PATHOLOGY OF INORGANIC AND ORGANIC SELENIUM TOXICOSIS IN YOUNG SWINE

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ABSTRACT

PATHOLOGY OF INORGANIC AND ORGANIC SELENIUM TOXICOSIS IN YOUNG SWINE

By

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A total of 47 young pigs and 6 yearling steers was used to determine the toxicosis of sodium selenite and selenomethionine and to compare the lesions produced by both selenium compounds with those produced by vitamin E-selenium deficiency in pigs. In feeding experiments using rations based either on Torula yeast or dried whole milk as dietary protein sources, pigs were fed comparable graded amounts of both selenium compounds in the ration.

In 1 yearling steer, 1 intravenous injection of 1 mg selenium/kg body weight as sodium selenite produced death in 8 hours and gross lesions of massive pulmonary edema, hydrothorax, and congestion of the gastrointestinal tract, while 0.5 mg selenium/kg body weight given intravenously into another steer had no effect in 4 days. In both cattle and swine, intramuscular injections of a selenite solution resulted in localized muscular necrosis. Two pigs given 3 mg selenium/kg body weight intravenously as sodium selenite or selenomethionine developed fatal selenium toxicosis in 2-1/2 and 14 hours, respectively. Pulmonary edema was the important lesion of intravenous selenite toxicosis. Twenty-one pigs fed 20 to 600 ppm selenium as

sodium selenite or selenomethionine developed the clinical signs of emesis, anorexia, loss of weight, cachexia, depression, respiratory distress, and coma with subnormal temperature and death. The lesions of selenosis were similar for both selenium compounds and included fatty metamorphosis and centrolobular necrosis in the liver; congestion of renal medulla; necrosis in lymphoid follicles; hemorrhagic necrosis of adrenal cortex; edema and degenerative changes in the cerebrum, cerebellum and spinal cord; edema and hemorrhagic necrosis of pancreas; serous atrophy of body fat and degenerative changes in skeletal muscles. Lesions in the central nervous system were generally more common with inorganic selenosis while pancreatic lesions were associated with organic selenosis. Microangiopathy was an important lesion of acute selenosis while degenerative lesions and cachectic changes of a wide variety of organs characterized selenosis of longer duration.

The selenium content in liver and kidney, while variable, was somewhat proportional to the level and duration of selenium in the feed. The diagnosis of selenium toxicosis in swine depends on both the nature of the lesions and selenium content of the liver and kidneys. Selenium toxicosis can be differentiated from vitamin E-selenium deficiency in swine by the characteristics of the hepatic lesions.

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By

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INTRODUCTION

Selenium as an element in animal and human nutrition has paradoxical biological functions which caused Krehl (1970) to describe it as "one of the most maddening, frustrating nutritional minerals to examine in the entire table of elements." In most animals it is highly toxic in relatively low amounts by toxicologic standards as well as an essential element in trace amounts by nutritional standards. This knowledge of selenium in animals has produced a dichotomy of philosophy between its present use as an additive in animal feeds and the future effects of its use on public health and the environment.

Workers in South Dakota and Wyoming documented the toxicity of selenium under natural and experimental conditions nearly 40 years ago (Franke, 1934a; Rosenfeld and Beath, 1964). Selenium toxicosis in the natural form primarily involved organic selenium compounds. Selenomethionine was one of the principal compounds in seleniferous feed grains (Allaway, 1968). Some question has existed in the literature concerning the interaction of the components of natural foodstuffs with selenium during toxicosis (Muth and Binns, 1964). Although the toxic effects of selenium, principally inorganic selenium, have been recorded by many workers, detailed studies on the experimental and diagnostic pathology of selenosis in domestic animals are very limited. Published information on the pathogenesis

of selenium toxicosis in swine was not available. In addition, no experimental work using synthetic organoselenium compounds to produce selenium toxicosis in swine was located. Moreover, in addition to limited information on the pathologic effects of abnormal amounts of selenium, the stigma of the possibility of selenium having carcinogenic properties has been raised (Anon., 1970).

In 1957, selenium was discovered as the essential component of "Factor 3" which prevented dietary hepatic degeneration in rats on a diet of Torula yeast (Schwarz and Foltz, 1957). Since that time numerous selenium-responsive diseases have been described in domestic animals and man (Hartley and Grant, 1961; Majaj and Hopkins, 1966; NAS-NRC Monograph, 1971). In fact, selenium has now been identified as an essential trace mineral element in cattle, sheep, swine, horses, chickens, turkeys, quail, dogs and subhuman primates (NAS-NRC Monograph, 1971; Muth *et al.*, 1971). Presently, there is an effort in the scientific community to convince the Food and Drug Administration to approve selenium as a feed additive for swine and poultry (NAS-NRC Monograph, 1971; Frost, 1971; Frost, 1972).

Although vitamin E-selenium responsive diseases are more common today than natural selenosis, occasions of inadvertent selenium toxicosis have been reported in sheep and cattle following the therapeutic use of selenium (Morrow, 1968; Lambourne and Mason, 1969; Gabbedy and Dickson, 1969; Shortridge *et al.*, 1971).

If or when selenium becomes approved as a feed additive in the United States, it can be speculated that a concern or need for more reliable information on selenium toxicosis not only in swine but also in other species of animals will be desired. In addition, if the widespread use of selenium is permitted in animal feeds, more

information is needed on the impact of this development upon the environment and upon public health and agronomic practices. In the past the detrimental effects of new drugs and chemicals, such as mercury and DDT, on the environment and public health were not fully realized until after many years of extensive use. Thus, there is public concern, for good reasons, about the widespread indiscriminate use of potentially toxic chemicals in our food supply and environment.

While considerable information is already available on the pathology of vitamin E-selenium deficiency in a variety of species, information on the pathology of selenium toxicosis would be valuable in differentiating these 2 syndromes and might also provide suggestions as to the biological role of both vitamin E and selenium at the cellular level.

Thus, additional research information on selenium toxicosis, not only as it applies to swine, but also other animal species and public health as well, would seem essential. It would serve as an important informational resource to the livestock industry, the biomedical professions and the public welfare.

REVIEW OF LITERATURE

The literature on the biological aspects and agricultural implications of selenium has been extensively reviewed in several books (Trelease and Beath, 1959; Rosenfeld and Beath, 1964; Muth *et al.*, 1967; Mills, 1970; Underwood, 1971; NAS-NRC Monograph, 1971) and review articles (Painter, 1941; Moxon and Rhian, 1943; Schwarz, 1961).

This review of the literature is limited primarily to selenium toxicosis in animals. Only a fundamental understanding of the chemistry, sources, industrial uses, and metabolism of selenium is presented. In addition, the general lesions of vitamin E-selenium deficiency disease are reviewed.

Chemistry

Baron Jons Jakob Berzelius, a Swedish chemist, discovered selenium in 1817 and named it after Selene, the Greek goddess of the moon (Ageton, 1970). Selenium has the atomic number 34 and an atomic weight of 78.96. It is found in naturally occurring materials such as rocks, soil, plants, and manure in 4 different oxidation states or valences: -2, the selenides; 0, elemental selenium; +4, the selenites; and +6, the selenates. Elemental selenium and the selenates tend to resist oxidation or reduction and theoretically are more stable in a given environment than selenides or selenites. The selenites as ready electron acceptors are reduced to elemental

selenium or organic selenides, while the organic selenides as electron donors are oxidized to those selenium compounds with higher valences (Allaway, 1968). The many forms of selenium have a complex environmental cycle.

In the periodic table of elements, selenium is in a group VI A below sulfur. Selenium, sulfur, arsenic and phosphorus are grouped together and have some similar chemical and biochemical properties. The organic chemistry of selenium resembles the organic chemistry of sulfur except that oxidized forms of selenium are more readily reduced in living systems and organic selenium compounds are less stable than their sulfur analogs. Many forms of selenium are volatile at low temperatures which complicate chemical analysis of feeds and animal tissues (Allaway, 1968).

Sources

Selenium is estimated to be the 69th most abundant element in the earth's crust (Ageton, 1970). It is rarely found in the elemental state. This element is associated with sulfide minerals of copper, iron, lead, and other metals. In the United States, it is mainly obtained as a byproduct of electrolytic copper refining. High-purity grades (99.95 to 99.99% selenium) and commercial grades (97 to 99.94% selenium) of material are used in industry.

Industrial Uses

According to a publication of the U.S. Department of Interior (Ageton, 1970), the current most important use of selenium is in flat-glass, pressed or blown glass and glassware industries which accounted for about 27% of the 1968 demand. Selenium removes color impurities in glass. Equipment for distribution of electrical power

such as specialty electrical transformers, semiconductors and related devices used approximately 23%. Another 23% is utilized in xerographic duplicating machines. The orange-red-maroon pigment, cadmium sulfoselenide, which is light stable and heat and chemical resistant, accounted for another 14% of the 1968 selenium demand. Other uses of selenium included applications in the manufacture of stainless steel and in the preparation of pharmaceuticals, such as niacin, cortisone, fungicides and deodorants. Compounds of selenium are also used as accelerators and vulcanizing agents in rubber products. Fat products are hardened by a selenium catalyst. Selenium has had application in lubricating oils; in extreme-pressure lubricants; in vegetable oils for painting and printing; in photographic photosensitizers and toners; and in mercury vapor detectors, fireproofing agents, insect repellents, phosphorescents, and luminescents.

Metabolism

The true metabolic function of selenium as a toxicant and as an essential micronutrient is unknown. Since selenium is present in varying amounts in all tissues of the body, the true metabolic function of selenium is probably very complex. Several theories have been proposed with the initial ones intimately coupled to vitamin E metabolism. Tappel (1965) demonstrated that selenium was a scavenger preventing free-radical lipid peroxidation damage similar to the antioxidant effect of vitamin E. Schwarz (1965a) proposed that the dehydrogenases of the tricarboxylic acid cycle, especially lipoyl dehydrogenase, was the site of inhibition during selenium and vitamin E deficiency. Although selenium has not been demonstrated to be a cofactor to this enzyme, it may protect the enzyme in a

reduced state (NAS-NRC Monograph, 1971). Diplock *et al.* (1971), who no longer regard the antioxidant theory of vitamin E and selenium as viable, suggest that the active form of selenium may be selenide and that the selenide may form part of the active center of an uncharacterized class of catalytically active non-heme-iron proteins that are protected from oxidation *in vivo* by vitamin E. Rotruck *et al.* (1972) recently suggested that selenium was an integral part of reduced glutathione peroxidase which catalytically prevents hydrogen peroxide- or ascorbic acid-induced oxidation of hemoglobin. Thompson and Scott (1970) indicated that selenium was important in pancreatic metabolism and lipase production since pancreatic fibrosis and impaired vitamin E absorption resulted in chickens on a purified selenium-deficient diet. Ganther (1966) reported the *in vitro* enzymic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts. In 1969, Ganther and Corcoran (1969) concluded that selenium was incorporated between 2 sulfur atoms to form an intramolecular selenotrisulfide linkage in place of the disulfide. Some of these theories on the chemical and biochemical reactions and functions of selenium are fundamentally diverse, and it appears that the true function of selenium will be controversial for some time to come.

Selenium from plants is usually in the organic form (Painter, 1941) which is apparently present mainly in analogs of the common sulfur amino acids: selenocystine, selenocysteine, selenomethionine and selenocystathionine (NAS-NRC Monograph, 1971). Allaway (1968) stated that in feed grains most of the selenium is present in the analog of methionine which is incorporated into proteins of the plants. The other organic forms of selenium along with some

selenate are more commonly found in selenium-accumulator plants: Astragulus (milk vetch), Xylorrhiza (woody aster), Oonopsis (golden weed), and Stanleya (prince's plume) species (Rosenfeld and Beath, 1964). Synthetic organic selenium compounds have only recently become available commercially. Thus, most of the experimental studies have used natural feedstuffs with a highly variable selenium content or purified inorganic selenium salts (Rosenfeld and Beath, 1964; NAS-NRC Monograph, 1971).

Absorption

Inorganic selenium compounds are synthesized into selenoamino acids by rumen microorganisms (Rosenfeld, 1962). There is a suggestion that selenite could be reduced to an insoluble unavailable form by the rumen microorganisms which is considered to be an important factor in the susceptibility of ruminants to selenium deficiency disease (Wright, 1967).

The amount of selenium absorbed from the intestine, and its retention and distribution within the body vary with the species and with the chemical form and amount of the element ingested (Underwood, 1971). Selenium compounds are absorbed from the distal 4/5 of the small intestine (Wright, 1967). Using an isotope of selenite-selenium and a chromium marker, Wright and Bell (1966) found that the net absorption was approximately 35% for sheep and 85% for swine when the respective rations contained 0.35 and 0.50 ppm selenium. Ehlig *et al.* (1967) compared the absorption of selenium as selenite or selenomethionine in lambs and reported no difference when 1 ppm selenium was fed. Direct data on the absorption of toxic doses of selenium are limited.

The mechanism of absorption for ionic selenium has not been reported, but L-selenomethionine is actively transported across the intestinal mucosa similar to and in competition with methionine (McConnell and Cho, 1967).

Jenkins *et al.* (1970) indicated that absorbed ^{75}Se is carried in the $alpha_2$- and $alpha_3$-globulins of plasma within 2 hours after a small dose and also in the albumin fraction of plasma after a large dose. This albumin activity with the large dose dropped to the level of the low dose after 28 hours. The selenium from the small dose was found with the $alpha_2$- and gamma-globulin fractions while the selenium with the large dose was inside the red blood cells associated with globin after 24 hours. This agreed with the work of Imbach and Sternberg (1966), who found that, 3 minutes after intravenous injection of ^{75}Se isotope from selenite into rats, 50% of the selenium was attached to the precipitable serum proteins suggesting a surface adsorption to the serum proteins. After absorption, selenium is widely distributed in all tissues with the largest amounts in liver and kidney (Smith *et al.*, 1937b).

Intermediary Metabolism

Cummins and Martin (1967) found no pathway for *in vivo* synthesis of selenocystine or selenomethionine from selenite in rabbits. From the *in vitro* studies of Ganther and Corcoran (1969), inorganic selenium compounds are probably firmly bound to sulfur compounds or amino acids in the form of selenotrisulfides although a previous report of McConnell (1963) suggested that selenium was present in animal proteins as selenoamino acids. Recently, McConnell and Hoffman (1972) concluded that selenomethionine is incorporated into

polypeptides of eukaryotic cells via the methionine pathway. Awwad *et al.* (1967) had demonstrated the *in vivo* convertibility of selenomethionine to selenocystine and selenocysteine. In addition to the above reports, Imbach and Sternberg (1966) found that ^{75}Se isotope from selenite was chiefly in the microsome fraction (65-76%), while the mitochondria and nuclei averaged 11 and 14%, respectively, in hepatocytes. This suggested the integration of the isotope into the ribosomal phase of protein synthesis. Also, ^{75}Se-methionine in the liver had a more rapid decrease than ^{75}Se from selenite which was probably related to the elaboration and release of ^{75}Se-labeled proteins. Thus, it would seem that there are 2 pathways for selenium metabolism and both involve protein metabolism. One pathway is used by selenium from inorganic compounds and forms selenotrisulfide complexes while the other incorporates sulfur-substituted selenoamino acids into polypeptides. Further work similar to that of Ganther (1966) on the enzymic synthesis of dimethyl selenide may identify the difference between these 2 pathways.

Retention

Underwood (1971) reviewed data indicating that selenium is more efficiently retained from selenium-deficient diets than from selenium-supplemented diets in several species. This indicates an adaptive reaction of the animal to the selenium content of the diet. In addition to this inverse relationship between the percent of selenium retained in the body for diets of low and high selenium content, Hopkins *et al.* (1966) found that the percent of selenium retained in the liver was relatively constant and did not depend upon the level of selenium fed. This observation suggested a

metabolic difference for selenium between the liver and the rest of the body.

A higher percentage of ingested selenium is retained from natural organic compounds than from inorganic selenium salts (Smith *et al.*, 1938). Ehlig *et al.* (1967) confirmed this observation in lambs supplemented with 1 ppm selenium as sodium selenite or selenomethionine in their feed. Thirteen days after initiation of differential treatments, tissues from lambs fed selenomethionine had significantly more selenium than tissues from lambs fed sodium selenite.

Excretion

Underwood (1971) reviewed reports which indicated that selenium excretion varies with the species, diet fed, and previous selenium status. Selenium is excreted primarily by 3 pathways: urine (50-80%), feces (10-20%), and expired air (10-30%) (Smith *et al.*, 1937b; Ganther *et al.*, 1966). Urinary excretion is the predominant pathway of elimination in non-ruminants when physiologic levels of selenium are fed, but selenium excretion in feces and expired air markedly increases when toxic levels are fed. Byard (1969) identified trimethyl selenide as the major excretory product which comprised 60% of urinary selenium in rats fed subtoxic doses of selenium. Palmer *et al.* (1969) found essentially the same result and termed their compound "trimethylselenonium", which accounted for 30 to 50% of urinary selenium in rats injected with selenite. Imbach and Sternburg (1966), using trace amounts of ^{75}Se from selenite and biliary cannulation in rats, demonstrated that 15.6% of the isotope was excreted in the bile in 3 hours, but there was a significant

reabsorption of the isotope through an enterohepatic cycle. Ruminants have a tendency to excrete more of the ingested selenium through the feces than do non-ruminants. The selenium compounds excreted in feces have not been characterized and are generally considered to be predominantly elemental selenium from reduction by the intestinal microflora. The selenium product in expired air has been characterized as dimethyl selenide (McConnell and Portman, 1952). Ganther *et al.* (1966) reported that selenium volatilization can be increased by increasing the selenium content of the basal diet, by increasing the protein and methionine content of the basal diet, or by feeding the appropriate crude materials. Ganther (1966) reported the *in vitro* enzymic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts which is probably an important pathway in selenium detoxification and metabolism.

Rosenfeld (1964) reviewed data on selenium excretion after administering toxic doses and concluded that: (1) there was a limit to the amount of ionic selenium that can pass through the urinary tract; (2) administration of toxic doses of selenium reduced the urinary excretion with subsequent increase in the feces; (3) the interference with excretion was probably due to functional damage to the kidneys; (4) the elimination of selenium by the respiratory tract increased with increased selenium administration; probably there was a threshhold that limited further increase in selenium elimination by the respiratory tract; (5) the selenium that was retained by tissues and entered in the various metabolic pathways subsequently was eliminated in the feces or reabsorbed from the intestinal content for a considerable length of time.

Selenium-Deficiency Disease

Selenium and vitamin E have a complex and somewhat unexplained interrelationship. In some cases the lesions of simple selenium deficiency have been experimentally produced in animals with adequate vitamin E and other nutrients in their diet, while in other cases animals have had natural nutritional diseases which responded to selenium therapy without any additional treatment. Some of the manifestations of selenium deficiency which fall into one or the other of the above classifications include: dietary liver necrosis, poor growth, and alopecia in rats; multiple necrotic degeneration in mice; hepatosis dietetica, edema and "nutritional muscular dystrophy" in swine; exudative diathesis, poor growth, poor feathering, and fibrotic degeneration of the pancreas in chicks; gizzard myopathy in turkey poults; generalized corporal wasting in Japanese quail; and white muscle disease of the skeletal and cardiac muscles and growth and reproductive inefficiency in ruminants (NAS-NRC Monograph, 1971). Although the status of selenium in human nutrition is unestablished, some reports have stated that a low level of selenium therapy resulted in a favorable response in children with kwashiorkor (Schwarz, 1965b; Majaj and Hopkins, 1966).

Trapp *et al.* (1970), at Michigan State University, outlined the clinical manifestations and pathology of vitamin E-selenium deficiency in swine. Sudden death of thrifty feeder pigs was the most characteristic history. Some pigs had been observed with clinical signs of icterus, weakness, and reluctance to move. The gross lesions were multifocal lobules of hepatic necrosis and hemorrhage; bilateral paleness of skeletal muscles; and edema of the lungs, submucosa of the stomach, serosa of the spiral colon, and subcutaneous tissues.

The histopathologic changes in the liver were characterized by cytoplasmic and nuclear degeneration and lysis throughout an entire lobule with massive intralobular hemorrhage. Adjacent lobules often appeared normal. The microscopic lesions in the skeletal muscles varied from Zenker's necrosis, possibly with mineralization, to a loss of myofibers and an accumulation of mononuclear cells. Microangiopathy of arterioles in affected organs was observed. These findings were similar to earlier experimental results of Michel *et al.* (1969).

The suggested requirement for selenium as a feed additive is 0.1 ppm in swine. Muth and Binns (1964) reported that this dose of selenium in sheep was somewhere in the order of 1/50 to 1/75 the minimum lethal dose when fed in daily oral doses. Earlier Schwarz (1960) suggested that the ratio between effective dose and toxic dose based on selenium from sodium selenite was in the order of 1:100. He was working with rats which are the species most resistant to selenium toxicosis (Smith *et al.*, 1937a).

Selenium Toxicosis

Moxon (1937) reviewed the early history of selenium toxicosis. In the story of his travels, Marco Polo may have been the first to describe selenium poisoning in his beasts of burden while traveling through the mountains of western China. In 1856, Dr. T. C. Madison, an Army surgeon, wrote a report describing a malaise in cavalry horses at Fort Randall, Territory of Nebraska. This is credited as the first authentic report of selenium poisoning although the toxic factor was undetermined until Robinson (1933) isolated selenium from toxic feedstuffs. The areas of south central South Dakota and

adjacent northern Nebraska which are just west of the Missouri River and parts of eastern Wyoming have the proper conditions to grow highly seleniferous vegetation. Early settlers in these areas had devastating losses of livestock which prompted workers from the South Dakota Experiment Station and United States Department of Agriculture to begin a joint effort in 1931 to identify, characterize, and answer the agricultural problem that existed. In addition to these researchers, workers at the Wyoming Experiment Station studied poisonous plants that were later shown to have a high content of selenium.

Several potentially toxic minerals, selenium, tellurium, arsenic, vanadium, and molybdenum, were investigated by the South Dakota workers. In 1934 K. W. Franke and others began a series of papers entitled "A New Toxicant Occurring in Certain Samples of Plant Foodstuffs" which incriminated selenium as the toxic agent after Robinson (1933) had identified the presence of selenium in toxic wheat. It was not until later that Franke and Painter (1938) were willing to assume that selenium was the sole toxicant in seleniferous cereals. More recently the question of unidentified phytotoxins as one of the many complicating factors in selenium poisoning was raised again (Muth and Binns, 1964).

Selenium poisoning has been divided into 3 forms in livestock: acute, subacute (blind staggers) and chronic (alkali disease) (Rosenfeld and Beath, 1964). Experimental selenium toxicosis has not always produced the same syndrome as under natural conditions because of the many modifying factors involved (Muth and Binns, 1964). It was extremely difficult to determine the toxic dose of

selenium from the literature because the experimental methods, selenium compounds used, and methods of reporting results have been so varied.

Acute Selenium Toxicosis

In an early report Franke and Moxon (1936) found that the intraperitoneal LD_{75} in 48 hours for rats was 3.25 to 3.50 mg selenium per kg body weight for sodium selenite and 5.25 to 5.75 mg selenium per kg body weight for sodium selenate. By subcutaneous, intraperitoneal, or intravenous injection, the minimum lethal dose of sodium selenite expressed as mg selenium per kg body weight was from 3.0 to 5.7 for rats, 0.9 to 1.5 for rabbits, and 1.5 to 2.0 for the dog (Smith *et al.*, 1937a; Painter, 1941; Anderson and Moxon, 1942). In studies on the toxicity of intravenous sodium selenite, Heinrich and MacCanon (1957) reported that the 24-hour LD_{50} in dogs was between 0.875 and 1.0 mg selenium per kg body weight; the 24-hour LD_{100} was between 1.0 and 1.5 mg selenium per kg body weight. From the conclusions of Franke and Painter (1938), the relative toxicity of selenium in the different diets fed to rats was in the order: wheat > corn > barley > selenate > selenite. Since the natural forms of selenium were organic, Moxon *et al.* (1938) studied the toxicity of β selenodipropionic acid, n-prophylseleninic acid, β diselinopropionic acid, ββ'diselenodipropionic acid and dibenzyldiselenide and found each of them to be much less toxic than selenite to rats. In a subsequent paper, Moxon *et al.* (1941) reported that the toxicity of L-selenocystine was similar to seleniferous wheat while D-selenocystine was less toxic to rats. In 1949 Klug *et al.* found that the 48-hour LD_{75} for rats was 4 mg selenium

per kg body weight for selenocystine and 4.25 mg selenium per kg body weight for selenomethionine. In addition, selenocystine was very toxic to the liver succinoxidase system while selenomethionine was relatively nontoxic.

Toxicity studies with farm animals are more limited than those with laboratory animals. The acute toxic oral dose of sodium selenite in 24 hours calculated as mg selenium per kg body weight was reported as 8.4 to 11.0 for cows, 3.3 for horses and mules, and 13.2 to 17.6 for pigs (Miller and Williams, 1940a; Anderson *et al.*, 1961). Diener (1961) confirmed the minimum lethal oral dose of sodium selenite reported by Miller and Williams (1940a) and found that 17.6 mg selenium per kg body weight killed young pigs in 48 hours. Orstadius (1960) injected sodium selenite subcutaneously into pigs weighing 30 to 70 kg at single dosages of 2.0 and 1.2 mg selenium per kg body weight, and the pigs died after 4 hours and 5 days, respectively. Dosages of 0.9 to 1.1 mg selenium per kg body weight caused no clinical signs of toxicosis. In a report on selenium toxicosis in calves resulting from an error in preparing a therapeutic solution of selenium, Shortridge *et al.* (1971) found that one subcutaneous injection of sodium selenite at a dosage of approximately 0.5 mg selenium per kg body weight was lethal within 24 hours for 18 of 557 stressed calves. This single injection of selenium resulted in 376 (67%) deaths in these 557 calves over a 5-week period. Eighty percent of the heifers which received both a brucellosis vaccination and an injection of selenium died, compared to 56% of the steers which only had the injection of selenium. Kuttler *et al.* (1961) used sodium selenite suspended in peanut oil containing 2% beeswax which was injected subcutaneously into sheep

at levels of 0.44, 0.88, 1.76 and 3.3 mg selenium per kg body weight. Two sheep receiving 3.3 mg selenium per kg body weight and 1 sheep receiving 1.76 mg selenium per kg body weight died in less than 24 hours. Muth found that 11 of 12 ewes tolerated 3.73 mg selenium per kg body weight when fed as sodium selenite, 12 of 12 ewes died from 5.03 mg selenium per kg body weight when injected subcutaneously in a sodium selenite-peanut oil-beeswax suspension, and 12 of 12 ewes died from 0.66 mg selenium per kg body weight when injected subcutaneously as an aqueous sodium selenite solution (Muth and Binns, 1964). Binns reported that 8 sheep fed 0.33 to 1.15 mg selenium per kg body weight per day as sodium selenite remained normal through 90 days, but 2 sheep fed 0.33 mg selenium per kg body weight per day from seleniferous *Astragalus prussii* died on the 5th and 6th day, respectively (Muth and Binns, 1964). In 2 reports, Caravaggi *et al.* (1970a, 1970b) found that the LD_{50} in lambs was 0.455 mg selenium per kg body weight when sodium selenite was administered as a single intramuscular injection and was 1.9 mg selenium per kg body weight when sodium selenite was fed as a single oral dose. Neethling *et al.* (1968) observed that 4 mg selenium (as selenium dioxide) per kg body weight when administered intravenously was fatal to 2 adult sheep in 20 minutes and that 3.4 mg selenium (as selenomethionine) per kg body weight when administered intravenously was fatal in approximately 10 hours. All reports have indicated that metallic selenium is non-toxic because of its insolubility. Young animals are more susceptible to acute selenium toxicosis than are adults when dosages based on body weight are used (Gabbedy and Dickson, 1969; Shortridge *et al.*, 1971).

Clinical signs and physiological data. The clinical signs and physiological data are primarily limited to the dog (Heinrich and MacCanon, 1957; Heinrich and MacCanon, 1960; Heinrich and MacCanon, 1961; Heinrich, 1963). Acute selenium toxicosis was characterized by vomiting, diarrhea, apprehension, respiratory stimulation often accompanied by rales, and cardiovascular changes such as arrhythmias and weak, irregular pulse; all of these clinical signs became more pronounced with increasing dosage. Physiological data after the intravenous administration of 2.0 mg selenium (as sodium selenite) per kg body weight included a decrease in systemic blood pressure and peripheral resistance, an increase in pulmonary arterial pressure and resistance, and an initial increase and subsequent depression of cardiac output. Electrocardiographic changes indicated cardiac damage. Increased minute volume and decreased tidal volume characterized the respiratory stimulation. The significant changes in the blood components were an increase in hematocrit and plasma protein, potassium, lactate, and pyruvate, and a decrease in venous pH. From these studies it was concluded that functional circulatory failure is a more plausible reason for the cause of death in acute selenium toxicosis rather than respiratory failure, as reported in the earlier literature (Beath *et al.*, 1935; Moxon and Rhian, 1943). Previously, Anderson and Moxon (1942) had characterized the changes in blood from dogs with acute selenium poisoning. These were summarized as a marked increase in hemoglobin and hematocrit; decrease in inorganic phosphorus, non-protein nitrogen, calcium, ascorbic acid, and blood sugar; and reduction in the glutathione content of the blood.

In the economic farm animals, the general clinical signs of acute selenium toxicosis have been the rapid onset of hyperpnea, dyspnea, cyanosis, salivation, depression, tremors, hypersensitivity to external stimuli, normal to slightly elevated temperature, opisthotonos, paresis, convulsions, coma, and death (Miller and Williams, 1940a; Neethling *et al.*, 1968; Shortridge *et al.*, 1971). In addition, vomition was reported in swine. Schneider (1936) cited Gmelin (1824) and credited him with the identification of a characteristic garlicky odor on the breath of selenium-poisoned animals. This observation has been established in the literature (Moxon and Rhian, 1943) but has been peculiarly omitted from the more recent reports (Glenn *et al.*, 1964a; Morrow, 1968; Neethling *et al.*, 1968; Caravaggi *et al.*, 1970a; Shortridge *et al.*, 1971).

Lesions. Descriptions of the gross morphologic lesions of acute selenium toxicosis in domestic livestock and laboratory animals have been reviewed (Moxon and Rhian, 1943; Rosenfeld and Beath, 1964) and have recently been reported for sheep and cattle (Caravaggi *et al.*, 1970a; Shortridge *et al.*, 1971), but the reports of toxicosis in swine are limited (Miller and Williams, 1940a; Diener, 1961). The gross lesions are summarized as: numerous petechial hemorrhages on serosal surfaces, congestion of the mesenteric blood vessels, hemorrhages and edema in the lungs, hydrothorax, subendocardial and subepicardial hemorrhages, enlargement of the heart, enlarged and pale yellow liver, congestion of lymph nodes, enlarged kidneys, congestion of the renal medulla, distention of the urinary bladder, hemorrhagic enteritis, and congestion of adrenals and pancreas. Rosenfeld and Beath (1946a) indicated the

general picture in acute selenosis was hemorrhages and smooth muscle atony, but more recent reports (Diener, 1961; Gabbedy and Dickson, 1969; Caravaggi *et al.*, 1970a; Shortridge *et al.*, 1971) emphasized the histopathologic changes of fatty metamorphosis and necrosis in liver and kidney as well as myopathy of the heart and skeletal muscles. Glenn *et al.* (1964b) reported that the most severe and consistent lesions of selenate toxicosis in sheep were focal to diffuse myocardial necrosis and fibrosis, and edema and interstitial hemorrhages in the lung.

Selenium content of tissues. Dudley (1936) noted that in animals fed inorganic or organic selenium compounds the selenium was found to be distributed throughout the entire carcass but in widely varying proportions. The liver, kidneys and spleen from animals with selenium poisoning had the greatest concentrations of selenium, ranging from 4.0 to 25.0 ppm. When lambs were drenched with an oral dose of 6.4 mg selenium (as sodium selenite) per kg body weight, Gabbedy and Dickson (1969) reported that the hepatic concentrations of selenium, expressed on a dry weight basis, in lambs dying within 2 days averaged 64 ppm, and in those dying 15 days later hepatic and renal concentrations of selenium averaged 26 ppm and 7.4 ppm, respectively. Shortridge *et al.* (1971) found that 2 calves dying in 48 to 72 hours had hepatic concentrations of selenium on a wet basis between 1.82 and 3.11 ppm and renal concentrations between 1.57 and 2.09 ppm, while a calf dying 1 month later had hepatic and renal concentrations of 0.76 and 0.89 ppm, respectively.

Subacute Selenium Toxicosis ("Blind Staggers")

Although selenium toxicosis from inorganic selenium salts and organic selenium compounds may be manifested in all stages from acute to chronic, "blind staggers" was thought to be the result of more modifying factors such as phytotoxins than any of the other syndromes involving selenium toxicity (Muth and Binns, 1964). "Blind staggers", a disease of cattle and sheep, was an inappropriate lay term that has been perpetuated especially by Beath and co-workers (Draize and Beath, 1935; Rosenfeld and Beath, 1946a; Rosenfeld and Beath, 1964). This type of poisoning results when moderate amounts of seleniferous plants have been consumed over a period of time. The amount of selenium consumed has been highly variable or virtually undetermined.

Clinical signs. Rosenfeld and Beath (1964) reviewed 3 stages of "blind staggers." In the early stage the affected animal wanders, circles, and disregards objects in its path as if it were partially blind. Anorexia is present. The body temperature and respiration are normal. The second stage is characterized by weakness and collapse of the front legs without other evidence of paralysis. The third stage is paralytic with dysphagia, amaurosis, dyspnea, abdominal pain, subnormal body temperature, and subsequent death. The exact cause of death remains unknown.

Lesions. The gross pathologic changes of "blind staggers" were described by Draize and Beath (1935) and Rosenfeld and Beath (1946a). This condition is characterized by congestion of the visceral blood vessels and lymph nodes, hemorrhages in heart and gastrointestinal tract, atony of the gastrointestinal tract,

urinary bladder and gallbladder, congestion of the renal medulla, focal necrosis of liver with cirrhosis, and variable erosions on the articular surfaces of long bones.

Histologically the changes were fibrosis, congestion and necrosis of the myocardium with serous atrophy of fat around the coronary vessels; thickened walls of alveoli in the lungs; fatty metamorphosis, necrosis and fibrosis in the liver; glomerulonephritis and tubular degeneration in the kidney; hyperplasia, fibrosis, and congestion of the spleen; epithelial desquamation, hemorrhages and frequently ulcerations in the gastrointestinal tract; and necrosis of the anterior pituitary gland.

Selenium content of tissues. The selenium content of tissues in the terminal stages of "blind staggers" has been directly related by Rosenfeld and Beath (1964) to the duration of life of the animal. If death occurred soon after the disease developed, the concentrations of selenium were high in all tissues. When the disease was prolonged, the selenium content was considerably lower; the kidney, liver, and blood contained 2.0 to 5.0, 1 to 8, and 1.5 to 4 ppm selenium, respectively.

Chronic Selenium Toxicosis

In chronic selenium toxicosis, it may take several weeks to months before mortalities are precipitated depending on numerous modifying factors such as composition of diet, age, sex, and species of animal. In his first publication on the "new toxicant", F. W. Franke (1934a) found that feeding toxic grain to rats resulted in deaths over an extended period. The toxicant was present in the protein fraction of these grains (Franke, 1934b). Diluting the toxic

grain with non-toxic grain so that only 17% was from the toxic source still resulted in growth depression and death of rats (Franke, 1935a). Alternate feeding of toxic and control diets gave rhythmic decreases and increases in growth and food consumption (Franke, 1935b). Smith *et al.* (1937a) reported that chronic toxicity of selenium varied in the different species and in different individuals of the same species. The rat was the most resistant, and the cat was the most susceptible of the species examined. Females of a species were more susceptible than males, and young animals were more susceptible to chronic selenium poisoning than older animals (Rosenfeld and Beath, 1964). Smith *et al.* (1937a) indicated that no acquired tolerance to selenium was demonstrated, but Heinrich and MacCanon (1957) stated that dogs developed a tolerance to selenite under suitable conditions. Wahlstrom and Olson (1959) also observed tolerance to selenium toxicity in pigs from sows that had been fed selenized rations over a long time.

The levels of selenium, expressed as ppm unless otherwise indicated, in the ration generally associated with chronic toxicity have been: 5 to 8 for chickens, 5 to 40 for cattle, 7.2 to 10 for dogs, 5 to 15 for rats, 10 to 15 for swine, and 10 mg selenium per day for sheep (Miller and Schoening, 1938; Rosenfeld and Beath, 1946b; Anderson *et al.*, 1961). Miller and Williams (1940b) fed sodium selenite to 2 horses at the rate of 24 ppm selenium equivalent in the feed for 24 weeks, 48 ppm for another 37 weeks, and 96 ppm for an additional 9 weeks before they died of an atypical selenium toxicosis. Schoening (1936) described 2 pigs that were fed seleniferous corn containing 10 ppm selenium for about 4 months and had the clinical signs of toxicosis. Schoening (1936) failed to observe

mortality in 2 pigs fed seleniferous corn containing 10 ppm selenium although the clinical signs began to develop after about 6 weeks. Seleniferous corn with 10 ppm selenium fed to 3 additional pigs and corn with 5 ppm selenium fed to 5 other pigs had no visible deleterious effect. Maag *et al.* (1960) fed selenium orally 3 times a week in varying dosages from 0.55 to 1.1 mg selenium (as sodium selenite) per kg body weight to 8 feedlot steers. They reported that 2 steers died within 8 weeks and 4 more died after 20 to 23 weeks. No cumulative effects from sodium selenite were demonstrated, and it was concluded that there were dosages of sodium selenite which an animal can tolerate without ill effects. The classic lesions of selenium poisoning did not develop; polioencephalomalacia was noted in 2 steers.

Clinical signs and physiological data. The clinical manifestations of natural chronic selenium toxicosis have been historically associated with another inappropriate lay term, "alkali disease." Domestic animals with this disease were identified by a lack of vitality, anemia, stiff gait, lameness, rough hair coat, loss of hair, especially mane and tail in horses, and hoof lesions and deformities (Rosenfeld and Beath, 1964). Appetite was markedly decreased with naturally seleniferous and artificially selenized feedstuffs in rats, swine, and horses (Munsell *et al.*, 1936; Miller and Schoening, 1938; Miller and Williams, 1940b). Specifically, in swine, additional clinical signs included loss of hair along the back, retarded growth, emaciation, lameness and hoof lesions followed by the shedding of the hoof wall (Schoening, 1936; Miller and Schoening, 1938). These clinical manifestations have been thought to be nearly pathognomonic.

Halverson *et al.* (1970) determined that the anemia which occurred with selenite-fed rats was attributable to hemolysis rather than a defect in blood synthesis. Neethling *et al.* (1968) isolated an abnormal hemoglobin C from sheep erythrocytes after feeding selenium. They postulated that this abnormal hemoglobin C which contained selenium was important in the pathogenesis of the anemia found in the South African ovine diseases, "geeldikkop" and "enzootic icterus." Rosenfeld and Beath (1946c) reported that sheep with selenium poisoning had less protein, sulfur, vitamin A and ascorbic acid in their livers although these parameters returned to normal in about 60 days after selenium withdrawal. During chronic selenosis, adenosine triphosphate and other labile phosphate compounds were decreased in the liver. Inorganic phosphate was elevated probably because of an insufficiency in renal function (Rosenfeld, 1964).

Lesions. Many of the characteristic findings in chronic selenosis have been mentioned in the section on clinical signs. Additional gross changes included a flabby decompensated heart and an atrophic cirrhotic liver. The renal medulla was congested, and the spleen was atrophic and dull brownish-yellow in color. Erosions of the articulating surfaces of long bones and miscellaneous other changes were present (Draize and Beath, 1935).

The histopathologic changes included atrophy and fibrosis of myocardial fibers, an accumulation of yellow pigment near myocardial nuclei; thickening and fibrosis in the alveolar walls of the lung; fibrosis, pseudolobulation, and proliferation of bile ducts in the liver; chronic cholecystitis; fibrosis and obliteration of glomeruli

and tubules in the kidney; and fibrosis and hemosiderosis of the spleen (Rosenfeld and Beath, 1964). In addition, Smith *et al.* (1937a) reported gastroenteritis and hematopoietic depression in rats.

Selenium content in tissues. The reported selenium content of the tissues from animals dying of chronic toxicosis have been variable. Rosenfeld and Beath (1945) indicated that liver and kidney accumulated greater quantities of selenium than did other body tissues. In sheep on high, medium, and low protein diets, the selenium concentration in the liver and kidneys of those dying from selenium toxicosis ranged from 9.10 to 33.17 ppm and 3.95 to 13.55 ppm, respectively, when fed between 15 and 30 mg selenium per day. Death occurred in these sheep after the concentration of selenium in the blood increased above 2 ppm selenium and selenium feeding continued (Rosenfeld and Beath, 1946a). Dinkel *et al.* (1957) confirmed the diagnostic value of the selenium content in blood and extended their observations to the selenium content of hair from cattle grazing seleniferous pastures. They noted that cattle having over 2 ppm selenium in the blood would likely develop the clinical signs of selenium toxicosis. They also reported that a selenium content above 10 ppm in hair is a reliable indication of toxicity to selenium in cattle if the samples of hair are collected from several animals.

Withdrawal of seleniferous feed for 60 days resulted in normally non-toxic tissue concentrations of selenium (Rosenfeld and Beath, 1945; Dinkel *et al.*, 1957). However, Munsell *et al.* (1936) noted the effects of selenium poisoning in rats even after the withdrawal

of seleniferous feed and the elimination of selenium residues in the tissues. This emphasized the seriousness of selenium poisoning.

Factors Influencing Toxicosis

Because selenium occurred in soils with numerous other possibly toxic elements, arsenic, molybdenum, tellurium, vanadium, and selenium salts were injected intraperitoneally and were fed to study their comparative toxicities (Franke and Moxon, 1936; Franke and Moxon, 1937). Selenium salts were the most toxic to rats. The combined toxicities of 11 ppm selenium from seleniferous wheat with vanadium, molybdenum, chromium, tungsten, fluorine, arsenic, cadmium, zinc, cobalt, uranium, and nickel, given as water-soluble salts at 5 ppm in the drinking water, indicated that these elements, with the exception of tungsten and arsenic, increased the mortality of rats (Moxon and DuBois, 1939). Rosenfeld (1964) confirmed increased selenium toxicity after zinc supplementation. While investigating the interrelationship between selenium and specific trace elements, McConnell and Carpenter (1971) reported evidence that there was a reaction at the site of injection between selenium and zinc or cadmium but not between selenium and arsenic or tellurium. It appeared that selenium prevented arsenic and tellurium from reaching target tissues.

Rosenfeld and Beath (1964) reviewed rather extensive data indicating that arsenical compounds prevented or reduced selenium toxicity when relatively low toxic amounts of selenium were present in the diet. Inorganic arsenic salts were too toxic for practical use. The organoarsenicals, arsanilic acid or 3-nitro-4-hydroxyphenylarsonic acid, were less toxic and have given partial protection

against selenium poisoning (Moxon, 1941; Hendrick *et al.*, 1953; Wahlstrom *et al.*, 1955; Dinkel *et al.*, 1957; Minyard *et al.*, 1960).

Numerous reports have indicated that increased protein in the diet partially reduced the incidence of selenium toxicosis (Smith, 1939; Gortner, 1940; Rosenfeld and Beath, 1946b; Rosenfeld and Beath, 1964). Linseed meal was especially effective when combined with an arsenical (Wahlstrom *et al.*, 1956), but the amount of linseed meal necessary to prevent selenium poisoning is so great that it is not considered a practical preventative substance (Olson, 1969b). Rosenfeld and Beath (1947) found that beet pectin gave some protection against selenium poisoning from selenite while vitamin C and potassium iodide did not. Two months after selenium and beet pectin feeding was discontinued, ascites due to portal obstruction in the liver became apparent.

Smith (1939) reported that rats fed high fat diets were more tolerant to selenium than those fed high carbohydrate diets. This observation was extended by Romanowski *et al.* (1958). With sodium selenite incorporated at a level which permitted growth, rats fed isocaloric diets with added fat but without added carbohydrate, in most instances, exhibited somewhat better growth and more normal livers than those fed isocaloric diets containing added carbohydrate. A better understanding of the interrelationship of selenium and the major dietary components is still needed.

When administered separately methionine and tocopherol were ineffective against selenium toxicity (Klug *et al.*, 1952; Morss and Olcott, 1967), but a combination of 0.5% methionine plus 0.01 to 0.05% alpha-tocopherol gave increasingly better protection in rats depending on the level of alpha-tocopherol added (Levander and Morris, 1970).

Bromobenzene has been used in an attempt to increase selenium excretion following ingestion of seleniferous feed (Moxon *et al.*, 1940). As reviewed by Rosenfeld and Beath (1964), some experimental results have indicated that the administration of bromobenzene increased the urinary excretion of selenium, but other reports have failed to confirm this observation. The inherent toxicity of bromobenzene must be pointed out. Koch-Weser *et al.* (1952) described the acute centrolobular hepatic necrosis observed following the parenteral injection of bromobenzene.

There are no truly effective means of preventing or treating selenium poisoning. Prevention through good management is the most practical answer to the problem of selenium poisoning in animals (Olson, 1969b).

Selenium and Human Health

Knowledge on actual selenium toxicosis in man has never been fully documented. The surveys of Smith *et al.* (1936) and Smith and Westfall (1937) from seleniferous areas of South Dakota and Wyoming did not identify selenium poisoning as a problem in man although selenium intake may have contributed to the gastrointestinal disturbances and hepatic dysfunctions that were noted. Urinary excretion was mentioned as a reliable index of selenium intake. Schroeder *et al.* (1970) recently estimated that the daily intake of selenium in a standard hospital diet was 62 µg. He also noted that wild animals normally carry a body-burden of selenium 2 to 3 times higher than that found in humans.

Selenium and Cancer

Frost (1971) indicated that the carcinogenic stigma of selenium has placed it under the Delaney Clause of the Pure Food Law and prevented its approval as a feed additive for animals. The experimental evidence on the interrelationship between selenium and cancer was reviewed (Anon., 1970). Although selenium was considered a highly toxic element that produced tumors when ingested over long periods, recent work has suggested that some selenium compounds may actually inhibit the development of tumors and that there may be an inverse relation between the level of selenium in the blood of human subjects and mortality from cancer. More experimental and epidemiologic studies are needed to clarify the relationship, if any, between selenium and cancer.

Summary

Although the literature on selenium is voluminous, the true metabolic function of selenium as a micronutrient or a toxicant is essentially unknown. Many seemingly contradictory and incomplete findings have been reported. Accurate minimum lethal dosages of the various forms of selenium for each species of animal, especially in swine, have not been recorded for application to current nutritional and agricultural practices. Studies on selenium toxicity as related to losses from swine diseases are limited.

It is evident from this survey of the literature that additional information on selenium toxicosis in swine is needed. This information would not only be valuable to the swine and general livestock industry, but it would also provide additional basic information on the role of selenium in biomedicine.

OBJECTIVES

The general objectives of this research were to obtain additional information on the clinical signs, lesions, and selenium residues due to feeding young pigs specific amounts of both sodium selenite and selenomethionine. This research was preceded by preliminary studies on the effects produced by feeding a vitamin E-selenium deficient diet to pigs and also by injecting selected amounts of selenium as sodium selenite into swine and cattle. The specific goals of the main research were:

1. To determine the general clinical signs and lesions when young pigs were fed specific amounts of sodium selenite or selenomethionine.

2. To interpret and record the macroscopic, microscopic, and ultrastructural changes produced by feeding different amounts of the compounds in Objective 1.

3. To determine the selenium content of liver and kidney after feeding different amounts of both selenium compounds and to correlate the selenium content of these organs with the lesions recorded in Objective 2.

4. To differentiate the pathologic changes of selenium toxicosis from those of vitamin E-selenium deficiency in pigs.

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5. To provide relevant information from a combination of the above objectives for the diagnosis of selenium toxicosis in swine and for possible implications in public health.

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MATERIALS AND METHODS

Preliminary studies on the toxicity of sodium selenite involved intramuscular and/or intravenous injections into 3 young pigs and 6 yearling steers. (The steers had been previously used on a leukemia project.) The essential procedures of these preliminary studies are included in the results.

Two principal experiments were conducted using a total of 44 pigs which were fed rations based on either Torula yeast (Experiment A) or whole milk powder (Experiment B) as protein sources. Graded amounts of either sodium selenite or selenomethionine were added to each of the above rations to produce various degrees of selenium toxicosis. In addition to feeding the selenium compounds, each of these 2 compounds was injected intravenously into 2 individual pigs from Experiment B to investigate peracute selenium toxicosis.

Animals, Facilities and Husbandry

A total of 50 genetically related 2-week-old Yorkshire-Hampshire pigs were purchased from the L. W. Cheney Farm, Mason, Michigan. Each pig had had an iron injection at the farm to prevent anemia. Thirty-eight pigs which were initially assigned to Experiment A were selected for size and sex from 4 litters in 1 farrowing and were received in January 1972, while 12 pigs which were assigned to Experiment B were selected for size from 2 litters in another farrowing and were received in March 1972. In addition to the pigs

from the Cheney Farm, four 2-week-old Yorkshire pigs were obtained from a litter which farrowed in March 1972 at Barn 5 (MSU Pathology Department) and were assigned to Experiment B.

On arrival each pig was weighed and identified by coded ear-notches. During a 2-week period of acclimation to the experimental environment and ration after weaning, 10 of the 38 pigs for Experiment A were selected and removed because of poor appetite, scours or death. The 16 pigs in Experiment B underwent a similar period of acclimation without problems.

All pigs were kept at Barn 5 on the MSU Veterinary Research Farm. They were placed in individual 21 x 36 inch galvanized metal metabolism cages equipped with wire floors and individual feed and water crocks. The feed and water crocks were thoroughly washed once each day. Each pig in Experiment A had a specifically identified and weighed feed crock to facilitate measurement of feed consumption. In Experiment B the unconsumed liquid was measured back in a graduated cylinder. The daily feed consumption was recorded in both experiments. Each pig was fed and observed 2 to 4 times per day after initiation of the experiments.

Experimental Design

The pigs in Experiment A were assigned according to the experimental design given in Table 1. Each group contained a male and a female pig; the pigs with better appetites were allotted to groups of pigs fed the higher concentrations of selenium. The experimental design of Experiment B is given in Table 2. These pigs were consigned to groups of 2 pigs each so that pigs 13B, 14B, 15B, and 16B from the litter born at Barn 5 were in different experimental groups. No attempt was made to allot these pigs by sex.

Table 1. Allotment of pigs according to sex, selenium compound, and selenium concentration of ration in Experiment A

Selenium added to ration (ppm)	Sodium selenite		Selenomethionine	
	Pig no.	Sex	Pig no.	Sex
0	4*	M**	31	M
	35*	F**	5	F
0.1	34	M	13	M
	24	F	30	F
5	11	M	12	M
	19	F	17	F
10	15	M	8	M
	32	F	9	F
20	23	M	3	M
	36	F	26	F
45	18	M	7	M
	6	F	20	F
100	1	M	14	M
	37	F	38	F

*These 2 pigs were not supplemented with 22 IU vitamin E/kg of ration to produce vitamin E-selenium deficiency; all other pigs were supplemented.

**M=male; F=female.

Table 2. Sex and allotment of pigs according to selenium compound and selenium concentration of dry ration in Experiment B

Selenium added to dry basal ration (ppm)	Sodium selenite		Selenomethionine	
	Pig no.	Sex	Pig no.	Sex
0	3B*	F**	4B	F
	13B	M**	14B*	F
60	5B	M	6B	F
	15B	M	16B	F
120	1B	F	2B	F
	11B	M	8B	F
600	7B	F	10B	F
	9B	M	12B	F

*After the principals of this experiment had died of selenium toxicosis, Pigs 3B and 14B were given 3 mg selenium/kg body weight intravenously as sodium selenite or selenomethionine, respectively, to produce peracute selenium toxicosis.

**F=female; M=male.

Rations

The rations of both experiments were calculated to provide 16% protein in the dry mixed ingredients from information provided by the suppliers. The basal ration to which the selenium compounds were added at each feeding for the pigs in Experiment A is given in Table 3. The general feeding regimen was a modified paired feeding method where all surviving pigs were fed 150 gm/pig twice daily for the first 3 weeks, 250 gm/pig twice daily for the second 3 weeks, and 1000 or 2000 gm/pig once daily until the end of Experiment A, which was as long as 13 weeks.

The basal ration to which graded amounts of selenium compounds were added at each feeding for the pigs in Experiment B is given in Table 4. The general feeding regimen was 250 gm reconstituted milk/pig fed 4 times a day. This amounted to the feeding of 167 gm dry basal ration/pig/day. No additional water was provided for the pigs in Experiment B.

There was one irregularity in the feeding regimen in Experiment B. Following profound anorexia, Pigs 2B, 5B, 15B, 6B and 16B were force-fed their daily allotments of selenized milk by syringe and stomach tube during their last 2 days (8 feedings) on experiment, since the pathologic changes of selenium toxicosis were primary and the changes from anorexia were to be avoided.

Selenium Compounds

Sodium selenite* (bacteriological grade) and seleno DL methionine* (chemically pure) were used as the sources of inorganic and organic selenium, respectively. Each of these selenium compounds was

*Purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio 44128.

Table 3. Composition of basal ration fed to pigs in Experiment A

Ingredients	Percent
Torula yeast, Type B (50% protein)	32
Cod liver oil*	5
Mineral mix**	6
Vitamin mix† ††	2
Cerelose	55
	100%

*Provided vitamin D_3, 600 ICU/gm; and vitamin A, 1500 USP units/gm.

**Prepared by Dr. E. R. Miller, Animal Husbandry Department, as used previously (J. Nutr., 85, (1965): 347), and contained the following compounds expressed as percentages of the total mineral mixture: KCl, 10.0; KI, 0.002; $FeSO_4 \cdot 2H_2O$, 0.7; $CuSO_4$, 0.1; $CoCO_3$, 0.1; $MnSO_4 \cdot H_2O$, 0.1; $ZnSO_4 \cdot H_2O$, 0.4; $MgCO_3$, 2.0; $NaHCO_3$, 25.0; $CaHPO_4 \cdot 2H_2O$, 36.0; $CaCO_3$, 12.5; and cerelose, 13.098.

†Provided the following compounds expressed as ppm of basal ration: thiamin HCl, 3.0; riboflavin, 6.0; nicotinic acid, 40.0; Ca pantothenate, 30.0; pyridoxine HCl, 2.0; PABA, 13.0; ascorbic acid, 80.0; inositol, 130.0; choline, 1300.0; folic acid, 0.260; d-biotin, 0.050; cyancobalamin, 0.10; and menadione Na bisulfite, 0.065.

††Provided 22 IU vitamin E/kg of basal ration for all pigs in experiment except Pigs 4 and 35.

Table 4. Composition of dry basal ration fed to pigs in Experiment B*

Ingredients	Percent
Whole milk powder**	60.5
Cerelose	39.5
	100.0%

*This dry basal ration was reconstituted for feeding in the following proportions by weight: 1 part dry basal ration to 5 parts tap water.

**Supplier's analysis expressed as percentages was: protein, 26.50; lactose, 38.00; fat, 27.00; ash, 6.05; water, 2.00.

accurately weighed and dissolved in distilled water so that stock solutions contained 1 mg selenium/ml. As needed, volumetrically measured amounts of each stock solution were diluted with distilled water to prepare 6 different working solutions for each selenium compound which contained 0.001, 0.05, 0.1, 0.2, 0.45, and 1.0 mg selenium/ml. When 1 ml of each working solution was added to 10 gm of dry ration, it provided selenized feed which contained 0.1, 5, 10, 20, 45, or 100 ppm selenium, respectively, in Experiment A. A similar amount of distilled water was added to the ration of those pigs that received no selenium. In Experiment B, 1 ml of working solution (0.1, 0.2, or 1.0 mg selenium/ml) was added to each 10 gm of reconstituted milk, and this provided concentrations of 60, 120, or 600 ppm selenium when calculated on a dry weight basis using the reconstituted milk. Separate and identified volumetric pipettes were used to measure the working solutions added to the feed at each feeding. Following addition of the selenium to the feed, both were mixed together by stirring.

Experimental Parameters and Procedures

When the pigs in Experiment A were placed on experiment, blood (20 ml) from the anterior vena cava was collected into tubes containing heparin, and accurate body weights were recorded to establish base lines. Additional bleedings were made just prior to euthanasia of healthy or moribund pigs. Body weights of surviving pigs were registered at weekly intervals for 2 months and at the termination of the experiment. From the pigs in Experiment B, blood (20 ml) without an anticoagulant was collected initially and terminally as in Experiment A. Initial and final body weights were also recorded.

From the blood collected in Experiment A, a small aliquot was used for determinations of hemoglobin, hematocrit, and total and differential leukocyte counts; the remainder was centrifuged and separated as plasma and packed erythrocytes. The plasma was frozen and stored for selected determinations of plasma tocopherol by the Emmerie-Engel method modified by Tsen (1961) and plasma ornithine carbamyl transferase (OCT) by the method of Sigma Chemical Company (1965). The 48-hour osmotic fragility of erythrocytes was determined by the method of Dacie (1953) cited and modified by Stowe (1962). After evaluating the results of this procedure during the majority of Experiment A, it was discontinued as an unreliable procedure in the pig. In Experiment B, the clotted whole blood was centrifuged for separation of the serum which was frozen for selected determinations of serum OCT by the method of Sigma Chemical Company (1965) and serum glutamic-oxaloacetic transaminase (GOT) by the method of General Diagnostics (1968). In addition to the above, selected determinations of urea nitrogen were made on either

plasma or serum in both experiments by the method of Hycel, Incorporated (1964).

From specimens of liver and kidney collected at necropsy, selected livers were analyzed for tocopherols by the Emmerie-Engel method modified by Tsen (1961); all livers and kidneys from all pigs were analyzed for selenium by the method of Olson (1969a).* All determinations of selenium were made in duplicate on samples weighing about 2 gm. The duplicates of each sample were analyzed on separate days. These results will be reported as average values of the duplicates.

The clinical signs of each pig were recorded after each feeding. Body temperatures were taken in pigs that had clinical toxicosis. When the body temperature was below normal and the pig appeared moribund, it was euthanatized by electrocution or bleeding depending on its state of consciousness. Some pigs with toxicosis died naturally. Healthy principals and controls were euthanatized by electrocution at selected periods during the experiments and at the termination of the experiments.

Postmortem Examination

At necropsy, postmortem weights of liver and kidneys were registered. A thorough gross examination of all organs was made, and lesions were recorded. The following tissues were routinely collected and fixed in buffered 10% formalin for histopathologic

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*In cooperation with Dr. O.E. Olson, Department of Experiment Station Biochemistry, South Dakota State University. This cooperation resulted from personal communication with Dr. Olson and his interest in this research.

examination by light microscopy: salivary glands, mandibular or portal lymph nodes, thymus, thyroid, lung, heart, diaphragm, semitendinosus muscle, esophageal and fundic portions of the stomach, duodenum, jejunum, ileum, colon, pancreas, liver, gallbladder, ovary or testicle, cerebrum, cerebellum, brain stem, pituitary gland, a small segment of spinal cord within the 13th or 14th thoracic vertebra, sciatic nerve, ventral part of the right 3rd rib including the costochondral junction, medial claw of the right hind foot, and skin. Selected tissues were also fixed in Zenker's and Bouin's fixatives. The fixed tissues were routinely embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin (H & E) (Luna, 1968). Selected sections of tissues were also stained with oil red O, Best's carmine, PAS, and Gomori's trichrome.

In addition to the primary postmortem procedures previously mentioned, 1 mm thick slices of the following organs were collected in cold buffered 4% glutaraldehyde for transmission electron microscopy from selected moribund and control pigs: liver, kidney, adrenal gland, myocardium from the septum of the left ventricle, diaphragm, cerebellar folia, spinal cord within the 13th or 14th thoracic vertebra, and sciatic nerve. After 4 hours of fixation under refrigeration in glutaraldehyde, the tissues were washed in 6 changes of cold Sorenson's buffer (pH 7.4) and stored in Sorenson's buffer in the refrigerator to await further processing. Only the thin slices of liver were processed further. Several (10 to 20) 1 mm cubes of hepatic tissue from 4 selected pigs were post-fixed in osmium tetroxide, dehydrated, embedded in epoxy resin, sectioned and examined by the methods described by Cockrell (1969) and Boam (1970).

Analysis of Data

Although the experimental design and results in this research did not always lend themselves to statistical considerations, whenever possible, these data were analyzed and observed for statistically significant differences by the Student's t-test and Snedecor's F-test as outlined by Lewis (1966).

RESULTS

Preliminary Data

The general results of preliminary trials involving selenium injections into 6 yearling steers and 3 young pigs are summarized in Table 5. The clinical signs of peracute selenosis in Steer 1 were salivation, respiratory distress, cyanosis and death in 8 hours. The characteristic gross lesions were massive pulmonary edema; hydrothorax; large fibrinous clots in thoracic cavity and trachea; endocardial ecchymoses in the left ventricle; congestion of the gastrointestinal tract, renal medulla, and urinary bladder; blood-tinged urine; and meningeal edema over the brain. The important histopathologic change was hyaline degeneration in the arterioles of heart and kidney. Following intramuscular injections of sodium selenite into 3 steers, the general gross and microscopic lesions were limited to the sites of injection and were characterized by muscular necrosis, thrombosis and vascular necrosis, hemorrhage, edema and an infiltration of neutrophils. For 1 steer, the selenium solution was neutralized to pH 7, but this did not noticeably change the character of the muscular lesions.

In Pig 2, the clinical signs of peracute selenosis were dyspnea, depression, coma and death in 10 hours. The principal gross lesions were petechiae on the pleura, hemorrhagic lymph nodes, subendocardial and subepicardial ecchymoses, excessive pericardial fluid and congestion of the renal medulla. Additional changes that were noted

Table 5. Summary of results using steers and pigs in preliminary trials of selenium toxicity

Steer no.	Wt. (kg)	Se* injection route	Se dosage	Total Se injected (mg)	Results		
					Clinical signs	Gross lesions	Histopath. changes
1	300	i.v.	1 mg/kg	300	Salivation, resp. distress, cyanosis, death in 8 hrs.	Pulmonary edema; hydrothorax; endocardial ecchymoses; congestion of GI tract & renal medulla; edema of brain	Hyaline degeneration of arterioles in heart and kidneys, necrosis in lymphoid follicles
2	310	i.v.	0.75 mg/kg	232	Depression & increased resp. rate at 2 hrs. Killed after 24 hrs.	None	Same as #1
3	270	i.v.	0.50 mg/kg	140	None. Killed after 96 hrs.	None	None
4	260	i.v. i.m.	30 mg/day/3 days 20 & 40 mg on 3rd day	150	None. Killed after 96 hrs. SGOT=480 S-F units/ml	Muscular necrosis at sites of i.m. injections	I.m. injection site: Zenker's necrosis, mineral, thrombosis & arteriolar necrosis, hemorrhage, edema, & PMN's
5	250	i.m.	5 & 10 mg once	15	None. Killed after 96 hrs.	Same as #4	Same as #4**
6	280	i.m.	5, 10 & 20 mg once	35	None. Killed after 144 hrs.	Same as #4	Same as #4**

Table 5 (cont'd.)

Pig no.	Wt. (kg)	Se* injection route	Se dosage	Total Se injected (mg)	Results		
					Clinical signs	Gross lesions	Histopath. changes
1	8.2	i.m.	1.1 mg/kg	9.0	None. Killed after 96 hrs.	None	Inject. site: mild Zenker's necrosis, hemorrhage, edema, fibroblast proliferation, PMN's
2	8.7	s.c.	1.1 mg/kg	9.6	None. Second injection after 12 days	None	None
	13.6	i.v.	2.02 mg/kg	27.5	Dyspnea, depression, coma, death in 10 hrs.	Hemorrhages in lungs, lymph nodes & heart; congestion of renal medulla	Congestion of adrenal cortex, necrosis in lymphoid follicles
3	9.1	i.v.	1.1 mg/kg	10.0	None. Second injection after 12 days	None	None
	13.6	i.v.	1.46 mg/kg	20.0	None. Killed after 96 hrs.	None	None

*As selenium from sodium selenite in distilled water (1 mg Se/ml); i.v.=intravenously; i.m.=intramuscularly; s.c.=subcutaneously.

**Control sites of intramuscular injection of distilled water, 20 or 40 ml, had fragmented myofibers, fibroblast and capillary proliferation which was not the necrotizing reaction as at the sites of selenium injection.

histopathologically were congestion of the adrenal cortex and necrosis of germinal follicles in lymph nodes and Malpighian corpuscles of the spleen. The intramuscular injection of sodium selenite into the thigh of Pig 1 produced a reaction similar to that found in the steers given intramuscular injections of selenium.

Experiments on Selenium Toxicosis

The pigs fed sodium selenite or selenomethionine had considerable variability in their individual response to toxic doses of selenium. While the objective of producing selenium toxicosis and vitamin E-selenium deficiency with the same basal ration was completed, the consumption of the Torula yeast basal ration was not as good as was anticipated. After 1 or 2 feedings, the appetites of pigs fed selenium were proportionally depressed with increased concentrations of selenium in their feed. That is, anorexia became apparent earlier in the pigs fed 100 ppm selenium than in those fed 45 ppm. Consequently, the pigs fed 45 ppm selenium ate a larger dose of selenium on a body-weight basis before the pigs on the highest level in their feed. These peculiarities in appetite and feed consumption must be remembered in evaluating the following results against practical rations and conditions. The increased feed consumption expected with natural rations may produce toxicosis at lower selenium concentrations.

In the pigs fed selenium, clinical toxicosis developed in 21 of 38 pigs (55.3%) fed either sodium selenite or selenomethionine at various levels in the rations of both experiments. In Experiment A (Torula yeast basal), Pigs 1, 37, 6, 18, and 23 which were fed sodium selenite and Pigs 14, 38, 7, and 20 which were fed selenomethionine developed clinical selenium toxicosis. These pigs

represented 9 of 24 pigs (39.1%) fed selenium in Experiment A. In Experiment B (dried milk basal), all pigs fed the selenium compounds developed toxicosis either as a result of natural ingestion or forced feeding of the prescribed amounts of selenium.

Growth, Duration and Amounts of Selenium Fed

A summary of the total amounts of selenium fed, days on experiment, and growth responses for each pig in Experiment A is given in Table 6. In general, the average daily gain of pigs with clinical toxicosis was markedly less or negative compared to pigs without clinical toxicosis. No real difference was identified between the toxicity of sodium selenite or selenomethionine at levels of 45 and 100 ppm selenium by comparing the average daily weight loss, the toxic dose, or the number of days to moribund toxicosis. Of the pigs fed 20 ppm selenium, only 1 developed the clinical signs of selenium toxicosis.

When the data of individual pigs were examined, it was revealed that Pig 18 died in 3 days after ingesting only 13.5 mg selenium. In contrast, Pigs 3 and 36 tolerated a total of 1709 and 1380.5 mg selenium, respectively, without evident toxicosis. Pig 6 was fed nearly 1150 mg total selenium but did develop clinical toxicosis.

A summary of the total amounts of selenium fed, time on experiment, and growth response for each pig in Experiment B is given in Table 7. The weight changes of all pigs with selenium toxicosis were markedly less or negative in comparison to the controls. The interval of time that the pigs fed selenium remained alive and on experiment was inversely related to the dosage of selenium fed except for Pig 2B. From the calculated total selenium fed, Pigs 2B

Table 6. Total amounts of selenium fed, days on experiment, weights, and growth responses of pigs fed sodium selenite or selenomethionine at various selenium concentrations in the ration in Experiment A

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Calculated total selenium fed (mg)	Days on experiment	Initial wt. (gm)	Final wt. (gm)	Average daily gain or loss (gm/day)
4*	0	---	---	4	4675	4540	- 34
35	0	---	---	84	6660	39600	392
5	0	---	---	91	5015	38925	373
31*	0	---	---	17	4785	6590	106
24	0.1	SeO_3**	1.48	39	4760	11400	170
34	0.1	SeO_3	1.41	35	4630	11040	183
13	0.1	SeMET**	1.37	38	3355	8810	144
30	0.1	SeMET	1.42	35	4610	10830	178
11	5	SeO_3	72.8	39	4145	9560	139
19	5	SeO_3	72.6	39	3620	9230	144
12	5	SeMET	69.4	38	3990	9950	157
17	5	SeMET	70.8	38	3620	9760	162
15	10	SeO_3	324.0	59	4045	17360	226
32	10	SeO_3	324.5	53	4305	19125	280
8	10	SeMET	324.5	53	6170	21150	283
9	10	SeMET	325.0	59	4110	17180	222
23†	20	SeO_3	73.1	32	4660	4160	- 15.6
36	20	SeO_3	1380.5	84	4990	30800	307
3	20	SeMET	1709.0	84	6135	36450	361
26	20	SeMET	750.7	63	4850	19460	232
6†	45	SeO_3	1148.8	63	5290	7620	37
18†	45	SeO_3	13.5	3	4240	3580	-220
7†	45	SeMET	74.9	9	4450	4420	- 3.3
20†	45	SeMET	24.1	5	3910	3000	-182
1†	100	SeO_3	222.0	28	4295	3840	- 16.2
37†	100	SeO_3	136.0	19	6635	4440	-116
14†	100	SeMET	84.5	20	5160	3410	- 87.5
38†	100	SeMET	55.0	11	4835	3300	-140

*These pigs had the lesions of hepatosis dietetica.

**SeO_3=sodium selenite; SeMET=selenomethionine.

†These pigs developed clinical selenium toxicosis.

Table 7. Total amounts of selenium fed, hours on experiment, weights, and growth responses of pigs fed sodium selenite or selenomethionine at various concentrations in the dry ration in Experiment B*

Pig no.	Selenium added to dry ration (ppm)	Selenium compound fed	Calculated total selenium fed (mg)	Hours on experiment	Initial wt. (gm)	Final wt. (gm)	Average daily gain or loss (gm/day)
4B	0	---	---	29	5330	5505	145
13B	0	---	---	192	4885	5740	107
5B	60	SeO_3**	39.2	142	4930	4720	- 35
15B	60	SeO_3	35.1	152	5460	4480	-157
6B	60	SeMET**	37.6	100	5050	5170	29
16B	60	SeMET	59.6	150	6170	5850	- 51
1B	120	SeO_3	10.4	56	4320	3570	-326
11B	120	SeO_3	12.8	53	4160	3365	-365
2B	120	SeMET	65.8	220	4530	3740	- 86
8B	120	SeMET	22.0	59	4940	4250	-283
7B	600	SeO_3	27.5	24	3830	3660	-170
9B	600	SeO_3	43.2	46	4545	4100	-232
10B	600	SeMET	34.0	29	3715	3770	44
12B	600	SeMET	32.0	46	3500	3460	- 21

Pig no.	Selenium dosage injected i.v.† (mg/kg BW)	Selenium compound injected i.v.	Total selenium injected i.v.				
3B	3	SeO_3	20.0	2.5	6680	6680	0
14B	3	SeMET	20.6	14	6880	6680	-200

*All pigs given selenium became moribund or died with toxicosis.

**SeO_3=sodium selenite; SeMET=selenomethionine.

†i.v.=intravenously.

and 16B were relatively more resistant to selenium than other pigs in the same groups. Selenium from sodium selenite was slightly, but not significantly, more toxic than selenium from selenomethionine. Also, when Pigs 3B and 14B were given 3 mg selenium/kg body weight intravenously as sodium selenite or selenomethionine, respectively, selenium from selenite produced moribund toxicosis more rapidly than that from selenomethionine.

Clinicopathologic Findings

The results of hemograms and determinations of hepatic and plasma tocopherols, plasma or serum GOT, OCT, and urea nitrogen were not particularly specific for selenium toxicosis but would aid in a differential diagnosis. The hemograms from initial and final blood samples from pigs in Experiment A are given in Tables 8 and 9, respectively. No pertinent differences could be associated with selenium toxicosis among the groups of pigs between the 2 sampling periods. The results of selected determinations of tocopherols in liver and tocopherols, OCT, and urea nitrogen in plasma from various groups of pigs in Experiment A are summarized in Table 10. Since these data were so irregular, the results are presented as ranges of values. In general, the values of hepatic tocopherols between control and selenium-fed pigs did not indicate any abnormal relationship. The values of plasma tocopherols were slightly higher in the final samples from all groups than were the values of plasma tocopherols in the initial samples. The values of plasma OCT and urea nitrogen from poisoned pigs were elevated above those from control pigs although there was considerable variability among the values from these groups of pigs.

Table 8. Initial hemoglobins, hematocrits, total and differential leukocyte counts from pigs in Experiment A

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Hb. (gm/ 100 ml)	Hct. (%)	Total WBC	Abs. differential leukocyte counts					
						Segs.	Stab.	Lymph.	Mono.	Eosino.	Baso.
4	0	---	13.8	42	22,350	15,198	894	6,034	---	224	---
35	0	---	7.3	24	10,280	3,906	206	6,168	---	---	---
5	0	---	12.5	40	27,350	21,060	820	5,196	---	274	---
31	0	---	9.7	30	20,180	11,300	2,220	6,660	---	---	---
24	0.1	SeO_3*	11.5	37	18,750	9,188	937	8,625	---	---	---
34	0.1	SeO_3	12.7	38	13,475	5,929	404	7,007	---	135	---
13	0.1	SeMET*	12.7	37	18,790	7,140	1,128	10,334	---	188	---
30	0.1	SeMET	8.5	26	12,715	6,485	635	5,214	---	381	---
11	5	SeO_3	13.5	40	20,900	12,540	418	7,315	209	418	---
19	5	SeO_3	11.8	35	11,465	5,618	1,032	4,600	---	115	---
12	5	SeMET	12.7	40	12,600	4,284	378	7,686	126	252	---
17	5	SeMET	13.0	37	14,875	6,843	892	7,140	---	---	---
15	10	SeO_3	12.9	37	14,150	3,962	284	9,622	141	141	---
32	10	SeO_3	8.0	24	18,640	9,880	2,796	5,964	---	---	---
8	10	SeMET	11.0	32	16,010	6,565	1,120	8,005	---	320	---
9	10	SeMET	13.0	39	18,200	10,738	364	6,552	---	546	---
23	20	SeO_3	14.3	42	24,025	8,890	480	13,935	---	480	240
36	20	SeO_3	7.0	23	13,700	5,480	548	7,535	---	137	---
3	20	SeMET	11.3	33	20,500	6,150	1,025	13,325	---	---	---
26	20	SeMET	---	---	---	---	---	---	---	---	---

Table 8 (cont'd.)

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Hb. (gm/ 100 ml)	Hct. (%)	Total WBC	Abs. differential leukocyte counts					
						Segs.	Stab.	Lymph.	Mono.	Eosino.	Baso.
6	45	SeO_3	11.5	37	26,475	10,325	530	15,355	---	265	---
18	45	SeO_3	12.5	34	13,515	7,840	540	5,135	---	---	---
7	45	SeMET	12.0	36	16,950	9,153	508	6,780	170	339	---
20	45	SeMET	11.0	30	15,510	8,065	1,706	6,980	---	310	---
1	100	SeO_3	10.8	---	30,100	15,050	2,107	12,642	---	301	---
37	100	SeO_3	9.8	29	8,010	3,605	---	4,085	---	320	---
14	100	SeMET	14.3	43	19,675	10,625	984	7,476	196	394	---
38	100	SeMET	9.8	30	17,950	7,539	538	9,693	180	---	---

*SeO_3=sodium selenite; SeMET=selenomethionine.

Table 9. Final hemoglobins, hematocrits, total and differential leukocyte counts from pigs in Experiment A

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Hb. (gm/ 100 ml)	Hct. (%)	Total WBC	Abs. differential leukocyte counts					
						Segs.	Stab.	Lymph.	Mono.	Eosino.	Baso.
4	0	---	---	---	---	---	---	---	---	---	---
35	0	---	14.0	40	15,075	7,840	150	6,784	---	301	---
5	0	---	14.0	41	21,320	11,726	213	8,528	---	640	213
31	0	---	---	---	---	---	---	---	---	---	---
24	0.1	SeO_3*	14.0	42	13,645	4,093	275	8,732	---	545	---
34	0.1	SeO_3	12.3	38	13,110	3,408	394	8,783	---	394	131
13	0.1	SeMET*	12.0	35	17,105	6,500	342	8,552	171	1,540	---
30	0.1	SeMET	12.0	38	11,530	3,114	---	8,186	115	115	---
11	5	SeO_3	12.0	36	16,830	6,395	170	8,245	---	2,020	---
19	5	SeO_3	11.7	36	16,265	7,810	325	7,156	162	650	162
12	5	SeMET	11.7	36	16,070	5,945	322	8,035	---	1,446	322
17	5	SeMET	12.9	40	15,170	6,675	151	7,435	---	606	303
15	10	SeO_3	12.8	39	22,215	10,663	222	10,663	---	445	222
32	10	SeO_3	12.7	39	26,775	15,800	535	9,906	---	267	267
8	10	SeMET	12.5	38	18,390	6,805	---	11,585	---	---	---
9	10	SeMET	12.5	39	19,545	10,555	585	8,210	---	195	---
23	20	SeO_3	12.8	38	11,145	6,352	335	4,450	---	---	---
36	20	SeO_3	13.8	41	21,870	11,590	656	8,530	---	438	656
3	20	SeMET	13.0	37	17,525	7,535	---	8,940	175	700	175
26	20	SeMET	---	---	---	---	---	---	---	---	---

Table 9 (cont'd.)

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Hb. (gm/ 100 ml)	Hct. (%)	Total WBC	Abs. differential leukocyte counts					
						Segs.	Stab.	Lymph.	Mono.	Eosino.	Baso.
6	45	SeO_3	17.8	52	19,365	6,390	---	12,780	195	---	---
18	45	SeO_3	13.5	40	14,520	6,535	580	7,115	290	---	---
7	45	SeMET	11.7	37	16,980	9,678	340	6,622	---	340	---
20	45	SeMET	13.0	43	26,525	14,324	2,122	9,549	265	265	---
1	100	SeO_3	12.5	38	23,255	17,675	1,395	3,953	232	---	---
37	100	SeO_3	13.5	43	16,605	9,630	166	6,477	166	166	---
14	100	SeMET	---	---	---	---	---	---	---	---	---
38	100	SeMET	11.2	37	12,750	3,698	765	8,287	---	---	---

*SeO_3=sodium selenite; SeMET=selenomethionine.

Table 10. Range of results from selected determinations for tocopherols in liver and tocopherol, OCT and urea nitrogen in plasma from various groups of pigs at initial and final periods during Experiment A

Experimental period and groups of pigs bled	Hepatic tocopherols (μg/gm)	Plasma tocopherols (μg/ml)	Plasma OCT (Sigma units/ml)	Plasma urea nitrogen (mg/100 ml)
Initial base-line samples from pigs	---	0.65 to 1.0(3)*	0 to 1050 (11)	6.0 to 31.5(11)
Final samples from pigs fed 0.1 ppm selenium	32 to 58 (4)	1.0 to 1.4(4)	0 to 250 (3)	5.8 to 9.0(4)
Final samples from pigs fed 5, 10 or 20 ppm selenium without toxicosis	17 to 87 (10)	1.0 to 2.1(4)	---	---
Final samples from moribund pigs with toxicosis	42 to 82 (8)	1.4 and 2.2(2)	75 to 3150 (7)	6.5 to 80.0(6)

*Number in parentheses indicates number of pigs involved in respective determinations.

The results of osmotic fragility of erythrocytes in Experiment A using the degree of layering hemolysis after 48 hours under refrigeration indicated that erythrocytes from all pigs in all groups had layering hemolysis in aqueous solutions of sodium chloride in the following gradations: no hemolysis, 0.85% saline; slight hemolysis, 0.7 to 0.6% saline; moderate hemolysis, 0.55 to 0.45% saline; complete hemolysis, 0.35 and 0.2% saline.

The results of determinations of GOT, OCT, and urea nitrogen in serum in Experiment B are given in Table 11. These data also had wide variability of values from different groups of pigs; therefore, only the ranges in values are presented. The high end of the values of serum GOT and OCT from poisoned pigs was larger than those from control pigs. Serum urea nitrogen was not elevated above normal in any of the groups.

Clinical Signs

The clinical signs of selenium toxicosis were arbitrarily divided into peracute (0-1 day), acute (1-30 days), and subacute (30-90 days) stages.* Chronic selenium toxicosis was not observed although 2 pigs were fed 20 ppm selenium for 84 days (Table 6). No differences were noted in the clinical toxicosis produced by sodium selenite or selenomethione.

Control and selenium-fed pigs without toxicosis had no clinical signs except Pig 26 which had a trauma-induced chronic bleeding ulcer on the bottom of its left hind foot. It was believed that this pig died from loss of blood and resultant anemia.

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*Veterinary Toxicology Training and Workshop Notes, Iowa State University, 1972.

Table 11. Range of results from selected determinations for GOT, OCT and urea nitrogen in serum from various groups of pigs at initial and final periods during Experiment B

Experimental period and groups of pigs bled	Serum GOT (Trans AC units)	Serum OCT (Sigma units/ml)	Serum urea nitrogen (mg/100 ml)
Initial base-line samples from pigs	22 to 63 (15)*	0 to 250 (7)	1.0 to 5.7 (8)
Final samples from pigs fed 0 ppm selenium	17 to 27 (4)	0 to 250 (4)	1.0 to 5.5 (4)
Final samples from moribund pigs with selenium toxicosis	54 to >1050(8)	0 to >5000(4)	7.4 to 25 (4)

*Number in parentheses indicates the number of pigs involved in respective determinations.

Peracute selenium toxicosis. Pig 3B given sodium selenite intravenously had the clinical signs of peracute toxicosis and died in 2-1/2 hours. This pig began vomiting in less than 15 minutes. One hour postinjection, profound depression, weakness, and a "thumping" respiration were present. The following measurements were recorded: rectal temperature, 100.9 F.; respiratory rate, 80/minute; cardiac rate, 80/minute; and cardiac sounds were faint. After another hour, the rectal temperature was 98.5 F.; respiratory rate, only 4/minute; cardiac rate, 180/minute. Although the pig appeared comatose, the palpebral reflex was present until just before death.

Pig 14B was given selenomethionine intravenously and had a longer period of clinical signs, but the basic clinical course was not different. After 1 hour, this pig was depressed, shivering, and recumbent. There was a tendency toward opisthotonos. The following

measurements were taken: rectal temperature, 106 F.; respiratory rate, 68/minute; and cardiac rate, 80/minute. The elevated temperature, shivering, and lethargy continued through the 2nd hour when emesis started and intermittently continued for about 3 hours. The rectal temperature was 103.5 F.; respiratory rate, 68/minute; and cardiac rate, 160/minute during the 3rd hour after the intravenous injection. The depression progressed into coma, and the temperature decreased to 98.5 F. by the 13th hour. The respiratory rate had slowed to 24/minute. The character of the breathing was regular but deep and very deliberate.

Acute selenium toxicosis. The clinical signs of Pigs 1, 7, 14, 18, 20, 37, and 38 in Experiment A and all pigs fed selenium in Experiment B were included in this group. The most characteristic clinical sign of acute selenium toxicosis was the profound and protracted anorexia that developed in these pigs after a relatively short period of ingesting selenized feed. Some pigs ate only a very small part of their daily ration. In Experiment A, some pigs did not eat at all for 4 to 7 days before they became weak and moribund.

Other clinical signs of acute toxicosis included vomition, lethargy, accelerated and gasping respiration, coma and death especially in the pigs of Experiment B. Some pigs had tense abdominal muscles and squealed abnormally when they were handled. Ataxia preceded recumbency. Convulsive signs of paddling and opisthotonos were also noticed. The ears and feet were cold on palpation. White hoofs appeared somewhat blue due to congestion. Rectal temperatures decreased from normal to subnormal. Although

the pigs in Experiment A had most of the above signs, they did not usually vomit. Cachexia and loss of body weight was most pronounced before the terminal period of dyspnea and coma. Pig 7, fed selenomethionine in Experiment A, became paralyzed before moribund signs developed. In addition, Pig 2B, with Hampshire markings, lost the hair from the white belt after several days of anorexia but the hair in the black areas was unaffected. In general, the duration of the moribund signs varied from 1 day to several days.

Subacute selenium toxicosis. Pigs 6 and 23 from Experiment A were included in this classification, and both were euthanatized when their general condition had deteriorated to a near terminal state of cachexia. During the initial 3 weeks of the experiment, these pigs ate their daily allotment of selenized feed fairly well. After the amount of feed was increased for the controls, Pigs 6 and 23 refused to eat the increased amount of daily ration. Pig 23 became severely cachectic while Pig 6 began eating slightly better after another 3 weeks.

The clinical signs in Pigs 6 and 23 were mainly anorexia and cachexia. These pigs were small and had rough hair coats, yellow-tinged skin in unpigmented areas, and muscular atrophy and weakness (Figure 1). No loss of hair or lesions of the hoofs were noted.

Hepatosis dietetica. Pigs 4 and 31 developed the lesions of hepatosis dietetica during the course of this research. No selenium had been fed to either pig, but Pig 31 had been supplemented 22 ppm vitamin E in its feed. Pig 4 was observed 2 hours before death, and the clinical signs were minimal. Both pigs had eaten their previous meal, and both died suddenly and unexpectedly.

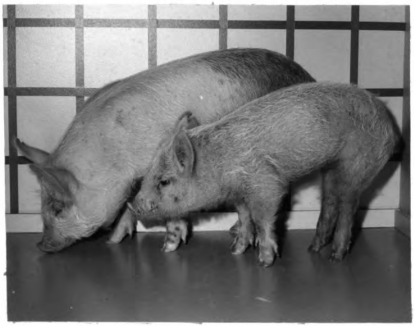

Figure 1. Comparison of control Pig 5 and Pig 6 after 60 experimental days. Notice smaller size, emaciation, rough hair, tucked-up appearance of abdomen and cocked appearance of hocks of Pig 6, in foreground, fed 45 ppm selenium as sodium selenite.

Gross Lesions

There were no essential differences between the gross lesions of selenium toxicosis in pigs poisoned with either sodium selenite or selenomethionine. No lesions in the gastrointestinal tract could be attributed to selenium toxicosis. Nearly all of the pigs in both experiments had some degree of mycosis in the esophagus and esophagogastric area of the stomach. Some pigs had esophagogastric ulcers which were unrelated to selenium toxicosis. Pigs in Experiment B that were force-fed their daily allotment of selenized milk developed foreign-body pneumonia in the anterior ventral portions of their lungs.

Organ weights of livers and kidneys, expressed as percentages of final body weights, from pigs in Experiment A and Experiment B are given in Tables 12 and 13, respectively. Although there was no apparent difference between the pigs with or without toxicosis as groups, it is suggested that the livers of Pigs 1 and 23 were slightly atrophic, but the degree of cachexia and dehydration markedly influenced these results.

Peracute selenium toxicosis. The pig given sodium selenite intravenously developed massive pulmonary edema (Figure 2). The heart had subendocardial ecchymoses. The renal cortex was normal, but the medulla was slightly congested. The appearance of the liver was normal which might have been expected since this pig lived only 2-1/2 hours.

The pig given selenomethionine intravenously had a yellow-brown mottled liver. The renal cortex was pale, and the medulla was congested (Figure 3). No other gross lesions were seen.

Table 12. Final weights of livers and kidneys expressed as percentages of final body weights of pigs in Experiment A

Sodium selenite				Selenomethionine			
Pig no.	Selenium added to ration (ppm)	Liver (%)	Kidney (%)	Pig no.	Selenium added to ration (ppm)	Liver (%)	Kidney (%)
4	0	3.27	0.71	5	0	2.01	0.55
35	0	2.13	0.52	31	0	4.28	0.84
24	0.1	2.55	0.56	13	0.1	2.88	0.51
34	0.1	3.22	0.61	30	0.1	2.73	0.52
11	5	3.30	0.62	12	5	2.68	0.56
19	5	3.25	0.64	17	5	2.86	0.54
15	10	3.16	0.63	8	10	2.83	0.68
32	10	3.57	0.74	9	10	2.46	0.62
23	20	1.44	1.09	3	20	3.07	0.67
36	20	3.26	0.63	26	20	3.34	0.84
6	45	2.34	0.87	7	45	3.55	0.73
18	45	3.76	0.77	20	45	2.90	0.72
1	100	1.74	0.76	14	100	2.30	0.64
37	100	6.36	1.24	38	100	2.30	0.72

Table 13. Final weights of livers and kidneys expressed as percentages of final body weights of pigs in Experiment B

Sodium selenite				Selenomethionine			
Pig no.	Selenium added to dry ration (ppm)	Liver (%)	Kidney (%)	Pig no.	Selenium added to dry ration (ppm)	Liver (%)	Kidney (%)
13B	0	2.56	0.56	4B	0	2.36	0.46
5B	60	3.67	0.80	6B	60	2.34	0.64
15B	60	3.04	0.67	16B	60	3.13	0.60
1B	120	4.45	0.95	2B	120	3.05	0.80
11B	120	3.82	0.88	8B	120	3.03	0.79
7B	600	2.90	0.74	10B	600	3.35	0.59
9B	600	3.24	0.60	12B	600	3.82	0.73
	Selenium injected i.v. (mg/ kg BW)				Selenium injected i.v. (mg/ kg BW)		
3B	3	2.27	0.52	14B	3	2.60	0.56

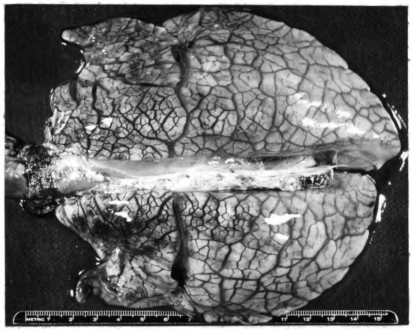

Figure 2. Pulmonary edema in a pig with peracute selenium toxicosis.

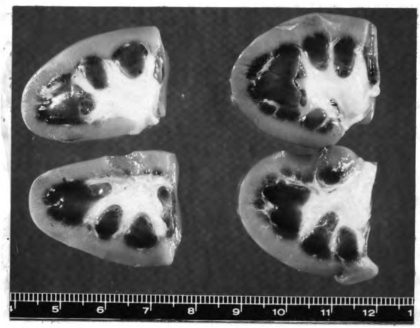

Figure 3. Pale renal cortices and congested renal medullae from a pig with selenium toxicosis. These were characteristic but non-specific changes in pigs with selenium toxicosis.

Acute selenium toxicosis. Selenium toxicosis in pigs fed the largest oral dosages of selenium was characterized by a liver which was pale yellow throughout its parenchyma compared to a normal liver (Figures 4 and 5). In acute toxicosis from a lower dosage of selenium, the liver had an obvious yellow-brown mottling on its external (Figure 6) and cut surfaces, but a few livers had to be inspected closely to detect this change (Figure 7). The gallbladder was smaller than normal, and the bile was so thick that it could not be expressed through the common bile duct. The renal medulla was congested. Distention of the urinary bladder with urine resulted in a turgid appearance of the ureters. In Experiment A, hemorrhage into the adrenal cortex was common. The hemorrhages, which were few, on the heart, lungs, and serosal surfaces were associated with agonal changes because hemorrhages were not noted in moribund pigs that were euthanatized. Hemorrhagic pancreatitis was more commonly associated with toxicosis from selenomethionine.

The cachectic changes were serous atrophy of fat in the coronary groove of the heart, in the perirenal area, and in the subcutis of the head. Some pigs also had edema of the pancreas, intestinal mesentery, spiral colon, and meninges of the brain. Skeletal muscles were occasionally pale and edematous. Atrophy of the thymus was also noted. Emaciation of the carcass was most pronounced in these pigs that survived the longest time.

Subacute selenium toxicosis. The 2 pigs in the classification of subacute selenium toxicosis had more advanced lesions of toxicosis and cachexia with a similar distribution as those previously described under acute selenium toxicosis. The livers of these pigs were slightly atrophic and less mottled.

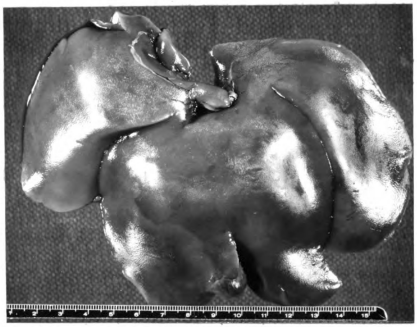

Figure 4. Normal liver from control pig. This liver from Pig 13B had a selenium content of 0.21 ppm.

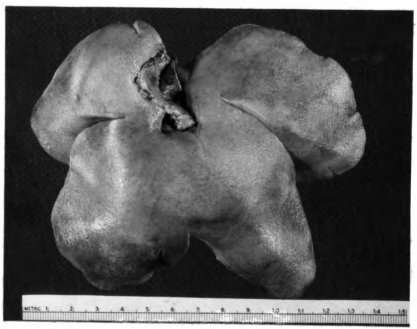

Figure 5. Very pale liver of acute selenium toxicosis in a pig fed 120 ppm selenium in Experiment B. This liver from Pig 1B had a selenium content of 5.62 ppm.

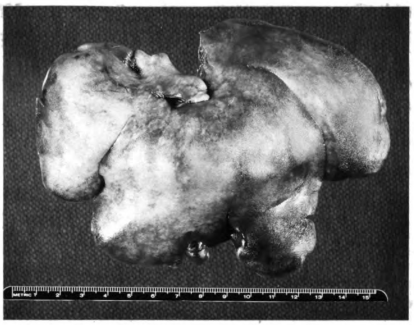

Figure 6. Pale mottled liver of acute selenium toxicosis in pig fed 60 ppm selenium in Experiment B. Compare with Figures 4 and 5. This liver from Pig 15B had a selenium content of 15.9 ppm.

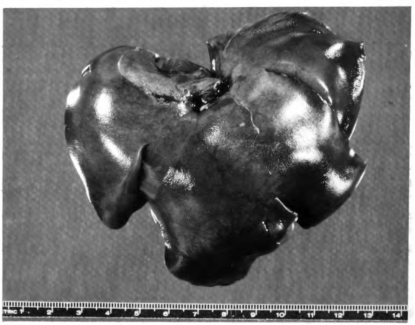

Figure 7. Subtly mottled liver of acute selenium toxicosis in pig fed 45 ppm selenium in Experiment A. Compare with Figures 4, 5 and 6. This liver from Pig 20 had a selenium content of 14.6 ppm.

Hepatosis dietetica. The acute dietary liver necrosis in 2 pigs was characterized by multifocal to diffuse areas of lobular hemorrhage, irregularities in Glisson's capsule, and hepatic swelling (Figure 8). Both pigs had hemorrhagic ileitis. One pig had subcutaneous hemorrhages behind the shoulders. It also had pale, edematous skeletal muscles. The other pig had epicardial hemorrhages as well as hemorrhagic portal and mesenteric lymph nodes.

Histopathologic Changes

Although the livers of euthanatized pigs fed no selenium and those fed 0.1 ppm selenium were considered normal, there were subtle histological changes of undetermined significance in the livers of pigs fed 5, 10, and 20 ppm selenium without clinical toxicosis in Experiment A. These livers had peculiar hepatocytes with basophilia of the cytoplasm near the nucleus and a lacy appearance of the peripheral cytoplasm. The hepatocytes were swollen, and the hepatic sinusoids were usually obliterated. The above changes were more pronounced in the pigs fed 10 or 20 ppm selenium.

There were histologic changes in other tissues of the experimental pigs that were not related to selenium toxicosis. The epithelium of the esophagus and esophagogastric portion of the stomach with mycosis had hyperkeratosis, acanthosis, fungal yeast and mycelial forms, and foci of neutrophils in the keratin, and accumulations of lymphocytes in the submucosa. The esophagogastric ulcers, when present, were characterized by denudation of the epithelium with fungal organisms, neutrophils, lymphocytes, and necrotic changes in the submucosal connective tissue. Histologic sections of the

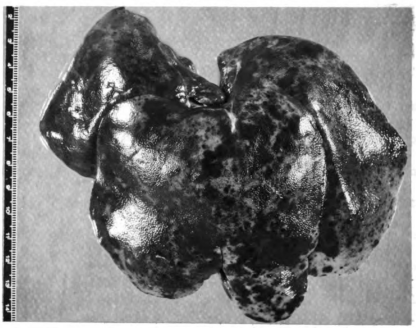

Figure 8. Hepatosis dietetica in liver of pig with vitamin E-selenium deficiency. Notice multifocal and multilobular hemorrhages (dark areas) seen through Glisson's capsule.

pulmonary lobes with foreign-body pneumonia were characterized by a bluish-pink foreign material in the bronchioles and alveoli with massive infiltration of neutrophils into the affected areas. Some lobules were extensively involved while other lobules were relatively normal.

Although the gross lesions of selenium toxicosis from sodium selenite or selenomethionine were, in general, similar, there were some differences noted in the histopathologic changes. The extent of changes and organs involved were somewhat different.

Peracute selenium toxicosis. In the pig given sodium selenite intravenously the pulmonary edema was characterized microscopically by extensive edema in the alveoli and interlobular septa with resultant distention of these structures. No cellular infiltrates were present. The heart had endocardial hemorrhage around the Purkinje fibers without visible changes in these fibers. The spleen had necrosis of lymphocytes in the Malpighian corpuscles. Germinal follicles in the lymph nodes and other lymphoid tissues had increased pyknotic nuclei and cellular fragmentation. No microscopic lesions were noted in the liver.

In the pig given selenomethionine intravenously, the most striking histopathologic change was in the pancreas. The lesion was characterized by necrosis, edema, and cellular lysis of entire pancreatic lobules while other lobules had fairly normal acini, but the nuclei were pyknotic (Figure 9). The sections of lung were considered normal. The centrolobular hepatocytes of the liver were swollen and had increased cytoplasmic eosinophilia compared to the peripherolobular hepatocytes. Fatty metamorphosis of the liver was not apparent.

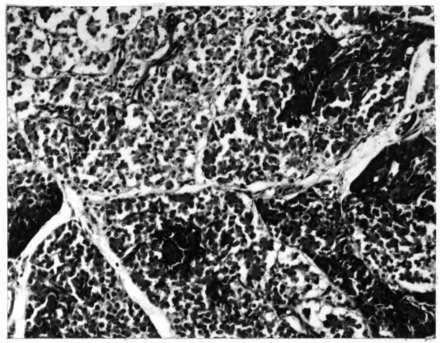

Figure 9. Necrosis, edema and cellular lysis in lobular areas in pancreas from the pig given seleno-methionine intravenously. H & E stain. x 180.

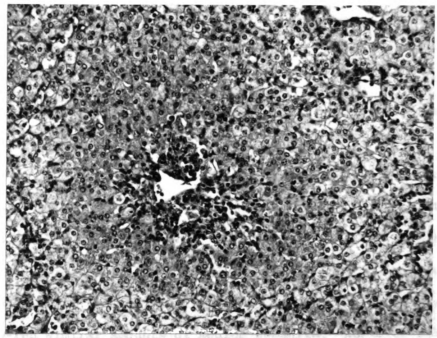

Figure 10. Fatty metamorphosis and centrolobular necrosis in liver from pig with acute selenium toxi-cosis. H & E stain. x 180.

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Acute selenium toxicosis. In general, livers from pigs with acute selenium toxicosis were characterized by fatty metamorphosis of entire hepatic lobules and by varying degrees of necrosis and increased centrolobular eosinophilia (Figure 10). This hepatic fat was identified by oil red O stain. Livers from pigs that died 24 hours after the initial ingestion of selenium had less centrolobular degeneration or necrosis than did those from pigs that died after 50 hours in Experiment B. The fat vacuoles in hepatocytes were, to a certain extent, slightly smaller with the shorter duration of toxicosis. Also, the hepatic fatty metamorphosis in pigs fed selenomethionine had smaller fat vacuoles than it did in pigs fed sodium selenite at the same dosage. In Experiment A, the fatty metamorphosis of the liver had a lacy appearance in the peripheral cytoplasm with basophilia near the nucleus of hepatocytes. Fatty metamorphosis was more extensive at the periphery of the hepatic lobules than in the centrolobular areas. A few of the livers from poisoned pigs fed selenomethionine had only cloudy swelling of the hepatocytes and obliteration of hepatic sinusoids. Kupffer cells were quite prominent in all livers with acute selenium toxicosis.

Although the liver appeared to be the primary target organ for both selenium compounds, there were several other significant changes in other organs. Some of the pancreases from pigs fed larger dosages of selenomethionine had extensive necrosis, hemorrhage and edema (Figure 9). This was not noted in the pancreases of pigs fed similar amounts of sodium selenite. But with the larger dosages of sodium selenite, histologic lesions of the central nervous system were more common. The granular layer of the cerebellum

had pyknosis of granule cells while at the tips of the cerebellar folia the granular layer had a peculiar loss of both stroma and granule cells (Figure 11). Edema or clear spaces were also noted around glial cells in the white matter of the cerebellum. The cerebral cortex had edema and possible early laminar necrosis between the layers of gray and white matter. Two pigs, 1 fed sodium selenite and 1 fed selenomethionine, had extensive poliomyelomalacia in different areas of the spinal cord. Hyaline degeneration and fibrinoid necrosis of arterioles was a common lesion in the brains as well as other organs of pigs fed either selenium compound (Figure 12).

Other histopathologic changes were adrenal cortical hemorrhage and pyknotic nuclei in the zona glomerulosa and zona reticularis (Figure 13). Pyknotic acidophils and pallid staining character of the cells in the anterior pituitary were noted in some pigs. Lymphocytes in the germinal follicles of lymph nodes and in the thymus and spleen were pyknotic and fragmented. The number of hematopoietic cells in the bone marrow was markedly depleted in pigs fed larger dosages of selenium for more than 1 week (Figure 14). The remaining cells in the bone marrow were mature polymorphonuclear cells. Skeletal muscles and diaphragm had degenerate myofibers without infiltration of mononuclear cells (Figure 15). The heart did not, in general, have significant histologic changes. No renal lesions, other than a mild hydropic change in the epithelium of the tubules, could be identified. The skin did not have any significant changes except edema or serous atrophy of fat in the subcutis. Hooves were considered histologically normal.

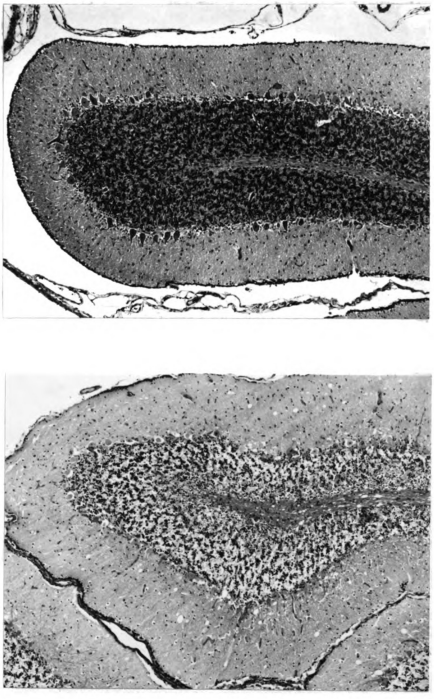

Figure 11. Comparison of the tips of cerebellar folia between control pig (top picture) and a pig fed 600 ppm selenium as sodium selenite (bottom picture). Notice the loss of stroma and granule cells in granular layer of cerebellum from pig with acute selenite toxicosis. H & E stain. x 70.

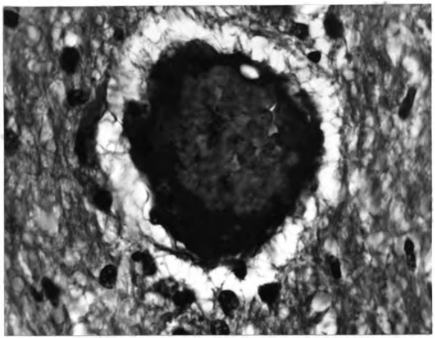

Figure 12. Fibrinoid necrosis of wall of arteriole in white matter of cerebellum from pig with acute selenium toxicosis. H & E stain. x 720.

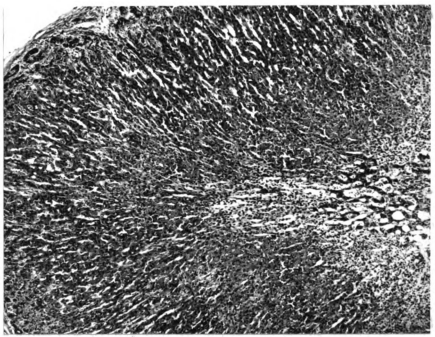

Figure 13. Hemorrhagic necrosis of adrenal cortex from pig with acute selenium toxicosis in Experiment A. H & E stain. x 70.

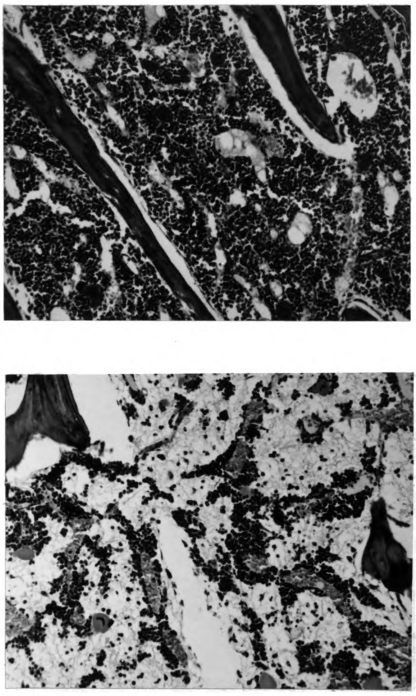

Figure 14. Comparison of normal bone marrow from control pig (top picture) and aplastic bone marrow from pig with acute selenium toxicosis lasting over 1 week (bottom picture). Both sections were taken from the comparable areas of ribs. Notice the loss of hematopoietic cells in the bone marrow from pig with selenium toxicosis. H & E stain. x 180.

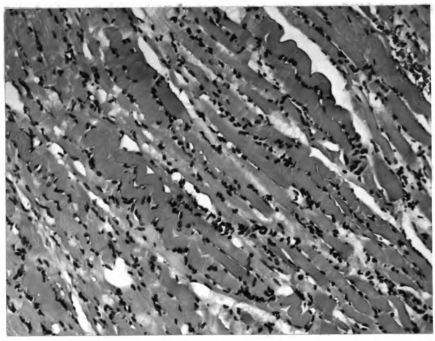

Figure 15. Degenerate myofibers in skeletal muscle from pig with acute selenium toxicosis. H & E stain. x 180.

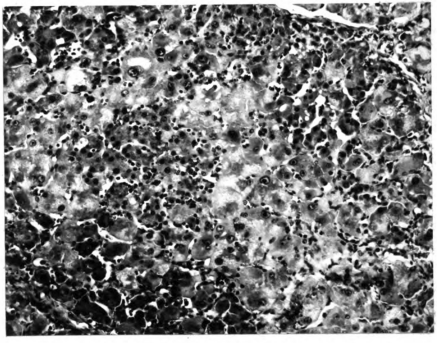

Figure 16. Hepatic lobule from pig with subacute selenium toxicosis. Notice variation in size of hepatocytes, loss of sinusoidal structure, and difference in staining of peripherolobular and centrolobular hepatocytes. H & E stain. x 180.

Subacute selenium toxicosis. The livers of pigs with subacute selenium toxicosis were characterized by degenerative and regenerative hepatocytes, loss of sinusoidal architecture without loss of lobular structure, increased sinusoidal and peripherolobular connective tissue, and increased numbers of lymphocytes and plasma cells in the periportal and peripherolobular areas (Figure 16). The hepatocytes at the periphery of the hepatic lobules were slightly larger than normal and had basophilia in the cytoplasm while in the centrolobular areas the hepatocytes were very large, as if coalesced, and had foamy, lightly eosinophilic cytoplasm. Mitotic figures, multinucleated hepatocytes and nuclei with multiple nucleoli were observed. Although cellular degeneration and pyknosis of nuclei were present, they had to be looked for carefully because the large regenerative hepatocytes hid the degenerative ones. The structure of hepatic lobules remained intact, but there was marked variation in the size of hepatic lobules. Fat vacuoles were demonstrated in some hepatocytes with oil red O stain; however, fat was not nearly as abundant as in the fatty metamorphosis of acute selenium toxicosis. Cirrhosis had not developed in these livers with subacute selenium toxicosis.

Additional histologic lesions in the pigs with subacute selenium toxicosis were similar to the more prolonged cases of acute selenium toxicosis. These included hemorrhagic necrosis of the adrenal cortex, edema of pancreas and brain, myopathy, cellular depletion of the bone marrow, degenerative changes and vacuolation of the anterior pituitary.

Hepatosis dietetica. The livers from pigs with dietary liver necrosis were characterized by hepatic lobules with extensive nuclear pyknosis, karyorrhexis and cytolysis of hepatocytes, loss of sinusoidal architecture, and massive hemorrhage into the affected lobules (Figure 17). Some adjacent hepatic lobules appeared relatively normal. There was also interlobular proliferation of connective tissue and bile ducts.

In the molecular layer of the cerebellum from 1 pig, there was fibrinoid necrosis of the walls of small blood vessels and perivascular hemorrhage (Figure 18). The skeletal muscles had early changes of myopathy characterized by hyalinization, loss of striations and fragmentation of myofibers. One section of heart also had hemorrhage between the myocardial fibers.

Ultrastructural Changes

After examining 114 electron photomicroscopic exposures (2 or 3 embedded blocks and 3 or 4 grids from each pig) of liver from Pigs 6, 37, 4 and 30 to compare the ultrastructural changes of selenium toxicosis with hepatosis dietetica, it is suggested that there is a difference bewteen the osmiophilic properties of large lipid bodies in the cytoplasm of hepatocytes in selenium toxicosis and hepatosis dietetica. The cytoplasm of hepatocytes from control Pig 30, fed 0.1 ppm selenium, did not have these lipid bodies. In Pig 6, with selenium toxicosis, the cytoplasmic lipid bodies in hepatocytes were smaller and less electron-dense (Figure 19) than those in Pig 4 with hepatosis dietetica. The large, densely osmiophilic lipid bodies in hepatocytes of Pig 4 contained smaller, less electron-dense bodies (Figure 20). The cytoplasmic membrane was

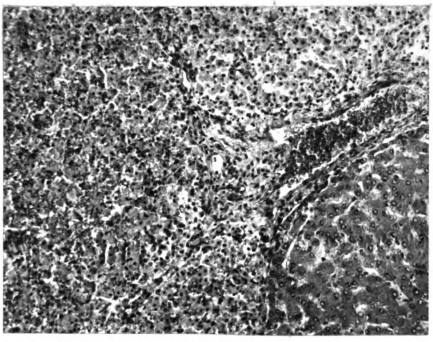

Figure 17. Necrotic, hemorrhagic lobules and normal adjacent lobule in liver from pig with hepatosis dietetica. H & E stain. x 180.

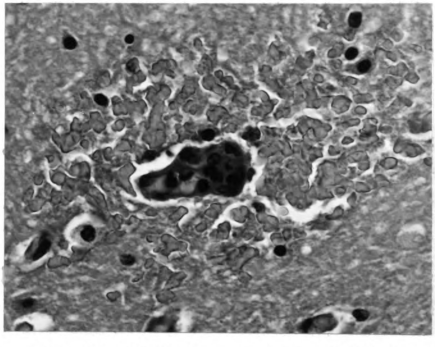

Figure 18. Fibrinoid necrosis of small blood vessel with perivascular hemorrhage in the molecular layer of cerebellum from pig with hepatosis dietetica. Compare with Figure 12. H & E stain. x 720.

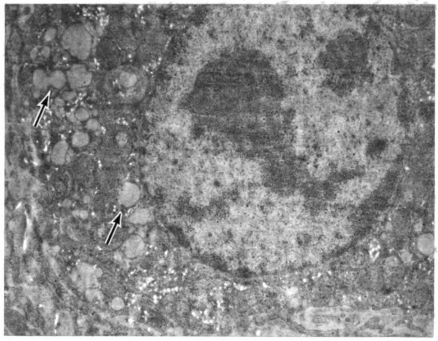

Figure 19. Hepatocyte from Pig 6 fed 45 ppm selenium as sodium selenite for 63 days. Notice lipid bodies (arrows) in cytoplasm. Stain: uranyl acetate and lead citrate. x 12,000.

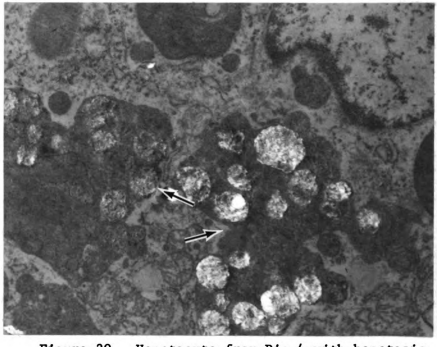

Figure 20. Hepatocyte from Pig 4 with hepatosis dietetica. Notice osmiophilic lipid bodies (arrows) in cytoplasm. Compare density of these lipid bodies with those in Figure 19. Stain: uranyl acetate and lead citrate. x 12,000.

more distorted with hepatosis dietetica probably because of crowding by the numerous erythrocytes. Swollen mitochondria and vacuolation of endoplasmic reticulum were seen in the hepatocytes with both selenium toxicosis and hepatosis dietetica.

Selenium Content in Tissues

The mean terminal selenium content, expressed as ppm selenium (wet weight basis), in liver and kidneys from each pig in both Experiment A and Experiment B is tabulated in Tables 14 and 15, respectively. In Experiment A, pigs fed 5 ppm selenium or more without clinical toxicosis had ranges of selenium residues of 2.27 to 18.0 ppm in livers and 2.19 to 20.1 ppm in kidneys. The pigs with clinical toxicosis had ranges of selenium residues of 6.7 to 36.2 ppm in livers and 2.73 to 22.4 ppm in kidneys. In Experiment B, the pigs with fatal toxicosis had ranges of selenium residues of 5.62 to 48.2 ppm in livers and 2.56 to 15.2 ppm in kidneys.

Statistical analyses of the above data substantiated the following conclusions when statistical differences were identified at the 0.05 or less level of probability. In Experiment A, the selenium content in livers from pigs with selenite toxicosis was not significantly different than that in livers from pigs with selenomethionine toxicosis. The selenium content in kidneys from pigs with selenite toxicosis was not significantly different than that in kidneys from pigs with selenomethionine toxicosis. Also, there was no significant difference in the selenium content between livers and kidneys from pigs with selenite toxicosis or from pigs with selenomethionine toxicosis.

Table 14. Terminal selenium content in liver and kidney from pigs fed selenium in Experiment A

Pig no.	Selenium added to ration (ppm)	Selenium compound fed	Selenium concentration (ppm, wet wt.) Liver	Kidney
4*	0	---	0.11	0.37
35	0	---	0.20	1.27
5	0	---	0.20	0.90
31*	0	---	0.09	0.43
24	0.1	SeO_3**	0.42	1.41
34	0.1	SeO_3	0.39	1.05
13	0.1	SeMET**	0.22	0.90
30	0.1	SeMET	0.23	0.89
11	5	SeO_3	2.45	2.19
19	5	SeO_3	2.27	2.64
12	5	SeMET	4.66	6.64
17	5	SeMET	5.13	6.58
15	10	SeO_3	3.36	4.07
32	10	SeO_3	3.88	3.14
8	10	SeMET	9.13	10.1
9	10	SeMET	10.7	10.7
23†	20	SeO_3	36.2	9.52
36	20	SeO_3	8.04	16.3
3	20	SeMET	6.90	20.1
26	20	SeMET	18.0	17.4
6†	45	SeO_3	8.19	11.9
18†	45	SeO_3	6.70	2.73
7†	45	SeMET	21.5	15.7
20†	45	SeMET	14.6	8.21
1†	100	SeO_3	36.0	10.3
37†	100	SeO_3	27.4	17.2
14†	100	SeMET	24.3	22.4
38†	100	SeMET	14.2	11.0

*These pigs had the lesions of hepatosis dietetica.

**SeO_3=sodium selenite; SeMET=selenomethionine.

†These pigs developed clinical selenium toxicosis.

Table 15. Terminal selenium content in liver and kidney of pigs given selenium in Experiment B*

Pig no.	Selenium added to dry ration (ppm)	Selenium compound fed	Selenium concentration (ppm, wet wt.) Liver	Kidney
4B	0	---	0.26	0.73
13B	0	---	0.21	0.45
5B	60	SeO_3**	6.05	4.44
15B	60	SeO_3	15.9	5.09
6B	60	SeMET**	15.3	12.1
16B	60	SeMET	20.6	15.2
1B	120	SeO_3	5.62	2.65
11B	120	SeO_3	9.60	2.98
2B	120	SeMET	48.2	14.1
8B	120	SeMET	20.8	8.08
7B	600	SeO_3	9.10	2.56
9B	600	SeO_3	9.54	3.35
10B	600	SeMET	28.5	14.4
12B	600	SeMET	26.2	11.1
	Selenium injected i.v. (mg/ kg BW)	**Selenium compound injected**		
3B	3	SeO_3	10.2	7.28
14B	3	SeMET	11.3	10.8

*All pigs given selenium became moribund or died with toxicosis.

**SeO_3=sodium selenite; SeMET=selenomethionine.

In Experiment B, the selenium content of both livers and kidneys from pigs with selenomethionine toxicosis was significantly greater ($P<0.01$) than that in these organs from pigs with selenite toxicosis. Also, the selenium content of livers from pigs with selenium toxicosis (both compounds) was significantly greater ($P<0.025$) than that in kidneys, but the selenium content in these livers had significantly more variation ($P<0.01$) than did the selenium content in kidneys.

In a comparison of Experiment A and Experiment B, there was no significant difference in the selenium content in livers from pigs with selenite toxicosis or in livers or kidneys from pigs with selenomethionine toxicosis between Experiment A and Experiment B. However, the selenium content in kidneys from pigs with selenite toxicosis in Experiment A was significantly greater ($P<0.025$) than that from pigs with selenite toxicosis in Experiment B.

DISCUSSION

This research supplied important new information on the pathology of selenium toxicosis in young pigs which was the primary purpose of these studies. Specifically, several clinical features and gross and microscopic lesions were determined and recorded for both inorganic and organic selenium toxicosis in 2 experiments. In addition to characterizing the lesions of selenium toxicosis using both sodium selenite and selenomethionine, these lesions were compared with those of vitamin E-selenium deficiency or hepatosis dietetica in the same experiment by feeding a Torula yeast basal ration. This latter disease was previously studied in more detail in this department by Michel *et al.* (1969).

Individual pigs had variability in their response to the amounts of selenium administered. Some of this variability may have been due to appetite, selenium tolerance and other inherent biological variation of each pig.

Consumption of the Torula yeast basal, semipurified diet was not as good as had been anticipated; however, the Torula yeast basal diet provided a ration to study the lesions of both selenium toxicosis and vitamin E-selenium deficiency in the same experiment. A much higher daily consumption of a practical or natural ration might be expected. With higher consumption, selenium toxicosis might have been induced at lower selenium levels in the feed than those used in Experiment A. However, a natural ration may have

afforded more protection against toxicosis than did the semipurified ration. In Experiment B, the consumption and palatability of the milk basal ration with added selenium probably was better than might be expected with a selenized natural ration. Consequently, the pigs in Experiment B ingested larger amounts of selenium more rapidly than could be expected under natural conditions. The pigs used in this research were quite young and perhaps were more susceptible to selenium toxicosis than older pigs would have been. For the above reasons, the information collected in this research must be used judiciously in its application to naturally occurring selenium toxicosis in swine.

The preliminary experiments in pigs and steers were informative and useful in designing the main experiments and in standardizing techniques. The amount of total selenium that could be administered intravenously to either species was unexpectedly large although the selenium dosages administered on a body-weight basis were quite small compared to those of the common veterinary drugs. The rather severe local reaction to intramuscular injections of moderate amounts of selenite solutions was interesting and suggested the need for multiple injections.

The results of the main experiments indicated that selenium from either sodium selenite or selenomethionine was highly toxic to young pigs. In Experiment A, Pig 18 died 3 days after consuming only 13.5 mg total selenium (3.2 mg selenium/kg body weight) in its feed. In Experiment B, the calculated mean lethal dose was 9.4 mg selenium/kg body weight in pigs fed sodium selenite and 13.6 mg selenium/kg body weight in pigs fed selenomethionine. One intravenous injection of 3 mg selenium/kg body weight as either sodium

selenite or selenomethionine into 2 pigs resulted in fatal toxicosis in 2-1/2 hours and 14 hours, respectively. The above results indicated that under the conditions of this research the minimum lethal dose of selenium as sodium selenite was lower than the 13.2 to 17.6 mg selenium/kg body weight reported by Miller and Williams (1940a) and confirmed by Diener (1961). The toxicity of selenomethionine in Experiment B more nearly paralleled the above reports.

No real difference was noted in the toxicity of selenium from sodium selenite or selenomethionine in Experiment A; however, in Experiment B it appeared that selenium as sodium selenite was slightly more toxic than selenium as selenomethionine. In earlier work the 48-hour LD_{75} in rats for selenomethionine and sodium selenite was 4.25 and 3.25 to 3.50 mg selenium/kg body weight, respectively (Franke and Moxon, 1936; Klug *et al.*, 1949). Thus, selenomethionine should probably be considered less toxic than sodium selenite although no statistically significant difference was evident in this research. This is contrary to the toxicity of organic selenium compounds found in natural cereals inasmuch as Franke and Painter (1938) indicated that the relative toxicity of selenium in the different diets was in the order: wheat > corn > barley > selenate > selenite. This order of relative toxicity of selenium assumed that selenium was the sole toxicant in selenized cereals. Some reports have mentioned a concern about unidentified phytotoxins with selenium during natural selenium toxicosis (Muth and Binns, 1964).

Stowe (1962) indicated that vitamin E-selenium deficiency disease in swine could be expected when the serum levels of tocopherols were below 0.65 µg/ml. On this basis, the pigs in

Experiment A were in a state of marginal vitamin E deficiency. Hepatosis dietetica observed relatively early in 2 pigs that were not fed selenium could be explained by marginal levels of plasma tocopherol. This marginal vitamin E deficiency may have enhanced selenium toxicosis in pigs fed selenium in the early part of Experiment A. While Morss and Olcott (1967) could not demonstrate an effect of tocopherol supplementation on acute oral toxicity of selenium in rats, vitamin E deficiency may increase susceptibility to selenium toxicity. In addition to the vitamin E status, the selenium was probably absorbed more efficiently in these marginally deficient pigs (Wright, 1967).

Selenium toxicosis in this research produced both hepatic and muscular degeneration. Thus, the elevated levels of serum or plasma GOT and OCT that were observed could be expected but could not be directly correlated with the lesions of selenium toxicosis, especially in acute cases. For the diagnosis of selenium toxicosis, plasma or serum levels of these enzymes were nonspecific. Michel *et al.* (1969) reported that the levels of serum OCT were elevated with dietary liver necrosis (vitamin E-selenium deficiency) in the pig. Orstadius (1960) found that plasma levels of GOT were increased but plasma levels of OCT were not increased and concluded after post-mortem examination that muscles were more vulnerable in selenium toxicosis than other organs including the liver. The results of the present research did not totally support the conclusions of Orstadius.

In addition to the above clinicopathologic findings, hemograms were not changed and, therefore, would be of little specific value in the diagnosis of selenium toxicosis in the pig but would aid in

a differential diagnosis. Diener (1961) also made a similar observation.

The selenium content in livers and kidneys was highly variable in pigs fed selenium with or without selenium toxicosis. There were selenium concentrations in liver and kidney of healthy pigs fed selenium that were greater than those in pigs that died of selenium toxicosis. Thus, there must have been a significant mechanism for tolerance to selenium in these pigs. Wahlstrom and Olson (1959) previously observed tolerance to selenium in pigs from sows fed selenium during gestation.

Although selenium accumulates in the body and death results, there was no concentration in liver or kidney below 20 ppm selenium that identified selenium toxicosis under the conditions of this research. Selenium analyses of these organs will readily differentiate between pigs fed selenium and pigs not fed selenium. In a comparison of the selenium content of livers and kidneys from pigs with selenium toxicosis, kidneys, as a group, had less variation in their selenium content than did livers which indicated that kidneys gave the more reliable or accurate estimation of selenium status. But in addition to this, the ratios of selenium content between livers and kidneys generally were: 1-4:1 in pigs with selenium toxicosis and 1:1-4 in pigs without selenium toxicosis, respectively. Glenn *et al.* (1964c) also observed a similar relationship between the selenium content of liver and kidney from sheep with selenate toxicosis. Thus, as an aid in establishing a diagnosis of selenium toxicosis, the selenium content in liver and kidney from a suspected animal should be evaluated not only by the amount in each organ but also by the relationship of the amounts between these 2 organs.

In general, the clinical signs of anorexia, emesis, loss of weight, weakness, depression, subnormal temperature and respiratory distress were in agreement with those of earlier reports on a fewer number of pigs (Miller and Williams, 1940a; Diener, 1961). The exceptions were that diarrhea was not common and rectal temperatures were elevated early in the course of peracute and acute selenium toxicosis.

Since the pigs readily consumed 1 or 2 feedings of the selenized rations, the anorexia that developed was probably due to a systemic reaction of selenium rather than the unpalatability of the rations. The loss of hair that occurred in 1 pig in Experiment B resulted only after profound toxipathic anorexia. Thus, it can be speculated that selenium toxicity may induce general and specific malnutrition which results in the classic clinical manifestations of chronic selenium toxicosis such as loss of hair and hoof lesions. Olson (1969b) mentioned that the rate and manner of selenium administration was probably important in determining the resultant clinical manifestations since some experimental reports have failed to confirm the natural manifestations of selenium toxicosis.

The pathologic changes of selenium toxicosis in this research involved many different organs. In general, the important histologic lesion was microangiopathy which probably contributed to the production of the lesions found in the lungs and brains of pigs with peracute and acute selenosis. In acute selenosis of longer duration and in subacute selenosis, the degenerative changes in the liver and other organs contributed to the demise of these pigs. Although the liver appeared to be the primary target organ from gross examination, histologic lesions were found in cerebrum, cerebellum, spinal

cord, pituitary gland, pancreas, lymph nodes, thumus, bone marrow, adrenal gland, diaphragm and skeletal muscles. These histopathologic changes had a wider distribution than previously described by others (Schoening, 1963; Miller and Schoening, 1936; Miller and Williams, 1940a; Diener, 1961). This distribution of lesions indicated that the pathogenesis and cause of death in selenium toxicosis may be more complex than previous research has suggested. It is believed that the hepatic lesion alone was not severe enough to result in death.

Histopathologic changes in the central nervous system have not been previously reported. Laminar edema between the white and gray matter of the cerebral cortex in a few pigs with selenite toxicosis gave some credibility to the previous association of polioencephalomalacia in cattle with the "blind staggers" of selenium poisoning in cattle (Maag *et al.*, 1960). In addition, the loss of granule cells in the cerebellar granular layer was similar to the cerebellar lesion of chronic methylmercury toxicosis in calves reported by Herigstad *et al.* (1972). A relationship between the metabolism of selenium and methylmercury has been suggested by others (Ganther *et al.*, 1972).

Selenium toxicosis and vitamin E-selenium deficiency have some comparable changes. The severe lesions in the pancreas especially with selenomethionine toxicosis are considered both interesting and important because pancreatic changes have also been suspected but not clearly established in vitamin E-selenium deficiency (Thompson and Scott, 1970). The vascular lesions of selenium toxicosis are comparable to the microangiopathy of vitamin E-selenium deficiency in swine mentioned by Trapp *et al.* (1970). Edema, myopathy, and

hepatic lesions are common to both syndromes. Jubb and Kennedy (1970) theorized that massive necrosis occurred in the liver with selenium toxicosis as a result of nutritional deficiency since selenium apparently replaces sulfur in sulfur-containing amino acids and possibly produces a conditional deficiency in essential amino acids. If this be true, it must be remembered that the massive hemorrhagic hepatic necrosis of vitamin E-selenium deficiency has also been considered to be a complex nutritional deficiency involving vitamin E, selenium, and sulfur amino acids. Although the biochemical pathogenesis of both selenium toxicosis and vitamin E-selenium deficiency disease remains undetermined, ultrastructural studies of advanced hepatic lesions in this research suggested that there may be a difference in the composition of lipid bodies in the cytoplasm of hepatocytes from livers of pigs with selenium toxicosis or hepatosis dietetica. These ultrastructural studies should be expanded to a separate project in and of themselves.

In a differential diagnosis of selenium toxicosis and vitamin E-selenium deficiency in swine, several differences can be emphasized. The clinical signs of selenium toxicosis were more prolonged while sudden death characterized vitamin E-selenium deficiency. Anorexia and resulting cachexia was usually profound in selenium toxicosis. On gross examination, the liver was pale to normal in selenium toxicosis. In vitamin E-selenium deficiency, if hepatosis dietetica was present, the liver had multifocal areas of lobular necrosis and hemorrhage with possible irregularities in Glisson's capsule. The cachectic changes of serous atrophy of fat were usually quite characteristic of selenium toxicosis of relatively short duration. Histopathologically, the liver in selenium

toxicosis was characterized by fatty metamorphosis and centrolobular eosinophilia and necrosis of all lobules without much congestion while in vitamin E-selenium deficiency the liver was characterized by massive lobular necrosis and intralobular hemorrhage with some lobules remaining relatively normal. The selenium residues were over 2 ppm selenium (wet weight basis) in selenium toxicosis while they were less than 0.5 ppm selenium in liver with vitamin E-selenium deficiency.

Selenium probably will become approved as a feed additive for livestock. As with other minor chemicals and some drugs, the full impact of this practice on practical livestock production, agronomic practices and public health probably will not be known for a number of years. Selenium is clearly established to be an essential micronutrient for livestock. Likewise, in small absolute amounts it can be very toxic to livestock. With judicious use in rations, selenium can be very valuable in minimizing livestock losses from selenium deficiencies. If the past misuse of other chemicals is any indication of the future, some misuse of selenium might be anticipated. In the past the prophylactic and therapeutic use of selenium preparations has been limited, but a number of reports have been published attesting to the nature of livestock losses due to inadvertent errors in formulating, mixing or administering these selenium preparations. One might wonder about the livestock losses that have been unidentified or have gone unreported. The information from this research will be most useful in diagnosing selenium toxicosis in livestock and equally important--or perhaps even more essential--in determining what is not selenium toxicosis.

SUMMARY

A total of 47 young pigs and 6 yearling steers was used to determine the toxicosis of sodium selenite and selenomethionine and to compare the lesions produced by both selenium compounds with those produced by vitamin E-selenium deficiency in pigs. In feeding experiments using rations based either on Torula yeast or dried whole milk as dietary protein sources, pigs were fed comparable graded amounts of both selenium compounds in the ration.

In 1 yearling steer, 1 intravenous injection of 1 mg selenium/kg body weight as sodium selenite produced death in 8 hours and gross lesions of massive pulmonary edema, hydrothorax, and congestion of the gastrointestinal tract, while 0.5 mg selenium/kg body weight given intravenously into another steer had no effect in 4 days. In both cattle and swine, intramuscular injections of a selenite solution resulted in localized muscular necrosis. Two pigs given 3 mg selenium/kg body weight intravenously as sodium selenite or selenomethionine developed fatal selenium toxicosis in 2-1/2 and 14 hours, respectively. Pulmonary edema was the important lesion of intravenous selenite toxicosis. Twenty-one pigs fed 20 to 600 ppm selenium as sodium selenite or selenomethionine developed the clinical signs of emesis, anorexia, loss of weight, cachexia, depression, respiratory distress, and coma with subnormal temperature and death. The lesions of selenosis were similar for both selenium compounds and included fatty metamorphosis and centrolobular

necrosis in the liver; congestion of renal medulla; necrosis in lymphoid follicles; hemorrhagic necrosis of adrenal cortex; edema and degenerative changes in the cerebrum, cerebellum and spinal cord; edema and hemorrhagic necrosis of pancreas; serous atrophy of body fat and degenerative changes in skeletal muscles. Lesions in the central nervous system were generally more common with inorganic selenosis while pancreatic lesions were associated with organic selenosis. Microangiopathy was an important lesion of acute selenosis while degenerative lesions and cachectic changes of a wide variety of organs characterized selenosis of longer duration.

The selenium content in liver and kidney, while variable, was somewhat proportional to the level and duration of selenium in the feed. The diagnosis of selenium toxicosis in swine depends on both the nature of the lesions and selenium content of the liver and kidneys. Selenium toxicosis can be differentiated from vitamin E-selenium deficiency in swine by the characteristics of the hepatic lesions.

REFERENCES CITED

REFERENCES CITED

Ageton, R. W.: Selenium. In *Mineral Facts and Problems*. U.S. Dept. of Interior, Bureau of Mines Bull., 650, (1970): 713-721.

Allaway, W. H.: The Chemistry of Selenium. Proc. A.F.M.A. Nutr. Council, (1968): 27-29.

Anderson, H. D., and Moxon, A. L.: Changes in the Blood Picture of the Dog Following Subcutaneous Injections of Sodium Selenite. J. Pharmacol. Exptl. Therap., 76, (1942): 343-354.

Anderson, M. S., Lakin, H. W., Beeson, K. C., Smith, F. F., and Thacker, E.: *Selenium in Agriculture*. U.S. Dept. Agric. Handbook, 200, (1961): 1-65.

Anon.: Selenium and Cancer. Nutr. Rev., 28, (1970): 75-80.

Awwad, H. K., Adelstein, S. J., Potchen, E. J., and Dealy, J. B., Jr.: The Interconversion and Reutilization of Injected ^{75}Se-selenomethionine in the Rat. J. Biol. Chem., 242, (1967): 492-500.

Beath, O. A., Eppson, H. F., and Gilbert, C. S.: Selenium and Other Toxic Minerals in Soils and Vegetation. Wyoming Agr. Expt. Sta. Bull., 206, (1935): 1-55.

Boam, G. W.: *Ultrastructural and Histochemical Studies of Mycoplasma Infected Turkey Sinus Epithelial Cells*. Michigan State University, M.S. Thesis, 1970.

Byard, J. L.: Trimethyl Selenide. A Urinary Metabolite of Selenite. Arch. Biochem. Biophys., 130, (1969): 556-560.

Caravaggi, C., Clark, F. L., and Jackson, A. R. B.: Acute Selenium Toxicity in Lambs Following Intramuscular Injection of Sodium Selenite. Res. Vet. Sci., 11, (1970a): 146-149.

Caravaggi, C., Clark, F. L., and Jackson, A. R. B.: Experimental Acute Toxicity of Orally Administered Sodium Selenite in Lambs. Res. Vet. Sci., 11, (1970b): 501-502.

Cockrell, B. Y.: *A Quantitative Description of Anthelmintic and Prednisolone-Induced Alterations in the Ultrastructure of Hepatic Parenchymal Cells of the Beagle*. University of Illinois, Ph.D. Thesis, 1969.

Cummins, L. M., and Martin, J. L.: Are Selenocystine and Selenomethionine Synthesized *in vivo* from Sodium Selenite in Mammals? Biochemistry, 6, (1967): 3162-3168.

Diener, R. M.: *Acute Selenium Toxicosis in the Baby Pig.* Michigan State University, M.S. Thesis, 1961.

Dinkel, C. A., Minyard, J. A., Whitehead, E. I., and Olson, O. E.: Agricultural Research at the Reed Ranch Substation--A Progress Report. South Dakota Agric. Expt. Sta. Circ., 135, (1957): 1-35.

Diplock, A. T., Baum, H., and Lucy, J. A.: The Effect of Vitamin E on the Oxidation State of Selenium in Rat Liver. Biochem. J., 123, (1971): 721-729.

Draize, J. H., and Beath, O. A.: Observations on the Pathology of "Blind Staggers" and "Alkali Disease." J.A.V.M.A., 86, (June, 1935): 753-763.

Dudley, H. C.: Toxicology of Selenium. I. A Study of the Distribution of Selenium in Acute and Chronic Cases of Selenium Poisoning. Am. J. Hygiene, 23, (1936): 169-180.

Ehlig, C. F., Hogue, D. E., Allaway, W. H., and Hamm, D. J.: Fate of Selenium from Selenite or Seleno-Methionine, With or Without Vitamin E, in Lambs. J. Nutr., 92, (1967): 121-126.

Franke, K. W.: A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs. I. Results Obtained in Preliminary Feeding Trials. J. Nutr., 8, (1934a): 597-608.

Franke, K. W.: A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs. II. The Occurrence of the Toxicant in the Protein Fraction. J. Nutr., 8, (1934b): 609-613.

Franke, K. W.: A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs. X. The Effect of Feeding Toxic Foodstuffs in Varying Amounts, and for Different Time Periods. J. Nutr., 10, (1935a): 223-231.

Franke, K. W.: A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs. XI. The Effect of Feeding Toxic and Control Foodstuffs Alternately. J. Nutr., 10, (1935b): 233-239.

Franke, K. W., and Moxon, A. L.: A Comparison of the Minimum Fatal Doses of Selenium, Tellurium, Arsenic, and Vanadium. J. Pharmacol. Exptl. Therap., 58, (1936): 454-459.

Franke, K. W., and Moxon, A. L.: Toxicity of Orally Ingested Arsenic, Selenium, Tellurium, Vanadium, and Molybdenum. J. Pharmacol. Exptl. Therap., 61, (1937): 89-102.

Franke, K. W., and Painter, E. P.: A Study of the Toxicity and Selenium Content of Seleniferous Diets: With Statistical Consideration. Cereal Chem., 15, (1938): 1-24.

Frost, D. V.: The Case for Selenite as a Feed Additive. Feedstuffs, 43, (July 31, 1971): 12-16; 33.

Frost, D. V.: Selenium Has Great Nutritional Significance for Man; Should be Cleared for Feed. Feedstuffs, 44, (Jan. 24, 1972): 8; 58-59.

Gabbedy, B. J., and Dickson, J.: Acute Selenium Poisoning in Lambs. Aust. Vet. J., 45, (1969): 470-472.

Ganther, H. E.: Enzymic Synthesis of Dimethyl Selenide from Sodium Selenite in Mouse Liver Extracts. Biochemistry, 5, (1966): 1089-1098.

Ganther, H. E., and Corcoran, C.: Selenotrisulfides. II. Cross-Linking of Reduced Pancreatic Ribonuclease with Selenium. Biochemistry, 8, (1969): 2557-2563.

Ganther, H. E., Goudie, C., Sunde, M. L., Kopecky, M. J., Wagner, P., Oh, S. H., and Hoekstra, W. G.: Selenium: Relation to Decreased Toxicity of Methylmercury Added to Diets Containing Tuna. Science, 175, (1972): 1122-1124.

Ganther, H. E., Levander, O. A., and Baumann, C. A.: Dietary Control of Selenium Volatilization in the Rat. J. Nutr., 88, (1966): 55-60.

Glenn, M. W., Jensen, R., and Griner, L. A.: Sodium Selenate Toxicosis: The Effects of Extended Oral Administration of Sodium Selenate on Mortality, Clinical Signs, Fertility, and Early Embryonic Development in Sheep. Am. J. Vet. Res., 25, (Sept., 1964a): 1479-1485.

Glenn, M. W., Jensen, R., and Griner, L. A.: Sodium Selenate Toxicosis: Pathology and Pathogenesis of Sodium Selenate Toxicosis in Sheep. Am. J. Vet. Res., 25, (Sept., 1964b): 1486-1494.

Glenn, M. W., Martin, J. L., and Cummins, L. M.: Sodium Selenate Toxicosis: The Distribution of Selenium Within the Body after Prolonged Feeding of Toxic Quantities of Sodium Selenate to Sheep. Am. J. Vet. Res., 25, (Sept., 1964c): 1495-1499.

Gortner, R. A.: Chronic Selenium Poisoning of Rats as Influenced by Dietary Protein. J. Nutr., 19, (1940): 105-112.

Halverson, A. W., Tsay, D., Triebwasser, K. C., and Whitehead, E. I.: Development of Hemolytic Anemia in Rats Fed Selenite. Toxicol. Appl. Pharmacol., 17, (1970): 151-159.

Hartley, W. J., and Grant, A. B.: A Review of Selenium Responsive Diseases of New Zealand Livestock. Fed. Proc., 20, (1961): 679-688.

Heinrich, M. A., Jr.: Effects of Sodium Selenite on Several Blood Components. Toxicol. Appl. Pharmacol., 5, (1963): 267-271.

Heinrich, M., and MacCanon, D. M.: Some Observations on the Toxicity of Intravenous Sodium Selenite in Dogs. Proc. South Dakota Acad. Sci., 36, (1957): 173-177.

Heinrich, M. A., Jr., and MacCanon, D. M.: Some Effects of Sodium Selenite on the Cardiovascular System. Toxicol. Appl. Pharmacol., 2, (1960): 33-43.

Heinrich, M. A., Jr., and MacCanon, D. M.: Respiratory Responses to Sodium Selenite. Toxicol. Appl. Pharmacol., 3, (1961): 174-181.

Hendrick, C. M., Klug, H. L., and Olson, O. E.: Effect of 3-nitro-4-hydroxylphenylarsonic Acid and Arsanilic Acid on Selenium Poisoning in the Rat. J. Nutr., 51, (1953): 131-137.

Herigstad, R. R., Whitehair, C. K., Beyer, N., Mickelsen, O., and Zabik, M. J.: Chronic Methylmercury Toxicosis in Calves. J.A.V.M.A., 160, (Jan. 15, 1972): 173-182.

Hopkins, L. L., Jr., Pope, A. L., and Baumann, C. A.: Distribution of Microgram Quantities of Selenium in the Tissues of the Rat, and Effects of Previous Selenium Intake. J. Nutr., 88, (1966): 61-65.

Hycel Urea Nitrogen Determinations. 2nd Revision. Hycel, Inc., Houston, Texas, 1964.

Imbach, A., and Sternberg, J.: Turnover Studies and Placental Transfer of Selenium-75 Components in Rats. J. Nucl. Med., 7, (1966): 368-369.

Jenkins, K. J., Dickson, R. C., and Hidiroglou, M.: Intravascular Transport of Selenium in the Chick. In *Trace Element Metabolism in Animals.* E & S Livingstone, Edinburgh, Scotland, 1970: 336-339.

Jubb, K. V. F., and Kennedy, P. C.: *Pathology of Domestic Animals,* 2nd ed. Academic Press, New York, Vol. 2, 1970: 211.

Klug, H. L., Harshfield, R. D., Pengra, R. M., and Moxon, A. L.: Methionine and Selenium Toxicity. J. Nutr., 48, (1952): 409-420.

Klug, H. L., Petersen, D. F., and Moxon, A. L.: The Toxicity of Selenium Analogues of Cystine and Methionine. Proc. South Dakota Acad. Sci., 28, (1949): 117-120.

Koch-Weser, D., de la Huerga, J., and Popper, H.: Hepatic Necrosis Due to Bromobenzene and Its Dependence upon Available Sulfur Amino Acids. Proc. Soc. Exptl. Biol. Med., 79, (1952): 196-198.

Krehl, W. A.: Selenium--The Maddening Mineral. Nutrition Today, 5, (1970): 26-32.

Kuttler, K. L., Marble, D. W., and Blincoe, C.: Serum and Tissue Residues Following Selenium Injections in Sheep. Am. J. Vet. Res., 22, (May, 1961): 422-428.

Lambourne, D. A., and Mason, R. W.: Mortality in Lambs Following Overdosing with Sodium Selenite. Aust. Vet. J., 45, (1969): 208.

Levander, O. A., and Morris, V. C.: Interactions of Methionine, Vitamin E, and Antioxidants in Selenium Toxicity in the Rat. J. Nutr., 100, (1970): 1111-1118.

Lewis, A. E.: *Biostatistics*. Reinhold Publishing Corporation, New York, 1966.

Luna, L. G. (ed.): *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. McGraw-Hill Book Co., New York, 1968.

Maag, D. D., Orsborn, J. S., and Clopton, J. R.: The Effect of Sodium Selenite on Cattle. Am. J. Vet. Res., 21, (Nov., 1960): 1049-1053.

Majaj, A. S., and Hopkins, L. L., Jr.: Selenium and Kwashiorkor. Lancet, 2, (1966): 592-593.

McConnell, K. P.: Metabolism of Selenium in the Mammalian Organism. J. Agr. Food Chem., 11, (1963): 385-388.

McConnell, K. P., and Carpenter, D. M.: Interrelationship Between Selenium and Specific Trace Elements. Proc. Soc. Exptl. Biol. Med., 37, (1971): 996-1001.

McConnell, K. P., and Cho, G. J.: Active Transport of L-Selenomethionine in the Intestine. Am. J. Physiol., 213, (1967): 150-156.

McConnell, K. P., and Hoffman, J. L.: Methionine-Selenomethionine Parallels in Rat Liver Polypeptide Chain Synthesis. Fed. Proc., 31, (1972): 691 ABS 2685.

McConnell, K. P., and Portman, O. W.: Excretion of Dimethyl Selenide by the Rat. J. Biol. Chem., 195, (1952): 277-282.

Michel, R. L., Whitehair, C. K., and Keahey, K. K.: Dietary Hepatic Necrosis Associated with Selenium-Vitamin E Deficiency in Swine. J.A.V.M.A., 155, (July 1, 1969): 50-59.

Miller, W. T., and Schoening, H. W.: Toxicity of Selenium Fed to Swine in the Form of Sodium Selenite. J. Agric. Res., 56, (1938): 831-842.

Miller, W. T., and Williams, K. T.: Minimum Lethal Dose of Selenium as Sodium Selenite, for Horses, Mules, Cattle, and Swine. J. Agric. Res., 60, (1940a): 163-173.

Miller, W. T., and Williams, K. T.: Effect of Feeding Repeated Small Doses of Selenium as Sodium Selenite to Equines. J. Agric. Res., 61, (1940b): 353-368.

Mills, C. F. (ed.): *Trace Element Metabolism in Animals*. Proc. of WAAP/IBP International Symposium, Aberdeen, Scotland, July, 1969. E & S Livingstone, Edinburgh, Scotland, 1970.

Minyard, J. A., Dinkel, C. A., and Olson, O. E.: Effect of Arsanilic Acid in Counteracting Selenium Poisoning in Beef Cattle. J. Anim. Sci., 19, (1960): 260-264.

Morrow, D. A.: Acute Selenite Toxicosis in Lambs. J.A.V.M.A., 152, (June 1, 1968): 1625-1629.

Morss, S. G., and Olcott, H. S.: Absence of Effect of Tocopherol on Acute Oral Toxicity of Sodium Selenite in the Rat. Proc. Soc. Exptl. Biol. Med., 124, (1967): 483-485.

Moxon, A. L.: Alkali Disease or Selenium Poisoning. South Dakota Agric. Expt. Sta. Bull. 311, (1937): 1-91.

Moxon, A. L.: The Influence of Arsenic on Selenium Poisoning in Hogs. Proc. South Dakota Acad. Sci., 21, (1941): 34-36.

Moxon, A. L., Anderson, H. D., and Painter, E. P.: The Toxicity of Some Organic Selenium Compounds. J. Pharmacol. Exptl. Therap., 63, (1938): 357-368.

Moxon, A. L., and DuBois, K. P.: The Influence of Arsenic Acid and Certain Other Elements on the Toxicity of Seleniferous Grains. J. Nutr., 18, (1939): 447-457.

Moxon, A. L., DuBois, K. P., and Potter, R. L.: The Toxicity of Optically Inactive, d- and l-Selenium-Cystine. J. Pharmacol. Exptl. Therap., 72, (1941): 184-195.

Moxon, A. L., and Rhian, M.: Selenium Poisoning. Physiol. Rev., 23, (1943): 305-337.

Moxon, A. L., Schaefer, A. E., Lardy, H. A., DuBois, K. P., and Olson, O. E.: Increasing the Rate of Excretion of Selenium from Selenized Animals by the Administration of p-Bromobenzene. J. Biol. Chem., 132, (1940): 785-786.

Munsell, H. E., DeVaney, G. M., and Kennedy, M. H.: Toxicity of Food Containing Selenium as Shown by its Effect on the Rat. U.S. Dept. Agric. Tech. Bull. 534, (1936): 1-25.

Muth, O. H., and Binns, W.: Selenium Toxicity in Domestic Animals. Ann. N.Y. Acad. Sci., 111, (1964): 583-590.

Muth, O. H., Oldfield, J. E., and Weswig, P. H. (ed.): *Symposium: Selenium in Biomedicine*. The AVI Publishing Co., Westport, Conn., 1967.

Muth, O. H., Weswig, P. H., Whanger, P. D., and Oldfield, J. E.: Effect of Feeding Selenium-Deficient Ration to the Subhuman Primate. Am. J. Vet. Res., 32, (Oct., 1971): 1603-1605.

NAS-NRC Monograph: *Selenium in Nutrition*. Nat. Acad. Sci., Washington, D.C., 1971.

Neethling, L. P., Brown, M. M. M., and DeWet, P. J.: The Toxicology and Metabolic Fate of Selenium in Sheep. J. S. Afr. Vet. Med. Assoc., 39, (1968): 25-33.

Olson, O. E.: Fluorometric Analysis of Selenium in Plants. J.A.O.A.C., 52, (1969a): 627-634.

Olson, O. E.: Selenium as a Toxic Factor in Animal Nutrition. Proc. Georgia Nutr. Conf., (1969b): 68-78.

Orstadius, K.: Toxicity of a Single Subcutaneous Dose of Sodium Selenite in Pigs. Nature, 188, (1960): 1117.

Painter, E. P.: The Chemistry and Toxicity of Selenium Compounds, With Special Reference to the Selenium Problem. Chem. Rev., 28, (1941): 179-213.

Palmer, I. S., Fischer, D. D., Halverson, A. W., and Olson, O. E.: Identification of a Major Selenium Excretory Product in Rat Urine. Biochim. Biophys. Acta, 177, (1969): 336-342.

Robinson, W. O.: Determination of Selenium in Wheat and Soils. J.A.O.A.C., 16, (1933): 423-424.

Romanowski, R. D., Larson, R. L., Halverson, A. W., and Olson, O. E.: Observations on the Protective Effect of Dietary Fat Against Selenium Poisoning in Rats. Proc. South Dakota Acad. Sci., 37, (1958): 76-80.

Rosenfeld, I.: Biosynthesis of Seleno-Compounds from Inorganic Selenium by Sheep. Proc. Soc. Exptl. Biol. Med., 111, (1962): 670-673.

Rosenfeld, I.: Metabolic Effects and Metabolism of Selenium in Animals. Wyoming Agric. Expt. Sta. Bull. 414, (1964): 1-64.

Rosenfeld, I., and Beath, O. A.: The Elimination and Distribution of Selenium in the Tissues in Experimental Selenium Poisoning. J. Nutr., 30, (1945): 443-449.

Rosenfeld, I., and Beath, O. A.: Pathology of Selenium Poisoning. Wyoming Agric. Expt. Sta. Bull. 275, (1946a): 1-27.

Rosenfeld, I., and Beath, O. A.: The Influence of Protein Diets on Selenium Poisoning. I. Am. J. Vet. Res., 7, (Jan., 1946b): 52-56.

Rosenfeld, I., and Beath, O. A.: The Influence of Protein Diets on Selenium Poisoning. II. The Chemical Changes in the Tissues Following Selenium Administration. Am. J. Vet. Res., 7, (Jan., 1946c): 57-61.

Rosenfeld, I., and Beath, O. A.: The Influence of Various Substances on Chronic Selenium Poisoning. J. Pharmacol. Expt. Therap., 91, (1947): 218-223.

Rosenfeld, I., and Beath, O. A.: *Selenium: Geobotany, Biochemistry, Toxicity and Nutrition.* Academic Press, New York, 1964.

Rotruck, J. T., Hoekstra, W. G., Pope, A. L., Ganther, H., Swanson, A., and Hafeman, D.: Relationship of Selenium to GSH Peroxidase. Fed. Proc., 31, (1972): 691 ABS 2684.

Schneider, H. A.: Selenium in Nutrition. Science, 83, (1936): 32-34.

Schoening, H. W.: Production of So-Called Alkali Disease in Hogs by Feeding Corn Grown in Affected Area. North Am. Vet., 17, (1936): 22-28.

Schroeder, H. A., Frost, D. V., and Balassa, J. J.: Essential Trace Metals in Man: Selenium. J. Chron. Dis., 23, (1970): 227-243.

Schwarz, K.: Factor 3, Selenium and Vitamin E. Nutr. Rev., 18, (1960): 193-197.

Schwarz, K.: The Nutritional Significance of Selenium--A Symposium. Fed. Proc., 20, (1961): 665-702.

Schwarz, K.: Role of Vitamin E, Selenium, and Related Factors in Experimental Nutritional Liver Disease. Fed. Proc., 24, (1965a): 58-67.

Schwarz, K.: Selenium and Kwashiorkor. Lancet, 1, (1965b): 1335.

Schwarz, K., and Foltz, C. M.: Selenium as an Integral Part of Factor 3 against Dietary Necrotic Liver Degeneration. J. Am. Chem. Soc., 79, (1957): 3292-3293.

Shortridge, E. H., O'Hara, P. J., and Marshall, P. M.: Acute Selenium Poisoning in Cattle. N.Z. Vet. J., 19, (1971): 47-50.

Sigma Technical Bulletin No. 108: *The Determination of Ornithine Carbamyl Transferase in Serum or Other Fluids, of Possible Value in the Diagnosis of Liver Damage.* **Sigma Chemical Co., St. Louis, Mo., 1965.**

Smith, M. I.: The Influence of Diet on the Chronic Toxicity of Selenium. U.S. Public Health Repts., 54, (1939): 1441-1453.

Smith, M. I., Franke, K. W., and Westfall, B. B.: The Selenium Problem in Relation to Public Health. A Preliminary Survey to Determine the Possibility of Selenium Intoxication in the Rural Population Living on Seleniferous Soil. U.S. Public Health Rept., 51, (1936): 1496-1505.

Smith, M. I., Stohlman, E. F., and Lillie, R. D.: The Toxicity and Pathology of Selenium. J. Pharmacol. Exptl. Therap., 60, (1937a): 449-471.

Smith, M. I., and Westfall, B. B.: Further Field Studies on the Selenium Problem in Relation to Public Health. U.S. Public Health Rept., 52, (1937): 1375-1384.

Smith, M. I., Westfall, B. B., and Stohlman, E. F.: The Elimination of Selenium and Its Distribution in the Tissues. U.S. Public Health Repts., 52, (1937b): 1171-1177.

Smith, M. I., Westfall, B. B., and Stohlman, E. F.: Studies on the Fate of Selenium in the Organism. U.S. Public Health Rept., 53, (1938): 1199-1216.

Stowe, H. D.: *Pathology of Tocopherol-Deficient Mink and Swine.* Michigan State University, Ph.D. Thesis, 1962.

Tappel, A. L.: Free Radical Lipid Peroxidation Damage and Its Inhibition by Vitamin E and Selenium. Fed. Proc., 24, (1965): 73-78.

Thompson, J. N., and Scott, M. L.: Impaired Lipid and Vitamin E Absorption Related to Atrophy of the Pancreas in Selenium-Deficient Chicks. J. Nutr., 100, (1970): 797-809.

TransAc-Reagent System for Colorimetric Assay of Serum Glutamic-Oxaloacetic Transaminase (GOT). General Diagnostics, Morris Plains, N.J., 1968.

Trapp, A. L., Keahey, K. K., Whitenack, D. L., and Whitehair, C. K.: Vitamin E-Selenium Deficiency in Swine: Differential Diagnosis and Nature of Field Problem. J.A.V.M.A., 157, (Aug. 1, 1970): 289-300.

Trelease, S. F., and Beath, O. A.: *Selenium: Its Geological Occurrence and Its Biological Effects in Relation to Botany, Chemistry, Agricultural Nutrition and Medicine.* Published by the authors, New York, 1949.

Tsen, C. C.: An Improved Spectrophotometric Method for the Determination of Tocopherols using 4,7-diphenyl-1, 10-phenanthroline. Anal. Chem., 33, (1961): 849-851.

Underwood, E. J.: *Trace Elements in Human and Animal Nutrition*, 3rd ed. Academic Press, New York, 1971: 323-368.

Wahlstrom, R. C., Kamstra, L. D., and Olson, O. E.: The Effect of Arsanilic Acid and 3-nitro-4-hydroxyphenylarsonic Acid on Selenium Poisoning in the Pig. J. Anim. Sci., 14, (1955): 105-110.

Wahlstrom, R. C., Kamstra, L. D., and Olson, O. E.: The Effect of Organic Arsenicals, Chlortetracycline and Linseed Oil Meal on Selenium Poisoning in Swine. J. Anim. Sci., 15, (1956): 794-799.

Wahlstrom, R. C., and Olson, I. E.: The Relation of Pre-Natal and Pre-Weaning Treatment to the Effect of Arsanilic Acid on Selenium Poisoning in Weanling Pigs. J. Anim. Sci., 18, (1959): 578-582.

Wright, P. L.: The Absorption and Tissue Distribution of Selenium in Depleted Animals. In *Symposium: Selenium in Biomedicine*. AVI Publishing Co., Westport, Conn., 1967: 313-328.

Wright, P. L., and Bell, M. C.: Comparative Metabolism of Selenium and Tellurium in Sheep and Swine. Am. J. Physiol., 211, (1966): 6-10.

VITA

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