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THE INFLUENCE OF VITAMINE E AND SELENIUM
ON GASTRIC ULCERS IN SWINE

presented by

DAVID MICHAEL BEBIAK

has been accepted towards fulfillment
of the requirements for

M.S. degree in ANIMAL HUSBANDRY

E. R. Miller

Major professor

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THE INFLUENCE OF VITAMIN E AND SELENIUM ON GASTRIC ULCERS IN SWINE

By

David Michael Bebiak

A THESIS

Submitted to
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ABSTRACT

Previous research has concluded that a primary lesion of vitamin E(E) and selenium (Se) deficiency is microangiopathy. Such vascular disruption and subsequent platelet aggregation may reduce blood flow and cause focal necrosis. These events would explain the broad range of lesions observed in E-Se deficiency. A study was conducted to evaluate the influence of E and Se supplementation of an ulcerogenic diet of swine. The basal ulcerogenic diet (BUD) in trial I consisted of corn starch, soybean meal and corn oil and was adequately fortified with minerals and vitamins except Se and E. A similar basal diet in which finely ground corn supplanted corn starch and corn oil was used in trial II. Pigs were randomly assigned from litters to four dietary groups, namely: BUD, BUD + 22 IU/kg of E, BUD + .1 ppm Se, BUD + 22 IU/kg of E + .1 ppm Se. Average daily gain of pigs in these respective groups from 6 to 22 weeks of age were: 591 g, 782 g, 630 g, and 661 g. The E supplemented group gained significantly faster than all other groups ($P < .01$). All pigs were

slaughtered at an average weight of 105 kg and the stomachs were inspected. Gastric ulcers were grossly characterized according to size, depth and color into the following classifications of increasing severity: epithelial change, acute erosion, subacute ulcer, chronic ulcer and scars. In the first trial, among 8 pigs fed BUD, 6 had ulcers of varied severity, 1 had an epithelial change and 1 was normal. Of 7 E supplemented pigs, 1 had a chronic ulcer, 2 had erosions and 4 were normal. Among 7 Se supplemented and 7 E + Se supplemented pigs, all had ulcers of varying severity. In the second trial, once again 6 of 8 pigs fed the BUD had ulcers or preulcerous lesions of varied severity and 2 were normal. Of 7 Se supplemented pigs, 1 had an acute erosion and 6 were normal. Among 7 E supplemented and 7 E + Se supplemented pigs, all were normal. All ulcers were located in the non-glandular, cardiac region. There was no consistent effect of dietary supplement on blood platelet aggregation. A hypothetical ulcerogenic mechanism is proposed.

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INTRODUCTION

The total nutrient intake of swine raised in confinement is provided by the compounded feeds as allocated at the discretion of the swine producer. The significance of complete nutrient provision by the producer intensifies as the incidence of nutrition-related health problems increases among pigs reared in confinement. An increased incidence of alpha-tocopherol (E) and Selenium (Se) deficiency is a manifestation of current trends in swine production toward confinement and over-processing of feeds.

Several researchers studying the E-Se-deficiency syndrome in swine (Grant, 1961; Michel et al., 1967; Nafsted, 1971) observed extensive vascular degeneration. Local vascular disruptions could explain the wide range of lesions occurring in active tissues among E-Se-deficient swine. Among other observations, Grant (1961), Michel et al. (1967) and Nafsted (1971) also noted a high incidence of gastric ulcers in these same animals. Awareness of the incidence of gastric ulcers (circumscribed necrotic area of gastric mucosa) among swine in field cases has parallelled

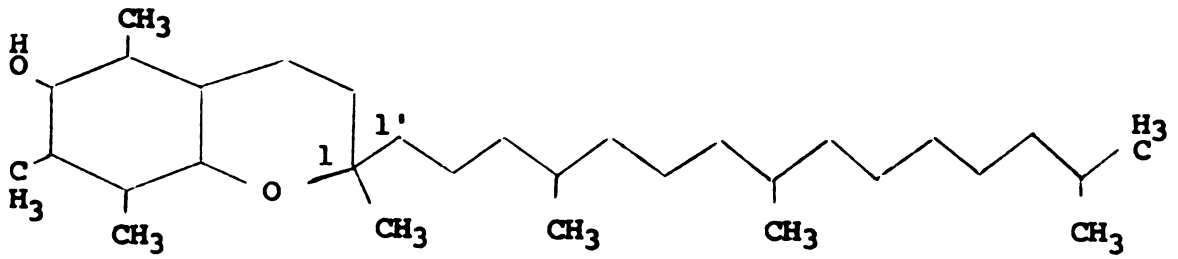
the trend toward confinement rearing, although, observations of gastric ulcers are by no means limited to pigs raised in confinement. In general, the pathogenesis of the mucosal lesions is attributed to several factors, including genetic selection of meat type swine, crowding, and the feeding of high energy finely ground rations for greater feed conversion.

Determining more precise pathogenic factors may be useful in assisting swine producers in diminishing this problem. The curious association, described above, between E-Se-deficiency and the appearance of gastric ulceration inspired this particular research effort toward more accurately defining the ulcerogenic process.

REVIEW OF LITERATURE

Vitamin E and Selenium

Chemistry. Alpha-tocopherol has three methyl groups (position 5, 7, 8-). Beta and gamma-tocopherols have two methyl groups (5, 8- and 7, 8- respectively). Delta-tocopherols have one methyl group occurring at position 8 (Tappel, 1969).



alpha-tocopherol

Selenium is an integral component of the enzyme glutathione peroxidase. Four Se atoms are associated with each mole of enzyme, an average of one Se per protein subunit of about 22,000 molecular weight (Oh et al., 1974).

Natural occurrence. Tocopherol composition changes with the stage of development in many grains. The changes vary with each plant. Vitamin E in grasses, as in leaves

and stems of cereal plants, is largely or entirely comprised of alpha-tocopherol. Prominent reductions in concentration accompany maturation and curing of forages. One of the factors responsible for the decrease in concentration which accompanies maturation is the increasing proportion of stem to leaf, the latter containing 20 to 30 times more tocopherol.

Animal food products are generally poor sources of tocopherols by comparison to cereals and vegetables (Tappel, 1969).

The fully methylated alpha-tocopherol is substantially more active in vivo than any of the other forms and, in general, dealkylation leads to progressive loss of activity. Taking the trimethyl alpha to be 100% potent, the biopotencies of the dimethyl beta and gamma homologues are approximately 33%. On the other hand, a synthetic hybrid 5, 7-dimethyl tocopherol has been reported to have 66% potency, suggesting that the presence of methyl groups in the positions ortho to the hydroxyl group is biologically important.

Levels of naturally occurring Se vary greatly between geographical regions. Consequently, the Se values of common feedstuffs exhibit great regional variation. Corn samples grown from the following states were assayed for Se content:

Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, and Wisconsin (Patrias, 1969). Selenium values ranged from a low of .01 ppm to a high of 2.03 ppm. North Dakota, South Dakota, and Nebraska had mean values of .2 ppm or higher and there were no values below .04 ppm. All other states had some samples with values of .03 ppm or less.

Methods of harvest, storage, and preparation have significant effects on the nutrient content of feeds. Much of the midwest corn is harvested at high moisture levels and artificially dried. Mechanical damage of the kernel and subsequent air exposure and heat exposure may result in significant destruction of tocopherols found in the corn germ.

Antioxidant role. Opinions vary as to the mode of action of vitamin E and Se in preventing specific diseases in animals. However, at this point, proposed etiologic factors of diseases related to either simple or compound deficiencies of these nutrients must be related to their one known role as biological antioxidants.

Phospholipid constituents of biological membranes are subject to oxidative degradation which leads to structural damage and ultimately to disruption of cellular integrity. Lability to lipid peroxidation in membranes is a function

of the polyunsaturated fatty acid content of constituent phospholipids. The rate of peroxidation varies directly with the number of double bonds in the fatty acids present in those phospholipids (Witting, 1965). Vitamin E plays an important role as an intracellular antioxidant, related especially to the stabilization of ingested fats and possibly of products arising in the metabolic synthesis and degradation of lipids. Thus the concept that vitamin E functions as a general protector of structural lipoproteins or of oxidizable lipid components explains the great diversity of histopathological lesions which can develop during vitamin E deficiency.

The relationship between Se and vitamin E was first demonstrated when Schwarz (1957) observed that sodium selenite prevented liver necrosis in rats, a sign previously thought to be characteristic of vitamin E deficiency. The recognition of the role of Se in the enzyme glutathione peroxidase further established the existence of a common function of this nutrient with that of vitamin E (Rotruck et al., 1973). Glutathione peroxidase is an important enzyme in destroying hydrogen peroxide and organic hydroperoxides. Therefore, like vitamin E, it protects against oxidative damage to cellular and subcellular membranes.

Nishikimi (1975) used 6-hydroxy-2, 5, 7, 8- tetramethyl-chroman -2- carboxylic acid as a model for alpha-tocopherol and found that it was oxidized by a xanthine oxidase system. Superoxide dismutase completely inhibited the reaction while catalase had no effect. The xanthine oxidase system is a common component of the purine catabolic process and is responsible for the production of superoxide anion, a partial reduction product of oxygen which is very reactive and capable of irreversible damage to various biomolecules. In particular, the superoxide anion radical had been shown to be involved in the process of lipid peroxidation (Rawls, 1970). The enzyme superoxide dismutase converts the superoxide anion to hydrogen peroxide, another toxic partial reduction product of oxygen. In turn, the enzyme catalase decomposes and detoxifies hydrogen peroxide. Specific inhibition of tocopherol-model oxidation by superoxide dismutase indicates that the oxidation is caused by the superoxide anion. The presence of alpha-tocopherol in biological membranes, specifically subcellular membranes, suggests that it scavenges those superoxide anions generated by membrane-bound enzymes participating in biological oxidation (Nishikimi, 1975).

Hoekstra's (1975) proposed scheme based on the inhibition of oxidant damage accounts for the interaction

between Se, vitamin E, and unsaturated lipids. Vitamin E acts as an antioxidant in preventing lipid hydroperoxide formation and consequent autocatalytic lipid peroxidation and potential cell damage. When lipid peroxidation is initiated, a lipid-free radical is generated. This radical combines with molecular oxygen to form a lipid peroxyl radical. The peroxyl radical is then capable of continuing the peroxidation cycle by removing a hydrogen from another lipid and so on. Alpha-tocopherol reduces the peroxyl radical, itself being oxidized to alpha-tocopherylquinone, thus breaking the chain reaction.

Selenium, as a component of glutathione peroxidase, converts hydrogen peroxides to less damaging alcohols. Glutathione peroxidase utilizes reducing equivalents from glutathione to reduce and detoxify hydrogen peroxide and fatty acid hydroperoxides that could otherwise initiate oxidative degradation of cellular components including membrane lipids. This activity is dependant upon the level of Se in the diet. Nishikimi's (1975) observation and Hoekstra's (1975) proposal are not mutually exclusive though clearly different. The biological roles assigned to alpha-tocopherol are respectively preventive and inhibitive of membrane peroxidation.

Dietary requirements. Because of the marked effect

of other nutrients on the need for tocopherols it is evident that no meaningful general estimate of vitamin E requirement can be formulated. With particular reference to the quantity of Se in the diet and proportion and constitution of fat, the tocopherol requirements of two animals of common species may differ by one or two orders of magnitude.

It is possible that 12 IU/day of vitamin E could be adequate for man when the tissues are low in polyunsaturates, and more than 45 IU/day may be needed when the tissues are high in linoleates. The requirement must be related in some manner to the amount of polyunsaturates in the diet. Any calculation of the vitamin E requirement based only on dietary polyunsaturated fatty acids (PUFA) would not be complete since the tissues would have considerable amounts of polyunsaturated lipids even when the diet was deficient in PUFA. Accordingly, Horwitt, (1974) suggested that 6 IU (4 mg of alpha-tocopherol) be allowed for tissue synthesis of peroxidizable compounds in the adult male. Horwitt (1974) incorporates this estimate into the following formula for determining the requirement of d-alpha-tocopherol equivalents: .25 x (% PUFA ÷ g PUFA) ÷ 4 mg ÷ mg d-alpha-tocopherol.

The current practice of growing pigs in confinement

offers a unique opportunity to estimate meaningful vitamin E requirements. The complete nutrient content of a diet which is controlled by the producer can be determined with adequate precision. In relation to known constant levels of dietary fat and Se, the vitamin E requirement can be established.

Ames (1956) determined the alpha-tocopherol content of several feedstuffs of which corn contained 4 mg/kg dry matter and solvent extracted soybean meal contained .79 mg/kg. Compared with the tocopherol content of a number of other plant foods, it is apparent that corn and solvent extracted soybean meal are poor sources of vitamin E. In many areas, corn and soybean meal are the principal components of swine diets. One mg of alpha-tocopherol represents 1.49 IU. There is an obvious potential for vitamin E deficiency problems among swine reared in confinement when these two feedstuffs are the primary ingredients.

Precise Se requirements are also difficult to establish. However, some indication of a margin of safety between an oral intake adequate to prevent deficiencies and an intake which will produce toxicity symptoms has been established. Groce et al. (1973) attempted to define the minimum dietary Se requirements of growing-finishing

swine fed corn-soybean meal diets. The diets used contained .05 ppm of natural Se and 4.2 mg of alpha-tocopherol per kg. Observations concerned the effects of incremental additions of Se (from sodium selenite) upon development of deficiency signs and tissue concentration. Supplements of .1 ppm Se prevented death losses, gross pathology and histopathological lesions of nutritional muscular dystrophy and dietary hepatic necrosis. Selenium concentrations in skeletal muscle, myocardium, liver, and kidney were increased proportionately to supplemental increases but tended to reach a maximum at .1 ppm supplemental Se (over a range of 0 to .5 ppm supplemental Se). Even the highest levels of tissue Se resulting from dietary sodium selenite were well below those tissues of pigs fed natural Se-adequate diets (Groce et al., 1971).

Levels of supplemental Se above .1 ppm were absorbed then excreted in greater proportions in urine to produce absolute Se retentions similar to that observed at .1 ppm supplemental Se. Supplementation of Se-deficient swine diets with sodium selenite resulting in a total Se level of about .15 ppm optimally prevents deficiency signs in confined, growing-finishing swine. This is about 1/50 of the continuously fed dietary Se level shown to produce

toxicity in swine.

Deficiency signs. Many clinical signs specifically related to deficiency of vitamin E are based on the one known role of the vitamin as a physiologic antioxidant. Characteristic lesions may be readily produced by prolonged feeding of a deficient diet containing high levels of oxidizable substances such as PUFA. Cod liver oil, corn oil, and lard have been employed in the diets of experimental animals for the induction of vitamin E deficiency (Nafsted, 1974; Wesoloski et al., 1975; Michel et al., 1969).

The clinical signs of general dietary deficiencies in swine to which vitamin E deficiency is known to contribute are: slow or interrupted growth, reduced appetite, lameness or stiffness and impaired reproduction. Clinical signs which are characteristic of specific deficiencies or marginal levels of vitamin E and Se include liver necrosis, yellowish brown discoloration of body fat, sudden death in young pigs, exudative diathesis in chicks, muscular degeneration, and erythrocyte hemolysis (Sondegaard, 1966)

Currently, it seems that the most important vitamin E-Se-deficiency signs which relate to practical swine nutrition problems are muscular degeneration, liver

necrosis and vascular damage.

Species-variant muscular degeneration occurs consequential to vitamin E deficiency. Muscular dystrophy occurs in deficient rats, hamsters, rabbits, swine, and monkeys and is associated with creatinurea. Chicks reared on fat-free, vitamin E-deficient diets low in protein develop muscular degeneration of skeletal muscle expressed as white striation (Dam et al., 1952).

Grossly the skeletal muscles are pale, ischemic, moist, and often grayish and gritty due to calcium deposition. Microscopically there is widespread interstitial edema, leucocyte infiltration and segmental fragmentation of muscle fibers.

Liver necrosis in swine fed vitamin E-deficient diets has recently become a problem in the swine industry. The disease may be accompanied by skeletal muscle degeneration and pulmonary hemorrhages in growing swine (Michel et al., 1969).

Grant (1961) investigated and described in detail vascular lesions in pigs designated as a microangiopathy and characterized by hyaline thrombi, endothelial swelling, and mural necrosis in myocardial arteries. He also demonstrated that the pathological changes of the experimental disease were prevented with dietary tocopherol.

Later, vascular changes in the myocardium of vitamin E-deficient pigs were studied by light and electron microscopy (Nafsted, 1971). Most vessels in the vitamin E-deficient pigs had swollen endothelial cells with reduced electron density of mitochondrial christae. Osmiophyllic lysosomes which occurred regularly in endothelial cells of control material were usually absent. There were discontinuities of the basement membrane in the areas of the subendothelial deposits. The endothelium was focally disrupted. Aggregates of platelets in various stages of degeneration, admixed with strands of fibrin, occurred adjacent to the discontinuities. In more advanced stages of vascular alteration, the endothelial lining was missing.

Arterioles and capillaries were often completely or partially filled with aggregated platelets and the platelets were apparently adherant to the vascular wall. Some platelets had retained their internal structures, but many were degranulated. Occasionally, red blood cells and cellular debris, including empty membranous loops, occurred with aggregated platelets.

Nafsted (1971) proposed that severe vascular changes begin with endothelial damage and end with thrombosis. Besides myocardial degeneration and hemorrhages, the most prominant feature of the lesions in pigs dying from

"mulberry heart disease" under natural conditions is multiple microthrombi in the myocardial vessels. The incidence of this disease is increased among pigs fed vitamin E and Se deficient diets.

Aggregation of platelets is known to occur in association with vascular damage in various conditions such as thrombosis. The principle factors causing platelets to adhere to each other are adenosine diphosphate (ADP), thrombin and collagen. These factors can be released from red blood cells, injured cells of the vascular wall, or from the platelets themselves (Weiss, 1975). Thus, discontinuities in the endothelial lining could account for the exposure of blood platelets to subendothelial tissue and result in the further development of stable thrombi.

Among experimental pigs fed vitamin E and Se-deficient rations, and in field cases, the most frequently observed gross lesions were liver necrosis and fibrosis, ulceration, and preulcerous lesions of the squammous epithelium of the stomach (Michel et al., 1969). Icterus, generalized edema, and transudation were not especially evident in experimental pigs but were consistently observed in field cases. Gastric lesions approached an incidence of 100 percent in experimental pigs, and frequently occurred in

field cases as well. A yellow-white granular pseudomembranous material on the squamous epithelium of the stomach and esophagus was characteristic in many of the pigs.

Ulcers are frequently noticed in pigs with other naturally occurring diseases, and are an important and constant feature of dietary liver necrosis.

At Michigan State University, it was observed (Ullrey, 1973) that of pigs fed practical corn-soybean meal diets unsupplemented with vitamin E or Se, a 20 percent death loss due to this deficiency could be expected. Of all pigs necropsied from one experiment, 25 percent had vitamin E-Se deficiency lesions.

Blood Platelets

Biological role. Platelets are produced in the megakaryocytes of the bone marrow. Under normal conditions 200,000 to 400,000 platelets/mm^3 circulate in the blood for 10 days as smooth disc shaped cells that are non-adherent to each other and to the vascular endothelium. Under conditions of endothelial disruption platelets interact with elements of the vessel wall or perivascular tissue (Weiss, 1975).

In response, platelets become adherent to various vascular tissue constituents such as collagen, basement

membrane, microfibrils associated with elastin, and eventually to other platelets. Adhesion initiates a secretory process during which substances found in the subcellular granules of platelets are extruded from the cell. Similar to the activity of endocrine and nerve cells, the membranes, cytoplasm, and mitochondria appear to remain intact. Among the granular substances released is adenosine diphosphate (ADP) which itself can aggregate platelets. The sequence of adhesion (first phase aggregation) - release reaction - ADP induced platelet aggregation (second phase aggregation) accounts for the primary arrest of bleeding following endothelial disruption (Figure 1).

The lipoprotein surface of the platelet catalyzes one or more of the reactions involved in the conversion of prothrombin to thrombin in the blood clotting mechanism. Thrombin induced consolidation of platelet fibrin completes the hemostatic process, and accounts for the permanent arrest of bleeding. Thrombin, like ADP, is capable of aggregating platelets.

The role of platelets in coagulation is summarized in Figure 2.

Structure. The trilaminar plasma membrane is similar to that of other cells in being covered with an atypical

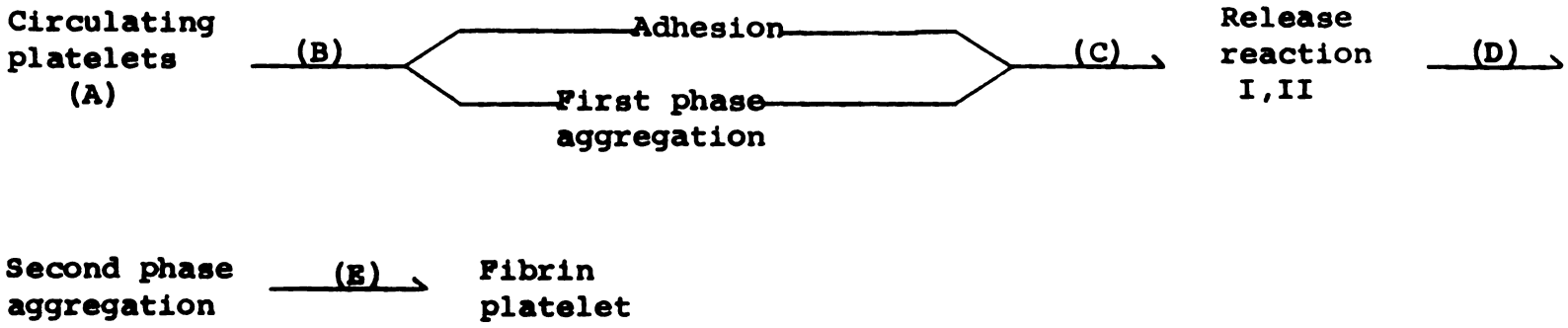


Figure 1. Role of platelets in hemostasis (Weiss, 1975).

(A). Circulating nonadherant platelets.

(B). Vessel injury causing platelet adherance to vascular tissue constituents and aggregation with other platelets.

(C). Degranulation of platelets and the extrusion of ADP.

(D). Endogenous ADP initiates second phase aggregation (primary arrest of bleeding).

(E). Fibrin-platelet formation (permanent arrest of bleeding) detailed in Figure 2.

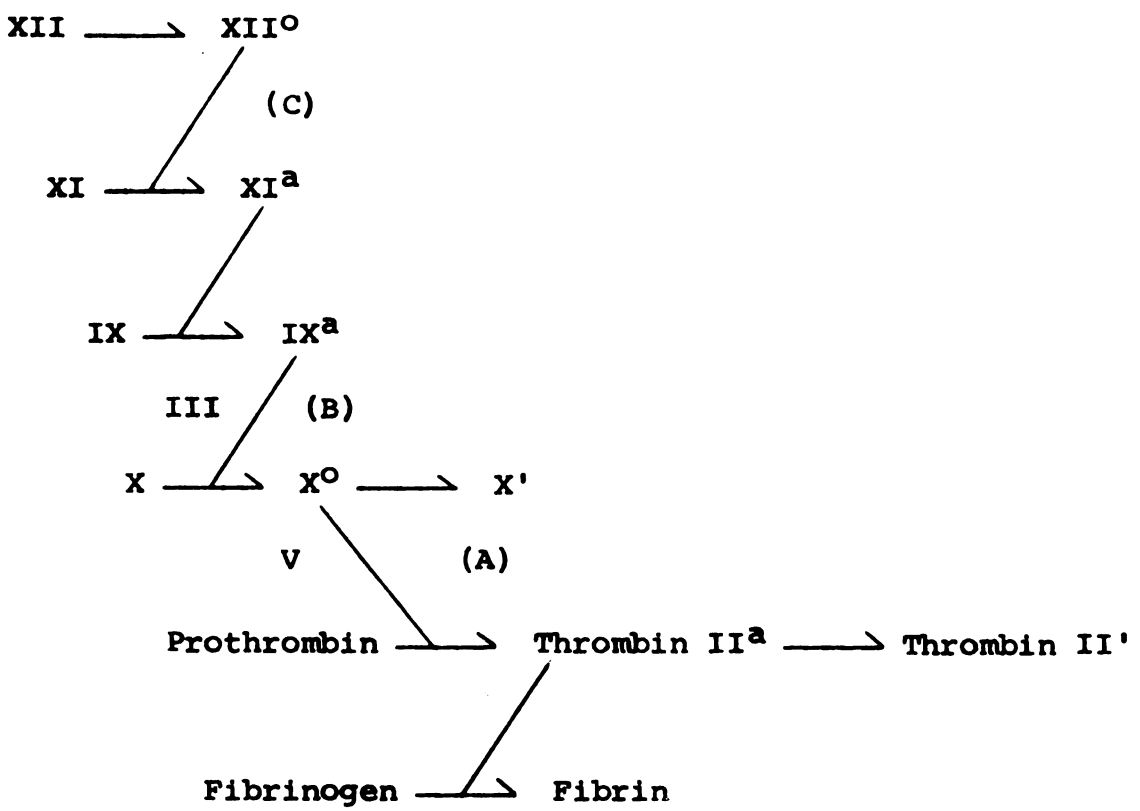


Figure 2. Role of platelets in coagulation (Weiss, 1975).

(A), (B), (C). Platelets accelerate these reactions. In addition, platelets may protect X^{o} from inactivation by plasma inhibitors.

II^{a}, II', V, X, X^{o}, X', III, IX, IX^{a}, XI, XI^{a}, XII, XII^{o}. Coagulation cofactors.

amorphous coat 10 to 20 nm thick and of unknown function (Weiss, 1975). However, some role in platelet aggregation has been inferred from the observation that a gap between aggregating platelets contains a substance similar to the amorphous coat of free circulating platelets. The plasma membrane itself differs from that of other cells in that it has many invaginations which channel through the platelet cytoplasm. This open-surface-connecting system provides a large surface area of membrane for interaction with the circulating vascular tissue constituents, and also provides an exit for granule-bound secretory substances extruded during the release reaction.

Another matrix of narrow membranous tubules known as the dense tubular system interacts with the surface connecting system. It has been suggested that this system may be derived from endoplasmic reticulum and that the relation of the dense tubular and surface connecting systems in platelets may be analagous to that of the sarcoplasmic reticulum and transverse tubular systems in muscle (White, 1972).

High electron-dense granules in human and rabbit platelets are likely storage sites for ATP, ADP, secrotonin, and calcium. Granule-bound ADP (storage ADP) as opposed to the metabolic pool of ADP is responsible for second

phase aggregation upon being extruded in the release reaction.

Platelets contain a circumferential band of hollow, cylindrical structures similar to microtubules found in many other cells. Their primary role is probably to maintain the highly assymmetric shape of the circulating platelet. An earlier study reports that microtubular protein may be related to actin suggesting that these elements provide a cytoskeletal structure that orients contractile activity within platelets (Zucker-Franklin, 1969).

Mechanism of adhesion. Platelets adhere to a variety of substances present in the vessel wall and perivascular tissue (Weiss, 1975). Collagen may be the most platelet-reactive substance in the vessel wall. Studies using a platelet aggregometer show collagen to be a potent stimulus for inducing both aggregation and the release reaction whereupon platelets become degranulated and are able to recruit other platelets in the thrombus formation. The adhesion of platelets to non-collagenous substances requires calcium, whereas collagen-induced platelet adhesion does not.

Roseman (1970) proposed a general theory of cell to cell adhesion. He suggested that cell surfaces contain

specific glycosyl transferases capable of transferring glycosyl units from nucleoside phosphates on one cell onto glycoprotein acceptors containing incomplete carbohydrate side chains on the surface of the second cell. The nucleoside phosphate-enzyme-glycoprotein complex provides adhesion. Glucose deficient galactosyl hydroxy-lysine groups in collagen could provide such a substrate for attachment of glycosyl transferase present in platelet membranes.

The biological role of ADP in mediating platelet aggregation has been discussed, though the mechanism is not known. ADP added to a suspension of platelets in an aggregometer causes a rapid isovolumic change in the shape of the platelets from discoid to spiny shperes which are then capable of aggregating in the presence of calcium and fibrinogen. This phenomenon may be potentiated by other plasma proteins, and inhibited by EDTA, prostaglandin E, (PGE), and cyclic AMP (Mustard, 1970). At concentrations of .2 to .1 uM ADP aggregation is reversible. At higher concentrations ADP induces the release reaction, and the release of endogenous ADP induces second phase aggregation which is not reversible.

The release reaction is the process whereby substances packaged and stored in the granules are specifically

extruded from the cell. In addition to ADP, platelets extrude serotonin and hydrolases. A release reaction inducer causes platelet membrane invaginations at many points forming a system of channels known as the surface connecting system. Platelet granules then cluster in the center of the cell and are encircled by a band of microtubules. Secretory products are then exocytotically extruded through the membranes of the surface connecting system and to the exterior of the cell by way of that system.

The mechanism governing the release of granule-bound substances in platelets may be different for different types of granules. The majority of granules are moderately dense, contain enzymes such as acid hydrolases and are called alpha granules. Another granule of high electron density called a dense body contains calcium, serotonin, and ADP. Release reaction induced by exogenous ADP causes release of only the dense body constituents serotonin and endogenous ADP. This selective secretory effect (Release I) may be inhibited by aspirin. In contrast, thrombin-induced release reaction causes the release of contents of both types of granules (Release II).

The prostaglandins apparently play a role in mediating Release I and consequently second phase aggregation. When

platelets are stimulated with a variety of release inducers, arachidonic acid, (probably made available by hydrolysis of membrane phospholipid) is converted to a labile cyclic endoperoxide that itself is a precursor of PGE_2 and PGF_2 (Smith et al., 1974). This "labile aggregation stimulating substance" (LASS), but not PGE_2 or PGF_2 , directly induces Release I. Aspirin inhibits the synthesis of LASS, explaining why it also inhibits second phase aggregation. LASS and other cyclic endoperoxide precursors of prostaglandins stimulate contraction of smoothe muscle, suggesting that they may contribute to contractile processes in platelets. LASS does not affect Release II.

The greatest quantity of arachidonate is present in esterified form with in tissue lipids (Furlow, 1975). In particular, arachidonate is the single most plentiful fatty acid in the phospholipid fraction of platelet membranes where it comprises nearly 20 percent of the total fatty acid content. Arachidonate binds to albumin as other free fatty acids do and normally circulates in blood as such. Albumin reduces the amount of arachidonate that is converted to prostaglandins by platelets and inhibits arachidonate-induced platelet aggregation. Silver et al. (1975) suggested that in an abnormal or pathological

situation *in vivo*, arachidonate could suddenly be released at a specific locus and momentarily exceed the capacity of albumin to bind it. Silver et al. (1975) injected sodium arachidonate into the ear veins of rabbits and found that a dose of 1.4 mg/kg proved lethal within 15 minutes of injection. Death was preceded by acute respiratory symptoms and platelet aggregates were found in the heart blood and in vessels of microcirculation of the lungs. The importance of aracidonate was further demonstrated by Kinlough-Rathbone et al. (1976) who reported evidence that sodium arachidonate causes shape change and aggregation of platelets independent of the release of ADP. The addition of sodium arachidonate to a suspension of human platelets previously induced to release 99 percent of their granule content initiated shape change and aggregation. This process was inhibited by aspirin and indomethacin (a compound known to block LASS formation).

Intravenous infusion of sodium arachidonate caused a dose dependent decrease in platelet counts and appearance of circulating platelet aggregates in the arterial blood of guinea pigs (Rafflenbeul, 1977). Platelet counts decreased gradually as long as the fatty acid was infused. A gradually developing thrombocytopenia in the microcirculation of the lungs caused death in the majority

of the guniea pigs during or shortly after infusion.

In keeping with the thoughts of Silver et al. (1975), it is possible that under abnormal conditions (such as microvasculature disruption observed under conditions of vitamin E deficiency) local release of large amounts of arachidonate could cause platelet aggregation leading to obstruction of vital organs such as lungs, brain, heart, or the stomach resulting in a disabling illness or death.

Influence of vitamin E. The precursors of prostaglandins such as LASS belong to the chemical class of peroxides. There is a measurable burst in lipid peroxidation associated with irreversible platelet aggregation primarily due to the formation of prostaglandin intermediates of endoperoxide structure. It has been suggested that alpha-tocopherol might inhibit platelet aggregation by blocking the formation of the endoperoxide LASS. Anostosi (1976) inhibited the platelet release reaction in a dose dependent manner with the addition of alpha-tocopherol to platelet rich plasma. Complete inhibition occurred at .9 to 1.5 mM alpha-tocopherol and was associated with a 50 percent reduction in lipid peroxidation. In vivo experiments indicated increasing reduction of the maximal aggregation response when alpha-tocopherol intake was raised to 1800 IU daily. At this

dosage aggregation was decreased 50 percent while increasing the dosage did not further depress aggregation.

In similar work Machlin et al. (1975) used a control group of rats fed 200 mg alpha-tocopherol per kg of diet. Platelet aggregation was depressed among animals fed vitamin E when collagen but not ADP was used as an aggregating agent.

Nafsted (1974) demonstrated a specific structural role for vitamin E in maintaining membrane integrity. Because vascular damage is a common denominator to several vitamin E deficiency lesions, Nafsted investigated the ultrastructural morphology of the endothelial lining of myocardial vessels in vitamin E-deficient pigs. The lesions of vitamin E deficiency syndrome in pigs comprise myocardial lesions accompanied with widespread thrombosis in the myocardial microcirculation and vascular necrosis in intramyocardial arteries. Edema, transudation, and hemorrhages indicative of a general permeability disturbance were observed.

Electron microscopical examination of capillaries and arterioles revealed endothelium cells with a washed-out appearance. The endothelium commonly protruded into the lumen and partly occluded it. In some cases the endothelium was destroyed and a thrombus consisting of

partly degranulated platelets and fibrin had formed.

These observations suggest that the mechanism of thrombogenesis appears to represent a response secondary to endothelial damage which leads to exposure of subendothelial structures and further induces platelet adhesion and thrombus formation.

Gastric Ulcers

Anatomy of the stomach. The general shape of the stomach is like that of a retort (Greenberger, 1976). The region where the esophagus opens into the stomach is known as the cardia (Figure 3). The cardia enters to the right of the uppermost pole of the stomach on the medial side distal to the uppermost pole of the fundus of the stomach. From the cardia, the outline of the stomach curves downward and to the right depicting the lesser curvature of the stomach merging into the pyloric region, ending at the pyloric valve. The proximal side of the stomach curves upward and left from the cardia to the uppermost pole of the stomach then down and to the right in a half circle ending again at the pyloric valve depicting the greater curvature. The greater curvature incorporates the fundus or body of the stomach. The entire surface of the stomach is covered with peritoneum and moves quite freely about the abdominal cavity.

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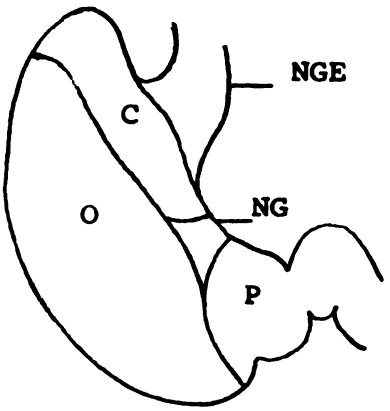

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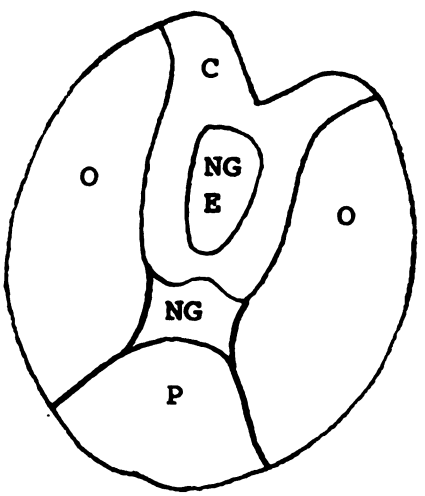

Figure 3. Gross morphology of the swine stomach.

NGE: nonglandular esophageal region
C : cardiac region
NG : nonglandular region
O : oxyntic region
P : pyloric region

Anteriorly, the human stomach rests against the anterior abdominal wall and against the inferior surface of the left lobe of the liver. Posteriorly, it's surface juxtaposes the pancreas and splenic vessels, and left kidney and adrenal gland. The upper margin of the greater curvature rests against the spleen to which it is attached by the gastrospenic ligament.

The stomach wall consists of three layers of smooth muscle, the exterior superficial longitudinal layer, the middle circular layer, and the interior oblique layer.

The gastric mucosa may be classified as either glandular or nonglandular (Ito, 1962). The glandular mucosa, present in all stomachs, is responsible for the secretion of gastric juice. Nonglandular mucosa is found only in certain species and is restricted to certain parts of the stomach.

A layer of simple columnar mucous cells lines the entire gastric mucosa. One or more simple or branched tubular glands open into the bottom of invaginations called gastric pits. The epithelium rests on a delicate basement membrane and the underlying lamina propria is composed of connective tissue cells and fibers as well as blood vessels, lymphatics, nerves and smooth muscle cells.

Three types of gastric glands are found in the

glandular mucosa. They are the oxyntic, cardiac, and pyloric glands. The oxyntic glands are the principal contributors to gastric juice and occupy most of the gastric mucosa. The oxyntic gland is characterized by the presence of oxyntic and chief cells in addition to the ubiquitous mucous and argentaffin cells.

The presence of cardiac glands near the esophageal orifice defines a cardial region which varies from a narrow rim of a few millimeters or less in the cat, dog, or human stomach to an extensive area in swine (Miyagawa, 1921). The principal function of the cardiac glands appears to be secretion of mucus.

Pyloric glands occupy a larger area than that of the cardiac gland region. Like cardiac glands the principal product of the pyloric gland is mucus, as indicated by the dominance of surface mucus cells lining the gastric pits. In addition to mucus the glands of the pyloric region secrete some gastrin.

The nonglandular gastric mucosa is a typical keratinized stratified squamous epithelium with a basal cell layer resting on the basement membrane. The nonglandular region ends abruptly at the junction with glandular cardiac and pyloric regions.

The lamina propria occupies the area beneath all

gastric epithelial cells and the muscularis mucosae. It contains collagenous and reticular fibers, some elastic fibers, connective tissue cells, blood and lymphatic vessels, and smooth muscle cells.

Gastric blood supply is derived from the celiac artery. The submucous arterial plexus sends arteries and arterioles through the muscularis mucosae to the lamina propria and the gastric mucosa. The lymphatic vessels in the gastric mucosa begin as lymphatic capillaries in the lamina propria. They are extensive but not always apparent in usual histological preparations because their walls tend to collapse. The endothelial cells of the lymphatic capillaries may have clefts or spaces between adjacent cells and have a very thin basement lamina (Majno, 1965). The venous blood from the stomach leaves by the portal vein to the liver.

Incidence. In the past decade there has been an increased awareness of the incidence of gastric ulcers in animals. These ulcers may develop under varying conditions of age, sex, diet, climate, and feeding and have been observed in cattle, dogs, foxes, cats, monkeys, rabbits, rats, mink, and swine.

The incidence of gastric ulcers in swine ranges from 13 to 23 percent and the economics of the disease

becomes significant when apparently normal pigs die suddenly (Boenker, 1967). Debilitating effects are difficult to establish since even a growing pig with an ulcer may eat and gain weight normally. The only positive method of diagnosing gastric ulcers in the pig is by necropsy.

Obrien (1969) in a review of gastric ulceration in the pig referred to this syndrome as ulceration of the pars esophagea. In accordance with his own findings, and those of others, this terminology differentiated between the nonglandular area (esophageal and pyloric) and glandular areas which are only rarely ulcerated (less than .5%).

Description. Muggenburg et al. (1966) differentiated between epithelial changes, acute erosions, subacute ulcers, and scars on the basis of gross and histopathologic characteristics. Muggenburg's (1966) histopathologic descriptions should not be confused with current etiologic terminology (Greenberger, 1976) distinguishing chronic gastric ulcers from acute (stress) ulcers. Chronic gastric ulcers must be distinguished from acute ulceration of the gastric mucosa which occurs during some form of environmental stress. Acute stress ulcers are superficial mucosal lesions which develop in either the stomach, duodenum or both within an hour to two weeks following any extreme

stress such as burns, trauma, and shock. The individual defect tends to be circular and small and characteristically it does not penetrate the muscularis mucosa but only the superficial mucosa.

The surface of the gastric mucosa of the normal swine stomach is white, smooth, and glistening.

Epithelial changes are characteristically roughened, irregular, elevated, and corrugated. Often this roughened surface can be peeled off, removing most of the epithelial layer. Surface color changes from white to yellow. Histological abnormalities often accompany epithelial changes. The epithelium becomes irregular in depth and in some places greatly thickened. True keratinization is rarely observed though nuclei persist in the epithelial cells to the pseudokeratinized surface suggesting a parakeratotic type change. Also swelling and vesiculation of the cytoplasm of the epithelial cells are frequently observed.

Acute erosions vary in size and are generally shallow with irregular edges. The base is often covered by necrotic tissue. The color may vary from dark brown, in the presence of digested blood, to red because of vascular engorgement. Swelling of surrounding tissues is often observed. The site most commonly occupied by the acute

erosion in the squamous epithelium is along its junction with the glandular epithelium of the cardiac or pyloric regions. Gradual erosion of the epithelium exposes the tips of the papillae of the tunica propria. Later, further erosion exposes entire areas of underlying tunica. The erosion is generally confined to the mucosa. The surface of the lesion is covered by necrotic tissue. Some minor hemorrhages occur near the necrotic edges of the ulcer, and the erosion is surrounded by a zone of congestion and edema. In tissue immediately underlying the ulcer there is no apparent increase in connective tissue.

Subacute ulcers occur along the border of the esophageal region of the stomach in the same areas as the acute erosions. The lesions appear oval to linear in shape, .5 to 4 cm in width. Characteristically, these ulcerations are regular in outline. Like the acute erosion the subacute ulcer is shallow extending only through the mucosa, and its base covered by a thin layer of necrotic tissue. Subacute ulcers tend to be red if active and gray white if healing. Unlike the acute erosion, the subacute ulcer is firm to the touch indicating an increase in connective tissue. Histologically, the subacute ulcer extends through the epithelium and lamina propria into the muscularis mucosa and occasionally into the submucosa.

Beneath the thin layer of necrotic material is a layer of granulation tissue and enlarged occluded veins. The inflammatory process, expressed as edema, extends through the submucosa to the muscle coats.

Ulcers are classified as chronic if they contain sufficient connective tissue to indicate that they have existed for quite some time. The chronic ulcer is round or oval and 6 to 8 cm in width. Apparently several smaller ulcers coalesce to form a single ulcer which may involve the entire esophageal region. The edge of the ulcer is distinct. The ulcer may be gray to brown in color and the crater itself covered by a layer of mucus-like material. This ulcer extends through the mucosa, submucosa, and into the muscular layer of the stomach wall. The floor of the chronic ulcer is covered by a thin layer of fibrinopurulent exudate. This layer, and often an accompanying necrotic layer, blends into a layer of granulation tissue. The connective tissue extends down between the bundles of muscle. Thrombosis and peri-arteritis can be seen in vessels beneath the crater of the ulcer. Most chronic ulcers contain areas of healing and active areas which are characteristic of acute erosions or subacute ulcers. The areas of healing have connective tissue forming in the base of the crater and regeneration

of the epeithelium at the periphery of the ulcer.

Finally, large ulcers which heal often result in the formation of scars. These scars are either stellate or linear in shape. They distort the area in which they appear. Tissue beneath the mucosa is replaced by connective tissue. The epithelium of the stomach regenerates to cover the defect.

The frequency with which each type of lesions occurred was recorded by Muggenburg et al. (1966). Epithelial changes of the esophageal region was concommitant with nearly all erosions, ulcers, and scars. Acute erosions were described in 62% of affected stomachs. Subacute ulcers were described in 27%, chronic ulcers in 6%, and scars in 6%.

This description of each group suggests a possible path by which gastric ulcers may develop morphologically in the esophageal region in swine.

Etiology. Ulcers have been observed in the pig under a number of circumstances and consequently a number of causes have been suggested.

Mahan et al. (1966) studied the effects of various physical properties of feed on the incidence of ulceration. Rations ground to a fine particle size (.16 cm) were associated with an increased incidence of ulcers, as were

rations containing expanded maize.

Workers at the University of Wisconsin (Reimann et al., 1968) also suggested that particle size of corn affected the development of gastric lesions. Rations composed of coarse cracked, medium (.95 cm), and fine (.15 cm) corn provided high, intermediate, and low protection, respectively.

Gastric hyperacidity may play an important role in gastric ulceration (Kowalczyk, 1963; Huber, 1965). Excessive secretion of gastric HCl and an absence of buffering material may be related to the incidence of ulcers.

The theory of pathogenesis of human peptic ulcers "no acid, no ulcer" was investigated using experimentally induced ulcers in swine prepared with gastric fistulas (Reimann et al., 1968; Muggenburg et al., 1966; Muggenburg et al., 1967a). Both histamine and reserpine are known to produce gastric ulcers in swine similar to those occurring naturally. Each substance alone increased free and total acid secretion, and given together further increased secretion. The pH of gastric secretions decreased while pepsin activity remained unchanged. A subnormal pH of the gastric contents of swine fed finely ground corn rations as well as subnormal pH in the esophageal region was

detected.

Robert (1973) in work with rats and dogs has prevented formation of duodenal ulcers by administration of PGE_2. Duodenal ulcers can consistently be induced by continuous 24 hour subcutaneous infusion of gastric secretegogues histamine, carbachol and pentagastrin. PGE_2 infused into animals simultaneously treated with duodenoulcerogenic doses of secretegogues prevented ulcer formation. It remains unclear whether that property of the PG which is implicated in the general antiulcer effect is antisecretory or cytoprotective.

Several authors (Kowalczyk, 1963; Curtin et al., 1963) suggest an analogy from cases of human ulceration, that pigs develop ulceration as a result of intensive management methods which may induce fear, pain, fatigue, and other stress factors. Kowalczyk (1963) reported that stomach lesions were more prevalent among pigs raised in confinement than in those raised on pasture. The observations of Curtin et al. (1963) refute this, and attribute a greater incidence of ulcers to rapid growth and highly variant ambient temperatures. Anxiety, tension, crowding and starvation all have been observed to increase both incidence and severity of ulcers (Muggenburg et al., 1966).

Hove (1950) suggested that when pigs are fed unsaturated fatty acids, as in excess cod liver oil supplementation the unsaturated fatty acids exert a detrimental effect in the absence of vitamin E, on the cutaneous mucous membrane by the formation of peroxides.

In separate studies of hepatosis dietetica (Obel, 1953) and microangiopathy (Grant, 1961) an elevated incidence of ulcerous lesions in pigs was encountered. Also 40 percent of the pigs submitted to the Michigan State University Diagnostic Laboratory, in a study of vitamin E-Se-deficient swine, exhibited gastric ulcers (Trapp et al., 1970).

In Norway, Nafsted (1967) reported a high incidence of ulcers in swine given casein, sugar, and potato starch, especially after large amounts of PUFA were added to the ration. Replacement of unsaturated with saturated fats resulted in the reduction of the severity of lesions. Adding vitamin E had no significant effect.

In Australia (Kinnaird, 1964), workers attributed the appearance of gastric ulcers in swine to vitamin E deficiency associated with feeding whey, pelleted rations, or the two together. Uninterrupted large doses of vitamin E given for six months prevented the disease.

OBJECTIVES

The general objective of this experiment was to establish a consistent model through which the occurrence of gastric ulceration, a common disease to both swine and man, might be investigated.

Specifically, this experiment was intended to examine the effects of vitamin E and Se supplementation on the incidence of gastric ulcers and platelet aggregation in swine.

In addition, this investigation may provide the basis for a long term in depth investigation on cardio-vascular disturbances.

METHODS AND MATERIALS

Experimental Design Trial I

Pigs (31) were randomly assigned from 6 litters to four dietary groups. Animals used in this experiment were born from sows which had been fed a low vitamin E-Se diet throughout gestation and lactation. These pigs were weaned to a low vitamin E-Se diet at 3 weeks of age.

The four experimental diets consisted of a control ration (Table 1) reported to be ulcerogenic in pigs (Wesoloski et al., 1974) and three test rations consisting of this basal ulcerogenic diet (BUD) supplemented with vitamin E (E) and/or selenium (Se). Namely: BUD, BUD + 22 IU/kg of E, BUD + .1 ppm Se, and BUD + 22 IU/kg of E + .1 ppm Se.

The pigs in all groups were maintained in confinement on slotted concrete floors without the imposition of any determinable environmental stress.

Periodically, blood was obtained from the anterior vena cava for serum E and Se analysis and for platelet aggregation determinations.

Table 1. Composition of Basal Ulcerogenic Diet. Trial I

Ingredient	Percentage
Corn starch	70.70
Soybean meal (.49 protein)	20.00
Corn oil	3.00
Cellulose	3.00
Defluorinated phosphate	2.00
Vitamin-Trace mineral supplement *	.50
Salt	.25
ASP-250 (antibiotic)	.25
Lysine (.78)	.20
D,L-Methionine (.98)	.10
	100.00

* Composition of the Vitamin-Trace mineral supplement.

Nutrient	Amount in 10 kg
Vitamin A as retinyl palmitate	6.6×10^6 IU
Vitamin D_3	1.3×10^6 IU
Choline as choline chloride	220.0 g
Niacin as nicotinic acid	35.2 g
D-Pantothenic acid as calcium pantothenate	26.4 g
Riboflavin	6.6 g
Pyridoxane	6.6 g
Thiamin	6.6 g
Vitamin K as menadione sodium bisulfate	4.4 g
Vitamin B_{12}	39.6 mg
Zinc as zinc oxide	149.6 g
Iron as ferrous sulfate	118.8 g
Manganese as manganous oxide	74.8 g
Copper as copper sulfate	19.8 g
Iodine as potassium iodate	5.5 g
Antioxidant as BHT	99.0 g
Corn starch carrier	the balance of 10 kg
	10.0 kg

All pigs were slaughtered at an average weight of 105 kg and the stomachs were inspected for the presence of ulcers.

Experimental Design Trial II

The experimental design of trial II was essentially the same as that of trial I. The four experimental diets fed to 29 pigs consisted of a control ration which was a modified version of the BUD employed in trial I (Table 1a). The substitution of finely ground corn (ground through a 1/4 inch mesh) for corn starch represents the principal modification. It was thought that the presence of ground corn adequately supplanted corn oil, corn starch, and cellulose and that this substitution would not affect the ulcerogenic quality of the ration. The motives for tampering with a successful dietary model were twofold. Elevated cost and inaccessability discourage the use of the corn starch-corn oil-cellulose ingredients, and the use of a ground corn ration to produce gastric ulcers in swine would emphasize the practical nature of this production problem.

Characterization of Ulcers Trial I, II

The stomach was cut open midway along the greater curvature, everted, and rinsed lightly with water. The entire mucosal surface was examined and the type of lesion

Table 1a. Composition of Basal Ulcerogenic Diet. Trial II

Ingredients	Percentage
Corn (fine grnd.)	76.70
Soybean meal (.49 protein)	20.00
Defluorinated phosphate	2.00
Vitamin-Trace mineral supplement *	.50
Salt	.25
Aureomycin (antibiotic)	.25
Lysine (.78)	.20
D,L-Methionine (.98)	.10
	100.00

* This Vitamin-Trace mineral supplement is the same as that used in Trial I, and is described in Table 1.

was recorded. The classification of gastric ulcers into five groups was based on the following gross characteristics (Muggenburg, 1966):

epithelial changes:	rough, corrugated, irregular shape, yellow
acute erosion:	rough, irregular, surrounding tissue swollen, red, shallow
subacute ulcer:	rough, regular-oval to linear shape, red, shallow-extending through the mucosa, mild keratinization surrounding ulcer
chronic ulcer:	rough, regular-round to oval shape, red or brown, deep-extending to the muscle layer, extensive keratinization
scar:	stellate to linear shape, distorted, tough

Serum Selenium Determination (Whetter: modified from Olson et al., 1975). Trial I, II

Preparation. All glassware was rinsed with concentrated HNO_3 then three times with deionized water. Only Saran Wrap is a suitable cover for flasks, and the work surface was always covered with paper. In duplicate, 1 ml of serum was placed into 50 ml erlenmeyer flasks, and a pyrex glass bead added to each. To each flask was added 3 ml of concentrated HNO_3 and 3 ml $HClO_3$. As a reference, duplicate blanks and .10, .20, and .30 ug/ml standards

were prepared, half at the beginning of the digestion and half at the end.

Digestion. Under ventilation the flasks were placed on a hot plate preheated to 320° C. The first flasks to show dense white fumes of $HClO_3$ were removed from the heat and the temperature lowered to 260° C. All other flasks were then removed subsequent to fuming. As the last flasks were removed all flasks were returned (first removed, first returned) to the hot plate in order to synchronize the digestion process among samples. All samples were then digested for 20 additional minutes and monitored in order to avoid vigorous boiling but to encourage active refluxing. The samples were colorless and the dense white fumes prominent at the end of the digestion period. The flasks were removed from the heat and when the fumes subsided 3 ml of 1 : 9 HCl v/v was added to each one. All samples were then heated at 50° C for an additional 15 minutes.

Neutralization, Chelation, Complexion. To each flask was added 10 ml ethylene diamino tetrahydrogen acetic acid (EDTA) and swirled, 1 ml concentrated NH_4OH and swirled, and one drop of cresol red solution (.01 g in 1 ml H_2O - one drop of 1 : 1 NH_4OH) v/v. Then concentrated NH_4OH was added, dropwise, to a color of orange-pink. overneutralization was remedied by dropwise addition of 1 : 9 HCl v/v

back to orange-pink. At this point the room lights were turned off for the remainder of the analysis.

To each flask was added 5 ml 2, 3- diamino napthalene (DAN) (1 mg/ml in .1N HCl). All flasks were incubated at 50° C for 10 minutes then the hot plate turned off and without removing the flasks, allowed to warm for 20 additional minutes before cooling.

Extraction, Clearing, Reading. To each flask was added 6 ml cyclohexane. All flasks were then placed in a shaker for 5 minutes. Upon removal of the flasks from the shaker, H_2O was forcibly added (with a squirt bottle) until the level of the solution reached the neck of the flask. Blanks, standards, and then sample supernatants were read on a previously adjusted spectrofluorometer.

Calculation. Fluorescence was linear and the curve passed through Ox, y. A linear regression analysis was performed and the slope multiplied by the percent emission.

Serum Vitamin E Determination (Whetter modified from Taylor et al., 1976). Trial I, II

Preparation. All glassware was rinsed with dilute HNO_3 (deionized water: HNO_3 5 : 1) three times with deionized water, and then twice with absolute ethanol (EtOH). As with Se determinations the work surface was always covered, and in addition an isolated well ventilated

area was necessary to avoid contamination. Dilutions of the 1 mg/ml alpha-tocopherol standard were prepared by adding .20 ml of the standard solution to 19.80 ml EtOH, a final concentration of 10 ug/ml. Working standard dilutions were developed by adding 1, 2, and 3 ml of the 10 ug/ml standard to 9, 8, and 7 ml absolute EtOH respectively, to final concentrations of 1, 2, and 3 ug/ml. As a reference, duplicate blanks of EtOH and water and duplicate standards of 1, 2, and 3 ug alpha-tocopherol/ml were prepared for each run. Duplicate test samples were developed by adding 1 ml of serum to 2 ml EtOH in 15 x 150 mm culture tubes which were then swirled for 3 seconds on a Vortex Genie Mixer at high speed.

Extraction. To all tubes, 2.5 ml cyclohexane was added and each tube was swirled as above for 10 seconds. All tubes were then centrifuged at 4° C for 10 minutes at 1.25 x G. With a Pasteur pipet as much of the cyclohexane was pulled off the top of the serum as possible and transferred to prenumbered 25 ml Erlenmeyer flasks. The extraction procedure was repeated once. After repeating the extraction process the samples and developed standards were evaporated to dryness at 37° C and 40 mm Hg.

Reading. Using a Selecto-pipet the dried sample was collected in 1 ml absolute EtOH. Into all flasks,

including non developed standards, was added .25 ml of .1% bathophenanthroline. After two minutes .10 ml of .55% $FeCl_3$ was added to each flask. (Bathophenanthroline reacts with tocopherol-reduced - $FeCl_3$ to form a pink color). After two minutes the reaction was stopped by the addition of .25 ml of 1% H_3PO_4. Relative tocopherol concentrations (not specific to alpha-tocopherol) were determined spectrophotometrically at 536 mu on a Gilford spectrophotometer.

Platelet Aggregation (Brett, 1976). Trial I, II

Only plasticware was used in this assay. Blood was collected and transferred to a centrifugation tube containing sodium citrate (3.8%), adjusted to pH 7.4 with citric acid, as an anticoagulant in the ratio of 9 : 1 v/v. This solution was centrifuged for 12 minutes x 1000 rpm in a 10" diameter rotor at room temperature and the platelet rich plasma (PRP) supernatant drawn and transferred. The remaining solution was centrifuged for 15 minutes at 3000 rpm (10" rotor) at room temperature and the platelet poor plasma (PPP) supernatant drawn and transferred. The PRP was allowed to stand at room temperature for 45 minutes.

Dilutions of a stock solution of ADP (2.7×10^{-3} M) with physiologic saline 1 : 9, 1 : 99, 1 : 999 v/v were

used as aggregating agents. Platelet aggregation was measured with a Chronolog aggregometer adjusted to 100 percent transmission with PPP and to 0 percent transmission with PRP. At 37°, 5 ml PRP was placed in a cuvette with a stirring bar. The aggregating agent (.02 ml) was introduced into the suspension and the degree of aggregation measured graphically by the aggregometer. An increase in light transmission through the stirred suspension indicated platelet aggregation. The small decrease in transmission upon addition of the aggregating agent reflects turbidity. Initial oscillations in light transmission reflect the fact that freely suspended platelets are disc shaped cells. Diminishing oscillations indicate a shape change (rounding) induced by the agent.

The relative occurence of aggregation among samples was indicated by the lowest concentration of ADP causing permanent aggregation, and the degree of disaggregation expressed as a percentage of the observed aggregation for common concentrations of ADP.

Tissue Selenium Determination (Whetter modified from Olson et al., 1975). Trial II

While still frozen, four .5 g samples of gastric mucosa were taken from the areas indicated in Figure 3 and hemogenized. The samples were predigested in 15 ml

HNO_3 overnight. After shaking, duplicate subsamples containing .5 to 1.0 g of wet tissue were used for Se analysis. From this point on (Digestion, Extraction, Clearing, Reading, and Calculating) the procedure for tissue Se determination is the same as that described under Serum Selenium Determination above.

Tissue Alpha Tocopherol Determination (Whetter modified from Bieri, 1969; Bushnell, 1967). Trial II

Preparation. The procedure for preparing glassware and standard dilutions was the same as that described earlier in this section for serum vitamin E determination. As a reference, duplicate blanks of 2 ml EtOH and duplicate standards of 1, 2 and 3 ug alpha-tocopherol/ml + 1 ml EtOH were added to .8 g ascorbate. Duplicate test samples were developed by adding .4 g of homogenized samples to .8 g ascorbate and 2 ml EtOH in 50 ml flasks. Subsamples of gastric mucosa were taken from each of the areas designated in Figure 3, that is, from each stomach, subsamples of cardial, pyloric, oxyntic, and nonglandular tissue were analyzed.

Saponification. To each flask was added 1 ml of 60% potassium hydroxide and the flask was then evacuated with N_2. For 20 minutes each flask was placed in a hot water shaker bath preheated to 60^{o} C. After 20 minutes all

flasks were immediately chilled in ice and to each was added 1 ml of deionized water and 1 ml of 6N HCl while swirling.

Extraction. Diethyl ether was added to a volume of 20 ml and the solution was poured into a 75 ml separatory funnel and shaken vigorously. The bottom layer was drained back into the saponification flask to which had been added 10 ml of diethyl ether. For each flask this solution was again poured into its respective separatory funnel. This time the bottom layer was discarded. The remaining ether phase was forcibly rinsed with 5 ml of deionized water and the resultant bottom layer discarded. Finally the ether extract was collected into clean 50 ml flasks and evaporated to dryness at 37^{o} C and 40 mm Hg.

Chromatographic Separation. Using 12 mm "Econo columns", 20 percent hydrated florisil was allowed to settle through 20 ml of Skellysolve. These columns were prepared during the evaporation period mentioned above for each analysis. The Skellysolve was allowed to drain to about 1 cm above the florisil packing. Dried samples were taken up into 2 ml of Skellysolve and loaded on to the column. Each flask was rinsed three times with 1 ml of Skellysolve and the rinse was added to the column. The tocopherol solution was lowered on to the column packing,

again draining the solvent to within 1 cm of the packing. Alpha-tocopherol was eluted with 35 ml of a 199 : 1 Skellysolve: diethyl ether eluant. The eluant was collected in clean 75 ml flasks and evaporated to dryness at 37° C and 40 mm Hg.

Reading. The further development and analysis of samples, blanks, standards and non treated blanks and standards was identical to that described earlier in this section for serum tocopherol analysis.

RESULTS AND DISCUSSION : TRIAL I

Performance (Trial I)

The average daily gain, from 6 to 22 weeks of age, of pigs in the groups fed BUD, BUD + 22 IU/kg of E, BUD + .1 ppm Se, and BUD + 22 IU/kg of E + .1 ppm Se were 591 g, and 661 g, respectively (Table 2). The E-supplemented group gained significantly ($P < .01$) faster than all other groups. A graph (Figure 4) plotting average group weights against age depicts an early separation of the E-supplemented group from the others.

Ulcers (Trial I)

Among 8 pigs fed the BUD, 6 had ulcers of varied severity, 1 had an epithelial change, and 1 was normal. Of 7 E-supplemented pigs 1 had a chronic ulcer, 2 had erosions, and 4 were normal. Among 7 Se-supplemented and 7 E - Se-supplemented pigs all had ulcers of varying

Table 2. Summary of results. Trial I

	Diet			
	BUD	+E BUD	+Se BUD	+E+Se BUD
Mean serum Se (ug/ml)	.025	.035	.090	.105
Mean serum E (ug/ml)	1.432	2.853	1.560	3.398
Ulcers (no. of pigs)	6	1	7	7
Preulcerous lesions (no. of pigs)	1	2	0	0
Normal (no. of pigs)	1	4	0	0
Average daily gain (g)	591	782	630	661

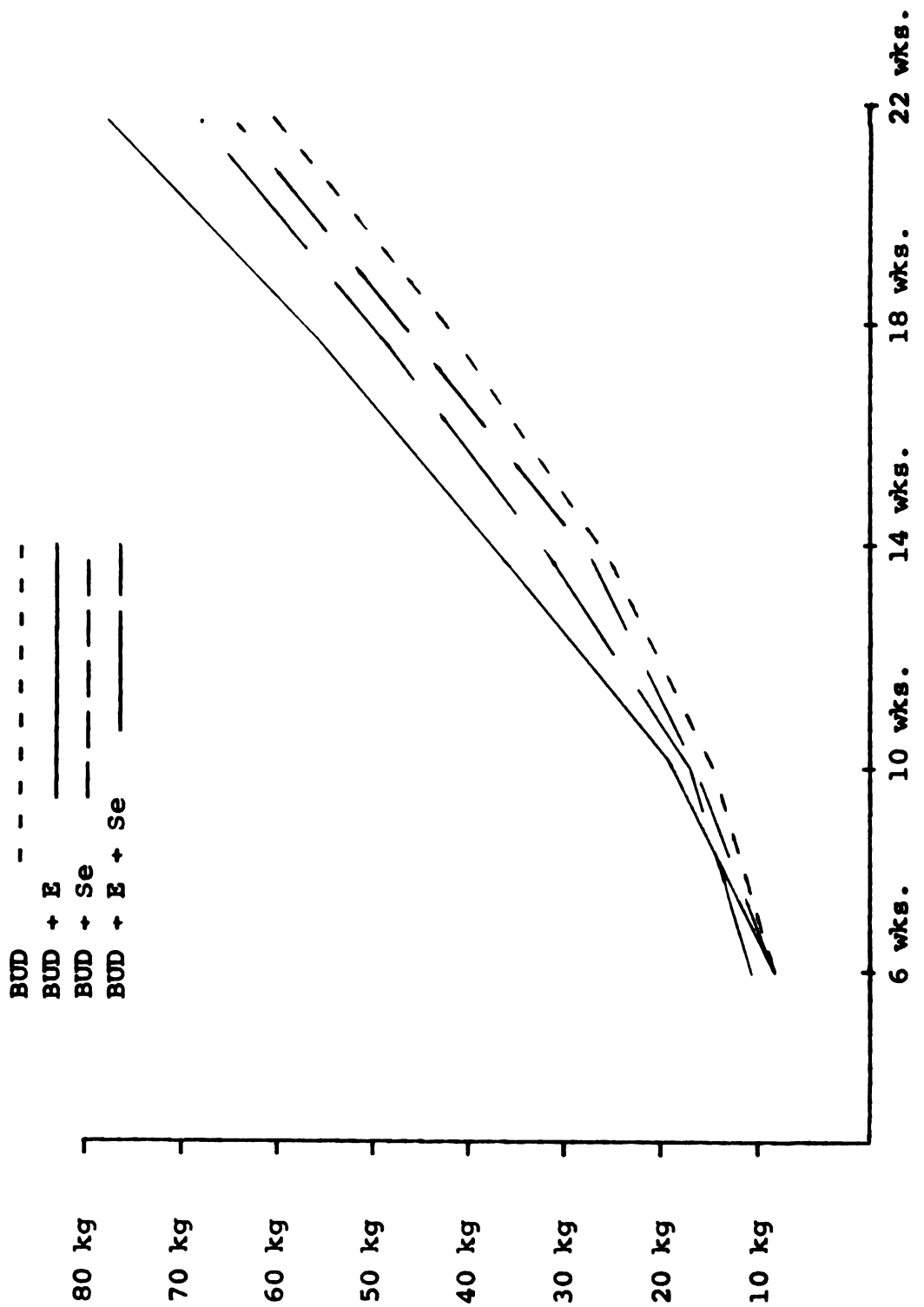

Figure 4. Average group weights from 6 to 22 weeks of age. Trial I

severity. All ulcers were precisely located in the non-glandular esophageal region along the lesser curvature nearest the cardia. A brief characterization of each stomach examined was recorded (Table 3), and summarized. (Table 2)

It should be noted that the two animals categorized as E supplemented pigs which both exhibited acute erosions 1.25 cm in diameter were in fact fed the BUD (unsupplemented) for 3 weeks before being slaughtered and examined. This deviation occurred as a result of an oversight and was not intentional.

Observing a significantly higher average daily gain among pigs in the group exhibiting a relative absence of gastric ulcers may suggest a performance, and or economic, differential.

In itself the appearance of acute erosions in the two animals unwittingly deprived of E supplementation for 3 weeks prior to slaughter is not significant. However, in light of the early separation of group weights (Figure 4), and assuming the separation reflects the presence of ulceration, it may be that the ulcerative process in this case occurs within a matter of weeks (perhaps 3 to 6). If this were the case, the absence of ulceration in the E-supplemented group would have been nearly absolute under

Table 3. Gross characterization of stomachs. Trial I

Group-Pig Number		Characterization of Stomach
BUD	133-01	normal
	130-03	epithelial change
	135-02	.75 cm subacute ulcer
	130-10	2 cm subacute ulcer
	133-12	2 cm subacute ulcer
	127-01	2.5 cm subacute ulcer
	135-10	1.25 cm chronic ulcer
	126-02	.75 cm x 5 cm chronic ulcer
BUD + E	130-13	normal
	135-01	normal
	133-10	normal
	127-12	normal
	130-05	1.25 cm acute erosion
	135-04	1.25 cm acute erosion
	130-01	1.25 cm x 3.75 cm chronic ulcer
BUD + Se	123-11	.75 cm subacute ulcer
	126-01	2.5 cm chronic ulcer
	130-13	2.5 cm chronic ulcer
	133-14	2.5 cm chronic ulcer
	127-13	3.75 cm chronic ulcer
	135-03	1.25 cm x 3.75 cm chronic ulcer
	133-13	.75 cm x 5 cm chronic ulcer
BUD + E + Se	135-11	scar
	126-10	2.5 cm chronic ulcer
	133-02	2.5 cm chronic ulcer
	133-04	2.5 cm chronic ulcer
	127-02	1.25 cm x 2.5 cm chronic ulcer
	130-04	2 cm x 2.5 cm chronic ulcer
	130-02	2 cm x 2.5 cm chronic ulcer

designed conditions.

Selenium, Serum (Trial I)

Serum selenium levels reflected dietary supplementation. The mean value (± S.E.) of pigs fed the BUD supplemented with .1 ppm Se (BUD + .1 ppm Se and BUD + 22 IU/kg of E + .1 ppm Se) was .098 ug/ml 1 .005. The mean serum selenium value of pigs fed the BUD unsupplemented with Se was .030 ug/ml ± .001 (Table 2). Serum Se levels were elevated among pigs fed diets supplemented with E suggesting a sparing effect of tissue Se by tocopherol previously described by Groce et al. (1973).

Vitamin E, Serum (Trial I)

Serum alpha-tocopherol levels generally reflected dietary supplementation (Table 2). Mean levels (± Se) pigs fed diets without tocopherol supplementation (BUD and BUD + Se) were 1.432 ug/ml ± .232 and 1.560 ug/ml .164, respectively. Mean levels among pigs fed the tocopherol supplemented diets (BUD + E and BUD + E + Se) were 2.853 ug/ml ± .346 and 3.398 ug/ml ± .752, respectively. Serum alpha-tocopherol levels were elevated among pigs fed diets supplemented with Se suggesting a sparing effect of tissue tocopherol Se (Groce et al., 1973).

Platelet Aggregation (Trial I)

Platelet aggregation determinations were inconsistent

and no inferences could be made from the data (Table 3[a], Appendix). It is believed that upon piercing the wall of the vena cava to collect blood into a syringe, the release of cell wall constituents such as collagen, fibrin, and ADP affected platelet behavior and denied reliable measurements.

RESULTS AND DISCUSSION : TRIAL II

Performance (Trial II)

The average daily gain, from 6 to 22 weeks of age, of pigs in the groups fed BUD, BUD + E, BUD + Se, and BUD + E + Se were 607 g, 594 g, 570 g, and 618 g respectively (Table 4). There were no significant differences among these values. A graph plotting average group weights against age is represented in Figure 5.

Ulcers (Trial II)

Among 8 pigs fed the BUD, 1 had a subacute ulcer, 2 had acute erosions, 3 exhibited epithelial changes and 2 were normal. One pig among the 7 Se supplemented pigs had an acute erosion while the other 6 were normal. All of the stomachs collected from the 7 pigs receiving the E-supplemented diet and of those 7 pigs receiving the E + Se-supplemented diet were normal. These results are detailed in Table 5 and summarized in Table 4.

The ulcerogenicity of the finely ground corn-soy diet was reduced in comparison to the corn starch diet used in the first trial. Yet, even this second trial demonstrates

Table 4. Summary of results. Trial II

	Diet			
	BUD	+E BUD	+Se BUD	+E+Se BUD
Mean serum Se (ug/ml)	.037	.042	.104	.110
Mean serum E (ug/ml)	.523	2.648	.869	3.069
Mean tissue Se (ppm)				
cardiac region	.081	.078	.129	.143
esophageal region	.069	.078	.135	.154
pyloric region	.089	.097	.124	.159
oxyntic region	.084	.084	.122	.153
Mean tissue E (ppm)				
cardiac region	1.682	2.727	1.762	3.296
esophageal region	1.167	2.393	1.820	3.569
pyloric region	1.299	1.804	1.854	2.174
oxyntic region	1.123	2.175	1.377	2.161
Ulcers (no. of pigs)	1	0	0	0
Preulcerous lesions (no. of pigs)	5	0	1	0
Normal (no. of pigs)	2	7	6	7
Average daily gain (g)	607	594	570	618

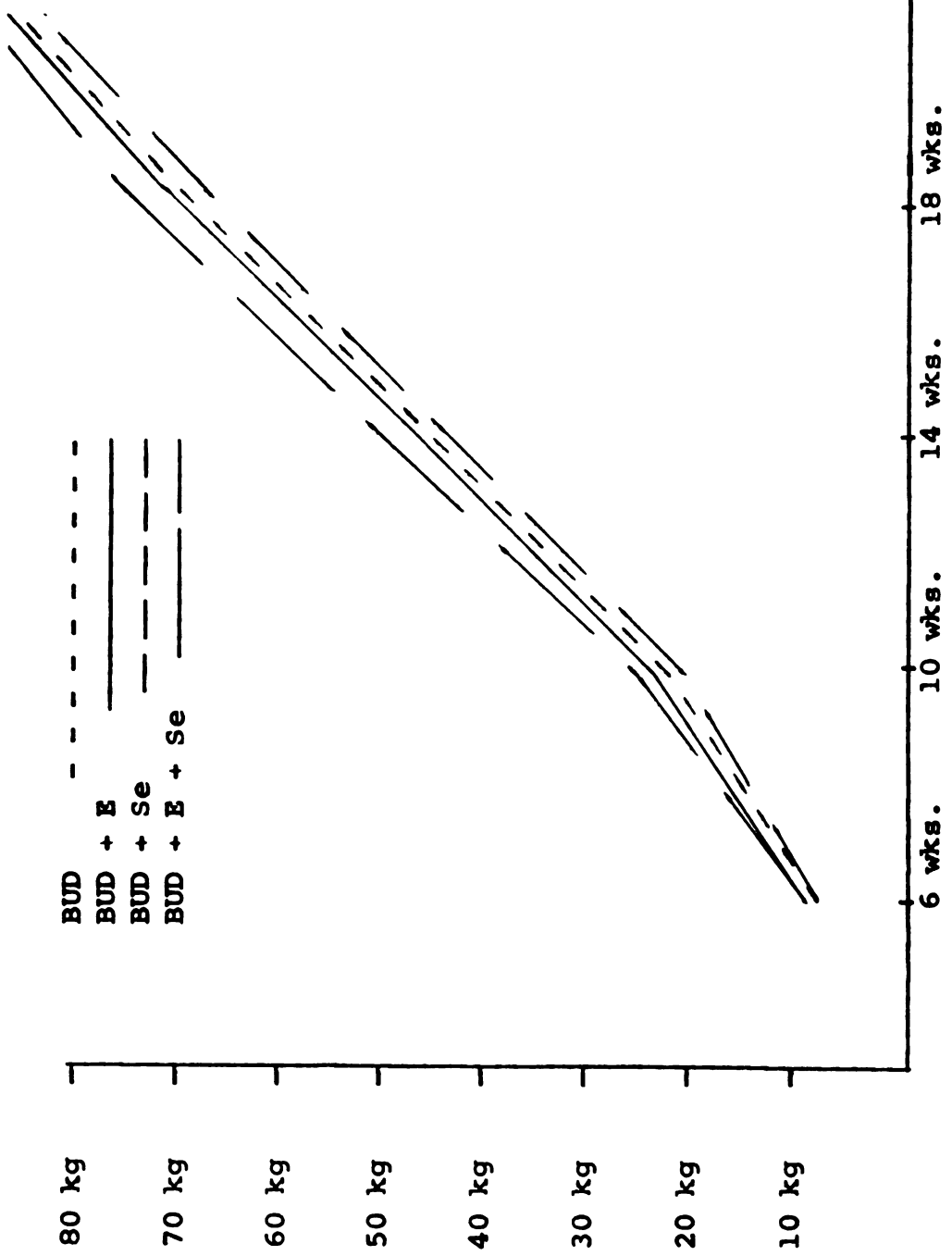

Figure 5. Average group weights from 6 to 22 weeks of age. Trial II

Table 5. Gross characterization of stomachs. Trial II

Group-Pig Number		Characterization of stomach
BUD	101-10	2.5 cm x 5 cm acute erosion
	106-01	2.5 cm x 5 cm acute erosion
	108-12	normal
	108-01	epithelial change
	115-01	2.5 cm x 5 cm subacute ulcer
	106-14	epithelial change
	115-10	epithelial change
	101-02	normal
BUD + E	106-10	normal
	108-10	normal
	106-02	normal
	101-16	normal
	108-03	normal
	101-03	normal
	101-06	normal
BUD + Se	106-13	normal
	108-11	normal
	101-13	normal
	108-02	normal
	101-04	normal
	106-04	normal
	115-12	2.5 cm acute erosion
BUD + E + Se	101-14	normal
	101-05	normal
	108-13	normal
	106-11	normal
	106-12	normal
	108-04	normal
	115-13	normal

the practical nature of this problem, particulary among pigs fed finely ground rations. While the severity of lesions decreased, the incidence of lesions among pigs fed the basal negative control diet remained the same as in the first trial. This observation, in addition to the nearly complete absence of lesions among pigs fed diets supplemented with E and or Se suggests a possible protective effect of these nutrients against ulcerogenesis. The effect of E and Se on ulcerogenesis exclusive of an exotic stress diet probably more accurately represents field conditions.

Selenium, Serum (Trial II)

As in trial I the general trends in serum Se levels reflected dietary supplementation of the mineral (Table 4). However, unlike in the previous trial, elevation of serum Se levels among pigs fed tocopherol-supplemented diets was significantly greater. The serum Se levels of pigs fed the BUD and BUD + E were .037 ug/ml ± .003 and .042 ug/ml ± .003 respectively, the latter being significantly greater ($P < .025$). The serum Se levels among pigs fed the BUD + Se and BUD + E + Se were .104 ug/ml ± .003 and .110 ug/ml ± .006 respectively, the latter being significantly greater ($P < .025$). As in trial I a sparing effect of tocopherol on tissue Se was suggested.

Vitamin E, Serum (Trial II)

As in trial I, and in keeping with trends observed in serum Se determinations, serum alpha-tocopherol levels were elevated in the presence of dietary Se; all else equal (Table 4). Values among pigs fed the BUD and BUD + Se were .523 ug/ml ± .120 and .869 ug/ml ± .158 respectively. The difference between these values was of little statistically significance ($P < .25$). The same degree of significance ($P = .25$) characterized the difference between serum alpha-tocopherol levels among pigs fed the BUD + E and BUD + E + Se. The average values for those groups were 2.648 ug/ml ± .205 and 3.069 ug/ml ± .293, respectively.

Selenium, Tissue (Trial II)

The Se content of subsamples of gastric mucosal tissue reflected dietary supplementation although seemingly with less sensitivity than did serum levels. Mucosal tissue of pigs fed E-Se-supplemented diets contained roughly twice as much Se as did pigs fed the negative control diet. In contrast, a similar comparison involving serum levels showed a three to four fold difference.

There were no significant differences between subsamples within any dietary group. The results of the tissue Se determinations are summarized in Table 4.

Vitamin E, Tissue (Trial II)

The tocopherol content of gastric mucosal subsamples reflected dietary supplementation of the vitamin, and are summarized in Table 4. Subsamples collected from cardial and nonglandular esophageal regions of the gastric mucosa exhibited elevated levels of marginal significance ($P < .25$) over samples from pyloric and oxyntic regions among pigs in the BUD + E + Se group. Similar trends appeared in other groups without statistical significance.

Hypothetical Ulcerogenic Mechanism

Formulation of the hypothetical ulcerogenic mechanism (Figure 6) is based on the observations of four independent authors and numerous related works of others as well as results of this experiment. With respect to chronological order, the principal observations, all of which are cited in the Literature Review section of this paper, are arranged in a logical etiologic sequence, the first four observations being linked by proposed mechanisms which are consistent with known biological events.

In this scheme, vitamin E deficiency is implicated, through its role as a biological antioxidant, in the ulcerogenic process both indirectly by two mechanisms and directly by a single different mechanism. Microvascular

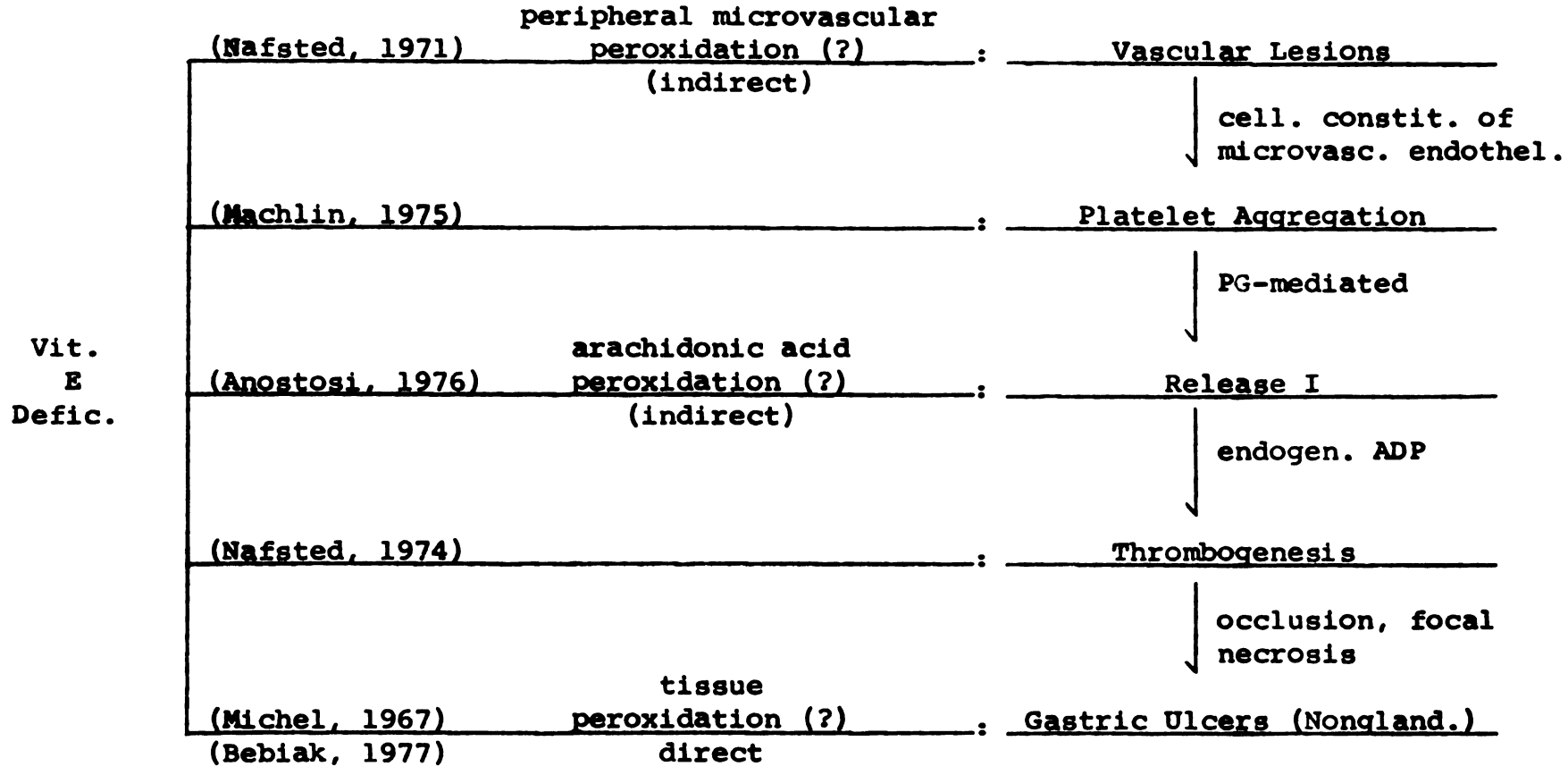

Figure 6. Hypothetical ulcerogenic mechanism.

endothelial peroxidative disruption of vessels perfusing the nonglandular region of the gastric mucosa represents the principal mechanism by which an absence of vitamin E could be indirectly predisposed to ulcerogenesis. Cellular constituents released by such a disruption would induce first phase platelet aggregation at the damaged site and initiate the thrombogenic process. The formation of the endoperoxide LASS by peroxidation of platelet arachidonic acid would accelerate the normal thrombogenic process. This represents the secondary mechanism by which an absence of vitamin E may indirectly contribute to ulcerogenesis. LASS is known to mediate Release I in which platelet extrusion of endogenous ADP occurs. The potential ulcerogenicity of these mechanisms is realized through the hypothesis that vascular occlusion occurs subsequent to thrombogenesis and that focal necrosis occurs in that tissue which was dependent upon the occluded vessles.

Peroxidative degradation of nonglandular gastric tissue could initiate, directly, the ulcerogenic process by exposing tissue underlying the nonglandular endothelium which is sensitive to gastric acidity. This idea is consistent with the "no acid, no ulcer" theory and with the suddenness of the ulcerative process. In addition, the predictability of location of the ulcers observed in

this experiment lends circumstantial evidence to this proposed direct mechanism of ulcerogenesis. This mechanism implies the existence of an inherent "weakness" to peroxidative damage peculiar to the nonglandular region of the stomach, in particular, that portion of the non-glandular esophageal region along the lesser curvature.

SUMMARY

Two experiments involving 60 pigs were conducted to evaluate the effects of vitamin E and selenium on the incidence and severity of gastric ulcers, and, on platelet aggregation. The pigs were fed four experimental diets consisting of a negative-control ration, and three test diets consisting of the basal diet supplemented with E and/or Se.

Conclusions: 1. The basal ulcerogenic diet used in this study provides a reliable and practical method of producing gastric ulcers in swine.

a. The basal diet used in trial I was more completely predisposed to ulcerogenesis. The extreme physical properties of this ration (talc-like fineness) may have overwhelmed the nutritional aspects of the experiment. That is, the protective effect, if any, of E and Se against ulcerogenesis may have been masked by the intensely stressful diet.

b. The basal diet used in trial II caused an equal number of lesions (of less severity) in the negative control group. However, this practical swine diet appeared to be responsive, with respect to antiulcerogenesis, to E and Se supplementation.

2. In each affected animal, exactly one ulcer occurred and in precisely the same location, that is, in the nonglandualr esophageal region along the lesser curvature of the stomach.

3. The presence of severe ulcers may reduce average daily gain.

4. Results of this study suggest a protective effect of E and to a lesser extent Se against ulcerogenesis.

5. Platelet aggregation was not shown to be affected by the presence of either nutrient.

APPENDIX[a]

Table 1[a]. Serum selenium determinations. Trial I

Group	Pig No.	% Emission	Slope x % Emission	Avg. of Duplicates
BUD	127-01	4, 3	.026, .020	.02
	126-02	5.5, 4	.036, .026	.03
	130-10	3, 3	.020, .020	.02
	127-10	6, 4	.040, .026	.03
BUD + E	127-12	4, 8	.026, .053	.04
	123-10	6, 3	.040, .020	.03
	130-05	7, 5.5	.046, .036	.04
	130-01	3, 4.5	.020, .030	.03
BUD + Se	123-11	18, 14	.119, .092	.11
	127-13	14, 13	.092, .086	.09
	127-01	13, 13	.086, .086	.09
	130-06	10.5, 11.5	.070, .076	.07
BUD + E + Se	130-02	14.5, 13	.096, .086	.09
	126-10	21, 16	.139, .106	.12
	130-11	18, 16	.119, .106	.11
	127-02	14, 17	.092, .112	.10
blank		0, 0		
stnd. .10 ug/ml		15, 16		
stnd. .20 ug/ml		31, 33		
stnd. .30 ug/ml		45, 45		

$r = .999$
$b_o = -.002$
$m = .007$

Table 2[a]. Serum Tocopherol Determination. Trial I

Group-Pig No.		Opt. Density	Slope x (Optical Density - Avg. Blank)	Avg. of Duplicates
BUD	135-10	.140, .169	1.260, 1.770	1.515 µg/ml
	133-12	.158, .174	1.580, 1.860	1.720 µg/ml
BUD + E	130-05	.219, .217	2.660, 2.620	2.640 µg/ml
	127-12	.267, .251	3.500, 3.220	3.360 µg/ml
BUD + Se	135-03	.145, .154	1.350, 1.500	1.425 µg/ml
	133-13	.158, .162	1.580, 1.650	1.615 µg/ml
BUD + E + Se	130-02	.244, .214	3.100, 2.570	2.835 µg/ml
	127-02	.346, .301	4.900, 4.110	4.505 µg/ml
Blank		.066, .072 (extrnl.)		
Stnd. 1 µg/ml		.138, .126 (extrnl.)		
Stnd. 2 µg/ml		.180, .195 (extrnl.)		r = .996
Stnd. 3 µg/ml		.242, .253 (extrnl.)		b_o = -.061
				m = 17.700
BUD	127-01	.123, .137	1.180, 1.250	1.215 µg/ml
	126-02	.127, .140	1.250, 1.302	1.276 µg/ml
BUD + E	123-10	.201, .199	2.547, 2.688	2.618 µg/ml
	130-01	.209, .221	2.688, 2.898	2.793 µg/ml
BUD + Se	123-11	.135, .151	1.390, 1.671	1.636 µg/ml
	127-13	.143, .147	1.530, 1.600	1.565 µg/ml
BUD + E + Se	130-11	.231, .217	3.074, 3.003	3.074 µg/ml
	126-10	.239, .241	3.214, 3.144	3.179 µg/ml
Blank		.052, .059 (extrnl.)		
Stnd. 1 µg/ml		.117, .125 (extrnl.)		
Stnd. 2 µg/ml		.160, .163 (extrnl.)		r = 1.000
Stnd. 3 µg/ml		.217, .231 (extrnl.)		b_o = .013
				m = 17.537

Table 3[a]. Platelet Aggregation

Group-Pig No.		(1:999 ADP) Aggn.	Disaggn.	% Disaggn.	(1:9 ADP) Aggn.	Disaggn.	% Disaggn.
BUD	127-01	86	0	.00	90	0	.00
	126-02	58	31	.53	79	11	.14
	130-10	48	48	1.00	62	29	.42
	127-10	50	8	.16	58	0	.00
average value		61	22	.42	72	10	.14
BUD + E	127-12	59	3	.05	86	0	.00
	123-10	55	55	1.00	69	34	.50
	130-05	79	24	.30	93	8	.09
	130-01	63	6	.10	77	0	.00
average value		64	22	.36	81	11	.15
BUD + Se	123-11	78	2	.03	67	1	.01
	127-13	36	36	1.00	57	38	.67
	126-01	45	19	.53	81	1	.01
average value		53	19	.52	68	13	.23
BUD + E + Se	130-02	57	8	.14	80	0	.00
	126-10	59	50	.85	53	23	.43
	127-02	19	15	.81	26	15	.59
average value		45	24	.60	53	13	.34

Table 4[a], Serum Selenium Determination. Trial II

Group-Pig No.		% Emission	Slope x % Emission	Avg. of Duplicates
BUD	108-12	4 , 4	.033, .033	.033
	101-02	4 , 5	.033, .039	.036
	106-14	5 , 5	.039, .039	.039
	115-01	4.5, 5.5	.036, .042	.039
BUD + E	101-03	6 , 5.5	.045, .042	.044
	115-11	5 , 5.5	.039, .042	.041
	108-10	4 , 6	.033, .045	.039
	106-02	6 , 5	.045, .042	.044
BUD + Se	106-13	14 , 16.5	.093, .108	.101
	101-13	17.5, 15.5	.114, .102	.108
	108-02	16 , 16.5	.105, .108	.107
	115-03	14 , 16.5	.093, .108	.101
BUD + E + Se	101-14	16.5, 15.5	.108, .102	.105
	108-13	17 , 18.5	.111, .120	.116
	115-13	16 , 16.5	.105, .108	.107
	106-11	16.5, 18	.108, .117	.113
Blank		0 , 0		
Stnd. .10 ug/ml		16 , 17		
Stnd. .20 ug/ml		32 , 35		
Stnd. .30 ug/ml		48.5, 46.5		

$r = .998$
$b_o = -.009$
$m = .006$

Table 5[a]. Serum Tocopherol Determination. Trial II

Group-Pig	No.	Opt. Density	Slope x (Optical Density - Avg. Blank)	Avg. of Duplicates
BUD	101-02	.120, .099	.629, .491	.560 µg/ml
	106-14	.089, .082	.264, .277	.271 µg/ml
BUD + E	115-11	.248, .250	2.365, 2.390	2.378 µg/ml
	101-03	.260, .273	2.516, 2.680	2.598 µg/ml
BUD + Se	106-13	.131, .101	.767, .642	.705 µg/ml
	108-02	.117, .129	.717, .868	.793 µg/ml
BUD + E + Se	101-14	.262, .281	2.541, 2.780	2.661 µg/ml
	108-03	.292, .317	2.919, 3.233	3.076 µg/ml
Blank		.051, .055 (extrnl.)		
Stnd. 1 ug/ml		.140, .132 (extrnl.)		
Stnd. 2 ug/ml		.195, .182 (extrnl.)		r = .984
Stnd. 3 ug/ml		.299, .280 (extrnl.)		b_o= .088
				m = 12.580
BUD	108-12	.068, .069	.601, .624	.613 µg/ml
	115-01	.082, .068	.693, .601	.647 µg/ml
BUD + E	108-10	.146, .157	2.815, 2.837	2.826 µg/ml
	106-02	.145, .182	2.707, 2.869	2.788 µg/ml
BUD + Se	101-13	.079, .083	.855, .948	.902 µg/ml
	115-03	.083, .094	.948, 1.202	1.075 µg/ml
BUD + E + Se	115-13	.176, .179	3.193, 3.263	3.228 µg/ml
	106-11	.180, .192	3.286, 3.332	3.309 µg/ml
Blank		.043, .032 (extrnl.)		
Stnd. 1 ug/ml		.062, .064 (extrnl.)		
Stnd. 2 ug/ml		.091, .099 (extrnl.)		r = .998
Stnd. 3 ug/ml		.170, .183 (extrnl.)		b_o= .094
				m = 23.151

Table 6[a]. Tissue Selenium Determination. Trial II

Group-Pig No.		% Emission C*	E*	P*	O*	Slope x % Emission (ppm) C	E	P	O
BUD	108-12	32	29	27	25	.090	.082	.076	.070
	101-02	29	29	31	28	.082	.082	.088	.079
	106-14	30	29	29	31	.084	.081	.081	.087
	115-01	29	25	32	30	.081	.069	.089	.084
group	avg.'s					.084	.079	.084	.080
BUD + E	101-03	29	30	29	33	.082	.085	.082	.085
	115-11	28	26	37	28	.079	.074	.104	.079
	108-10	31	33	29	30	.089	.092	.082	.084
	106-02	28	28	35	30	.078	.078	.097	.084
group	avg.'s					.082	.082	.091	.083
BUD + Se	106-13	45	42	58	58	.126	.115	.163	.163
	101-13	33	41	46	47	.093	.115	.130	.132
	108-02	53	52	47	46	.146	.143	.130	.127
	115-03	43	49	45	44	.120	.135	.124	.122
group	avg.'s					.121	.127	.137	.136
BUD + E + Se	101-14	60	52	60	55	.168	.146	.168	.154
	108-13	59	56	56	54	.165	.157	.157	.151
	115-13	61	53	58	52	.168	.146	.160	.143
	106-11	52	56	54	61	.143	.154	.151	.168
group	avg.'s					.161	.151	.159	.153

*
C - cardiac region tissue
E - nonglandular esophageal region tissue
P - pyloric region tissue
O - oxyntic region tissue

Table 7[a]. Tissue Tocopherol Determination. Trial II

Group-Pig No.		O.D. C*	O.D. E*	O.D. P*	O.D. O*	Slope x O.D. C	Slope x O.D. E	Slope x O.D. P	Slope x O.D. O
BUD	108-12	.101	.163	.054	.081	.970	1.585	.504	.771
	101-02	.101	.143	.094	.099	.999	1.423	.928	.978
	106-14	.114	.091	.072	.083	1.523	1.208	.948	1.098
	115-01	.117	.082	.079	.091	1.682	1.167	1.123	1.299
group avg.'s						1.294	1.346	.876	1.037
BUD + E	115-11	.149	.429	.116	.113	1.797	5.171	1.400	1.345
	101-03	.211	.207	.169	.201	4.200	4.124	3.360	4.000
	108-10	.171	.151	.142	.173	3.621	3.195	3.004	3.663
	106-02	.215	.189	.172	.143	2.727	2.393	2.175	1.804
group avg.'s						3.086	3.721	2.485	2.703
BUD + Se	106-13	.333	.145	.065	.230	3.189	1.373	.611	2.183
	108-02	.094	.095	.064	.088	.417	.421	.296	.393
	101-13	.143	.147	.122	.136	1.714	1.804	1.408	1.630
	115-03	.117	.124	.092	.123	1.762	1.870	1.377	1.854
group avg.'s						1.771	1.367	.923	1.515
BUD + E + Se	101-14	.291	.260	.095	.104	3.283	2.988	1.416	1.502
	108-03	.115	.131	.098	.097	3.301	3.739	2.836	2.808
	115-13	.132	.152	.099	.103	3.367	3.880	2.521	2.624
	106-11	.177	.201	.096	.102	3.234	3.670	1.870	1.761
group avg.'s						3.296	3.569	2.161	2.174

*

C - cardiac region tissue
E - nonglandular esophageal region tissue
P - pyloric region tissue
O - oxyntic region tissue

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