Two concurrent studies were conducted to evaluate the effect
of selenium (Se) supplementation on beef cows. Trial 1 was designed
to evaluate the safety and efficacy of two intrareticular Se supple-
mentation delivery systems. Trial 2 was designed to evaluate the
transfer of Se across the placenta and through the colostrum in beef
cows.

One hundred fifty, selenium (Se) deficient, pregnant crossbred
beef cows were assigned to 1 of 4 treatment groups: group A, negative
control; group B, 1 Dura-Se bolus at 0 and 119 d; group C, 1 Dura-se
at 0 d; and group D, 2 Permasel pellets at 0 d. Cattle were main-
tained on selenium deficient pastures or forages prepared from these
pastures. Blood samples were collected from cows before treatment
administration (0 time) and thereafter at 28, 52, 119, and 220 d.
Calves from cows selected for data collection above were sampled at
birth. Blood samples were collected from calves, prior to suckling,
and were analyzed for whole blood selenium (WBSe) concentration.
Colostrum samples were collected from dams and analyzed for total Se
concentration. Additional samples were collected from calves 24-48

hours post suckling and analyzed for WBSe concentration and serum creatine kinase (CK) level. Weight and health data were recorded on all cows at each blood collection date. Birth weight, sex, and health were recorded on all calves at parturition.

Whole blood selenium concentration of cows from all supplemented groups were significantly higher (P<.001) than controls at all sample dates after treatments began. By the end of the 220 d study, treatment group B attained significantly higher (P<.001) WBSe concentrations than any other group. Calves from all Se treated groups had significantly (P<.001) higher WBSe concentrations, both pre- and post-suckling, than controls. Post-suckle WBSe concentration of calves were not significantly (P>.05) different than pre-suckle concentrations in any of the groups. Colostrum selenium concentration from Se supplemented cows were significantly (P<.001) higher than from control cows. No difference (P>.05) was determined in plasma CK activities or birth weights between groups of calves. Seven animals died, two of which showed signs of Se deficiency. Necropsy of 5 calves provided no evidence linking these deaths to selenium treatments. No differences (P>.05) in mortality between groups were determined.

Effects of Two Forms of Intrareticular Selenium
Supplements on Beef Cow Selenium Status and on
the Transfer of Selenium From Dam to Offspring

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF TWO FORMS OF INTRARETICULAR SELENIUM SUPPLEMENTS ON BEEF COW SELENIUM STATUS AND ON THE TRANSFER OF SELENIUM FROM DAM TO OFFSPRING

CHAPTER 1

INTRODUCTION

Historically, selenium (Se) was identified as a new chemical element in 1818 by Berzelius in Gripsolm, Sweden. The substance was coincidentally purified as a residue during the oxidation of sulfur dioxide from copper pyrites. From this humble beginning commercial demand for selenium has risen to an annual production of approximately 266 metric tons of selenium in the United States and a world wide production of 1,559 metric tons (Anonymous, 1979a, 1979b). After selenium's discovery, its biological significance went unnoticed until 1934 when Franke identified selenium as the toxic principle causing lameness and death of livestock grazing in the Dakotas and Wyoming (Franke, 1934). The possibility that selenium was an essential element for animals went widely unnoticed until Schwarz and Foltz in 1957 demonstrated that it was the effective component of "factor 3" preventing liver degeneration in rats fed torula yeast diets. In the same year, selenium was reported to prevent exudative diathesis in chicks (Schwarz and Foltz, 1957) and hepatosis dietetica in swine (Eggert et al., 1957). Nutritional muscular dystrophy in calves (Muth et al., 1958) and lambs (Hogue, 1958) was soon shown to

be prevented by increased levels of dietary selenium. The enzyme glutathione peroxidase (glutathione:H_2O_2 oxidoreductase, E.C. 1.11.1.9.) was discovered by Mills (1957), who found that this enzyme in the presence of reduced glutathione would protect erythrocytes against H_2O_2-induced and ascorbate-induced hemoglobin oxidation and hemolysis. Hence, glutathione peroxidase is said to possess antioxidant properties. Rotruck and coworkers (1971, 1972a) integrated previous information about the effect of added vitamin E and selenium on glutathione peroxidase's ability to protect erythrocytes (Mills and Randall, 1958; Cohen and Hochstein, 1963; Dam, 1957; Christensen et al., 1958; Gitler et al., 1958) and demonstrated that dietary selenium protected erythrocytes from ascorbic acid-induced hemolysis only if glucose was included in the incubation medium. Rotruck and coworkers (1972b, 1973) then focused on glutathione peroxidase and discovered that it was a selenoenzyme.

Currently, the only known biochemical function of selenium is as a component of glutathione peroxidase. The selenium deficiency signs observed in animals can be partially explained by a lack of glutathione peroxidase (Hoekstra, 1975), but this does not eliminate the possibility of other roles for selenium in animals. Recent work by Hawkes and coworkers (1985a) provides increased probability of other roles for selenium. He showed that the majority of an individual's total selenium content is not found in glutathione peroxidase but has been identified in numerous proteins and components of cell membranes (Black et al., 1978; Hawkes et al., 1985a). Complete characterization of the proteins and elucidation of

their function has been difficult and incomplete. Moreover, a non-seleno-glutathione peroxidase exists (Lawrence and Burk, 1978), raising questions as to the precise role of seleno-glutathione peroxidase.

Selenium deficiency in livestock provokes a myriad of diseases with a potential to cause enormous yearly economic loss to producers. These diseases range from the well recognized, severe condition of nutritional myodegeneration (NMD), "white muscle disease", to the numerous less explicit conditions often referred to as selenium-associated or selenium-responsive diseases. Some of these diseases are characterized by muscular weakness of the newborn, unthriftiness, reduced weight gain, diarrhea, stillbirth, abortion and diminished fertility. Perhaps the greatest loss to the producer results from the unrealized gains of unthrifty animals having subclinical disease symptoms.

Recent advances of delivery systems for drugs and feed additives have made it more practical for beef cattle producers to manage the health and nutrition of their herds. The research reported here was designed to evaluate the safety and efficacy of two delivery systems for selenium supplementation and the transfer of selenium from cows to calves.
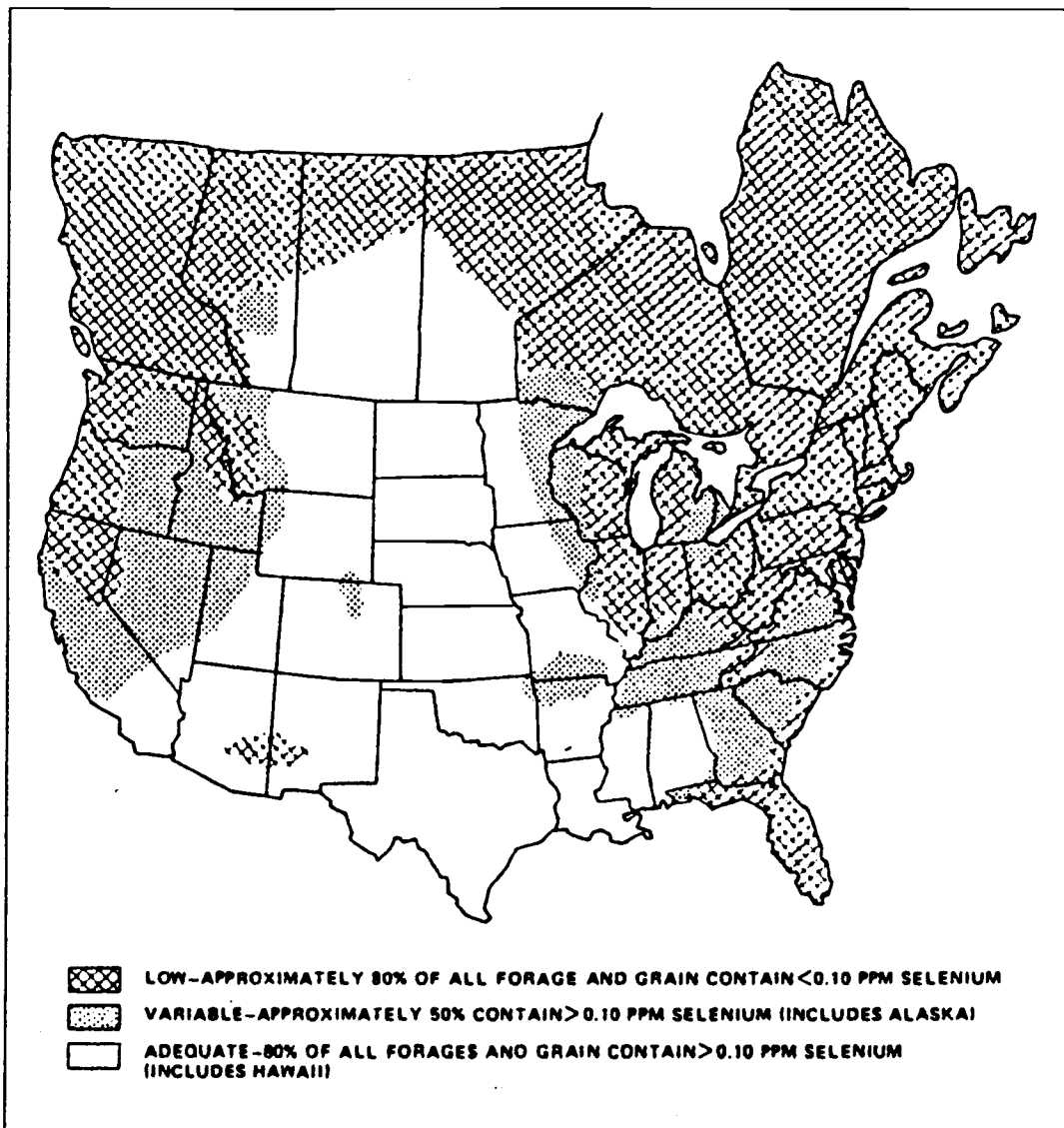
CHAPTER 2

LITERATURE REVIEW

Selenium in Feedstuffs

The distribution of selenium levels in forages and grains in various areas of North America is shown (Figure 1). The most deficient regions are the Pacific Northwest, Northeast, the Atlantic Coastal Area, Florida, and regions surrounding the Great Lakes. The prevalence of selenium deficiency diseases in animals throughout the United States correlates closely with the areas having low ($\leq$ 0.05 ppm) plant selenium content (Kubota et al., 1967). Grains and forages sampled between the Rocky Mountains and the Mississippi River were generally adequate ($\geq$ 0.1 PPM) in selenium and, in some cases, contained excessive levels of the element (Kubota et al., 1967). Research by Hoffman et al. (1973) in Canada showed that selenium in tissues from lambs and calves generally was lower in the eastern area and higher in the central to western portion of the country. Thus, the geographic pattern and the distribution of selenium apparently is similar in the United States and Canada.

Selenium moves through a soil-plant-animal cycle (Lawrence and Burk, 1978; Allaway et al., 1967; Allaway, 1973). Sedimentary rocks provide most of the selenium that becomes incorporated into soils. Alkaline and well-aerated soils provide much higher amounts of selenium available to growing plants than acid, poorly aerated soils. This difference in availability from soils is related to the chemical

Figure 1.1 Selenium concentrations in feedstuffs in the United
States and Canada.

LOW-APPROXIMATELY 80% OF ALL FORAGE AND GRAIN CONTAIN<0.10 PPM SELENIUM

VARIABLE-APPROXIMATELY 50% CONTAIN>0.10 PPM SELENIUM (INCLUDES ALASKA)

ADEQUATE-80% OF ALL FORAGES AND GRAIN CONTAIN>0.10 PPM SELENIUM
(INCLUDES HAWAII)

form of selenium and not to the selenium concentration in the soil. Soluble selenates predominate in alkaline soils, and sparingly soluble selenites complexed with iron salts are in acid soils.

The "apparent" distribution of selenium in North American soils could be misleading if one drew conclusions from figure 1 alone. Nevertheless, it is the amount of selenium available to the animal that is important nutritionally. In research cited by Ullrey (1974) 20 different corn hybrids grown in the same location in Michigan had an average selenium content of 0.014 parts per million (ppm) with a range of 0.007 to 0.024 (dry matter basis), suggesting differences in the capability of various hybrids to concentrate selenium in the grain. Effect of location was pronounced in the same study when a single hybrid grown in 17 locations within the state yielded in the grain an average of 0.03 and a range of 0.013 to 0.089 ppm selenium on a dry matter basis.

Dietary Forms

A number of organic selenium compounds have been identified in plants or plant products. These compounds include selenocystine, selenocysteine, Se-methyl-selenocysteine, seleno-homocystine, seleno-methionine, Se-methylselenomethionine, selenomethionine selenoxide, selenocystathionine, and dimethyl diselenide (Shrift, 1969). There is some evidence for the presence of selenite and selenate in plants (Butler and Peterson, 1967; Olsen et al., 1970). Selenocystine, selenocysteine, selenomethionine, and Se-methylseleno-methionine, however, appear to be the major selenium compounds in seeds or

forages commonly consumed by livestock (Peterson and Butler, 1962; Shrift, 1969; and Olson et al., 1970). Thus, organic selenium is the major form for animals consuming natural feeds.

Feeds and forages grown in certain areas of North America, however, do not contain enough selenium to meet livestock requirements (Figure 1). One way to correct this deficiency has been to mix grains grown in high selenium areas with selenium-deficient feeds (Ullrey et al., 1977). Approval has been given to add inorganic selenium to feeds deficient in this element, and the most common form used is sodium selenite. Selenized yeast tablets, containing primarily organic selenium, are available as human supplements and have been shown to increase blood selenium levels (Schrauzer and White, 1978).

Absorption

The absorption of selenium is significantly lower in ruminants than in monogastric animals. The retention of selenium taken orally as selenite was found to be 77 percent in swine as compared to only 29 percent for sheep (Wright and Bell, 1966). Essentially, no absorption of selenium occurred in the rumen and abomasum, and the greatest absorption of selenium occurred in the small intestine and the cecum of sheep. In swine, no absorption occurred in the stomach and the first part of the small intestine, and the greatest absorption occurred in the last part of the small intestine, the cecum, and the colon (NRC, 1983). The rumen microorganisms are probably responsible for the lower absorption of selenium in

ruminants than in non-ruminants. Much of the dietary selenium is reduced to insoluble forms by rumen microbes (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Whanger et al., 1968). A greater percentage of inorganic rather than organic selenium is converted into these insoluble forms, and a high-carbohydrate diet is more favorable to selenium conversion into insoluble forms than a high-roughage diet (Whanger et al., 1968).

Even though rumen microbes convert a portion of the selenium into insoluble forms, they also incorporate selenium into their proteins. Selenomethionine was shown to be incorporated into bacterial proteins when rumen fluid was incubated *in vitro* with this compound (Paulson et al., 1968b). Characterization of ^{75}Se-containing compounds in rumen microbes revealed the presence of ^{75}Se-selenomethionine after incubation with ^{75}Se-selenite in vitro (Hidiroglou et al., 1968). Thus, rumen microbes appear to be able to convert inorganic selenium to organic selenium compounds, as well as to incorporate organic selenium compounds into bacterial proteins.

By use of everted intestinal sacs of hamsters, McConnell and Cho (1965) found that selenomethionine was transported against a concentration gradient, whereas selenite and selenocystine were not. The transport of selenomethionine was inhibited by methionine, but the transport of selenite and selenocystine was not inhibited by their respective sulfur analogues.

Vascular Transport

From 75 to 85% of the selenium in ovine erythrocytes is associated with glutathione peroxidase (GSH-Px) (Oh et al., 1974). Essentially all of the selenium in erythrocytes of rats is associated with GSH-Px, but only about 10% of the selenium is associated with this enzyme in human erythrocytes (Behne and Wolters, 1979). Even less of the selenium (1.5%) in human plasma is associated with this enzyme. Patterns similar to those in humans have been noted for the rhesus monkey (Whanger, unpublished observation). Thus, the major proportion of selenium is associated with GSH-Px in rat or sheep erythrocytes, but not in primate erythrocytes.

Even though there are differences in the amount of selenium associated with GSH-Px between primates and other animals, the metabolism of selenite by blood *in vitro* is similar. The uptake and release of selenite by bovine (Jenkins and Hidiroglou, 1972) or human erythrocytes (Lee et al., 1969) involve sulfhydryl groups, and the binding of selenite to plasma protein is dependent upon the presence of erythrocytes (Lee et al., 1969; Sandholm, 1974, 1975). This binding of selenium to plasma proteins is not energy dependent and does not require protein synthesis (Porter et al., 1979).

Body Retention and Tissue Distribution

The tissue distribution of selenium in various animals using either ^{75}Se or stable selenium has been reported by several investigators. With required dietary selenium intake, the kidney contains the highest concentration of selenium, followed by the liver

and other glandular tissue such as the spleen and pancreas.

Intestinal and lung tissue can have relatively high concentrations of

Se. Cardiac muscle contains appreciably more selenium than skeletal

muscle. Wool and hair may have relatively high concentrations of Se,

but nervous tissue has low concentrations (Allaway et al., 1966; Ewan

et al., 1968b; Oh et al., 1976b; Paulson et al., 1968a; Ullrey et

al., 1978; Ammerman et al., 1980; Kincaid et al., 1977).

Not only is the tissue content of selenium dependent upon the

level in the diet, but also upon the chemical form. In general,

selenium is deposited in tissues at higher concentrations when

present in diets as organic rather than as inorganic selenium.

The content of selenium in human tissues appears to show

patterns similar to tissues from animals fed diets with required

levels of selenium. The mean selenium content (wet-weight basis) was

highest in kidney (1.09 ppm) followed by liver (0.54 ppm), spleen

(0.34 ppm), and testes and pancreas (0.30 ppm), with the lowest

content in brain (0.13 ppm) for humans ranging from 9 months to 68

years of age (Schroeder et al., 1970). The peak of selenium

retention on a whole-body basis (Muth et al., 1967) and in tissue

such as blood, liver, muscle, kidney, spleen, and lung (Brown and

Burk, 1973) is reached within 24 hours after the injection of ^{75}Se as

$SeO_3^=$.

Glutathione Peroxidase

In 1957, Mills discovered an enzyme, glutathione peroxidase

($GSH:H_2O_2$ oxidoreductase, EC 1.11.1.9), which protected erythrocytes

against hemoglobin oxidation and hemolysis (Mills, 1957). Vitamin E, supplemented in the diet or added to the incubation medium, protected erythrocytes against hemolysis (Dam, 1957), but dietary selenium was at first reported to be without effect (Christensen et al., 1958; Gitler et al., 1958). Addition of glucose to the medium was shown to protect erythrocytes against hemolysis by maintaining glutathione (GSH) levels and thus providing reducing substrates for glutathione peroxidase (GSH-Px) (Mills and Randall, 1958; Cohen and Hochstein, 1963). Rotruck et al. (1971, 1972a, 1972b, 1973) integrated these facts and demonstrated that dietary selenium would protect erythrocytes from hemolysis, but only if glucose was included in the medium. The ability of vitamin E to prevent hemolysis was not affected by glucose, and glucose had no protective effect if the erythrocytes were from Se-deficient rats. Rotruck et al. (1973) focused on glutathione peroxidase and discovered that it was a selenoenzyme.

In the intervening years much has been learned about the biochemistry of selenium and GSH-Px. The selenium deficiency signs observed in animals can, for the most part, be logically explained by a lack of GSH-Px (Hoekstra, 1975). Nevertheless the full extent of the role of GSH-Px *in vivo* is not known, and the possibility of other roles for selenium in animals cannot be eliminated.

Structure of Glutathione Peroxidase

Glutathione peroxidase is an enzyme with a molecular weight of about 80,000 consisting of four apparently identical subunits.

Sedimentation equilibrium determinations have indicated that the molecular weight of GSH-Px differs from species to species 76,000 $\pm$1,000 for rat liver, 83,800 $\pm$1,200 for bovine erythrocyte, 95,000 $\pm$ 3,000 for human erythrocyte (Nakamura et al., 1974; Flohe et al., 1971a; Awasthi et al., 1975). The molecular weight of GSH-Px also can vary from tissue to tissue in the same species (Sunde et al., 1978; Awasthi et al., 1979). After Rotruck et al. (1973) reported at least 2 g-atoms of selenium per mole of GSH-Px, Oh et al. (1974) and Flohe et al. (1973) independently demonstrated that GSH-Px from ovine and bovine erythrocytes contained 4 g-atoms of selenium per mole of GSH-Px. This value has been confirmed by Nakamura et al. (1974) and Awasthi et al. (1975) for rat liver and human erythrocytes GSH-Px. In contrast to other peroxidases, GSH-Px contains no heme or flavin moieties (Flohe et al., 1971a), and neutron activation analysis of the crystalline enzyme indicates that GSH-Px contains no metals other than selenium (Flohe et al., 1973).

Forstrom et al. (1978) and then Wendel et al. (1978) reported that the selenium in reduced GSH-Px is present as selenocysteine. Amino acid analysis of rat liver GSH-Px showed that each subunit also contains two cysteine and three methionine residues out of a total of 153 amino acids (Nakamura et al., 1974). The amino acid composition of bovine erythrocyte GSH-Px is similar, with 178 amino acid residues per subunit. A tentative amino acid sequence has been published (Ladenstein et al., 1979).

The GSH-Px tetramere, composed of two dimers, appears to be an almost flat, planar molecule with dimensions of 90.4Å x 109.5Å x

58.6Å. The active sites are located in flat depressions on the surface, and each active site is probably built up of regions from two subunits. Unlike other peroxidases, GSH-Px has high specificity for its donor substrate, GSH (Mills, 1959). Flohe et al. (1971c) found that no other thiol substrate studied had more than 30% of the activity and most had less than 10% of the activity of GSH. In contrast to catalase, GSH-Px will destroy a variety of hydroperoxides at rates similar to those of H_2O_2 destruction (Little and O'Brien, 1968). Various lipid hydroperoxides, steroid hydroperoxides, thiamine hydroperoxide, nucleic acid hydroperoxides, prostaglandin hydroperoxides and a hypothetical vitamin K hydroperoxide have been shown to be substrates for GSH-Px (Gunzler et al., 1972; Little, 1972; Christophersen, 1969; Nugteren and Hazelhof, 1973; Larson and Suttie, 1978). Only cholestrol-25- and cholestrol-7alpha-hydroperoxide have been reported to be poor substrates (Little, 1972). Because almost all peroxidase substrates elicit similar maximal velocities, an acceptor-enzyme complex is most likely not formed during catalysis.

The kinetic mechanism of GSH-Px is best described as a series of three biomolecular steps (Flohe et al., 1972; Flohe and Gunzler, 1974). The first step of this ter uni ping pong mechanism is the oxidation of the enzyme by the peroxide substrate and release of the corresponding alcohol (or water in the case of H_2O_2). This is followed by two successive additions of GSH, and then GSSG release. This mechanism takes into account the inability to saturate the enzyme with either substrate, and the similar V_{MAX} for most peroxide

substrates. The formation of tertiary or quaternary complexes during catalysis is not predicted from the kinetic data.

Function of Glutathione Peroxidase

The discovery that organic hydroperoxides as well as H_2O_2 are substrates for GSH-Px (Little and O'Brien, 1968) provided an important clue as to the biochemical function of GSH-Px, and thus of selenium. The erythrocyte possesses both catalase and GSH-Px activity. The rate of H_2O_2 reduction per heme or per Se, respectively, is nearly identical for these two enzymes in the erythrocyte (Flohe et al., 1972). Because of the greater concentration of catalase in the erythrocyte, catalase would seem to be far more important than GSH-Px for H_2O_2 destruction. GSH-Px deficient erythrocytes, however, are susceptible to hemolysis when exposed to oxidizing agents, indicating that the ability to reduce organic hydroperoxides may be of critical importance to the erythrocyte.

Except in degenerate cells like the erythrocyte, catalase and GSH-Px often are localized in distinct compartments (catalase in the peroxisomes and GSH-Px in the cytosol and mitochondrial matrix space) such that there is little direct overlap in the competition for H_2O_2 (Flohe et al., 1976). In human and guinea pig leukocytes, catalase activity is high and GSH-Px activity is low, whereas in rat and mouse leukocytes catalase activity is low and GSH-Px activity is high (Higgens et al., 1978). This observation further demonstrates that these two enzymes apparently complement each other in the protection

of cells against peroxidation.

Vitamin E deficiency in animals, like selenium deficiency, causes degenerative lesions in tissues. The nature and location of the lesion depends on the species and the animal's nutritional status with respect to other nutritional factors. For example, in rats a combined deficiency of vitamin E and selenium results in liver necrosis (Schwarz and Foltz, 1957). Vitamin E deficiency alone causes fetal death and resorption (Evans and Bishop, 1922), and selenium deficiency alone results in poor growth and failure to reproduce in second generation, Se-deficient rats (McCoy and Weswig, 1969). Chicks develop muscular dystrophy, encephalomalacia, exudative diathesis or pancreatic degeneration, depending on the presence or absence of vitamin E, Se, sulfur amino acids, synthetic lipid antioxidants and excess dietary unsaturated fatty acids (Scott, 1978).

The effects of vitamin E and selenium deficiency have been postulated to result from loss of membrane integrity which leads to cell death. Addition of polyunsaturated fatty acids to the diet tends to exacerbate these deficiency defects, whereas synthetic antioxidants in many cases will alleviate the signs of vitamin E and selenium deficiency.

Hydrogen peroxide, hydroperoxides, superoxide, various free radicals including hydroxy radical and possibly singlet oxygen are products of normal cellular reactions as well as products of the metabolism of toxic substances (Sunde and Hoekstra, 1980). The effectiveness of an enzyme (or alpha-tocopherol) to contain and

ultimately destroy these reactive species depends on both the specificity of the enzyme (or alpha-tocopherol) and its subcellular location. Thus in a typical animal cell, lipid-soluble alpha-tocopherol scavenges free radicals and possibly quenches singlet oxygen in the membranes (McCay et al., 1978). Glutathione peroxidase and superoxide dismutase react with peroxides and superoxide, respectively, in the cytosol and mitochondrial matrix space, and catalase destroys H_2O_2 in the peroxisomes. The relative concentration and the importance of these protective species vary from tissue to tissue and from species to species, and probably result in the variety of selenium and/or vitamin E deficiency signs observed in different species.

In deficiency diseases that can be prevented by either vitamin E or selenium, such as liver necrosis in the rat, the cells seemingly have alpha-tocopherol and GSH-Px organized in serial arrangement McCay et al. 1976). The radicals or pro-oxidant species in such cases appear to originate in or diffuse through the soluble portion of the cell with their ultimate molecular target being the membrane. If GSH-Px does not destroy the harmful species, presumably a peroxide, then alpha-tocopherol can still protect the membrane. Although lipid hydroperoxides are excellent substrates for GSH-Px, this soluble enzyme was reported not to reduce lipid hydroperoxides (within membranes) to the corresponding alcohols in vitro, but instead GSH-Px was postulated to prevent the peroxidation by destroying H_2O_2 and thus prevent the formation of hydroxy radical (McCay et al., 1976).

In deficiency diseases that are prevented only by selenium and not vitamin E, such as pancreatic degeneration in the chick, apparently both the origin and target of the destructive species are in the water-soluble portion of the cell, which is not protected by lipid-soluble alpha-tocopherol. In deficiency diseases, such as encephalomalacia in the chick, which can be prevented by vitamin E but not selenium, both the origin and the target of the pro-oxidant species presumably lie within the hydrophobic regions of the cell membranes. The differential ability of various antioxidants to replace vitamin E and prevent deficiency diseases has been related to their differential ability to quench these species, and to the differential solubility of the antioxidants in the hydrophobic tissues and membranes of the body.

In 1976, Lawrence and Burk (1976) discovered that selenium deficient rat liver had non-Se-dependent GSH-Px activity that was found to be due to one or more of the GSH-S-transferases (Prohaska and Ganther, 1977). These enzymes have no catalytic ability to destroy H_2O_2 but will reduce organic hydroperoxides such as cumene- and t-butyl hydroperoxide. Burk et al. (1980) demonstrated that semi-purified GSH-S-transferase blocked the formation of malondialdehyde, a breakdown product of peroxidized fatty acids, in an NADPH-microsomal lipid peroxidation system, but GSH-Px did not. Thus, the GSH-S-transferases also may be important in the protection of cells against peroxidation.

Selenium may have other biochemical functions in higher animals not resulting from the ability of GSH-Px to serve as a biological

antioxidant. A 10,000 dalton mammalian selenoprotein has been reported to be present in muscle of Se-adequate lambs but absent in lambs suffering from nutritional muscular dystrophy (Pedersen et al., 1972; Whanger et al., 1973; Black et al., 1978). The claim that it is a selenoprotein must remain tentative until the protein is characterized and the selenium stoichiometry determined. Spermatozoa have an as yet uncharacterized, 15,000 to 20,000 dalton selenoprotein that may serve as a mitochondrial structural protein, or as an enzyme, although the possibility that this protein is a GSH-Px subunit or its derivative has not been eliminated (Calvin, 1978; Pallini and Bacci, 1979; McConnell et al., 1979b). Selenium has been shown to protect against the toxicity of heavy metals such as mercury, cadmium and silver, apparently by altering the nature, retention and distribution of these metals within the body (Parizek et al., 1974; Ganther et al., 1972; Wagner et al., 1975). Selenium may have a role in the regulation of heme catabolism, (Correlia and Burk, 1976) as a component of rare purine or pyrimidine bases (Hoffman and McConnell, 1974; Chen and Stadtman, 1980) and in resistance to infection (Yarrington et al., 1973; Serfass and Ganther, 1975, 1976) or cancer, (Schrauzer et al., 1978; Poirier and Milner, 1979) although the biochemical mechanisms behind these interactions remain unknown.

In addition to the role of GSH-Px as an antioxidant, it is attractive to postulate that GSH-Px may have a specific function in the metabolism of certain hydroperoxides, such as prostaglandin hydroperoxides (Nugteren and Hazelhof, 1973; Christ-Haselhof and

Nugteren, 1978). However, no evidence has been produced to prove that GSH-Px is essential for any biosynthetic pathway in vivo.

Nature of Selenium Deficiency Problems

A wide variety of selenium deficiency symptoms are observed in numerous species with specific species differences. These uniquenesses among species are related to the management and environmental conditions encountered by the animals. Therefore while most readily observable symptoms may vary from species to species, many of the symptoms do occur across the various species involved with variation in the apparent magnitude and consequence of the symptom.

The occurrence of selenium deficiency in the diets of domesticated ruminants is associated largely with muscular degeneration or weakness. Most prominent among the conditions is nutritional myodegeneration (NMD) a disease that has occurred most widely in sheep (Muth, 1963), but also occurs in cattle.

This disease appeared as a prominent economic condition in the improved grazing areas of the United States and Canada after World War II (Muth, 1955, 1963). Its association with selenium deficiency came immediately after the discovery of the nutritional importance of selenium (Hogue, 1958; Muth et al., 1958). Selenium has effectively prevented the disease when administered to young cattle (Hartley and Grant, 1961) or to pregnant cows (Oksanen, 1965). Examples of the forms of the disease observed on farms are described by Hartley and Grant (1961) and Andrews et al. (1968). Lambs affected with the

congenital form are either born dead or die suddenly after physical exertion a few days following birth. Myocardial, liver, and body cavity lesions are observed, but skeletal musculature is rarely affected. The "delayed" form occurs mostly between 3 and 8 weeks of age. The predominant sign is muscular weakness. The lambs walk with a stiff gait and arched back, avoid movement, lose condition, and die. Skeletal muscles show degeneration, but cardiac lesions are not always present. Acute clinical signs can be exacerbated by physical and/or mental stress. Cawley and Bradley (1978) reported that 2-month-old calves on four different farms died suddenly after a period of excitement. Histopathological examination revealed acute myocardial degeneration. Biochemical examination of other animals in the herd showed selenium deficiency. Selenium injections and feeding corrected the problem.

Lesions of nutritional myodegeneration in cattle and sheep are primarily calcification and degeneration of skeletal muscle and myocardium (Bonucci and Sadun, 1973). The affected areas are the left ventricular wall and the interventricular septum of the heart. The most active skeletal muscles having the greatest work requirement are the common sites of lesions. The abductors of the thigh are commonly involved in the neonate, and the longissimus dorsi and triceps are affected in the 3 to 8 week old.

The activities of lactic dehydrogenase, creatine kinase, 5'-nucleotidase, and glutamic, oxalic, and pyruvic transaminase in the serum or plasma are increased (Buchanan-Smith et al., 1969; Whanger et al., 1976). Hoffman et al. (1973) concluded that a

selenium concentration below 5 ppm in kidney cortex (dry matter) and 0.5 ppm in muscle of calves and lambs might be used as an indication of selenium deficiency in ruminants, although lower muscle selenium concentrations have been observed in apparently normal calves and lambs (Ullrey et al., 1977).

Nutritional myodegeneration is a multietiological problem, and this is manifested in the histopathology of the sarcomere. The initial histological lesions seen by Muth (1966) in affected animals are microscopic deposits of calcium midway between the Z-bands of the sarcomere. In Canada, treatment with both vitamin E and selenite were most successful (Hoffman et al., 1973). In northern Europe, alpha-tocopherol has been considered the most effective component of the selenium-vitamin E mixture in preventing nutritional muscular dystrophy. In these countries, the problem is associated with poor curing of lightly fertilized grass forages and with an alteration of the unsaturated fat components in the cured hays. As a result the primary lesion of the sarcomere is degeneration and calcification is secondary if seen at all (Oksanen, 1967).

Other Selenium Responsive Diseases

Cattle, as well as other species, have shown reduced weight gain due to selenium deficiency and respond to selenium supplementation by increasing gain (Allaway and Hodgson, 1964; Shirley et al., 1966; Andrews et al., 1968; Ammerman et al., 1980; Johnson et al., 1981; Koller et al., 1984). While numerous studies have considered the relationship between selenium status and GSH-Px, no precise mechanism

has been established for the decreased weight gain associated with selenium deficiency. The proposed antioxidant theory has been assumed responsible but no specific site of action has been determined as critically significant toward reduced weight gain and the subsequent selenium responsiveness. Although apparently no published trials are available, improved feed efficiency without increased weight gain has been observed (Drake, personal comm.).

Other growth and development symptoms associated with selenium deficiency include general unthriftness (illthrift), weak calf syndrome and scours or diarrhea. Illthrift has been best characterized in sheep by Andrews et al. (1968) as variable, ranging from subclinical inability to maintain optimum growth rate to clinical unthriftiness and increased mortality. Affected lambs may thrive for several months only to show reduced weight gain. Anorexia and diarrhea may be associated, and the fleece is harsh and dry. There may be no increases in serum aspartate transaminase (AST) levels. Post mortem findings are emaciation and osteoporosis with no characteristic microscopic lesions. In contrast, Harsch (1976) found increased serum AST levels in yearling cattle identified as suffering from illthrift and were selenium deficient and responsive to supplemental selenium. Williams et al. (1981), in Northern California, found that cattle from ranches historically suffering from illthrift had whole blood selenium concentrations below 0.04 ppm. No such association was found with the weak calf syndrome (Willliams et al. 1981). Kendall (1960) identified cattle with diarrhea from Northern California as selenium deficient and

responsive to supplemental selenium.

Selenium deficiency effects have been observed in reproductive performance of both male and female bovine. Trinder et al. (1969) observed a beneficial effect of selenium with vitamin E on reducing the incidence of retained placenta in dairy cattle. Blood selenium concentrations were also lower in dairy herds with a higher incidence of retained placenta. Julien et al. (1976) using a selenium and vitamin E injection significantly reduced retained placenta in four large commercial dairy herds. Williams et al. (1981) did not find an association between selenium levels and retained placenta or abortions in beef cattle. Differences in response between beef and dairy cattle may relate to ration components. Reinhardt et al. (1978) found that feeding dairy cattle alfalfa silage reduced the effectiveness of selenium and vitamin E injections that typically are effective in rations without alfalfa silage.

The importance of selenium in male reproduction is indicated by the concentration of ^{75}Se as $SeO_3^=$ in the reproductive organs. In contrast to other tissues, the maximum incorporation of selenium into testes and epididymis of rats was reached 2 to 3 weeks after injection (Brown and Burk, 1973; and McConnell et al., 1979b). As an indication of the accumulation of selenium in male reproductive organs, about 40 percent of the total body ^{75}Se was found in testes plus epididymis of rats 3 weeks after injection (Brown and Burk, 1973). Within the sperm, ^{75}Se became associated primarily with the midpiece of the sperm tail. Calvin (1978) extended these studies with rat sperm and found the selenium to be primarily in the tail

keratin, a disulfide-stabilized fraction obtained by extracting isolated tails with sodium dodecyl sulfate. The name selenoflagellin was proposed for this selenium-binding polypeptide of 17,000 daltons. Calvin suggested that this molecule may be essential for proper assembly of the rat sperm tail. In bulls, the peak in accumulation of ^{75}Se in whole semen was reached 40 days after injection (Smith et al., 1979), which is similar to results with a ram in which the maximum incorporation of ^{75}Se in sperm was reached 49 days after injection (Tripp et al., 1979). In the bull, 23 days after injection, the epididymis and testis retained the greatest amount of ^{75}Se (cpm/g basis), except for the kidney. Selenium additions (1 ppm) to diluted semen increased motility and oxygen consumption in 13 of 15 ejaculates (Julien and Murray, 1977; Pratt, 1978). Wu et al. (1979) demonstrated that selenium is necessary for the production of morphologically normal spermatozoa in rats. Segerson et al. (1981) found no changes in sperm morphology or viability in boars fed a diet containing 0.025 ppm selenium and 33 IU vitamin E/kg from about 77 to 250 days of age. It's important to note that these boars may not have been on the selenium deficient diet long enough to become selenium deficient.

Selenium's involvement with the immune system has been measured in several different species experimentally and may be supported by its widespread use as both a preventive and treatment for calf scours. Dietary supplements of either selenium, vitamin E or both did not reduce the incidence of mastitis in dairy cows but did shorten the duration of clinical symptoms (Smith et al., 1984).

Norman and Johnson (1976), using a selenium and vitamin E injection, observed increased antibody titers to Leptospira pomona vaccine in supplemented calves. Experimentally induced swine diarrhea was less prevalent in animals given dietary selenium and vitamin E supplementation (Teige et al., 1978). Increased responses to various vaccines by selenium supplementation were seen in rats (Spallholz et al., 1973, 1974, 1975), swine (Peplowski et al., 1981), and rabbits (Berenstein, 1972). Selenium supplementation increased weight gain of chickens exposed to coccidiosis (Eimeria tenella) whether immunized or not (Colnago et al., 1984).

There is strong evidence that selenium functions biochemically in neutrophils of steers (Boyne and Arthur, 1979). There was no detectable GSH-Px activity in selenium-deficient neutrophils, whereas activity was detected in the selenium-adequate group. Selenium deficiency in steers does not affect the ability of neutrophils to phagocytize bacterial cells. The deficiency does cause a significant reduction in the ability of the phagocytic neutrophils to kill ingested bacteria (Boyne and Arthur, 1979). A similar decrease in antimicrobial activity has also been reported in the neutrophils of selenium-deficient rats (Serfass and Ganther, 1975). Alterations of microtubular function in GSH-Px-deficient polymorphonuclear leukocyte has been reported by McCallister et al. (1980), which may explain why selenium deficiency impairs the killing ability of phagocytic cells.

Delivery Systems for Supplemental Selenium

The primary selenium compounds consumed by livestock from natural plant foodstuffs are selenocystine, selenocysteine, selenomethionine and se-methylselenomethionine (Peterson and Butler, 1962; Shrift, 1969; and Olson et al., 1970). Inorganic forms of selenium, usually as sodium selenite or selenate, are available, efficacious and approved for use as selenium supplementation by various routes in several countries including the United States.

The efficiency of supplementation with various forms of selenium is variable and depends on the parameter for determining efficiency, stage of production and previous selenium status. Selenium in alfalfa was comparable to sodium selenite for prevention of liver necrosis in rats (Mathias et al., 1965) and selenium in milk was as effective as sodium selenite for liver necrosis protection in rats but milk was superior for prevention of exudative diathesis (Mathias et al., 1967). Miller et al. (1972) also found differences in the effectiveness of various selenium forms for prevention of exudative diathesis, decreasing from selenomethionine to selenite and fishery products. Similarly, Cantor et al. (1975a) found selenomethionine more effective than sodium selenite for prevention of pancreatic fibrosis in the chick.

Consistently higher tissue selenium levels have been observed in cattle, sheep, swine and poultry when organic selenium is fed, compared to sodium selenite. Selenium from grain results in higher muscle, liver, kidney and plasma contents than selenite when both were fed at 0.2 or 0.3 ppm in the diet of sheep and cattle (Ullrey et

al., 1977). Mahan and Moxon (1978), reporting on swine fed selenium at 0.4 ppm of the diet found muscular selenium content was higher from fish meal or brewers' grains than selenite, but nonmuscular tissues, liver, kidney and testes, were similar. Organic selenium such as selenomethionine, has also been more effective than selenite at raising tissue concentrations of muscle, liver and eggs of poultry (Latshaw and Osman, 1975; Osman and Latshaw, 1976; Latshaw and Biggert, 1981).

While organic selenium forms are superior to inorganic forms in increasing tissue selenium levels, selenite is at least equal to or perhaps superior at low levels, to organic selenium in increasing GSH-Px levels. Selenium as a single large oral dose of selenite or selenomethionine in rats (Pierce and Tappel, 1977) and the same selenium forms in the chick (Omaye and Tappel, 1974; Cantor et al., 1975b) resulted in similar increases in liver, kidney, and small intestine GSH-Px activity. Noguchi et al. (1973) found that at lower levels of selenium, 0.1 ppm dietary Se, selenite was twice as effective as selenomethionine at raising plasma, liver and heart GSH-Px. in the chick. In selenium deficient rats, Sunde et al. (1981) found dietary supplementation with selenite unaffected by dietary methionine whereas selenomethionine below 0.5 ppm selenium was less potent with suboptimal dietary methionine. Addition of adequate methionine restored selenite equivalent potency of selenomethionine.

Practical methods of providing additional selenium to that occurring in the natural diet of beef cattle is through parental or

oral administration. Oral routes may include supplying selenium through drenches, as a component of a mineral or feed, or by boluses and pellets that remain in the rumeno-reticulum area releasing selenium to the gastrointestinal tract.

Sodium selenite is the most frequent selenium form used in parental administrations. Experimentally, parental solutions may be prepared and contain only selenium; however, commercially available products commonly contain mixtures of selenium and vitamin E. For example, Mu-Se, a product of Burns-Biotic Laboratories Div., Chromalloy Pharmaceutical Inc., Oakland, California, contains 5 mg/ml of selenium as sodium selenite and 10.95 mg/ml of alpha tocopherol.

Selenium added to the diets of deficient beef cows, whether in the form of sodium selenite or organically in linseed meal (1186 ppb Se) increased selenium in the plasma, milk and liver of cows and plasma, muscle and liver of their nursing calves (Ammerman et al., 1980). In this study linseed meal diets produced higher selenium levels than comparable dietary selenium levels supplied as soybean meal plus sodium selenite in calves but not cows. Milk selenium levels were increased shortly after calving; but by 2 and 8 weeks after calving, dietary selenium supplementation did not increase milk selenium levels. In goats, radioactive selenium was identified in milk, after intravenous jugular injection, peaking two hours after plasma peak selenium concentration. Selenium was primarily associated with the casein in milk (Yoshida et al., 1981). Seven days after dosing, both kidney and liver had higher selenium levels than the mammary gland. Major changes in selenium metabolism

affecting selenium concentration in milk near parturition apparently occur. In ewes that were parentally supplemented with barium selenate, milk selenium levels were twice as high just prior to lambing as compared to the first day after lambing and one week after lambing (Overnes et al., 1985).

Gleed et al. (1983) used sodium selenate injections subcutaneously at 0.15 mg Se/Kg bodyweight and observed increased serum selenium and serum GSH-Px in Hereford/Friesian and Charolais steers. No breed effects were observed. Similarly, Thompson et al. (1981) using 0.1 mg Se/Kg BW on nine month old calves found a rapid increase in serum and liver selenium concentrations exhibiting an exponential decline with half-lives of 22.1 and 28.3 days, respectively. Whole blood selenium levels responded similarly but declined more slowly. Selenium concentration and GSH-Px activity in erythrocytes increased more slowly and remained elevated for several months after liver and whole blood selenium concentrations had declined. Injections every two months at 0.1 mg Se/Kg BW were suggested as necessary to maintain calves on selenium deficient forage (0.018 mg Se/Kg forage). Calves injected with 0.078 mg Se/Kg BW and 5.4 IU of vitamin E/Kg BW at birth were 87% higher in serum selenium at day 14 but 2 injections (double the dose) were required for serum selenium levels to be 10% higher by day 28 (Weiss et al., 1983). A lag of 4-5 weeks occurred before erythrocyte glutathione peroxidase activity increased. Decline of serum selenium in calves not given supplemental selenium was linear from birth to 56 days of age.

Scholz et al. (1981) investigated the effects of oral selenium on selenium concentration and glutathione peroxidase activity in various tissues and their response to varying levels of supplementation. Calves from birth to twelve weeks of age were fed graded levels of selenium (0.03, 0.23 and 0.53 microgram Se/gm of solids) in whole milk diets. Selenium concentrations on a fresh tissue-weight basis were highest in kidney cortex, intermediate in kidney medulla, testes, liver and spleen and lowest in striated muscle, adipose and blood plasma. Increased dietary selenium caused increased selenium concentrations in liver, kidney cortex, spleen and heart but not in testes and adipose. Glutathione peroxidase activity measured by hydrogen peroxide was highest in erythrocyte and testis and lowest in thymus, brain, striated muscle, adipose tissue and plasma.

Work with sodium selenite in salt mixes indicates increased and adequate blood selenium concentration and glutathione peroxidase activity when administered free choice at 90 ppm but not at 20-30 ppm selenium (Williams et al., 1981; Koller et al., 1983). Supplementation of first calf Hereford heifers on alfalfa hay rations with either soybean meal (0.313 mg Se/Kg) or 90 ppm sodium selenite salt resulted in female whole blood selenium concentrations of 0.250, 0.162 and 0.052 ppm at parturition with corresponding calf levels of 0.242, 0.175 and 0.81, indicating some concentrating of selenium in the calf in utero, when the dam is relatively low in selenium (Koller et al., 1984). Glutathione peroxidase activity showed a similar response to supplementation of the dam.

Sodium selenate has been added to drinking water of cattle to receive 0.5, 1.0 or 2.0 mg selenium per day (Morris et al., 1984). Blood selenium concentration of first calf Santa Gertrudis heifers were significantly increased and linearly related to the level of selenium supplementation. However after one year of supplementation all supplement levels were similar and about six times higher than control deficient animals. Supplementation at 1.0 mg selenium per day resulted in blood selenium levels generally considered adequate (5-8 microgram/dl).

Parental preparations of barium selenate have been determined to be efficacious in elevating blood selenium concentrations in cattle and sheep for six months to two years at rates of approximately 1.2 mg Se/kg BW (Cawley and McPhee, 1984; Overnes et al., 1985).

Hidiroglou et al. (1971, 1972) reported the use of subcutaneous silastic and magnesium stearate implants in sheep and calves as increasing the selenium concentration in blood.

Pellets of various selenium compounds and carriers, such as iron, given orally and remaining in the reticulum or rumen have been reported to be effective in increasing tissue selenium levels (Kuchel and Buckley, 1969; Judson et al., 1980; Hudson et al., 1981; Hunter et al., 1981; Judson and McFarlane, 1984). Selenium sources have included calcium selenate, barium selenate and elemental selenium (Kuchel and Buckley, 1969). Elemental selenium pellets (10%) with 90% iron granules are now available commercially (ICI, Australia). Radiolabelled selenium pellets in sheep have yielded detectable amounts of selenium in plasma by 7 hours after administration

(Handreck and Godwin, 1970). Over one-half of the total plasma selenium was bound to globulin and albumin with the predominate amount on globulin. Tissue distribution and urinary excretion of labelled selenium from the pellets followed the same pattern as seen with oral sodium selenite supplementation. Hunter et al. (1981) observed maximum plasma selenium concentrations by 2 weeks after administration of either elemental selenium pellets or oral sodium selenate; however, the selenate declined rapidly thereafter while the pellets maintained selenium concentration for 1 year in sheep. The authors did find differences in actual selenium concentrations between 3 brands of pellets tested but all maintained adequate selenium levels for 1 year. A major factor on long-term effectiveness of rumen pellets is grain size of the selenium (Hudson et al., 1981). In their studies commercial sources of pellets varied in grain size from 4 to 40 microns with larger grain selenium pellets maintaining adequate selenium levels for longer periods of time. After 28 days, only a small percentage of elemental selenium remained in pellets composed of small grain selenium, while approximately 50% remained in coarser grained pellets. It was postulated that the rapidly released selenium occurred as a result of iron oxidation and concomitant alteration of elemental selenium to iron selenide. Selenide is the most reduced ionized state (-2) of selenium (NRC, p.5). Subsequent work (Peter et al., 1981) with various non-Se and Se/Fe pellets has shown electrochemical reactions as the likely events leading to release of selenium from the pellets and establishment of an anode/cathode relationship between pellets.

However, the process appears complex and the precise chemical forms of selenium that are released and subsequently absorbed by the animal are still unknown.

Supplementation of livestock through pasture fertilization has also been successful. Pasture sprays of sodium selenite (Grant, 1965), or selenious acid (Davies and Watkinson, 1966a), or top dressing with superphosphate containing sodium selenate (Grant, 1965), elemental selenium (Grant, 1965; Davies and Watkinson, 1966a), and selenite with sodium, barium, iron or zinc (Grant, 1965; Davies and Watkinson, 1966b; Hartley, 1967) has proven efficacious.

This chapter has been a brief review of the role that selenium plays in animal health and how a producer might benefit from evaluating the status of their animals. The following two chapters are research projects articles submitted to the American Journal of Veterinary Research for publication.

CHAPTER 3

SAFETY AND EFFICACY OF TWO SUSTAINED-RELEASE
INTRARETICULAR SELENIUM SUPPLEMENTS IN BEEF COWS

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SUMMARY

One hundred fifty selenium deficient, pregnant, crossbred beef cows were assigned to one of four treatment groups: group A, negative control; group B, 1 Dura-Se bolus at 0 and 119 days; group C, 1 Dura-Se bolus at 0 days; and group D, 2 Permasel pellets at 0 days. Cattle were maintained on selenium deficient pastures or forages prepared from these pastures. Blood samples were collected prior to treatment administration (time 0) and at 28, 52, 119, and 220 days thereafter and analyzed for whole blood selenium (WBSe) concentration. Individual body weights were recorded at each sampling date. Whole blood selenium concentration of cows from all supplemented groups were significantly higher (P<0.01) than controls at all sample dates after treatments began. By the end of the 220

day study, treatment group B attained significantly higher (P<0.01) WBSe concentrations than any other group. Body weights of treatment groups fluctuated throughout the study but did not differ (P>0.05) between groups. One cow and six calves born to cows during the experimental period died. Necropsy of 5 calves provided no evidence linking these deaths to selenium treatments. No difference (P>0.05) in mortality between groups was detected.

INTRODUCTION

Selenium (Se) has been recognized as an essential trace element for animals (NRC, 1983). In the United States, forages from several regions have inadequate selenium concentrations to meet these requirements. The regions with the lowest amounts of Se in soils and plants are the Pacific Northwest, Northeast, Southeast, and areas of the Midwest which adjoin the Great Lakes (Ammerman, 1981; Carter et al., 1968; Kubota et al., 1967). Several delivery systems have been successfully used to supplement Se to animals which are housed in confined areas. Selenium has been added to total rations of confined cattle either as sodium selenite or in linseed meal to increase plasma Se concentrations (Ammerman et al., 1980). Subcutaneous injection of sodium selenate at 0.15 mg Se/kg body weight effectively increased serum Se in Hereford/Friesian and Charolais steers (Gleed et al., 1983). Sodium selenite has been added to the drinking water of Santa Gertrudis heifers and significantly increased their blood Se concentrations (Morris et al., 1984).

Complications of supplementation arise when animals are not

confined to small areas. Such is the case for many beef cattle production systems. Delivery systems have also been used in this management system. Sodium selenite in salt mixes has been used to increase blood selenium concentration when administered free choice at 90 mg Se/kg salt mixture (Koller et al., 1983). Sporadic intake, individual animal variability, mixing and solubility factors are problems with this system. Selenium containing pellets have been used in Australia, New Zealand and California as a method to supply Se under extensive management. These iron-based pellets weigh approximately 30 grams and contain 10% elemental selenium. They remain in the reticulum and supply Se for approximately 3 years. The exact mechanism of bioavailable Se release from these pellets is uncertain. Presently, these pellets are available only for experimental purposes in the United States outside of California. A bolus designed to release 3 mg/d of selenium as sodium selenite and to last for 4 months was also evaluated. The purpose of the study was to evaluate the safety and efficacy of these 2 intrareticular Se-containing boluses.

MATERIAL AND METHODS

Experimental Animals: One hundred fifty crossbred beef cows and heifers were stratified by age and assigned to 1 of 4 treatment groups in a completely randomized design. The animals were known to be selenium deficient, and they were in mid-gestation. Group A cows (n=50) served as selenium deficient controls and received no supplemental selenium. Group B cows (n=40) were administered one

supplemental selenium. Group B cows (n=40) were administered one Dura-Se[a] bolus orally at time 0 and again at 119 days. Group C cows (n=10) were administered one Dura-Se bolus orally at time 0 only. Group D cows (n=50) were administered two Permasel[b] pellets orally. Throughout the 220 day trial all animals were maintained on a diet of grass silage, grass hay, pasture and selenium-free salt. All forages were harvested from Oregon State University's Soap Creek Ranch, where the experiment was conducted.

All animals in group C (10) and 26 animals from each of groups A, B, and D were selected for blood sample collection. Blood samples were collected before treatment administration (0 time) and thereafter at 28, 52, 119, and 220 days. Blood samples were collected by venipuncture from the jugular vein into trace mineral free vacutainer[c] tubes. Samples were refrigerated until analyzed for whole blood selenium (WBSe) concentration. Cow weight and health data were recorded for all animals at each blood collection date.

Selenium Analysis: WBSe concentrations were determined by semi-automated fluorometric measurement of the Se-2,3-diaminonapthalene (DAN) complex (Olson et al., 1975).

Statistical Analysis: Data were analyzed statistically by conventional least-squares procedures. The general linear models procedure of Statistical Analysis Systems was used (SAS, 1982).

RESULTS

The whole blood Se concentration of cows are shown (fig 1). Group A (control) cows maintained extremely low WBSe concentrations which decreased throughout the experimental period. Groups B, C, and D obtained significantly higher ($P<0.01$) WBSe concentrations than did controls at all sample dates after treatment administration. After 28 days, group D had reached significantly higher ($P<0.01$) WBSe concentrations than all other groups. At the 52 and 119 day sample dates there were no differences ($P>0.05$) between the supplemented groups. At the 220 day sample date only group B cows continued to increase their WBSe concentrations which was significantly higher ($P<0.01$) than all other groups. Group D cows' WBSe concentration dropped slightly but was significantly higher ($P<0.01$) than group C at 220 days.

Body weights of groups fluctuated throughout the study but did not differ ($P>0.05$) between groups.

One cow and six calves died during the experiment (Table 1). The cow was from group A and died as a result of post surgical complications following a caesarean section. The fetus of this cow was dead and necrotic *in utero*. Neither of these animals were available for necropsy. No significant ($P>0.10$) differences in mortality of newborn calves were seen between the treatment groups. Necropsy of the five available calves provided no evidence linking these deaths to Se treatments.

DISCUSSION

Whole blood Se concentration averaged 0.02 ug/ml for all groups at the initiation of the study. Control cows maintained low WBSe concentrations throughout the study and WBSe concentrations were decreased at 220 days in the control group cows. Possible explanations for the decrease over time include seasonal variation of available Se in forages and increased physiological demands during gestation and lactation. The 220 day sample was collected on June 4, 1987, at which time the cows had been on pasture for approximately 56 days. Selenium uptake by plants is a nonselective process; therefore, in rapidly growing forages the Se concentration of the dry matter is less (NRC, 1983). Annual fluctuations in Se requirements may result from gestation and lactation. Transplacental transfer of Se from the cow to the fetus has been demonstrated during late gestation (Koller et al., 1984). Transfer of Se in colostrum and milk from cows to calves has also been demonstrated (Koller et al., 1984; Conrad and Moxon, 1979). The net effect of these physiological activities is an increased demand for Se during gestation and lactation. With a concurrent decrease in forage Se concentrations, the decreased WBSe concentration observed in control cows at 220 days would be expected.

Supplementation of Se to treated beef cows by both delivery systems effectively increase WBSe concentrations in this study.

This study supports previous data regarding the safety and efficacy of the Permasel pellets (Judson et al., 1980; Judson and McFarlane, 1984). These pellets caused a rapid increase in WBSe

concentration, from 0.02 ug/ml to 0.08 ug/ml in 28 days. Whole blood Se concentration peaked at 119 days and then declined slightly by 220 days. These findings suggest that Se supplementation from these pellets is characterized by rapid increases in WBSe concentration peaking at 4-5 months and then decreasing with time.
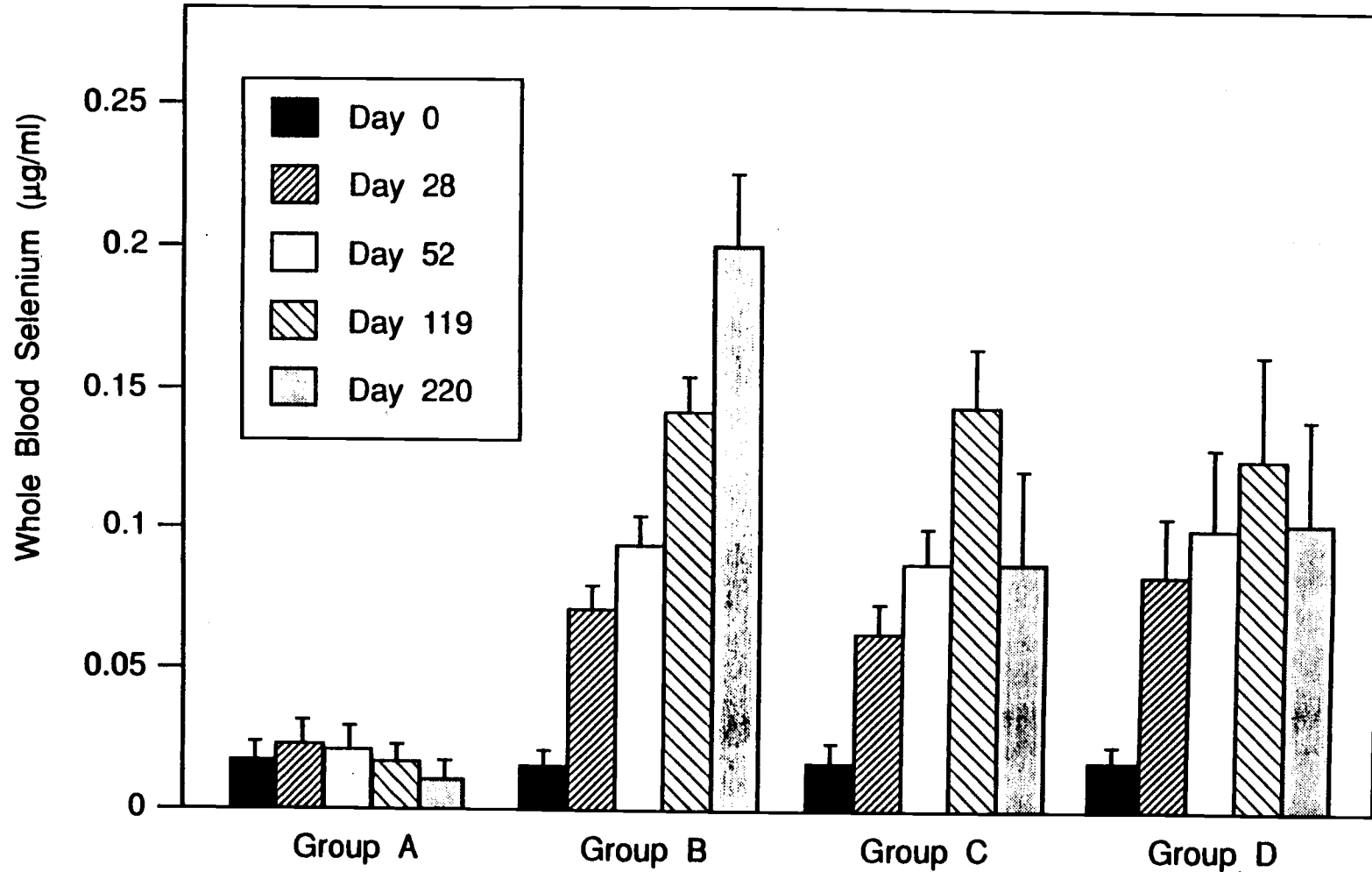
This study indicates that the Dura-Se bolus was safe and effective in supplying Se to cows. Evidence for the efficacy of the Dura-Se bolus is shown by the linear increase in WBSe concentration of group B animals reaching 0.199 ug/ml at 220 days. Additional evidence for efficacy and safety is demonstrated by the observed WBSe concentrations of group C cows. Supplementation to this group ceased at 4 months. Therefore, as expected, WBSe concentrations were lower at the 220 days sample than at the 119 days sample; dropping rather than increasing as with treatment group B.

The Dura-Se bolus is designed to provide 3 mg Se/d for 120 days.[a] Comparison of WBSe concentration of treatment groups showed that treatment with the Permasel pellets resulted in slightly higher WBSe concentration initially; but by day 52 and day 119 WBSe was similar to those in group B where release was 3 mg Se/d. The Se released from the Permasel pellets appeared to decrease substantially after 119 days resulting in decreased WBSe concentration at 220 days versus cows in group B. This decrease was not as large as that seen in group C which had no Se supplementation after 120 days. This indicates that Se released from the pellets to cows in group D was less than 3 mg/day after day 119 and more that 3 mg/day prior to day 52. This is concluded by comparing the WBSe concentrations of group

D with those of group B in which Se release was constant[a].

During the study, 1 cow and 6 calves died. There was no evidence to link these deaths to Se treatments. Furthermore, the mortality rate (2.3%) was within the historic range for this herd. There were no other untoward signs observed in treatment group (groups B, C, and D) cows. The absence of detectable differences in individual cow body weights between the groups also indicated the safety of those supplementation methods.

Figure 3.1 Mean whole blood selenium (WBSe) concentration of cows.

Bar graph expressed as mean WBSe concentration ± Standard deviation. Group A, selenium deficient cows receiving no supplemental Se (negative control group); Group B, Dura-Se bolus at day 0, and a second Dura-Se bolus at day 119; Group C, 1 Dura-Se bolus at day 0; Group D, 2 Permasel pellets at day 0.

Table 3.1 Mortality data summary.

Group	Animal Number	Pathologic findings and etiology	Hepatic selenium concentration ug/g (DMB)	Selenium status
A	calf 0010	Autolytic fetus	NA	NA
A	cow 0010	Dystocia, caesarian section	NA	NA
A	calf 5090	Stillborn, no evidence of dystocia	0.53	Deficient
B	calf 1042	Dystocia, calf died during birth	1.71	Normal
B	calf 2200	Enterotoxemia, _Clostridium perfringens_, Type C	2.24	Normal
C	calf 4097	Dystocia, calf was still-born, (atelectic lungs)	2.29	Normal
D	calf 5136	Dystocia, caesarian section degenerative myopathy	0.93	Deficient

ug/g = ppm (parts per million); DMB = dry matter basis; NA = not available

CHAPTER 4

PLACENTAL AND COLOSTRAL TRANSFER OF SELENIUM IN BEEF CATTLE

D.T. Campbell, BS; J. Maas, DVM, MS; D.W. Weber, PhD;
O.R. Hedstrom, DVM, PhD; B.B. Norman, DVM, PhD

SUMMARY

One hundred fifty selenium (Se) deficient, pregnant, crossbred beef cows were assigned to one of four treatment groups: group A, negative control; group B, 1 Dura-Se bolus at 0 and 119 days; group C, 1 Dura-Se bolus at 0 days; and group D, 2 Permasel pellets at 0 days. Cattle were maintained on selenium deficient pastures or forages prepared from these pastures. Blood samples were collected from calves, prior to suckling, and were analyzed for whole-blood selenium (WBSe) concentration. Colostrum samples were collected from dams and analyzed for total Se concentration. Additional blood samples were collected from calves 24-48 hours post-suckling and

analyzed for WBSe concentration and serum creatinine kinase (CK)

activity. Birth weight, sex, and health were recorded on all calves

at birth. Calves from cows in Se treated groups had significantly

(P<0.001) higher WBSe concentrations, both pre- and post-suckling,

than did controls. Post-suckle WBSe concentrations of calves were

not significantly (P>0.05) different than pre-suckle concentrations

in any of the groups. Selenium concentrations in colostrum from

Se-supplemented cows were significantly (P<0.001) higher than from

control cows. No difference (P>0.05) was determined in serum-CK

activities or birth weights between groups.

INTRODUCTION

Nutritional myodegeneration (NMD) commonly affects calves and

lambs. Two etiologic forms of the disease have been proposed,

congenital and juvenile. The congenital form usually results in

abortion, stillbirths or death shortly after birth (Andrews et al.,

1968; Hartley and Grant, 1961; Hidiroglou, 1980). The major

pathologic observations are degenerative changes of the myocardium

(Andrews et al., 1968; Hartley and Grant, 1961; NRC, 1983).

The juvenile or "delayed" form occurs most commonly between

three and eight weeks of age. The predominant sign is muscular

weakness. The neonates walk with a stiff gait and arched backs,

avoid movement, lose condition, and die. Skeletal muscles show

degeneration, but cardiac lesions are not always present (NRC, 1983).

The use of Se supplementation in cows during gestation and/or

lactation to prevent NMD in their calves has provided variable

results. This variation in part has resulted from differences in the

time, duration, level, and form of Se supplementation.

The purpose of this study was to evaluate the transfer of Se across the placenta and through colostrum in beef cattle under extensive pasture conditions.

MATERIAL AND METHODS

Experimental Animals: One hundred fifty crossbred beef cows (N=117) and heifers (N=33) were stratified by age and assigned to 1 of 4 treatment groups in a completely randomized design. The animals were known to be selenium deficient at mid-gestation. Group A cows (n=50) served as selenium deficient controls and received no supplemental selenium. Group B cows (n=40) were administered one Dura-Se[a] bolus orally at time 0 and 119 days. Group C cows (n=10) were administered one Dura-Se bolus orally at time 0 only. Group D cows (n=50) were administered two Permasel[b] pellets orally. Throughout the 220 day trial all animals were maintained on a selenium-deficient diet of grass silage, grass hay, pasture and selenium-free salt. All forages were harvested from Oregon State University's Soap Creek Ranch, where the experiment was conducted.

All animals in group C (10) and 26 animals from each of groups A, B, and D were selected for blood sampling and data collection at parturition. Blood samples were collected before treatment administration (0 time) and thereafter at 28, 52, 119, and 220 days.

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[a] Schering Animal Health, 1011 Morris Avenue, Union, New Jersey
[b] ICI Australia Operation Pty Ltd, 1 Nicholson St., Melbourne, Victoria, Australia

Calves from cows selected for data collection were sampled at birth. Blood samples were collected from calves, prior to suckling, and were analyzed for WBSe concentration. Colostrum samples were collected from dams and analyzed for total Se concentration. Additional samples were collected from calves 24-48 hours post-suckling and analyzed for WBSe concentration and serum creatinine kinase (CK) activity.

Blood samples for WBSe concentration determination were collected by venipuncture from the jugular vein into trace mineral free vacutainer[c] tubes and refrigerated until analyzed. Blood samples for CK analysis were collected by venipuncture from the jugular vein into vacutainer[d] tubes and allowed to clot. Tubes were centrifuged to separate the serum which was transferred to sample tubes[e] and stored at -20 °C until analyzed. Colostrum was collected into sterile specimen containers[e] and frozen until analyzed for Se concentration. Cow weight and health were recorded on all animals at each blood collection date. Birth weight, sex, and health were recorded on all calves at birth.

Selenium Analysis: Selenium concentration of whole blood and colostrum samples were determined by semi-automated fluorometric measurement of the Se-2,3-diaminonapthalene (DAN) complex (Olson et al., 1975; Cousins, 1960).

CK Analysis: Serum CK activity was assayed by the Szasz method (Szasz et al., 1976).

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[c] Becton-Dickinson (No. 6527), 1950 Williams Drive, Oxnard, California
[d] Becton-Dickinson (No. 6430), 1950 Williams Drive, Oxnard, California
[e] Premium Plastics, Inc., 465 West Cermak Road, Chicago, Illinois

Statistical Analysis: Data were analyzed statistically by conventional least-squares procedures. The general linear models procedure of Statistical Analysis Systems was used (SAS, 1982).

RESULTS

Calves from all Se-treated groups had significantly (P<0.001) higher WBSe concentrations, both pre- and post-suckle, than did controls (Table 1). Post-suckle WBSe concentrations of calves were not significantly (P>0.05) different than pre-suckle concentrations in any of the groups. The post-suckle WBSe concentrations did tend to be decreased versus the pre-suckle value in 3 of 4 groups.

Selenium concentrations of colostrum from Se-supplemented cows, groups B, C, and D, were significantly (P<0.001) higher than from control cows (Table 1).

The birth weight of calves from all groups averaged approximately 43 kg and were not different (P>0.05) for calves from Se-treated cows or control cows (Table 1). There were no differences in birth weight by sex between groups.

Serum CK activity from all groups varied widely and were not different (P>0.05) for calves from Se-treated cows versus control cows (Table 1). The average weight gain for calves from all groups was approximately 65 kg during the trial period, and the rate of gain for Se-treated groups and the control group did not differ (P>0.05).

The relationship of cow WBSe concentration at 119 days post-treatment to calf WBSe concentration at birth is shown (fig 1). The correlation coefficient (r) = 0.872 (r^2 = 0.76). The value of y

(calf WBSe concentration) could be predicted from the equation y = 0.02 + 1.03(X) if X (cow WBSe concentration) is known.

The median calving date was 152 days after treatment administration with a range of 54 days.

DISCUSSION

Evaluation of the transfer of Se across the placenta and through colostrum and milk has provided differing results. The dynamics of placental transfer of Se was first studied in pregnant dogs and showed that Se administered intravenously rapidly enters the umbilical circulation (McConnell and Roth, 1964). Hidiroglou observed that radioselenium passes across the placental membranes of the ewe, but that maternal tissue concentrations had higher radioselenium concentrations than the corresponding fetal tissues (Hidiroglou et al., 1969). Wright observed in sheep that the ratio of the concentrations of radioselenium in the maternal plasma to fetal plasma is 11.7:1 for a single fetus and 21.8:1 for twin fetuses (Wright and Bell, 1964) and this suggests that only a limited fraction of the Se in the maternal tissues is in a form which could traverse the placental membranes. Others have reported findings in beef cattle that Se readily crosses the placenta and that the fetus can actually attain WBSe concentrations greater than the mother (Koller et al., 1984). Furthermore, it was observed that if dams were deficient in Se, a higher WBSe concentration would be observed in the calf (Koller et al., 1984).

Our data indicated significant placental transfer of Se and accumulation in fetal blood in beef cattle. In group A calves' WBSe concentrations at birth were higher than their respective dams. These control cows were Se deficient and there was apparent fetal sequestering of Se resulting in higher WBSe concentration in calves at birth than in the dams. Calves from group B attained WBSe concentration near those of their dams assuming a linear increase in dam WBSe concentrations from the 119 day to the 220 day samples. This suggests that as dam Se status approaches normal, there may be a decrease in the apparent percentage of Se that is transferred. Nevertheless, the actual amount of Se transferred is probably much greater than in the deficient animals. Calves from groups C and D attained higher levels than their dams and maintained these levels while dams' WBSe concentrations were declining. These observations suggest many possibilities. The transfer of Se may be a one-way process across the placental membranes from cow to fetus. The metabolism of Se in the fetus may be minimal prior to birth resulting in a reserve. There may be a specific time period during gestation in which Se can be transferred across the placenta and after that it is trapped in the fetus. All of these possibilities need further study with animals which are both deficient and normal in Se status.

Conrad and Moxon found that only a small proportion of dietary Se was transferred to milk (Conrad and Moxon, 1979). They concluded that there would be no important increase in Se intake from consuming milk provided by cows eating feeds supplemented with sodium selenite in amounts adequate to prevent Se deficiency (Conrad and Moxon,

1979). Subsequent studies found that only 1.5 percent of radioactive sodium selenite accumulated in milk after an oral dose (Conrad et al., 1976; Waite et al., 1975). Maus and co-workers found that increasing Se intake from about 2 mg/day to about 6 mg/day will elevate milk Se concentration significantly from .03 to .06 ug/ml (Maus et al., 1978). However, increasing Se intake from 6 to 12 mg/day will not significantly increase milk Se (Maus et al., 1978).

Our study supports these observations. All Se supplemented group cows (groups B, C, and D) had higher colostral Se concentrations than did controls. Selenium concentration in colostrum relative to WBSe concentrations were very close to values observed by others (Koller et al., 1984). Even though the Se concentrations of colostrum and milk are higher in supplemented groups, the actual amount of Se in colostrum and expecially in milk appears to be too little to be of any nutritional importance in a deficient neonate.

Others have shown results which suggest that there is some factor, presumably Se and/or vitamin E, which can be effectively transferred through the milk which can effectively decrease NMD in lambs (Young et al., 1961). In these studies, two types of hay rations were fed to ewes at different stages of gestation and lactation to determine the interaction of period of feeding on incidence of NMD. One hay was known to cause NMD; the other was known not to cause NMD. If the non-NMD-causing hay was introduced for the early lactation period of 3 weeks it decreased the incidence of NMD substantially (Young et al., 1961). Likewise, feeding of the

NMD-causing hay to ewes only in early lactation was followed by an incidence as high as that following feeding the NMD-causing hay throughout gestation. The highest incidence of NMD occurred when the NMD-causing hay was fed both in the late gestation and early lactation (Young et al., 1961). These results indicate a correlation between feeding the NMD-causing hay and NMD occurrence; unfortunately, no evaluation was reported for the hay's or animal's micromineral or vitamin status to allow evaluation of Se or vitamin E metabolism. These observations, however, indicate that factors central to the cause or prevention of NMD can pass the placenta or be present in colostrum or milk.
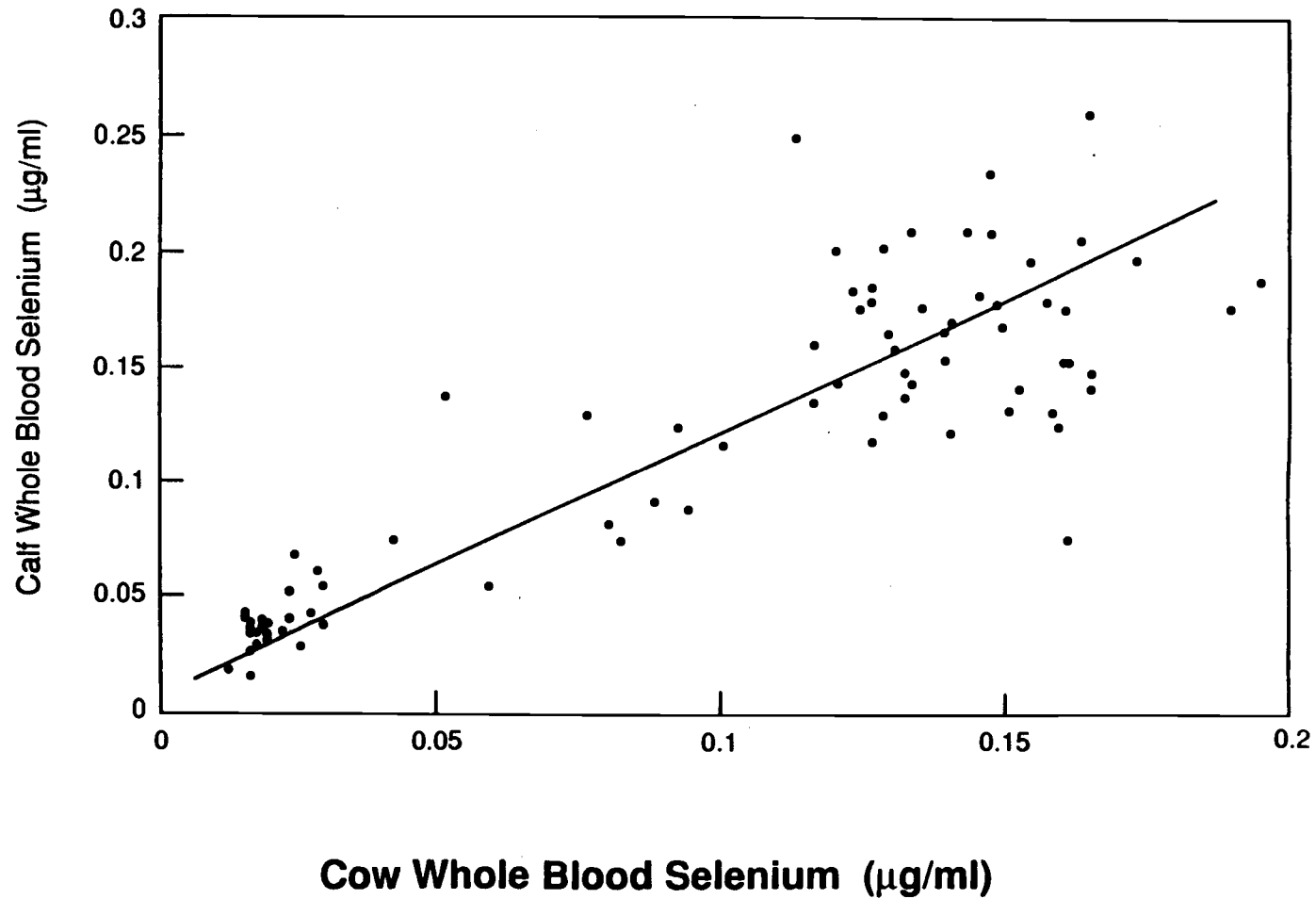
Our data indicates that Se readily crosses the placenta in beef cattle and that when dams are Se deficient the fetus can partially compensate by apparently sequestering Se from its mother. The Se concentration in colostrum is significantly increased by supplementing; but, the actual amounts of Se are very small and are probably of little significance in the prevention of juvenile NMD. The correlation between the WBSe concentration of cows and the WBSe concentration of calves at birth was fairly high. This would allow the clinical diagnostician to utilize either blood from cows or calves as an appropriate specimen for analysis.

TABLE 4.1 Selenium concentrations of whole blood of calves, pre-suckle and post-suckle; Se concentrations of colostrum; birth weight of calves; serum creatinine kinase (CK) activity of calves at 24-48 hours of age.

Group	Whole blood Se concentration ug/ml (ppm)		Colostrum Se concentration ug/ml	Calf Birth-weight kg	Calf Serum CK IU/L
	Pre-suckle	Post-suckle			
A	0.035 ±0.007	0.027 ±0.006	0.017 ±0.005	42.7 ±1.4	85 ±10.8
B	0.180 ±0.008	0.165 ±0.007	0.072 ±0.005	43.0 ±1.4	62 ±11.1
C	0.154 ±0.014	0.156 ±0.012	0.052 ±0.009	44.9 ±2.0	99 ±16.9
D	0.124 ±0.008	0.110 ±0.006	0.060 ±0.005	40.3 ±1.7	73 ±11.3

Se = Selenium (ug/ml = ppm) ppm = parts per million
Data are expressed as mean values ± standard deviation.

Figure 4.1 Correlation between cow whole blood selenium (WBSe) concentration and WBSe concentration of their calf at birth.

Cow Whole Blood Selenium (μg/ml)

Cow WBSe concentration determined at 119 days post treatment. Correlation coefficient (r)=0.872, r^2=0.76. Formula to predict y (calf WBSe) if x (cow WBSe) is known is y=0.02+1.03x.

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