EFFECTS OF VITAMIN E AND SELENIUM DEFICIENCIES

IN SHEEP FED A PURIFIED DIET DURING GROWTH

AND REPRODUCTION

By

JOCK GORDON BUCHANAN-SMITH

Bachelor of Science
University of Aberdeen
Aberdeen, Scotland
1962

Bachelor of Science
Iowa State University
Ames, Iowa
1963

Master of Science
Texas Technological College
Lubbock, Texas
1965

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
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Thesis Approved:

Allen D. Tillman
Thesis Adviser

Eldon C. Nelson

Gwynn Jones

Irvin T. Omtvedt

Robert Totusek

O. O. Durham
Dean of the Graduate College

724766

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CHAPTER I

INTRODUCTION

It was demonstrated in 1922 that fetal resorption could be pre-
vented by supplementing synthetic diets with lettuce or alfalfa for
female rats (Evans and Bishop, 1922). The active principle was iso-
lated and the compound named tocopherol and assigned to vitamin E.
Tocopherols are ubiquitous, occurring in all plant and animal life.
However, there is no unifying hypothesis for the function of the vita-
min. Among invertebrates, it differentiates sex in the rotifer (Gil-
bert and Thompson, 1968), and increases spermatogenic activity in
crickets (Meikle and McFarlane, 1965). In vertebrates, vitamin E-
deficiency symptoms are more protean than deficiency symptoms of most
micronutrients. In ruminants, vitamin E was found to prevent muscle
dystrophy (Willman et al., 1945), and it is to this property that much
attention has been focused. Sufficiently critical experiments to deter-
mine if vitamin E might be involved in other physiological mechanisms
in these animals, such as reproduction, have not been performed
(Blaxter, 1962b).

Selenium was discovered by Berzelius in 1812. Up to 1940, atten-
tion in nutrition was given to its toxicity. In 1942, Schwarz found
that selenium was the active principle of a compound that could prevent
liver necrosis in the rat. Selenium is of great economic importance in
the livestock industry because it has been found that it, and not

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vitamin E, will protect against some of the naturally-occurring muscular dystrophies (Hartley and Grant, 1961). Other dystrophies respond to either vitamin E or selenium and some to vitamin E only. Thus it is clear that the roles of these two micronutrients are probably interrelated.

The study described herein is concerned chiefly with the relationship of vitamin E and selenium to reproduction in sheep. In addition, pathological and biochemical measurements were taken.

CHAPTER II

LITERATURE REVIEW

Tocopherols and Selenium

Properties and Structure

Tocopherols are composed of a 6-hydroxychroman derivative with a phytol side chain. The general structure is represented:
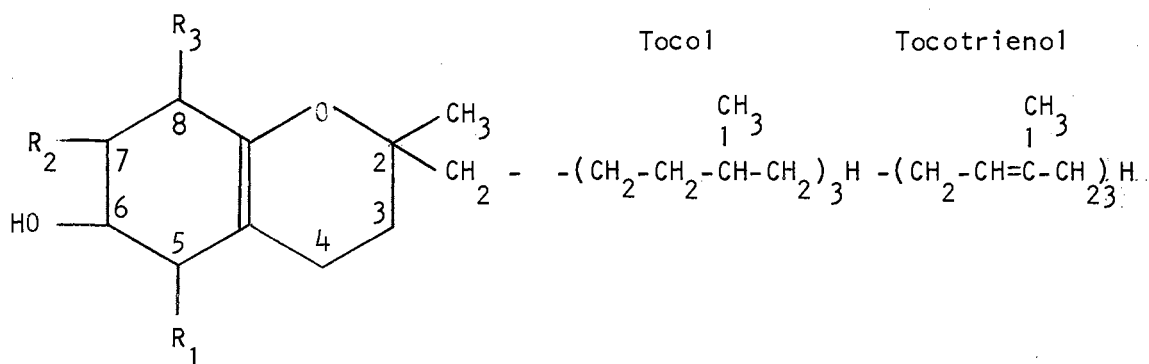
(Kofler et al., 1962)

Alpha-tocopherol, the most potent form of vitamin E, is a tocol containing methyl groups on all three positions: five, seven and eight. Tocopherols are liposoluble compounds with melting points around $0^{\circ}C$. (Kofler et al., 1962). They absorb light (290-300 mμ) and the intensity of absorption depends on the derivative (Kofler et al., 1962). Tocopherols may be oxidized reversibly to tocopherylquinones:
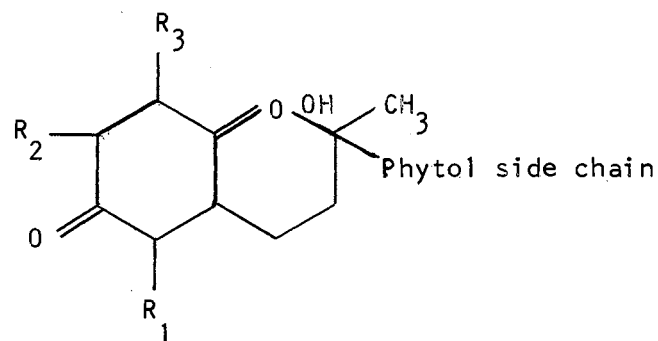
Two other metabolites of tocopherols have been isolated from urine (Simon et al., 1956).

Selenium is the element from Group VI of the periodic table, next in increasing atomic weight to sulfur. More metallic than sulfur in its physical-chemical properties, selenium nevertheless resembles sulfur in many ways. For example, it is found in trace quantities in most proteins, where it is believed to replace sulfur (Shrift, 1961).

Biochemical Functions

Degeneration of subcellular particles is one outstanding characteristic of vitamin E-deficiency lesions. For example, swelling of mitochondrial cristae precedes rupture of the mitochondrion in vitamin E-deficient rabbit muscle (Van Vleet et al., 1968). Cystic degeneration of microsomes occurs in vitamin E-deficient rat liver (Schwarz, 1958). Under these conditions it is difficult to determine the primary event. At present there are two schools of thought. One school believes that vitamin E and selenium are simply non-specific intracellular antioxidants (Tappel, 1962). The other school believes their functions are more fundamental but no satisfactory unifying hypothesis is available (Green et al., 1967).

The antioxidant theory states that vitamin E and selenium act as scavengers of free radicals; for example, peroxides of unsaturated

fatty acids (Tappel, 1962). Irradiation produces free radicals and it has been found that irradiated alcohol dehydrogenase is protected by the selenium-containing amino acids (Shimazu and Tappel, 1964). In vivo experiments have shown that the replacement of vitamin E in rat diets with synthetic antioxidants may prevent all the deficiency symptoms (Moore, 1962). Concerning comparisons made between vitamin E and synthetic antioxidants in feeding trials, the following two points should be considered: firstly, the antioxidant may only be conserving dietary stores of vitamin E (Roels, 1967a). Secondly, the antioxidant may not be available to all parts of the cell (Wiss et al., 1962).

The antioxidant hypothesis explains many observed secondary effects. Increases of lysosomal enzymes in rabbit (Zalkin et al., 1962) and lamb muscle (Desai, 1966) are thought to result from rupture of the lysosomal membrane by free radicals. Hemolysis of the erythrocyte is a similar event (Tsen and Collier, 1960). Fracture of the cell membrane by free radicals could cause the release of soluble enzymes into blood plasma (White and Hess, 1958; Blincoe and Dye, 1958). It has been suggested that the activity of gulonolactone-oxidase, the enzyme which converts gulonolactone to ascorbate, is depressed in vitamin E deficiency as a result of free radical proliferation (Kitabchi et al., 1960).

Many observations refute the antioxidant hypothesis. In the presence of an anti oxidant couple (ascorbic acid) in high concentration, tocopherols were still metabolized by in vitro systems (Green et al., 1967). The tissue levels of polyunsaturated fatty acids do not decline in vitamin E deficiency as would be expected if they were being utilized to form peroxides (Bunyan et al., 1967a). Five percent cod

liver oil in diets of rats aggravates vitamin E deficiency, yet levels of cod liver oil in excess of five percent are required before an increase in peroxide concentration of tissues is noticed (Bunyan et al., 1968). There are additional observations on accumulation of peroxides in tissues which are pertinent. Respiratory decline in rat liver homogenates is prevented by vitamin E or synthetic antioxidants yet peroxides continue to appear after respiration has been restored to normal (Corwin, 1960). In dialuric acid-induced hemolysis of the rat erythrocyte, peroxides appear only after the erythrocyte has been treated with dialuric acid (Tsen and Collier, 1960). If peroxides are the antagonistic intermediates in vitamin E deficiency, all unsaturated fatty acids which are equally liable to oxidation should aggravate deficiency of the vitamin. However this is not always the case. It has been found that linolenic acid and not linoleic acid aggravates exudative diathesis in chickens (Hutcheson et al., 1963).

There are grave doubts about the role of selenium as an antioxidant. In myopathies of young lambs or calves induced by feeding cod liver oil, selenium provides only partial protection (Welch et al., 1960). If protection given by vitamin E resides in its antioxidant properties, selenium should be able to replace the vitamin in the diet since selenium has 50 to 100 times the in vitro antioxidant activity of vitamin E (Tappel, 1962).

What are some of the available alternatives to the antioxidant hypothesis? The tocopherols have similar structure to the ubiquinones, for example ubiquinone-50:
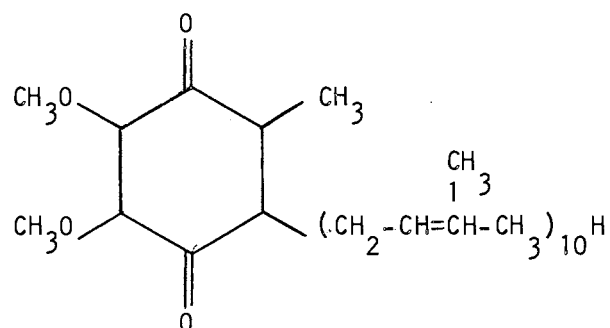
which may be reduced to form the ubichromenols:
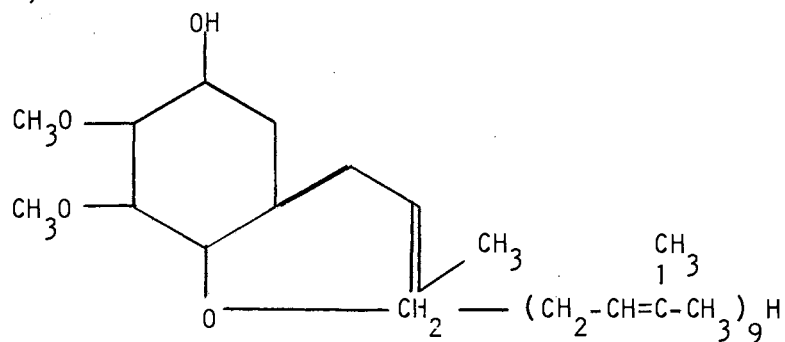
Attempts to demonstrate a metabolic pathway between the tocopherols and ubiquinones have failed (Roels, 1967b). With such a metabolic pathway, tissue levels of the ubiquinones would decline in vitamin E deficiency. This has been demonstrated by Edwin et al. (1961), and Bird and Szabo (1966). Others have failed to show this (Lee et al., 1965). A synthetic intermediate between alpha-tocopherol and ubichromenol-50, ubichromanol-50, has vitamin E activity (Smith et al., 1963). Ubichromanols do not occur naturally (Roels, 1967b). Ubiquinone-50 cannot replace vitamin E in the diet (Seidel and Harper, 1960).

Ubiquinone-50 may be a component of the electron transport scheme (Green and Wharton, 1963), and in view of the oxidation-reduction states of tocopherols, it would appear a role might exist in the electron transport scheme for them also. Vasington et al. (1960) demonstrated the presence of alpha-tocopherol in NADH-cytochrome c reductase; however, these results have not been confirmed. A failure for vitamin E-

deficient tissue to respire has not been demonstrated, although respiration of whole homogenates of vitamin E-deficient rat liver does decline after one hour (Chernick et al., 1955).

Prevention of respiratory decline appears to be the result of the fact that tocopherols protect critical sulfhydryl groups of liver enzymes. This protective function extends beyond their role as antioxidants (Corwin, 1960). Lipoic acid, a cofactor for alpha-ketoglutaric acid dehydrogenase, is one of the compounds protected. A deficiency of of sulfur-amino acids and selenium as well as vitamin E results in liver necrosis in the rat. Sulfur-amino acids are required for the synthesis of compounds containing sulfhydryl groups (Corwin and Schwarz, 1963). Bull and Oldfield (1967) have demonstrated that selenium increases oxygen consumption of mitochondria with alpha-ketoglutarate as substrate.

Some believe vitamin E plays a more fundamental role in relation to enzymes. It has been indicated vitamin E acts as a repressor in the syntheses of catabolic enzymes (Olson and Carpenter, 1967), an observation which awaits ratification by others. A further effect of vitamin E at the level of protein synthesis is indicated by the observation that levels of DNA and RNA increase in vitamin E-deficient tissues (Dinning, 1962).

No role for selenium that is entirely independent of vitamin E has yet been demonstrated. Selenium increased retention of vitamin E (Desai and Scott, 1965), indicating a synergism exists between the two. Both nutrients are associated with a lipoprotein of plasma, specifically a gamma-globulin, indicating retention of vitamin E to the protein in the presence of selenium. This observation is significant in support of the fact that chicks will die on selenium-deficient diets if the

diets are not supplemented with extremely high levels of vitamin E (Thompson and Scott, 1968).

It must be concluded that there are insufficient data available to suggest a unifying role for vitamin E and selenium. In view of the antagonism between polyunsaturated fats and vitamin E or selenium and the inclusion of polyunsaturated fats in phospholipids of membranes, further elucidation of the structure and function of membranes may clarify the area.

Nutrition: General

In contrast to selenium, very high levels of vitamin E have been administered to both man (Hillman, 1957) and swine (Bratzler et al., 1950) without deleterious effects. Selenium is toxic for livestock in doses slightly higher than the minimum requirement (Rosenfeld and Beath, 1964) and is concentrated by certain plants to levels of over 100 ppm. The poisoning of ruminants with such plants results in blind staggers, or alkali disease (Rosenfeld and Beath, 1964). Selenium has a terato- genic effect if administered by injection at a high level (Kuttler et al., 1961). The sulfur-amino acids give protection against some vita- min E- and selenium-deficiency diseases. Table I presents some of the deficiency symptoms of vitamin E, selenium and the sulfur-amino acids in various mammals and the chicken.

Deficiencies in chicks and rodents result in lesions throughout the body. It is outstanding that in pigs, cattle and sheep, lesions are confined mainly to muscle and liver.

TABLE I

VITAMIN E-, SELENIUM- AND SULFUR-AMINO ACID- DEFICIENCY SYMPTOMS
IN VARIOUS MAMMALS AND THE CHICKEN

	Tissue/organ affected (and symptom of deficiency)									
Item	Skeletal Muscle (Dystrophy)	Heart (Dystrophy)	Kidney (Necrosis)	Liver (Necrosis)	Fetus (Resorption)	Testis (Sterility)	Connective Tissue (Exudative diathesis)	Brain (Encephalo-malacia)	Red Blood Cell (Hemolysis)	Teeth (Depig-mentation)
Species										
Rat	$a_?$(b)	a_4(b)	a,b	a,b	a	a			a	a
Mouse	a,b	a,b	a,b	a,b						
Rabbit	a	a		b						
Pig	a,b	a,b		a,b	(a)					
Sheep	a,b	a,b								
Cow	a,b	a,b								
Horse	a,b									
Chicken	a,b,c				a,b	a	a,b	a		

a,b,c Indicates if the deficiency symptom responds to vitamin E, a; selenium, b; or sulfur-amino acids, c.
Parenthesis indicates that the deficiency is suspected. A blank space indicates either that the relationship is
non-existent or has not been examined.

Nutrition: Cattle, Sheep and Pigs

There are two nutritional classes of muscle dystrophies in these
species. One has been found on natural diets whereas the other has
been induced artificially. The pathology and symptoms of the two dis-
eases are similar. Naturally-occurring dystrophies have been reported
from New Zealand, parts of Australia, the Pacific and Atlantic coasts
of North America and Scotland. The dystrophy is associated with low
selenium in the indigenous forages and it appears most readily in the
newborn. In most cases, successful prophylaxis results from the ad-
ministration of selenium and not vitamin E (Hartley and Grant, 1961).
However, vitamin E or selenium treatment for the newborn is successful.
The artificially-induced dystrophies, by contrast, are responsive to
vitamin E; selenium treatment serves merely to delay the onset of the
deficiency (Blaxter, 1962a). The artificially- but not the naturally-
induced dystrophies are responsive to dietary antioxidant (Blaxter,
1962a; Hogue, 1958). The artificially-induced dystrophy is aggravated
by unsaturated fat in the diet (Maplesdon et al., 1960) and the
naturally-induced dystrophy by sulfate-sulfur (Muth et al., 1961).

Corn oil, fish oil and coconut oils have all been used as antag-
onists to vitamin E in synthetic diets. Lard failed to produce dystro-
phy in a calf diet (Maplesdon et al., 1960), but it has been used suc-
cessfully in lamb diets (Hopkins et al., 1964). Among herbivores,
lambs are less resistant than calves to the dietary stress imposed by
fat (Davis and Maynard, 1938). Corn oil was used in a skim milk diet
for lambs, in which clinical signs of dystrophy were produced that did
not respond to vitamin E (Boyd, 1968). The dystrophy in lambs produced
by Erwin et al. (1961) with coconut oil could be prevented by the

antioxidant, ethoxyquin. The sulfur-amino acids and selenium did not prevent the dystrophy. Petechial hemorrhages, resembling exudative diathesis in chickens appeared on the ethoxyquin-deficient diet in this trial. Besides ethoxyquin, N,N-Diphenyl-p-phenylene diamine (DPPD) and methylene blue are other antioxidants which may replace vitamin E (Blaxter, 1962a); ascorbic acid, butylated hydroxytoluene and ethyl gallate cannot replace vitamin E (Blaxter, 1962a).

Linoleic acid is the antagonistic compound in fats which induce muscular dystrophy in the chicken (Hutcheson et al., 1963). It appears likely that muscular dystrophy in the immature ruminant is associated with unsaturated fat (Blaxter, 1962a) although it is not known which compound is responsible. Fat is hydrogenated by the microflora of the ruminant stomach (Shorland et al., 1957). Thus it is interesting to ask the question: is it possible to produce muscular dystrophy in the mature ruminant?

A powerful antagonist to the naturally-occurring dystrophies is sulfur. Sulfide fertilization of pastures in Finland (Andersson, 1960) and gypsum fertilization of pastures in New Zealand and Oregon increased the incidence of dystrophy (Schubert et al., 1961; Hartley and Grant, 1961). The Oregon workers demonstrated that only 0.053 percent additional sulfate-sulfur in the diets of dry lot-fed ewes was sufficient to reduce protection afforded by 0.2 ppm supplementary selenium (Muth et al., 1961). Cornell workers have demonstrated the same effect (Hintz and Hogue, 1964); and in later experiments they found a correlation between the level of sulfur in the milk and symptoms of dystrophy in the lambs (Gardner and Hogue, 1967). In contrast, levels of sulfate-sulfur in natural diets for ewes of 0.7 percent and 1.0 percent did not

antagonize the protective effect of vitamin E and selenium to dystrophy in their lambs surviving over 24 hours (Boyazoglu et al., 1967). The sulfate supplementation, however, was discontinued at lambing time although the lambs were fed a creep feed containing 2.0 percent elemental sulfur. These workers did not indicate the milk levels of sulfur in their experiments.

Attempts have been made to determine other specific antagonists to the naturally-occurring dystrogenic rations. Gardner and Hogue (1967) found that autoclaving kidney beans destroys their dystrogenic activity. Autoclaving the beans increased digestibility of selenium and the content of vitamin E and selenium in the ewe's milk was increased. This heat labile factor is alcohol insoluble. An alcohol soluble factor with unsaturated fatty acid properties has also been extracted (Hintz and Hogue, 1964).

No doubt, one predisposing factor for naturally-occurring muscle dystrophies is the tocopherol status of the newborn. Vitamin E is poorly transported across the placenta (Dancis, 1960). Bratzler et al. (1950) and Parrish et al. (1950) have reported that vitamin E supplementation of the dam does not significantly improve tocopherol status in the newborn. It appears that factors affecting placental transport of vitamin E are presently unknown.

It has been demonstrated that vitamin E enhances liver storage of vitamin A in laboratory animals (Moore, 1940). It is controversial whether or not the same relationship exists in farm animals. Connecticut workers found that vitamin E will conserve vitamin A at high but not low levels of supplementary vitamin A (Dicks et al., 1959). In another experiment vitamin E increased plasma levels but did not affect

liver storage of vitamin A (Rousseau et al., 1957).

The effects of vitamin E in the rumen have not been studied extensively. It appears probable that rumen microorganisms do not synthesize vitamin E (Hungate, 1966). High levels of vitamin E prolong the life span of rumen protozoa (D'Agostino, 1951). A significant finding has recently been made in regard to selenium utilization in the rumen. Rumen microorganisms could synthesize proteins containing selenomethionine from dietary selenomethionine, but not from dietary selenate or selenite (Paulson et al., 1968). The selenate or selenite was bound tightly to the proteins but the selenium was not incorporated into the protein.

It is not surprising that the requirements of vitamin E for farm animals depend on a variety of conditions. Since vitamin E appears to be non-toxic, levels of 80 I.U. per 45.4 kg. body weight is the recommended daily allowance for beef cattle (N. R. C., 1963). Cornell workers have obtained a response from selenium in chickens with only 0.02 ppm in the diet (Scott, 1968). Selenate and selenite are both satisfactory salts of selenium to administer and benefit is obtained from oral treatment as well as injection (Hartley, 1967). No additional benefit has been obtained in farm animals using y y-di seleno divaleric acid (Hartley, 1967). The latter had greater potency than selenate in prevention of liver necrosis in rats (Schwarz, 1961). Eighty mg. of the sodium salt and 1080 mg. of the barium salt is toxic in one dose by injection (Kuttler et al., 1961). Dietary selenium levels that are considered toxic are between 5 and 10 ppm. on a chronic basis and over 20 ppm. on a shorter period (Scott, 1968).

Reproduction

Dystrophy of muscle and other tissues are lesions mostly of grow-
ing animals. As stated above, there are significant lesions in repro-
ducing vitamin E-deficient rats; namely, fetal resorption in the female
(Evans and Bishop, 1922) and testicular degeneration in the male (Evans
and Burr, 1927). In the female, resorption of the fetus takes place be-
tween day nine and 11 or midway through gestation. It is not known why
vitamin E-deficiency results in fetal resorption. Testicular degenera-
tion subsequent to vitamin E deficiency results from atrophy of germinal
epithelium (Evans and Burr, 1927). Similar responses to vitamin E-
deficiency have been demonstrated in the chicken. If the hen is sub-
jected to deficiency of vitamin E, embryonic death occurs during the
first (Card et al., 1930) or last half (Jensen and McGinnis, 1960) of
incubation. Antioxidants were found to prevent fetal resorption in the
female rat consequent upon vitamin E deficiency (Draper et al., 1964).
Antioxidants have a similar effect in chickens, but in this instance it
appears likely that the antioxidant is merely conserving vitamin E
(Jensen, 1968). In prophylaxis of fetal resorption in the vitamin E-
deficient rat, selenium is ineffective (Harris et al., 1958); however,
it was found to improve hatchability in quail (Jensen, 1968). In the
hamster, vitamin E-deficiency results in reduced litter size (Soderwall
and Jacklet, 1963). There are no reports in the literature that vita-
min E deficiency clearly affects reproductive capacity in other species.

The reproductive failure in rats is irreversible after the first
two or three days of pregnancy in the female (Cheng et al., 1961) and
early in the life of the male (Evans and Burr, 1926). Vitamin E ad-
ministered to vitamin E-deficient female rats after day two or three of

pregnancy caused fetal monstrosities, in which the liver and other tissues were degenerated (King and Verma, 1967).

Early work by Gullickson and Fitch (1944) and Gullickson et al. (1948, 1949) and by Thomas et al. (1942) did not implicate vitamin E in reproduction of farm animals. Nevertheless, reports are available that implicate vitamin E in reproductive failure of farm animals. Field observation on farms in an area of California where muscular dystrophy was prevalent, demonstrated that vitamin E and selenium treatment prevented abortions and improved neonatal vitality (Mace et al., 1963). Vitamin E therapy on similar farms in Rumania improved fertility (Lunca and Musetescu, 1966). In Minnesota, 4,400 I.U. of vitamin E in four equal doses was sufficient to prevent abortions which occurred on treatments in which the animals did not receive vitamin E (Boyazoglu et al., 1967).

Using purified diets, Virtanen (1966) found that cattle did not cycle regularly on a ration in which the only vitamin E came from a small amount of plant oils. In another purified diet that contained only 10.27 mg. alpha-tocopheryl acetate per kg. of feed, the same result was obtained (Matrone et al., 1965). This level of vitamin E may be insufficient, but it was found that the addition of only 5.0 percent alfalfa meal to the diet cured the reproductive difficulty. It is improbable that such a small amount of alfalfa meal would add sufficient vitamin E. Sheep maintained from weaning on a purified diet with approximately 4.4 I.U. vitamin E per kg. failed to reproduce normally (Erlinger, 1968). The ewes conceived but the lambs were either stillborn, or born so weak that they could not survive more than a few days. In this trial, one ewe was treated with 500 I.U. of vitamin E 23 days

before parturition and she produced a healthy lamb. In ewes which were maintained on a vitamin E- and selenium-deficient ration from the initiation of gestation, reproduction was normal (Wright and Bell, 1964). However, in those that were maintained into a second gestation, reproductive failure was encountered (Bell, 1967).

There is some evidence in the literature incriminating selenium in reproductive problems. In New Zealand, infertility in sheep has been associated with the incidence of white muscle disease (Hartley and Grant, 1961). In field trials, it was found that the administration of 5 mg. selenium or more per ewe was sufficient to reduce the incidence of barren ewes to less than 5 percent. This dose of selenium was inadequate to prevent the white muscle disease. In subsequent experiments, it was found that the infertility did not respond to vitamin E (Hartley, 1963). Similar work in Oregon has demonstrated improved fertility by selenium treatment but the improvement was not so significant as that found in New Zealand (Oldfield et al., 1963).

Although there is much applied data that indicate vitamin E and selenium may be involved in reproduction, conclusive results are lacking because experiments conducted have been insufficiently critical (Blaxter, 1962b). Yet economic loss to livestock producers through reproductive failure remains quite significant and many of the factors affecting this are not resolved. Thus the primary purpose of these investigations was to re-evaluate the roles of vitamin E and selenium in reproduction of sheep. In order to do this, a purified diet essentially devoid of both vitamin E and selenium was used.

CHAPTER III

PROCEDURE

Introduction

A two x two factorial design was used in two trials to determine the effect of vitamin E and selenium on growth and reproduction in sheep. The two levels of intake of vitamin E and selenium were none and normal. Diets were common to both trials and were as follows: all animals were fed a basal purified diet with urea as the sole nitrogen source (Table II) and with less than 0.005 ppm selenium by analysis[1]. The diet of the vitamin E-deficient animals contained corn oil which was molecularly distilled to remove vitamin E[2]. A synthetic anti-oxidant, ethoxyquin, was included in all diets and reagent grade minerals were used.

Trial 1

Forty-eight ewe lambs (average weight, 24 kg.; average age, four months) were randomly assigned to the four treatments. Vitamin E[2] (700 I.U.) and selenium (one mg.) were given weekly by subcutaneous

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[1] Method of Watkinson (1966). Courtesy W. H. Allaway, U. S. Plant, Soil and Nutrition Laboratory, U.S.D.A., Ithaca, N. Y.

[2] Provided gratis by Distillation Products Industries, Rochester, New York, courtesy Dr. Stanley R. Ames, Director, Biochemical Research Laboratories.

TABLE II

COMPOSITION OF THE BASAL PURIFIED DIET

Ingredient	%
Starch	34.37
Dextrose	24.37
Cellulose	31.00
Urea[a]	4.20
Corn Oil	1.00
Minerals and Vitamins[b]	5.06

[a]Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[b]Prepared so as to provide (% of diet); K, 1.25; Ca, 0.39; P 0.30; Mg, 0.09; S, 0.1; Na, 0.33; Cl, 0.38; Fe, 0.016; Mn, 0.005; B, 0.003; Zn, 0.006; Cu, 0.001; I, 0.00005; Co, 0.00001; F, 0.0001; Mo, 0.0002; Cr, 0.00001; Choline Chloride, 0.1; 1,2 Dihydroxy-6-ethoxy, 2,2,4,trimethyl quinoline (ethoxyquin), 0.0125. The diet contained 4,400 I.U. vitamin A and 550 I.U. vitamin D_3 per kg.

injection as dl alpha-tocopheryl acetate in a water miscible base, and sodium selenate in physiological saline, respectively. The weekly selenium dose was later increased to five mg.

The animals were purchased locally in June, 1967 and treated with vermifuge. The sheep were housed indoors on slatted floors in wooden pens. No attempt was made to keep the environmental temperature constant. Feed assignment was ad libitum throughout the growth phase but was not recorded. Water was provided ad libitum and was that provided for human consumption by the city.

Growth rate of the ewes was determined over the initial 140 days of the trial. Blood samples from half the ewes in each treatment were taken from the jugular vein into heparin at biweekly intervals. The blood was immediately placed in ice.

At the end of the growth phase (October, 1967) several animals on the vitamin E-deficient diets died. Selenium delayed but did not prevent this. Thus, observations on reproduction were made only on those ewes that received either vitamin E or those vitamin E-deficient ewes in which selenium delayed death. Six extra ewes were maintained on the vitamin E-deficient diet supplemented with selenium to compensate for early death losses on this treatment. All surviving ewes were mated to rams during November and December[3]. The rams were fertility tested before use and were rotated among treatments at three or four day intervals. The rams were taken from the ewes for short periods twice daily and fed alfalfa pellets. Brisket paint was used to identify mated ewes and results were recorded every twelve hours. All ewes in the trial,

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[3]Rams courtesy Dr. Robert L. Noble, Department of Animal Science.

except one, were mated within 54 days.

At different stages of gestation, six animals on each of the three treatments in which vitamin E or selenium was given, were sacrificed. Death was by exsanguination and immediately upon dissection, selected maternal and fetal tissues were removed, blotted and placed in ice-cold 0.25 M sucrose for immediate enzyme assays. Additional tissue samples were taken for vitamin assays and stored at -10°C. A thorough inspection of all tissues was made for pathological lesions and the uterus, placenta and contents were examined for abnormalities. Selected tissues were fixed in ten percent buffered formalin. They were embedded in paraffin, sectioned at 6μ and stained with hematoxylin-eosin for histopathological observations.

During gestation, blood samples were taken as before from the remaining six ewes on each treatment. These ewes were not sacrificed and if pregnant were allowed to carry fetuses to term. Milk samples were taken from these ewes two weeks after lambing.

Prior to weaning at approximately ten weeks of age, no lamb obtained any supplement to their dam's milk. At ten weeks of age, the lambs were then placed on the same diet and treatment as their dam. Surviving ewes were maintained on the same treatments on limited feed intake through the summer of 1968.

Trial 2

Twelve ram lambs (average wt., 30 kg.) were assigned to this trial in May, 1968. Each treatment contained three individuals. Feeding and management of the rams was as described for the ewes in Trial 1, except selenium supplementation was five mg. per week throughout the

trial. Weights were recorded for 140 days. Blood samples were collected from the rams as described in Trial 1. In October 1968, when the surviving rams had received the experimental diet for 5 months, semen was collected by electro-ejaculation at weekly intervals for five weeks and examined as described in a paragraph below.

Methods of Analyses

Microhematocrit, hemoglobin (by cyanmethemoglobin reagent[4]) and red blood cell counts (by mechanical counter[5]) were determined on fresh blood. Plasma was harvested by centrifuging whole blood at 5000 x g for 20 minutes at 5° C. and then stored at -10° C.

Total free tocopherols of plasma were determined by a procedure adapted from Duggan (1959). Duplicate 6 ml. plasma samples (or a volume less than 6 ml. made up to 6 ml. with deionized water) were shaken in glass stoppered centrifuge tubes for 20 minutes with 6 ml. ethanol[6] and 14.4 ml. petroleum ether[7]. Blanks (two per seven samples) were prepared identically except plasma was replaced by deionized water. Twelve ml. of the petroleum ether layer from blanks and samples were evaporated to dryness under a stream of nitrogen and the residue dissolved in 1.5 ml. hexane[8]. The latter was carefully transferred to a

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[4]Hycel, Inc., Houston, Texas.

[5]Coulter Electronics, Inc., Hialeah, Florida.

[6]Once distilled over KOH.

[7]Nanograde, b.p. 30-60°C., Mallinckrodt Chemical Works, St. Louis, Mo.

[8]Nanograde, Mallinckrodt Chemical Works, St. Louis, Mo.

quartz cuvette in a spectrophotofluorometer[9]. Fluorescence was deter-

mined with the excitation wavelength set at 294 mµ and emission at 342

mµ. A suitable aliquot of standard alpha-tocopherol[10] was added to the

sample and fluorescence intensity of sample plus standard recorded.

Calculations of total tocopherol concentrations using this internal

standard were according to Duggan (1959). The spectrophotofluorometer

was adjusted so that there was a linear relationship between tocopherol

concentration and fluorescence intensity.

Vitamin A and carotene of plasma were determined by the procedure

of Kimble (1939).

With the exception of ornithine transcarbamylase (OCT) and iso-

citrate dehydrogenase (ICDH), plasma enzyme assays were performed

spectrophotometrically by measuring the disappearance of NADH at 340

mµ. A spectrophotometer[11] with a continuous recorder and a constant

temperature water bath set at 25°C. was used. Glutamate-oxaloacetate

transaminase (GOT) and lactic acid dehydrogenase (LDH) were assayed ac-

cording to Henry (1964) with the exception that dichromate blanks were

not used. Alpha-hydroxy butyric acid dehydrogenase (HBD) was assayed

according to Elliot and Wilkinson (1961); creatine phosphokinase (CPK)

by Wiesmann et al. (1966); diphosphofructoaldolase (FDA) by Bruns and

Bergmeyer (1965); phosphofructoaldolase (PFA) by Wolf (1965); glutamate

dehydrogenase (GDH) by Schmidt (1965) and glutamate-pyruvate transami-

nase (GPT) by Bergmeyer and Bernt (1965). ICDH was assayed spectro-

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[9]Aminco Bowman, American Instrument Company, Inc., Silver Springs, Md.

[10]Distillation Products Industries, Rochester, N. Y.

[11]Gilford Model 240, Gilford Instrument Labs.,Oberlin, Ohio.

photometrically by measuring the formation of NADPH at 340 mμ according to Freedland et al. (1965) and OCT colorimetrically by measuring the formation of citrulline according to Carper and Roesler (1968).

Prior to performing any of the enzyme assays, conditions described in the procedures were confirmed such that zero order kinetics existed and the enzyme was the only rate-limiting step in the reaction. Heparin was shown not to interfere with any of the enzyme assays.

Plasma protein concentration was determined using the biuret method (Layne, 1957).

Total tocopherols of tissues were also assayed fluorimetrically The tissues were extracted in a high-speed omnimixer[12] using a modification of the technique for vitamin A described by Thompson et al. (1949). In preliminary work using thirteen tissue samples from three different sheep, it was established that one extraction for a duration of eight minutes followed by a second for four minutes removed an average of 96 percent of the total that could be obtained by extracting for a longer time. Thus the following procedure was adopted: tissues were first prepared by one of three methods. Adult tissues were ground to a fine particle size. Fetal tissues were either chopped to a fine particle size with scissors or were partially homogenized by expulsion through a 1 mm. stainless steel screen by means of a screw press[13]. One to two grams of the thoroughly mixed tissue were introduced into the solvent

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[12]Sorvall, Inc., Norwalk, Conn.

[13]Harvard Apparatus, Inc., Cambridge, Mass.

mixture of 3.5 ml. deionized water, 7 ml. ethanol[14] and 23.5 ml. hexane[15] and extracted in the omnimixer at 10°C. for eight minutes. The organic solvent layer was removed and replaced by an additional 15 ml. hexane and the sample rextracted for four minutes at 10°C. Total tocopherols were estimated in 2 ml. aliquots of the combined organic solvent layers using the method identical to that employed for plasma. In addition, esterified tocopherols were determined in the hexane extracts of the tissues of adult sheep by the lithium aluminum hydride procedure described by Duggan (1959). Liver vitamin A and carotene were determined by the procedure of Bunnel et al. (1954).

The activities of two lysosomal enzymes, acid protease and beta-glucuronidase, were determined in liver, skeletal muscle and uterus. Liver was first partially homogenized by expulsion through a 1 mm. stainless steel screen using a screw press[16]. Ten and 20 percent homogenates of liver and uterus, respectively, were prepared in ice-cold 0.25 M sucrose. Initially, the muscle homogenate in 0.25 M sucrose was 20 percent, but this was later increased to 40 percent. Homogenization of liver was completed with three up and down strokes of a Potter-Elvehjem tissue grinder with a motor driven, grooved teflon piston. Muscle and uterus were homogenized in an omnimixer[17] at high speed for 30 seconds. Total activities of the enzymes in all tissues were

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[14]See footnote 6.

[15]See footnote 8.

[16]See footnote 13.

[17]See footnote 12.

determined by adding detergent[18] to the homogenate to a final concentration of 0.5 percent. The homogenate was centrifuged at 18,000 x g for 30 minutes and the assays performed on suitable aliquots of the supernatant solution. Free activity of the enzymes was determined on the homogenate in the same way but without the inclusion of detergent. All tissue homogenization was carried out at 0-5° C.

In addition, liver homogenates without detergent were centrifuged at 600 x g for 10 minutes to determine the nuclear and cell wall bound activity. A suspension of the lysosomal rich fraction of liver harvested at 18,000 x g was incubated in 0.25 M sucrose at 37°C. for 0, 1 hr. or 2 hr. to test stability of the lysosome. The values obtained for enzyme released were divided by the total amount of enzyme in the lysosomal rich fraction, as found using detergent, and expressed as a percent.

Acid protease assay was performed according to Dingle et al. (1966) and one unit of activity is expressed as the amount of enzyme required to release one mcg. of tyrosine in one hour with hemoglobin as substrate. Beta-Glucuronidase was assayed according to Giannetto and de Duve (1955) using phenolphthalein glucuronide as substrate and measuring the released phenolphthalein by the method of Tappel et al. (1962). Protein of homogenate supernatants was determined according to Lowry et al. (1951).

CPK, LDH and HBD were determined on suitable aliquots of the muscle and uterus supernatants obtained with and without the addition

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[18]Triton X-100, Rohm and Haas, Philadelphia, Pa.

of detergent[19]. Procedures used for these assays were identical to those applied for plasma.

Dry matter and ash of milk samples were determined according to AOAC (1960). Protein was by Kjeldahl and fat by the Babcock test.

A qualitative examination of semen was made for sperm concentration and presence of any major abnormalities. Sperm cells were stained by the nigrosin-eosin method to determine the percent live and percent with normal morphology (Hancock, 1952). Seminal plasma citrate was determined according to Saffran and Denstedt (1948) and fructose by Roe (1934).

Analyses of variance were used to determine standard errors on treatment means. Differences between treatment means were tested by multiplying the standard error with the appropriate significant studentized range.

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[19] See footnote 18.

CHAPTER IV

RESULTS AND DISCUSSION

Growth and Survival

All the ewes on the basal treatment died between the 140th. and 230th. days of Trial 1 (Figure 1). Selenium increased survival time but did not prevent death; three ewes died by the 230th day and a cumulative total of six by the 305th. day. Only one of the original 18 ewes placed on this treatment survived 500 days. A similar result was obtained in Trial 2 in which all the rams on the basal treatment died between the 80th. and 155th. day. One ram treated with selenium died after 178 days and others survived beyond 200 days. Treatment with vitamin E prevented the deaths of three of the ewes on the basal ration in Trial 1. All animals showed very few outward symptoms of vitamin E deficiency until shortly before death; i.e., there was no stiffness, lameness or other sign of muscular degeneration. Animals dying at a younger age did so after being prostrate for a period of from twelve to forty-eight hours. During this time, they continued to eat and drink. In older animals death was more sudden.

External condition of the animals prior to death appeared to be normal, other than for weight loss in the last few days of life. Postmortem examination revealed muscle necrosis in all dead animals, but other tissues were involved, especially in the older animals. It appeared that the ultimate cause of death was heart failure. However,
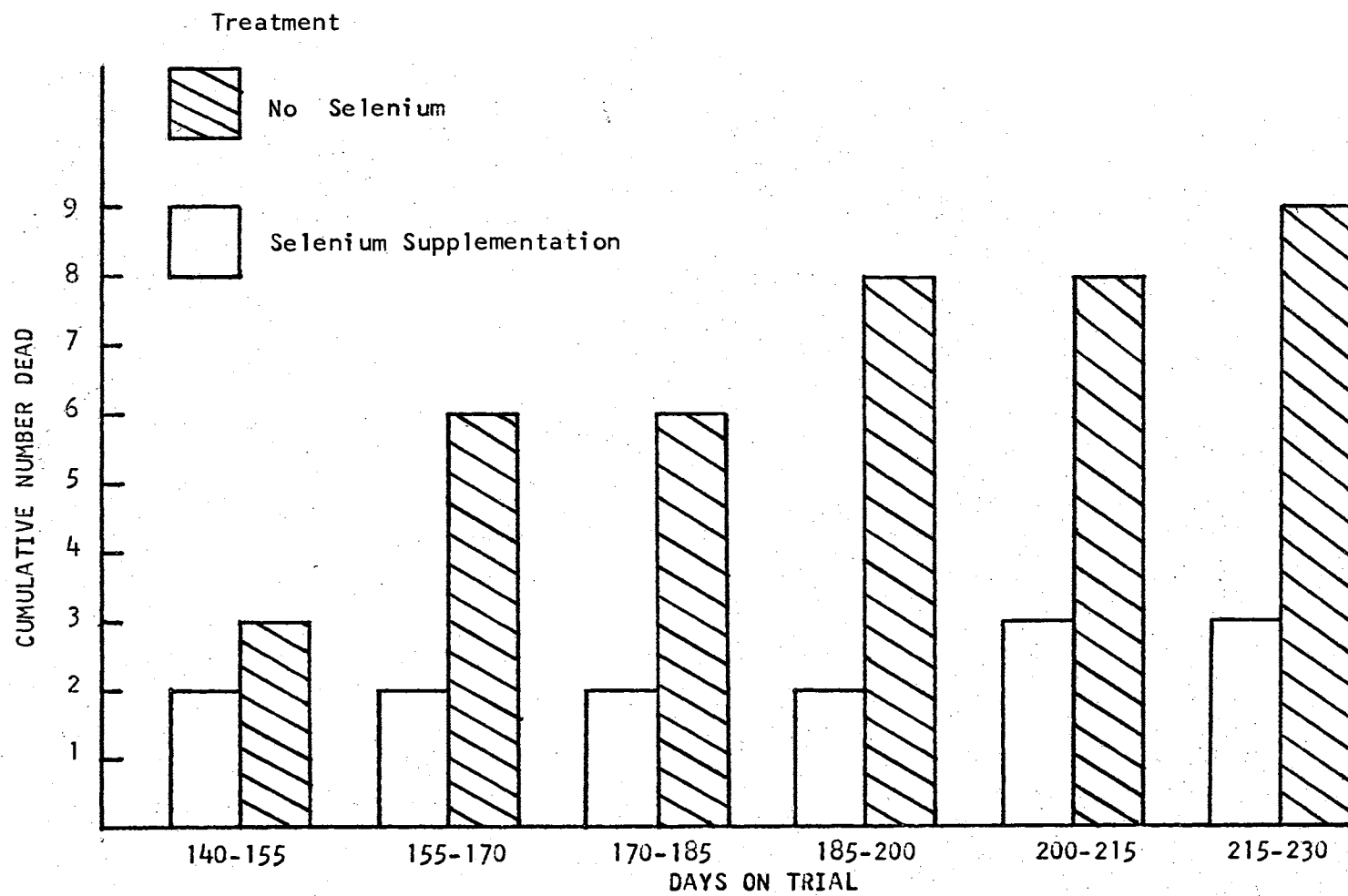
Figure 1. The Effect of Time on Trial on the Occurrence of Deaths in the Vitamin E-Deficient Ewes (Twelve ewes were originally placed on each treatment. Three ewes on the basal treatment which were given vitamin E survived.).

necrotic heart tissue was clearly identified only in animals on the basal treatment. Detailed description of the pathology of these animals is given below.

Although no adult animal receiving both vitamin E and selenium died in this trial, two ewes treated with vitamin E died at the time of lambing. The cause of death seemed to be complicated but heart muscle necrosis was identified in one ewe.

In Trial 1 (Table III), selenium improved gain (P < .10) while vitamin E depressed it (P < .05). There was no interaction between vitamin E and selenium. Selenium appeared to exert a beneficial but not significant effect on yearling weights of the ewes. Rams treated with selenium gained faster (P < .05) than those rams treated with both vitamin E and selenium.

Reproduction in Ewes and Performance of Their Lambs

Conception was lower in the vitamin E-deficient treatments (Table IV). This result is a reflection upon the lack of survival of the ewes. A greater percentage of ewes treated with vitamin E conceived during the first estrus than ewes on the other treatments.

The percentage of sacrificed ewes containing live fetuses was lower for ewes treated with both vitamin E and selenium than for other ewes. An explanation concerns the fact that three of the sacrificed ewes treated with vitamin E and selenium were not pregnant; however, all of those that were not sacrificed were pregnant. All fetuses taken in this trial except one were alive; the dead one was from a ewe treated with selenium. All three ewes, which were originally on the basal treatment and then were given vitamin E conceived. Although one

TABLE III

THE EFFECT OF VITAMIN E AND SELENIUM ON THE AVERAGE
DAILY GAIN OF THE EWES AND RAMS DURING THE
INITIAL 140 DAYS OF THE EXPERIMENTS

	Treatment				
Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
Ewes					
Number	12	12	12	12	
Av. daily gain, gm.	107.1[b,c]	120.2[b]	92.8[c]	105.8[b,c]	6.7
Rams					
Number	—[d]	3	3	3	
Av. daily gain, gm.		153.1[b]	134.3[c]	87.6[c]	11.9

[a]Standard error of treatment means.

[b,c]Horizontal values on the same line bearing different super-scripts differ significantly ($P < .05$).

[d]Rams who were on the basal treatment died.

TABLE IV

THE EFFECT OF VITAMIN E AND SELENIUM ON THE
REPRODUCTIVE PERFORMANCE OF THE EWES

Item		Treatment		
	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se
Number of ewes	12	12	12	12
Conception in:				
First estrus	1	3	9	4
Second estrus		3	1	4
Third estrus		1		1
Total	1	7	10	9
Post-conception[a]				
Fetuses alive at sacrifice	0	4	5	3
Lambs alive at birth	0	2	3	5
Lambs weaned	0	0	1	5

[a] Six ewes were sacrificed during gestation.

of these three ewes gave birth to a live lamb, another produced an ab-
normally developed one, weighing only 273 gm., at term. The third ewe
aborted her fetus after 70 days of pregnancy. One other ewe treated
with vitamin E also aborted a lamb after 109 days of pregnancy. It
appeared probable that one ewe, on the basal treatment, resorbed her
fetus. This ewe died 63 days after conception. No ewe had multiple
births. The mean gestation times were estimated to be 145.5, 144.3
and 142.0 days, respectively, for ewes treated with selenium, vitamin
E and both vitamin E and selenium. These are slightly shorter than
values reported in the literature (Forbes, 1967).

Fetal size and birth weight of lambs did not differ among treat-
ments and values obtained agreed with those in the literature (Winters
and Feuffel, 1936).

No lambs survived beyond weaning except those from ewes treated
with both vitamin E and selenium. There were two lambs born to ewes
treated with selenium and one died after seven days while the second
died after 56 days. Stiffness was noticeable in the second lamb only.
Both of these lambs had extensive myocardial necrosis. Three of four
lambs raised from ewes treated with vitamin E died before weaning. The
fourth lamb survived to weaning but died one day later at the age of
102 days. Unfortunately, two of these lambs were infected with coccidia
and death was attributed to this infection. However, in the other two,
myocardial necrosis was identified and death was attributed to this in-
volvement. This appears to be the first report in the literature of
tissue necrosis related to selenium deficiency in experimental diets.

Lambs surviving long enough to permit a comparison of pre-weaning
gains (Table V), indicated no difference among those receiving vitamin

TABLE V

THE EFFECT OF VITAMIN E AND SELENIUM ON THE AVERAGE
DAILY GAIN OF THE LAMBS PRE- AND POST-WEANING[a]

Item	Treatment			
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Pre-weaning, 56 days				
Number of lambs	1	4	5	
Av. daily gain, gm.	41.1	142.0	168.8	45.3
Post-weaning, 111 days				
Number of lambs	—[c]	—[c]	5	
Av. daily gain, gm.			112.6	17.8

[a]No lambs were born to ewes who were on the basal treatment.

[b]Standard error of the treatment mean(s).

[c]Lambs who were not treated with both vitamin E and selenium died.

E, but the lamb from one of the ewes on the selenium treatment gained poorly.

Composition of ewe's milk differed only slightly between treatments, and values obtained (See Appendix Table XXVII) were in accord with those in the literature (Petersen, 1950).

Ram Fertility

There were no significant (P $>$ .05) differences in semen characteristics (Table VI). In each of the selenium and vitamin E treatments, one ram was unsatisfactory. The unsatisfactory animal on the selenium treatment never produced any live sperm. Upon examination of the testes, it was concluded they either were infected or otherwise injured. Live sperm from the unsatisfactory animal on the vitamin E treatment were obtained on one ejaculate, and his testes were not infected or injured. One other animal on the selenium treatment produced an unsatisfactory ejaculate on one occasion. The proportions of live and normal sperm observed in semen samples taken from rams treated with selenium or vitamin E are lower than that from rams treated with both vitamin E and selenium but this is a direct reflection upon the infertility encountered. There were fluctuations in the concentrations of seminal citric acid and fructose. This would be a result of taking the samples by electro-ejaculation. Seminal citric acid and fructose concentration reflect upon testosterone levels in the blood (Mann, 1964). Thus it appears vitamin E or selenium does not directly affect the secretion of testosterone. A similar finding has been made in the rat (Evans and Burr, 1927).

TABLE VI

THE EFFECT OF VITAMIN E AND SELENIUM
ON SEMEN CHARACTERISTICS[a]

	Treatment			
Item	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of rams[c]	3	3	3	
Sperm				
Concentration[d]	1.93	2.13	3.00	0.75
Live, %	38.1	43.1	69.0	17.3
Normal, %	52.5	49.7	83.3	21.0
Seminal citric acid, mg./100 ml.	484.3	382.1	403.6	37.9
Seminal fructose, mg./100 ml.	497.6	386.1	237.6	82.0

[a]Rams who were on the basal treatment died.

[b]Standard error of the treatment means.

[c]Five semen samples were obtained from each ram.

[d]Concentration score: 3, satisfactory; 1, 2, subnormal; 0, unsatisfactory.

Pathology

The outstanding lesion of the vitamin E-deficient animals was muscle degeneration and necrosis. Gross lesions were not observed in animals sacrificed before the terminal stages of the disease. Also, these lesions were not observed in adult animals receiving vitamin E. The subcutaneous and interfacial tissue surrounding the affected muscle masses in the scapula and loin were infiltrated with yellow gelatinous edema. The muscles were pale or mottled and contained many 1-2 mm. gray-white foci. The latter were also evident in some of the muscles from the vitamin E-treated animals. The extent of the gross lesions in the muscle mass varied from animal to animal (Muth, 1955; Blaxter and Brown, 1952). The heavy muscles of the limbs and tongue were the most severely affected. Lesions were bilaterally symmetrical as has been previously reported (Muth, 1955; Boyd, 1964). Microscopic examination revealed typical Zenker's degeneration. Figure 2 illustrates the very early changes which are encountered. Located among some of the more normal appearing muscle fibers are swollen muscle fibers that show a loss of cross striations and fragmented sarcoplasm characteristic of hyalin degeneration. Figure 3 illustrates a section in which muscle lesions are more extensive. There are three prominent features: hyalin necrosis and mineralization of affected fibers, disappearance of fibers and hypercellularity involving macrophages, sarcolemmal nuclei and a few lymphocytes. The body's attempts at regeneration of sarcolemmal tubes were also observed in necrotic tissue.

Lesions in the myocardium were not as widespread as in skeletal muscle. In the vitamin E-deficient and selenium-treated ewes the only heart lesions occurred in one of the animals which died after being on
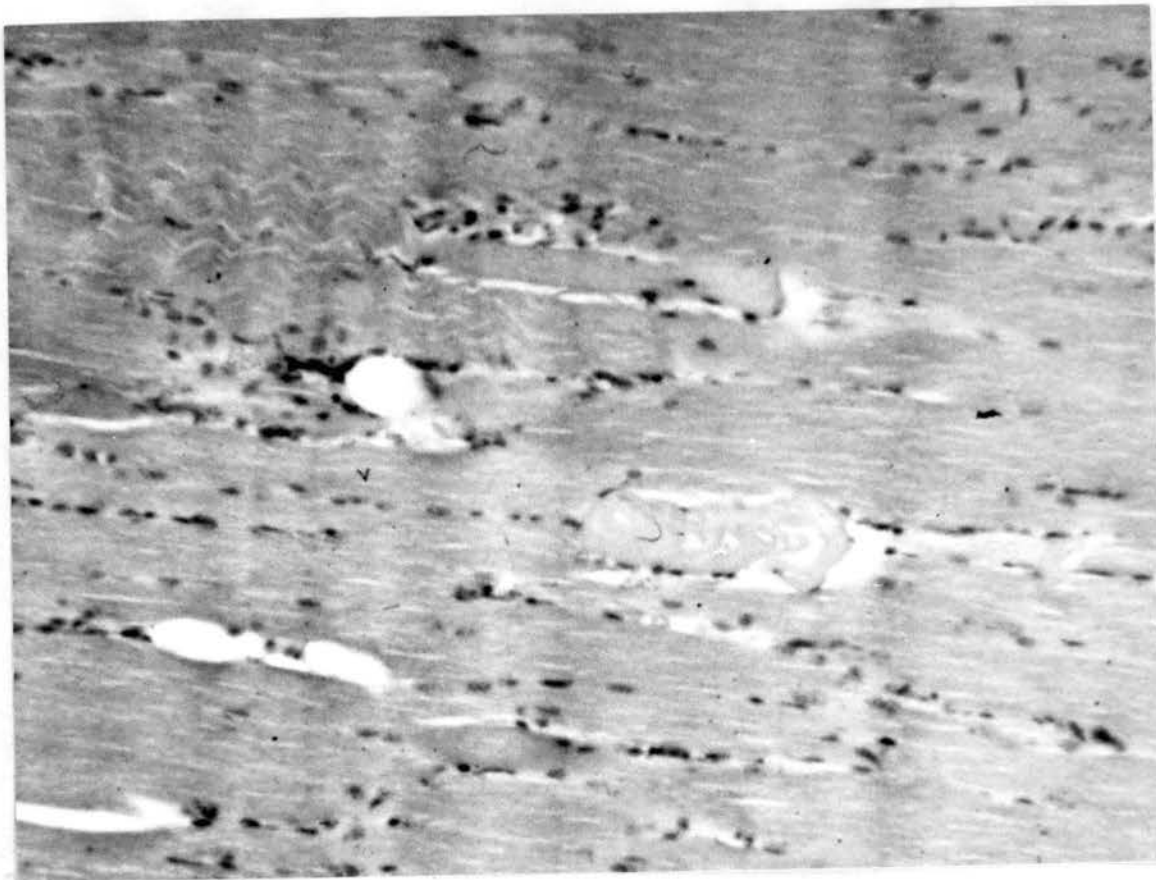
Figure 2. Photomicrograph of Skeletal Muscle from One of the Ewes
Showing Initial Changes Encountered in Vitamin E
Deficiency (X 700) (For preparation of photomicro-
graphs, see page 21).
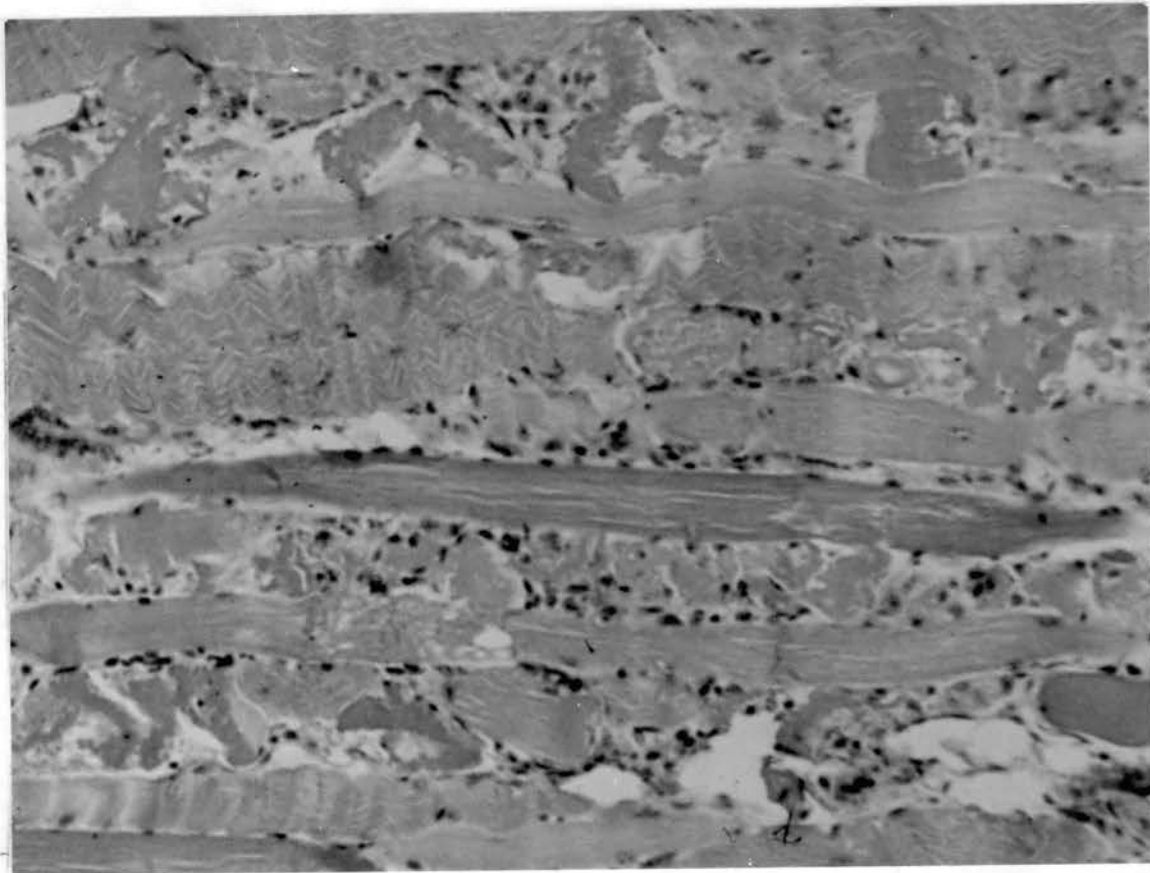
Figure 3. Photomicrograph of Skeletal Muscle from One of the Ewes
Showing Extensive Lesions Encountered in Vitamin E
Deficiency (X 700) (For preparation of photomicro-
graphs, see page 21).

experiment for one year. Several vitamin E-deficient animals died
without demonstrable heart lesions. The greatest occurrence of heart
lesions were in the ewes fed the basal diet alone; however, there was
no consistency between the extent of the disease in skeletal muscle and
the occurrence of necrosis in the heart. These results confirm those
of Muth (1955) and Blaxter and Brown (1952). The heart lesions ap-
peared as 2-3 mm. by 4-5 mm. white or whitish-yellow subendocardial
plaques. Petechial hemorrhages in the subendocardium were occasionally
noted. In those hearts having noticeable lesions, there appeared to
be arrest in diastole Microscopic examination of the heart and skele-
tal muscle lesions was similar; hyalin degeneration and necrosis ap-
pearing as a prominent feature.

Gross lesions appeared in liver but were not confined to the vit-
amin E-deficient animals. The livers of the most severe cases were
occasionally friable, mottled and otherwise discolored and usually con-
gested. The latter was confirmed by microscopic examination. In many
livers there was evidence of fatty infiltration and areas of focal
necrosis. The latter occurred in many of the animals on the vitamin E-
deficient treatments which died from the disease; however, it was also
observed among animals treated with vitamin E and both vitamin E and
selenium.

Glomerulonephritis occurred in animals from all treatment groups.
In almost all the kidneys examined in this experiment, congestion, min-
eralization and protein depositions in the tubules were prominent.
Nephrosis was clearly described only in vitamin E-deficient animals
with muscle lesions. The relation of nephrosis to white muscle disease
is not clear (Muth, 1955). However, since lesions similar to these

have been produced by globin, it seems plausible to assume that these lesions are the result of the release of myoglobin from muscle (Menefee et al., 1964).

With few exceptions, lesions of other tissues were not so prominent. There were no irregularities of placentation in pregnant ewes and no testicular lesions were noticed in the rams. In the severely affected animals on the basal treatment, the spleen and thymus were often congested. In no case was necrosis of nervous tissue noticed. However, necrosis did extend to the abomasum and colon in one adult animal treated with selenium, which died after being on trial for one year.

Two minor abnormalities of irregular size to muscle fiber were noticed in fetuses from ewes treated with selenium. Otherwise, the fetuses of sacrificed animals were normal. The extensive necrosis of liver that occurs in the fetuses of vitamin E-deficient rats was not observed here (King and Verma, 1967).

Blood Tocopherols and Vitamin A Levels

In both Trial 1 (Figure 4) and Trial 2 (Table VII), there were significant increases ($P < .01$) in plasma free tocopherols after vita-E treatment. The responses occurred soon after the first injection of 700 I.U. vitamin E, given at seven days. In both trials, selenium significantly ($P < .05$) increased the tocopherol concentration in vitamin E-treated sheep soon after the first injection. This may reflect upon a greater uptake of tocopherols from the site of injection in the presence of selenium.

In contrast, selenium treatment resulted in lower ($P < .05$) plasma tocopherol levels in vitamin E-treated ewes after 70 and 140 days on
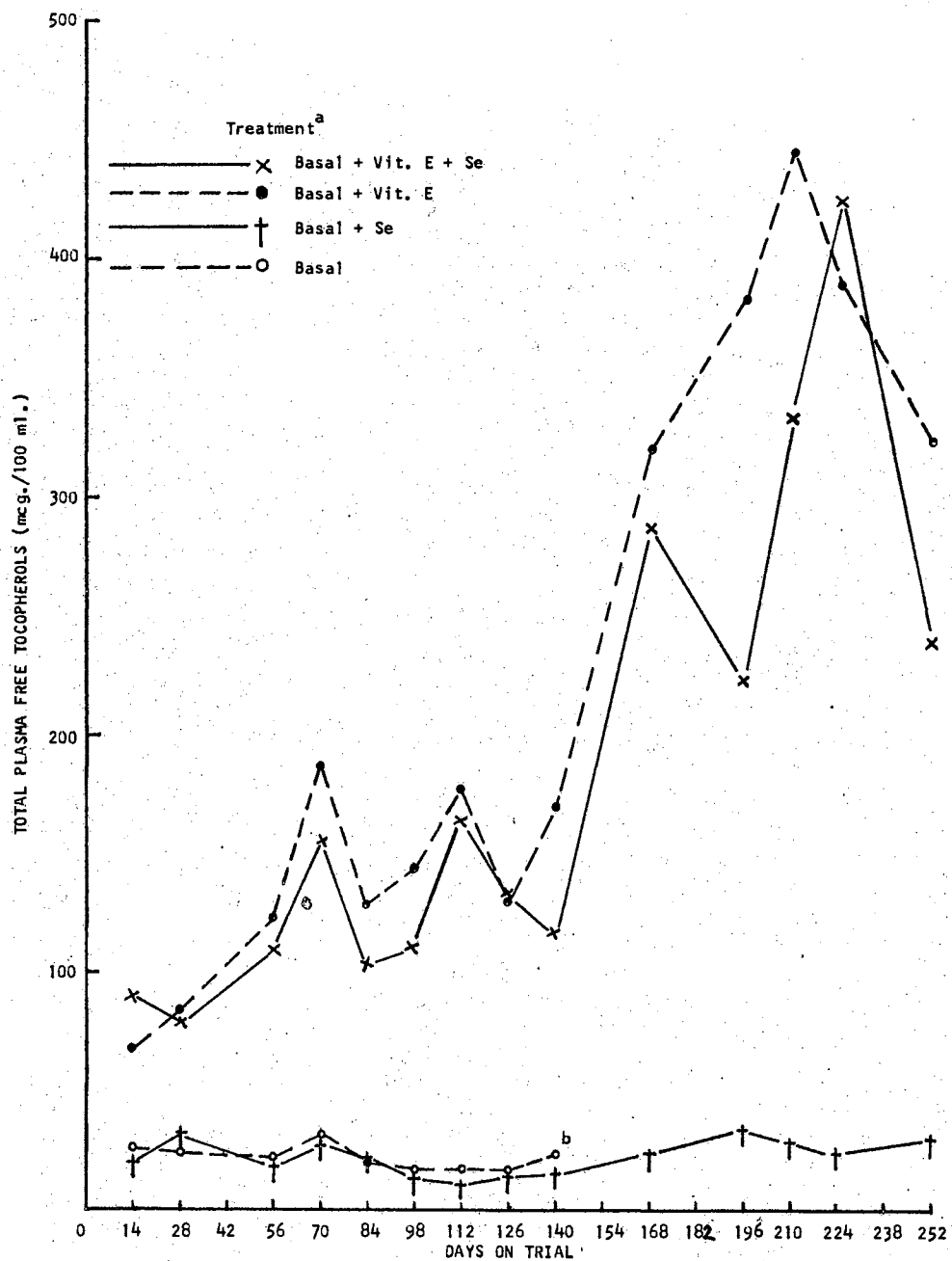
Figure 4. The Effect of Vitamin E and Selenium on the
Concentration of Total Free Tocopherols in
Blood Plasma of the Ewes ([a]Standard errors
of treatment means (each point represents
a mean of six observations) are in the Ap-
pendix (Table XXIX). [b]Ewes which were on
the basal treatment died.)

TABLE VII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF TOTAL FREE TOCOPHEROLS IN BLOOD PLASMA OF THE RAMS

Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
			Treatment		
Number of rams	3	3	3	3	
Concentration, mcg./100 ml.					
On day:					
0	35.0	39.3	23.7	50.0	10.5
14	31.2^b	29.1^b	74.2^b	178.3^c	29.4
28	28.6^b	23.1^b	143.7^c	147.4^c	20.8
98	$-^d$	15.5^b	114.1^c	134.7^c	18.8

[a] Standard error of the treatment means.

[b,c] Horizontal values on the same line bearing different superscripts differ significantly ($P < .01$).

[d] Rams who were on the basal treatment died.

the trial. The latter effect was not found in Trial 2. Fluctuations in plasma tocopherol levels after the 140th day may be a reflection on pregnancy which began at this time.

To test the effect of vitamin E treatment upon body storage of the vitamin, tissue tocopherols were analyzed. In addition, observations were made on the decline in plasma tocopherol when vitamin E treatment ceased. Five vitamin E-treated ewes, which were of no further use in the reproduction study, were placed on a vitamin E-deficient diet after 300 days of Trial 1. The plasma tocopherol level declined gradually, thus the method of least squares was used to fit the date of Figure 5. It appears that it would have taken between 200 and 250 days for the plasma tocopherol to have declined to the level found in sheep not treated with vitamin E during Trial 1.

The initial levels of plasma tocopherol found in the sheep which were fed a ration of natural feedstuffs prior to the initiation of these experiments were low enough to indicate vitamin E deficiency. Thus, it appears vitamin E deficiency may be quite prevalent under local conditions and further work should be initiated to investigate just how far this might extend.

The uptake of tocopherols from the injectible vitamin E preparation used in these experiments was tested in two wethers, in which the plasma tocopherol levels at the time of the injection were below 30 mcg./100 ml. There was a response within 24 hours: 700 I.U. of vitamin E resulted in plasma total tocopherols of 3000 to 5000 mcg./100 ml. The blood tocopherol level gradually declined from this point and after one week assumed a stationary level at approximately 100 mcg./100 ml. plasma.
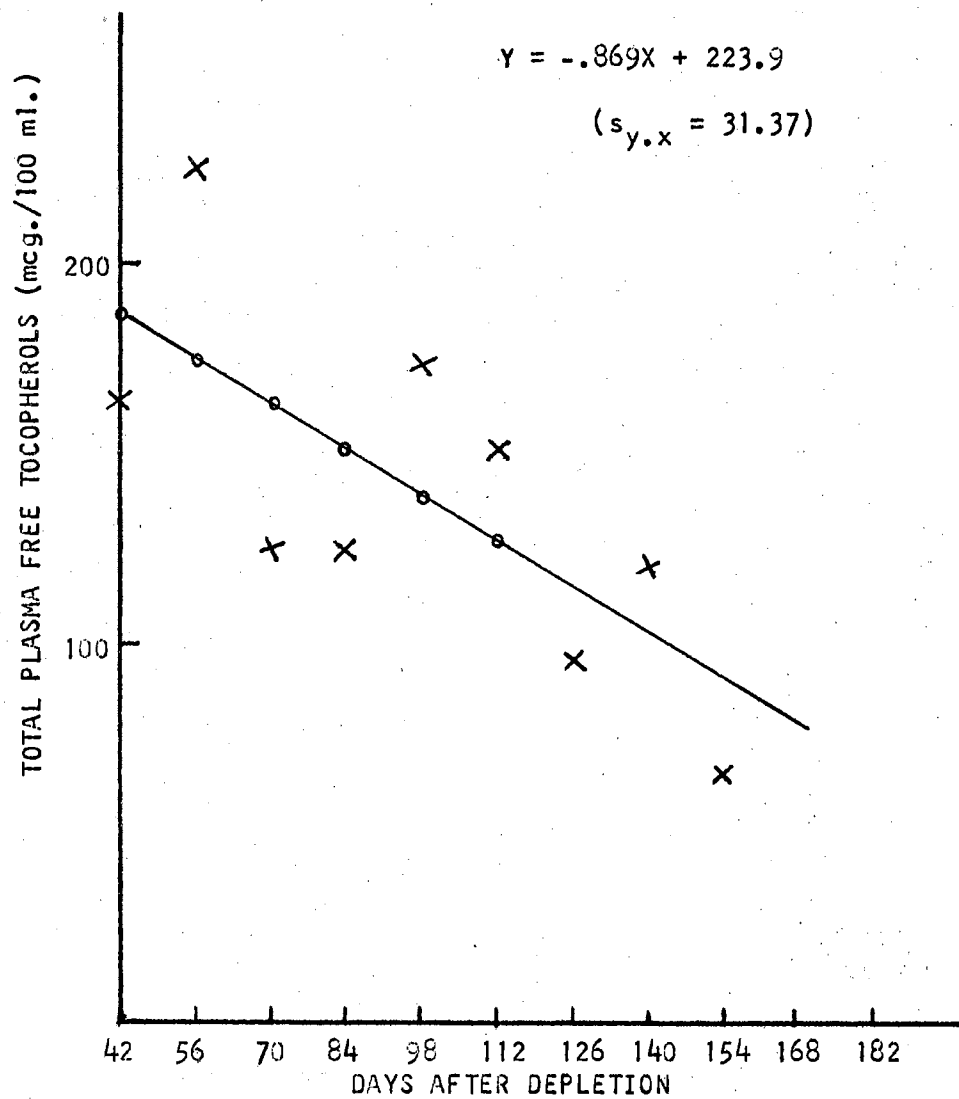
Figure 5.   The Rate of Depletion of Total Free
            Tocopherols in Blood Plasma of Five
            Ewes After the Cessation of Vitamin
            E Treatment That Lasted 300 Days.

Vitamin E and selenium both increased (P $<$ .05) plasma vitamin A levels (Table VIII). Carotenes were not found in any plasma in these experiments. Plasma levels of vitamin A found in this trial were not so low as to indicate uncomplicated vitamin A-deficiency in any animal.

Blood Hematocrit, Hemoglobin, Red Cell Counts and Plasma Protein

In the early stages of both trials, there was depression of hematocrit, hemoglobin and red blood cell concentration in all treatments (Appendix Tables XXI - XXV). This is probably a reflection upon the transition to feeding a purified diet. In Trial 1, it was calculated that the corpuscular hemoglobin concentration increased, which reflected upon the fact that the hematocrit was the most severely affected. Although this phenomenon did occur among animals on all treatments, hematocrit values were significantly lower (P $<$ .05) among animals treated with selenium after 28 days in Trial 1 and 14 days in Trial 2. Blood hemoglobin was lower among animals on the same treatments. These phenomena could be a reflection upon greater water content in the extracellular compartment. Since this appeared as an early response in the trials, and no explanation for it is apparent, further investigation in this area will be required.

After these parameters returned to normal, there were no consistent differences according to treatment, even after 300 days on the trial. In Trial 2, however, blood hematocrit and hemoglobin rose markedly (P $<$ .01) just before the animals fed the basal diet died. There were no consistent differences found in plasma protein values and these results are in the Appendix (Table XXVI).

TABLE VIII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF VITAMIN A IN BLOOD PLASMA OF THE EWES

	Treatment				
Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
Number of ewes	6	6	6	6	
Concentration, mcg./100 ml.					
On day					
140	33.4^{b}	$39.2^{b,c}$	49.7^{c}	51.3^{c}	4.6
154	43.0	50.4	56.0	63.9	7.8

[a]Standard error of the treatment means.

[b,c]Horizontal values on the same line with different superscripts differ significantly ($P < .05$).

It was observed that vitamin E deficiency resulted in several hemolysed blood samples while selenium appeared to have no effect. There is controversy concerning the phenomenon of peroxide-induced hemolysis of the erythrocyte in sheep on vitamin E-deficient diets (Boyd, 1968). It is possible that the apparent hemolysis observed in these experiments was not a result of fracture of the red blood cell but was related to myoglobin release from dystrophic muscle.

Plasma Enzymes

General increases (P < .05) occurred in the plasma levels of GOT (Figures 6 and 7), CPK (Figure 8), HBD and LDH (Figures 9 and 10), FDA and GPT (Table IX). All of these enzymes were elevated in response to vitamin E- not selenium-deficiency. Elevated enzyme levels were not found in any of the lambs born to ewes receiving vitamin E, which ultimately died of heart necrosis, but blood was not sampled near the time these animals died. The data on tissue distribution of these enzymes indicate that muscle was the primary source of the enzymes in plasma (Boyd, 1962, 1964; Bruns and Bergmeyer, 1965; Wolf, 1965; Carper and Roesler, 1967).

Changes in the ratio, HBD/LDH, reflect upon changes in the isoenzyme pattern of LDH (Elliot and Wilkinson, 1961). A significant decrease in the ratio (P < .01) occurred at 56 days in animals on the basal treatment in Trial 2 (Table X). This is indicative of an increase in proportion of the isoenzyme from skeletal muscle as opposed to cardiac muscle; thus confirming the observations of Boyd (1964) and Paulson et al., (1966).

Boyd (1964; 1968) has reported increases of GDH in vitamin E-
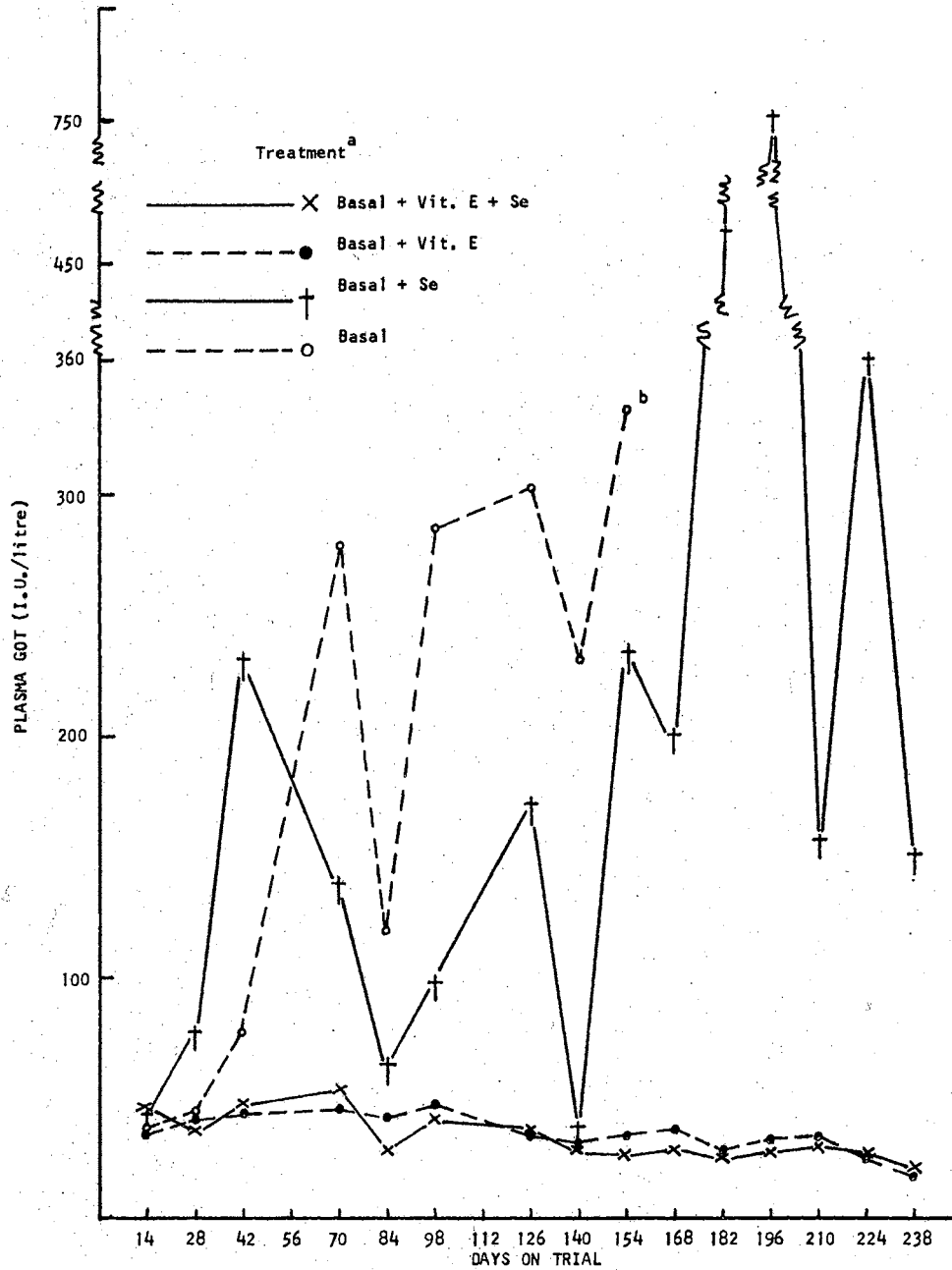
Figure 6. The Effect of Vitamin E and Selenium on the Activity of Glutamate-Oxaloacetate Transaminase in Blood Plasma of the Ewes. ([a]Standard errors of treatment means (each point represents a mean of six observations) are in the Appendix (Table XXX). [b]Ewes which were on the basal treatment died.)
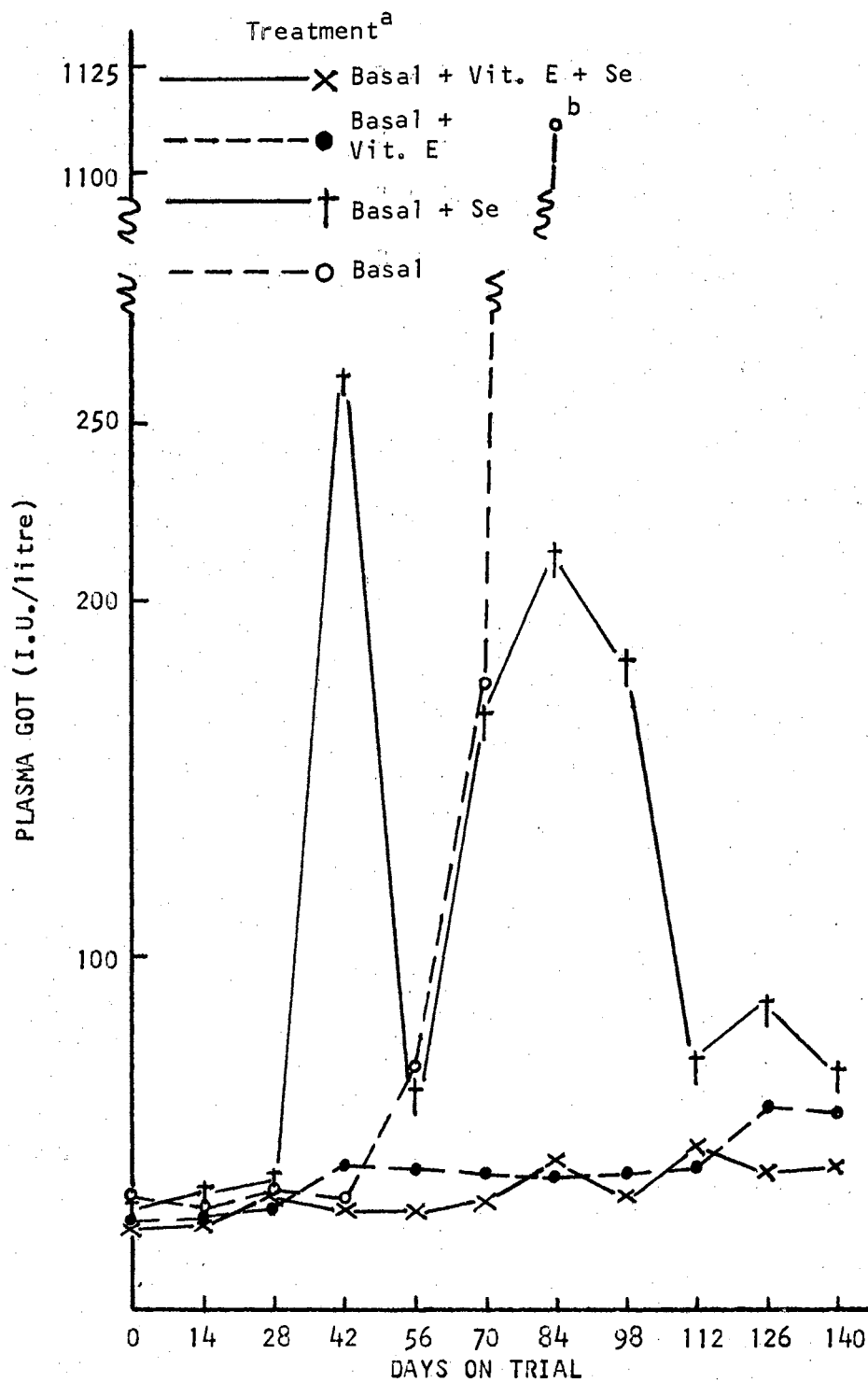
Figure 7. The Effect of Vitamin E and Selenium on the Activity of Glutamate-Oxaloacetate Transaminase in Blood Plasma of the Rams. ([a]Standard errors of treatment means (each point represents a mean of three observations) are in the Appendix (Table XXXI). [b]Rams which were on the basal treatment died.)
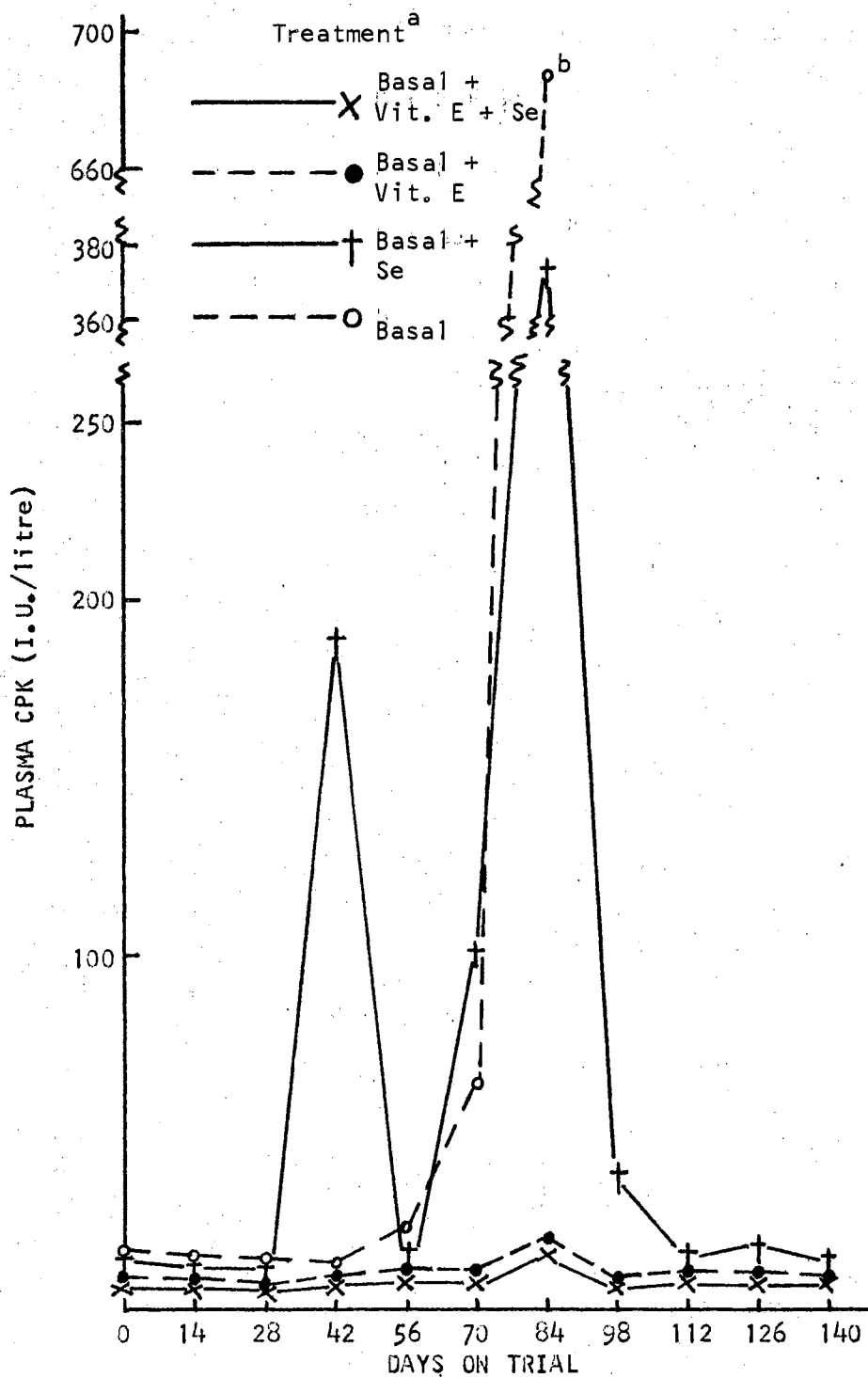
Figure 8. The Effect of Vitamin E and Selenium on
the Activity of Creatine Phosphokinase
in Blood Plasma of the Rams ([a]Standard
errors of treatment means (each point
represents a mean of three observations)
are in the Appendix (Table XXXII).
[b]Rams which were on the basal treatment
died).
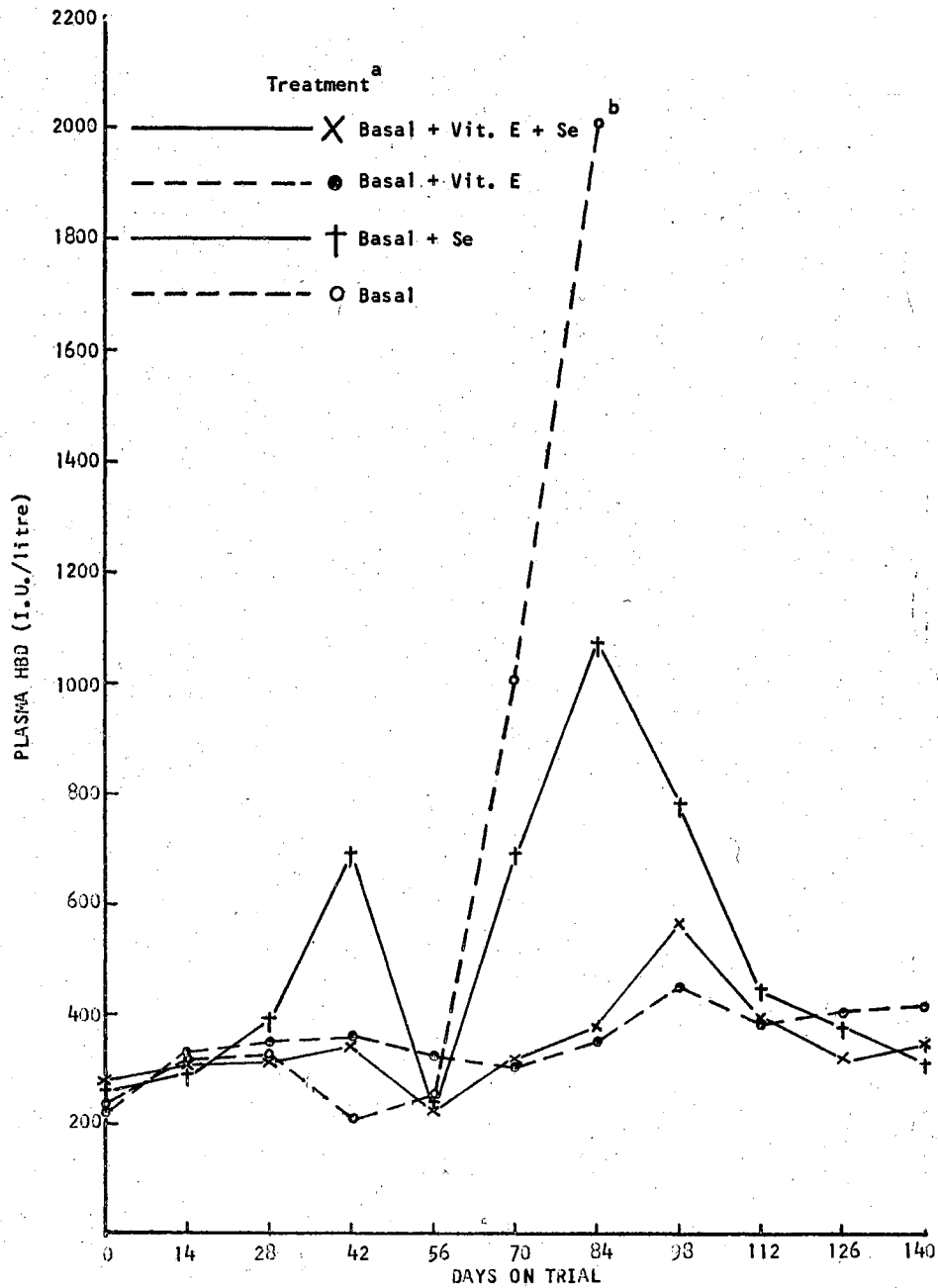
Figure 9. The Effect of Vitamin E and Selenium on
the Activity of Alpha-Hydroxy Butyric
Acid Dehydrogenase in Blood Plasma of
the Rams ([a]Standard errors of treat-
ment means (each point represents a
mean of three observations) are in
the Appendix (Table XXXIII). [b]Rams
which were on the basal treatment
died.)
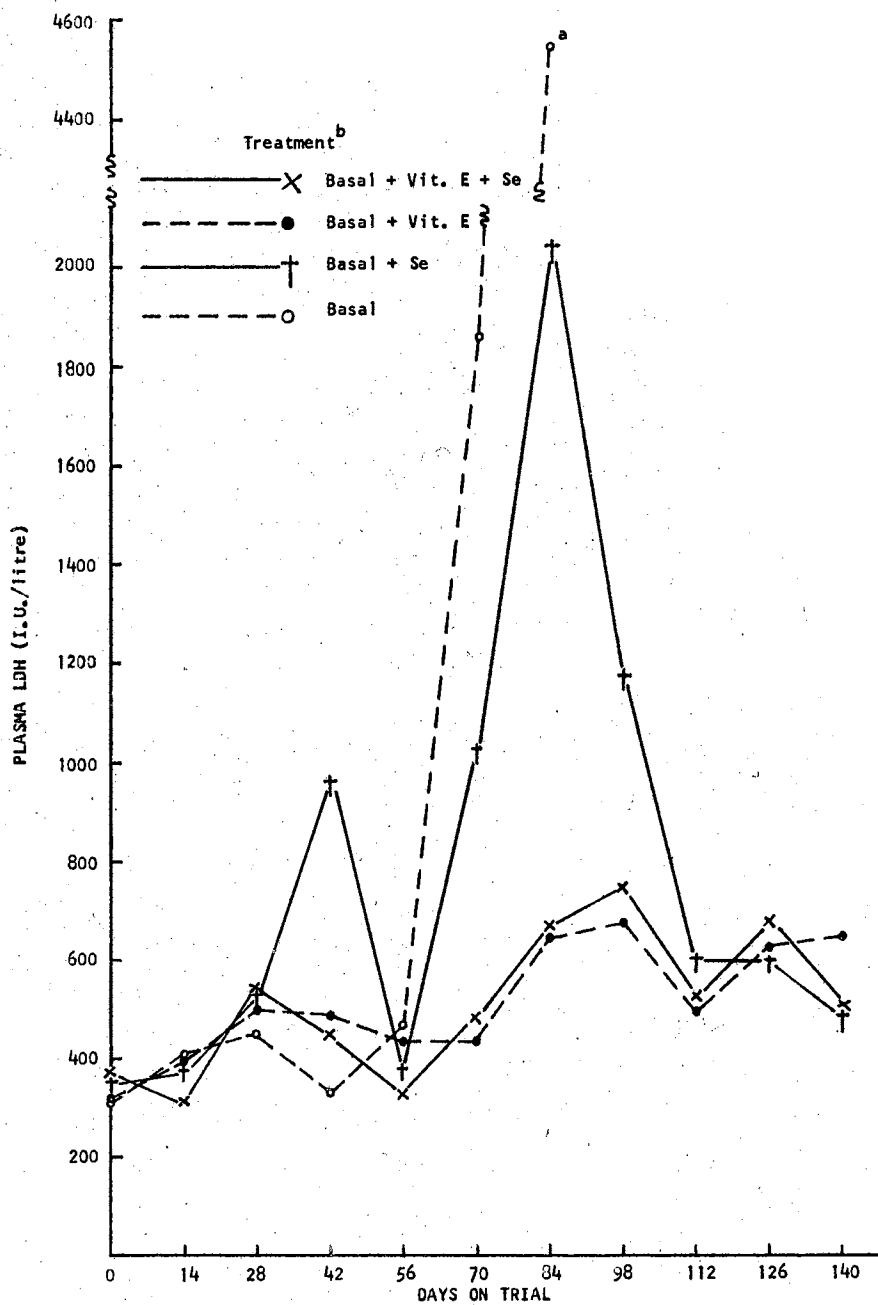
Figure 10. The Effect of Vitamin E and Selenium on the Activity of Lactic Acid Dehydrogenase in Blood Plasma of the Rams. ([a]Standard errors of treatment means (each point represents a mean of three observations) are in the Appendix (Table XXXIV). [b]Rams which were on the basal treatment died.)

TABLE IX

THE EFFECT OF VITAMIN E AND SELENIUM ON THE ACTIVITIES
OF SOME ENZYMES IN BLOOD PLASMA OF THE RAMS[a]

| Item | Days on Trial | Treatment | | | SE[b] |
		Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	
Number of rams		3	3	3	
Activity, I.U./1.					
Glutamate-	98	24.5^c	12.3^d	10.2^d	2.9
Pyruvate	112	20.4	9.3	15.3	3.7
Transaminase	126	19.7	11.7	14.1	3.9
	146	13.8	30.0	12.0	5.7
Glutamate	98	16.8	17.6	17.1	4.6
Dehydrogenase	112	25.2	10.1	6.9	8.5
	126	19.6	31.9_f	15.4	10.7
	140	15.3^e	33.5^f	12.6^e	2.3
1,6 Diphospho-	98	57.5	24.2	32.2	10.0
fructoaldolase	112	34.5_c	25.1_d	26.5_d	3.1
	126	98.7^c	40.7^d	33.9^d	10.8
	140	59.2^c	$47.7^{c,d}$	34.9^d	5.9
1, Phospho-	112	15.2	16.0	16.7	2.1
fructoaldolase	126	6.9	11.7	12.0	1.9
Isocitrate	126	7.7	7.0	4.8	1.3
Dehydrogenase	140	1.8	4.1	7.3	2.4
Ornithine	112	0.36	0.38	0.32	0.01
Transcarbamylase	126	0.24	0.25	0.23	0.01

[a]Rams who were on the basal treatment died.

[b]Standard error of the treatment means.

[c,d]Horizontal values on the same line bearing different superscripts differ significantly (P < .05).

[e,f]Horizontal values on the same line bearing different superscripts differ significantly (P < .01).

TABLE X

THE EFFECT OF VITAMIN E AND SELENIUM ON THE RATIO OF THE
ACTIVITY OF ALPHA-HYDROXY BUTYRATE DEHYDROGENASE (HBD)
TO THE ACTIVITY OF LACTATE DEHYDROGENASE (LDH) IN
BLOOD PLASMA OF THE RAMS

	Treatment				
Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
Number of rams	3	3	3	3	
Ratio, %					
On day					
0	72.0	73.1	69.8	68.2	2.0
14	79.2	80.3	80.5	77.9	5.1
28	72.3	72.9	69.9	60.5	5.2
42	63.7	73.5	73.2	72.8	6.9
56	56.1[b]	63.6[b,c]	72.7[c]	69.2[c]	2.5
70	62.6	62.3	68.9	65.0	6.3
84	45.3	54.3	55.9	57.6	3.2
98	—[e]	67.1	66.8	75.3	5.0
112		73.1[c]	77.1[d]	74.3[c]	0.3
126		63.8	66.7	52.2	7.5
140		64.6	63.9	68.9	2.9

[a] Standard error of the treatment means.

[b-d] Horizontal values on the same line with different superscripts differ significantly (P < .01).

[e] Rams who were on the basal treatment died.

deficiency in young lambs and has attributed this to transient periods of necrosis in GDH-rich hepatic tissue. This was not observed in the present experiments.

Selenium was without effect upon plasma enzyme levels ($P > .05$); a similar observation has been made by others (Wright and Bell, 1964; Hopkins et al., 1964). As all diets contained ethoxyquin, it is apparent that it was also without effect upon this phenomenon, which is contrary to the findings in young lambs made by Kuttler and Marble (1959) and Erwin et al. (1961). However, Erwin et al. (1961) applied a higher level of ethoxyquin (0.1 percent versus 0.0125 percent) in the diets used in their experiments.

Elevation of plasma enzymes in both trials occurred at an early stage; for example, GOT was elevated ($P < .05$) after only 28 days in the ewes. Although all enzymes appeared elevated after 42 days of Trial 2, this difference was not significant ($P > .05$); the elevation occurred in only one animal. Thus in Trial 2, the elevation of plasma enzymes was only significant after 70 days (GOT, $P < .05$) and agrees with the findings of Wright and Bell (1964). If very young sheep are used, this response to vitamin E-deficiency occurs much earlier (Boyd, 1968).

In both of these trials, it was found that the elevation of plasma enzymes occurred first among those vitamin E-deficient animals receiving selenium and is contrary to the findings of Wright and Bell (1964) and Hopkins et al., (1964), who found that selenium delayed an increase in plasma GOT in vitamin E deficiency.

Evaluation of data collected on individual sheep revealed a tendency for elevated enzyme levels in plasma to be transient. The fact

that the resultant peaks in plasma enzyme levels did not coincide among
different sheep on the same treatment and also that the peaks were of
different duration in different animals is largely responsible for the
extensive variation in these data. The variation is not due to analyt-
ical error, which was found to be low. The transiency in plasma enzyme
levels was not so pronounced in vitamin E-deficient animals not receiv-
ing selenium. In only two of the six ewes on this treatment in Trial 1,
did values return to normal before death. In Trial 2, all the sheep on
this treatment died once the plasma enzyme levels became elevated.
These results considered alongside those of animal survival indicate
that selenium could temporarily satisfy some of the demands of the
vitamin E-deficient animal. It is important to note that as a result
of the transient nature of elevated enzyme levels, a normal value of
an enzyme in plasma does not by itself confirm adequate vitamin E
status.

To test the effect of vitamin E on restoration of plasma enzyme
levels to normal, blood was sampled from the three ewes on the basal
ration in Trial 1, seven days after vitamin E was administered. The
GOT level found was similar to that in ewes treated with vitamin E and
selenium. This experiment was repeated in two wethers which were vit-
amin E deficient and the results are shown in Figure 11. The level of
GOT in plasma was reduced rapidly; the half-time for disappearance of
the enzyme being 1.6 to 2.1 days.

The phenomenon of elevated enzyme levels is worthy of some dis-
cussion since it is an early response to the condition of vitamin E-
deficiency. Blincoe and Marble (1960) obtained a correlation of 0.97
between LDH and GOT in plasma of sheep with muscular dystrophy, the
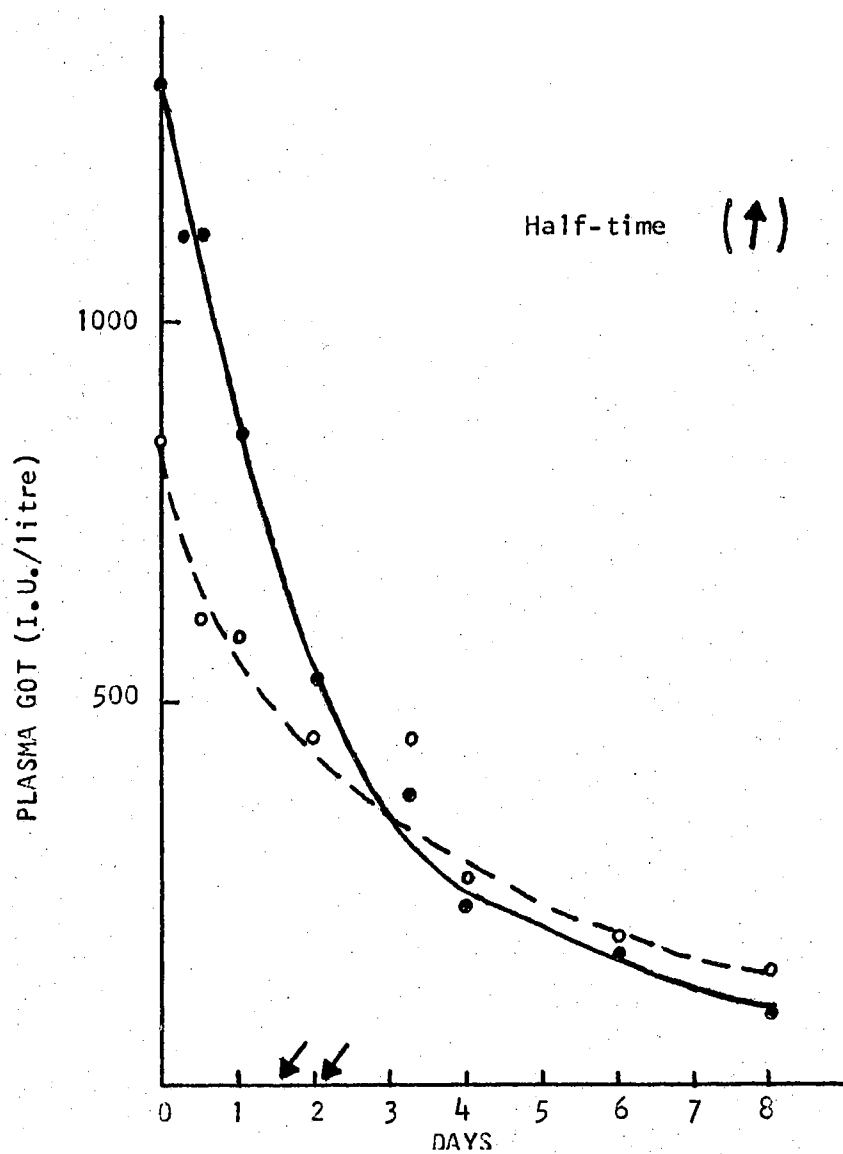
Figure 11. The Effect of Vitamin E Treatment
on the Activity of Plasma Gluta-
mate-Oxaloacetate Transaminase
in Vitamin E-Deficient Sheep.
(Two vitamin E-deficient wethers
were injected with 700 I.U.
Vitamin E at Day 0).

corresponding correlation for this trial is 0.70. The correlation co-efficient between GOT and CPK is 0.45. The following are factors which may be responsible for an inconsistent relationship between the activities of one enzyme to another. First, the activity of an enzyme may not be constant throughout the muscular system. Secondly, plasma enzymes may be removed from the circulatory system at different rates. Boyd (1967) demonstrated that the LDH isoenzyme which predominates in skeletal muscle is removed from plasma at a greater rate than the heart isoenzyme. Third, varying proportions of different tissues may be affected by vitamin E at different stages of deficiency.

Undoubtedly, necrotic tissue is the major source of plasma enzymes. However, it might be an oversimplification to ascribe this as the only mechanism resulting in the phenomenon (Henley et al., 1966). In a review on plasma enzymes, Hess (1963) states many conditions which cause an increase in cell permeability with consequent leakage of enzymes into the extracellular space. A fundamental cause is an oxygen deficiency and a reduction in ATP. It is possible therefore that vitamin E may have a role at some subcellular site which is directly responsible for maintaining the cell membrane. There is a need to evaluate whether or not elevated plasma enzyme levels in vitamin E deficiency precede the appearance of any necrotic tissue.

There are few reports available in the literature on standard plasma enzyme levels in sheep. Thus the values obtained in these experiments for sheep treated with vitamin E and selenium have been collected together and are given in the Appendix (Table XXVIII).

Relative Weight of Organs

Vitamin E deficiency resulted in an increase in the relative weight of liver ($P < .05$) and kidneys (Table XI). This observation is most pronounced for those animals which died from the vitamin E deficiency, in which the average relative liver, heart and kidney weights were 17.9, 4.8 and 3.4 gm./kg., respectively. The increase in relative organ weight could be a result either of enlargement of the organ itself or a reduction in peripheral body tissues. It would appear that it is a result of enlargement of the organ itself, because there were no significant differences in body weight among the animals. Thus, the effect of different treatments used in this trial upon activities of enzymes and concentrations of vitamins in the tissues is expressed both on a per gram and a per organ basis.

Lysosomal Enzymes in Tissues

Vitamin E deficiency affects the activities of lysosomal enzymes in tissues of many animals. There appears to be only one report on sheep and this was on muscle (Desai, 1966). The values obtained for protease activities in liver are shown in Table XII. Total liver protease was greater ($P < .05$) in vitamin E-treated ewes than in selenium- and vitamin E-treated ewes. There were no differences ($P > .05$) between treatments regarding the proportion of the total enzyme activity which could be associated with the different subcellular fractions. The total proteolytic activity of sheep liver appears to be greater than in chicken (Thayer and Nelson, 1968) and rat liver (Dingle et al., 1966; Bunyan et al., 1967c). Muscle necrosis is the outstanding lesion in the vitamin E-deficient sheep or rabbit. Proteolytic activity in liver of

TABLE XI

THE EFFECT OF VITAMIN E AND SELENIUM ON THE RELATIVE WEIGHTS
OF LIVER, HEART AND KIDNEYS IN THE EWES[a]

Item	Treatment			
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of animals	6	6	6	
Live weight, kg.	55.2	48.8	53.0	3.21
Liver, gm./kg. live wt.	12.0^c	$11.3^{c,d}$	9.7^d	0.65
Heart, gm./kg. live wt.	3.8	3.6	3.7	0.20
Kidneys, gm./kg. live wt.	2.0	2.0	1.6	0.15

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c,d]Horizontal values on the same line bearing different super-
scripts differ significantly ($P < .05$).

TABLE XII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE TOTAL ACTIVITY, SPECIFIC ACTIVITY AND SUBCELLULAR DISTRIBUTION OF ACID PROTEASE IN LIVER OF THE EWES[a]

Item	Treatment			SE[b]
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	
Number of animals	6	6	6	
Total activity				
Units/mg. liver[c]	$17.7_{d,e}$	24.6_e	17.7_d	2.87
Units/liver/gm. live wt.[c]	$215.5^{d,e}$	273.8^e	166.9^d	30.2
Specific activity				
Units/mg. soluble protein[c]	16.5	22.2	15.2	3.44
Nuclear fraction[c]				
Units/mg. liver[c]	7.8	10.1	8.0	1.46
% of total activity	46.3	41.6	43.0	4.49
Lysosomal fraction[c]				
Units/mg. liver[c]	6.5	9.3	6.6	1.08
% of total activity	38.5	37.7	39.0	3.97
Supernatant fraction[c]				
Units/mg. liver[c]	3.3	5.3	3.1	1.11
% of total activity	15.2	20.7	18.0	3.91

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c]For preparation of fractions and definition of units, see page 26.

[d,e]Horizontal values on the same line bearing different superscripts differ significantly ($P < .05$).

rabbits fed vitamin E-deficient diets was increased two-fold compared to the controls (Zalkin et al., 1962). Results of the present experiment indicate that this is not true for sheep.

Figure 12 illustrates the effect of treatment upon stability of the liver lysosomes. It was found that greater proportions of protease were released from liver lysosomes taken from the vitamin E-treated ewes than the others. As slopes of the lines are similar, the differences appear to be a result of greater lability from the origin of the incubation rather than a greater rate of release. In vitamin E-treated ewes, it was found that a slightly greater proportion of subcellular liver protease was present in the supernatant fraction (Table XII).

The proportion of enzyme released after a two hour incubation of the lysosomes agrees with results obtained from chicken liver (Thayer and Nelson, 1968) and rat kidney (Moore et al., 1967).

The finding that vitamin E deficiency results in greater total activity for skeletal muscle protease and beta-glucuronidase (Table XIII) supports the results of Desai (1966), who used young lambs. Total activities of protease and beta-glucuronidase were 10.5- and 8.5-fold greater, respectively, for selenium- compared to vitamin E- and selenium-treated ewes. In two ewes, which were sampled in the terminal stages of vitamin E deficiency, total skeletal muscle protease and beta-glucuronidase activity were elevated 82- and 19-fold, respectively, which indicate the activities of the enzymes are greatly increased at this time.

Protease, as free enzyme, was found in only one vitamin E-deficient ewe. A greater proportion of the total beta-glucuronidase
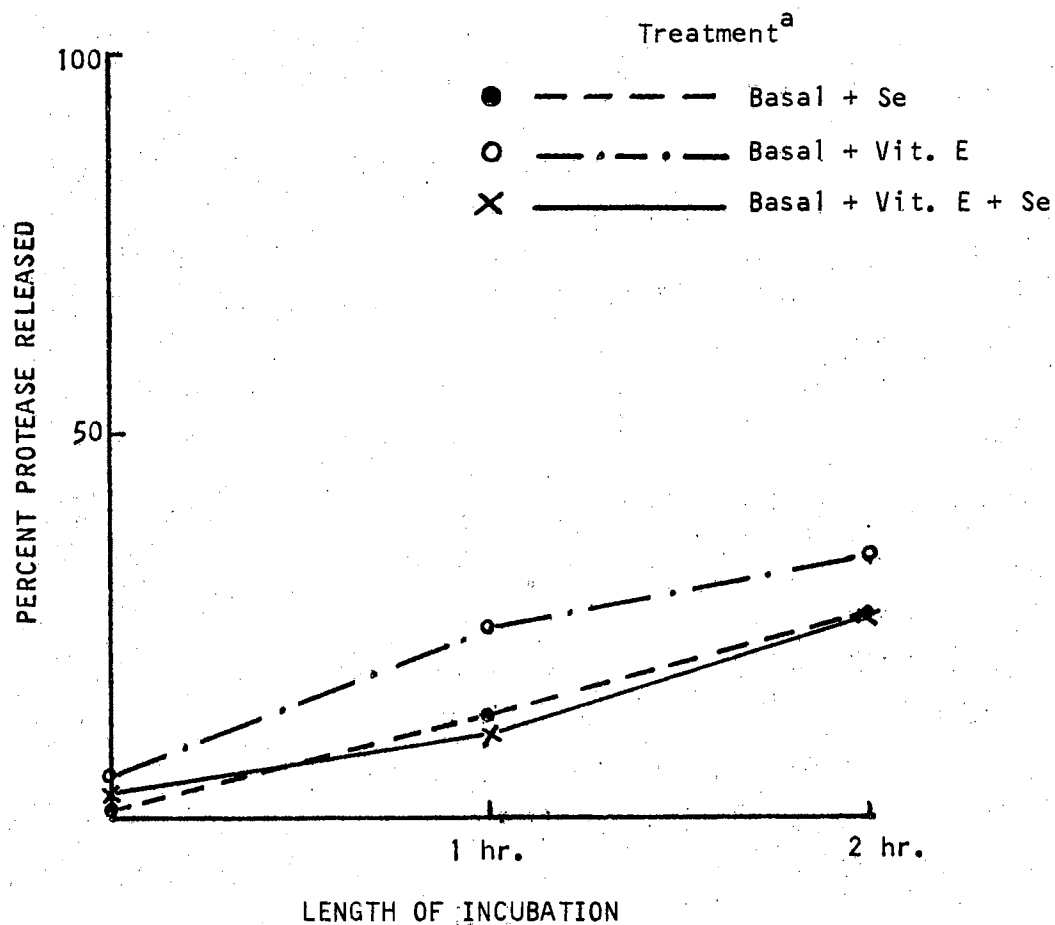
Figure 12. The Effect of Vitamin E and Selenium on the
Stability of Liver Lysosomes as Measured
by the Release of Acid Protease (For ex-
perimental technique, see page 26. [a]Each
point is the mean of six observations and
the standard error of the combined means
of eighteen observations per treatment is
2.6%)

TABLE XIII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE TOTAL AND FREE
ACTIVITY OF ACID PROTEASE AND BETA-GLUCURONIDASE IN
SKELETAL MUSCLE OF THE EWES[a]

| Item | Treatment | | | |
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Acid protease				
Number of animals	6	6	6	
Total activity, units/mg.[c]	0.443^d	0.046^e	0.042^e	0.091
Free activity, units/mg.[c]	0.022	ND	ND	0.013
Free activity, % of total activity	2.0			1.2
Beta-Glucuronidase				
Number of animals	5	4	5	
Total activity, m-I.U./gm.	4.34^d	1.06^e	0.51^e	0.315
Free activity, m-I.U./gm.[c]	1.87^d	0.18^e	0.15^e	0.373
Free activity, % of total activity	44.5	11.2	12.6	13.3

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment mean(s).

[c]For determination of free activity and definition of acid protease units, see page 26.

[d,e]Horizontal values on the same line bearing different superscripts differ significantly ($P \leqslant .01$).

ND, None detectable.

activity was free, indicating a difference in the subcellular localiza-
tion of these two enzymes in sheep skeletal muscle. Nevertheless, it
is clear that the amount of lysosomal bound enzyme was increased sig-
nificantly in vitamin E-deficiency. The proportion of free lysosomal
enzyme activity was not determined by Desai (1966). In work on muscle
lysosomes from vitamin E-sufficient and -deficient rabbits, a variation
among enzymes in the proportion of free enzyme was also found (Zalkin
et al., 1962). Contrary to results reported here, these workers showed
that 87 percent of the protease activity from vitamin E-deficient rab-
bit muscle was free, thus indicating a species difference.

Results of the present experiment indicate that the hydrolytic
enzymes of sheep muscle are structure linked. Activities of hydrolytic
enzymes in normal sheep muscle are extremely low; this is in agreement
with results obtained on the rabbit (Zalkin et al., 1962). It appears
that the species, which exhibit muscle lesions in vitamin E deficiency,
simultaneously exhibit increases in lysosomal enzyme activities (Zalkin
et al., 1962; Desai, 1966; Bunyan et al., 1967a ; Koszalki et al.,
1961).

Owing to the subjective nature of evaluating the extent of muscle
necrosis in these sheep, it appears impossible to ascribe a relation-
ship between the extent of necrosis and the elevation of lysosomal en-
zymes. Severe muscle necrosis and greatly elevated enzyme levels
existed in the skeletal muscle of two animals which were in terminal
stages of the vitamin E-deficiency syndrome.

Due to the involvement of lysosomal enzymes in vitamin E-deficient
muscle, it was decided to analyse uterine and placental tissue for the
same enzymes. However, no differences ($P > .05$) were found in the

activities of beta-glucuronidase and protease in the uteri and placentae from sheep (Table XIV). The activities of both enzymes were greater in the uterus and placenta than in skeletal muscle. Also, a consistently higher proportion of both of these enzymes were in the free form as compared to muscle.

In work on lysosomal enzymes in rat uterus, no differences could be attributed to vitamin E deficiency except three days after fetal resorption had taken place, at which time beta-glucuronidase increased (Bunyan et al., 1967b). These results indicate that the increase in activity was a requirement for involution of the uterus. Results in the present experiment support the hypothesis (Figure 13). A general increase in activities of lysosomal enzymes of uterus and placenta was observed toward the end of pregnancy.

The results of the present experiments indicate that vitamin E deficiency affects only the activity of the lysosomal enzymes in skeletal muscle, the tissue which is pathologically most severely affected. There was a relationship between selenium deficiency and the activity of protease in sheep liver but its significance is not clear; there were no obvious pathological lesions of this tissue associated.

Creatine Phosphokinase in Tissues

Elevated levels of several enzymes were found in blood plasma of vitamin E-deficient sheep. If this phenomenon is a consequence of tissue necrosis, then necrotic tissue should contain depressed quantities of the same enzymes. There were no significant differences (P > .05) in activity of CPK in any one tissue but values were lowest in skeletal muscle, heart and uterus and placenta of the vitamin E-deficient animals

TABLE XIV

THE EFFECT OF VITAMIN E AND SELENIUM ON THE TOTAL AND FREE
ACTIVITY OF ACID PROTEASE AND BETA-GLUCURONIDASE IN
UTERUS AND PLACENTA OF THE EWES[a]

| Item | Treatment | | | |
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Acid protease				
Number of animals	6	6	6	
Total activity, units/mg.[c]	9.0	10.7	5.2	3.30
Free activity[c], units/mg.	5.7	5.9	2.1	1.65
Free activity[c], % of total activity	64.9	65.4	73.6	11.7
Beta-glucuronidase				
Number of animals	5	4	5	
Total activity, m-I.U./gm.	22.6	17.8	15.1	5.93
Free activity[c], m-I.U./gm.	15.4	11.6	7.3	3.44
Free activity[c], % of total activity	67.1	73.0	62.7	15.2

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c]For determination of free activity and definition of acid
protease units, see page 26.
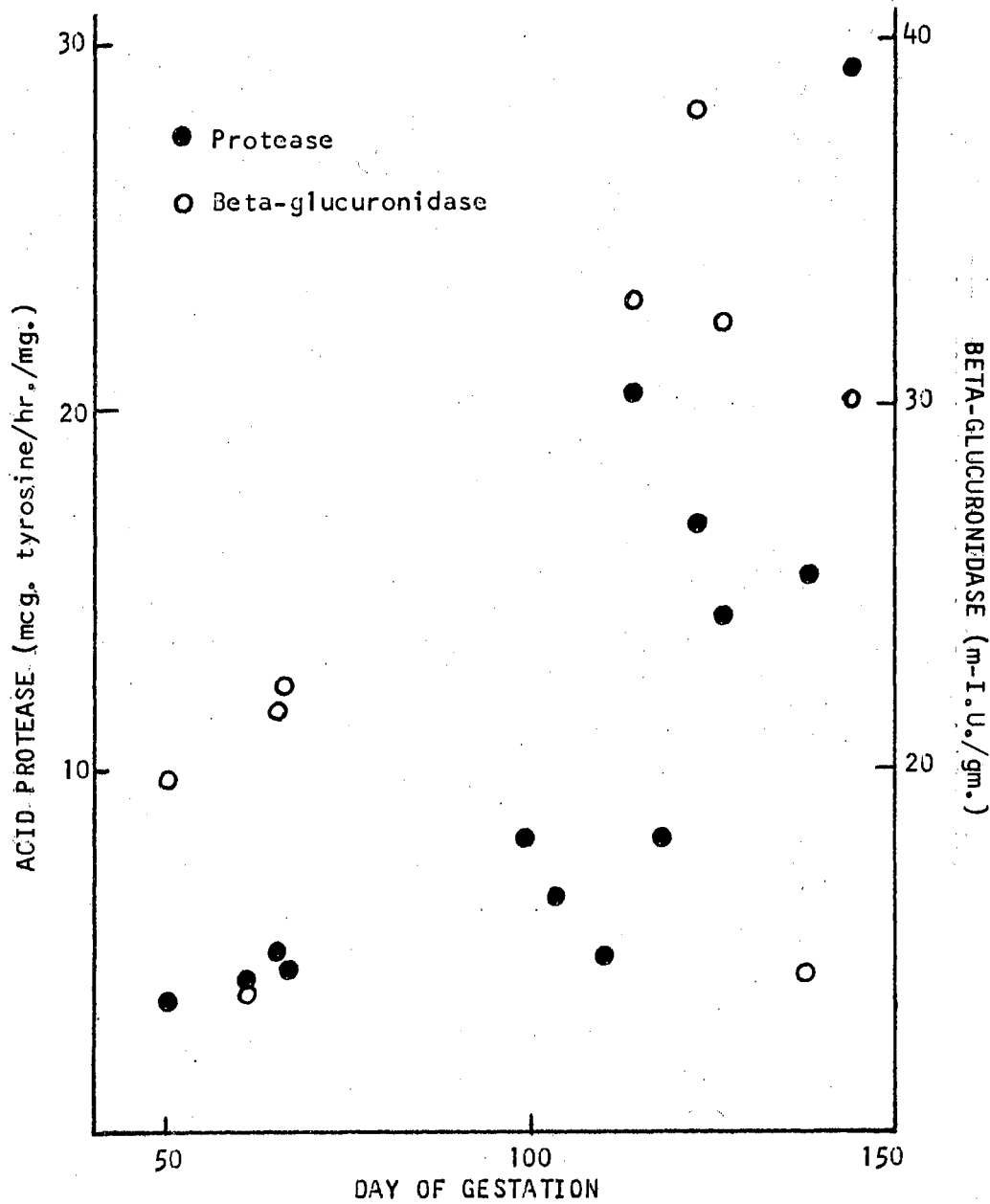
Figure 13. The Effect of Day of Gestation on the
Activities of Acid Protease and Beta-
Glucuronidase in the Uterus and Pla-
centa of the Pregnant Ewes. (Each
point represents one observation.
Mean values determined for non-
pregnant ewes: Acid protease - 1.1
mcg. tyrosine/hr/mg.; Beta-Glucuroni-
dase - 6.9 m-I.U./gm.)

(Table XV). Upon storage, detergent depressed activity of CPK, thus results presented are those obtained from homogenates without the inclusion of the detergent. Depression in skeletal muscle CPK activity was most striking in samples obtained from two animals, which were dying from vitamin E deficiency. The mean skeletal muscle CPK activity for these animals was determined to be 119.6 I.U./gm. On the contrary, the activity of CPK in the hearts of the same animals was increased to a mean value of 388 I.U. per heart per kg. live wt. Elevated activity of CPK in plasma was associated with depressed activity of CPK in muscle.

Activities of CPK in skeletal muscle and heart of sheep fetuses were below that obtained in adult animals but there were no differences (P > .05) between treatments. Values determined for muscle in the control adults of this experiment agree closely with values obtained in the horse (Cardinet et al., 1967).

Lactate Dehydrogenase and Alpha-Hydroxy Butyrate Dehydrogenase in Tissues

LDH is another prominent enzyme found in the soluble portion of the cell. As mentioned previously, at least three and maybe four of the five isoenzymes of LDH are not only active on the natural substrate of the enzyme, lactic acid, but also on alpha-hydroxy butyric acid. Different isoenzymes of LDH are present in different tissues of the sheep (Boyd, 1964). Thus changes in the proportion of activity, HBD/LDH, reflect changes in the composition of LDH in the different tissues.

Activities of HBD and LDH in skeletal muscle were lowest in vitamin E- and selenium-treated ewes but the difference was not significant (P > .05) (Table XVI). However, in one ewe, sampled in the terminal stage of the vitamin E-deficiency disease, the HBD and LDH values in

TABLE XV

THE EFFECT OF VITAMIN E AND SELENIUM ON THE ACTIVITY OF
CREATINE PHOSPHOKINASE IN SKELETAL MUSCLE, HEART AND
UTERUS AND PLACENTA OF THE EWES[a]

Item	Treatment			
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of animals	4	4	4	
Skeletal muscle, I.U./gm.	348.1	379.9	362.0	25.9
Heart				
I.U./gm.	31.8	35.2	42.5	4.6
I.U./heart/kg. live wt.	124.7	119.2	155.4	13.7
Uterus and placenta, I.U./gm.	5.3	8.2	6.2	1.2

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

TABLE XVI

THE EFFECT OF VITAMIN E AND SELENIUM ON THE ACTIVITIES OF
ALPHA-HYDROXY BUTYRATE DEHYDROGENASE (HBD) AND LACTATE
DEHYDROGENASE (LDH) IN SKELETAL MUSCLE, HEART AND
UTERUS AND PLACENTA OF THE EWES[a]

| | Treatment | | | |
Item	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of animals	4	4	4	
Skeletal muscle				
HBD, I.U./gm.	119.6	133.3	105.5	13.0
LDH, I.U./gm.	471.4	547.7	378.0	69.8
HBD/LDH, %	25.3	25.0	28.7	1.2
Heart				
HBD, I.U./gm.	95.0	83.8	100.8	6.7
HBD, I.U./heart/kg. live wt.	371.6[c]	283.2[d]	375.6[c]	25.1
LDH, I.U./gm.	123.0	113.0	130.9	10.4
LDH, I.U./heart/kg. live wt.	480.4	383.6	486.4	38.5
HBD/LDH, %	77.6	74.6	77.2	2.2
Uterus and placenta				
HBD, I.U./gm.	33.6	39.4	9.7	19.9
LDH, I.U./gm.	68.2	67.0	16.0	40.7
HBD/LDH, %	54.9	57.7	63.2	7.5

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c,d]Horizontal values on the same line bearing different super-
scripts differ significantly (P < .10).

skeletal muscle were depressed to 49.9 and 98.4 I.U. per gm., respec-
tively. Total HBD and LDH per heart were significantly lower (P $<$ .10)
in vitamin E-treated animals.

There were no treatment differences in the ratio of HBD/LDH, for
skeletal muscle, heart or uterus and placenta. These results support
the observations of Boyd (1964), who found that vitamin E deficiency
did not affect the spread of LDH isoenzymes in lamb tissues. In con-
trast, the ratio of HBD/LDH in the skeletal muscle of the sheep which
died from vitamin E-deficiency, was increased to 50.7 percent and this
result supports the observations of Paulson et al. (1966). Perhaps the
severity of vitamin E deficiency affects this phenomenon.

The work reported here on the ratio of HBD/LDH indicates that
there are major differences between sheep tissues, presumably reflect-
ing a change in spread of the isoenzymes of LDH. Like LDH, HBD is
simple to assay. This method should be explored as an alternative to
the somewhat laborious separation of the LDH isoenzymes by electro-
phoresis.

HBD and LDH were determined in the skeletal muscle and hearts of
the sheep fetuses and no treatment differences were found. LDH activity
increased with age of the fetus (Figure 14). As with CPK, prenatal
values determined were lower than values found in adult sheep. The
ratio of HBD/LDH was similar in the fetal heart (71.3) to the adult
heart, but in the fetal skeletal muscle the ratio was correspondingly
greater (38.5). No noticeable reduction occurred in the ratio, HBD/LDH,
as the fetus grew older.

There were trends toward lower activities of CPK, HBD and LDH in
vitamin E-deficient animals and differences were pronounced in those
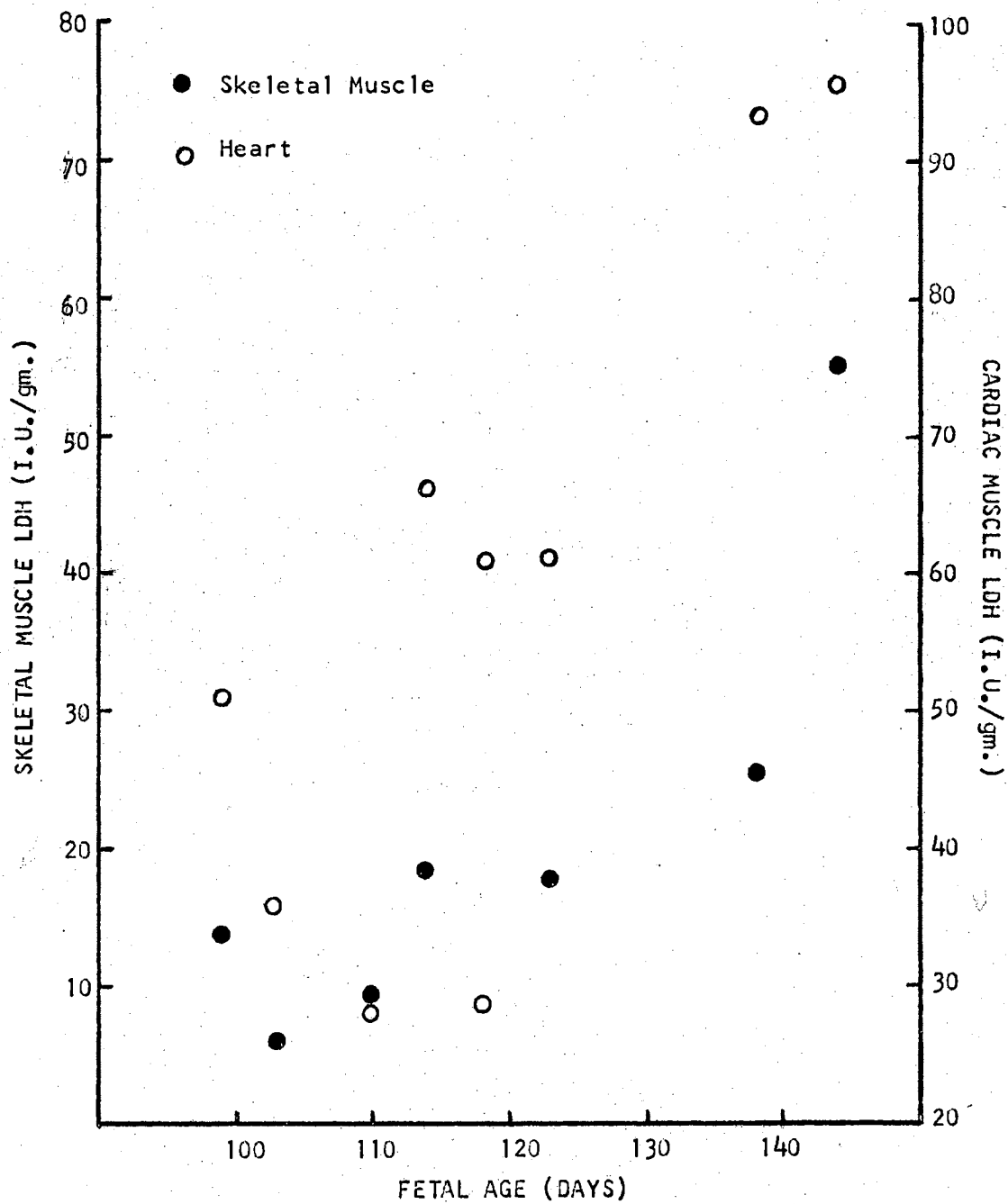
Figure 14. The Effect of Age on the Activity of Lactic
Acid Dehydrogenase in Skeletal and Cardiac
Muscle of the Ewes' Fetuses. (Each point
represents one observation.)

animals dying from the deficiency. Activities of CPK, HBD and LDH in the hearts were lowest in selenium-deficient animals but the significance of this observation is not clear.

Tocopherols and Vitamin A in Tissues

Tocopherol concentrations (Table XVII) in all tissues except liver were lower in the vitamin E-deficient ewes than in those ewes treated with vitamin E. There was great variation in liver tocopherol levels. A possible explanation concerns the fact that the tocopherol level in one vitamin E-treated sheep was 345 mcg./gm.; the sheep did not grow normally and weighed only 25 kg. at sacrifice, but no serious pathological changes were noted. If this value is removed from the statistical analysis, then the effect of vitamin E deficiency on tocopherol concentration in liver is significant (P $<$ .005). If the tocopherol values are expressed on an organ basis, differences between vitamin E-deficient and vitamin E-treated ewes are also significant (P $<$ .05).

Selenium had no effect (P $>$ .05) on the tissue tocopherol levels of vitamin E-treated ewes. Greater concentrations of tocopherol shown in this experiment in favor of the ewes not receiving selenium is largely a reflection of high tocopherol concentration in all tissues of the one ewe, which had an extremely high liver value. The results of the present experiment do not affirm the hypothesis that selenium enhances retention of vitamin E (Desai and Scott, 1965). Selenium dosage to vitamin E-treated rats increased the concentration of alpha-tocopherol in heart and liver, but the results were not consistent (Edwin et al., 1961). Liver tocopherol values for the vitamin E-sufficient sheep in the present experiment are in agreement with values reported by

TABLE XVII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF TOTAL TOCOPHEROLS IN LIVER, SKELETAL MUSCLE, HEART,
KIDNEYS AND UTERUS AND PLACENTA OF THE EWES[a]

Item	Treatment			
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of animals	6	6	6	
Liver, mcg./gm.	21.3	93.8	47.2	29.2
mcg./liver/kg. live wt.	261.	1102.	455.	358.
Skeletal muscle, mcg./gm.	3.7^c	12.1^d	9.8^d	1.6
Heart, mcg./gm.	5.2^c	24.0^d	19.4^d	1.9
mcg./heart/kg. live wt.	19.6^c	89.8^d	71.4^d	9.8
Kidneys, mcg./gm.	5.7^c	16.4^d	15.6^d	1.7
mcg./kidneys/kg. live wt.	11.7^e	36.0^f	25.4^{e,f}	6.6
Uterus and placenta mcg./gm.	2.7^e	9.1^f	6.6^f	1.3

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c,d]Horizontal values on the same line bearing different super-
scripts differ significantly (P < .005).

[e,f]Horizontal values on the same line bearing different super-
scripts differ significantly (P < .05).

Rousseau et al. (1957), but they did not measure liver tocopherols in vitamin E-deficient sheep. The results obtained for liver tocopherol in vitamin E-deficient animals in the present experiment appear high. This could be a fault in technique in which another compound in liver was extracted besides tocopherol which fluoresced as does tocopherol. A compound was found in liver which severely inhibited the fluorescence of tocopherol. This is probably vitamin A (Duggan, 1959). Because of this inhibition, extremely dilute concentrations of liver extracts had to be used for measurements.

The results of the present experiment support the findings in rats (Edwin et al., 1961) and rabbits (Green et al., 1961) which show that tocopherol is widely distributed throughout the body. In rats, the uterus is especially susceptible to vitamin E deficiency as indicated by concentrations of tocopherols (Edwin et al., 1961). In the rabbit, skeletal muscle is the more susceptible tissue (Green et al., 1961). The results of the present experiment indicate that both of these tissues have lower tocopherol concentrations than any of the others examined.

Esterified tocopherols (Table XVIII) were not present in liver samples and were found in one kidney sample. With the exception of one skeletal muscle sample, esterified tocopherols were not present in vitamin E-deficient ewes. The amounts of esterified tocopherols found in tissues of vitamin E-treated ewes were very low and could be detected in 50 percent of the samples only.

The total free tocopherol levels of fetal tissues are presented in Table XIX and values are lower than those found in the adult tissues. There were no significant differences (P $\geqslant$ .05) between treatments

TABLE XVIII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE RATIO OF
ESTERIFIED TOCOPHEROLS TO TOTAL TOCOPHEROLS IN
SKELETAL MUSCLE, HEART, KIDNEYS AND UTERUS
AND PLACENTA OF THE EWES[a,b]

	Treatment			
Item	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[c]
Number of animals	6	6	6	
Skeletal muscle, %	6.6	6.0	1.3	4.2
Heart, %	0	7.0	5.1	2.5
Kidneys, %	0	2.7	0	1.6
Uterus and placenta, %	0	4.4	8.2	4.1

[a]Ewes who were on the basal treatment died.

[b]No esterified tocopherols were found in the liver of any animal.

[c]Standard error of the treatment means.

TABLE XIX

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF TOTAL FREE TOCOPHEROLS IN LIVER, SKELETAL MUSCLE,
HEART AND KIDNEYS OF THE EWES' FETUSES[a]

	Treatment			
Item	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Liver				
Number of fetuses	5	4	3	
Concentration, mcg./gm.	5.0	7.9	8.6	1.5
Skeletal muscle				
Number of fetuses	5	3	2	
Concentration, mcg./gm.	2.8	4.0	3.6	0.9
Heart				
Number of fetuses	2	5	3	
Concentration, mcg./gm.	2.3	7.5	5.2	3.6
Kidneys				
Number of fetuses	3	3	1	
Concentration, mcg./gm.	1.8	5.8	3.6	2.6

[a]No fetuses were obtained from ewes who were on the basal treatment.

[b]Standard error of the treatment means.

although fetuses from vitamin E-deficient ewes contained lower concentra-
tions than fetuses from vitamin E-treated ewes. The ratio of total free
tocopherol concentration in adult tissues for ewes on the vitamin E and
selenium treatment over those on the selenium treatment varied from 2.2
for liver to 3.7 for heart. The corresponding ratio was reduced in the
fetuses to 1.7 for both liver and skeletal muscle to 2.7 for heart.
These findings agree with those of Whiting and Loosli (1948) and Par-
rish et al. (1950), who measured the effect of vitamin E supplementa-
tion to the dam on concentration of tocopherol in the liver and plasma
of the newborn. Tocopherols pass across the placenta, but only with
difficulty, and the factors affecting this are not known. In this
trial with sheep, selenium had no observable effect upon the phenomenon.

Concentration of vitamin A in liver was increased by vitamin E but
treatment differences were not as pronounced as found in plasma (Table
XX). Liver vitamin A values obtained here are tenfold greater than
those reported by Rousseau et al. (1957); hence, no animal in this ex-
periment should have suffered directly from vitamin A deficiency. The
data for liver vitamin A in sheep fetuses indicate that vitamin E and
selenium treatment might enhance the vitamin A status of the newborn,
but treatment differences found in these experiments were not signifi-
cant (P > .05).

General Disscusion

No live lambs were born to ewes on the basal diet, and successful
production of weaned lambs occurred only in those ewes treated with
both vitamin E and selenium. There were no observable pathological
lesions of the reproductive tracts of ewes on any treatment. Work on

TABLE XX

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF VITAMIN A IN LIVER OF THE EWES AND THEIR FETUSES[a]

Item	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Ewes				
Number	6	6	6	
Concentration				
mcg./gm.	155.2	183.2	207.2	19.2
mcg./liver/kg. live wt.	1858.	2075.	2001.	211.
Fetuses				
Number	5	4	3	
Concentration				
mcg./gm.	0.32	0.51	0.70	0.18

Header spanning "Treatment" over first three treatment columns.

[a] Ewes who were on the basal treatment died.

[b] Standard error of the treatment means.

the vitamin E-deficient pregnant rat indicates that resorption may take place as a result of fetal degeneration (King and Verma, 1967). In the present experiments, no degeneration or similar abnormality of vitamin E-deficient sheep fetuses was noticed, although there was a resorbed fetus from one ewe on the basal diet. It appears probable that the abortions from cows, bred on pastures in California associated with vitamin E deficiency, probably were not the result of simple vitamin E deficiency (Mace et al., 1951). The same might apply to the work of Virtanen (1966) and Matrone et al. (1965) who encountered irregular estrus cycles. Matrone et al. (1965) prevented the reproductive difficulty with the addition of five percent alfalfa meal to the purified diet and it appears highly improbable that this alone would supply sufficient vitamin E. Similarly, it would appear that the weak or dead lambs, born to ewes on a vitamin E-low diet, would be consequent to the chronic state of vitamin E deficiency in the same animals (Erlinger, 1968).

If the effect of vitamin E on reproduction in sheep is an indirect one, it may be mediated through another nutrient. The results of these experiments indicated that vitamin E and selenium additively increase vitamin A concentration of tissues especially plasma. There is a need to investigate the effect of vitamin E and selenium on transfer of vitamin A across the placenta.

No significant effect of vitamin E or selenium on fertility of rams is demonstrated in these experiments. It is unfortunate that the rams used were not fertility tested before being placed on the experiment. If this had been done, it would have been easier to determine the cause of sterility in two of the rams used.

Muscular dystrophy symptoms in the sheep used in these trials were encountered after the animals had developed functional rumens, and this confirms the earlier results of Erlinger (1968). Dietary unsaturated fatty acids are necessary to produce muscular lesions in immature calves or lambs (Blaxter, 1962a). Unsaturated fat is hydrogenated by the microflora of the rumen (Shorland et al., 1957). The proportion of unsaturated fat to total fat leaving the rumen is extremely small but this increases with passage through the gut (Lennox et al., 1968). The source and origin of unsaturated fat in the lower gut are the phospholipids of bile. Linoleic acid is the unsaturated fatty acid antagonistic to vitamin E for muscular dystrophy in the chicken (Hutcheson et al., 1963). Linolenic acid prevents deposition of arachidonic acid (formed from linoleic acid) in rat tissues and thus alleviates the linoleic acid-vitamin E antagonism (Witting and Horwitt, 1964). Amounts of linoleic and linolenic acids entering the body from the gut of the adult ruminant animal are small and in equal amount (Lennox and Garton, 1968). Therefore, the question undoubtedly arises as to what unsaturated fatty acid, if any, is antagonistic to vitamin E in these ruminant tissues. Future investigations should concern the possibility that biohydrogenation of unsaturated fatty acids does not take place in the rumens of adult sheep fed synthetic diets similar to the one used in these experiments. Another possibility is to raise the level of dietary antioxidant to investigate if antioxidants will completely replace vitamin E in these rations. Erwin et al., (1961) used diets containing 0.1 percent ethoxyquin for immature lambs and prevented dystrophy. Only 0.0125 percent ethoxyquin was used in the diets of these experiments.

Further research is indicated for confirmation of selenium-

deficiency symptoms in ruminants fed synthetic diets. The purified

diet used in the present experiments contained less than 0.005 ppm

selenium and the minimum requirement for selenium appears to be 0.02

ppm (Thompson and Scott, 1968). Therefore, this diet, fed to sheep,

would certainly have application in studying the biological role of

selenium.

CHAPTER V

SUMMARY

The primary objective of this study was to determine the effect of vitamin E and selenium on reproduction in sheep. Forty-eight ewe and twelve ram lambs (four months of age) were fed a purified diet containing urea as the sole nitrogen source, corn oil (one percent) and ethoxyquin (0.0125 percent). The experimental design was a two by two factorial. Subcutaneous injections of vitamin E (700 I.U. as dl alpha-tocopheryl acetate) and selenium (five mg. as sodium selenate) were given separately and in combination, at weekly intervals. After 140 days, one half the ewes were bred and treatments continued until lambing and for several additional months. The remaining ewes were bred and sacrificed at various stages of pregnancy to obtain tissues for pathological and biochemical analyses as well as to observe fetal development. Various fertility tests were conducted in the rams after 140 days on trial. Blood samples were taken biweekly from half the ewes and all the rams for chemical analyses.

Sheep fed the basal diet died between the 80th and 230th days of the trials. Treatment with vitamin E prevented the deaths of some of these animals. Selenium treatment delayed but did not prevent death. Death was sudden. Skeletal muscle necrosis was observed in all vitamin E-deficient sheep, post-mortem, and pathology was evident in the heart and kidneys of some of these animals. Growth was greatest in sheep

treated with selenium. Satisfactory reproductive performance was only
obtained in ewes treated with a combination of vitamin E and selenium.
Abortions occurred in some ewes on the selenium-deficient treatment.
Myocardial necrosis was noted in lambs from other ewes on the same
treatment. Lambs from selenium-treated ewes did not survive beyond 56
days of age. One ewe fed the basal diet resorbed a fetus. No pathology
was noted in the reproductive tracts or fetuses of any ewes, or in the
testes of any rams. There were no significant differences in fertility
of the rams.

Vitamin E and selenium increased tocopherol concentration of blood
plasma one week after the first injections. This response to selenium
was reversed later in the trial. Vitamin A concentration in blood plas-
ma was increased by both vitamin E and selenium. Blood hematocrit,
hemoglobin and red cell concentrations were depressed in all animals at
the start of the experiments and were lowest in the selenium-treated
sheep. Elevated activities of glutamate-oxaloacetate transaminase,
lactate dehydrogenase, alpha-hydroxy butyrate dehydrogenase, creatine
phosphokinase and 1,6 diphosphofructoaldolase were found in blood plasma
of vitamin E-deficient sheep after 28 days on trial. The ratio of the
activity of alpha-hydroxy butyrate dehydrogenase to the activity of
lactate dehydrogenase in blood plasma was lower in selenium-treated
sheep. Elevated activities of enzymes in blood plasma were found first
in selenium-treated sheep but these elevations were transient. In most
of the sheep on the basal treatment, activities of enzymes in blood
plasma remained elevated until death.

Creatine phosphokinase, lactate dehydrogenase and alpha-hydroxy
butyrate dehydrogenase activities were lower in skeletal muscle of

selenium-treated ewes. In fetal muscle, treatment had no effect on the activities of these enzymes. Total acid protease activity in liver was greater in vitamin E treated ewes than in ewes treated with both vitamin E and selenium. Activities of bound acid protease and beta-glucuronidase were greater in skeletal muscle of vitamin E-deficient ewes than in other ewes and greatest in ewes in the terminal stages of vitamin E deficiency. Treatment had no effect on the bound or free activities of these enzymes in uterus and placenta. Concentration of tocopherol in skeletal muscle, heart, kidneys and uterus and placenta of ewes was increased by vitamin E but not selenium. A similar response was observed in fetal tissues but differences were not significant.

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APPENDICES

APPENDIX A

TABLE XXI

THE EFFECT OF VITAMIN E AND SELENIUM ON
THE HEMATOCRIT IN BLOOD OF THE EWES

Item		Treatment			
	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE^a
Number of ewes	6	6	6	6	
Ratio, %					
On day					
14	28.0	27.2	28.1	26.9	3.32
28	24.5^b	$22.8^{b,c}$	28.1^d	21.7^c	0.76
42	$31.3^{b,c}$	$28.8^{b,d}$	32.3^c	27.8^d	1.18
56	32.5	32.5	35.2	34.0	1.25
70	37.0	36.2	36.7	36.8	1.26
84	$35.9^{b,c}$	33.4^b	37.7^c	$34.2^{b,c}$	1.29
98	35.9	38.6	36.0	36.0	1.20
112	37.0	37.1	36.3	35.6	1.35
126	40.2	37.4	36.6	38.6	1.30
140	37.1	37.0	37.9	38.1	1.26

[a]Standard error of the treatment means.

[b-d]Horizontal values on the same line bearing different superscripts differ significantly (P $\leqslant$ .01).

TABLE XXII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF HEMOGLOBIN IN THE BLOOD OF THE EWES

Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
					Treatment
Number of ewes	6	6	6	6	
Concentration, gm./100 ml.					
On day					
14	12.1	11.8	12.3	11.3	0.49
28	9.7	9.6	9.7	9.9	0.55
42	12.0^{b}	$10.9^{b,c}$	$11.2^{b,c}$	9.9^{c}	0.49
56	10.8^{b}	$11.6^{b,c}$	12.9^{c}	$11.1^{b,c}$	0.60
70	11.6	11.3	10.5	11.8	0.48
84	12.3	11.6	13.4	12.3	0.51
98	13.2	12.5	13.0	13.4	0.48
112	13.7	12.4	12.4	12.4	0.51
126	14.5	14.7	13.3	14.7	0.58
140	13.1	13.4	12.9	12.8	0.64

[a]Standard error of the treatment means.

[b,c]Horizontal values on the same line with different superscripts differ significantly (P $\leq$ .05).

TABLE XXIII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF RED BLOOD CELLS IN THE BLOOD OF THE EWES

Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
			Treatment		
Number of ewes	6	6	6	6	
Concentration, Cells($\times 10^6$)/mm^3					
On day					
14	12.6	12.6	12.1	11.4	0.47
28	10.7[b,c]	11.1[b]	11.5[b]	10.1[c]	0.27
42	11.2	10.6	10.9	9.8	0.88
56	11.3	11.6	12.1	11.3	0.47
70	11.9	11.9	11.8	11.8	0.79
84	11.3	11.7	12.1	11.1	0.40
98	11.0	11.6	12.0	12.2	0.37
112	10.6[b]	11.9[c]	10.7[b]	10.4[b]	0.40
126	11.5	11.8	11.6	12.1	0.43
140	12.0	12.3	12.5	11.9	0.45

[a]Standard error of the treatment means.

[b,c]Horizontal values on the same line bearing different superscripts differ significantly (P < .05).

TABLE XXIV

THE EFFECT OF VITAMIN E AND SELENIUM ON HEMATOCRIT
IN THE BLOOD OF THE RAMS

		Treatment			
Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE^a
Number	3	3	3	3	
Ratio, %					
On day					
0	34.7	32.6	35.8	33.9	2.02
14	32.8^b	30.1^c	$32.1^{b,c}$	30.1^c	0.74
28	30.5	30.0	30.3	29.0	1.34
42	31.1	27.8	31.0	30.4	1.74
56	32.7^b	27.2^c	$32.2^{b,c}$	$28.4^{b,c}$	1.71
70	32.3	30.7	32.9	28.3	1.82
84	44.9^d	32.0^e	33.0^e	31.0^e	2.48
98	$—^f$	34.6	34.4	31.1	1.61
112		33.9	33.6	32.8	1.09
126		38.2	37.6	34.2	1.29
140		38.3	35.0	32.5	1.81

[a]Standard error of the treatment means.

[b,c]Horizontal values on the same line bearing different superscripts differ significantly (P < .05).

[d,e]Horizontal values on the same line bearing different superscripts differ significantly (P < .01).

[f]Rams who were on the basal treatment died.

TABLE XXV

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION
OF HEMOGLOBIN IN THE BLOOD OF THE RAMS

Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
			Treatment		
Number of rams	3	3	3	3	
Concentration, gm./100 ml.					
On day					
0	12.2	11.8	13.2	12.0	0.95
14	10.5^b	$9.8^{b,c}$	10.2^b	9.1^c	0.32
28	10.6	9.8	11.0	9.8	0.45
42	9.2	8.7	9.6	9.0	0.58
56	9.5	8.4	9.5	9.4	0.57
70	10.5	10.3	9.9	9.3	0.61
84	13.7^d	9.9^e	10.7^e	10.4^e	0.56
98	$—^f$	11.0	9.4	10.8	0.84
112		10.9	11.7	11.1	0.52
126		12.1	12.0	10.6	0.45
140		11.7	11.8	10.8	1.06

[a]Standard error of the treatment means.

[b,c]Horizontal values on the same line bearing different superscripts differ significantly (P < .05).

[d,e]Horizontal values on the same line bearing different superscripts differ significantly (P < .01).

[f]Rams who were on the basal treatment died.

TABLE XXVI

THE EFFECT OF VITAMIN E AND SELENIUM ON THE CONCENTRATION OF
PROTEIN IN BLOOD PLASMA OF THE EWES

Item	Basal	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[a]
			Treatment		
Number	6	6	6	6	
Concentration, gm./100 ml.					
On day					
70	$9.4^{b,c}$	10.0^{b}	10.0^{b}	9.0^{c}	0.30
84	8.6	9.0	8.0	8.9	0.54
98	$7.7^{b,c}$	7.4^{b}	9.2^{d}	$8.8^{c,d}$	0.50
112	7.6	7.1	7.5	7.3	0.24
126	7.2	7.4	7.5	7.4	0.17
140	6.6	6.2	7.0	6.7	0.22
154	7.1	7.6	7.7	7.8	0.24
168	$-^{g}$	7.0	7.1	7.1	0.13
182		7.5	7.7	7.6	0.14
196		9.6^{e}	8.0^{f}	7.6^{f}	0.34
210		8.4	8.3	8.0	0.27

[a]Standard error of the treatment means.

[b-d]Horizontal values on the same line bearing different super-scripts differ significantly ($P < .05$).

[e,f]Horizontal values on the same line bearing different super-scripts differ significantly ($P < .01$).

[g]Ewes who were on the basal treatment died.

APPENDIX B

TABLE XXVII

THE EFFECT OF VITAMIN E AND SELENIUM ON THE PROXIMATE
ANALYSES OF THE EWES' MILK[a]

Item	Treatment			
	Basal + Se	Basal + Vit. E	Basal + Vit. E + Se	SE[b]
Number of ewes[c]	1	3	5	
Proximate analysis				
Protein, %	5.3	3.8	4.2	0.3
Fat, %	0.9	5.4	5.1	0.7
Ash, %	0.96	0.93	0.86	0.01
Total solids, %	12.3	15.2	15.1	0.6

[a]Ewes who were on the basal treatment died.

[b]Standard error of the treatment means.

[c]One milk sample was obtained two weeks post-partum from each ewe.

APPENDIX C

TABLE XXVIII

NORMAL MEAN VALUES AND RANGES OBTAINED FOR THE ACTIVITIES OF
SEVERAL ENZYMES IN BLOOD PLASMA OF SHEEP[a]

| | Sex | | | | | |
| | Ewes | | | Rams | | |
Enzyme[b]	Number of values	Mean	Range[c]	Number of values	Mean	Range[c]
GOT	102	32.0	2.6 - 61.4	30	33.8	15.0 - 52.6
CPK	18	3.3	0 - 11.3	30	7.0	0 - 16.6
LDH	12	243.0	134.6 - 351.4	30	521.4	117.2 - 925.6
HBD	12	179.3	102.1 - 256.5	30	342.6	91.8 - 593.4
GDH				12	12.8	0 - 42.4
GPT				12	12.9	4.3 - 21.5
FDA				12	31.9	14.9 - 48.9
PFA				6	14.4	4.8 - 24.0
ICDH				6	6.0	0 - 14.8

[a]Values are from samples obtained in sheep fed the basal diet and treated with both vitamin E and selenium.

[b]GOT: Glutamate-oxaloacetate transaminase; CPK: Creatine phosphokinase; LDH: Lactate dehydrogenase; HBD: Alpha-hydroxy butyrate dehydrogenase; GDH: Glutamate dehydrogenase; GPT: Glutamate-pyruvate transaminase; FDA: 1,6 Diphosphofructoaldolase; PFA: 1,Phosphofructoaldolase; ICDH: Isocitrate dehydrogenase.

[c]Range = Mean $\pm$ Two standard deviations.

APPENDIX D

TABLE XXIX

STANDARD ERRORS ON TREATMENT MEANS FOR TOTAL PLASMA
FREE TOCOPHEROL VALUES PLOTTED IN FIGURE 4

Item	
Time on Trial	Standard Error
Days	mcg./100 ml. plasma
14	6.5
28	10.3
56	9.9
70	8.4
84	8.8
98	11.0
112	15.4
126	13.8
140	14.8
168	29.2
196	77.0
210	58.1
224	37.4
252	34.2

TABLE XXX

STANDARD ERRORS ON TREATMENT MEANS FOR PLASMA
GLUTAMATE-OXALOACETATE TRANSAMINASE VALUES
PLOTTED IN FIGURE 6

Item	
Time on Trial	Standard Error
Days	I.U./1. plasma
14	4.0
28	10.7
42	21.6
70	71.0
84	25.4
98	48.1
126	61.3
140	67.1
154	51.4
168	38.5
182	188.6
196	161.8
210	26.5
224	97.1
238	35.9

TABLE XXXI

STANDARD ERRORS ON TREATMENT MEANS FOR PLASMA
GLUTAMATE-OXALOACETATE TRANSAMINASE VALUES
PLOTTED IN FIGURE 7

Item	
Time on Trial	Standard Error
Days	I.U./l. plasma
0	3.5
14	3.3
28	6.3
42	88.6
56	21.8
70	39.9
84	271.6
98	18.4
112	6.9
126	11.6
140	18.8

TABLE XXXII

STANDARD ERRORS ON TREATMENT MEANS FOR PLASMA
CREATINE PHOSPHOKINASE VALUES PLOTTED
IN FIGURE 8

Item	
Time on Trial	Standard Error
Days	I.U./1. plasma
0	0.8
14	1.1
28	1.6
42	91.3
56	7.1
70	22.0
84	175.6
98	3.9
112	0.7
126	2.8
140	3.8

TABLE XXXIII

STANDARD ERRORS ON TREATMENT MEANS FOR PLASMA
ALPHA-HYDROXY BUTYRATE DEHYDROGENASE VALUES
PLOTTED IN FIGURE 9

Item	
Time on Trial	Standard Error
Days	I.U./l. plasma
0	32.4
14	23.3
28	39.1
42	248.9
56	42.2
70	292.5
84	400.4
98	101.7
112	46.4
126	34.3
140	34.8

TABLE XXXIV

STANDARD ERRORS ON TREATMENT MEANS FOR PLASMA
LACTATE DEHYDROGENASE VALUES PLOTTED
IN FIGURE 10

Item	
Time on Trial	Standard Error
Days	I.U./l. plasma
0	42.2
14	21.7
28	66.2
42	342.7
56	69.0
70	591.5
84	793.2
98	145.1
112	62.8
126	140.1
140	60.4

VITA

Jock Gordon Buchanan-Smith

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF VITAMIN E AND SELENIUM DEFICIENCIES IN SHEEP
FED A PURIFIED DIET DURING GROWTH AND REPRODUCTION

Major Field: Animal Nutrition

Biographical:

Personal Data: Born in Edinburgh, Scotland, March 9, 1940, the
son of Alick Drummond and Mary Kathleen Buchanan-Smith.

Education: Graduated from Trinity College, Glenalmond, Scotland
in 1958; received the Bachelor of Science degree in Agri-
culture from the University of Aberdeen, Scotland in 1962;
received the Bachelor of Science degree in Animal Science
from Iowa State University, Ames, Iowa in 1963; received
the Master of Science degree with a major in Animal Hus-
bandry from Texas Technological College, Lubbock, Texas in
1965; completed the requirements for the Doctor of Philosophy
degree at Oklahoma State University in May, 1969.

Professional Experience: Research Assistant in the Department of
Animal Husbandry, Texas Technological College, Lubbock, Texas,
1964-65; Research and Teaching Assistant in the Department of
Animal Science, Oklahoma State University, Stillwater, Okla-
homa, 1965-1969.

Professional Societies: Sigma Xi; Phi Kappa Phi; Alpha Zeta;
American Society of Animal Science; American Association
for the Advancement of Science.