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A series of four trials were conducted to determine the influence of selenium supplementation of a selenium- and vitamin E-deficient basal diet on the absorption, excretion, tissue distribution and placental transfer of a single dose of tritium-labeled α-tocopherol in the rat.

In the first trial, the effect of supplementation of the basal diet with 1.0 ppm selenium, 500 IU vitamin E per kg diet, or 1.0 ppm selenium and 500 IU vitamin E per kg diet, on the placental transfer of a dose of labeled tocopherol was examined. Twenty-four hours after dosing, the fetuses of the basal group contained a significantly higher ($P < 0.005$) percentage of the dose than did those of any of the other groups. Approximately equal fetal uptake of the dose occurred in those groups receiving supplementation with either vitamin E or selenium, while the lowest uptake was observed in the group receiving

both vitamin E and selenium supplementation. These data indicate that the provision of selenium reduced the fetal tocopherol requirement.

The second trial was concerned with the effect of supplementation of the basal diet with 0.5, 1.0, and 2.0 ppm selenium on the absorption and excretion of a dose of tritium-labeled α-tocopherol. Neither the urinary nor fecal excretion over a 72 hour period after dosing was affected by the addition of selenium. A significant inverse correlation was found between urinary excretion of the radioactivity and body weight. About 1.5% of the dose was excreted in the urine over the 72 hour experimental period, while 45-50% was excreted in the feces. No effect of level of selenium supplementation on the absorption of the radioactive tocopherol was found.

The effect of supplementation of the basal diet with either 2.0 ppm selenium or 500 IU vitamin E per kg diet on the absorption, excretion and distribution of a dose of labeled tocopherol was examined in the third trial. As in the second trial, no effect of supplementary selenium on the absorption or excretion of the radioactive material was observed. Supplementation with vitamin E resulted in a slight increase in fecal excretion of the dose, probably because of reduced absorption. The urinary excretion of radioactivity was markedly affected by vitamin E supplementation; with almost twice as much activity excreted by this group as by the basal

and selenium-supplemented animals. The plasma level of radioactivity was significantly higher ($P < 0.01$) in the basal group than in the selenium-supplemented rats, while the plasma radioactivity was significantly lower ($P < 0.01$) in the vitamin E-supplemented group than in the basal or selenium-supplemented groups. The level of protein-bound plasma radioactivity was also significantly higher ($P < 0.01$) in the basal group than in those receiving selenium, and significantly lower ($P < 0.01$) in the vitamin E supplemented group than in the others. The percentage of the plasma radioactivity that was protein-bound was not affected by treatment. The results indicate that selenium may have a role in promoting the withdrawal of vitamin E from the blood. The radioactivity content of the heart and liver tended to be higher in the selenium-supplemented groups than in the basal animals but the difference was not statistically significant.

The fourth experiment compared the distribution of labeled vitamin E at intervals of 12, 24, 48 and 72 hours after dosing in rats on the basal diet and a group receiving supplementation with 2.0 ppm selenium. The plasma level of radioactivity was higher at all time intervals in the basal group, substantiating the results of the third trial which suggested that selenium may promote the withdrawal of vitamin E from the plasma. The levels of radioactivity in the heart and liver were not significantly different between the two groups.

The rate of uptake and depletion of the labeled tocopherol varied among the tissues examined; the liver and plasma reached the peak level prior to 12 hours after dosing, and declined to less than half the 12 hour level by 24 hours. The radioactivity in the heart did not show appreciable variation over the experimental period.

These experiments indicate that in the rat, selenium does not influence the absorption or excretion of α-tocopherol. Selenium does appear to modify tissue distribution of vitamin E; it was found that the plasma level of vitamin E was reduced when the basal diet was supplemented with selenium. The placental transfer of labeled vitamin E was reduced when the basal diet was supplemented with selenium, suggesting that the fetal requirement for α-tocopherol may be reduced in the presence of selenium.

Influence of Dietary Selenium on the Distribution of Tritium-Labeled Vitamin E in the Rat

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INTRODUCTION

Interest in selenium as an essential nutritional factor began more than ten years ago when Schwarz and Foltz (1957) discovered that minute amounts of selenium would protect against the development of liver necrosis in rats fed a diet based on torula yeast. Discovery of other selenium-responsive conditions quickly followed. For example, research at Oregon State University established the efficacy of selenium in preventing white muscle disease in sheep (Muth *et al.*, 1958), while such conditions as exudative diathesis (Patterson *et al.*, 1957) and muscular degeneration (Dam and Søndergaard, 1957) in chicks were found to be responsive to selenium supplementation. The fact that most of these pathologies also respond to vitamin E, and often to synthetic antioxidants, has led to the contention that vitamin E and selenium are interchangeable in the diet, both serving as biological lipid antioxidants as their sole metabolic function. In spite of intensive studies conducted in the past decade, no definitive examples of specific metabolic roles other than an antioxidant function have been established for vitamin E and selenium.

The research embodied in this thesis is an effort to establish

a function for selenium in the metabolism of vitamin E. Some evidence exists indicating that selenium may function by enhancing the activity of α-tocopherol, perhaps by aiding its transport to its cellular site of action, or by influencing the retention or distribution pattern in animal tissue. When added to a vitamin E deficient diet, selenium is only partially effective in preventing muscular dystrophy in chicks (Nesheim and Scott, 1958); a combination of selenium and low level of vitamin E, however, completely prevents the disorder (Desai and Scott, 1965). Similarly, in muscular dystrophy in lambs, Hopkins et al. (1964) found that selenium in combination with vitamin E was fully effective in preventing symptoms of dystrophy, while selenium alone delayed but did not prevent the condition. In studies at Oregon State, a level of vitamin E much higher than contained in most diets was required to prevent white muscle disease when "dystrophogenic" diets were fed (Schubert et al., 1961), whereas a small amount of selenium was effective. This can be construed as evidence that the presence of selenium might enhance the activity of a low level of vitamin E, and that to prevent pathology in the absence (or deficiency) of selenium, requires a high level of vitamin E. The annual variation in occurrence of white muscle disease that is commonly observed (Muth, 1963) would be explained by this concept. Studies in Finland have established that the incidence of nutritional muscular dystrophy in cattle in selenium deficient areas corresponds

with the nature of the hay-making season the previous year (Oksanen, 1967). Prolonged wetting of the hay, resulting in a marked lowering of the vitamin E content, is followed the next year by a high rate of incidence of muscular dystrophy (Thafvelin and Oksanen, 1966). The low level of vitamin E, in conjunction with a marginal level of selenium, presumably precipitates the onset of dystrophy. A tenable explanation for the role of supplementary selenium in such cases is the enhancement of activity of the low level of vitamin E present. Further evidence of a selenium involvement in vitamin E metabolism is provided by the work of Desai and Scott (1965), who found evidence for a role of selenium in the blood transport of α-tocopherol, and possibly in the absorption, retention, and transport across membranes of vitamin E. The study reported herein was intended to further explore the possible role of selenium in influencing the metabolism of α-tocopherol. The effect of dietary supplementation with selenium on the absorption, excretion and distribution of a dose of tritium-labeled α-tocopherol in rats on a vitamin E and selenium deficient diet was examined. Also, an investigation of the role of selenium in the placental transport of tritium-labeled α-tocopherol in rats was included.

LITERATURE REVIEW

Since the discovery of the value of both selenium and vitamin E in preventing or curing various pathological conditions in a number of animal species, intensive efforts have been made to elucidate the mode of action of these compounds. While the role of vitamin E as an effective biological lipid antioxidant is well established, some investigators maintain that evidence exists suggesting additional roles besides the antioxidant effect. The value of selenium as an antioxidant is much less firmly established; while it is claimed by some that such activity could account for its biological effects, others are convinced that the function of selenium is unrelated to any antioxidant activity that selenium-containing compounds may possess. This review will be concerned mainly with the theories that have been proposed to account for the metabolic action of vitamin E and selenium, and with interactions between these compounds that are pertinent to the experimental work described in this report.

Absorption, Distribution and Metabolism of Selenium

A significant difference in the efficiency of selenium absorption apparently exists between monogastrics and ruminants. For example, in an experiment comparing sheep and swine, Wright and Bell (1966) found that in sheep about 35% of a dose of radioselenium

was absorbed, while in swine the figure was 85%. Reduction of selenium to an insoluble unavailable form by the rumen microorganisms, as suggested by Butler and Peterson (1961), would account for the lower absorption of selenium compounds in ruminants. After introduction via the rumen of ^{75}Se-selenious acid into sheep, about 50% of the dose was recovered in the feces (Butler and Peterson, 1961). Most of the fecal radioactivity was insoluble in water, suggesting the formation of unavailable compounds. Hidiroglou et al. (1968) have recently examined the metabolism of inorganic selenium by rumen microorganisms, and observed the incorporation of elemental selenium into microbial protein. Whanger et al. (1968) also reported the synthesis of seleno-amino acids by rumen microorganisms. When the selenium was provided as selenite, selenocystine was the predominant compound produced; incubation with selenomethionine led to the incorporation of selenocystine and selenomethionine. The metabolism by sheep of organic selenium compounds synthesized by plant tissue has been examined by Jenkins and Hidiroglou (1967), who found that seleno-amino acids were effectively absorbed and retained, while lipid-bound selenium was poorly absorbed. Another report of seleno-amino acid absorption in ruminants is that of Ehlig et al. (1967) who demonstrated the absorption of selenomethionine in sheep. The existence in intestinal tissue of an active transport mechanism for selenium compounds has been

postulated; McConnell and Cho (1965) observed that L-selenomethionine, but not selenite or DL-selenocystine, was actively transported across hamster intestine *in vitro*. The full significance of the formation of selenium-containing organic compounds in the gastrointestinal tract presumably awaits the discovery of the biologically active form(s) of selenium at the cellular level.

The distribution of selenium in animal tissue is of interest, since concentration of the element within specific tissues might provide clues to its metabolic function. High levels of both selenium and α-tocopherol in the same tissues might suggest a common mode of action of the two nutrients, or at least a close interrelation in their function.

The liver and kidney are important sites of selenium localization in animal tissue. Jones and Godwin (1963) found that in mice receiving powdered alfalfa grown in a nutrient solution containing ^{75}Se, the highest concentrations of ^{75}Se were in the liver and kidney, while the pancreas and stomach wall also contained high concentrations. Intracellularly, the cell nuclei were found to be the predominant site of ^{75}Se localization. Similarly in rats, the liver and kidney are reported to be important sites of selenium concentration (Burk *et al.*, 1968). The liver, kidney, pancreas and spleen retained the largest quantity of a dose of ^{75}Se administered to chicks in studies reported by Jensen *et al.* (1963). A similar pattern of

distribution of selenium occurs in ruminants. Wright and Bell (1964) found the greatest retention of ^{75}Se administered to sheep in the kidney, liver, spleen, plasma and whole blood. The apparent concentration of selenium by the liver may be of functional importance, especially in view of the liver necrosis observed in selenium-deficient rats. Another possibility is that the tissues containing high levels of selenium such as the liver and kidney may be sites of synthesis of biologically active selenium-containing organic compounds. Wright (1965) has suggested that the spleen may be active in incorporating selenium into blood constituents during hematopoiesis.

Several studies have been made to determine the influence of various dietary regimes on the distribution of selenium in the tissues. Previous intake of selenium seems to be the major determinant of the pattern of distribution. Selenium-deficient animals, as would be expected, retain larger quantities of a given dose than those on a selenium-adequate diet. Wright and Bell (1964) found that the kidney and spleen of selenium deficient ewes accumulated very high concentrations of an oral dose of ^{75}Se. Similar results were obtained with chicks by Jensen _et al._ (1963). Hopkins _et al._ (1966) working with rats, found that the retention of a dose of radioselenium increased with decreasing levels of selenium in the diet; however, the liver tended to retain a relatively constant percentage of the dose regardless of the previous selenium intake. The apparent vitamin

E-selenium interrelationship in nutrition would suggest that the dietary status of α-tocopherol would influence the retention or distribution of selenium, but this is not the case. No effect of various dietary levels of vitamin E on selenium metabolism was reported in sheep (Wright and Bell, 1965), in rats (Hopkins et al., 1966; Burk et al., 1968), or in chicks (Jensen et al., 1963).

Other mineral elements have been found to influence selenium metabolism. Early studies of selenium toxicity revealing that arsenic (arsenite, arsenate and organic arsenicals such as arsenilic acid) is effective in reducing the toxicity of selenium are reviewed by Underwood (1962). More recent studies by Ganther and Baumann (1962a) have shown a marked effect of arsenite on increasing fecal selenium excretion (presumably by decreasing absorption) and decreasing tissue selenium levels. Cadmium exerted opposite effects; the amounts of selenium in the blood, liver and carcass increased in the presence of cadmium, while excretion via the feces, urine and expired air decreased. Inorganic sulfate was found to increase the excretion of selenium in the urine; the effect was more pronounced when selenium was provided as selenate rather than selenite (Ganther and Baumann, 1962b). Similar results were reported by Halverson et al. (1962) who found that sodium sulfate in the diet increased the urinary excretion of selenium from rats fed selenate. Fecal selenium excretion was not affected. The addition of sodium

sulfate to diets containing 10 ppm of selenium reduced the toxicity to rats when the selenium was added as selenate, but not as selenite or as wheat containing selenium. Experiments concerned with nutritional muscular dystrophy also indicate an interference with selenium metabolism by inorganic sulfur. Hintz and Hogue (1964b) observed that sodium sulfate (0.33%) in the diet of ewes increased the incidence of muscular dystrophy in the lambs, and when given in combination with selenium prevented the usual beneficial effects of selenium. Oregon State data similarly indicate an interference by inorganic sulfur with the protective action of selenium against white muscle disease in lambs (Muth et al., 1961).

Incorporation of selenium into organic compounds within the body has been observed. Because of the chemical similarity of selenium and sulfur, greatest attention has been paid to the selenium analogs of the sulfur-containing amino acids. Synthesis of selenium-containing amino acids has been assumed because of the occurrence of selenium in tissue proteins. Hidiroglou et al. (1965) found that the selenium content of hair of calves receiving selenium treatment or from selenium-treated dams was greater than that of selenium-deficient animals. Thus incorporation of selenium into proteins of the hair is suggested. Rosenfeld (1962) isolated ^{75}Se-selenomethionine and ^{75}Se-selenocystine from the wool of a sheep given repeated doses of ^{75}Se-selenite. Selenium is found in association with serum

proteins (McConnell, 1963; Desai and Scott, 1965), but it has not yet been clearly established if it is merely bound to the circulating proteins in some manner or if it is actually an integral part of the protein. McConnell (1963) has reviewed the question of synthesis of selenium-containing compounds in animal tissues, and cites evidence for selenium incorporation into such compounds as cytochrome c, nucleoproteins, serum lipoproteins, and the muscle enzymes aldolase and myosin. Definite evidence has not yet been obtained to indicate if the incorporation of selenium into various organic compounds in animal tissue is due to simple substitution for sulfur in the biosynthetic pathways, or if specific enzymes for selenium metabolism exist. The concept of selenium as an essential nutrient implies the existence of at least one selenium-containing compound that serves an essential function, and that a specific pathway for its synthesis exists. In spite of intensive research in this area, such a compound has yet to be discovered.

Absorption, Distribution and Metabolism of Vitamin E

Vitamin E is apparently absorbed in a similar manner to other fat-soluble vitamins (Wiss, *et al.*, 1962); the process is not confined to any particular segment of the intestinal tract (Sternberg and Pascoe-Dawson, 1959). It is absorbed unchanged into the lymphatic system (Johnson and Pover, 1962). In the blood, α-tocopherol is

transported mainly in association with the plasma proteins. In a study of vitamin E in human blood, Lewis et al. (1954) observed an average of 74% of the total blood tocopherol bound to the α- and β-lipoprotein fractions. McCormick et al. (1960) and Desai and Scott (1965) also reported the transport of vitamin E in association with the serum lipoproteins.

Krishnamurthy and Bieri (1963) found in both rats and chicks that the tissues vary in their rate of uptake and depletion of α-tocopherol. Using ^{14}C-labeled tocopherol, they found that the liver and blood rapidly reached their peak concentrations. Other organs, such as the lung, kidney and heart had a slow turn-over rate, retaining significant concentration of radioactivity 21 days after dosage. The livers of vitamin E-deficient rats in their experiment accumulated their maximum uptake of ^{14}C-labeled tocopherol at 2.5 hours after dosing, and after 24 hours, the liver contained more activity than all the other organs combined. Mellors and Barnes (1966) also investigated the fate of radioactive tocopherol in the rat; at 24 hours after dosage, the liver contained much higher levels of activity than other organs, while the spleen, kidney, testis and adrenals contained appreciable levels. Edwin et al. (1961) examined the distribution of vitamin E in the rat, and reported high concentrations of vitamin E in adrenal, heart, uterus and nerve tissue. They also investigated the rate of loss of tocopherol from various tissues when

rats were put on a vitamin E deficient diet; the adrenal gland and nerve tissue retained high concentrations, while the uterus and liver readily lost tocopherol. The variation in tocopherol retention by various tissues is probably related to their susceptibility to damage in vitamin E deficiency, since the liver and uterus are among the first tissues to undergo degeneration. Wiss et al. (1962) reported similar data on the distribution of labeled vitamin E, with the concentration highest in the adrenals, liver, heart and kidney. The apparent concentration of tocopherol in the adrenals is of interest; whether this is of functional importance is as yet undetermined.

Draper of Illinois has been active in attempting to elucidate pathways of metabolism of vitamin E in animal tissue. In a recent report from his laboratory, a proposed scheme for tocopherol metabolism is summarized (Chow et al., 1967). These workers proposed that as a result of oxidation of α-tocopherol by lipid free radicals or peroxides, α-tocopherol quinone is produced. This compound, they suggest, is reduced to α-tocopheryl hydroquinone in the liver, where it is conjugated with glucuronic acid and secreted in the bile. They propose that in the kidney, α-tocopheryl quinone undergoes reduction and oxidative degradation of the side chain to form α-tocopheronic acid, which is then excreted in the urine as a conjugate of glucuronic acid. Simon et al. (1956) reported the excretion in rabbit urine of a quinone (tocopheronolactone) conjugated with

glucuronic acid. Krishnamurthy and Bieri (1963) were unable to find evidence of tocopheryl quinone or other oxidation products in the urine of rats that had been dosed with ^{14}C-labeled α-tocopherol. However, the radioactivity in the urine was essentially all water-soluble, indicating that degradation of the α-tocopherol molecule probably had occurred. With regard to excretion of metabolites via the bile, it is not firmly established if biliary excretion is a significant pathway for the removal of tocopherol or its metabolites. Chow et al. (1967) suggested the excretion of α-tocopheryl hydroquinone via the bile. Krishnamurthy and Bieri (1963) found that the gastro-intestinal tracts of rats given a single dose of ^{14}C-labeled tocopherol had significant levels of radioactivity 21 days after dosing. They attributed the activity to excretion of tocopherol or its metabolites in the bile. However, Mellors and Barnes (1966), also working with radioactive tocopherol, found no evidence of excretion via the bile; they suggested that coprophagy could account for the presence of radioactivity in the gastro-intestinal tract. The work of both Mellors and Barnes (1966) and Krishnamurthy and Bieri (1963) indicates that α-tocopherol is relatively stable in animal tissue, and that degradation of the molecule is accompanied by rapid removal of the end products of its metabolism.

Metabolic Functions for Vitamin E and Selenium

Function of α-tocopherol as an effective lipid antioxidant is well established. Tappel and his colleagues have been especially active in elucidating mechanisms by which both vitamin E and selenium may exert protective effects against autooxidation in animal tissue. Vitamin E apparently acts by reacting with free radicals produced in the initial stages of lipid autooxidation, thus preventing initiation of an autocatalytic chain reaction (Tappel and Caldwell, 1967). The action of tocopherol is essentially, then, competition with an autooxidizable unsaturated fatty acid for free radicals produced by reaction of an unsaturated fatty acid with oxygen. When these free radicals react with vitamin E or other antioxidants, they are neutralized and the reaction ceases; in the absence of an antioxidant, free radical attack of unsaturated fatty acids produces more free radicals, thus initiating a chain reaction. *In vivo* activity of vitamin E as an antioxidant is indicated by several observations. Most vitamin E-responsive conditions can be prevented by replacement of dietary tocopherol with structurally dissimilar synthetic antioxidants. For example, encephalomalacia in chicks is prevented by vitamin E, and a wide variety of synthetic antioxidants (Søndergaard, 1967). The level of tocopherol necessary in the diet to prevent deficiency symptoms is related to the content of unsaturated fatty acids present,

further supporting an antioxidant role for vitamin E. Century and Horwitt (1960) found that the length of time necessary for muscular dystrophy to develop in chicks depended on the dietary content of unsaturated fatty acids. The level of linoleic acid seems to be of particular importance. Calvert, Desai and Scott (1964) found that linoleic acid is the only fatty acid in lard that influences the development of muscular dystrophy in chicks.

A role for selenium as a biological antioxidant has been proposed, and is particularly championed by Tappel and his group. These workers have used ionizing radiation to produce free radicals in animal tissue, and then measured the effects of various organic selenium compounds on reducing lipid peroxidation and cellular breakdown in such tissues. In a recent summary of this work (Tappel and Caldwell, 1967) the ability of selenomethionine and selenocystine to protect against free radical damage was reported. However, a major factor preventing complete acceptance of this theory of selenium action is that the sulfur analogs of the selenium compounds used are almost as effective as antioxidants, and are present in the tissues at much higher concentrations. The very small amount of selenium necessary to prevent a deficiency state suggests a catalytic role for the element. Tappel has proposed that selenium compounds may serve a catalytic function in stimulating the action of sulfhydryl compounds in neutralizing free radicals (Tappel and Caldwell, 1967).

Caldwell and Tappel (1965) examined the effects of seleno-amino acids in a number of oxidation-reduction reactions, and found that selenocystine accelerated the oxidation of cysteine, homocysteine and glutathione by peroxides and protected sulfhydryl enzymes from oxidative inactivation. They proposed that these reactions involve cycling of selenium among different oxidation states with sulfhydryl groups acting as reducing agents. Such a cycling reaction of selenium compounds would account for the low levels of the element required to prevent disease conditions.

Most of the selenium and vitamin E responsive pathologies can be explained, in theory at least, as the result of an antioxidant deficiency. Unsaturated fatty acids play a structural role in cellular and intracellular membranes; free radical attack of these fatty acids, with their subsequent degradation, could lead to profound cellular damage. Increased susceptibility of erythrocytes to hemolysis in vitamin E deficient animals (Søndergaard, 1967) is probably the most obvious example of how a disturbance in membrane integrity could account for the effects of a tocopherol deficiency. Vitamin E deficiency is associated with an increase in lysozyme enzymes in tissue; these enzymes hydrolyze cellular components. The increase in lysosomal enzymes is apparently a consequence of tissue breakdown, serving to remove degradation products, rather than being a causative factor (Desai _et al._, 1964). The etiology of muscular dystrophy

induced by vitamin E or selenium deficiency may be considered a consequence of muscle degeneration following cell membrane destruction initiated by free radical attack of unsaturated fatty acids in the lipoprotein complex of which the membrane is comprised. Disturbances in metabolism, such as the "respiratory decline" observed in liver homogenates from vitamin E deficient rats (Corwin and Schwarz, 1960), may be attributable to peroxidation damage to the mitochondrial membrane (Tappel, 1962). Thus, the antioxidant theory of vitamin E and selenium function has support in that, in theory at least, it can account for the pathologies observed in deficiency states of either of these compounds.

Influence of Selenium on Vitamin E Metabolism

Desai and Scott (1965) advanced the theory that one function of selenium may be the enchancement of the biological activity of d-α-tocopherol, either by improved absorption or decreased excretion of the vitamin in the presence of selenium. They found that when tritium-labeled α-tocopherol was administered to chicks on a selenium-deficient diet, the plasma level of activity was markedly higher in those chicks receiving selenium supplementation of the basal diet. The enhancement was greater when the d-form, rather than the l-isomer, of α-tocopherol was administered. Simultaneous administration of ^{75}Se with tritium-labeled d-α-tocopherol revealed

that the radioactivity in the plasma of both the selenium and vitamin E was predominantly associated with the lipoprotein fraction, particularly the γ-globulin. On the basis of this similar pattern of distribution of tocopherol and selenium in the blood, and the elevation of the serum tocopherol level in the presence of selenium, Desai and Scott (1965) proposed that selenium, as an essential constituent of a seleno-lipoprotein, may function in the transport of d-α-tocopherol in the blood, aiding in the retention of the vitamin. The significance of the fact that the elevation of blood tocopherol with selenium supplementation was more pronounced when d-α-tocopherol was administered is apparent from the earlier observation of Scott and Desai (1964) that d-α-tocopheryl acetate is approximately four times as effective as the l-form in the prevention of nutritional muscular dystrophy in chicks. It was found that for the prevention of muscular dystrophy in the chick a plasma tocopherol level of 900-1000μg/100 ml is necessary for complete protection, regardless of the form of tocopherol administered. Since there is apparently little difference in the absorption of d- and l-α-tocopherol in chicks (Desai et al., 1965), the suggestion of a difference in their retention is favored. A higher blood level of tocopherol, because of a specific effect of selenium on the retention of the d-form, would account for its greater effectiveness in prevention of muscular dystrophy. Weber et al. (1964) compared the absorption and distribution of d- and

l-α-tocopherol in the rat, and found that the l- form was both absorbed and excreted more rapidly. Twenty-four hours after dosage, various tissues that were examined contained higher levels of the d-α-tocopherol. Fitch and Biehl (1965) found that in the rabbit, l-α-tocopherol was only one-fifth as potent as the d- form in curing nutritional muscular dystrophy; the lower activity of the l-form was associated with a more rapid loss from the body. The serum concentration fell off more rapidly and reached much lower levels in rabbits given the l- form than in those given an equivalent amount of d-α-tocopherol. These reports provide additional evidence of a specific blood transport system for d-α-tocopherol, with the work of Desai and Scott (1965) suggesting that selenium is concerned with this system. Additional evidence for a role of selenium in maintaining a high plasma level of the d- form was reported recently by Scott (1967). In a comparison of plasma levels of d-α-tocopherol following its administration to poults receiving selenium-deficient or selenium-supplemented diets, there was a rapid peak at three hours after dosing in the selenium-deficient group, but after six hours the plasma level was higher in the supplemented group and remained higher for the duration of the 72 hour experimental period. Effects of selenium on the whole-body retention of vitamin E have been observed. Witting et al. (1967) reported a tendency for supplementary selenium to increase the carcass retention of vitamin E

by rats. Thompson and Scott (1968) found that the tissue uptake of labeled tocopherol was up to 100 times greater in chicks receiving dietary selenium than in those receiving a selenium-deficient purified diet.

There are various reports in the literature that tend to support the theory that selenium may enhance the biological activity of a low level of vitamin E. In the case of white muscle disease in calves and lambs, several investigators have found that a combination of selenium and vitamin E often gives a better response than selenium alone. Hidiroglou et al. (1965) observed that supplementation of pregnant beef cows with both selenium and vitamin E gave better protection against white muscle disease than did treatment with selenium only. Ewan et al. (1968) obtained better results in preventing muscular dystrophy in lambs with a combined treatment of vitamin E and selenium than when either was added alone. Survival time, daily gain and the blood levels of two enzymes, serum glutamic-oxaloacetic transaminase and lactic acid dehydrogenase, were used in the preparation of a score for the severity of muscular dystrophy. The optimal responses with vitamin E supplementation were obtained with 22 mgm dl-α-tocopherol per kgm of body weight when no supplemental selenium was provided, 11 mgm per kgm with 0.1 ppm selenium, and 2.2 mgm per kgm with 1.0 ppm selenium. These data support a role of selenium in improving the utilization of

α-tocopherol; selenium alone at a level of 1.5 ppm did not give as satisfactory a response as did the lower selenium levels in conjunction with tocopherol supplementation. If selenium does exert its effects by influencing α-tocopherol metabolism, animals on a diet completely devoid of vitamin E should not respond to selenium. While it is difficult to rid a diet completely of vitamin E, Calvert et al. (1962) reported that with a diet which they considered to be very deficient in vitamin E, selenium supplementation had no beneficial effect in reducing the incidence of muscular dystrophy in chicks. Supplementation with selenium of a diet containing a low level of vitamin E was, however, effective in preventing the disorder.

An interaction of selenium and vitamin E would offer an explanation for the seasonal variation in the incidence of white muscle disease that has been reported (Muth, 1963). In areas of selenium deficiency, adequate levels of vitamin E in the forage presumably prevent the onset of muscular dystrophy. Oksanen and co-workers in Finland have studied this aspect. A relationship between the incidence of nutritional muscular dystrophy in cattle and the quality of the forage was observed. In all cases the forage had a very low level of selenium (Oksanen, 1967), but in hay that had been rain-damaged, the vitamin E content was markedly lowered (Thafvelin and Oksanen, 1966). It was the rain-damaged hay, low in vitamin E, that provoked the occurrence of muscular dystrophy. A possible explanation of

these observations is that the simultaneous deficiency of both vitamin E and selenium provokes the disease condition, and that the effect of supplementary selenium is the enhancement of the activity of the low level of vitamin E. In studies of white muscle disease in Oregon, alfalfa hay was the feed most commonly associated with the incidence of muscular dystrophy in lambs (Muth, 1963). This is of interest since a vitamin E antagonist in alfalfa has been demonstrated. Pudelkiewicz and Matterson (1960) reported that only about 25% of the tocopherol of alfalfa was available for utilization by the chick. The antagonistic factor was contained in the ethanol-extractable portion of the plant material. Subsequent work by these investigators has led to a partial chemical identification of a compound that markedly interferes with tocopherol utilization by the chick (Olson et al., 1966). Apparently no studies of this nature have been made with species other than the chick; it would be of interest to determine if the same vitamin E antagonism is found in ruminants. This presents another possible explanation for the cause of white muscle disease; the presence of a vitamin E antagonist in alfalfa in conjunction with a low selenium level, could provoke the deficiency symptoms of vitamin E. Besides the reports of a vitamin E antagonist in alfalfa, there is evidence of such a compound in kidney beans. Hintz and Hogue (1964) reported an apparent anti-vitamin E effect when kidney beans were included in the diet of chicks. Desai (1966) found

that raw kidney beans in chick diets caused a significant reduction in the plasma tocopherol level; autoclaving the beans reduced the effect. Hogue et al. (1962) reported production of white muscle disease in lambs from ewes receiving raw kidney beans. Autoclaving of the beans reduced the incidence of muscular dystrophy, suggesting the presence of a heat-labile vitamin E antagonist. Recent studies (Gardner and Hogue, 1967) confirm the value of autoclaving kidney beans in reducing the occurrence of white muscle disease. These reports indicate that certain feedstuffs may contain vitamin E antagonists, which can exert their effects in both monogastric and ruminant animals. In addition, it has been commonly assumed that such products as dehydrated alfalfa meal and apparently well-cured alfalfa hay contain adequate amounts of vitamin E. This assumption may not be correct. Bunnell et al. (1968) have examined the distribution of α-tocopherol in various feed-stuffs using several improved techniques. They stress that many forage materials, and especially alfalfa, contain substances which give positive Emmerie-Engel reactions, thus making a measurement of the total reductants meaningless. Many determinations of "tocopherol" in previous studies have not included isolation of α-tocopherol, so that what were considered to be adequate vitamin E levels may in fact have been due to substances other than tocopherol. Thus the occurrence of low vitamin E levels in "dystrophogenic" forages may be more widespread

than formerly believed.

Since, as discussed above, selenium may have a role in improving the utilization of vitamin E, some selenium-responsive conditions might be interpreted as being, in fact, vitamin E deficiency states. For example, white muscle disease in calves and lambs might be considered to be a condition provoked by a low dietary level of vitamin E in conjunction with very low intakes of selenium. Supplementation with selenium might improve the retention or utilization of the low level of vitamin E present, and thus overcome a vitamin E deficiency state. The marginal level of vitamin E in selenium-deficient "dystrophogenic" forages could result either from destruction of the tocopherol during the harvesting process, or from the presence in the forage of compounds antagonistic to vitamin E utilization, or from a combination of both factors.

Desai and Scott (1965) speculated that selenium may enhance the transport of α-tocopherol across membranes. Such a proposal, if correct, may have implications in the etiology of white muscle disease. The role of selenium might be one of increasing the transfer of vitamin E to the young, either across the placenta to the fetuses, or via the milk. Oldfield *et al.* (1960) reported that vitamin E administered orally to lambs protected against development of white muscle disease, while in earlier studies (Muth *et al.*, 1958), supplementation of the ewes with vitamin E prior to parturition was not

effective. Gardner and Hogue (1967) found that supplementation of a "dystrophogenic" diet based on raw kidney beans with approximately 1.0 ppm selenium increased the level of α-tocopherol in the milk by about one-third. The possibility that selenium exerts its protective effect against white muscle disease by enhancing placental transfer and/or mammary transfer of vitamin E is intriguing, and deserves full investigation. Such an effect would become of importance only in cases of marginal vitamin E intake by the dam, which, as indicated earlier in this discussion, may be a common situation in outbreaks of white muscle disease.

EXPERIMENTAL PROCEDURE

Trial I

The intent of this experiment was to examine the proposal of Desai and Scott (1965) that selenium may function by enhancing the transport of vitamin E across membranes. The influence of selenium supplementation of a selenium- and vitamin E-deficient Torula yeast diet on the placental transfer of a dose of tritium-labeled α-tocopherol was investigated. Pregnant rats were administered the radioactive tocopherol by stomach tube on the 19th day of pregnancy; the total fetal content of radioactivity 24 hours after dosing was used as a measure of the extent of placental transfer of the vitamin.

Twenty-four female Long-Evans rats[1] averaging approximately 275 gm body weight were fed for three weeks on a basal diet deficient in selenium and vitamin E but containing 0.0125% of the synthetic antioxidant Ethoxyquin[2]. They were then divided into four equal groups and given the following dietary treatments:

1. Basal (selenium and vitamin E deficient)
2. Basal + 500 IU vitamin E/kg diet

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[1] Simonsen Laboratories, Gilroy, California.

[2] 1, 2-dehydro-6-ethoxy-2, 2, 4-trimethylquinoline, Monsanto Company, St. Louis.

3. Basal + 1 ppm selenium (2.13 mg Na_2SeO_3/kg diet)

4. Basal + 500 IU vitamin E/kg diet + 1 ppm selenium)

The composition of the selenium- and vitamin E-deficient basal diet is shown in Table 1.

Table 1. Composition of the basal diet used in Trial I.

Ingredient	grams
Torula yeast[1]	300
Corn starch	574
Glucose	6
B-vitamin mixture[2]	10
Mineral mixture[3]	40
Stripped lard[4]	50
Cellulose[5]	18
Methionine	1.9
Ethoxyquin[6]	0.125
Vitamin A	2000 IU
Vitamin D	555 IU

[1] Lake States Yeast and Chemical Division, St. Regis Paper Company, Rhinelander, Wisconsin.

[2] Bull and Oldfield (1967)

[3] Jones and Foster (1942)

[4] Distillation Products Industries, Rochester, New York.

[5] Alphacel, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[6] 1, 2-dehydro-6-ethoxy-2, 2, 4-trimethylquinoline, Monsanto Company, St. Louis, Missouri.

The animals were housed in groups of six in wire-screen bottom cages. Feed and distilled water were provided ad libitum. Following a six week period on their respective diets, the animals were bred. Males previously proven fertile were used; they were put in with the females at night and removed the following morning. Vaginal smears were examined for the presence of sperm each day to establish the time of breeding. On the 19th day of pregnancy, 12.88 μc (0.0299 μmoles) of ^{3}H-DL-α-tocopherol (5-methyl-T)[3] were administered by stomach tube. This dosage was contained in 0.5 ml of 95% ethanol. In order to calculate the administered dose for each animal, the syringes used in stomach tubing were rinsed with ethanol, the washings counted, and the appropriate corrections applied. Twenty-four hours after the administration of labeled α-tocopherol the animals were killed with CO_2, and the fetuses and maternal gastro-intestinal tracts removed. These tissues were freeze-dried and extracted for 24 hours in a Soxhlet apparatus with 95% ethanol. The alcohol extracts were reduced to a volume of 25 ml; one ml of each of these solutions was diluted to 25 ml. One-half ml samples of the dilute solutions were evaporated to dryness in counting vials, and 15 ml of the scintillation fluid (5 gm PPO[4]

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[3]Nuclear-Chicago Corporation, Des Plaines, Illinois.

[4]PPO = 2, 5-diphenyloxazole

and 0. 3 gm POPOP[5] per 1 of toluene) were added to each vial. Duplicates of each extract were counted for ten minutes in a liquid scintillation counter[6].

The radioactivity in the maternal gastro-intestinal tract was subtracted from the administered dose so that the uptake of labeled material by the fetuses was expressed on the basis of the apparent absorbed dose.

Thin layer chromatography of some of the fetal extracts was performed using the method of Bieri and Prival (1965) to establish if the radioactivity in the fetuses was associated with α-tocopherol. The determination was qualitative, so that specific activity of α-tocopherol in the fetuses was not established.

Trial II

Some evidence has been reported indicating that a metabolic role of selenium may reside in effects of selenium on the retention of vitamin E in the tissues. Witting *et al.* (1967) observed somewhat higher levels of α-tocopherol retained in the carcasses of rats receiving dietary selenium supplementation than in deficient animals. On the basis of higher plasma vitamin E levels observed in chicks

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[5]POPOP = 1, 4-bis-2-(5-phenyloxazoyl)-benzene.

[6]Nuclear Chicago Liquid Scintillation Spectrometer Model 6766.

receiving selenium supplementation, Desai and Scott (1965) proposed that selenium may play a role in increasing absorption or decreasing excretion of vitamin E, thus enhancing the biological activity of a low level of tocopherol intake. In this experiment, the effect of various levels of selenium supplementation on the fecal and urinary excretion by rats of a dose of tritium labeled α-tocopherol was examined.

Thirty-two male Long-Evans rats (Simonsen Laboratories) of about 60 gm body weight were randomly distributed into four equal groups. They received ad libitum the basal diet (Table 2) for three weeks, after which time they were given one of the following diets:

Table 2. Composition of basal diet used in Trial II.

Ingredient	grams
Torula yeast	300
Sucrose	580
Vitamin mixture[1]	10
Mineral mixture[2]	40
Cellulose	18
Stripped lard	50
Methionine	2
Ethoxyquin	0.125
Vitamin A	2000 IU
Vitamin D	555 IU

[1] Bull and Oldfield (1967).

[2] Jones and Foster (1942).

1. Basal (vitamin E- and selenium-deficient)
2. Basal + 0.5 ppm selenium
3. Basal + 1.0 ppm selenium
4. Basal + 2.0 ppm selenium

The animals were kept on these diets for six weeks, and were then placed in metabolism cages. Each rat was given by stomach tube a dose of 15 μc (1.44 mgm) of ^{3}H-DL-α-tocopherol-(-5-methyl-T) in a 50% ethanol solution containing 2% Tween 40. The dose was contained in a volume of one-half ml. The administered doses were corrected for radioactivity remaining in the syringes used for stomach tubing. The rats were kept in the metabolism cages for 72 hours, with feces and urine collected at 24 hour intervals. The animals were then killed with CO_2, the gastro-intestinal tracts removed, and the carcasses and gastro-intestinal tracts freeze-dried. The urine samples were filtered through glass wool, and diluted to a volume of 50 ml with a 50% ethanol solution containing 2% Tween 40. One-half ml samples were evaporated to dryness in counting vials, and 15 ml of the scintillation fluid added. Fecal samples were prepared for counting by extraction with 95% ethanol for 24 hours in a Soxhlet apparatus. The extracts were concentrated to a 50 ml volume; one ml of these solutions was diluted to 10 ml with 95% ethanol. One-half ml samples of the dilute solutions were evaporated to dryness in counting vials. The dried gastro-intestinal

tracts were treated in the same manner as the fecal samples. Fifteen ml of the scintillation fluid (5 gm PPO and 0. 3 gm POPOP per l of toluene) were added to each counting vial, and the count rate determined in a liquid scintillation counter. All samples were counted in duplicate.

Trial III

The effect of dietary selenium on the retention and excretion of a single dose of tritium-labeled α-tocopherol was further examined in this experiment. Replications of the treatments in the presence and absence of the synthetic antioxidant Ethoxyquin were included to determine if the effect, if any, of selenium on the retention of α-tocopherol was due to antioxidant properties of selenium. Also included was a group receiving dietary supplementation with vitamin E to determine if any effect of selenium on the distribution of the administered dose of labeled tocopherol was different from the effect observed with supplementary vitamin E.

Twenty-four female Long-Evans rats (Simonsen Laboratories) of about 60 gm body weight were divided into three equal groups, receiving *ad libitum* one of the following treatments:

1. Basal (vitamin E and selenium deficient)
2. Basal + 2. 0 ppm selenium
3. Basal + 500 IU vitamin E/kg diet.

The basal diet, containing 0.0125% ethoxyquin, was that used in Trial II. The animals were kept on these diets for six weeks, and were then placed in metabolism cages and administered tritium-labeled α-tocopherol as described for Trial II. An additional eight animals received the basal and selenium-supplemented diets (four rats on each diet) for the six-week period; three days before the administration of the labeled tocopherol, the ethoxyquin was removed from the diets. According to tracer studies, the rat body is essentially completely depleted of ethoxyquin three days after its removal from the diet (Wiss et al., 1962). These animals were intended to provide information on whether any effect of selenium on tocopherol retention might be due to its antioxidant activity.

Collection of fecal and urine samples and their preparation for counting was as described for Trial II. In addition, radioactivity in blood plasma was determined in this experiment, using a modification of the method of Mahin and Lofberg (1966). Blood was collected by heart puncture at the time of killing, and plasma prepared. One-half ml samples of plasma were digested for one hour at 90° C with 0.5 ml of 70% perchloric acid. One ml of hydrogen peroxide was added, and heating at 90° C continued until the samples turned colorless. Conical-tipped centrifuge tubes (15 ml capacity) were used for the digestion procedure. The digested and bleached samples were then extracted five times with toluene, and the toluene extract made up to a volume of 10 ml. Duplicate two ml samples of the toluene extracts were placed in

scintillation vials, and 15 ml of the scintillation fluid added. POPOP was omitted from the scintillation fluid used for the plasma samples, since its inclusion results in a yellow coloration when added to samples digested with perchloric acid (Mahin and Lofberg, 1966). The protein-bound portion of the plasma tocopherol was determined by adding a 0.5 ml sample of plasma to 15 ml of 10% perchloric acid. Following centrifugation, the protein precipitate was prepared for counting as described above for whole plasma samples. For each group, the plasma remaining after aliquots had been taken for measurement of radioactivity was pooled, and the tocopherol contents of the pooled samples were measured by the method of Quaife, Scrimshaw and Lowry (1949). Triplicates of each sample were run; the amount of plasma used was 0.5 ml, rather than the 0.06 ml specified in the method. Amounts of reagents used were increased in the same proportion.

The heart and liver of each animal were also examined for their content of radioactivity. The hearts were opened, washed twice with 20 ml of 0.85% sodium chloride solution to remove any remaining blood, blotted on filter paper and weighed. They were digested for two hours at 90° C with two ml of 70% perchloric acid. An equal volume of hydrogen peroxide was added, and heating continued until the solutions were colorless. The digested samples were extracted five times with approximately 10 ml of toluene per extraction;

the toluene extract was made up to a volume of 50 ml. Duplicate four ml samples of each extract were added to scintillation vials containing 15 ml of the scintillation fluid (5 gm PPO per l of toluene). The livers were digested and extracted in the same manner as the hearts, except that the volumes used were increased. Ten ml of perchloric acid and hydrogen peroxide were used; the final volume of the toluene extract was 100 ml. Duplicate one ml samples of the toluene extract were counted in a liquid scintillation counter.

The distribution of the radioactivity of the urine between water and benzene was examined; the water soluble activity presumably would be associated with the excretion of water-soluble metabolites, and thus may be an indicator of the extent of metabolism of the administered dose. Urine samples from two rats from each group were examined. Twenty-five ml of each urine sample were added to 20 ml of water in a separatory funnel. This mixture was extracted five times with 20 ml aliquots of benzene; the benzene extract was washed twice with 10 ml portions of water, and both the water and benzene extracts made up to a volume of 100 ml. Duplicate one ml samples were counted for radioactivity.

Trial IV

The objective of this experiment was to study in further detail the influence of selenium on the distribution of a dose of

tritium-labeled α-tocopherol in rats receiving a diet deficient in both selenium and vitamin E. The radioactivity in selected tissues was determined at various intervals after dosing.

Thirty-two male Long-Evans rats (Simonsen Laboratories) of about 150 gm body weight were randomly assigned to two equal groups. One group received ad libitum the basal diet of Trial II, while the other received the basal diet supplemented to contain an added level of two ppm selenium. Neither diet contained ethoxyquin. The animals were kept on these diets for a four week period; then they were administered labeled tocopherol. On the basis of the results of the previous trials of this report, liver necrosis would not be produced in the four week period, but according to the work of Burk et al. (1968) the tissue selenium level would be low at this time. These investigators used a torula yeast-based diet very similar to that used in this experiment, and found a rapid decline (within two weeks) of the tissue selenium levels. The rats were administered by stomach tube a dose of 5 μc of ^{3}H-DL-α-tocopherol -(5-methyl-T) in a 50% ethanol solution containing 2% Tween 40. The dose was contained in a volume of one-half ml. Radioactivity remaining in the syringes was determined and subtracted from the total activity of the dose. Four animals from each group were killed at intervals of 12, 24, 48 and 72 hours after dosing. Blood was collected in heparinized tubes by heart puncture, and plasma prepared. Two ml

samples of plasma were digested for one hour at 90° C with 1.0 ml of 70% perchloric acid; one ml of 30% hydrogen peroxide was then added, and heating continued until the samples turned colorless. Conical-tipped centrifuge tubes were used for the digestion procedure. After digestion and bleaching, the samples were extracted five times with toluene, and the toluene extract made up to a volume of 10 ml. The toluene extracts were added to scintillation vials each containing 5 ml of the scintillation fluid (15 gm PPO per liter of toluene) and counted in a liquid scintillation counter. The remaining plasma for each group was pooled and the tocopherol content measured as described in Trial III.

Radioactivity in the heart and liver of each animal was also determined. The hearts were cut open and washed twice with 20 ml of 0.85% sodium chloride solution to remove any remaining blood. They were digested for two hours at 90° C with two ml of 70% perchloric acid, and then bleached with two ml of hydrogen peroxide. Following this process, the samples were extracted five times with toluene, and the toluene extract made up to a volume of 10 ml. The toluene extracts were then prepared for counting as described above for plasma. The livers were digested and bleached with 15 ml of perchloric acid and hydrogen peroxide, and then extracted five times with toluene. The toluene extracts were made up to 50 ml, and duplicate 10 ml samples of each extract prepared for counting as described.

RESULTS AND DISCUSSION

Trial I

The objective of this study was to determine if the placental transfer of vitamin E is influenced by the dietary level of selenium. Tritium-labeled α-tocopherol was administered to pregnant rats receiving various treatments of dietary selenium and vitamin E; placental transfer of the radioactivity was estimated by determining the level of fetal uptake of the administered dose.

Reproductive difficulties due to the deficiency of vitamin E in the basal diet were not anticipated (and not observed) because of the inclusion of the synthetic antioxidant ethoxyquin in the rations. Crider *et al.* (1961) found earlier that ethoxyquin maintained the fertility of female rats on a vitamin E-deficient diet through two generations. The breeding performance was poorest in the groups receiving supplementary selenium; mechanical inducement of pseudopregnancy during the taking of vaginal smears was the main problem. No instances of pseudopregnancy occurred in the basal group and the vitamin E supplemented group, but pseudopregnancy was observed in both groups receiving supplementary selenium. The level of selenium used (1 ppm) is not in the range (5-10 ppm) usually considered to be detrimental to female reproduction (Underwood, 1962). The apparently increased sensitivity of the female reproductive tract to

a mating response in the selenium-supplemented groups may be a factor in the improvement of ewe fertility noted in New Zealand when selenium-deficient animals were administered selenium (Hartley and Grant, 1961).

Pertinent data on the transfer of labeled α-tocopherol to the fetuses are given in Table 3. Association of the radioactivity of the fetuses with α-tocopherol was confirmed by thin layer chromatography using the method of Bieri and Prival (1965). Greatest transfer occurred in the basal group, in which the dpm per gram wet fetal weight was significantly higher ($P < 0.005$) than for the other groups. The higher transfer was also reflected in the proportion of the dose found in the fetuses. The percentage of the apparent absorbed dose (dose administered minus labeled material in the maternal gastrointestinal tract) in the fetuses was significantly higher ($P < 0.005$) in the basal group than in the others. In comparing the basal and selenium-supplemented groups, it is apparent that supplementary selenium resulted in a significant reduction in the fetal uptake of α-tocopherol.

Two interpretations may be advanced for the reduction in the transfer of α-tocopherol to the fetuses of the selenium-supplemented rats. First, it may be hypothesized that the presence of selenium in the diet reduces the fetal requirement for α-tocopherol. This concept is supported by the observation that fetal uptake of labeled

Table 3. Fetal uptake of ^{3}H-DL-α-tocopherol - Trial I.

Treatment	Fetal Wet Weight [1]	Total Fetal Radioactivity	Radioactivity per gram Fetal Wet Weight	Apparent Absorbed Dose Retained in Fetuses [2]
	gm	dpm	dpm/gm	%
Basal				
Rat #1	47.4 (12)	1,277,562	26,953	6.05
Rat #2	35.8 (8)	1,077,076	30,086	5.12
Rat #3	44.5 (11)	1,102,244	24,770	5.19
Rat #4	38.8 (10)	1,024,452	26,403	5.19
Rat #5	36.2 (10)	1,098,240	30,338	5.04
Mean	40.5 ±5.2	1,115,915	27,710 ±2,423 [3]	5.32 ±0.41 [3]
Basal + Vitamin E				
Rat #1	52.1 (13)	764,478	14,673	3.56
Rat #2	43.4 (12)	750,178	17,285	3.56
Rat #3	36.3 (11)	824,252	22,707	4.24
Rat #4	34.8 (10)	719,004	20,661	3.65
Rat #5	40.6 (10)	795,738	19,604	3.96
Mean	41.4 ±6.9	770,770	18,986 ±3,104	3.79 ±0.30
Basal + Selenium				
Rat #1	36.8 (9)	741,026	20,136	3.61
Rat #2	35.9 (8)	583,154	16,244	3.03
Rat #3	29.3 (7)	710,710	24,256	3.84
Rat #4	25.0 (6)	294,580	11,783	1.68
Mean	31.8 ±5.6	582,368	18,105 ±5,335	3.04 ±0.97
Basal + Selenium + Vitamin E				
Rat #1	30.2 (7)	333,476	11,042	2.08
Rat #2	32.4 (9)	454,740	14,035	3.08
Mean	31.3 ±1.6	394,108	12,539 ±2,116	2.58 ±0.71

[1] Numbers in parentheses refer to number of fetuses.

[2] Apparent absorbed dose = radioactivity administered minus radioactivity of maternal gastrointestinal tract.

[3] These means are significantly higher ($P < 0.005$) than those for the other groups.

α-tocopherol was approximately equal when the basal diet was supplemented individually with vitamin E or selenium, and was lowest when both selenium and vitamin E were supplemented coincidentally. Selenium may, therefore, have a sparing effect on the tocopherol requirement of the rat fetus. Such effects have been reported; for example, Desai and Scott (1965) observed that dietary selenium reduced the level of vitamin E required for prevention of nutritional muscular dystrophy in chicks. Such a sparing effect may be the result of direct substitution of selenium for vitamin E in its metabolic function. The role of vitamin E as a biological lipid antioxidant is well established; one theory of selenium function is that it also acts as an antioxidant (Tappel and Caldwell, 1967). Hence, the "sparing effect" of selenium for vitamin E might be simply a contribution of selenium to the "antioxidant pool" of the animal. Alternatively, selenium may function by enhancing the activity of a given amount of α-tocopherol, perhaps by promoting its retention as Desai and Scott (1965) suggest, or by modifying its distribution in the tissues.

Another possible interpretation of the differences in fetal uptake of labeled tocopherol observed in this experiment is that since selenium has been reported to promote retention of α-tocopherol in the tissues of the rat (Witting _et al._, 1967), less of the administered dose might have been available for fetal transfer in the groups

receiving supplementary selenium. Results obtained in Trials II and III of this study do not support that interpretation. A significant effect of supplementary selenium on the retention of vitamin E was not observed.

The level of labeled α-tocopherol in the maternal blood plasma was not measured, but in Trial III of this report, it was found that three days after dosage with tritiated vitamin E, the radioactivity in the plasma of deficient animals was significantly higher than in selenium supplemented rats. Similar results were found in Trial IV. The greater placental transfer of vitamin E in the deficient animals might, therefore, be a reflection of a higher maternal blood level of labeled tocopherol in the absence of supplementary selenium. Thus, the lower fetal uptake of α-tocopherol in the selenium supplemented groups may not be the result of an effect on the fetal vitamin E requirement *per se*, but could be the result of a selenium influence on the distribution of α-tocopherol in the maternal tissues.

The hypothesis of Desai and Scott (1965) that selenium may function in the transport of α-tocopherol across membranes was not supported by this study. It should be noted, however, that transport across the placental barrier may not be representative of membrane transport in general.

Trial II

The influence of three levels of supplementary selenium on the fecal and urinary excretion of a dose of tritium-labeled α-tocopherol by rats was examined in this experiment.

Growth and Mortality

The average final weights for the rats in each group were as follows:

Basal	178.9 gm ± 27.7
Basal + 0.5 ppm selenium	204.5 gm ± 36.8
Basal + 1.0 ppm selenium	240.2 gm ± 20.2
Basal + 2.0 ppm selenium	261.5 gm ± 60.4

The final weight figure for the group receiving 2.0 ppm selenium was markedly skewed by the fact that one of the animals was extremely obese; when this animal is omitted, the final weight for this group averages 239.1 gm. Justification for omitting the obese rat is based on the fact that its final weight (396 gm) was almost twice that of the others in its group; upon dissection it was observed to have a very thick layer of subcutaneous fat, and massive depots of internal fat, which probably are evidence of an endocrine disturbance rather than of a direct dietary effect.

A favorable growth response to the presence of selenium was

observed. Growth responses to selenium supplementation have been reported for sheep (Hartley and Grant, 1961; Oldfield et al., 1960; Slen, et al., 1961), and in chicks and quail (Scott and Thompson, 1968). The response in this experiment upon increasing the selenium level from 0.5 ppm to 1.0 ppm was unexpected, since the level of selenium required to prevent liver necrosis in rats is only 0.04 ppm (Schwarz and Foltz, 1957).

Fifty percent mortality occurred in the basal group, whereas there were no deaths in the selenium-supplemented groups. This differs from the results of Bull and Oldfield (1967) who found that the presence of the synthetic antioxidant Ethoxyquin in a vitamin E and selenium-deficient diet prevented mortality and allowed normal growth. Schwarz (1958) found that ethoxyquin at the 0.25% level exerted a protective effect against liver necrosis in rats, but was not effective at the 0.125% level. Autopsy established gross liver necrosis as the cause of death of some of the rats in the basal group, while in others the cause of death was not discernable.

Urinary Excretion of Radioactivity

The excretion of radioactivity via the urinary route followed an exponential pattern over the three day period. The urinary excretion, expressed in terms of total cpm and percentage of dose per 24 hour interval, is tabulated in Tables 4 and 5. The cumulative

excretion was highest in the basal group, and decreased with each increasing level of dietary selenium. The suggestion of Desai and Scott (1965) that selenium promotes the retention of α-tocopherol by the body would account for the differences in excretion observed in this experiment. The reduced excretion with each level of selenium would suggest a role in tocopherol retention. However, an obscuring factor is that the mean body weight increased with each level of selenium; the differences in excretion might be due solely to a body weight effect. When expressed on the basis of radioactivity excretion per gm of body weight (Table 6) the cpm/gm was highest for the basal group, and decreased with each level of selenium. This would be the relationship expected if the capacity of the tissues to retain tocopherol was the same across treatments, i. e., the retention capacity of a small animal would be reached at a lower dosage level than for a large animal. Correlation coefficients were calculated to determine the relationship between body weight and various expressions of urinary excretion of radioactivity; these are summarized in Table 7.

These coefficients, all statistically significant ($P < 0.01$), indicate a strong relationship between the urinary excretion and body weight. Expression of the excretion in terms of body weight raised to the 0.75 power, or the so called "metabolic body size", increased the significance of the correlation coefficients. The highest

Table 4. Urinary excretion of radioactivity - Trial II.

Treatment	0-24 hrs cpm	24-48 hrs cpm	48-72 hrs cpm	0-72 hrs cpm
Basal	84, 425	58, 201	32, 936	175, 562
0. 5 ppm selenium	70, 675	40, 073	23, 161	133, 909
1. 0 ppm selenium	74, 196	29, 178	17, 165	120, 539
2. 0 ppm selenium	61, 081	27, 931	20, 259	109, 271

Table 5. Percent of dose excreted in urine - Trial II.

Treatment	0-24 hrs %	24-48 hrs %	48-72 hrs %	0-72 hrs %
Basal	0. 70	0. 49	0. 27	1. 46
0. 5 ppm selenium	0. 58	0. 33	0. 19	1. 10
1. 0 ppm selenium	0. 61	0. 24	0. 14	0. 99
2. 0 ppm selenium	0. 51	0. 23	0. 17	0. 91

Table 6. Urinary excretion of radioactivity per gram of body weight - Trial II.

Treatment	0-24 hrs cpm/g BW	24-48 hrs cpm/g BW	48-72 hrs cpm/g BW	0-72 hrs cpm/g BW
Basal	470. 4	347. 9	196. 3	1014. 6
0. 5 ppm selenium	362. 6	207. 9	176. 1	746. 6
1. 0 ppm selenium	316. 6	124. 7	73. 5	514. 8
2. 0 ppm selenium	243. 3	113. 6	79. 4	436. 3

Table 7. Correlations between body weight and urinary excretion of radioactivity - Trial II.

Relationship	Correlation Coefficient
Body weight x % of dose in urine	r = -0. 724
Body weight x urine cpm/gm body weight	r = -0. 876
Body weight $^{0.75}$ x % of dose in urine	r = -0. 736
Body weight $^{0.75}$ x urine cpm/gm body weight	r = -0. 891
Body weight x cpm/gm body weight*	r = -0. 882
Body weight $^{0.75}$ x cpm/gm body weight*	r = -0. 897

*cpm excreted corrected to a dosage level of 12 x 10^6 cpm.

correlation was obtained upon correction of the administered dose to an arbitrary 12×10^6 cpm. This correction was based on a linear extrapolation of the percent of dose excreted in the urine.

Because body weight differences among treatments influenced the amount of excretion via the urinary route, a correction of the urinary radioactivity excretion to a body weight of 200 gm was made. A tabulation of urinary loss of radioactivity corrected to a common basis of a 200 gm body weight is presented in Table 8.

Table 8. Urinary excretion of radioactivity corrected to a 200 gm body weight basis - Trial II.

Treatment	% of dose in urine	Total cpm in urine
Basal	1.35	154,270
Basal + 0.5 ppm selenium	1.09	132,738
Basal + 1.0 ppm selenium	1.18	142,453
Basal + 2.0 ppm selenium	1.03	123,088

Although when expressed on this basis the selenium supplemented groups tended to excrete less radioactivity than the deficient animals, the differences were not statistically significant.

It is evident, from the high correlation between urinary excretion and body weight, that the differences in excretion among the treatments are primarily the result of body weight differences, rather than to the supplementation with selenium. These results suggest that effects of selenium on increasing tocopherol retention as reported by Desai and Scott (1965) and Witting et al. (1967) are not

mediated through a reduction in urinary vitamin E excretion. The total urinary excretion over the three day period amounted to only 1 - 1.5% of the administered dose; hence, the urinary route probably would not be a major site of control of tocopherol retention.

The extent of urinary loss of the radioactivity in this experiment can be compared with published reports. Krishnamurthy and Bieri (1963) administered ^{14}C-labeled tocopherol to rats, and found about 0.5% of the dose was excreted in the urine within 24 hours. Less than 0.5% of a dose of ^{14}C-labeled tocopherol was recovered in the urine of rats during an eight day interval after dosing in an experiment conducted by Johnson and Pover (1962). These reports substantiate the findings of this experiment that urinary excretion is a minor path of tocopherol loss. On the other hand, Simon et al. (1956) reported that 70-80% of the activity excreted by rabbits given a dose of ^{14}C-labeled tocopherol was lost in the urine. Sternberg and Pascoe-Dawson (1959) found a loss of 37% of a dose of labeled tocopherol in the urine of rats 64 hours after administration. Presumably the level of urinary excretion is a function of the administered dose and the tocopherol status of the individual. In this experiment, the animals were tocopherol-deficient and the administered dose was low; under these conditions a low level of excretion would be expected.

Fecal Excretion of Radioactivity.

The pattern of fecal excretion of radioactivity was similar to that observed for urine, with the bulk of the excretion occurring in the first 24 hour period. Total fecal excretion and percent of dose excreted were not markedly affected by treatment (Tables 9 and 10). The differences are attributable in most part to the variation in mean body weight among treatments. When expressed on the basis of body weight (Table 11), the fecal cpm/gm body weight decreased with each level of selenium. That this is probably due to body weight differences is suggested by the fact that the correlation coefficient for the relationship body weight vs. cpm/gm body weight is -0.63, indicating that the smaller animals excreted proportionately more of the dose. These results do not indicate a significant influence of selenium on the fecal excretion of α-tocopherol.

The level of excretion compares well with that observed by Krishnamurthy and Bieri (1963), who found about 36-38% of an oral dose of ^{14}C-labeled α-tocopherol administered to rats was excreted in the feces within the first 24 hours. They determined that the excreted activity was due entirely to unchanged α-tocopherol, indicating that degradation of the molecule in the gastro-intestinal tract does not occur.

Approximately 1.5% of the dose remained in the

Table 9. Fecal excretion of radioactivity - Trial II.

Treatment	0-24 hrs cpm	24-48 hrs cpm	48-72 hrs cpm	72 hr Total cpm
Basal	3, 719, 488	1, 520, 625	218, 825	5, 485, 938
0. 5 ppm selenium	4, 426, 871	1, 025, 607	276, 200	5, 728, 678
1. 0 ppm selenium	5, 446, 888	526, 781	247, 263	6, 220, 932
2. 0 ppm selenium	3, 982, 136	702, 021	270, 450	4, 954, 607

Table 10. Fecal excretion of radioactivity per gram of body weight

Treatment	0-24 hrs cpm/g BW	24-48 hrs cpm/g BW	48-72 hrs cpm/g BW	72 hr Total cpm
Basal	20, 791	8, 500	1, 223	30, 514
0. 5 ppm selenium	21, 647	5, 015	1, 351	28, 013
1. 0 ppm selenium	22, 676	2, 193	1, 029	25, 899
2. 0 ppm selenium	15, 228	2, 685	1, 034	18, 947

Table 11. Percent of dose excreted in feces - Trial II.

Treatment	0-24 hrs %	24-48 hrs %	48-72 hrs %	72 hr Total %
Basal	31. 3	12. 6	2. 0	45. 9
0. 5 ppm selenium	36. 6	8. 5	2. 3	47. 4
1. 0 ppm selenium	45. 7	4. 4	2. 0	52. 1
2. 0 ppm selenium	33. 4	5. 9	2. 3	41. 6

gastro-intestinal tract at the end of the three day period, and this proportion did not differ significantly among treatments. Since coprophagy was not completely prevented, undoubtedly some recycling of the labeled tocopherol did occur. Small amounts of α-tocopherol are apparently excreted into the gastro-intestinal tract, probably via the bile, since Krishnamurthy and Bieri (1963) found detectable levels in the intestine 21 days after dosage of labeled tocopherol to rats fitted with tail cups to prevent coprophagy.

A summary of the fecal and urinary excretion of radioactivity is presented in Table 12.

Table 12. Total 72 hour excretion of radioactivity - Trial II.

Treatment	% of Dose Excreted		
	Urine	Feces	Total
Basal	1.48	45.79	47.27
Basal + 0.5 ppm selenium	1.10	47.52	48.62
Basal + 1.0 ppm selenium	1.00	52.16	53.16
Basal + 2.0 ppm selenium	0.92	41.73	42.65

In summary, there was no apparent effect of level of dietary selenium on the excretion of a dose of tritium-labeled α-tocopherol, implying little effect of selenium on the retention of the vitamin.

Trial III

The objectives of this experiment were to examine the effects of selenium supplementation of a selenium- and vitamin E-deficient

diet on the fecal and urinary excretion of a dose of tritium-labeled α-tocopherol by rats, and on the distribution of radioactivity in the blood, heart and liver.

Growth and Mortality

The average final weights for each group were as follows:

Basal	185.6 gm ± 12.0
Basal + selenium	225.0 gm ± 21.5
Basal + selenium (-ethoxyquin)	209.4 gm ± 16.7
Basal + vitamin E	215.9 gm ± 27.4

As in Trial II, a positive growth response to selenium supplementation was observed. The vitamin E supplemented group showed a similar response. There were no mortalities in the supplemented groups, while on the basal diet 50% mortality occurred. Liver necrosis was established as the cause of death in most cases. An additional four animals in each of the basal and selenium-supplemented groups received their respective diets for six weeks; three days before administration of the labeled tocopherol, ethoxyquin was omitted from the diet. The intention was to determine if any effect of the dietary selenium on the distribution of the dose was due to antioxidant activity of selenium. Ethoxyquin is rapidly excreted from the body; negligible quantities would be retained three days after its removal from the diet (Wiss et al., 1962). Only one animal in the

basal group survived this treatment. One of the rats died within the three day pre-dosing period, and the other two died before the three day period after dosing was completed. They appeared normal until a few hours before death, when they became lethargic. The urine was deeply pigmented, presumably with blood or blood pigments. Autopsy revealed extensive internal hemorrhaging. It was not established if the urine pigmentation was due to rupture of blood vessels or to hemolysis, a symptom provoked by antioxidant deficiency (Søndergaard, 1967). No adverse effects from the omission of the synthetic antioxidant were observed in the selenium supplemented group.

Urinary Excretion of Radioactivity

The urinary excretion of a single dose of tritium followed a similar pattern to that observed in Trial II --viz. -- a progressive decrease over the three day period. In Table 13 is listed the total cpm found in the urine; the vitamin E supplemented group excreted almost twice as much as the other groups, in each of the 24 hour periods. This higher excretion rate is also reflected in the percent of dose (Table 14) and cpm/gm body weight (Table 15) lost in the urine. The amount of radioactivity excreted in the urine of the vitamin E supplemented animals suggests that the urinary pathway can represent a significant route for the elimination of vitamin E

Table 13. Urinary excretion of radioactivity - Trial III.

Treatment	0-24 hrs cpm	24-48 hrs cpm	48-72 hrs cpm	0-72 hrs cpm
Basal	271, 900	83, 850	38, 500	394, 250
Basal (-Ethoxyquin)	159, 820	52, 370	21, 545	233, 735
Basal + Selenium	216, 898	98, 087	79, 157	394, 142
Basal + Selenium (-Ethoxyquin)	226, 374	118, 153	47, 585	392, 112
Basal + Vitamin E	401, 855	199, 511	123, 559	724, 925

Table 14. Percent of dose excreted in urine - Trial III.

Treatment	0-24 hrs %	24-48 hrs %	48-72 hrs %	0-72 hrs %
Basal	2. 30	0. 71	0. 32	3. 33
Basal (-Ethoxyquin)	1. 43	0. 46	0. 19	2. 08
Basal + Selenium	2. 01	0. 90	0. 73	3. 64
Basal + Selenium (-Ethoxyquin)	2. 04	1. 07	0. 43	3. 54
Basal + Vitamin E	3. 62	1. 79	1. 09	6. 50

Table 15. Urinary excretion of radioactivity per gram of body weight - Trial III.

Treatment	0-24 hrs cpm/gm	24-48 hrs cpm/gm	48-72 hrs cpm/gm	0-72 hrs cpm/gm
Basal	1465	451. 8	207. 4	2124. 2
Basal (-Ethoxyquin)	881. 0	288. 7	118. 8	1288. 5
Basal + Selenium	964. 0	435. 9	351. 8	1751. 7
Basal + Selenium (-Ethoxyquin)	1081. 1	564. 2	227. 2	1872. 6
Basal + Vitamin E	1861. 3	924. 1	572. 3	3357. 7

or its metabolites. There was no significant difference between the basal and selenium-supplemented groups. Unlike the situation in Trial II, there was not a significant correlation between urinary excretion and body weight. This may be a reflection of the smaller range of body weights in this experiment.

The distribution of the urine radioactivity between water and benzene was examined. Activity in the water phase may represent that of water-soluble metabolites; Simon et al. (1957) reported the presence of a quinone (tocopheronolactone) excreted in the urine as a glucuronide. Benzene-soluble activity, on the other hand, may be due to the excretion of unmetabolized tocopherol. The distribution of activity between the two solvents is shown in Table 16. Activity in the water phase was predominant, being approximately 70-75% of the total activity in most cases. It is of interest that in the vitamin E supplemented group, almost one-half of the activity was in the benzene phase, suggesting the excretion of unchanged tocopherol. There was no difference between the basal and selenium-supplemented groups in the distribution of activity in the water and benzene phases.

Table 16. Distribution of radioactivity in urine - Trial III.

Treatment	% of water-soluble activity in urine		
	Day 1	Day 2	Day 3
Basal	75.8	75.0	77.7
Basal (-ethoxyquin)	60.8	75.1	63.9
Basal + selenium	70.1	70.2	77.6
Basal + selenium (-ethoxyquin)	69.0	69.6	60.9
Basal + vitamin E	64.0	55.3	54.2

The activity in the urine as expected, was inversely related to the plasma activity at 72 hours. The percent of the dose in the urine was significantly correlated ($P < 0.01$) with the cpm/ml plasma ($r = -0.65$), as was the urine cpm/gm body weight with the cpm/ml plasma ($P < 0.05$, $r = -0.49$).

Fecal Excretion of Radioactivity

As in Trial II, the fecal excretion of activity dropped off rapidly over the three day period, with about 25% of the dose being excreted in the first 24 hours, and about 3% excreted in the last 24 hour period. The excretion via the feces is tabulated in Tables 17, 18, and 19. The basal group tended to excrete relatively more of the activity in the first 24 hours than the others; the first day's output was about 80% of the total excretion for the basal animals, while for the selenium and vitamin E supplemented groups, about 50% of the total fecal excretion occurred in the first 24 hours.

The total fecal excretion of radioactivity was highest in the vitamin E supplemented group, but the difference from the other groups was not statistically significant. There were no significant differences due to treatment. These data, along with the results of Trial II, suggest that selenium does not influence fecal excretion, or, by implication, absorption of α-tocopherol. Since the bile may be a route of tocopherol excretion (Krishnamurthy and Bieri, 1963),

Table 17. Fecal excretion of radioactivity - Trial III.

Treatment	0-24 hrs cpm	24-48 hrs cpm	48-72 hrs cpm	0-72 hrs cpm
Basal	3, 389, 913	716, 438	228, 638	4, 334, 989
Basal (-ethoxyquin)	2, 635, 850	724, 450	427, 650	3, 787, 950
Basal + Selenium	1, 391, 725	1, 591, 013	466, 906	3, 449, 644
Basal + Selenium (-ethoxyquin)	2, 872, 725	1, 298, 938	392, 450	4, 564, 113
Basal + Vitamin E	2, 708, 725	1, 437, 088	645, 375	4, 791, 188

Table 18. Fecal excretion of radioactivity per gram of body weight - Trial III.

Treatment	0-24 hrs cpm/gm BW	24-48 hrs cpm/gm BW	48-72 hrs cpm/gm BW	0-72 hrs cpm/gm BW
Basal	18, 265	3860	1232	23, 357
Basal (-ethoxyquin)	14, 531	3994	2358	20, 882
Basal + Selenium	6, 185	7072	2075	15, 332
Basal + Selenium (ethoxyquin)	13, 719	6203	1874	21, 796
Basal + Vitamin E	12, 546	6656	2989	22, 192

Table 19. Percent of dose excreted in feces - Trial III.

Treatment	0-24 hrs %	24-48 hrs %	48-72 hrs %	0-72 hrs %
Basal	28. 46	6. 15	1. 89	36. 50
Basal (-ethoxyquin)	23. 62	6. 49	3. 83	33. 94
Basal + Selenium	17. 06	12. 68	3. 03	32. 77
Basal + Selenium (-ethoxyquin)	25. 65	11. 52	3. 56	40. 73
Basal + Vitamin E	21. 53	16. 78	5. 98	44. 29

it is possible that differences in absorption could exist but remain undetected with the technique used in this experiment. However, with rats equipped with tail cups to prevent coprophagy, Mellors and Barnes (1966) found no evidence that the bile is a significant pathway of tocopherol excretion.

Blood Plasma Levels of Radioactivity

The mean radioactivity per ml of plasma for each group is tabulated in Table 20. The activity was significantly higher ($P < 0.01$) in the basal group than in the others, and significantly lower ($P < 0.01$) in the vitamin E supplemented group. The protein-bound activity was also determined, and was significantly lower ($P < 0.01$) in the vitamin E supplemented group than in the others. The percent of the activity that was protein-bound did not differ significantly among treatments. The differences were not due to body weight variations, since there was no correlation between plasma cpm and body weight; hence the differences may be ascribed to treatment effects. The total plasma level of radioactivity was calculated on the basis that the blood volume represents 6% of the body weight. On this basis, the basal group had the highest percent of the dose in the plasma (0.80%), while the vitamin E supplemented group had a very low level (0.07%).

Table 20. Plasma and plasma protein-bound radioactivity - Trial III.

	Basal	Basal + Selenium	Basal + Selenium (-ethoxyquin)	Basal + Vitamin E
Plasma cpm/ml	9438 ± 2760	5220 ± 484	3696 ± 881	654 ± 358
Protein-bound cpm/ml plasma	3003 ± 885	2140 ± 768	1442 ± 633	162 ± 81
% protein-bound	35.5 ± 14.5	43.4 ± 17.9	43.6 ± 22.9	29.1 ± 12.9
Total plasma cpm*	96,421	69,527	45,764	8,817
% of dose in plasma*	0.80	0.63	0.40	0.07
mg tocopherol/100 ml plasma	1.40 ± 0.04	1.25 ± 0.02	---	2.31 ± 0.02

*Calculated on the basis that blood volume is 6% of body weight.

The effect of supplementary selenium on the blood plasma tocopherol level was in direct contrast to the results of Desai and Scott (1965) who found that with added selenium the blood tocopherol level was elevated in chicks. On the basis of these findings, they postulated that selenium may function in the blood transport of α-tocopherol. The relationship between dietary selenium level and plasma tocopherol has not been examined in detail by other workers, but incidental observations by various investigators substantiate the results of this experiment. Burk et al. (1968), working with rats, reported that with a diet containing a low level of vitamin E, addition of 0.5 ppm selenium to the diet resulted in lower plasma vitamin E levels than when selenium was omitted. They suggested that selenium may enhance the disappearance of vitamin E from the plasma when the diet contains a low level of tocopherol. Working with sheep, Boyazoglu et al. (1967) also found that dietary selenium significantly depressed serum vitamin E levels, in what they describe "may have been a sparing action of selenium in mobilizing tissue tocopherols". Similarly, in Oregon State studies of white muscle disease in sheep, Schubert et al. (1961) reported data indicating that lambs from ewes receiving either selenium injections or dietary selenium supplementation had lower serum vitamin E levels that lambs from selenium-deficient ewes. These studies agree with the findings of this experiment--viz.--that selenium appears to

promote tocopherol withdrawal from the plasma, presumably by stimulating tissue uptake. On the other hand, Buchanan-Smith et al. (1968) reported that selenium supplementation significantly increased plasma vitamin E levels in ewes; it was not clear if these animals were also receiving supplementary α-tocopherol.

The plasma protein-bound radioactivity was measured to investigate the proposal of Desai and Scott (1965) that a selenium-containing plasma protein may transport α-tocopherol in the blood. If this theory is correct, presumably the protein-bound fraction would be greater in selenium supplemented animals. The proportion of the activity that was protein-bound did tend to be greater in the selenium-supplemented group (Table 20) but the difference was not statistically significant. A high degree of variability in the percent protein-bound activity was observed within all treatments. The percent that was protein-bound was approximately 40%, indicating that an effect of selenium on the plasma proteins probably would not constitute a major mechanism of controlling plasma tocopherol levels. The proportion of the plasma vitamin E that was protein bound is lower than observed by Lewis et al. (1954), who reported a value of 74% of the tocopherol in human serum carried by the α- and β-lipoprotein fractions. In rats, Sternberg and Pascoe-Dawson (1959) reported a value of 58% of the blood radioactivity associated with a dose of ^{14}C-labeled tocopherol was carried by the

lipoproteins; there was none in the albumin fraction.

The very low radioactivity of the plasma of the vitamin E-supplemented group is of interest. While the urinary excretion of radioactivity was greatest in this group, it would not seem of sufficient magnitude to account for the low plasma level. The fact that the retention of the administered dose in the plasma was low indicates that the blood is not a significant reservoir of α-tocopherol, and that some other tissue(s) in the body has the capacity to remove large amounts of vitamin E from the circulatory system. The liver is probably the major site of initial storage, since Wiss et al. (1962) found a direct correlation between tocopherol intake and liver tocopherol over a wide range of dietary levels.

Plasma protein-binding of α-tocopherol is associated with the α- and β-lipoprotein fractions (Lewis et al., 1954; McCormick et al., 1960; Desai and Scott, 1965). Erwin et al. (1961) suggested that an antioxidant shortage could lead to damage of the lipoprotein transport system. They proposed that specific serum proteins could be damaged by peroxides resulting from autooxidation of lipids bound to circulating proteins. Nishida and Kummerow (1960) demonstrated the specific in vitro destruction of the β lipoprotein fraction, which binds tocopherol, by the hydroperoxide of methyl linoleate. Erwin et al. (1961) hypothesized that selenium, as an integral part of the lipoprotein, may function as an antioxidant to maintain the

lipoprotein integrity. The more recent work of Desai and Scott (1965), who reported an association of ^{75}Se with the tocopherol-binding lipoproteins, supports this concept. An alteration of the serum protein pattern in dystrophic animals has been observed; Oppenheimer et al. (1958) found an altered lipoprotein distribution in vitamin E deficient rabbits. The results of this experiment do not support the idea of a role of selenium in facilitating protein-binding of tocopherol, since the protein-bound tocopherol was higher in the basal group than in the selenium-supplemented animals. The omission of the synthetic antioxidant ethoxyquin from the diet did result in a lowering of the plasma level of radioactivity; this could be a reflection of either a protective effect of the antioxidant on the transport system, or a greater tocopherol requirement of the tissues.

The chemically measured tocopherol levels in the pooled plasma samples of each group followed the same pattern as did the levels of radioactivity. The plasma level in the pooled sample of the basal group was 1.40 mgm per 100 ml, whereas it was 1.25 mgm per 100 ml for the selenium-supplemented group. These results, in conjunction with the data on the plasma radioactivity, do not support the concept of a role for selenium in maintaining a high blood level of vitamin E, but rather suggests a function of selenium in promoting the withdrawal of vitamin E from the circulatory system.

Radioactivity in Heart and Liver Tissue

Since liver necrosis is a symptom of vitamin E deficiency in rats, indicating a selective susceptibility of this tissue to tocopherol deficiency, the liver was selected for examination in this experiment. The liver reportedly accumulates tocopherol in direct proportion to tocopherol intake in animals receiving various dietary levels of vitamin E (Wiss et al., 1962). It may be a regulatory organ in vitamin E metabolism since Krishnamurthy and Bieri (1963) found that the livers from vitamin E-deficient rats accumulated maximum uptake of ^{14}C-labeled α-tocopherol within 2.5 hours after the administration of the dose. At 24 hours after dosing, there was more labeled tocopherol in the liver than in all other organs combined. There was a fairly rapid loss of tocopherol after 24 hours, suggesting a role of the liver in accumulating tocopherol rapidly and then releasing it as required to maintain the blood tocopherol level. The lack of prolonged retention of tocopherol in the liver compared to other organs may account for its susceptibility to necrosis in vitamin E deficiency states. It was of interest, then, to determine the influence of dietary vitamin E and selenium on the retention of a dose of labeled tocopherol by the liver.

Heart tissue was also investigated, since it is a representative of muscle tissue, and is frequently subject to damage in white

muscle disease (Muth, 1963). Krishnamurthy and Bieri (1963) found that the heart retained an almost constant amount of a dose of labeled tocopherol for 14 days following dosing, after which there was a gradual decline.

The distribution of radioactivity in the heart and liver for each group is given in Table 21. There was considerable variability within treatments, with a tendency for the selenium-supplemented group to have higher levels of radioactivity in both organs, but the difference was not statistically significant. The retention of radioactivity in the heart and liver of the vitamin E-supplemented group was significantly lower ($P < 0.01$) than in the basal and selenium-supplemented groups. When the data were adjusted for differences in organ weight and body weight by multiplying the total organ cpm by the ratio of organ weight to body weight, the same pattern was observed. Thus dietary supplementation with vitamin E resulted in reduced retention of the administered dose, while supplementary selenium was without significant effect.

The percent retention of the dose in heart and liver tissue was lower than that observed by Krishnamurthy and Bieri (1963) with rats killed 24 hours after dosage. They reported values of 5.5% for liver and 0.37% for heart tissue. While these figures are somewhat higher than those found in this experiment, approximately the same ratio between organs was observed.

Table 21. Distribution of radioactivity in heart and liver tissue - Trial III.

	Basal	Basal + Selenium	Basal + Se (Eq)*	Basal + Vit E
		Heart		
% of dose	.03	.07	.04	.01
Total cpm	4693	7785	5435	2068
cpm/gm tissue	7957	10293	7929	2983
$\frac{\text{Organ wt}}{\text{Body wt}}$ x organ cpm	15.75	26.82	18.54	6.38
		Liver		
% of dose	0.11	.14	.07	.03
Total cpm	14258	16484	8432	3529
cpm/gm tissue	1758	1926	1165	492
$\frac{\text{Organ wt}}{\text{Body wt}}$ x organ cpm	693.4	643.0	309.4	126.1

*Selenium (-ethoxyquin).

A number of correlations between heart, liver and plasma levels of radioactivity were calculated. There was not a significant correlation between the percent of dose in the heart and in the liver, nor between the percent of dose in either organ with the cpm/ml of plasma. The only statistically significant correlations were those relating liver cpm with plasma cpm. The cpm/gm of liver tissue was significantly correlated ($P < 0.05$) with the cpm/ml plasma ($r = 0.50$), and the total liver cpm multiplied by the ratio of liver weight to body weight was significantly correlated ($P < 0.05$) with

the cpm/ml plasma (r = 0.59). One interpretation of these data would be that a role of the liver in maintaining the blood tocopherol level is suggested. The liver may rapidly accumulate tocopherol after the administration of a dose, and then release it into the blood gradually. The observation of Krishramurthy and Bieri (1963) that the liver reaches its peak concentration of tocopherol very soon after dosage, and then loses it fairly rapidly, supports this suggested role of the liver in tocopherol metabolism. This aspect was examined in more detail in Trial IV, in which the relationship between liver and blood tocopherol levels was examined over varying intervals after dosage.

Trial IV

This experiment was intended to provide information on the effect of selenium on the time distribution of a dose of tritium-labeled α-tocopherol in the rat. Blood plasma, and heart and liver tissue were examined for their content of radioactivity at intervals of 12, 24, 48 and 72 hours after dosing.

Radioactivity in Plasma and Organs

The radioactivity associated with the plasma, heart and liver for each group is tabulated in Tables 22 and 23. To more clearly indicate the nature of the time changes in distribution of the labeled material, the percentage of the dose in the plasma, heart and liver

Table 22. Radioactivity in plasma and organs - Trial IV.

Group	Plasma cpm/ml	Heart Total cpm	Heart cpm/gm	Liver Total cpm	Liver cpm/gm
Basal					
12 hrs*	5668 ± 633	912 ± 81	1188 ± 62	59685 ± 26033	6277 ± 2870
24 hrs	1962 ± 407	730 ± 119	959 ± 157	43416 ± 9076	4183 ± 767
48 hrs	869 ± 198	1072 ± 179	1265 ± 237	36083 ± 8575	3228 ± 886
72 hrs	998 ± 131	893 ± 139	1177 ± 242	46182 ± 6272	3989 ± 257
Basal + Selenium					
12 hrs	3296 ± 811	537 ± 70	682 ± 100	52426 ± 12019	5049 ± 1068
24 hrs	1622 ± 520	637 ± 152	817 ± 192	53756 ± 27271	4523 ± 2255
48 hrs	425 ± 94	363 ± 62	460 ± 38	21080 ± 4754	2096 ± 615
72 hrs	759 ± 402	555 ± 250	753 ± 359	18705 ± 8338	1901 ± 931

*Hours after administration of the dose.

Table 23. Percentage of dose[1] in plasma and organs - Trial IV.

Group	Plasma per ml	Heart Total	Liver Total	Heart per gm	Liver per gm
Basal					
12 hrs[2]	12.03 ± 1.31	1.94 ± 0.17	124.94 ± 50.63	2.52 ± 0.11	13.15 ± 5.62
24 hrs	4.53 ± 0.99	1.68 ± 0.28	100.34 ± 22.53	2.21 ± 0.36	9.66 ± 1.90
48 hrs	1.97 ± 0.33	2.45 ± 0.35	81.50 ± 14.82	2.88 ± 0.44	7.27 ± 1.54
72 hrs	2.14 ± 0.25	1.91 ± 0.30	99.21 ± 14.82	2.52 ± 0.51	8.56 ± 0.67
Selenium					
12 hrs	7.62 ± 1.65	1.26 ± 0.19	121.96 ± 28.12	1.59 ± 0.24	11.72 ± 2.37
24 hrs	3.59 ± 1.41	1.44 ± 0.37	120.60 ± 59.58	1.85 ± 0.49	10.21 ± 5.11
48 hrs	0.96 ± 0.19	0.81 ± 0.09	47.55 ± 10.96	1.04 ± 0.03	4.72 ± 1.41
72 hrs	1.61 ± 0.86	1.20 ± 0.58	40.01 ± 18.49	1.61 ± 0.78	4.02 ± 1.94

[1] All figures refer to percent of dose x 10^2.

[2] Hours after administration of the dose.

at each time period is shown for the two groups in Figures 1 and 2. The peak level of radioactivity for both plasma and liver was at 12 hours, and by 48 hours had reached a fairly stable level. The content of radioactivity in the heart did not show much variation, and was approximately constant over the entire 72 hour period. The differences between the selenium-supplemented and the basal groups were not statistically significant, but in all cases the levels of radioactivity were higher in the basal group. The blood plasma level of activity is of interest, since it was found in Trial III that selenium supplementation resulted in a lowered retention of the dose in the plasma, suggesting an effect of selenium in promoting withdrawal of vitamin E from the blood. The same pattern was observed in this trial, except that the magnitude of the difference was not as great. This is probably due to the fact that the period on the deficient diet was shorter in Trial IV, so the animals may not have been as selenium deficient as in the previous experiment. The tocopherol levels of the pooled plasma samples were 1.46 ± 0.01 mg per 100 ml for the basal group and 1.24 ± 0.03 mg per 100 ml for the selenium-supplemented group, further indicating a greater plasma retention of vitamin E by the basal group. The plasma levels of radioactivity in both groups fell off markedly between 12 and 24 hours; in each case the value at 24 hours was less than half of the 12 hour level. The highest level of activity was probably reached prior to 12 hours

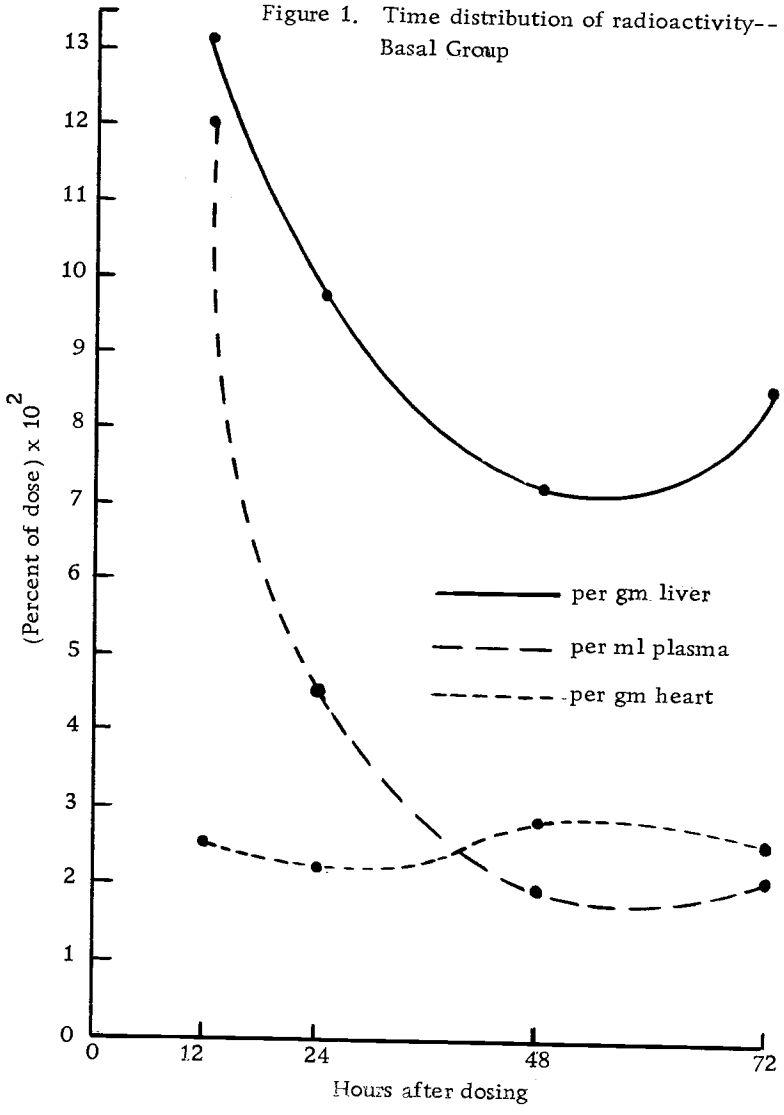

Figure 1. Time distribution of radioactivity--Basal Group

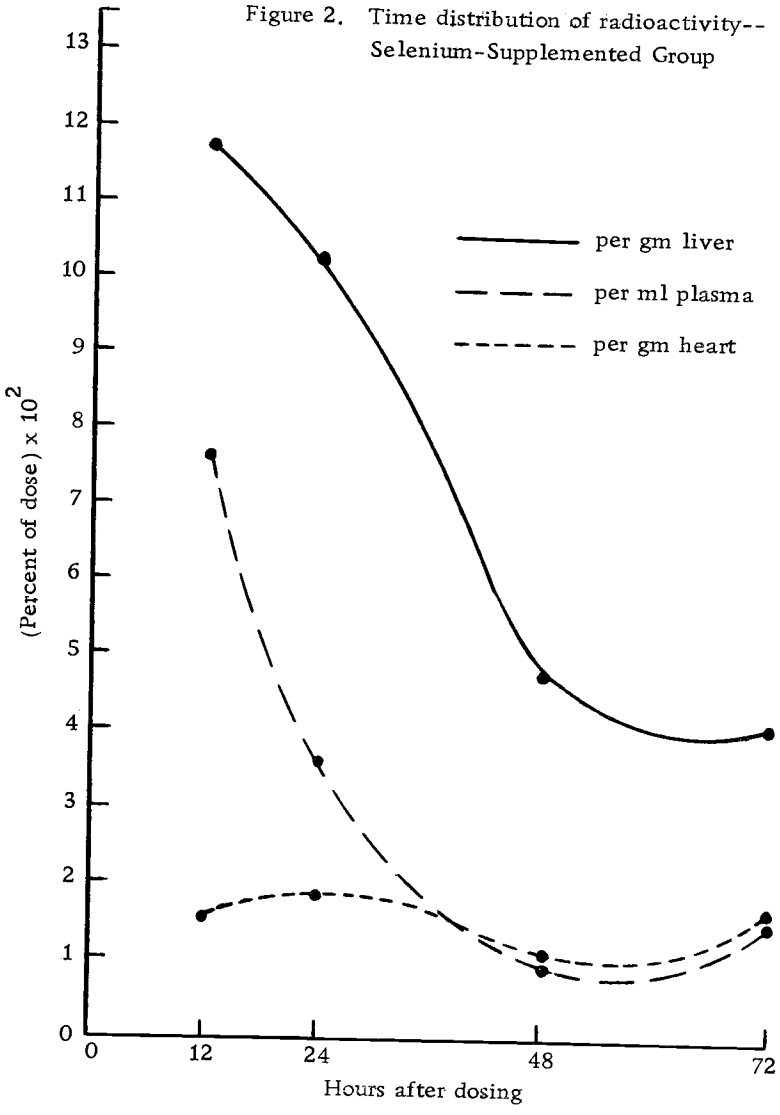

Figure 2. Time distribution of radioactivity--Selenium-Supplemented Group

after dosing; Scott (1967) found the maximum level in poults at about six hours after the dose was given. In the case of the liver, the peak activity was also probably attained in less than 12 hours; Krishnamurthy and Bieri (1963) reported the maximum liver level at 2.5 hours after dosing in their experiment. As can be seen from Figures 1 and 2, the change with time of the plasma level of radioactivity fairly closely paralleled that of the liver. It was suggested in the discussion of the results of Trial III that the liver might have a function in accumulating vitamin E rapidly after its administration or ingestion, and then releasing it as required to maintain a fairly constant blood level. The results of this experiment do not support such a role.

The rapid depletion of labeled tocopherol from the plasma and liver suggests that examination of these tissues would not provide information on the status of tocopherol stores in the body.

The results of this experiment generally support the findings of Trial III, indicating that the plasma level of vitamin E is reduced when a selenium-deficient basal diet is supplemented with selenium. This effect was apparent at each time interval from 12 to 24 hours after dosing with radioactive vitamin E.

GENERAL DISCUSSION

The objective of this experimental program was to examine the effect of selenium supplementation of a selenium- and vitamin E-deficient basal diet on the distribution of a single dose of tritium-labeled α-tocopherol in the rat. This study was prompted by reports indicating that a biological role of selenium may reside in its effects on the distribution of α-tocopherol in animal tissue. Desai and Scott (1965) found that in the chick, supplementary selenium caused a marked increase in the retention of a dose of radioactive tocopherol in the plasma, and that the distribution of radioactive tocopherol and radioselenium in the plasma proteins followed a similar pattern. On the basis of these results, they speculated that a specific selenium-containing lipoprotein may function in the transport of vitamin E in the blood, and possibly enhance the transport of tocopherol across membranes. Subsequently, Thompson and Scott (1968) reported that the whole body retention of a dose of labeled tocopherol was up to 100 times greater in chicks receiving supplementary selenium than in those on a selenium-deficient diet. Witting et al. (1967) observed that with rats receiving two levels of dietary selenium, the α-tocopherol content of the carcasses was greater in the group receiving the higher level of selenium. These reports indicate a role of selenium in improving the retention of vitamin E.

In this study, results in direct contrast to those of Desai and Scott (1965) were obtained. Whereas they reported that supplementary selenium increased the blood level of vitamin E in chicks, it was observed in both Trials III and IV that in the rats receiving added selenium, the plasma level of activity was lower than in the basal groups. This was determined both by measuring the radioactivity and by determining chemically the level of α-tocopherol. These data suggest a role for selenium in removal of vitamin E from the blood, rather than in increasing its retention. There are reports in the literature supporting both findings. Burk et al. (1968) found that in rats the addition of 0.5 ppm selenium to a basal diet containing a low level of vitamin E resulted in a lowering of the plasma vitamin E level. Similarly, Boyazoglu et al. (1967) found that in sheep, supplementation with selenium of a deficient diet significantly depressed the serum vitamin E levels. Paulson et al. (1968) also reported that selenium supplementation tended to lower plasma tocopherol levels in ewes. In support of the work of Desai and Scott (1965), Buchanan-Smith et al. (1968) reported that selenium supplementation significantly increased plasma vitamin E levels in ewes. These opposing findings might be explained on the basis of the dosages or dietary levels of vitamin E. Burk et al. (1968) found that with a low dietary level of tocopherol, supplementary selenium resulted in reduced plasma vitamin E levels in

rats, but added selenium was without effect when 200 mg vitamin E per kg diet was provided. In the work of Desai and Scott (1965) a dosage of five mg of tocopherol per chick was used, while in Trials III and IV of this study, dosages of 1.44 mg and 0.48 mg tocopherol per rat were given. On a body weight basis, the differences in dosage per unit of weight would probably be greater. Thus an explanation for the contrasting effects of selenium on the plasma vitamin E levels between this study and that of Desai and Scott (1965) may reside in the different dosage levels of α-tocopherol used. Another possible explanation might be that species differences exist, although evidence of contrasting responses in sheep is cited above.

According to the hypothesis of Desai and Scott (1965), selenium may function as a constituent of a selenolipoprotein which transports α-tocopherol in the blood. If this were the case, one would anticipate that the percentage of the plasma tocopherol that is protein-bound would be greater in selenium-supplemented animals than in deficient ones. This was examined in Trial III of this report; no evidence supporting this concept was obtained. The amount of activity that was protein-bound was highest in the basal group; the percentage of the plasma activity that was protein-bound was not affected by treatment.

The results obtained in these experiments do not support the concept of a role of selenium in increasing plasma retention of

tocopherol, and in fact suggest a role of selenium in promoting removal of tocopherol from the blood, presumably by stimulating tissue uptake.

The effect of selenium on the placental transfer of tritium-labeled vitamin E was investigated in Trial I. This aspect was of interest because of possible implication of a selenium-vitamin E interaction in placental transfer as a factor in white muscle disease. Oldfield et al. (1960) found that when vitamin E was administered orally to lambs, white muscle disease was prevented, but tocopherol supplementation of the ewes prior to lambing was only partially protective (Muth et al., 1958). Selenium administered to the dams is, however, fully protective. A role of selenium which would account for both observations is one of enhancing the placental transfer of vitamin E, thus preventing a tocopherol deficiency in the lambs. The objective of Trial I was to determine if selenium supplementation of a selenium- and vitamin E-deficient diet promoted the placental transfer of radioactive α-tocopherol using the rat as an experimental animal. The fetal uptake of the tritium-labeled tocopherol was significantly lower in rats receiving supplementary selenium than in those on a selenium-low diet; thus the presence of selenium reduced, rather than enhanced, the placental transfer of vitamin E. There are several possible interpretations of these results. Selenium might reduce the fetal requirement for tocopherol,

either by direct substitution for vitamin E in its metabolic mode of action, or by a "sparing" effect, perhaps by enhancing the biological activity of a given amount of tocopherol. Another interpretation of the effect of selenium in reducing the fetal uptake of vitamin E might be that in those animals supplemented with selenium, less of the administered dose of tocopherol may have been available for transfer to the fetuses. Thompson and Scott (1968) reported that in chicks, selenium promoted tissue retention of vitamin E, while Witting et al. (1967) reported similar findings with rats. In Trial I, if the maternal tissues of the selenium-supplemented rats retained a greater percentage of the dose than the basal group, less of the tocopherol would have been available for fetal uptake. This idea is not supported by the results of Trials II and III, in which no effect of selenium on the retention of vitamin E was observed. Since in these experiments, the same strain of rats, the same dosage level, and essentially the same diet was used as in Trial I, it can be concluded that it is unlikely that the lower placental transport of vitamin E in the selenium-supplemented group was due to greater maternal retention of the dose. Finally, the results of Trial I might be explained by an effect of selenium on the maternal blood levels of vitamin E. In Trials III and IV it was found that in selenium supplemented rats, the plasma tocopherol level was lower than in selenium-deficient animals. Thus the observed effect of selenium on the fetal uptake of vitamin E might be

the indirect result of a lower maternal plasma tocopherol level. The results of Trial III, while not directly applicable, do not support this view. In Trial I, approximately equal fetal uptake of the labeled tocopherol was observed in the rats receiving the basal diet supplemented with either 1 ppm selenium or 500 IU vitamin E per kg. In Trial III, in which essentially the same basal diet was used, the plasma radioactivity level was eight times higher at 72 hours after dosing in the rats receiving selenium than in those receiving 500 IU vitamin E per kg. If it is assumed that the ratio was of about the same magnitude at 24 hours, then the placental transfer of the labeled tocopherol would not have been proportional to the plasma level. This question would have been resolved if the maternal plasma level of radioactivity had been determined in Trial I, but unfortunately this was not done. Thus, while it was demonstrated that selenium supplementation of a selenium-deficient diet reduced the fetal uptake of a dose of α-tocopherol, it cannot be definitely concluded that this was a result of a "sparing effect" of selenium in reducing the fetal tocopherol requirement.

In Trials II and III, the effect of selenium on the excretion of a dose of tritium-labeled α-tocopherol was examined. Neither the urinary nor fecal excretion of the dose over a three day period was influenced by the level of supplementary selenium in the diet. The urinary excretion averaged about one to three percent of the dose,

and the fecal loss was 35-40% of the dose. These figures are in close agreement with those reported by Krishnamurthy and Bieri (1963). Supplementation of the diet with 500 IU of vitamin E per kg resulted in an increased loss of radioactivity via both the feces and urine; the effect on the urinary loss was the more pronounced, with almost twice as much activity excreted as compared to the basal group. The absorption of the labeled tocopherol was not directly determined, but since Mellors and Barnes (1966) reported that very low amounts of radioactivity were found in the bile following administration of labeled tocopherol to rats, it can probably be assumed that the radioactivity in the feces was due almost entirely to that which was not absorbed. Since the amount of radioactivity in the feces was not influenced by the level of selenium in the diet, it can be concluded that selenium did not influence the absorption of the labeled tocopherol. Similarly, the retention of the dose in the carcass was not directly measured, but can be assumed to be approximated by the difference between the administered dose and the amount excreted. Since there were no significant differences in excretion due to selenium supplementation, it is inferred that no differences in whole body retention of the dose occurred. Thus it is concluded that in the rat, selenium does not influence absorption, excretion or retention of vitamin E.

While this study was concerned mainly with the effect of selenium level on the retention of a dose of labeled tocopherol, some

information on the tissue distribution was also obtained. As previously discussed, a pronounced effect of selenium on the plasma level of radioactivity was observed. In Trial III, the heart and liver levels of radioactivity were measured; the selenium-supplemented group tended to have higher levels of activity in both organs, but the difference from the basal group was not statistically significant. In Trial IV, it was found that the liver activity followed that of the plasma, with the highest level at 12 hours after dosing. The radioactivity in the heart was approximately constant over the 12 to 72 hour interval. The susceptibility of the liver to necrosis in the rat in vitamin E deficiency states is probably due to its apparent low capacity to retain tocopherol. The rapid loss of vitamin E from both the plasma and liver suggests that they would not be suitable tissues for sampling to determine an individual's tocopherol status. The vitamin E level of both plasma and liver would reflect the current tocopherol intake, but would be subject to large variations depending upon the immediate previous intake of the vitamin.

In conclusion, the experimental work has indicated that in the rat, selenium does not influence the absorption, excretion or retention of α-tocopherol. It may modify the distribution of vitamin E in the body, since it was found that supplementation with selenium resulted in a significant lowering of the plasma vitamin E level, and tended to increase the amount found in the heat and liver. Placental

transfer of vitamin E was significantly reduced when the basal diet was supplemented with selenium. Possible interpretations of this finding are discussed.

SUMMARY

The placental transfer of a dose of tritium-labeled α-tocopherol was significantly lower in rats receiving one ppm selenium in the diet than in those on a selenium- and vitamin E-deficient basal diet. It was concluded that the most likely explanation of this finding was that selenium reduced the fetal tocopherol requirement, either by direct substitution or by enhancing the activity of the vitamin E. In two trials, no effect of selenium on the absorption or excretion of radioactive vitamin E was observed. This is in contrast to published reports indicating that in the chick selenium promotes the retention of vitamin E. The plasma level of labeled vitamin E was significantly lower in rats receiving supplementary selenium than in those on a deficient diet, indicating that selenium may have a role in promoting withdrawal of vitamin E from the blood. This result is in contrast to a report that in chicks selenium increases the plasma level of α-tocopherol. The liver and heart of selenium-supplemented animals tended to take up more of a dose of labeled tocopherol than those of deficient rats, but the differences were not statistically significant. The plasma, liver and heart exhibited contrasting patterns of retention of radioactivity; both the plasma and liver reached their maximum levels of radioactivity within 12 hours after administration of the labeled tocopherol, and between 12 and 72 hours the

level declined exponentially. The radioactivity of the heart remained virtually constant between 12 and 72 hours after dosage. These results indicate differences in retention time of tocopherol among various tissues, and suggest that while the blood and liver levels of tocopherol would provide a measure of current vitamin E intake, they would be unsatisfactory tissues for sampling to ascertain tocopherol stores.

The results are not in agreement with published reports indicating that selenium may promote the retention of vitamin E and enhance plasma tocopherol levels. Possible interpretations of the findings are discussed.

BIBLIOGRAPHY

Bieri, J. G. and E. L. Prival. 1965. Serum vitamin E determined by thin-layer chromatography. Proceedings of the Society for Experimental Biology and Medicine 120:554-557.

Boyazoglu, P. A., R. M. Jordan and R. J. Meade. 1967. Sulfur-selenium-vitamin E interrelationships in ovine nutrition. Journal of Animal Science 26:1390-1396.

Buchanan-Smith, J. G. et al. 1968. Vitamin E and selenium in ewe reproduction. (Abstract). Journal of Animal Science 27:1176.

Bull, R. C. and J. E. Oldfield. 1967. Selenium involvement in the oxidation by rat liver tissue of certain tricarboxylic acid cycle intermediates. Journal of Nutrition 91:237-246.

Bunnell, R. H., J. P. Keating and A. J. Quaresimo. 1968. Alpha-tocopherol content of feed stuffs. Journal of Agricultural and Food Chemistry 16:659-664.

Burk, R. F., Jr. et al. 1968. Tissue selenium levels during the development of dietary liver necrosis in rats fed torula yeast diets. Journal of Nutrition 95:420-428.

Butler, G. W. and P. J. Peterson. 1961. Aspects of the fecal excretion of selenium by sheep. New Zealand Journal of Agricultural Research 4:484-491.

Caldwell, K. A. and A. L. Tappel. 1965. Acceleration of sulfhydryl oxidations by selenocystine. Archives of Biochemistry and Biophysics 112:196-200.

Calvert, C. C., M. C. Nesheim and M. L. Scott. 1962. Effectiveness of selenium in prevention of nutritional muscular dystrophy in the chick. Proceedings of the Society for Experimental Biology and Medicine 109:16-18.

Calvert, C. C., M. C. Nesheim and M. L. Scott. 1962. Effectiveness of selenium in prevention of nutritional muscular dystrophy in the chick. Proceedings of the Society for Experimental Biology and Medicine 109:16-18.

Calvert, C. C., I. D. Desai and M. L. Scott. 1964. Effect of linoleic acid on nutritional muscular dystrophy in the chick. Journal of Nutrition 83:307-313.

Century, B. and M. K. Horwitt. 1960. Role of diet lipids in the appearance of dystrophy and creatinuria in the vitamin E-deficient rat. Journal of Nutrition 72:357-367.

Chow, C. K. et al. 1967. The metabolism of C^{14}-α-tocopheryl quinone and C^{14} - α-Tocopheryl hydroquinone. Lipids 2:390-396.

Corwin, L. M. and K. Schwarz. 1960. Prevention of decline of alpha-ketoglutarate and succinate oxidation in vitamin E-deficient rat liver homogenates. Journal of Biological Chemistry 235:3387-3392.

Crider, Q. E., P. Alaupovic and B. Connor Johnson. 1961. On the function and metabolism of vitamin E. III. Vitamin E and antioxidants in the nutrition of the rat. Journal of Nutrition 73:64-70.

Dam, H. and E. Søndergaard. 1957. Prophylactic effect of selenium dioxide against degeneration (white striation) of muscles in chicks. Experentia 13:494.

Desai, I. D., C. C. Calvert and M. L. Scott. 1964. A time-sequence study of the relationship of peroxidation lysosomal enzymes and nutritional muscular dystrophy. Archives of Biochemistry and Biophysics 108:60-64.

Desai, I. D., C. K. Parekh and M. L. Scott. 1965. Absorption of d- and l-α-tocopheryl acetates in normal and dystrophic chicks. Biochemica et Biophysica Acta 100:280-282.

Desai, I. D. and M. L. Scott. 1965. Mode of action of selenium in relation to biological activity of tocopherols. Archives of Biochemistry and Biophysics 110:309-315.

Desai, I. D. 1966. Effect of kidney beans on plasma tocopherol level and its relation to nutritional muscular dystrophy in the chick. Nature 209:810.

Edwin, E. E. et al. 1961. Studies on vitamin E. 6. The distribution of vitamin E in the rat and the effect of α-tocopherol and dietary selenium on ubiquinone and ubichromenol in tissues. Biochemistry Journal 79:91-105.

Ehlig, C. F. et al. 1967. Fate of selenium from selenite or selenomethionine, with or without vitamin E, in lambs. Journal of Nutrition 92:121-126.

Erwin, E. S. et al. 1961. Etiology of muscular dystrophy in the lamb and chick. Journal of Nutrition 75:45-50.

Ewan, R. C., C. A. Baumann and A. L. Pope. 1968. Effects of selenium and vitamin E on nutritional muscular dystrophy in lambs. Journal of Animal Science 27:751-756.

Fitch, C. D. and J. F. Diehl. 1965. Metabolism of l-α-tocopherol by the vitamin E deficient rabbit. Proceedings of the Society for Experimental Biology and Medicine 119:553-557.

Ganther, H. E. and C. A. Baumann. 1962a. Selenium metabolism. I. Effects of diet, arsenic and cadmium. Journal of Nutrition 77:210-216.

Ganther, H. E. and C. A. Baumann. 1962b. Selenium metabolism. II. Modifying effects of sulphate. Journal of Nutrition 77: 408-414.

Gardner, R. W. and D. E. Hogue. 1967. Milk levels of selenium and vitamin E related to nutritional muscular dystrophy in the suckling lamb. Journal of Nutrition 93:418-424.

Halverson, A. W., P. L. Guss and O. E. Olson. 1962. Effect of sulfur salts on selenium poisoning in the rat. Journal of Nutrition 77:459-464.

Hartley, W. J. and A. B. Grant. 1961. A review of selenium-responsive diseases of New Zealand livestock. Federation Proceedings 20:679-688.

Hidiroglou, M., R. B. Carson and G. A. Brossard. 1965. Influence of selenium on the selenium contents of hair and on the incidence of nutritional muscular dystrophy in beef cattle. Canadian Journal of Animal Science 45:197-202.

Hidiroglou, M., D. P. Heaney and K. J. Jenkins. 1968. Metabolism of inorganic selenium in rumen bacteria. Canadian Journal of Physiology and Pharmacology 46:299-332.

Hintz, H. F. and D. E. Hogue. 1964a. Kidney beans (Phaseolus vulgaris) and the effectiveness of vitamin E for prevention of nutritional muscular dystrophy in the chick. Journal of Nutrition 84:283-287.

Hintz, H. F. and D. E. Hogue. 1964b. Effect of selenium, sulfur and sulfur amino acids on nutritional muscular dystrophy in the lamb. Journal of Nutrition 82:495-498.

Hogue, H. F. et al. 1962. Relation of selenium, vitamin E and an unidentified factor to muscular dystrophy (stiff-lamb or white muscle disease) in the lamb. Journal of Animal Science 21: 25-29.

Hopkins, L. L., Jr., A. L. Pope and C. A. Baumann. 1964. Contrasting nutritional responses to vitamin E and selenium in lambs. Journal of Animal Science 23:674-681.

Hopkins, L. L., A. L. Pope and C. A. Baumann. 1966. Distribution of microgram quantities of selenium in the tissues of the rat, and effects of previous selenium intake. Journal of Nutrition 88:61-65.

Jenkins, K. J. and M. Hidiroglou. 1967. The incorporation of ^{75}Se-selenite into dystrophogenic pasture grass. The chemical nature of the seleno compounds formed and their availability to young ovine. Canadian Journal of Biochemistry 45:1027-1040.

Jensen, L. S., E. D. Walter and J. S. Dunlap. 1963. Influence of dietary vitamin E and selenium on distribution of Se^{75} in the chick. Proceedings of the Society for Experimental Biology and Medicine 112:899-901.

Johnson, P. and W. F. R. Pover. 1962. Intestinal absorption of α-tocopherol. Life Sciences 4:115-117.

Jones, G. B. and K. O. Godwin. 1963. Studies on the nutritional role of selenium. I. The distribution of radioactive selenium in mice. Australian Journal of Agricultural Research 14: 716-723.

Jones, J. H. and C. Foster. 1942. A salt mixture for use with basal diets either high or low in phosphorus. Journal of Nutrition 24:245-256.

Krishnamurthy, S. and J. G. Bieri. 1963. The absorption, storage, and metabolism of α-tocopherol-C^{14} in the rat and chicken. Journal of Lipid Research 4:330-336.

Lewis, L. A., M. L. Quaife and I. H. Page. 1954. Lipoproteins of serum, carriers of tocopherol. American Journal of Physiology 178:221-222.

McConnell, K. P. 1963. Metabolism of selenium in the mammalian organism. Journal of Agriculture and Food Chemistry 5:385-388.

McConnell, K. P. and G. F. Cho. 1965. Transmucosal movement of selenium. American Journal of Physiology 208:1191-1195.

McCormick, E. C., D. G. Cornwell and J. B. Brown. 1960. Studies on the distribution of tocopherols in human serum lipoproteins. Journal of Lipid Research 1:221-228.

Mahin, D. T. and R. T. Lofberg. 1966. A simplified method of sample preparation for determination of tritium, carbon-14 or sulfur-35 in blood or tissue by liquid scintillation counting. Analytical Biochemistry 16:500-509.

Mellors, A. and M. McC. Barnes. 1966. The distribution and metabolism of α-tocopherol in the rat. British Journal of Nutrition 20:69-77.

Muth, O. H., J. E. Oldfield, L. F. Remmert and J. R. Schubert. 1958. Effects of selenium and vitamin E on white muscle disease. Science 128:1090.

Muth, O. H., J. R. Schubert and J. E. Oldfield. 1961. White muscle disease (Myopathy) in lambs and calves. VII. Etiology and prophylaxis. American Journal of Veterinary Research 22:466-469.

Muth, O. H. 1963. White muscle disease, a selenium-responsive myopathy. Journal of the American Veterinary Medical Association 142:272-277.

Nesheim, M. C. and M. L. Scott. 1958. Studies on the nutritive effects of selenium for chicks. Journal of Nutrition 65:601-618.

Nishida, T. and F. A. Kummerow. 1960. Interation of serum lipoprotein with the hydroperoxide of methyl linoleate. Journal of Lipid Research 1:450-457.

Oksanen, H. E. 1967. Selenium deficiency: Clinical aspects and physiological responses in farm animals. In: First International Symposium: Selenium in Biomedicine, Corvallis, Ore., 1966, ed. by O. H. Muth, J. E. Oldfield and P. H. Weswig. Westport, Conn., AVI Publishing Company. p. 215-229.

Oldfield, J. E., O. H. Muth and J. R. Schubert. 1960. Selenium and vitamin E as related to growth and white muscle disease in lambs. Proceedings of the Society for Experimental Biology and Medicine 103:799-800.

Olson, G., W. J. Pudelkiewicz and L. D. Matterson. 1966. Isolation of a compound from alfalfa lipids that inhibits tocopherol deposition in chick tissues. Journal of Nutrition 90:199-206.

Oppenheimer, H. et al. 1958. Serum proteins, lipoproteins, and glycoproteins in muscular dystrophy of vitamin E deficiency. Proceedings of the Society for Experimental Biology and Medicine 97:882-886.

Patterson, E. L., R. Milstrey and E. L. R. Stokstad. 1957. Effect of selenium in preventing exudative diathesis in chicks. Proceedings of the Society for Experimental Biology and Medicine 95:617-620.

Paulson, G. D. et al. 1968. Effect of feeding sheep selenium fortified trace mineralized salt-effect of tocopherol. Journal of Animal Science 27:195-202.

Pudelkiewicz, W. J. and L. D. Matterson. 1960. A fat-soluble material in alfalfa that reduces the biological availability of tocopherol. Journal of Nutrition 71:143-148.

Quaife, M. L., N. S. Scrimshaw and O. H. Lowry. 1949. A micromethod of assay of total tocopherols in blood serum. Journal of Biological Chemistry 180:1229-1235.

Rosenfeld, I. 1962. Biosynthesis of seleno-compounds from inorganic selenium by sheep. Proceedings of the Society for Experimental Biology and Medicine 111:670-673.

Schubert, J. R., O. H. Muth, J. E. Oldfield and L. F. Remmert. 1961. Experimental results with selenium in white muscle disease of lambs and calves. Federation Proceedings 20:689-694.

Schwarz, K. and C. M. Foltz. 1957. Selenium as an integral part of factor 3 against necrotic liver degeneration. Journal of the American Chemical Society 79:3292-3293.

Schwarz, K. 1958. Effect of antioxidants on dietary necrotic liver degeneration. Proceedings of the Society for Experimental Biology and Medicine 99:20-24.

Scott, M. L. and I. D. Desai. 1964. The relative anti-muscular dystrophy activity of the d- and l-epimers of α-tocopherol and of other tocopherols in the chick. Journal of Nutrition 83:39-43.

Scott, M. L. 1967. Selenium deficiency in chicks and poults. In: First International Symposium: Selenium in Biomedicine, Corvallis, Ore., 1966, ed. by O. H. Muth, J. E. Oldfield and P. H. Weswig. Westport, Conn., AVI Publishing Company. p. 231-238.

Scott, M. L. and J. N. Thompson. 1968. Selenium in nutrition and metabolism. In: Proceedings of the Maryland Nutrition Conference for Feed Manufacturers, p. 1-4.

Simon, E. J., C. S. Gross and A. T. Milhorat. 1956. The metabolism of vitamin E. I. The absorption and excretion of d-α-tocopheryl-5-methyl-C^{14}-Succinate. Journal of Biological Chemistry 221:797-805.

Slen, S. B., A. S. Demiruren and A. D. Smith. 1961. Note on the effects of selenium on wool growth and body gains in sheep. Canadian Journal of Animal Science 41:263-265.

Søndergaard, E. Selenium and vitamin E interrelationships. In: Selenium in Biomedicine, Corvallis, Ore., 1966, ed. by O. H. Muth, J. E. Oldfield and P. H. Weswig. Westport, Conn., AVI Publishing Company, 1967. p. 365-381.

Sternberg, J. and E. Pascoe-Dawson. 1959. Metabolic studies in atherosclerosis. I. Metabolic pathway of C^{14}-labeled alpha-tocopherol. Journal of the Canadian Medical Association 80:266-275.

Tappel, A. L. 1962. Vitamin E and selenium in the in vivo lipid peroxidation. In: Symposium of foods: Lipids and their oxidation, ed. by W. H. Schultz, E. A. Day and R. O. Sinnhuber. Westport, Conn. AVI Publishing Company. p. 367-386.

Tappel, A. L. and K. A. Caldwell. 1967. Redox properties of selenium compounds related to biochemical function. In: First International Symposium: Selenium in Biomedicine, Corvallis, Ore., 1966, ed. by O. H. Muth, J. E. Oldfield and P. H. Weswig. Westport, Conn., AVI Publishing Company. p. 345-361.

Thafvelin, B. and H. E. Oksanen. 1966. Vitamin E and linolenic acid content of hay as related to different drying conditions. Journal of Dairy Science 49:282-286.

Thompson, J. N. and M. L. Scott. 1968. Selenium deficiency in chicks and its effect on the requirement for vitamin E. (Abstract). Federation Proceedings 27:417.

Underwood, E. J. 1962. Trace elements in human and animal nutrition. New York, Academic Press. 429 p.

Weber, F. et al. 1964. Studies on the absorption, distribution and metabolism of l-α-tocopherol in the rat. Biochemical and Biophysical Research Communications 14:189-192.

Whanger, P. D., P. H. Weswig and O. H. Muth. 1968. Metabolism of ^{75}Se-selenite and ^{75}Se-methionine by rumen microorganisms. (Abstract) Federation Proceedings 27:418.

Wiss, O., R. H. Bunnell and U. Gloor. 1962. Absorption and distribution of vitamin E in the tissues. Vitamins and Hormones 20:441-455.

Witting, L. A., E. M. Harmon and M. K. Horwitt. 1967. Influence of dietary selenium on whole body levels of α-tocopherol. (Abstract) Federation Proceedings 26:475.

Wright, P. L. and M. C. Bell. 1964. Selenium-75 metabolism in the gestating ewe and fetal lamb: effects of dietary α-tocopherol and selenium. Journal of Nutrition 84:49-57.

Wright, P. L. 1965. Life span of ovine erythrocytes as estimated from ^{75}Se kinetics. Journal of Animal Science 24:546-550.

Wright, P. L. and M. C. Bell. 1966. Comparative metabolism of selenium and tellurium in sheep and swine. American Journal of Physiology 211:6-10.