VITAMIN E AND THE IMMUNE SYSTEM IN CALVES

by

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INTRODUCTION

The vitamin E requirement of the calf is not well established. The quantity of vitamin E required to protect calves against vitamin E deficiency varies depending not only on the amount of vitamin E in the diet or body reserves but also on other factors, especially the amounts of unsaturated fat and selenium in the diet. Vitamin E deficiency has been observed in calves under research and field conditions. It is difficult to produce vitamin E deficiency in calves when selenium is present in the diet and unsaturated fat is absent. Under such conditions the vitamin E requirement of calves appears to be very low.

The function of vitamin E as a lipid antioxidant has been well established. Research implicates the need for vitamin E in more specific metabolic functions in the animal body. One of the lesser known functions of vitamin E is its role in the function and development of the immune system. Vitamin E enhances the immune system of many species but this relationship has not been adequately studied in the bovine.

The neonatal calf is born essentially devoid of serum immunoglobulins and therefore depends upon the absorption of immunoglobulins from colostrum for passive immune protection as soon as possible after birth. As passive immunity declines, the active immune system of the calf is initiated. Calves are more susceptible to disease during the first few months of life before their active immune system is fully developed. Low levels of serum immunoglobulins in young calves are related to increased incidence of disease. Vitamin E supplementation may be a means of reducing the incidence of disease among calves by increasing the protection provided by the immune system.

This research was conducted to study the effect of vitamin E on the humoral and cell-mediated immune responses in calves.

PHYSIOLOGICAL IMPORTANCE OF VITAMIN E - A REVIEW

Physical and Chemical Properties of Vitamin E

Vitamin E is a fat soluble vitamin which was first discovered in 1922 as a factor in vegetable oils required for normal reproduction in rats. Later studies have led to the recognition of vitamin E deficiency as the cause of many different pathological conditions in numerous animal species. The deficiency symptoms tend to be degenerative in nature including fetal degeneration, testicular and liver necrosis, kidney and muscular degeneration, erythrocyte destruction and others (Hoekstra, 1975; Scott et al., 1969).

Vitamin E was isolated by Evans et al. (1936) from wheat germ oil. Several different chemically-similar compounds having vitamin E activity have been isolated from plants and named the "tocopherols" (Hjarde et al., 1973; Lehninger, 1975). The name tocopherol comes from the Greek words tokos (offspring) and pherein (to bear). All of the tocopherols are biologically active but show very different degrees of potency (Hjarde et al., 1973).

Tocopherols are methyl-substituted hydroxychromans with an isoprenoid side chain (Parrish, 1980). They are composed of two homologous series: the tocopherols, with a saturated side chain; and the tocotrienols, with an unsaturated side chain. In each series four members have been identified in plant sources and are designated as alpha, beta, gamma, and delta, depending on the methyl group substitutions in the chroman ring (Hjarde et al., 1973; Parrish, 1980). These eight naturally occurring compounds are the dextro-rotatory isomers called the "d forms" (Hjarde et al., 1973).

The best known member of the group is d-alpha-tocopherol since it is the most abundant form found in nature (Lehninger, 1975) and the most biologically active within the animal (Hjarde et al., 1973; Lehninger, 1975; Parrish, 1980). Its structural name is 2,5,7,8,-tetramethyl-2-(4',8',12'-trimethyltri-

decyl)-6-chromanol, or 2-methyl-2(4',8',12'-trimethyl-tridecyl)chroman-6-ol (Figure 1) (Parrish, 1980).

The tocopherols are pale yellow, fat soluble, slightly viscous oils. They are more stable to heat and alkalies in the absence of oxygen and more stable to acid than alkali (Parrish, 1980; Stecher, 1968). They are slowly oxidized by atmospheric oxygen (Stecher, 1968) and readily oxidized by a number of ions (Parrish, 1980). The tocopherols are much more stable to visible light than to ultraviolet (Parrish, 1980). They have antioxidant activity stemming from the presence of the 6-OH group on the chroman ring (Diplock, 1973; Parrish, 1980).

Esters and ethers of tocopherols can be synthetically prepared. The esters have no antioxidant activity until hydrolyzed (Parrish, 1980). The commonly used supplementary form of vitamin E in animal diets is the synthetic racemic d,l-alpha-tocopherol acetate. It is practically unaffected by the oxidizing influence of air, light and ultraviolet light (Stecher, 1968). The International Unit for expressing "vitamin E activity" of various tocopherols is defined as the activity of 1 mg of d,l-alpha-tocopherol acetate (Hjarde et al., 1973; Parrish, 1980).

Selenium and its Interrelationship with Vitamin E

Selenium (Se) is a metallic element with properties similar to those of sulfur (S), both belonging to group VI of the periodic table. Se is often found associated with S in organic and inorganic compounds (Scott et al., 1969). Several Se analogues of S compounds of biological significance have been identified including selenomethionine, selenocystine, and seleno-coenzyme A. Inorganic selenite seems to be well incorporated into organic S compounds, possibly by reduction to H_2Se and then -SH:SeH exchange (Olson, 1965).

The initial concern with Se in animal feeds was due to toxicities resulting from excess Se. It was found that certain soils in certain areas of the

U.S. contain excess Se, while others may be deficient (Scott et al., 1969). The essentiality of Se as a nutrient was first established through its interrelation with vitamin E. In the 1950's an unidentified factor called Factor 3 was discovered in dried brewers yeast that was as effective as vitamin E in preventing liver necrosis in rats (Schwartz, 1951) and exudative diathesis in chicks (Patterson et al., 1957; Scott et al., 1969). Schwartz and Foltz in 1957 reported Se to be the active component of Factor 3 (Hoekstra, 1975; Schwartz, 1972; Scott, 1979). Se was also shown to be effective in preventing other vitamin E related defects such as white muscle disease in farm animals under some conditions (Hartley and Grant; 1961; Muth et al., 1958; Proctor et al., 1958; Schubert et al., 1961). By 1969, work clearly demonstrated that Se is required at low levels (around 0.1 ppm) in animal diets even in the presence of large amounts of vitamin E (McCoy and Weswig, 1969; Thompson and Scott, 1969). Although these findings led to intensive and widespread study on the metabolic interaction between vitamin E and Se, there is still no unifying concept acceptable to all (Leach, 1975). The interaction is complicated due to their further interrelationships with S amino acids, synthetic antioxidants, polyunsaturated fatty acids (PUFA), and other nutrients (Hoekstra, 1975).

Functions of Vitamin E and Se

There are two main hypotheses regarding the mechanisms of action of vitamin E and Se. The biological antioxidant theory, elaborated by Tappel (1962, 1972) holds that both substances act simply as nonspecific biological antioxidants. The metabolic function theory (Schwartz, 1965, 1972; Schwartz and Baumgartner, 1970; Scott et al., 1974) proposes that the functions of vitamin E and Se are entirely different, and that vitamin E is needed in specific metabolic roles in the animal body in addition to its role as a lipid antioxidant.

Biological Antioxidant Theory. The antioxidant theory of the function of vitamin E, although not accepted by all investigators (Diplock, 1973; Green, 1962, 1972; Lucy, 1972; Nair, 1972; Schwartz, 1951, 1965, 1972; Schwartz and Baumgartner, 1970; Scott, 1970, 1979) is the only attempt to unite vitamin E into a basic concept (Hoekstra, 1975; Sheffy and Schultz, 1979). The theory relates vitamin E to the stabilization of PUFA that are in danger of destructive peroxidation. Vitamin E inhibits the formation of destructive peroxides by reacting as a chain breaking antioxidant with free radical intermediates of lipid peroxidation (Tappel, 1962, 1972). The hydrogen of the 6-OH group, which is the group responsible for the antioxidant function of alpha-tocopherol, combines with a peroxide free radical and forms an inactive product which breaks the peroxide chain. Thus, alpha-tocopherol is oxidized while PUFA are protected. The oxidation products of alpha-tocopherol are complex. In the simplest reaction mechanism, alpha-tocopherol is oxidized to alpha-tocopherolquinone (Tappel, 1962, 1972) with a loss in its biological activity and the animal is unable to reconvert it into alpha-tocopherol (Scott et al., 1969).

Because all biological membranes contain phospholipids that are subject to oxidative degradation, which leads to structural damage of cells (Combs et al., 1975), vitamin E appears to be essential for the integrity and optimum function of many systems, including the reproductive, muscular-skeletal, nervous, circulatory, and hematopoietic systems (Hoekstra, 1975; Sheffy and Schultz, 1979). The antioxidant theory serves to explain the great diversity of vitamin E deficiency lesions tending to be degenerative in nature and the many factors that can influence them (Hoekstra, 1975).

Se also functions as an antioxidant. However, studies have demonstrated that the mechanism of Se is dissimilar to that of vitamin E (Hoekstra, 1974; Noguchi et al., 1973; Rotruck et al., 1972, 1973). Se is a component of the enzyme glutathione peroxidase, a non-heme iron protein (Hoekstra, 1975). Highly purified glutathione peroxidase contains 4 g of Se per mole of enzyme

(Rotruck et al., 1972, 1973). This selenoenzyme protects cellular and subcellular membranes from peroxidative damage by converting fatty acid hydroperoxides to the less damaging alcohols before they can undergo chain reactions and cause malfunctions in the membranes (Hoekstra, 1974; Scott, 1979; Tappel, 1972). The selenoenzyme is associated primarily with the aqueous phase of the cytosol and plasma, working synergistically with vitamin E in the membrane itself (Noguchi et al., 1973; Lucy, 1972). Dietary Se has been shown in vitro to protect both the erythrocyte membrane and its contents, hemoglobin, against oxidative damage while vitamin E protects only the erythrocyte membrane. The effects of dietary Se were dependent on the presence of glucose while the effects of vitamin E were not. Glucose metabolism is necessary for the generation of reduced glutathione within the cell (Hoekstra, 1974; Rotruck et al., 1972, 1973). The selenoenzyme utilizes glutathione, a tripeptide containing cysteine, to reduce damaging hydroperoxides (Hoekstra, 1974; Rotruck et al., 1972, 1973; Tappel, 1972).

The selenoenzyme has the dual role of destroying hydrogen peroxide (H_2O_2) by this reaction: $2\ GSH + H_2O_2 \xrightarrow{\text{glutathione peroxidase}} GSSG + 2\ H_2O$, where GSH is reduced glutathione and GSSG is oxidized glutathione (Hoekstra, 1974, 1975; Rotruck et al., 1972, 1973; Scott, 1979). Hydrogen peroxide may react with superoxide ion in the tissues to form the highly membrane-destructive hydroxyl free radical (Scott, 1979; Scott et al., 1969). The superoxide ion and hydrogen peroxide are the most important free radical intermediates of lipid peroxidation as they are extremely reactive and capable of irreversible damage to cell membranes. They are formed along with other toxic parital reduction products of oxygen during electron transport to molecular oxygen and in various hydroxylation and oxygenation reactions (Lehninger, 1975).

The antioxidant theory ties together the roles of vitamin E, Se, other antioxidants, and the S amino acids into the protection of cell membranes as well as proteins and enzymes from lipid peroxidation. Free radical inter-

mediates of lipid peroxidation react with proteins and enzymes, especially those with reactive sulfhydryl groups, to cause inactivation. Sulfhydryl compounds such as GSH and cysteine react in small amounts as free radical scavengers and peroxide decomposers. Small amounts of methionine can also do this. Selenoamino acids react similarly and are powerful catalysts of sulfhydryl-disulfide exchange (Tappel, 1970).

The biological antioxidant theory of vitamin E function was challenged by those who had difficulty detecting lipid peroxides in vitamin E deficient animals (Green, 1972). However, lipoperoxides have been found in adipose tissues of vitamin E deficient animals but not in phospholipids of muscle, kidney, or testes (Glavind, 1973). Increased rates of *in vitro* peroxidation have been demonstrated in homogenates of several tissues of Se- and vitamin E-deficient animals (Combs *et al.*, 1975). Also, lipid peroxidation was found *in vivo* in vitamin E- and Se-deficient rats by ethane evolution. The peroxidation process greatly accelerated during the terminal phase of the fatal disease caused by vitamin E and Se deficiency (Hafeman and Hoekstra, 1977). Furthermore, the studies which demonstrated a role for Se in glutathione peroxidase (Rotruck *et al.*, 1972, 1973) and two distinctly separate antioxidant functions of vitamin E and Se (Noguchi *et al.*, 1973), provided additional evidence for the antioxidant theory.

Metabolic Function Theory. In a review of the evidence for and against the biological antioxidant theory as an explanation of all functions of vitamin E, Green (1972) stated that the theory cannot possibly explain all functions of the vitamin. Schwartz (1972) stated that it seems unlikely an organism should depend on vitamin E, a structurally highly specific, essential dietary agent, for the simple prevention of random peroxidation in tissues. In the process each free radical would destroy a vitamin E molecule by converting it into a useless inactive byproduct. However, there is no doubt that tocopherol has antioxidant properties (Schwartz, 1972). Scott (1970) believes vitamin E,

in addition to its role as an antioxidant, is related to the control of oxidation-reduction potentials in one or more metabolic systems. The nature of these systems is unknown. Green (1972) suggested vitamin E may act as a mediator of specific hydrogen transfer or electron transfer reactions at particular membrane sites, possibly involving free radicals or ions.

The most definitive function for Se is its essential role as a component of glutathione peroxidase, although the specific role of Se in the enzyme remains to be elucidated. It is postulated to have a redox or electron-transferring role (Hoekstra, 1974). Glutathione peroxidase may have a biosynthetic role, such as in steroid synthesis or other hydroxylation reactions (Hoekstra, 1975). The role of Se in the enzyme does not exclude other possible roles for this element in animal tissues (Hoekstra, 1974).

Vitamin E may function as a membrane-bound substance directed specifically towards oxidation-sensitive proteins that contain S or Se or both (Diplock, 1973; Lucy, 1972). Experimental observations have demonstrated that Se is present in the mitochondria and microsomes obtained from normal rat liver as protein-bound selenide, a reduced form of Se, and its occurrence is directly related to the vitamin E status of the animal. Therefore, in a Se deficiency, liver necrosis may develop because of a metabolic failure caused by the inadequacy of a selenide-depleted protein in the respiratory chain. The active form of Se as selenide may form a part of the active center of non-heme iron proteins, and vitamin E may function by protecting the selenide from oxidation. The redox function of vitamin E is located in the chromanol ring and is associated with polar glycerol moieties of the membrane phospholipids. It is then able to interact with polar regions of membrane-associated proteins, specifically oxidation-sensitive proteins containing Se and/or S (Diplock, 1973). The results of Noguchi et al. (1973) do not appear to support a redox function of vitamin E for prevention of exudative diathesis since this disease appears to be prevented by either glutathione peroxidase, which destroys peroxides, or by

vitamin E which prevents peroxide formation.

Lucy (1972) suggested the hydrophobic chain of vitamin E may form a stable complex with arachidonic acid residues of phospholipids in the membrane bilayer, with interactions specifically between methyl groups of the phytol side chain and the cis double bonds of the fatty acid. Such a complex could stabilize membranes by preventing peroxidative destruction of the PUFA in cell membranes and reducing the permeability of biological membranes containing high levels of PUFA, particularly arachidonic acid. Results presented by Scott et al. (1974) lend support to this suggestion.

An attempt has been made to define the function of vitamin E and its relation to Se through the study of livers from animals undergoing the latent phase of liver degeneration of which respiratory decline is the characteristic phenomenon (Schwartz, 1965, 1972; Schwartz and Baumgartner, 1970). Vitamin E deficient liver tissue was unable to maintain normal respiratory activity in vitro. This respiratory decline was not related to the rate of peroxide formation. Vitamin E protected against respiratory decline when supplemented in the diet, injected, or added to in vitro systems. Se protected against respiratory decline only when applied in vivo (Schwartz, 1972).

Studies with inhibitors of individual electron transport reactions and other data showed that the initial impairment leading to respiratory decline in the mitochondria during liver necrosis does not involve oxidative phosphorylation or the cytochrome chain but those dehydrogenase systems which connect the citric acid cycle to the cytochrome chain (Schwartz, 1965). Vitamin E supplementation to deficient liver homogenate prevented the rapid decline of oxygen uptake with alpha-ketoglutarate and oxalacetic acid as substrates. Using the alpha-ketoglutarate oxidase system, which involves intermediate steps and cofactors in the conversion of alpha-ketoglutarate to succinyl CoA, to delineate the positions at which vitamin E, Se, and S amino acids may be effective, Schwartz (1965) proposed that vitamin E may interact with the sulfhydryl sites

on lipoyl dehydrogenase; Se may be involved with the decarboxylation reaction considering that Se is a potent inorganic catalyst of carbonic anhydrase which releases carbon dioxide from carbonic acid; and S amino acids may be involved in the overall supply of coenzyme A and lipoic acid or on the total amount of available sulfhydryl-containing enzyme protein in one or the other of these steps.

NADH oxidase, which catalyzes the transfer of reducing equivalents from the citric acid cycle to the cytochrome chain, declined markedly preceding respiratory breakdown in vitamin E deficient liver homogenates *in vitro* suggesting that vitamin E may affect respiration directly by involvement with this enzyme (Schwartz and Baumgartner, 1970). Cytochromes, heme-containing enzymes of the electron transport chain, were increased by vitamin E or by methionine supplementation. Vitamin E appears to have a direct effect on mitochondrial oxygen consumption *in vitro* and an indirect effect on cytochrome levels *in vivo* (Schwartz, 1972).

Shapiro *et al.* (1981) found about a 30% decrease in the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) binding to the erythrocyte membrane in vitamin E deficient monkeys. Since no differences were found in the activity or binding affinity for substrate of GAPDH from vitamin E supplemented or deficient animals, a modification of the GAPDH binding site may have resulted from vitamin E deficiency. GAPDH catalyzes the first redox reaction in glycolysis, has sulfhydryl groups that must be reduced for catalytic activity, and requires NAD^+ specifically as oxidant (Lehninger, 1975). It appears that the proper function of this dehydrogenase system requires the protective action of vitamin E on the erythrocyte membrane.

An active site of Se in preventing respiratory decline during liver necrosis has been proposed by Levander *et al.* (1973). Dietary Se, unlike dietary vitamin E, had no protective effect against reagents (ascorbate, iron, or GSH plus GSSG) which caused mitochondrial swelling associated with lipoperoxidation.

It was observed that the swelling of Se-deficient mitochondria induced by GSH could be accelerated by the addition of selenite *in vitro*. This finding could be related to a role for Se in catalyzing the transfer of electrons from GSH to cytochrome c *in vivo*. In a vitamin E deficient animal receiving Se, the Se could permit the transfer of electrons directly from GSH to cytochrome c. Thus, the potentially damaging hydrogen peroxide-producing step of the respiratory chain could be bypassed and the animal would not succomb to liver necrosis (Levander *et al.*, 1973).

Coenzyme Q_3 or ubiquinone, participates in the transport of electrons from organic substrates to oxygen in the mitochondrial respiratory chain. This coenzyme is structurally similar to vitamin E, consisting of a reversibly reducible quinone with a long isoprenoid side chain, and has vitamin E-like activity (Lehninger, 1975). Vitamin E has been reported to control coenzyme Q biosynthesis, although this role has not been widely confirmed (Green, 1962; Frost and Poucke, 1972; Moore, 1962; Olson, 1965). Se has been shown, without a doubt, to be needed for coenzyme Q biosynthesis but the part of coenzyme Q biosynthesis catalyzed by Se is not known. The role of vitamin E may be indirect and lie in its ability to maintain Se in its reduced functional state (Frost and Poucke, 1972).

The association of vitamin E deficiency with anemia in pigs, rats, and primates involving a reduced synthesis of porphyrins and heme by the erythroid cells of the bone marrow led to the study of a role for vitamin E in heme biosynthesis. Studies by Nair (1972) led to the observation that vitamin E functions as a regulator of heme biosynthesis in an inducer-repressor type system in liver at the levels of two important enzymes, delta-aminolevulinic acid (ALA) synthetase and ALA dehydratase. These enzymes are necessary for the synthesis of protoporphyrin IX which accepts iron to form heme proteins, including cytochromes, hemoglobin, catalase, microsomal mixed function oxidases, and many more, that have a diversity of functions. Heme proteins are fundamen-

tal to all living cells. In vitamin E deficient animals the activity of hepatic ALA dehydratase was significantly lower than controls (Caasi et al., 1972; Nair, 1972), heme proteins were significantly decreased (Caasi et al., 1972) and bone-marrow ALA synthetase was depressed (Nair, 1972). Allylisoprophylacetamide, a porphyrinogenic agent that affects ALA synthetase, induced RNA synthesis specific for ALA synthetase and also stimulated vitamin E uptake into rat liver nuclei. Vitamin E blocked this induced RNA synthesis suggesting that vitamin E regulates the synthesis of this RNA (Hauswirth and Nair, 1972), thus regulating synthesis of heme.

Vitamin E has been suggested to be involved in the regulation of other enzyme systems (Carpenter, 1972; Catignani et al., 1974). In vitamin E deficient rats the liver microsomal drug hydroxylation system has been shown to be depressed. This enzyme system requires NADPH, oxygen, and a heme protein, cytochrome P-450. Oral administration of alpha-tocopherol to deficient rats reversed the response of liver microsomes within 12 hr. Actinomycin D prevented the increase in drug hydroxylation when administered to vitamin E deficient rats 15 min before feeding vitamin E. The effect of actinomycin D on the response of rats to alpha-tocopherol appeared to be a specific inhibition. A mechanism involving RNA synthesis and/or turnover was implied in which vitamin E may regulate the activity of a repressor (Carpenter, 1972). In the liver of vitamin E deficient rabbits, it was shown that xanthine oxidase activity increased due to an elevated synthesis of the enzyme rather than loss of an inhibitor or presence of an activator. Therefore, it was suggested that vitamin E might regulate the synthesis of the enzyme (Catignani et al., 1974).

In summary, the role of vitamin E in membrane structure and integrity may be related both to its role as an antioxidant in preventing PUFA peroxidation in the inner hydrophobic part of the membrane and to its redox function in the hydrophilic portions of the membrane. Evidence exists for the implication of both vitamin E and Se at several key steps in respiration. For vitamin

E these are: NADH oxidase; heme-containing enzymes of the cytochromes; and both coenzyme Q and non-heme iron proteins where vitamin E may prevent the oxidation of selenide. Se may have a role as an active center of non-heme iron proteins in both the mitochondrial and microsomal systems of the liver, in catalyzing cytochrome c reduction by glutathione, and in coenzyme Q biosynthesis. Vitamin E may be involved in the regulation of RNA synthesis/turnover of several enzyme systems. These include the enzymes of heme biosynthesis, the liver microsomal drug hydroxylation system, and xanthine oxidase.

VITAMIN E, SELENIUM, AND THE IMMUNE SYSTEM - A REVIEW

A lesser known function of vitamin E and selenium (Se) is their role in the function and development of the immune system. The fact that vitamin E and Se prevent peroxidative damage to cells and subcellular elements could be a mechanism by which they aid to maintain the body's normal defense mechanism against disease and stress (Scott, 1979).

The subcellular organelles whose membranes are protected by vitamin E and Se from peroxidative damage include mitochondria, microsomes, and lysosomes. Lysosomes are less labile to lipid peroxidation than mitochondria and microsomes because their membranes contain less lipid and do not contain hemoprotein prooxidants of lipid peroxidation (Combs et al., 1975). Since mitochondria, microsomes, and ribosomes act to produce antibodies and other defense mechanisms, it is clear that adequate vitamin E and Se are necessary for preservation of the organelles responsible for building defense mechanisms against disease and other stresses (Scott, 1979).

It has been well established that vitamin E and Se function to maintain erythrocyte membrane integrity. Erythrocyte membranes are quite labile to lipid peroxidation due to their high polyunsaturated fatty acid (PUFA) content and to their direct exposure to molecular oxygen. Vitamin E protects the erthrocyte plasma membrane from peroxidative hemolysis (Combs et al., 1975). Since erythrocytes and lymphoid cells originate from common pluripotential stem cells, there is a basis provided for the concept that vitamin E and Se may be associated with membrane fluidity of lymphoid cells as well as immune response mechanisms (Sheffy and Schultz, 1979).

Effect on Humoral Immunity

Studies have demonstrated in vivo (Tengerdy et al., 1972, 1973; Tengerdy and Heinzerling, 1972; Tengerdy and Nockels, 1972) and in vitro (Campbell et al., 1974) that the humoral immune response (HIR) of chickens and mice immunized

with sheep red blood cells (SRBC) increased when their rations were supplemented with vitamin E. Seven-day-old chicks were immunized with SRBC and fed a normal diet containing the recommended level of vitamin E, approximately 22 mg/kg, plus a vitamin E supplement of 132 mg/kg diet. The HIR of these chicks, compared to the controls without supplement, was increased 20-25%, as measured by the antibody (AB) plaque forming cell (PFC) and hemagglutination tests (Tengerdy et al., 1972; Tengerdy and Heinzerling, 1972; Tengerdy and Nockels, 1972). Similar results were obtained when the same experiment was repeated using one year old laying hens (Tengerdy and Heinzerling, 1972).

To determine if vitamin E would stimulate immune response in another animal species, Tengerdy et al. (1973) conducted similar studies with mice. Two different antigens (AG) were used in these studies: SRBC, a good particulate AG which does not stimulate a true primary response since it is not completely foreign for mice; and tetanus toxoid which does stimulate a true primary response. The two antigenic stimuli were used as a means of comparison to distinguish whether vitamin E supplementation affects IgG and IgM production differently. Results supported their earlier finding with chicks that vitamin E supplementation stimulates the HIR. Eight to 10 week old mice were fed semisynthetic or natural commercial diets containing 60-180 mg vitamin E/kg. In the primary immunization with either AG, the enhancing effect was the same, that being a 30-40% increase in the HIR measured by PFC or hemagglutination tests. Vitamin E particularly affected the IgG response. When SRBC was used, a significant increase in the spleen weights of immunized and non-immunized vitamin E supplemented mice was noted. This indicates, perhaps, the general cell differentiation effect of vitamin E which is in addition to that caused by SRBC in this lymphopoietic organ. Following reimmunization with tetanus toxin 30 days later, the effect of vitamin E was much stronger on the primary response than the secondary. Since IgG production is more pronounced than IgM, the main effect of vitamin E on the primary response apparently is