance ratio	.29 ± .12	.19-.49	.32 ± .05	.23-.38

DISCUSSION

I. Introduction.

The purpose of these experiments was to determine the effects of continual monensin consumption on growth, Se balance, serum leakage enzymes and tissue concentrations of selected elements in growing pigs. It was theorized that monensin might precipitate peroxidative myopathy, similar to nutritional myodegeneration, in animals of marginal antioxidant status. It was our intention to study the effect of monensin on Se status, rather than vitamin E status, so vitamin E was supplemented at a standard rate of 44 IU/kg diet (44 mg/kg diet).

In Experiment 1 we chose 150 mg monensin/kg diet as a moderately high dose to be fed with or without .1 mg supplemental Se/kg diet. We anticipated that this regimen might produce clinical, but not fatal, toxicosis in pigs not receiving supplemental Se. Since monensin toxicosis was not observed in Experiment 1, we decided to increase the level of monensin fed in Experiment 2 to 200 and 400 mg/kg diet, and again included .1 mg supplemental Se/kg diet. Current regulations allow the addition of .3 mg supplemental Se/kg to feed for all ages of pigs (FDA, 1987).

In Experiment 3, feed containing .1 mg supplemental Se/kg was included with or without 300 mg monensin/kg. While monensin had been consumed readily at concentrations up to 400 mg/kg diet in the previous experiments, palatability seemed to be a problem in weanling pigs in

metabolism cages. Feed consumption improved after monensin concentration in the feed was reduced from 300 to 150 mg/kg, but this may have been related to acclimation to the feeding procedure as well.

A second balance trial, in which supplemental Se would have been omitted, was not performed due to potential problems with the use of monensin in a species for which it was not approved. For this reason, a withdrawal period of at least 35 days was observed before slaughter in all experiments to allow tissue monensin residues to decline. Tissue monensin residues were not determined, nor were the effects of monensin on the prevalence of coccidiosis infestation investigated.

No signs of monensin toxicosis or nutritional myopathy were seen in any of the pigs. However, lesions of cardiac and skeletal myopathy compatable with monensin toxicosis were present in one hypo-Se animal necropsied after consuming 200 mg monensin/kg diet for 42 days. Lesions were most pronounced in the right atrium, which is in contrast to the report of left atrial tissue as a predeliction site in monensin toxicosis (Van Vleet and Ferrans, 1984a). Serum Se and GSH-Px values in this animal had been within acceptable ranges. Another pig consuming 400 mg monensin/kg diet had no detectable lesionsat the time of its death, but had been on this treatment for only 28 days.

II. Body Weight.

All animals, except those in the balance trial, were group-fed; feed efficiency was not analyzed. Total feed consumption among groups was comparable at the end of Experiments 1 and 2. Consumption of feed containing up to 200 mg monensin/kg was previously reported to have no detrimental effect on weight gain or feed efficiency of growing pigs (Anon., 1980).

In our experiments, monensin had no significant effect on body weight, although pigs fed 400 mg/kg diet were 5 kg lighter at the end of Experiment 2 ($p>.05$). All pigs in Experiment 3 lost weight, presumably due to the combined stress of weaning, placement in metabolism cages and slow acclimation to twice-daily gruel feeding.

Hyper-Se pigs were, on average, 3.4 kg heavier at the start of Experiment 2 and 8.6 kg heavier at the end. Stowe and Miller (1982, 1985) had reported similar, but smaller, differences in the F1 generation of the same populations of hypo-Se and hyper-Se pigs. Relative hyperselenemia would appear to represent an advantage in growth (Stowe and Miller, 1982, 1985).

Castrated males were consistently heavier than intact females in Experiments 1 and 2, as would be expected.

III. Selenium Concentrations.

A. Overview.

Prior to supplementation, serum Se concentrations in most groups of pigs were below expected levels (Appendix B) and below the level (100 ng Se/ml) at which vitamin E-Se deficiency diseases can occur when vitamin E concentrations are inadequate (Sankari, 1985). With time mean serum Se concentrations increased in most groups, although to different degrees, regardless of treatment. However, only the hyper-Se group in Experiment 2 reached satisfactory plateau concentrations of serum Se.

The increases in serum Se values observed in response to supplementation were not tested for linearity, but it was evident that groups with lower initial mean serum Se values experienced a more rapid increase in serum Se concentration than did those with higher initial values, regardless of monensin treatment. In no case did serum Se concentrations in monensin-fed pigs approximate toxic Se concentrations as Wheeler (1984) had suggested.

Serum Se values (overall range 26-250 ng Se/ml) were similar to those reported for other experimental work within this herd (Whitehair et al., 1982a; Groce et al., 1973a, 1973b; Stowe and Miller, 1982, 1985). Groce et al. (1973a) reported that weanling pigs from sows that had received no supplemental Se during gestation or lactation had initial serum Se values of 36 ng Se/ml, which increased to 112 ng Se/ml after nine days of supplementation with .2 mg Se/kg diet as selenite. In another experiment (Groce et al., 1973b), three groups of growing swine consuming a basal diet (.052 mg Se/kg) supplemented with 0, .05, .1 or .2 mg Se/kg, had mean serum Se values of 46, 150, 164, and 168 ng Se/ml, respectively, after 19 weeks.

B. Influence of Monensin.

Monensin treatment had no significant effects on serum Se status in Experiments 1, 2 or 3. After a 35-day monensin withdrawal period, Se concentrations in liver and kidney tissue from pigs in Experiment 2 were similar to those reported by Groce et al. (1973b) and were not affected by monensin treatment. No significant differences in Se intake, apparent retention of Se, Se excretion or creatinine:Se clearance ratio (fractional excretion ratio) were observed in Experiment 3, perhaps since wide individual animal variation occurred.

Costa et al. (1985, 1987) reported that cattle treated with monensin had enhanced Se absorption and retention and increased blood and plasma Se values. However, Jensen (1986) found no effect of monensin on plasma Se values in chicks and suggested that simple-stomached animals may differ from ruminants in the effects of monensin on Se balance. Our work does not support the idea that Se balance in swine is influenced by monensin treatment. Although higher concentrations of monensin might affect Se balance, it would appear that the lesions seen in monensin toxicosis are not the result of direct interference with Se metabolism.

C. Influence of Genetic Se Status.

Genetic Se status had a marked effect on serum Se concentrations in Experiment 2. In these F2 generations of hypo-Se or hyper-Se pigs, serum Se concentrations differed by an overall average of 37 ng Se/ml from 76 to 181 days of age. Despite monensin feeding, serum Se values in the F2 generation pigs increased more rapidly and reached higher concentrations than they did in the F1 generation when supplemented with .1 mg Se/kg diet (Stowe and Miller, 1982, 1985). In the F1 pigs, average serum Se

concentrations for hypo-Se and hyper-Se groups differed by about 26 ng Se/ml from 58 to 152 days of age (Stowe and Miller, 1985). Thus, it would seem that the average difference between hypo-Se and hyper-Se populations of pigs may increase from the F1 to F2 generation; such genetic divergence is expected.

Hyper-Se pigs had significantly greater kidney, but not liver, Se contents than hypo-Se pigs. Based on expected serum Se values (Appendix B), both groups of pigs had adequate body Se status; perhaps increased renal tissue Se concentrations in hyper-Se animals reflected higher urinary excretion rates.

IV. Serum Glutathione Peroxidase Activity.

A. Overview.

Rotruck et al. (1973) first proposed that GSH-Px might serve as an indicator of Se status; Levander (1983) suggested that GSH-Px activity would better indicate Se bioavailability. Most reports have substantiated these predictions (Jansen, 1977; Ewan, 1976; Sivertsen et al., 1977; Hakkarainen, 1978; Adkins and Ewan, 1984), but it is also clear that the correlation between GSH-Px activity and Se values is more consistent at lower concentrations of each (Sivertsen et al., 1977; Chavez, 1979a; Sankari, 1985). This is because GSH-Px activity tends to plateau at the level of physiologic requirement, while body or serum Se content can continue to increase past that point. Based on the literature, it would seem that the best correlations between serum Se and GSH-Px in swine are reached at serum or plasma Se concentrations of less than 100 ng Se/ml. This would explain the poor correlation reported by Thompson et al. (1976) for swine where all values were above 93 ng Se/ml.

Ganther et al. (1976) pointed out that results of GSH-Px assays cannot be compared among laboratories due to differences in sample source, assay technique and units of measurement. There appears to be considerable debate as to the most appropriate sample source, but Stowe and Miller (1985) found a more reliable correlation of serum or dietary Se values with plasma GSH-Px than with erythrocyte GSH-Px in swine. Consequently, serum GSH-Px activity is reported here.

Enzyme activity is affected by other factors, such as sample damage due to improper storage or handling techniques. Zhang et al. (1986) suggested that plasma GSH-Px activity be determined immediately after

separation to avoid the decline in activity observed under different conditions of storage. Rice et al. (1987) found that storage at -20°C decreased blood GSH-Px concentrations but that enzyme activity could be restored by preincubation with reduced glutathione (GSH). Our enzyme assays were performed after storage at -20°C for 6-8 months, but all samples from a given day were handled identically, so that effects should not vary appreciably among treatment groups.

On a single day (day 14 in Experiment 1) the serum GSH-Px values were noticeably altered, apparently due to heat damage of the samples when aliquots were taken for the multielement assay. Virtually all mean group GSH-Px values were greater than those reported by Zhang et al. (1986) for nursing pigs from this herd (.43-.56 EU/ml plasma). Hemolysis was present in many samples and was a potential source of error for GSH-Px activity and Se concentration; in cattle 98% of the whole blood GSH-Px activity was found within erythrocytes (Scholz and Hutchinson, 1979).

In general, serum GSH-Px activity correlated fairly well with serum Se content for these experiments. The correlation coefficients (r) were .83 in Experiment 1 (without day 14 values), .77 in Experiment 2, .65 in Experiment 3, and .86 if values from Experiment 1 and 2 were combined (Figure 25). Mean serum GSH-Px activity (range .22-2.44 EU/ml) increased over time with all treatments in parallel with mean serum Se concentrations.

B. Influence of Monensin.

Anderson et al. (1983) reported an additive effect of Se and monensin on blood GSH-Px activity in ewes with marginal Se status and their lambs. Costa (1987) found monensin increased plasma Se, but not GSH-Px, values in cattle although plasma Se concentrations in that report

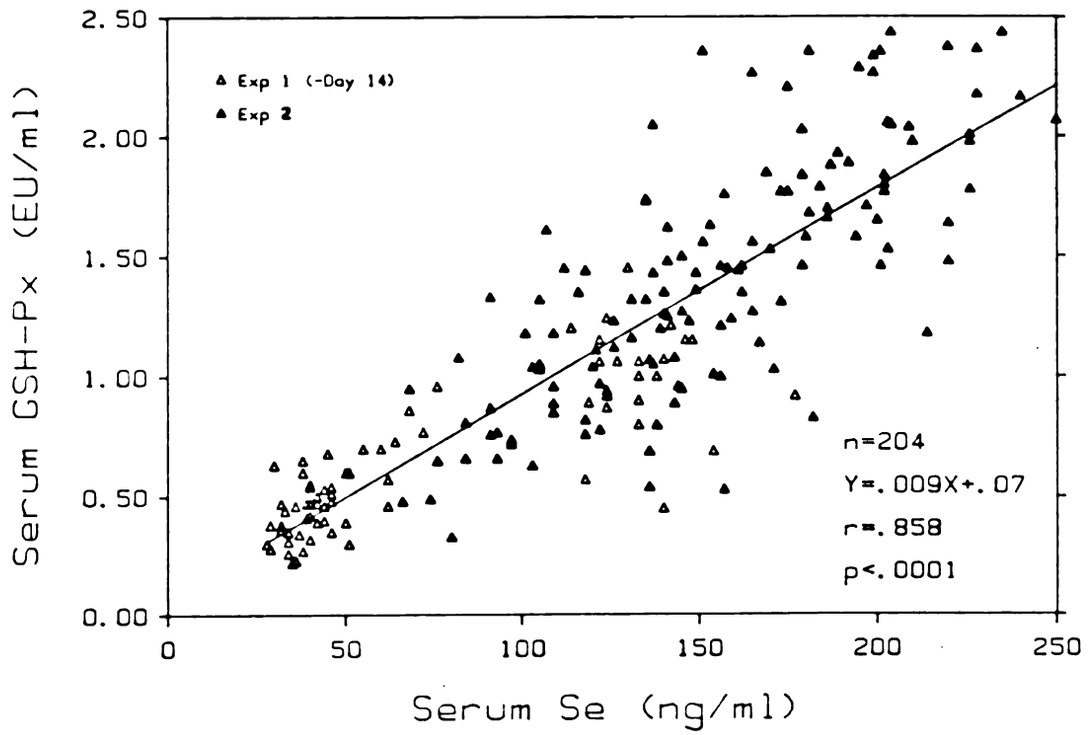

Figure 25. Regression analysis of serum GSH-Px activity on serum Se concentration for Experiment 1 (excluding day 14) and Experiment 2.

were low and supplemental Se was not added to the diet. In contrast, supplementation of monensin (150 mg/kg diet) and Se (.1 mg/kg diet) to growing pigs of marginal Se status in Experiment 1 did not significantly affect serum GSH-Px concentrations. On the other hand, monensin feeding (200 or 400 mg/kg) increased serum GSH-Px values over those of control pigs on all days except day 0 in Experiment 2.

The reason for the increases in serum GSH-Px activity seen with monensin feeding are unknown. Scholz and Hutchinson (1979) showed that hemolysis released the stores of GSH-Px from erythrocytes and increased serum or plasma GSH-Px activities. Hemolysis can occur due to defects in the glutathione, glutathione reductase and glutathione peroxidase system (Flohe, 1982), and was reported in Se-deficient cattle (Morris et al., 1984). The pigs in Experiment 2 had adequate Se levels by day 28, so hemolysis as a result of Se deficiency was unlikely.

Monensin has the capacity to induce membrane damage in a wide variety of tissues and may disrupt erythrocyte membranes sufficiently to allow GSH-Px leakage. Lipid peroxidation is also known to induce hemolysis (Fontaine and Valli, 1977). While hemolysis was noted in many serum samples, it was most likely related to our blood sampling technique and seemed to occur independently of treatment group.

Costa et al. (1985) showed that monensin feeding increased Se absorption, suggesting that more Se might be available for incorporation into tissue and plasma GSH-Px. Animals of marginal Se status consuming monensin might benefit from enhanced Se absorption by synthesizing more GSH-Px, but this did not occur in cattle (Costa, 1987). Monensin was not associated with increased Se absorption in our balance trial.

The source of plasma GSH-Px is not known. Omaye and Tappel (1974b) suggested that it is probably synthesized in the liver; increased serum GSH-Px activity might be due to leakage from the liver, much like that seen with AST in liver disease (Sankari, 1985). While Cohen et al. (1987) showed, in humans, that plasma GSH-Px is distinct from liver, erythrocyte, neutrophil, or platelet GSH-Px, this does not rule out liver leakage as a source of increased serum GSH-Px with monensin feeding.

Subclinical monensin toxicosis may damage hepatocytes and allow leakage of liver GSH-Px into serum. Monensin is apparently largely metabolized in the liver and excreted in bile (Herberg et al., 1978; Davison, 1984). Hepatocytes of sheep (Anderson et al., 1984) and ponies (Mollenhauer et al., 1984) with experimental monensin toxicosis had changes suggestive of increased activity of the mixed-function oxidase system, probably in response to monensin. We did not examine liver tissue histologically.

Lastly, an attractive explanation for the increase in serum GSH-Px activity seen with monensin feeding is that GSH-Px is an adaptive enzyme whose activity increases during times of lipid peroxidation (Chow and Tappel, 1972, 1973; Chow et al., 1973). As oxidant stressors are applied to an animal system, the ensuing lipo-peroxidation apparently stimulates synthesis of GSH-Px, provided adequate Se is available. Van Vleet and Ferrans (1984a) first suggested that peroxidative mechanisms might be involved in monensin toxicosis since the ultrastructural lesions were so similar.

Monensin is not known to be an oxidant, but its ionophoretic properties are believed to cause mitochondrial and cellular membrane

damage or lysis, due to Ca^{+2} overloading (Shlafer and Kane, 1980). It is, therefore, possible that monensin-induced membrane damage elicits GSH-Px synthesis and increases serum GSH-Px activity, much as peroxidation does.

Disruption of cellular membranes may be the pathophysiologic event underlying a number of diseases which might be thought of as "membrane diseases". The similarity of the lesions in iron toxicosis (Cook et al., 1982), other acute mineral toxicities (Van VLeet et al., 1981), VESD (Van Vleet et al., 1970), monensin toxicosis (Van Vleet et al., 1983a) and even genetic muscular dystrophy (Wrogeman and Pena, 1976) suggests that all occur due to membrane destabilization. Animals with limited antioxidant (i.e., membrane stabilizing) capacity would be more susceptible to membrane diseases. The protective effect of pretreatment with vitamin E/Se against many of these syndromes seems to support this idea.

C. Influence of Genetic Se Status.

Genetic Se status was closely identified with serum GSH-Px activity, as illustrated by Stowe and Miller (1982, 1985). In the present study, hypo-Se pigs reached a plateau in serum GSH-Px activity later than, and at lower levels than, hyper-Se pigs, although these values were considered adequate. Genetic control of Se status was first demonstrated in erythrocyte GSH-Px activity in Finn sheep (Atroshi et al., 1981; Sankari and Atroshi, 1983).

In pigs, elevated serum GSH-Px activity has been thought of more as a reflection of the relative selenemic status. It is unclear whether the differences in genetic Se status and serum GSH-Px concentrations are functions of alterations in Se absorption, incorporation in GSH-Px or in GSH-Px synthesis. Stowe and Miller (1986) found decreased apparent

retention and decreased apparent absorption of Se in hypo-Se pigs compared to hyper-Se pigs, but the differences were small. Individual animal variation in Se balance appears to be considerable, even within genetically-selected populations, and may reflect inadequacies of the balance trial method. Perhaps whole body ^{75}Se retention studies would give more accurate estimates (Costa, 1987).

Genetic variation in enzymatic activity has been documented for a wide variety of enzymes in human medicine, including some associated with the GSH-Px and HMP shunt systems (Ganther et al., 1976; Cooper and Bunn, 1987). It is easy to believe that enzymatic activity, rather than absorptive capacity, is responsible for genetic Se status since serum GSH-Px activity continued to increase above those values considered to be physiologically adequate in hyper-Se pigs. If Se absorption were responsible, GSH-Px synthesis ought to plateau at the same serum Se value in both hypo-Se and hyper-Se pigs once physiologic requirements were satisfied.

Hyper-Se (hyper-GSH-Px) pigs tended to grow more rapidly than hypo-Se pigs as Stowe and Miller (1985) had reported for the F1 generation pigs. This is in contrast to the report of Langlands et al. (1980) of little correlation between whole blood Se or GSH-Px values and live weight gains in cattle and sheep.

Increased serum GSH-Px activity should not necessarily be construed as an advantage. While Friendship and Wilson (1985) found a strong association between body weight and survivability, there was no relationship between body weight and blood GSH-Px activity in day-old piglets and only a weak association between GSH-Px values and

survivability. Atroshi et al. (1981) noted fewer live births per ewe, and lower weight gains and wool production, in sheep with increased erythrocyte GSH-Px activity. These workers theorized that low GSH-Px values may represent an adaptive mechanism selected to cope with the endemic low dietary Se contents. In a subsequent report, they suggested the difference in erythrocyte GSH-Px was due to differences in Se absorption or incorporation but could not determine which (Sankari and Atroshi, 1983).

In contrast, other workers suggest that increased GSH-Px values represent an advantage. Jorgensen and Wegger (1979) reported that disease frequency was less in swine with high erythrocyte GSH-Px values (i.e., increased Se content). The participation of Se and GSH-Px in immune mediation has been reviewed (Kiremidjian-Schumacher and Stotsky, 1987) and will not be discussed here. Omaye and Tappel (1974a) found increased GSH-Px and Se concentrations and evidence of lipid peroxidation in muscle from genetically dystrophic strains of chicks and mice. They interpreted this to mean that GSH-Px enzyme activity had been induced by peroxidation. It is tempting to speculate that the poor performance observed in hyper-GSH-Px (hyper-Se) sheep (Atroshi et al., 1981) was a manifestation of increased peroxidative damage. However, other reports (Stowe and Miller, 1985; Langlands et al., 1980), as well as this one, do not support this concept.

Wrogeman and Pena (1976) developed their theory of mitochondrial Ca^{+2} overload as the common mechanism of muscle disease while studying genetic muscular dystrophy. A membrane defect of unknown origin was believed to allow Ca^{+2} influx, possibly contributing to the increased

lipid peroxidation seen in human muscular dystrophies (Kar and Pearson, 1979). Similarily, monensin-induced Ca^{+2} overloading of myocytes, with subsequent mitochondrial and plasma membrane damage, might precipitate lipid peroxidation. Tissue and serum GSH-Px activity would be increased in response to the damage. Selenium concentrations would increase, secondarily, as a result of antioxidant demand and GSH-Px synthesis, rather than by passive increases in absorption. This explanation would seem to fit some of the available data on monensin-Se interactions, including the increase in serum GSH-Px activity measured in Experiment 2.

Animals with marginal antioxidant status ought to be more susceptible to monensin toxicosis since they already have compromised membrane integrity. Monensin might gain easier access to the internal organelles of such cells (Shlafer and Kane, 1980). At higher dosages, monensin could overwhelm the antioxidant capacity of an animal, and produce lesions of peroxidative disease, similar to those of VESD.

V. Serum Vitamin E Concentrations.

It is considered appropriate to supplement vitamin E when investigating the influence of Se concentrations on GSH-Px activity. Sankari et al. (1985) added 40 mg vitamin E/kg to Se-deficient feed and completely prevented increases in serum AST and ALT activities seen in pigs with low Se status. Bengtsson et al. (1978) found that 45 mg vitamin E/kg provided complete protection against disease in Se-deficient pigs. In our study supplemental vitamin E was added to all diets at 44 IU/kg (44 mg/kg) to facilitate the study of the effects of monensin on the Se-dependent portion of antioxidant status.

Despite addition of vitamin E to the diet, mean values for pigs in Experiments 1 and 2 were below expected ranges (Appendix B) on almost every sampling day in the study period suggesting that vitamin E-dependent antioxidant status was less than optimal. Since these pigs had marginal serum vitamin E/Se values it is even more perplexing that monensin-treated pigs did not develop significant indications of disease (i.e., increased serum leakage enzyme activity). Tolerance to monensin was greater than anticipated, perhaps because antioxidant status was sufficient even though low.

In Experiments 1 and 2, serum vitamin E content was not affected by monensin treatment or genetic Se status and increased over time. Serum vitamin E content was not measured in Experiment 3.

VI. Serum Leakage Enzyme Activities.

A. Creatine Phosphokinase.

In swine, tremendous elevations in serum CPK activity due to skeletal or cardiac muscle damage occurred with acute monensin toxicosis (>100,000 EU/L) (Van Vleet et al., 1983b); CPK values did not increase as markedly in cases of nutritional myopathy (<1,000 EU/L) (Fontaine et al., 1977). The difference appears to be related to the severity of muscle insult; most acute monensin toxicosis studies involve mortality, but many reports of VESD are of subclinical myopathy.

Other variables will affect serum CPK measurements. Van Vleet et al. (1975) have shown that plasma CPK activity is increased by heat stress and handling of pigs. Furthermore, although erythrocytes contain little CPK, hemolysis falsely elevates serum CPK values because of the release of other enzymes or substrates which interfere with the assay technique (Anon., 1983b).

In these experiments, mean serum CPK values were only moderately elevated (<1,500 EU/L) and were highly variable among individual pigs, suggesting that subclinical disease may have been present but was not equally severe. Monensin had no effect on serum CPK values in any experiment. Time had an effect in Experiment 1, where CPK values for all groups peaked on day 14, and in Experiment 2, where CPK activity tended to increase over time in all groups. An environmental factor, such as handling or fighting for social order, may have contributed to the variation in CPK activity with respect to time. Sample hemolysis may also have been a source of variation.

If monensin toxicosis develops more readily in animals with poor antioxidant reserves, then hypo-Se pigs might have evidence of myopathy, such as increased serum leakage enzyme activity. Interestingly, hypo-Se pigs had higher serum CPK activities on all days except day 0. Because CPK isoenzymes were not determined, it is unclear what proportion of this was due to enzyme leakage from damaged skeletal or cardiac muscle. Regardless, animals with lower serum Se (and GSH-Px) values appear prone to enzyme leakage, presumably due to membrane damage.

In theory, increased membrane peroxidation ought to induce GSH-Px synthesis and a positive correlation between serum CPK and GSH-Px activities might be expected in hypo-Se pigs. Likewise, if hyper-Se (hyper-GSH-Px) pigs are better protected against membrane damage, a negative correlation between serum GSH-Px and CPK activities might be found. Such was not the case, perhaps due to the variability in CPK activity or to the plateau in GSH-Px activity.

B. Aspartate Aminotransferase.

Elevations in serum AST activity are not organ specific and can be increased by liver or muscle damage or by "stress" (steroid-mediated hepatic effect) (Kramer, 1980). Additionally, hemolysis falsely elevates serum AST values since erythrocyte AST activity is 15 times that of serum (Anon., 1983a).

Increased AST activity has been seen with subclinical VESD in swine (Van Vleet et al., 1975) but was not detected in fatal VESD (Simensen et al., 1979) and was inconsistently increased in another report (Bengtsson et al., 1978). With acute monensin toxicosis in swine, AST values were increased (Anon., 1980; Van Vleet et al., 1983b) but the simultaneous

increases in CPK activity suggested that damage to striated muscle was the source. On the other hand, monensin toxicosis produced ultrastructural hepatic damage compatable with detoxification processes (Mollenhauer et al., 1981; Anderson et al., 1984) which implies that hepatic metabolism of monensin could cause hepatocellular damage and AST leakage.

Serum AST activity in these experiments showed substantial variation among animals and over time, perhaps due to sample hemolysis or stress. Monensin treatment at 400 mg/kg tended to increase serum AST activity in Experiment 2, primarily on days 28 and 56. However, the mean values observed (<250 EU/L) were much less than the 9,000 EU/L seen in acute monensin toxicosis studies (Van Vleet et al., 1983b). It is of interest that the greatest AST activity was not particularly associated with increased serum CPK values; correlations between serum AST and CPK activities were weak in all experiments. Increased AST activity is probably of liver origin and may be related to increased monensin metabolism.

In contrast to the increases in CPK activity, hypo-Se pigs did not have increased serum AST values. This suggests that monensin may be more toxic to myocytes than hepatocytes in hypo-Se pigs; perhaps differences in the innate antioxidant status of liver and striated muscle are responsible. This idea would be compatible with reports of muscle, but not liver, involvement in nutritional myopathy in pigs (Van Vleet et al., 1975; Fontaine et al., 1977).

Liver tissue may be more resistant to peroxidation than muscle due to the higher Se content. Groce et al. (1973b) reported .333, .815, 1.362 and 9.033 mg Se/kg dry weight in longissimus dorsi muscle, myocardium, and

liver and kidney tissue, respectively, of slaughter weight pigs fed .1 mg supplemental Se/kg diet. It is also possibile that tissues with relatively little non-Se-dependent GSH-S-Tr activity may be more susceptible to oxidation if Se-dependent GSH-Px activity were depleted.

Lastly, Wendel and Reiter (1985) found that Se-deficient mice had alterations in the pattern and activity of xenobiotic-metabolizing enzymes within liver tissue. If the same were true of pigs with low Se status, metabolism of monensin might be altered. If this change were to reduce monensin clearance, toxicosis might occur more easily, especially within hepatocytes.

VII. Serum, Liver, and Kidney Mineral Concentrations.

A. Overview.

The effect of monensin on mineral metabolism in pigs has not been reported, primarily because monensin is not approved for use in pigs. Studies in cattle, sheep and poultry revealed that differences may exist between simple-stomached and ruminant species in response to monensin (Jensen, 1986), but the direction and degree of influence of monensin on individual ion balance was unpredictable (Elsasser, 1984; Van Vleet, 1986). The numerous mineral interactions within an animal system (W. J. Miller, 1984; E. R. Miller and Kornegay, 1983) present opportunities for indirect, as well as direct, monensin effects.

Few reports of the effect of monensin on serum (plasma) or tissue mineral concentrations in other species exist. In this study, liver and kidney tissue was harvested 35 days after withdrawal of monensin, so results do not necessarily reflect the tissue mineral status of pigs still consuming monensin. Additionally, the dietary monensin concentrations tested (150, 200, 400 mg/kg) are not those commonly used in swine (100 mg/kg), and mineral balance trials were not performed.

Nevertheless, persistent alterations in tissue mineral content 35 days after monensin withdrawal were found and may indicate that monensin residues continue to exert their ionophoretic effects on mineral metabolism within selected tissues. Further study of the distribution of monensin residues in swine would seem necessary since unapproved use of monensin in commercial hogs is common. This would be especially important if concerns about the potentially adverse effects of monensin contamination of the food chain are warranted (Pressman and Fahim, 1982, 1983).

B. Macrominerals.

1. Sodium.

Greene et al. (1986) measured higher serum Na concentrations in sheep treated with monensin, but overall Na balance was unaffected. In other studies, monensin did not increase plasma Na in cattle (Starnes et al., 1984) or sheep (Kirk et al., 1985a) although Na balance was affected. In our experiments, monensin had no effect on serum Na concentrations. However, 200 mg monensin/kg diet increased liver, but not kidney, Na content. Kirk et al. (1985a) had reported no increase in liver or kidney Na content in sheep fed 20 mg monensin/kg diet.

2. Potassium.

Supplementation of K to monensin-fed steers (Ferrell et al., 1983) or chicks (Cervantes et al., 1982) enhanced growth, suggesting that K may be limiting when monensin is fed. However, monensin feeding did not affect plasma K values in steers (Starnes et al., 1984) or serum, liver or kidney K content in sheep despite increased K retention (Kirk et al.,1985a).

We found that serum K concentrations were lower for pigs consuming monensin in Experiment 2, but that tissue K concentrations (35 days after withdrawal) were not affected by monensin. Monensin tended to lower serum K values in Experiment 3 also.

It is not clear why serum K, but not Na, values were decreased since the affinity of monensin for Na is ten times that for K (Pressman, 1976). Monensin may have influenced serum K because of the relative differences in the concentrations of Na and K ions in serum; an equimolar change in ion concentrations would represent a larger portion of the serum content

of K than Na.

Hypo-Se pigs had consistently higher serum K values than hyper-Se pigs. The reason for this difference is unknown and it is not clear that it would be of any physiologic advantage. In fact, in contrast to other reports (Cervantes et al., 1982; Ferrel et al., 1983), the increased serum K values in hypo-Se pigs were not associated with increases in body weight.

3. Calcium.

Monensin had no effect on serum Ca values in bulls (Dvorak et al., 1980) or on plasma Ca values in sheep, although it tended to increase apparent absorption and retention of Ca in sheep (Starnes et al., 1984; Greene et al., 1986). In contrast, Elsasser (1984) reported that monensin lowered serum Ca content in sheep and altered intestinal Ca transport in chickens. We found that monensin decreased serum Ca values only in Experiment 3 and did not affect liver or kidney Ca contents. Kirk et al. (1985b) reported that monensin decreased Ca content of liver, but not kidney, tissue in sheep.

4. Phosphorus.

Serum P content was not affected by monensin treatment. This is consistent with reports that monensin did not influence serum or plasma P content in steers (Starnes et al., 1984) or sheep (Kirk et al., 1985a; Greene et al., 1986). However, 200 mg monensin/kg diet did lower liver, but not kidney, P concentrations. In contrast, Kirk et al. (1985a) found no effect of monensin on liver P content.

Serum P concentrations were higher in hyper-Se than hypo-Se pigs, particularly on day 7, but the reason is unclear.

5. Magnesium.

Monensin reportedly increased the apparent absorption and retention of Mg without affecting plasma Mg values in steers (Starnes et al., 1984) and sheep (Elsasser, 1984; Kirk et al., 1985b; Greene et al., 1986). We also found no effect of monensin on serum Mg content but liver Mg content was lower in pigs consuming 200 mg monensin/kg diet.

C. Microminerals.

1. Copper.

Monensin increased plasma Cu values in sheep (Starnes et al., 1984), but not cattle (Costa, 1987), and altered intestinal transport of Cu in chickens and hepatic Cu storage in chickens and sheep (Elsasser, 1984). We found that serum Cu values increased over time in Experiments 1 and 3, and decreased in Experiment 2, but that serum and tissue Cu contents were not affected by monensin treatment.

It was surprising to find that hypo-Se pigs were also relatively hypocupremic. Genetic regulation of Cu metabolism has been shown for different breeds of sheep in which differences in plasma Cu values were reportedly due to enhanced Cu absorption in hyper-Cu sheep, rather than to differences in systemic Cu utilization (Wiener et al., 1978). Selective breeding increased the difference between mean plasma Cu values and revealed that mortality (due to infectious disease and "swayback") was three times as high in hypo-Cu sheep (Jones et al., 1985; Weiner et al., 1985). When grazing Cu-deficient pastures, growth and wool production suffered in the hypo-Cu sheep.

Although both hypo-Cu and hyper-Cu populations of sheep were shown to be Se-deficient, there was no interactive effect of Cu and Se status

on disease prevalence (Jones et al., 1985). Hypo-Cu sheep had lower blood concentrations of the Cu-dependent enzyme, superoxide dismutase (SOD), than did hyper-Cu animals. Low erythrocyte SOD activity was found to be an even better predictor of the probability of increased mortality than was low plasma Cu content (Suttle et al., 1987).

The differences reported for mean plasma Cu values in the F1 hypo-Cu and hyper-Cu breeds of sheep were .2-.3 mg Cu/L (ug/ml) (Wiener et al., 1978), which are comparable to the differences in serum Cu concentrations observed for pigs in this study. Over all days, hyper-Se pigs had, on average, 2.0 ug Cu/ml serum, while hypo-Se pigs only had 1.8 ug Cu/ml serum. This would seem to be preliminary evidence for genetic control of serum Cu concentrations in pigs.

Since relative hypocupremia apparently occurred simultaneously with relative hyposelenemia, hypo-Cu pigs might have lower serum SOD as well as GSH-Px activities. Such pigs might be more susceptible to deficiencies in trace element-dependent enzyme activities if consuming inadequate dietary concentrations of Cu and/or Se. This would seem relevant to current interests in mineral - disease interactions (E. R. Miller, 1984) and to the controversial practice of supplementing 250 mg Cu/kg to swine diets (E. R. Miller and Kornegay, 1983). While it is tempting to postulate a single defect in divalent mineral uptake, Cu and Se are not known to share transport systems.

Oddly, hyper-Se/hyper-Cu pigs had lower liver Cu content than hypo-Se/hypo-Cu animals. This might imply an antagonistic role for hepatic storage of Cu and Se, even though liver Se content was not influenced by genetic Se status.

2. Iron.

Dvorak et al. (1980) and Costa (1987) found no effect of monensin on plasma Fe concentrations in cattle, but Elsasser (1984) reported altered intestinal Fe transport and hepatic Fe storage in chickens.

We found that monensin tended to increase serum Fe content in Experiment 2, where groups fed 0, 200 or 400 ppm monensin had average serum Fe concentrations of 2.81, 3.47, and 3.99 ug/ml, respectively, over all days. Tissue Fe content was not affected by monensin.

3. Zinc.

While Dvorak et al., (1980) reported no effect of monensin on plasma Zn values in cattle, others observed increased plasma Zn concentrations (Costa, 1987; Starnes et al., 1984). In sheep monensin influenced Zn balance without affecting plasma, liver or kidney Zn concentrations (Kirk et al., 1985b). Monensin increased hepatic storage of Zn in chickens and sheep in another study (Elsasser, 1984).

We found no effect of monensin on serum or tissue Zn concentrations, with the exception of lowered serum Zn values in Experiment 3.

SUMMARY

Monensin was fed with and without .1 mg supplemental Se/kg to growing pigs that had initial serum Se and vitamin E concentrations below expected ranges. No clinical abnormalities were noted in any of the pigs, although skeletal and cardiac myodegeneration was present in one of two pigs necropsied after death during the course of the study.

Serum Se concentrations and GSH-Px activity increased rapidly in Se-supplemented animals so that they approached acceptable ranges after 2-3 weeks and appeared to plateau. On the whole, serum Se values (range 26-250 ng/ml) and serum GSH-Px activity (range .22-2.44 EU/ml) were fairly well correlated. Serum vitamin E concentrations remained below the expected ranges despite supplementation (44 IU/kg).

Monensin had no significant effects on serum Se values or selenium balance, although individual animal variation in Se absorption, retention and excretion was considerable.

Monensin treatment significantly increased serum GSH-Px activities only in pigs fed 200 or 400 mg monensin/kg diet.

Serum leakage enzyme (CPK, AST) activities showed considerable variability among individual animals and sampling periods and were 2-4 times higher than the values expected for growing pigs. Monensin treatment had no significant effect on serum CPK values but tended to increase serum AST activity when fed at 400 mg/kg diet.

Serum concentrations of several minerals (Ca, Fe, K, P and Zn) were significantly affected by monensin. However, these effects were inconsistent among experiments, and may not have been of physiologically significant magnitude.

Although still within acceptable ranges, 35 days after withdrawal of monensin, the liver concentrations of Mg and P were decreased and hepatic Na content was increased in pigs which previously had consumed 200 mg monensin/kg diet.

There were no significant interaction effects of monensin and genetic Se status for any variable. Pigs from genetically hypo- and hyper-selenemic populations retained their relative Se status over the period of study and had significantly different serum Se concentrations and GSH-Px activities. Mean serum Se values for hypo-Se pigs were marginally adequate and responded more slowly to Se supplementation than those of hyper-Se pigs. Serum Se and GSH-Px values tended to plateau but at different levels in each genetic Se status group. Kidney Se content was greater in hyper-Se pigs than in hypo-Se pigs.

Hyper-Se pigs grew faster than hypo-Se pigs.

Hypo-Se pigs had higher serum CPK values, although no difference was seen in serum AST activity.

Serum concentrations of several minerals were influenced by genetic Se status; hypo-Se pigs had higher serum K concentrations and lower serum Cu concentrations. The difference in serum Cu values between hypo-Se and hyper-Se pigs was similar to that reported for hypo- and hypercupremic sheep. Hypo-Se pigs had higher Cu and lower Zn liver tissue concentrations than hyper-Se animals.

CONCLUSIONS

On the basis of the results obtained in the present investigation, the following conclusions can be drawn:

1. Many pigs raised on a diet believed to be adequate in Se and vitamin E content had initial serum Se and vitamin E concentrations below expected ranges for their age. Supplementation of .1 mg Se/kg and 44 IU vitamin E/kg increased serum Se, but not vitamin E, concentrations to acceptable values.

2. Serum GSH-Px activity was fairly well correlated with serum Se concentrations; both appeared to plateau after 3-4 weeks of Se supplementation.

3. Monensin may influence serum Se and GSH-Px values, but not consistently. In Experiment 1 monensin feeding, with or without Se supplementation, did not affect serum GSH-Px activity or serum Se values. In Experiment 2, monensin increased serum GSH-Px activity and tended to increase serum Se concentrations, but Experiment 3 failed to reveal any effect of monensin on Se metabolic balance.

4. Growing pigs fed .1 mg supplemental Se/kg in a basal diet tolerated a considerable amount of oral monensin from weaning to slaughter. Absence of clinical disease and only moderate increases in serum CPK and AST activities, despite marginal serum Se and vitamin E concentrations, suggest that the dietary Se and vitamin E provided were

sufficient to protect against monensin-induced muscle damage.

5. The effect of monensin, if any, on liver or kidney tissue Se content was not apparent at 35 days after monensin withdrawal.

6. Monensin influenced serum Ca, Fe, K, P and Zn concentrations in all experiments but not consistently. The magnitude of change in serum mineral values of treated animals was minor and may not be of physiological importance; the direction of change seemed unpredictable.

7. Some liver tissue mineral concentrations (Mg, Na, P) were altered by monensin when measured 35 days after monensin withdrawal, suggesting that monensin has long-term effects on mineral distribution and balance.

8. Genetically selected pigs maintained their relative Se status throughout the study. The average difference in serum Se concentrations (37 ng Se/ml) between F2 generation hypo-Se and hyper-Se groups was greater than that reported in the F1 generation groups in this herd. Hyperselenemia was associated with faster growth rate. Kidney Se concentration reflected genetic Se status since hyper-Se pigs had higher kidney Se concentrations.

9. Higher serum CPK activity in hypo-Se pigs treated with monensin is compatable with increased striated muscle damage, perhaps due to decreased GSH-Px membrane stabilization. Serum AST activities were comparably elevated in hypo-Se and hyper-Se pigs, suggesting that liver metabolism of monensin was similar for these two groups.

10. Serum concentrations of Cu, K, and P were affected by genetic Se status. Hypo-Se pigs had consistently lower serum Cu concentrations than hyper-Se pigs. Thus, serum Cu concentrations appear to be

genetically controlled in pigs, as has been shown in sheep.

11. Genetic Se status affected liver concentrations of Cu and Zn, as well as Se, suggesting that metabolism or storage of these elements may be different between these populations of pigs.

12. Based on tissue concentrations of vitamin E and Se, it is likely that similarities in clinical signs, clinicopathologic changes and pathologic lesions of monensin toxicosis and vitamin E-Se deficient disease are not the result of direct nutritional antagonism of monensin towards these two nutrients. However, both disease syndromes may share a common myopathic process: membrane damage, mediated through Ca^{+2} overloading and/or peroxidative events.

13. The unapproved use of monensin in swine may produce subclinical striated muscle damage and may precipitate clinical disease in animals with inadequate antioxidant status.

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APPENDICES

APPEN

Table

Groun

Soybe

Drie

Calc

Mono

Salt
Copp
L-ly
Vita
Vita
Sele
Mone
Tota

---

[a]Bas

[b]Wei

[c]Con

[d]Con

[e]Con

[f]Con

APPENDIX A.

Table 15. Experimental diet formulations.

------Ingredients------	Diets[a] Starter	Grower	Finisher
	(kg)		
Ground shelled corn (IFN 4-02-992)[b]	559.25-563	725.5-728.5	806.5-809.5
Soybean meal 44% (IFN 5-04-604)	250	235	165
Dried whey (IFN 4-01-182)	150	---	---
Calcium carbonate (IFN 6-01-632)	10	11	10
Mono-dicalcium phosphate (IFN 6-26-137)	15	15	7.5
Salt	2.5	5	2.5
Copper sulfate ($CuSO_4$-$5H_2O$)	0.5	---	---
L-lysine HCl (78.4% L-lysine)	1.5	---	---
Vitamin-trace mineral premix[c]	7.5	5.0	5.0
Vitamin E[d]	0.088	0.088	0.088
Selenium premix[e]	0-1.5	0.5	0.5
Monensin premix[f]	0-2.25	0-3.0	0-3.0
Total	1,000	1,000	1,000

[a]Basal diet has been previously reported to have natural Se content of 0.068 to 0.073 ppm (Stowe,1985).

[b]Weight of corn included in ration was adjusted after addition of Se and/or monensin to give a final total weight of 1000 kg.

[c]Containing the following vitamins and trace minerals per kg of premix: vitamin A, 660,000 IU; Vitamin D3, 132,000 IU; menadione sodium bisulfite, 440 mg as menadione; riboflavin, 660 mg; nicotinic acid, 3.5 g; D-pantothenic acid, 2.6 g; choline, 22 g; vitamin B12, 4 mg; Zn, 15 g; Fe, 12 g; Mn, 7.5 g; Cu, 2 g and I, 100 mg.

[d]Containing 500,000 IU vitamin E/kg. Vitamin E supplementation rate equals 44 IU/kg.

[e]Containing 200 mg Se/kg of premix as Na_2SeO_3. Premix was added at rates to give final concentrations of 0.1 or 0.3 mg Se/kg.

[f]Containing 130 g monensin/kg of premix. Premix was added at a rates to give final concentrations of 100, 150, 200, 300 or 400 mg monensin/kg.

APPEND

Table

Ag

*Val

APPENDIX B.

Table 16. Expected ranges for serum Se and vitamin E concentrations in pigs, based on age.

Age (days)	Serum Se (ng/ml)	Serum vitamin E (ug/ml)
fetal	70-90*	.3-.5
1-30	70-120	1.0-1.3*
31-70	100-160	.8-1.1
71-180	140-190	.9-1.2
>180	180-220	.9-1.2

*Values represent the expected range for serum samples from pigs of a given age but different backgrounds submitted for analysis to the Clinical Nutrition Department of The Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI.; Dr.Howard D. Stowe, personal communication.

APPEN

Table

Mult elem
Ca
Cu
Fe
K
Mg
Na
P[d]
Zn

*Va

+Va

[a]He
[b]In
[c]Se
[d]To
[e]Co

APPENDIX C.

Table 17. Expected ranges for serum, liver and kidney mineral concentrations in pigs, determined by inductively-coupled argon plasma emission spectroscopy.

	Serum (ug/ml=ppm)		Liver (ug/g wet wt)		Kidney (ug/g wet wt)	
Multi-element	range*	geometric means+	range*	geometric means+	range*	geometric means+
Ca	70-150	101	30-180	57	30-400	70
Cu	>0.7	1.9	7-125	15.8	1-15	3.9
Fe	>20[a]	2.9	30-1700[b]	216	14-206	62
K	100-300	---[c]	1400-3800	2587	1100-4200	2428
Mg	15-30	22.7	80-235	164	85-200	146
Na	2000-3600	3150	1000-2000	1032	950-2100	1298
P[d]	80-200	151	1600-4300	3203	1500-4000	2705
Zn	0.5-2.0[e]	1.2	30-210	64.0	10-40	18.7
	n = 321	289	890	961	817	912

*Values represent the expected range, based on samples from pigs of all ages and different backgrounds presented for postmortem examination to the Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI; Dr. Michael Slanker, personal communication.

+Values represent the geometric mean (n^{th} root of the product of n observations), based on samples from pigs presented to the Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI.; Dr. Michael Slanker, personal communication.

[a]Hemolysis of sample results in elevated serum Fe levels. Non-hemolyzed samples should be >5.0 but <20 ug/ml.

[b]Injection of young pigs with iron-containing products results in elevated liver Fe values.

[c]Serum K levels are usually not reported due to rapid postmortem changes and because they require use of a special "outrigger" channel.

[d]Total phosphorus.

[e]Contact of serum with rubber stoppers of collection tubes will contaminate the sample and elevate Zn levels (2.0-8.9 ug/ml).

APP

Tab

APPENDIX D.

Table 18. Error terms (PR>F) for main effects and interaction effects for variables in Experiment 1.

Variable	Group	Animal Within Group	Time	Group X Time
Body Weight	NS	.0001	.0001	NS
Serum Se	.0001	.15	.0001	.0001
Serum GSH-Px	.005	.0005	.0001	.0001
Serum CPK	NS	NS	.01	NS
Serum AST	NS	.0001	NS	.10
Serum Vit E	NS	.10	.0001	NS
Serum Ca	NS	.01	.0001	NS
Serum Cu	NS	.0001	.0001	NS
Serum Fe	NS	NS	.01	NS
Serum K	NS	.001	.0001	.05
Serum Mg	NS	.10	.0001	NS
Serum Na	NS	NS	.0001	NS
Serum P	.05	.05	.0001	.05
Serum Zn	NS	.01	.10	NS

Appendix E.

Table 19. Error terms (PR>F) for main effects and interaction effects for variables in Experiment 2.

Variable	Monensin Treatment	Se Status	Monensin X Se Status	Animal Within Monensin X Se Status	Time	Monensin X Time	Se Status X Time	Monensin X Se Status X Time
Body Weight	NS	.05	NS	.0001	.0001	.05	NS	NS
Serum Se	NS	.001	NS	.0001	.0001	.10	.001	NS
Serum GSH-Px	.01	.001	NS	.0001	.0001	.01	.05	NS
Serum CPK	NS	.01	NS	NS	.05	NS	.05	NS
Serum AST	.10	NS	NS	.0001	.001	.05	NS	NS
Serum Vit. E	NS	NS	NS	NS	.0001	.10	NS	.05
Serum Ca	NS	NS	NS	NS	.0001	.10	.05	NS
Serum Cu	NS	.01	NS	.0001	.0001	NS	.05	NS
Serum Fe	.10	NS	NS	NS	.05	NS	NS	NS
Serum K	.0001	.005	NS	NS	.0001	.0001	NS	NS
Serum Mg	NS	NS	NS	NS	.0001	NS	.10	NS
Serum Na	NS	NS	NS	NS	.0001	NS	NS	NS
Serum P	NS	.10	NS	NS	.0001	.05	NS	NS
Serum Zn	NS	NS	NS	.0001	NS	.01	.01	NS

Appendix F.

Table 20. Error terms (PR>F) for main effects and interaction effects for variables in Experiment 3.

Variable	Group	Animal Within Group	Time	Group X Time
Body Weight	NS	.0001	.0005	NS
Serum Se	NS	.05	.01	NS
Serum GSH-Px	NS	NS	NS	NS
Serum CPK	NS	NE	.10	NS
Serum AST	NS	.05	.01	NS
Serum Ca	.01	NS	NS	NS
Serum Cu	NS	NS	NS	NS
Serum Fe	NS	.005	NS	.005
Serum K	.05	.05	.0001	NS
Serum Mg	NS	NS	NS	NS
Serum Na	NS	.0005	NS	.10
Serum P	.05	NS	NS	NS
Serum Zn	.005	NS	NS	NS