THE EFFECTS OF VITAMIN E DEFICIENCY IN THE LAMB

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INTRODUCTION

Vitamin E is a dietary factor necessary to prevent the appearance of structural and functional derangement in the rat, mouse, guinea pig, rabbit, hamster, sheep, goat, kangaroo, dog, duckling, turkey, chick and guppy fish (Mason 1944).

In spite of the striking similarity between the pathological findings of chronic muscular dystrophy produced in rabbits, guinea pigs and rats by vitamin E deficiency, and the histopathological picture of progressive muscular dystrophy and other muscular disorders of human beings, it has been impossible to demonstrate with certainty vitamin E deficiency in man (Pappenheimer 1948).

In sheep, vitamin E deficiency has not been directly demonstrated, even though alpha tocopherol was found to prevent and cure "stiff-lamb" disease. This malady is a spectacular nutritional disorder producing paralysis and degeneration of the skeletal muscles, and eventually the death of lambs. Sometimes it causes 30% mortality in crops of young lambs.

The investigation about this disease had been carried out on lambs fed natural rations and, therefore, it was questionable whether the deficiency of tocopherol was the unique agent capable of causing the "stiff-lamb" disease.

The answer to this question had been possible since purified diets were formulated for experimentation with young

lambs (Luecke et al. 1950). And so, the present work was initiated to study the effect of vitamin E deficiency on the lamb.

The results of the first experiment (trial I) proved the identity of "stiff-lamb" disease and vitamin E deficiency. In several respects, these findings were extremely interesting. First, because they showed that vitamin E has a special significance in the nutrition of the lamb, since it occurs even when they are fed natural rations. Therefore, the lamb should be an ideal animal for the study of vitamin E. And second, because heart lesions appeared constantly, along with muscular dystrophy, in lambs fed a vitamin E-deficient diet.

Since there is an urgent need for better knowledge of the progressive muscular dystrophy and cardiac diseases affecting humans, the experimental results of the first trial suggested the continuation of further research on the vitamin E deficiency in lambs.

Thus two more experiments (trials II and III) were carried out in an attempt to elucidate the possible mechanisms of action of vitamin E.

REVIEW OF LITERATURE

Occurrence of Vitamin E Deficiency

Rats:

The existence of a vitamin necessary for reproduction had been suspected in 1920 by Matill and Conklin and confirmed in 1922 by Matill and in 1923 by Sure (Bicknell 1948).

However, it was Evans and co-workers who, in 1923, working with rats, proved that certain diets lacked a nutritional factor necessary to prevent the death and resorption of embryos and testicular degeneration. The newly discovered nutrient was called vitamin E and was named specifically as the "anti-sterility vitamin", or "fertility factor".

Soon after, the same workers found that vitamin E not only possessed an "anti-sterility" action, but was able to cure paretic symptoms of young suckling rats whose mothers had been maintained, before and after lactation, on a vitamin E deficient regimen (Pappenheimer 1943 and 1948).

Slow progressing muscular dystrophy was reported by Pappenheimer (1942) to be another symptom of vitamin E deficiency in the adult white rat.

Rabbits and Guinea pigs:

Muscular dystrophy is the main manifestation of vitamin E deficiency in rabbits and guinea pigs. It was first reported

by Goettsch and Pappenheimer (1931), who named the disease "nutritional muscular dystrophy". Under certain experimental conditions Pappenheimer et al. (1948) showed that in guinea pigs, as in the rat, a lack of sufficient dietary vitamin E resulted in testicular degeneration. Fetal death was also noted to occur around the middle of the gestation period. In rabbits, the same workers found that the roung may be born dead or survive but a few days.

Dogs:

Anderson et al. (1940) produced vitamin E deficiency in pups by feeding the mother a mineralized milk diet. Pups developed hemorrhages in the pericardia, lungs, brains and subcutaneous tissues. When the same mineralized milk was given to the pups, nutritional muscular dystrophy developed. Elvehjem et al. (1944) found later that young pups whose mothers had been fed a mineralized milk were unable to reproduce.

Vitamin E deficiency in the adult dog is manifested by muscular and testicular degeneration according to Brinkhous and Warner (1941). They were able to produce the deficiency by means of a chronic biliary fistula, which reduced the absorption of vitamin E due to the lack of bile in the intestinal tract.

Monkeys:

The monkey (macaca rhesus) has also been found to develop muscular dystrophy due to lack of vitamin E (Mason 1947). A more comprehensive study of the macaca rhesus during the development of chronic vitamin E deficiency showed that the only manifestations were alteration in the electrocardiogram and pneumocardiogram (Filer et al. 1949).

Birds:

Vitamin E is necessary during all periods of the chicken's life. Embryos, pullets, and adults are susceptible to the deficiency.

Hens fed a vitamin E free diet laid eggs whose embryos died at the fourth day of incubation. The cause of death was found to be the disintegration of blood vessels of the blastoderm, which was apparent by the formation of a typical dense ridge-like lethal ring (Adamstone 1931).

In pullets, vitamin E deficiency is manifested in three different ways: exudative diathesis, generalized edema and nutritional encephalomalacia (Mason 1944). Bird and Culton (1940) described the exudative diathesis, which was characterized by the accumulation of plasma-like fluid in localized areas of the subcutaneous tissues, muscles, adipose tissues and generally throughout the connective tissues.

Crazy chick disease, observed for the first time in 1928, was recognized by Pappenheimer and Goettsch (1931) as one of

nutritional origin and accordingly named it nutritional encephalomalcia. The symptoms were muscular incoordination, resting of the chicks on the pelvic bone, retraction of the head, somersaults or rotation in a laterally prone position.

In the adult chicken, symptoms of vitamin E deficiency are slight muscular dystrophy and testicular degeneration (Mason 1944).

Pappenheimer (1939) reported that the deficiency of vitamin E in ducks was manifested by dystrophy of skeletal muscles. In turkey poults, however, he did not find any specific outward sign of the vitamin E deficiency.

Sheep:

Vitamin E deficiency has been postulated as the cause of the condition known as "stiff-lamb" disease, "lamb rheumatism", "lamb paralysis", "stiff-neck" (Willman et al. 1934 and 1945) and "white muscle disease" (Cheng 1945). The disease is known to occur in different sections of this country (Metzger and Hagan 1927; Lee 1935; Sholl 1939; Thorp 1942) and in Europe (Slagsvold and Lund-Larsen 1934). It is a common disease among lambs, the losses being sometimes as high as 10-15% (Metzger and Hagan 1927) or even 30% (Sholl 1939). Adult sheep have never been reported affected by this disease.

Symptoms of the stiffness appear the day after the lambs are turned out on pasture in the spring. The lambs show a

disturbance in the locomotion, stiffness of the hind and fore legs, paralysis and death (Metzger and Hagan 1927). The coincidence of the appearance of the stiff lambs with unusually long winters, the time of weaning, and the translocation of lambs from pens to range have been speculated upon by many workers.

Sholl (1939) noted that the disease did not appear every year with the same intensity. Whenever the springs were cold and rainy and the previous fall had been long, the incidence of the "stiff-lamb" disease was highest.

The exercise factor was suggested as the cause of the disease by Welch et al. (1929); later Willman et al. (1934) discarded this possibility and instead proved that the "stiff-lamb" disease was consistently produced in animals fed certain rations. This work marks the turning point of the study of the "stiff-lamb" disease, since early suggestions on the pathological origin of the disease, as a secondary effect of coccidiosis (Jungherr and Welch 1927), umbilical infection (Marsh 1932) and the action of certain bacteria (Lee 1935) were shelved and the disease accepted as one of nutritional disturbance.

Lambs reared on open ranges or under semi-range conditions have never been observed to develop the "stiff-lamb" disease. Vawter (1939) thought that such conditions probably afford a

better opportunity for the ewes to balance their diet than would be the case under restricted diets.

The occurrence of "stiff-lamb" disease is very frequent in lambs whose dams have been fed a ration containing second cutting alfalfa, four parts of cull beans, three parts of barley and three parts of oats (Willman et al. 1936). They called this ration the "stiff-lamb ration" and it has proved to be very useful in the study of the disease (Willman et al. 1939).

The study of Sholl (1939) in the State of Michigan pointed also to a correlation between the disease and the nutrition of the ewe. Stiff lambs were observed on flocks fed the following rations: first cutting alfalfa, ear corn, and oats; clover, alfalfa, corn and oats; alfalfa, mixed hay, bundle corn, bran, oats, and soybean meal; hay alone and alfalfa; clover hay, no grain; alfalfa, clover hay, cornstalks silage, wheat and oats; clover hay, oats and corn fodder; alfalfa hay and barley; alfalfa, timothy hay, barley, oats and cornstalks.

Cattle:

Slagsvold and Lund-Larsen (1934) reported that calves, heifers as well as lambs, developed myositis as a consequence of the feeding regimen. The disease was more noticeable during the spring, a few days after animals, fed indoors, were let out to grass for the first time. The same disturbances have been found occasionally in calves raised in farms of Michigan (Thorp,

Barner, Johnston, and Huffman, personal communication).

In adult cattle, maintained on a vitamin E free ration for a prolonged period, the only symptom of the deficiency was the sudden death of the animals (Gullickson et al. 1946).

Humans:

Symptoms of vitamin E deficiency in man have not as yet been discovered. Pappenheimer (1948) believes, however, that it must exist: "Lesions which point unequivocally to deficiency of vitamin E have not yet been discovered in man. And yet, as we shall see, the lesions in many species of animals brought about by this deficiency are striking and often pathognomonic. Man is a curious animal in many respects, but there is no reason to think that he alone should be immune to the effects of this nutritional lack, and it is a challenging task for the pathologist, with the necessary aid of the biochemist and the clinician, to uncover the pathology of vitamin E deficiency".

Nature of Vitamin E

Wheat germ oil was the first product discovered to offer protection and cure for animals deficient in vitamin E (Evans and Bishop 1923).

Years later, the active principles of wheat germ oil were isolated by chemical procedures and were found to be three closely related higher alcohols. Because of their effect in preventing reabsorption of fetuses, the compounds were called

alpha, beta and gamma tocopherols, a Greek word meaning "to bear offspring" (Mason 1944).

Tocopherols have been used successfully to prevent and cure the diseases produced by the absence of vitamin E in the diet (Pappenheimer et al. 1939 and 1948; Willman et al. 1945).

Abundant evidence has shown that alpha, beta and gamma tocopherols have different biological values. Rats assays carried out by Gotlieb et al. (1943); Hove and Harris (1947a) proved that alpha tocopherol was more active than beta, and beta more than gamma. The difference of activity, reasoned Hickman and Harris (1946), should represent the ability of the depleted organism to methylate the co-vitamins. Similar results were obtained by Hove and Hove (1944), who demonstrated that the relative biological activities of alpha, beta and gamma tocopherol, at elevated temperatures, are of the same order as their antioxidant potencies in vitro.

Natural alpha, beta and gamma tocopherols were found by Harris and co-workers (1944) to have more potency than the synthetic dl compounds of alpha, beta and gamma tocopherols. This, according to Wachs (1949) is comparable to the antioxidant activity of the compounds, as measured by a special redox-electrometric apparatus.

Another compound with specific vitamin E activity has been prepared by Milhorat et al. (1949). They discovered that dl alpha tocopherylhydroquinone reduced creatinuria in human

patients with muscular dystrophy and also cured muscular dystrophy in standardized, vitamin E-deficient rabbits.

All compounds with vitamin E activity were studied by Boyer, Rabinovitz and Liebe (1951) in an effort to relate the chemical structure to the vitamin E function. They found that vitamin E activity was limited to certain substituted 6-hydroxychromans, or 5-hydroxycoumarans, or to compounds which can be readily converted to the substituted 6-hydroxychromans by hydrolysis or reduction.

Administration of curative and preventive doses of tocopherols have been successfully accomplished in various ways. Hove and Harris (1947a) injected alpha tocopheryl phosphate intramuscularly and found that in this way it had a slow but prolonged action. Willman and co-workers (1946) injected subcutaneously 100 mg. dose of disodium salt of dl-alpha tocopherol phosphoric acid ester dissolved in sterile water, and cured E deficient lambs. The most common way to supply vitamin E is by oral administration either mixed with oils or with the rations (Willman et al. 1945; Pappenheimer et al. 1939).

Synergists and Antagonists of Vitamin E

Synergism of the vitamin A and E was suggested by the work of Quackenbush and collaborators (1942). They were unable to rectify the vitamin A deficiency of rats with carotene unless

alpha tocopherol was given at the same time. This action seemed to be specific to vitamin E since at the 0.02% level vitamin K, pyrogallol, catechol and hydroquinone were inactive.

Opposite results, however, have been recently reported by Kachmar et al. (1950) who analyzed the blood plasma for vitamin E, vitamin A, and carotene, and concluded that the low vitamin E rations did not impair the utilization of carotene and vitamin A.

Dam and Glavind (1940) related that vitamins K and C were unable to repair a faulty capillary permeability produced by vitamin E deficiency.

Ascorbic acid, according to Willman, Morrison and Olafson (1935) did not possess a synergistic action in the case of "stiff-lamb" disease. However, the same vitamin has been reported recently by Dam and co-workers (1948a) to be effective in the reduction of the incidence of exudative diathesis of chickens. Cystine, nordihydroguairetic acid and choline also had a similar action (Dam et al. 1948; Vivanco et al. 1948).

Xanthophyll, when given in massive doses, was shown by Goldhaber et al. (1950) to protect chickens from vitamin E deficiency. It appeared to them that the protection of tocopherols by xanthphyll was confined to the gastrointestinal tract. This mechanism of protection, they thought, was different from the one of ascorbic acid, since vitamin C presumably protects the tocopherol of the tissues from oxidation.

Antagonistic factors to vitamin E have been found in cod liver oil, which have been identified as unsaturated fatty acids. This will be reviewed in detail under the heading of "Interrelationship of Vitamin E and the Lipids".

The most specific antagonist reported up to the present time is the di-O-cresyl succinate. Meunier and Chenavier (1949) fed rabbits a normal diet to which they added 100-166 mg. of the di-o-cresyl succinate. The rabbits first developed diarrhea, then became paralyzed and died within seven days. Administration of 50 mg. of synthetic tocopherol to similarly poisoned animals was curative and allowed them to regain their former weight.

A less specific vitamin E antagonist had been reported previously by Woolley (1945). He found that alpha tocopherol quinone seemed to have an antivitaminic action to both vitamins E and K.

Hove (1948) observed, in his work on the effect of factors influencing the mortality of rats after carbon tetrachloride poisoning, that 6 methyl thiouracil had an obliterating action on the activity of alpha tocopherol.

Macro and Micropathological Alterations in Vitamin E Deficiency

"The most outstanding characteristic of experimental vitamin E deficiency, which has no parallel in any other vitamin deficiency state, is the multiplicity of structural and functional alterations exhibited sometimes by closely related species,

and the similarity of certain changes in quite unrelated species." (Mason 1944).

Structural alterations have been observed in every system of the organism. Harris and Kujawski (1950) referred in their bibliography to alterations produced in the following systems as a result of vitamin E deficiency: reproductive, skeletal, muscular, central nervous, circulatory, respiratory, digestive, excretory, endocrine, skin and sensory organs.

Reproductive System:

In the female rat Martin and Moore (1939) reported that the failure to induce pregnancy was due to alterations in the structure of the uterine wall, as shown by the degeneration of the smooth muscle and a very distinctive pigmentation due to yellow brown granules deposited in the cytoplasm of the smooth muscle fibers.

In the male rat Pierangeli et al. (1949) has reported the presence of fluorescent pigments, yellow or luminous and whitish in the testes of rats rendered atrophic by avitaminosis E. This fluorescent lipo-pigment was not found in normal testes.

Mason (1926) pointed out that male rats severely depleted of vitamin E from early life show an irreversible degeneration of the seminiferous epithelium at the onset of sexual maturity, but no injury during adolescence. He also found that the spermatozoa show the first alteration, and these are followed by de-

generative changes in spermatids and spermatogonia. In the later stages, the tubules were found to be lined with indifferent Sertoli cells.

The experiments of Escudero et al. (1942) demonstrated that the vitamin E deficiency damages not only the germinal epithelium but also the intersticial cells of the testes. Seminal vesicles and prostate glands of rats decreased in weight during the six months on a low vitamin E diet.

Muscular System:

The first description of the histological alteration of the musculature of the guinea pig and rabbit was done by Goettsch and Pappenheimer (1931). They observed that at the earliest stage of the degeneration there is a very extensive hyaline necrosis of the muscle fibers. All the fibers were not found to degenerate at the same time, some remained normal while others rapidly degenerated. Pappenheimer, in 1948, in his lecture "On Certain Aspects of Vitamin E Deficiency" reviewed some of the characteristics of nutritional muscular dystrophy in the rat, and described the muscular necrosis selectively affecting the contractile sarcoplasma. The nuclei were left with their adjacent cytoplasm viable. He thought that this change makes possible the extraordinarily rapid and successful regeneration. Following the loss of striation of the coagulated muscle substance, he stated, "there occurs a strong inflammatory response,

with edema and cellular infiltration, in which, during the first 24 hours, polymorphonuclear leucocytes predominate. As the process progresses, the edema subsides, the polymorphonuclears disappear and are replaced by large mononuclears. . . . . An important feature of the lesions is that the necrosis affects chiefly the contractile portion of the muscle fibers. . . . . The necrotic material becomes surrounded by multinucleate giant cells, derived either from fused histiocytes or from the intact young myoblasts; it may become calcified, in which case it persists for some time. For the most part, it undergoes rapid digestion and disappears completely after four or five days".

Among the curious features of the disease, he mentioned that not all the fibers degenerate at the same time, some remained normal while others rapidly degenerate, and that the degeneration never extends to the surface of the muscle. He speculated about these last observations and reasoned that the superficial fiber may have a richer blood supply, or perhaps are partially immobilized by their attachment to the muscle sheath.

Similar histological changes to those already described have been confirmed elsewhere in other species affected by nutritional muscular dystrophy (Olcott 1938; Davis et al. 1938; Cheng 1945; Culik 1951).

The observed histological changes are essentially those well known to the human pathologist as hyaline, waxy or Zenker's degeneration usually associated with acute infections and fever (Mason 1944).

Sometimes, the most striking findings at autopsy of guinea pigs and lambs with nutritional muscular dystrophy are the whitish patches and streaks observed scattered throughout the skeletal muscles (Willman et al. 1934; Pamuckcu 1948). The white appearance of the lambs' muscles have justified naming the disturbance as the "white muscle disease".

Nervous System:

The experiments of Pappenheimer and Goettsch (1940) in which they prevented the occurrence of the nutritional muscular dystrophy in young rats, by denervation, has given rise to the question whether the syndrome is primarily a myogenic, neurogenic disease, or a combination of both.

Since that time, many research workers have studied the relation of the disease to the central nervous system and motor end plates.

The central nervous system was first reported to be affected in rats depleted in vitamin E by Lipshutz (1936). He stated that generally the first and largest site of the degeneration process occurred in the lumbo-sacral part of the spinal cord.

Other investigators have attacked the same problem and have found different distribution and characters of the lesions, or no lesions at all. Pappenheimer (1943) carefully reviewed the work done on vitamin E up to 1943 and concluded that the vitamin E deficiency does not alter the structure of the central nervous

system, and that the different results found by different workers were due to variations in technique, wrong interpretations, and lack of adequate control material for comparison.

However, recent work conducted independently by Lutrell and Mason (1949) and by Malamud, Nelson and Evans (1949), designed to re-evaluate the effects of vitamin E deficiency, demonstrated that the central nervous system is actually affected. Lutrell and Mason (1949) found that rats reared on a vitamin E deficient ration for 9-12 months consistently showed evidence of demyalinization, gliosis, and distortion of the axon pattern of the posterior columns and proximal parts of the posterior roots of the cervical, thoracic and lumbar segments of the spinal cord. This confirmed earlier observations of Einarson and Reigsted and others.

Malamud et al. (1949) essentially made the same observations and doubted the validity of the criticism of Pappenheimer in the case of lesions of the posterior columns, since, in their study, staining methods yielded the same positive proof of the latter and contrasted sharply with the normal findings in the control animals.

The studies of Telford (1941) indicated that the damage of motor end plates was secondary to the degeneration of the muscles. When there was a slight degree of muscular degeneration, there was no appreciable alteration of nerve endings; if the degeneration was considerable, there was also considerable reduction of

the nerve terminals and if the necrosis was widespread and of long standing, the nerve terminations were completely lost.

Circulatory System:

Circulatory disturbance has been found to be the primary factor in the production of nutritional encephalomalcia of chicks. Wolf and Pappenheimer (1931) and Pappenheimer et al. (1939) have observed that, due to circulatory failure, ischemic necrosis was produced in the cerebellum, cerebrum and medulla, and that there was hemorrhagic edema, capillary thrombosis, and dead ganglion cells and glial elements.

Exudative diathesis has been interpreted by Dam (1944b) and Hepding (1947) as a disturbance of the vascular wall. Dam and Glavind (1940) found that in these conditions there was increased capillary permeability, which can be checked by addition of vitamin E to the diet but not by addition of vitamin K, citrin or ascorbic acid. According to the experiments of Ames and co-workers (1951), vitamin E also offers significant protection against the increased fragility produced by a Radon ointment.

Mason (1943) observed that, prior to the exitus of rat fetuses, there appeared abnormalities in the vascular system. They were characterized by variable degrees of dilatation and thrombosis of peripheral and deeper vascular channels, and by either localized or diffuse areas of hemorrhages.

Hove (1949a and 1949 b) has described another vascular disturbance in rats when depleted of vitamin E. Within 6-12 weeks

75% of the rats died from massive lung hemorrhages, with centrilobular and sometimes extensive necrosis of the liver and distension of the subcutaneous blood vessels.

The role of vitamin E in the prevention of arteritis in dogs was demonstrated by Holman (1947 and 1949) who found that vitamin E retarded the incidence of arterial lesions in dogs which had experimentally produced renal insufficiency.

Heart lesions have been observed in lambs suffering from vitamin E deficiency. These lesions, however, were inconstant. When any abnormality was found, it consisted of whitish patches beneath the endocardium and usually in the right ventricle of the heart (Metzger and Hagan 1927). Years later Willman et al. (1945) again reported that a small percentage of stiff lambs showed heart lesions, usually in the endocardium of the right ventricle. The reports of other investigators agreed that the vitamin E deficiency did not always produce lesions in the heart of the lamb (Slagsvold and Lund-Larsen 1934; Cheng 1945).

Slagsvold and Lund-Larsen (1934) described that calves and lambs showed essentially the same heart abnormalities due to vitamin E deficiency. Gullickson et al. in 1946 made a histological study of hearts obtained from cows that had died of vitamin E deficiency. He found atrophy and scarring of the cardiac muscle fibers, increase in cellular elements, and in some instances nodules which resembled the Aschoff nodules seen in human endocarditis.

Anatomical injury of the heart has been reported in other species as a result of feeding a diet low in vitamin E. Gatz and Houchin (1947) noted that the portion of the heart which shows necrosis of cardiac muscle are in order of frequency and severity: the papillary muscles, columnae carnae, apex of heart, ventricular walls and septum and infrequently atria. The Purkinje fibers appeared unaffected.

Mason and Emmel (1945) found myocardial lesions in rats deficient in vitamin E. They noted that rats showed this symptom only after a year or more of the deficiency, which explained the absence of this symptom in previous experiments of Olcott (1938). The study of Butturini (1949) on the heart in vitamin E deficiency pointed out that the symptomatology of the disease in rats is rather peculiar, since the lesions were predominantly localized in the left ventricle.

Digestive system:

As a rule the gastro-intestinal tract is immune to the effects of vitamin E deficiency, even in the presence of severe dystrophic symptoms in other organs (Mason 1944). However, Pappenheimer et al. (1939) have shown that, in turkey poults, vitamin E deficiency can produce severe myopathy of the gizzard. Pathological changes were similar to the changes of skeletal muscles and were characterized by hyaline necrosis of the muscle fibers with secondary inflammatory reaction; this was followed by fibrosis or regeneration.

Physiological Changes

Creatine and Creatinine Excretion:

Creatinuria has been considered the first clinical sign of the vitamin E deficiency (Bicknell 1948). Melville and Hummel (1951), in their studies on the metabolism of creatine and glycocyamine in rabbits deficient in vitamin E, pointed out that creatinuria preceded not only the external signs of paralysis but also any observable histological changes. However, they found no correlation between the severity of the disease and the amount of creatine in the urine.

Butturini (1949) concluded from his experiments that hypercreatinuria was an unspecific symptom, since it was found in all the vitamin deficiencies, and was merely the first pathological manifestation prior to any loss in body weight. This hypercreatinuria, he stated, can be corrected only by adding the specific vitamin deficient in the diet.

Creatinine excretion has also been reported to be altered as a result of vitamin E deficiency. Hove (1947a) noted a sharp increased excretion and reduction in creatinine excretion, similar to that observed in rats poisoned with carbon tetrachloride. Bauer and Berg (1943) suggested an abnormal relationship between creatine and creatinine during vitamin E depletion. They proposed that the creatinuria of nutritional muscular dystrophy could be due to hydration of creatinine to creatine. He based his proposition on the fact that alteration of the normal

water balance of tissues is a consistent finding in the biochemical and histological examination of tissues affected by vitamin E depletion.

Effect of Vitamin E on Growth:

Vitamin E has not been shown to be a growth factor for the young rat. Emerson and Evans (1937) observed that vitamin E deficiency affected the growth of rats only after the fourth month of life. The growth was reestablished by adding vitamin E to the diet. During early age, vitamin E does not alter the normal growth of rats (Olcott 1937).

In mice, Vogt-Møller (1942) obtained the same growth curves regardless of the presence or absence of vitamin E in the ration. In cattle, Gullickson et al. (1949) were unable to detect any effect of the vitamin E deficiency on growth. Willman et al. (1934) made the interesting observation that in field cases the lambs that were better developed were more susceptible to "stiff-lamb" disease.

The growth of monkeys is not affected by a lack of dietary vitamin E (Filer et al. 1949); however, chickens, ducks, and turkey poults required vitamin E for adequate growth (Pappenheimer 1939).

Changes in the Blood, Milk and Colostrum Produced by Vitamin E Deficiency:

One of the first changes noted in the composition of the blood plasma of lambs deficient in vitamin E, was the low

creatinine and high protein nitrogen (Sholl 1939). Filer et al. (1949), however, was unable to detect any change in hematological studies, blood glucose, blood non-protein nitrogen, and plasma protein of monkeys affected with chronic vitamin E deficiency.

Until recently it had been very laborious and expensive to analyze blood samples for alpha tocopherol by means of the biological method. Newer physical and chemical assay methods to determine tocopherols have greatly facilitated the investigation, thus allowing for an extensive determination of tocopherols in animal and vegetable materials (Mason 1944).

Kachmar et al. (1950) found that cattle dying of cardiac failure, due to vitamin E deficiency, had a calculated tocopherol level of less than 100 micrograms per 100 ml. of blood plasma. Animals that received mixed tocopherol in their diets had a calculated tocopherol level of 685 micrograms per cent.

Ferrando and co-workers (1949) reported that the blood plasma tocopherol level of Tarantaise cows, receiving 50-75 gm. of cod liver oil daily for two months was lowered about 100 micrograms per cent. In one cow, which received no cod liver oil the tocopherol value fell only from 348 to 275 and rose again to 316 micrograms per 100 ml. of plasma during the same period.

Athanassiu (1947) measured the tocopherol content of the human maternal serum, the venous and arterial blood of the umbilical cord, and concluded that the vitamin E passes freely through the placenta and is absorbed by the fetus.

Whiting and Loosli (1948b) found that the total milk production was not significantly affected by feeding either tocopherol or cod liver oil. The feeding of 1 gm. of tocopherol per cow daily, over a four week period during winter feeding, slightly increased the percentage of fat in the milk. Cod liver oil fed at the rate of five ounces per cow daily decreased the fat percentage approximately 11%; this could not be counteracted by tocopherol feeding.

The effect of tocopherol feeding on the tocopherol level of the colostrum and the early milk of cows was studied by Parrish et al. (1947). It was found that the content of the former is much larger than the latter. When no tocopherols were given in the ration of cows the average tocopherol level in the colostrum fat was 98 micrograms per cent, and that of the milk fat 20 micrograms per cent. When 0.5 to 1.0 gm. of tocopherols were given daily in the ration, the level of tocopherol changed to 150 micrograms per cent in the colostrum fat and 17 micrograms per cent in the milk fat. When 10 gm. of tocopherols were given daily, the level increased in the colostrum to 489 and to 39 micrograms per cent in the milk fat.

Similar results were obtained later by Whiting and Loosli (1948b) in sheep and goats. Their milk was much less rich than the colostrum.

Electrocardiogram:

The electrocardiographic technique was put to use in the field of animal nutrition a little over 20 years ago. Agduhr and Stenstrom (1929) used this method to detect the alterations produced in the heart of animals fed an excess of cod liver oil.

Since some heart lesions were later discovered to be caused by the absence of vitamin E in the diet of animals, it was thought that the electrocardiogram could give valuable information for the understanding of the disease.

Gullickson and Calverly (1946) studied electrocardiographic changes produced in cattle by a vitamin E-free ration. The PR interval increased and the potential of the various deflections was modified four months prior to the death of the animal. The electrical axis of the heart was also changed. They concluded that the results showed a decreased functional activity of the myocardium in the terminal stages of the deficiency.

In rats, Ensor (1946) did not find a definite difference between animals maintained for a year on a diet free of vitamin E and those kept on the same diet plus vitamin E. He reported that, perhaps, a slight widening of the QRS complex, which occurred in five out of 15 rats, could be the only noticeable effect of the deficiency.

E-deficient rabbits were submitted to various functional tests by Houchin and Smith (1944). They found that deficient

animals had a greatly increased sensitivity to posterior pituitary extract, a higher resistance to the toxic effect of cardiac glycosides, and that the heart probably became dilated. Later, Gatz and Houchin (1947) pointed out that rabbits having myocarditis produced by vitamin E deficiency also gave abnormal EKG record. The records of deficient animals as compared with those of normal rabbits showed a right axis deviation, an inverted potential of QRS in leads 1 and 3; there was nothing frequently occurring in T1 and T2 and inversion of T3. No significant variations appeared in the PR and QRS intervals. The work of Bragdon and Levine (1949) did not fully reproduce the results reported by Gatz and Houchin (1947). E-deficient rabbits showed elevated ST segments and inversion of the T waves in lead II. These changes were only observed during the latter part of the period of observation.

In monkeys with chronic vitamin E deficiency, Filer et al. (1949) pointed out that there is a reduction in the amplitude of the R and T waves and inversion of the T waves. He compared his results with the results obtained in vitamin B1 deficiency of monkeys by Waisman and McCall (1944) and concluded that they were quite similar.

Interrelationships:

The study of the effects of vitamin E deficiency on the chemical composition of the tissues have shown that it influences

the lipid, protein, carbohydrate and mineral constituents. These changes presuppose the previous alteration either directly or indirectly, of the metabolism of the tissues. Although we are far from understanding the complex mechanisms involved in these changes, they will be briefly reviewed in the following paragraphs, taking into consideration the influence of vitamin E on the metabolism of proteins, lipids, carbohydrates and minerals.

Interrelationship of Vitamin E and the Lipids:

There is no doubt that vitamin E must be concerned with the lipid metabolism since certain fatty materials (rancid fat and cod liver oil especially) given in the diet precipitate the symptoms of the vitamin E deficiency (Mackenzie et al. 1941), and, the vitamin E deficiency produces derrangements of the lipid composition of the tissues.

Rancid fats, materials having a rank smell and high content of free fatty acids, aldehydes, ketones and peroxides were found by Matill (1938) to be highly destructive to vitamin E. This drastic action was due to rapid oxidative process.

Excessive feeding of cod liver oil has been known for a long time to be detrimental to animals (Agduhr and Stenstrom 1929) and many workers have used it to facilitate the study of the muscular dystrophy (Davis et al. 1938). The same authors made an effort to identify the factors in cod liver oil res-

ponsible for such action. Their results indicated that neither arsenic nor cholesterol were concerned with the production of muscular dystrophy.

More recent investigations point towards other constituents of cod liver oil. Dam (1944a, 1944b and 1948) and Goldhaber et al. (1950) thought that probably the unsaturated fatty acids were the necessary factor for production of vitamin E deficiency in the chicken. Holman (1947) also stated that fatty acids probably were the toxic substances contained in the cod liver oil which contributes to the development of arteritis in dogs.

However, the review of Cormier (1948) on the apparent antagonism of cod liver oil towards vitamin E in experiments with rats, rabbits and guinea pigs led to the conclusion that the specific nature of the toxicity of cod liver oil was not yet defined.

Cod liver oil was not the only fatty material that has shown antagonism to the vitamin E. Dam (1944b) showed that hog liver fat also had an effect on the production of vitamin E deficiency of the chick. Furthermore, he found, by separating three fractions of hog liver fatty acids of different iodine number, that: the least unsaturated fraction (I.N. 5) had little effect and gave only exudates; the intermediate fraction (I.N. 104) gave both exudates and encephalomalacia, whereas the most unsaturated fraction (I.N. 241) caused a rapid onset of severe encephalomalacia and early death of the animals.

More evidence on the interrelation of alpha tocopherol and essential unsaturated fat acids was given by Hove and Harris (1946) who showed that E-deficiency symptoms were aggravated when tocopherol but no essential fat was fed to fat-deficient rats.

The influence of vitamin E on the lipid composition of the tissues was studied by various investigators. Escudero et al. (1942) found that in vitamin E deficiency the lipid content of the adrenals was diminished after rats were on a low E diet for six months. The extensive work of Dam on the vitamin E deficiency of chicks (1944a, 1944b and 1949) gave some evidence that the E deficiency decreased the ratio of phospholipid to other lipids in the blood plasma of chicks, but he thought that the difference was not great enough to be of diagnostic value. He also showed (Dam 1948b) that the development of exudates in the adipose tissue of chicks, due to lack of tocopherols in the diet, coincided with the time when peroxides could be demonstrated in the depot fat. Later, the peroxides increased in the tissues, which was apparent through the brown coloration of the fat tissues. These last findings were essentially the same as reported by Criddle and Morgan (1947) who, besides, observed that the meat of turkeys, which were not fed tocopherols in their ration, developed unpleasant flavors during prolonged storage.

Bratzler et al. (1950) analyzed the body fat of pigs depleted in tocopherols and found a high percentage of oleic acid. The excess was produced at the expense of the saturated fatty acids.

Interrelationship of Vitamin E and the Nitrogen Metabolism:

The influence of the deficiency of vitamin E in the diet on the creatine-creatinine excretion in the urine has already been reviewed. Alpha tocopherol also has been demonstrated to modify the creatine, creatinine, glyocyamine content of tissues of animals deficient in vitamin E.

Goettsch and Brown (1932) found that the creatine content of the rabbit muscle decreased, but not in either heart or brain. Melville and Hummel (1951) also made the analysis of tissues of E depleted rabbits and found that the creatine content of the liver increased five times over normal, that of the kidney and blood four times, whereas the content of creatine in the muscle was lower than normal. These findings suggested to them that perhaps there is some storage in the kidneys cells, and the same may be true in the liver. Both organs would temporarily store some of the creatine released from degenerating muscles.

Vitamin E not only has interrelations with the nitrogenous compounds just mentioned, but also with the amino acids and protein metabolism.

Herschel (1946) made a theoretical study of the action of alpha tocopherol on the animal physiology. He concluded that there must be a relation of vitamin E to the amino acids, especially cystine and isoleucine.

On the other hand, other workers arrived at similar conclusions when investigations were carried out about the origin of creatine. It was found that creatine was formed from several amino acids, but especially from glycine and arginine, the specific biological precursors of creatine (Sahdu 1947; Almquist et al. 1941; Bloch and Schoenheimer 1941).

Glutamine of the muscle was found by Roderuck (1949) to be reduced in vitamin E deficient muscle of guinea pigs and rabbits. Notwithstanding the composition of the non-glutamine amino acids remained unchanged.

The possibility that vitamin E might be related to protein metabolism has been suggested by several workers (Dam 1944b; Hove 1946; Hove and Harris 1947b; Moore 1948, 1949). Moore (1948) found that in rats having a combined deficiency of vitamin E and protein, failure of growth was mainly due to lack of protein, and dental depigmentation to lack of vitamin E. Working on the same problem, Hove and Harris (1947b) pointed out that the efficiency of utilization of protein was not affected by adding alpha tocopherol to the diet.

Other studies, which combined the effect of protein and alpha tocopherol, demonstrated certain interesting interrelations.

The occurrence of hepatic necrosis in rats, produced by a deficiency of sulfur containing amino acids, was found by Lindan and Himsworth (1950) to be delayed by alpha tocopherol feeding. Alpha tocopherol, however, could not completely prevent the fatal injuries caused by a diet deficient in methionine (Kemeny et al. 1949). Yet, Moore (1949) found that tocopherol prevented liver necrosis, anaemia and persistent loss of weight due to a protein deficient ration. But tocopherol could not prevent the heavy infiltration of the liver with fat.

Prosperi and Lottini (1950) suggested that the formation of the protein thrombin from prothrombin could be influenced by the administration of alpha tocopherol, since vitamin E is a coagulant factor in hemophilia therapy, has no influence in the formation of fibrin, and does not act on the platelets.

Vitamin E and the Carbohydrate Metabolism:

Little work has been done on the relation of vitamin E to the carbohydrate metabolism. Most of the knowledge on this matter at present comes from clinical observations on the effects of vitamin E on certain diseases. Butturini (1949) showed that alpha tocopherol helped the formation and deposit of glucose in the skeletal muscle, in the heart and in the liver. He treated some cases of diabetis mellitus with vitamin E, and found that it increased the difference between arterial and venous glycemia and reduced the hyperglycemia and glycosuria. He con-

cluded from his experience that it was possible to cure mild cases of diabetis mellitus by only the use of vitamin E. In severe cases, he wrote, vitamin E still was helpful since it increased the intensity and duration of the hypoglycemic effect of insulin. The more complete utilization of carbohydrates in diabetis was demonstrated by the disappearance of glycosuria, when the glycemic values were still over the usual renal threshold.

In clinical cases of hypo and hyperfunctional states of the pituitary-diencephalic system, Heinsen (1951) reported that the administration of alpha tocopherol had a normalizing or regulatory effect on the carbohydrate and water metabolism.

It must be admitted, however, that critical data on the interrelationship of vitamin E and carbohydrates are lacking.

Vitamin E and the Mineral Metabolism:

The investigation of Fenn and Goettsch (1937) indicated that mineral changes in the composition of the muscles occurred in association with nutritional muscular dystrophy of rabbits. They found that dystrophic muscles had less potassium, less magnesium, and more sodium and chlorine than muscles of normal rabbits. When the muscles showed histological evidence of calcification there was also found, by the chemical method, high concentrations of calcium and phosphorus.

Calcification of necrotic fibers was observed by Pappenheimer (1942) and Morgulis and Jacobi (1946) in vitamin E deficiency.

The phosphorus metabolism was found to be disturbed by the absence of vitamin E by Lu, Emerson and Evans (1941). It was noticed that in paralyzed rats there was a slight decrease of inorganic phosphorus and creatine phosphorus, and a marked decrease of total acid soluble phosphorus, pyro, and organic ester of phosphorus. However, in these rats pronounced dystrophy was not observed and no change occurred in the calcium content. When the rats became markedly dystrophic the muscles had a white and waxy appearance, which was accompanied by an increase of 50-180% in calcium and by a decrease in phosphocreatine phosphorus.

Pappenheimer (1943), in his review of the muscular disorders associated with deficiency of vitamin E, stated that the interpretation of the overall changes in the electrolyte content is difficult, because of the complex variations in composition of the dystrophic muscles, as regard normal, necrotic and regenerating fibers, wandering cells, interstitial fat and connective tissue, and pathologic calcification of dead fibers.

Early investigators were suspicious that an imbalance of the ratio of calcium to phosphorus had some connection to the "stiff-lamb" disease (Marsh 1932). Since rations containing cull beans and alfalfa contained more phosphorus and produced high incidence of "stiff-lamb" disease, Willman et al. (1946) fed rations known to be satisfactory to sheep, but to which enough dibasic sodium phosphate

was added, in order to make the phosphorus content equal to that of the "stiff-lamb ration". The results indicated that phosphorus by itself was not a factor in the vitamin E deficiency of the lamb.

Iron had been used to destroy the vitamin E content of diets and produce the E deficiency. Zacharias et al. (1950) were interested to know whether or not iron per se had any influence in the development of the vitamin E deficiency. They found that the inclusion of iron, either in the ferric or ferrous form, had no effect on the plasma level of the chick.

Theory of Physiological Functions:

The theories about the physiological functions of vitamin E were reviewed by Mason (1944) in a chapter of his work: "Physiological Action of Vitamin E and Its Homologues". "The antioxidant function of the tocopherols, important as it may be", he said, "cannot represent the prime role of vitamin E. . . . ." Among the reasons given to support his point of view were: the inability of other antioxidants to substitute vitamin E, the inverse relationship between antioxidants and biological actions, lack of antioxidant activities of tocopherols after ingestion, and the manner in which changes unrelated to phenolic hydroxyl group in the molecule alter biological activity of the tocopherols.

"Adamstone", continued Mason, "postulated the theory that vitamin E may exert a controlling influence over cell prolifera-

tion. This idea inspired other workers to suggest that vitamin E might be necessary either for the synthesis of nuclear chromatin or the maintenance of the physico-chemical state of the latter."

A number of investigators proposed the thesis that arginine could be involved in the metabolic dysfunction of dystrophic muscles. Mason (1944) described these hypotheses, which had been deducted from the known facts, that arginine is an important component of nucleoproteins, and that vitamin E deficiency produces special symptoms in rats, animals known to have different abilities to synthesize arginine.

"In conclusion," Mason stated, "the fact that different organs or tissues are affected by vitamin E deficiency, and that there are striking species differences in this regard, can be interpreted in two ways: 1) vitamin E takes part in a specific metabolic process which is common to a variety of tissues, or 2) it plays a part in a variety of enzymic processes."

Since that time, many investigations have been carried out to disclose the physiological role of vitamin E. In the bibliography collected by Harris and Kujawski (1950) the following enzymes have been reported to have a close relationship to the vitamin E: carotene oxidase, choline esterase, succinoxidase, coenzyme I, cytochrome oxidase, cytochrome reductase, lipase, hyaluronidase, asparticglutamic transaminase, liver acid phosphatase, muscle alkaline phosphatase, muscle acid phosphatase and phosphorylases.

This extensive action of vitamin E on the enzyme system might indicate that perhaps the most important role of vitamin E is played in the enzymic processes of the animal organism.

Recently, Hickman (1949) has postulated a hypothesis about the highly polyfunctional properties of vitamin E. He proposed that vitamin E plays a predominant role in the in transitu chemistry performing an organic housekeeping and tidiness job which should be closely related to the aging problem.

EXPERIMENTAL PROCEDURE

Animals Used

A total of 51 lambs was used to study vitamin E deficiency in this species. Most of them were obtained from the Michigan State College's experimental flock and some from neighboring farms. The main criterion for the selection of the experimental animals were age and health.

It was thought that lambs three to four days old would be the most desirable to use in this experiment. At this age they would have received enough colostrum to develop normally but not quite enough to build up a great storage of tocopherols, which would have either increased the time of depletion, or would have not made possible the production of vitamin E deficient lambs.

The lambs were purebred or crossbred, but all belonged to the type of mutton and fine wool. Females and males, single and twin lambs were used in this experiment.

Composition and Preparation of the Purified Diet

Essentially, the purified diet used in this investigation was similar to that reported by Luecke et al. (1950). They found that their diet was very satisfactory for the development and growth of the young suckling lamb. However, for the present

work, it was felt necessary to make certain changes in this diet in order to eliminate the possibility of early gastro-intestinal synthesis of vitamin E, which could alter the results of the experiment.

The modification consisted in the elimination of lactose, a sugar known to promote the intestinal synthesis of vitamins in many animals. Lactose was replaced by glucose; thus, glucose constituted the only source of carbohydrate in the diet of the lambs. The diet was called diet A which had the composition shown in Tables I, II and III.

The experience with the first 10 animals of the trial I (diet A) indicated that perhaps the diet was not absolutely free of tocopherols, since the symptoms of vitamin E deficiency even after an extended period were rather mild. This was not the aim of the investigation, which, as it has been stated, was to attempt to duplicate the acute syndrome of vitamin E deficiency found in the field. Therefore, it was thought that the cause of production of moderate symptoms of vitamin E deficiency was the result of the presence of a small amount of tocopherols in the diet. If this was the reason, then, there were two choices for production of vitamin E deficiency. One was to feed a vitamin E antagonist along with the E-low diet, and the second was to use ingredients freer of tocopherols for the preparation of the liquid diet. Both of the approaches were successively employed.

TABLE I

COMPOSITION OF THE PURIFIED DIET

Component	Diet A Per cent		Diet B per cent	
Solids	16.05		16.05	
Casein (Labco)		4.80		4.80
Glucose		6.08		6.08
Lard	(commercial)	4.16	(molecular distilled)	4.16
Salt mixture		0.96		0.96
Vitamins		0.05		0.05
Distilled water	83.95		83.95	
	100.00		100.00	

TABLE II

VITAMIN CONTENT OF THE PURIFIED DIET

Vitamins	Mg. per lt. of artificial milk	Vitamins	Mg. per lt. of artificial milk
Thiamine	1.4	Inositol	40.00
Riboflavin	3.0	Biotin	0.04
Nicotinic acid	4.0	Choline	400.00
Calcium pantothenate	5.0	α-Tocopherol acetate	2.00
Pyridoxine	2.0	2-Methyl naphthoquinone	0.4
Pteroyl glutamic acid	0.2		
p-Amino benzoic acid	4.0	Vitamin A	4000 I.U.
		Vitamin D	500 I.U.

TABLE III

COMPOSITION OF THE SALT MIXTURE

Component	Per cent
NaCl	11.88
K_2HPO_4	25.76
$CaHPO_4$	33.80
Ca lactate $Ca(C_3H_5O_3)_2 \cdot 5H_2O$	22.88
$MgSO_4.7H_2O$	3.54
$FeSO_4.7H_2O$	1.93
KI	0.06
$MnSO_4.H_2O$	0.10
$ZnCl_2$	0.02
$CuSO_4 . 5H_2O$	0.02
$CoCl_2 .6H_2O$	0.01
	100.00

Cod liver oil was selected as the product for supplementation of antagonistic factors to vitamin E. Three animals, that were already fed a vitamin E-low diet for 40 days and two controls, which were fed a vitamin E containing diet for the same time, were daily supplemented with 5 ml. of cod liver oil by mouth for a period of 25 days.

Of all the ingredients of the diet A, commercial lard was the only material suspected to contain small but significant quantities of tocopherol tending to delay the appearance, or attenuate the E deficiency syndrome. Thus, the commercial lard was substituted by an equal amount of a molecular distilled lard (low tocopherol animal fat) containing less than five micrograms of tocopherols per gram. The rest of the ingredients remained unaltered. The new diet was called B which was identical to diet A in every respect except for the nature of the lard.

Some of the chemical characteristics of the commercial and molecular distilled lard have been determined by the Distillation Production Industries[1] which are summarized in Table IV.

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[1] Rochester 3, N.Y. Division of Eastman Kodak Company.

TABLE IV

SOME CHEMICAL CHARACTERISTICS OF COMMERCIAL AND MOLECULAR DISTILLED LARD

Characteristic	Commercial lard	Molecular distilled lard
Iodine number	65.9	65.0
Saponification Number	195.0	195.3
Percent Unsaponifiable	0.3	0.3
Acid Value	0.9	0.02
Peroxide number	14.0	23.0
Peroxide number after four hour of aereation at about 98°C.	361.0	418.0

The preparation of the liquified diet was carried out in the following manner. Distilled water (29.5 liters) was measured into a 10 gallon milk can and warmed with a steam coil to 80-85°C. Sodium bicarbonate (82 gm.) was dissolved in the warm water, and to this solution casein (1728 gm.) was added very slowly and in small portions. In this way casein was transformed to sodium caseinate, which is the form that casein is present in the natural milk. The formation of this compound and the preparation of a homogenous suspension was aided by rapid stirring. After all of the casein was added, the suspension was continuously stirred for about 30-45 minutes to assure uniformity and incorporate the small lumps of casein to the suspension.

Then glucose (2189 gm.) was added and the preparation was ready to receive the fat constituents. The lard (1498 gm.) was melted on the steam bath and 36 ml. of cod liver oil, reinforced with 0.4 mg. per ml. of menadione (Vitamin K), was added.

This liquified fat mixture was slowly poured into the rapidly stirred preparation. At this point the suspension of casein, sugars and lard was homogenized in a Manton-Gaulin homogenizer. The operation was done at 600 pounds per square inch in the first stage and 3200-2500 pounds pressure per square inch in the second stage.

A portion of this homogenized product was removed to make a suspension of 345 gm. of salt mixture. About 50 gm. portion of the salt mixture was placed in Waring blendors, filled to one-third of the capacity with the homogenized preparation. After seven to ten minutes of fast stirring, the salts were finely suspended and incorporated into the bulk of the homogenized preparation with the aid of electrical stirrers.

The last step of the milk preparation consisted in the addition of sufficient quantities of B vitamins which were contained in 360 ml. of a 20% alcohol solution. The mixture was completed by another two to three minutes of stirring, after which the purified liquid diet was ready to be poured into clean sterile gallon bottles.

The milk was stored under refrigeration at 4°C. until its use, but never for more than 15 days to eliminate any possibility of alteration.

Care and Feeding

The experiment was carried out during the winter and spring of 1951. The lambs were kept in a concrete-floored room maintained at 60°F. Fine wire mesh fenders subdivided the room in small pens of approximately 4x12 feet. Each pen was provided with two beds made of burlap bags filled with a small quantity of wood shavings. Two or three animals were placed in each pen. Every day the group of animals in one pen was moved to a clean one, which also was provided with clean bedding. The dirty pens were then scrubbed and cleaned with hot water and disinfected with a lye solution to make the place as aseptic as possible. The slight slope of the floor aided in the cleaning of the pens.

The lambs were fed the purified milk diet four times a day, by means of clean sterile nursing bottles. The artificial milk was thoroughly shaken to put back in suspension the minerals that settled during the storage period. It was poured into the nursing bottles and warmed up to about 80°F. After one or two days the lambs were able to drink from the bottle and became adjusted to the new method of feeding. Previous experience had indicated that overfeeding was one of the usual troubles in raising young lambs; thus, special care was taken to regulate the consumption of the artificial milk diet.

Individual records of each of the lambs were kept concerning milk consumption, growth (weight every three days), and clinical observations of the health and behavior of the animals.

The whole experiment was conducted in three successive trials. A total of 19 lambs was used during the trial I. Eleven of them were placed on diet A to find if the liquid diet was useful in the production of vitamin E deficiency. A second group of lambs was used as controls; these also received the diet A and in addition were given orally every other day, 100 mg. of dl-alpha tocopherol diluted in corn oil (Mazola). The oil was administered by means of a syringe fitted with a rubber hose. To test the usefulness of cod liver oil in accelerating the development of vitamin E deficiency another group of five animals was used in this first trial. The technique was described above under the heading of Composition and Preparation of the Purified Diet.

The second trial was carried out with a total of 16 animals. The purpose of this second trial was to produce an acute syndrome of vitamin E deficiency. For this reason diet B was used. Twelve lambs were separated for the deficient group and a group of four lambs was kept as controls. The controls also received the diet B, and were supplemented with dl-alpha tocopherol in similar manner as the controls of the first trial.

The object of trial III was to study the electrocardiographic changes of the lambs produced by the deficiency of vitamin E and, also, to study the recovery of E-deficient lambs. Diet B was again used in this trial. Three animals were kept as controls, which received dl-alpha tocopherol in an identical

manner to the previous controls. Thirteen lambs were placed on the deficient diet without any supplementation. Recovery was attempted in six of the thirteen deficient lambs by making use of dl-alpha tocopherol acetate[1] and cortisone[2]. Two vitamin E depleted lambs were treated with prostigmine[3] to test the effect of this drug on the vitamin E deficiency.

Recovery with dl-alpha tocopherol was tried on four lambs. The therapy was started when one of the animals was severely deficient and the three others were beginning to show the first symptoms. The treatment consisted in feeding an initial dose of 500 mg. of alpha tocopherol followed by 100 mg. doses every other day. Vitamin E was diluted in corn oil and administered in the same manner as described above for the control animals.

The number of days of therapy was gradually increased from one day for the first animal to 40 days for the last one. The lambs were sacrificed immediately after the therapy ended, in order to follow pathologically and chemically the process of recovery of the E-deficient lambs.

The experience with alpha tocopherol therapy indicated that it is more successful to start the medication as early as the symptoms were noticed. Therefore, four other lambs that

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1 Alpha Tocopherol Acetate Merck. Merck & Co., Inc. Rahway, N. J.

2 Cortone Acetate. Merck & Co., Inc. Rahway, N. J.

3 Prostigmine Bromide Merck. Merck & Co., Inc. Rahway, N. J.

began to show the vitamin E symptoms were immediately treated with the other drugs. Two animals were treated with prostigmine. It was injected subcutaneously every day in doses of 0.5 mg. The other two lambs were administered cortisone, which was injected intramuscularly at the rate of 50 mg. per day.

Blood Studies

The main objective of the blood studies was to determine the progressive effect of the vitamin E deficiency on the total tocopherol content of the blood plasma, the hemoglobin content of the blood, and the differential white count.

About 20 ml. of blood were collected from the jugular vein, placed into oxalated tubes and, as soon as possible, the initial values of tocopherol and other constituents were determined. Then, blood samples were drawn every ten days and when the animals were older, the blood analysis was carried out every week until the end of the experiment.

The procedure for the determination of tocopherols in the blood plasma was the one described by Quaife and Harris (1944) and Quaife and Biehler (1945). Most of the time the blood samples were analyzed immediately after collection; however, in a few instances extracts of blood plasma were kept for a week under refrigeration until the analysis was performed. This, according to the literature already cited and previous trials in this laboratory, showed that petroleum ether-alcohol

extracts of samples kept under refrigeration for 20 days did not alter the content of tocopherols.

The hemoglobin concentration was determined by making an exact dilution of a blood sample and determining colorimetrically the optical density of the solution. The "Cenco-Sheard-Sanford Photelometer" was the instrument used in these analyses.

The total leukocyte count was determined by using a 1% solution of 0.1 N HCl to which one or two drops of gentian violet were added. A hemocytometer with Neubauer ruling was used for the actual enumeration. The oil immersion objective was used to classify the leukocytes.

The findings of the first and second trials on vitamin E experiments suggested that perhaps the circulatory system was the first system to be affected as a result of the vitamin E deficiency. Thus, it was thought that the determination of blood pressures in lambs of the third trial (fed diet B) could give some support to the validity of this hypothesis.

After testing several methods for measuring blood pressure it was concluded that the only accurate way to determine the lambs' blood pressure was by using an electronic device. It consisted of three parts: a strain gage, an amplifier and an oscillograph. The strain gage[1] was connected to the arterial system by needle puncture and transformed the pressure pulse

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[1] Statham Blood Pressure Strain Gage. Statham Laboratories, Los Angeles, California.

to collect the urine at definite ages of the animals. Therefore, in some cases it was possible to have only two urine collections during the whole experiment, in the majority, three collections, and in a few lambs four collections were made.

The volume of the urine was measured, filtered and stored under refrigeration for about 20 days until there were enough samples to run an analysis.

Creatine and creatinine were determined by using the analytical method of Peters (1942). The spectrophotometer employed for measuring the density of the colored solutions was the "Beckman Model B Spectrophotometer".

The sodium and potassium content of the urine was also determined in an effort to detect other changes that might take place due to the vitamin E deficiency.

The analysis of the urine for these two minerals was carried out by the flame photometer method and using the "Flame Photometer Model 52 A" of the Perkin-Elmer Corporation. The "internal standard" method was the analytical technique employed in this determination. It consists of adding to the samples and standards the same amount of a known element (lithium for the mentioned photometer) and measuring the relative optical density of samples as compared to that of the standard. The photometer is built to measure on one tube the amount of light emitted by the constant amount of lithium, while other tube measures the amount of light emitted by the

unknown element. The method is very accurate (about 1%) because many of the variables, such as sample viscosity, rate of atomization, flame temperature, foreign materials, gas and air pressure tend to affect both the lithium and unknown in the same manner. Therefore the ratio tends to be constant. (The Perkin-Elmer Corporation 1949).

Two ml. of urine were placed in a 50 ml. volumetric flask, then 1.25 ml. of a solution containing 2000 ppm of lithium was added, and the mixture made up to volume with distilled water. By this dilution, the sodium content of the sample fell between 75-300 ppm and that of the potassium between 50-200 ppm.

Studies of the Gross Pathology

The lambs were closely examined once a day to detect the changes that vitamin E deficiency was inducing. Whenever it was noticed that the death of a lamb was imminent, the animal was sacrificed by exsanguination under ether anesthesia and necropsied. In cases where lambs died suddenly, they were autopsied as soon as they were found. The remaining animals, including the controls, were sacrificed at the end of the experimental period to find the extent of the damage produced by the deficiency, to check whether the diet containing tocopherols was suitable for the nutrition of the lambs and that there was no error in the process of the milk manufacturing, and, finally, in order to have material for the chemical analysis of the muscles.

The carcasses of the lambs and all the organs were carefully examined for gross lesions, with special attention paid to the alterations of the muscular system. Particular importance was given to the bilateral distribution of the lesions, the color of the muscles, the presence of exudates, and the relation of nerves and blood vessels to the lesions.

Since few lesions were noticed in the heart, little attention was paid to this organ during the first trial. However, in the second trial more lesions appeared and, consequently, several preliminary studies were started on this organ. First, diagrams and photographs of the heart were taken to study the most commonly affected areas. Then, india ink was injected into the coronary arteries in order to find if there was any relationship between the coronary arteries and the necrotic areas. Later on, carmine gelatin was used for the same purpose, after the heart had been perfused with isotonic 0.9% NaCl. It must be pointed out that this last technique was applied only after some samples of heart muscles had already been removed for the amino acid and mineral analysis.

Histological Studies

The histological studies were confined to the skeletal system, heart, liver, kidney and adrenals. Sections of different muscles were taken, but with more frequency from the various muscles of the rear and front legs, and from the heart. Samples

from the abdominal muscles, diaphragm, tongue and masseter were collected with less frequency. Zenker's fluid was used as a fixative for the muscular tissues. They were imbedded in paraffin and then cut in sections of seven micra in thickness and stained with hematoxylin-eosin.

Liver, kidney and adrenal tissues were also fixed in Zenker's fluid and stained with hematoxylin-eosin. But, in addition, tissues of these organs, and the heart, were also fixed in 10% formol saline and stained with Sudan IV for studies on fat.

Electrocardiographic Studies

It was mentioned in the review of literature that lesions in the heart of the lamb produced by vitamin E deficiency were reported as sporadic by most of the research workers. However, the results of the first and second trials indicated that these lesions were rather frequent in the present investigation. Therefore, it would seem of interest to determine by means of the electrocardiograph whether any functional changes were also present.

The first electrocardiograms were recorded within five days of the start of the experiment and then were taken every week until the end of the experiment.

The electrocardiograph used in the present investigation was a Cardiotron, Model PC-1A[1]. The three standard dipolar leads I, II and III were successively recorded in each one of the group of ten animals of the third trial. In lead I, the difference in electrical potential is measured between the right front leg and the left front leg; in lead II between the right front leg and the left rear leg; and in lead III, between the left front leg and the left rear leg.

The area of application of the electrodes was situated a little above the knees in the front legs, and a little above the hocks in the rear legs. In order to establish intimate contact of the electrodes with the skin, the area was clipped, rubbed with a cheese cloth saturated with ethyl ether and electrode paste was rubbed on the area. A small quantity of the paste was placed on the surface of the electrodes, prior to their application to the skin. The composition of the electrode paste was as follows:

Sodium Chloride USP	1500 gm.
Potassium bitartrate USP	112 gm.
Pumice Powdered	1800 gm.
Tragacanth USP (Ribbons)	225 gm.
Glycerine USP	360 ml.
Phenol liquid USP	15 ml.
Distilled water	7000 ml.

[1] Electro-Physical Laboratories Inc., New York.

It was prepared in the following manner. The glycerine was diluted with half the water and heated to boiling. The tragacanth was soaked in this solution for 6-12 hours. The salts and the bitartrate were dissolved in the rest of the water and added to the swollen gum with stirring. They were mixed thoroughly until the mixture became smooth. Finally the phenol and the pumice powder were added and mixed.

The first electrocardiographic recordings were difficult to take due to nervousness of the lambs. This happened even though considerable time was allowed for them to become accustomed to the electrodes and to the wooden box in which they were placed for better handling and for insulation from the ground. But after the first two or three records had been taken, the behavior of the animals improved considerable and it was much easier to obtain good records.

The nomenclature of Einthoven (Pardee 1936), commonly used in human electrocardiography was employed to describe the electrocardiogram of the lambs. Thus, the deflections were named P, Q, R, S and T which were considered positive when appearing above the base line, negative when below, and diphasic when evenly distributed on both sides of the base line. The segments ST and TP were regarded as elevated or depressed when they did not have the same level as the base line.

The following measurements were studied in the analysis of the electrocardiogram of the lamb: heart rate, the PR, QRS

and QT intervals, the systolic index, potential of the QRS complex, shape and character of the deflections, and arrythmias.

The systolic index or, the ratio of the systole to the heart rate was calculated according to the Bazett's formula (Pardee 1936) : $K = \frac{QT}{\sqrt{RR}}$ where K is a constant in the human electrocardiogram; QT is the time interval in seconds from the beginning of the complex QRS to the end of the T wave, and RR is the time interval in seconds between two successive R waves.

Due to the scarcity of information about the electrocardiogram of sheep and related species such as cattle (Alfredson 1942) a true calculation of the electrical axis of the lamb's heart was not possible.

Many authors have experienced similar limitations but they have resorted to the knowledge of the human electrocardiogram and used the methods for the determination of the human's cardiac electrical axis, to estimate the electrical axis of several species (Bovine, Alfredson 1942; Rats, Ensor 1946; Birds, Sturkie 1949). Likewise in this study, one of the methods for measuring the electrical axis of the human heart has been applied to the lamb's electrocardiogram.

The approximate electrical axis of the lamb's heart was estimated with an error of 30° by the method of Wilson (1938). The determinations of the electrical axes, according to this

method, were done by matching the relative QRS potentials of the electrocardiograms to a pattern of QRS_1, QRS_2, QRS_3 known to occur at various degrees of axis deviation (Table V).

TABLE V

MODIFIED WILSON'S TABLE FOR THE APPROXIMATE DETERMINATION OF THE ELECTRICAL AXIS OF THE HUMAN HEART

Electrical Axis of the Human's heart	Approximate number of degrees	Relative potentials of QRS		
		Lead I	Lead II	Lead III
	±180°	-2	-1	+1
	-150°	-1	-1	0*
	-120°	-1	-2	-1
	- 90°	0*	-1	-1
Left Axis	- 60°	+1	-1	-2
Deviation	- 30°	+1	0*	-1
	0°	+2	+1	-1
Normal Axis	+ 30°	+1	+1	0*
Deviation	+ 60°	+1	+2	+1
	+ 90°	0*	+1	+1
Right Axis	+120°	-1	+1	+2
Deviation	+150°	-1	0*	+1
	±180°	-2	-1	+1

* Either diphasic or zero potential.

Studies on the Chemical Changes of the Muscles

The thigh muscles and part of the ventricles of the lambs' heart were removed at the time of necropsy, put into jars, closed tightly and kept in a deep freezer (-22°C.) for two to three months. After this time, the samples were defrosted and prepared immediately for chemical analysis.

The chemical analysis consisted in the determination of the following minerals: calcium, phosphorus, magnesium, potassium and sodium, and the determination of the amino acids: glycine, proline, leucine and isoleucine.

The muscle samples were prepared in the following manner for the mineral analysis. The heart and thigh muscles were freed of the muscular sheaths, tendons and fat. Then they were cut in small pieces, placed on a previously weighed crucible and a sample of about 10 gm. was weighed accurately on the precision balance.

The crucibles were placed on a hot plate in order to dehydrate the samples as slowly as possible because, otherwise, according to the experience of the Agricultural Chemistry laboratory of this Institution, there would be splattering of the sample and quick foaming, which would introduce large errors in the quantitative determinations.

Muscle samples treated in such a manner, however, were still not ready for ashing, they needed further treatment. A few drops of sulfuric acid were added to each sample and the

crucibles carefully heated with the free flame of a Bunsen burner. Each sample was treated in this way, each time carefully avoiding the tendency to foam while the organic matter was being oxidized.

This processing left a black, dry residue sticking to the bottom of the crucibles, which, after cooling, were treated with a few drops of concentrated nitric and sulfuric acid. The crucibles were placed overnight in a muffle furnace, regulated to give a maximum temperature of 500°C. which is low enough to prevent the volatilization of sodium or potassium salts. Most of the samples required two ashings before a white ash was obtained; yet, in a few cases three and four ashings were necessary to get a white ash.

The still warm crucibles were placed in a desicator until they reached room temperature and were weighed again to determine the amount of sulfated ash. Then, 5 ml. of concentrated hydrochloric acid was added to the ashes and boiled for one minute. The solutions were filtered through an ashless filter paper into 50 ml. volumetric flasks. The crucibles were thoroughly cleaned with the policeman and rinsed with distilled water at least three times. In some cases it was necessary to add more hydrochloric acid to dissolve some of the ash that still remained. The solutions were then made up to volume and ready to be used for the mineral determinations.

An aliquot of the solution was employed for the calcium determination, which was done according to the method of Shohl (1922). The residuary supernatant left after the precipitation of the calcium oxalate was used for the determination of magnesium which was performed following the method of Briggs (1924).

Another aliquot was taken to make the determination of potassium and sodium by the flame photometer method. Since the maximum sensitivity of the apparatus for potassium and sodium is about 300 ppm, the aliquot was diluted until it contained less than 300 ppm of sodium and potassium. The usual dilution in the present situation was about 100 mg. of sulfated ash in 100 ml. of solution. However, sometimes it was necessary to have a dilution of 100 mg. of ashed sample in 50 ml. and, in other cases, 100 mg. of ash in 200 ml. of solution. The procedure and apparatus used in the determination were the same as those described in the quantitative analysis of sodium and potassium in the urine.

It was impossible to determine phosphorus by using the previously described ashing method because of the volatility of most of the phosphorus compounds. It would have been necessary to form, before ashing, non-volatile compounds of phosphorus at the temperature of 500°C., which could have been accomplished by addition of $Ba(OH)_2$. The other choice was the wet-ash procedure which was selected because the samples could be drawn from the acid hydrolysates of muscles prepared for the deter-

mination of amino acids, which is described below. The method employed for the determination of phosphorus was the one described by Fiske and Subbarrow (1925).

The procedure for the preparation of the muscle samples for the assay of amino acids was as follows. Twenty gm. of thigh muscle, or 5 gm. of heart muscle, were cleaned and freed from fat, finely cut and placed in a small warring blendor (capacity of about 300 ml.). Fifty and 25 ml. of distilled water respectively were added to the Waring blendors and the mixture stirred for about three to four minutes. Pieces of connective tissue that were sticking to the blades were cut again into small segments and the stirring continued until there was a rather homogenous suspension of the muscle tissues. Then the suspension was quantitatively transfered to a graduated cylinder and made up to a volume of 200 ml. in the case of thigh muscle, and to a volume of 50 ml. in the case of heart muscle. The cylinder was inverted several times to be sure of equal dispersion. Immediately after this, aliquots containing 2 grams of sample were taken, with wide tipped calibrated pippetes, and placed in Erlemmeyer flasks for hydrolysis.

The method of Stokes *et al.* (1945) was used for the acid hydrolysis which was accomplished by eight hours of autoclaving with 50 ml. of 6 N HCl. The amount of HCl acid was calculated by taking into consideration the dilution of the muscle samples. The hydrolysates were filtered, neutralized to pH 6.8 with NaOH,

and stored under toluene and refrigeration.

The determination of the amino acids were carried out microbiologically. The method of Schweigert et al. (1944) was used for the determination of leucine and isoleucine, and the method of Sauberlich et al. (1946) was employed for the determination of proline and glycine.

Field Cases of Stiff Lamb Disease

Cases of vitamin E deficiency occurring in lambs and in calves were reported on farms in Michigan while this experiment was being carried out. This coincidence gave another opportunity to study the symptoms of the E-deficiency and to confirm the beneficial value of the tocopherol therapy.

Several lambs in the Michigan State College Flock also developed stiffness. Most of them were rapidly cured by the administration of dl-alpha tocopherol, but three of them died before treatment was established. They were autopsied and submitted to the same pathological and chemical studies performed on the experimental lambs, in order to check the results of the experiment and, also, to find the similarities or dissimilarities of vitamin E deficient lambs fed two different rations.

RESULTS

Growth and Health

The growth criterion in the present experiment was regarded as secondary, since many workers (Olcott and Matill 1937; Emerson and Evans 1937; Vogt-Møller 1948; Willman et al. 1934) had demonstrated in several animal species that vitamin E is not required for growth of young animals. Even though vitamin E is recognized as a growth factor in some adult animals (Nelson et al. 1940; Vogt-Møller 1942) however, only a few have found that vitamin E is necessary for adequate growth of young animals (Kaunitz and Johnson 1946).

For this reason it was considered opportune to make an attempt to simplify the laborious job of rearing the lambs, by reducing the number of feeding times from five times a day (every four hours), to four times a day (every five hours). This, on the other hand, was also justifiable since it was a well-known fact that calves can grow at a normal rate by feeding them milk only twice a day (Warner and Sutton 1948).

However, the results of the growth data suggested that for maximum growth of the lambs on a milk diet, a five times a day regimen of feeding was a necessity. It was found that the rate of growth of animals fed diet A four times a day was low, 0.16 pounds per day, as compared to 0.36 pounds per day made by

other lambs fed an identical diet five times a day (Bacigalupo, non-published data). The growth curves are shown in Figure 1.

The rate of growth of lambs on diet B was found to be even lower (0.10 pounds per day). This, perhaps, was due to the scouring effect of diet B, as well as the reduced number of feedings (four times a day).

Pneumonia was again the most malignant disease of the young lambs, especially those of the deficient groups. Control lambs were also observed to have symptoms of pneumonia, but never as severely or as frequently as deficient lambs. The usual symptoms of pneumonia were coughing and labored respiration, which always coincided with an increased number of leukocytes.

Penicillin was successfully used to combat the disease, but it was rarely able to erradicate the pneumonia from the animal. The affected lambs were treated with 100,000 units of procaine penicillin by injection in the neck region. The treatment had to be given every other day until the end of the experiment to keep the animals alive.

In spite of the penicillin therapy, three lambs died of pneumonia. Another eight lambs died during the experiment due to reasons not related to pneumonia or to the vitamin E deficiency. The deaths were diagnosed as overeating (two lambs), intestinal obstruction (two lambs), and coccidiosis (one lamb). The cause of death of the other three lambs was not identified.

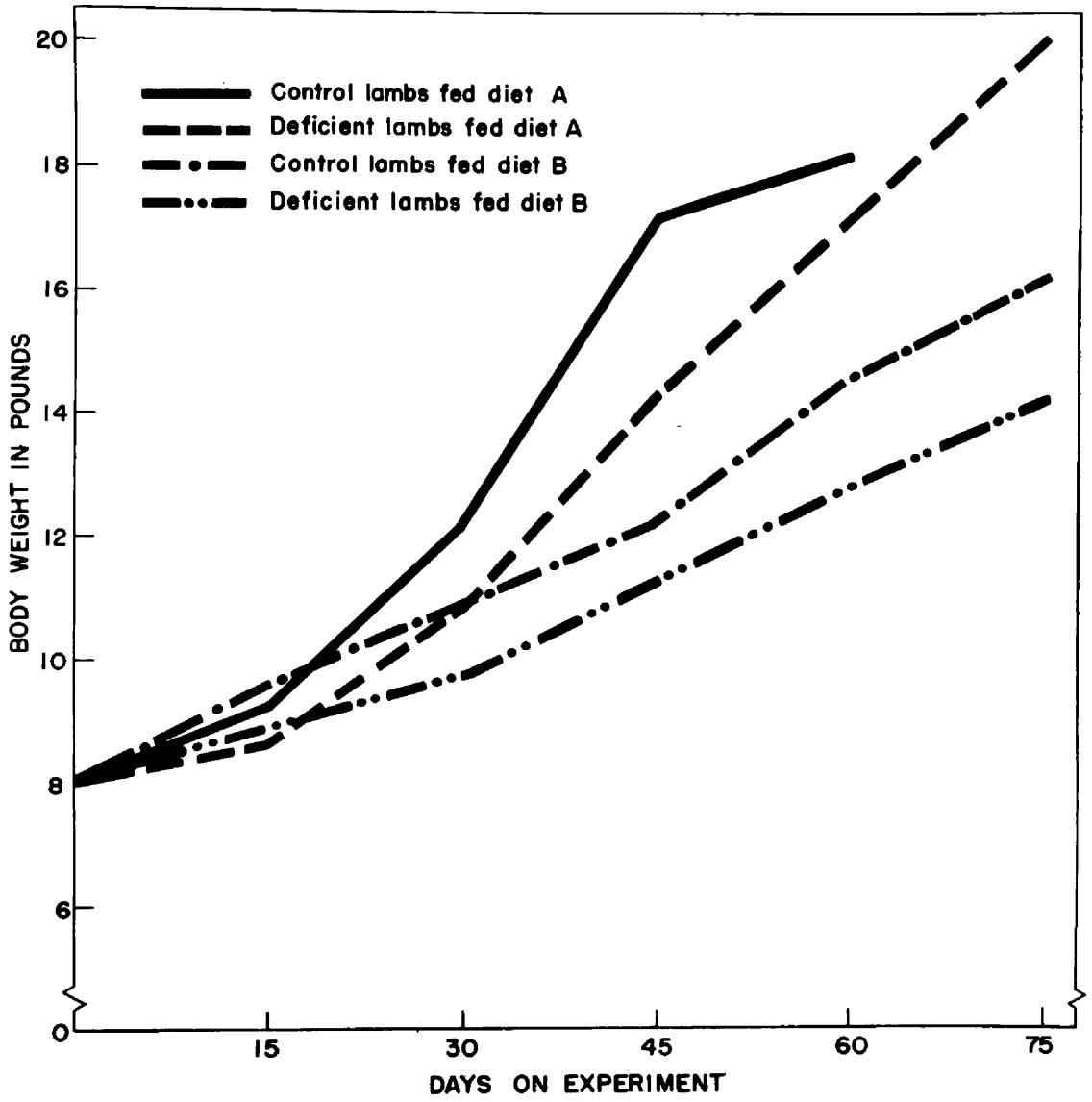


Figure 1. Growth Curves of Lambs Fed Diets Deficient in Vitamin E (Diets A and B) and of Lambs Fed the Same Diets Supplemented with dl-α - tocopherol.

Clinical Observations on the Vitamin E Deficiency

It is impossible to summarize step by step the sequence of symptoms produced by vitamin E deficiency, since the deficiency did not produce the same symptomatologic picture in each one of the animals.

The following description is a condensation of the significant symptoms observed in the lambs that became deficient in vitamin E. Those symptoms have been arranged according to the severity of the disease and, consequently, it can be considered that the description is representative of the vitamin E deficiency syndrome found in the present work.

The first indications that the lambs were being affected by the absence of vitamin E in the diet were: lack of vigor, locomotor incoordination, leg weakness and tendency to lay down most of the time. It was apparent that the animals were abnormal because of their poor appearance, reduced activity, and difficulty in crossing small obstacles. In spite of their poor condition, the lambs did not lose their appetites.

As the disease progressed, it was observed that the lambs had to make repeated efforts before they could get up. Their walk at this time became more incoordinated. It was evident that the muscle of the front legs was affected first because several lambs were unable to straighten their front legs. As a consequence, they adopted the kneeling position for a long time. Also, during this period, three lambs could not open

their mouths, apparently due to paralysis of the masticatory muscles.

When the muscular weakness involved the hind legs too, the lambs were unable to get up or to remain kneeling, in spite of struggling and kicking with their legs. However, after they had been helped to a standing position, they were able to walk.

As the deficiency progressed, the lambs were unable to step more than a few times without falling. Usually they remained standing with their legs spread, their backs arched and their rumps lowered, which gave them an appearance typical of vitamin E deficiency (Fig. 2).

In the final stages of the depletion, the lambs were unable to stand even when held (Fig. 3); they seemed like a flabby mass of muscle and bone, except in one single case of stiffness shown by paralysis of the locomotor muscles.

Lambs reaching this degree of deficiency appeared to be susceptible to pneumonia, perhaps because their bodies were wet all the time, due to urine. Further, at this time, a characteristicly strong odor was noticeable, which could have been due to an altered urinary excretion (probably increased creatine or creatinine) or to the reaction of such products with the constituents of the wool coat.

The diet A, containing a commercial lard as the source of fat, produced in most of the cases the above described symptomatology, which was called mild vitamin E deficiency. On the

Figure 2. Advanced symptoms of vitamin E deficiency in lamb No. 39. Note the poor appearance of the animal, the leg weakness and the dystrophy of thigh muscles.

Figure 3. Terminal symptoms of vitamin E deficiency in lamb No. 39. The animal was unable to stand due to the extreme weakness of the locomotor system.

other hand diet B, containing a molecular distilled lard, produced essentially the same disturbances, but in addition caused several other symptoms. Due to the fact that less time was required to produce vitamin E deficiency with diet B, the symptoms were called acute vitamin E deficiency. Animals on the diet B showed deficiency symptoms in a period between the 20th and 55th day, whereas those on diet A had become deficient between the 30th and 80th day.

Lambs with acute vitamin E deficiency showed, besides the forementioned symptoms, first a reduced volume of the thigh muscles and afterwards dystrophy of the other locomotor muscles as well. While the lambs were still able to walk, there was noticeable stiffness of the legs, wobbling joints, dragging feet and a peculiar marching gait. When these lambs were forced to run, they ran with an excessively balancing movement from the front to the back and made very little progress. In several instances they fell on their knees and were not able to stand again.

Abnormalities were also noticed when the animals tried to turn around. The turning was difficult and like a pivot, since the lambs rotated themselves with their rear legs kept together.

A test was devised to measure the degree of weakness of the legs. It consisted of pushing the lambs down until they fell; first on the rump to measure the degree of resistance of the rear legs, and then on the withers to measure the resistance

of the front legs. These two measurements were compared to the controls and a degree of weakness was established on a scale that ran from 0 (control animals) to four(lambs falling after a very weak push). This criterion was important for the estimation of the state of deficiency of the lambs and the performance of the animals on recovery.

Two of the lambs showed symptoms of goose-stepping and two others repeatedly kicked the floor, but in both cases for only a short period of two to three days. This was rather special since such symptoms had never been reported before in the vitamin E deficiency, and it lasted for such a short time. Locomotor incoordination (goose-stepping) is the characteristic symptom of pantothenic acid deficiency in the pig (Luecke et al. 1949). Therefore, even though pantothenic acid was supplied in the diet, it was important to determine the plasma level of pantothenic acid of these animals and compare them with the plasma level of the deficient and control animals. For that reason, pantothenic acid determinations were run in the blood plasma of all the experimental animals according to the procedure outlined by Skeggs and Wright (1944). The results of this analysis are summarized in Table XI under the heading of Blood Studies.

Alpha tocopherol was found very effective for the cure of two young lambs showing deficiency of vitamin E (lambs nos. 42 and 49). The therapy was more successful when the treatment

was started soon after the appearance of the first symptoms. After a few days of treatment, the lambs showed great improvement in general vigor and strength of the legs. The resistance to fall increased day by day until it was of the same degree as that of the controls. The therapy in a third lamb (lamb no. 50) was started when the animal was in the final stages of deficiency. The animal was unable to stand up and was very emaciated. Even though there was little hope, if any, for the cure of this dying animal, the treatment was started. It was amazing to find, however, that the lamb was able to get up with some help the day after the treatment was started, and two days later was able to get up without assistance and walk, dragging his feet. The test of leg resistance was applied every day and it indicated some improvement in the animal, though it was not steady. Thus, some days the lamb looked very well and appeared to be gaining strength, yet days later seemed weaker and had poor leg resistance. The same thing was true of the tests of running, walking and turning. After 40 days of treatment, it was found that there was some improvement of muscular strength of this animal, and of the ability to walk and run. However, the state of emaciation of the animal was improved very little by the treatment.

The therapeutic use of prostigmine and cortisone in the cure of the symptoms of vitamin E deficiency was not successful. Even though these drugs were used when the deficiency symptoms began

to appear, the lambs only showed a temporary relief which disappeared after five to eight days. Afterwards, the lambs relapsed and showed more marked signs of vitamin E depletion.

The feeding of cod liver oil to E-deficient animals for 25 days did not accelerate or accentuate the symptoms of the vitamin E deficiency.

Control animals fed diet A plus alpha tocopherol, diet B plus alpha tocopherol, or diet A plus alpha tocopherol plus cod liver oil did not show any of the signs described above, caused by the vitamin E deficiency.

Gross Pathological Results

It was very surprising that none of the diets or treatments produced either consistently or clearly the whitening of the lambs' muscles, a symptom considered by many to be typical of the vitamin E deficiency. The fact that the muscles had just a pale color gave the impression that the vitamin E deficiency was not being produced. This was complicated even more by the natural difference in color shades of skeletal muscles.

However, a closer observation of the musculature and the histological studies distinctly showed that most of the skeletal muscles were affected by the lack of vitamin E. The damaged muscles of a region were recognized by the pale yellow-grayish color, and the presence of a sticky exudate which sometimes was gelatinous.

Both sides of the body were affected, but it was very difficult in all cases to establish the bilaterallity of the disease.

The muscles most severely affected were those of the locomotor system. In advanced cases of deficiency muscles of the rump, the neck and loin were likewise found degenerated. When the deficiency of vitamin E had produced the greatest damage to the animal, even the diaphragm, intercostal, masseter and tongue muscles were also injured. Two lambs in this stage were observed to have very edematous muscles, which were found to be almost completely soluble in ethyl ether and water. It was found in the same animals and another showing goose-stepping, that the sciatic nerve was surrounded by a thick fatty coat, yellow in color.

Heart lesions were more common in animals fed the diet B than in the diet A. When diet A was fed, lesions of the heart were observed only after a rather prolonged time.

The heart lesions produced by diet B were outstanding in many respects. First, they were easy to observe due to the white or grayish color of the lesions and their peculiar shape, which varied from pin points to rods and rounded areas, and differed in size from one to four mm. These rods and discs in some instances fused to make big patchy spots of about 12 mm. in diameter. The second and not less outstanding feature was the location of these injured areas. Earlier observations indicated that the right ventricle was the only place where these damaged

areas were present. However, later on, it was also found that the left ventricle was affected and, in very few instances (two lambs), the right ventricle was found without injury.

Less frequent signs of heart alteration caused by the E deficiency were the presence of hemorrhages and an excess of pericardial fluid.

A very unusual observation was made at autopsy of lamb 47, which was fed the vitamin E-deficient diet B. It was a strong ammoniacal odor noticeable at the time of opening the carcass which vanished in a very short time (less than one minute).

There were no typical gross changes in the liver, kidney or adrenals, produced by vitamin E deficiency in the diet. Occasionally, several degrees of fat infiltration and necrosis were found in these organs.

Pneumonia was a complicating and, at times, a masking factor to the vitamin E deficiency. This happened in the case of lamb 14, which had pneumonia and was being treated with penicillin. Before death, it was observed that the lamb had very laborious respiration, presumably due to the pneumonia, which was assumed to be the cause of death. Nevertheless, the electrocardiogram showed that actually the lamb was suffering from heart failure, which eventually produced the death of the animal. This was corroborated by the post-mortem lung examination which did not reveal severe enough damage to justify the death of the animal. Instead the heart showed extensive damage by the macro and histopathological examination.

There was nothing characteristic about the pneumonia that could be correlated to the vitamin E deficiency. Both the unilateral and bilateral type of broncho-pneumonia were present in varying degrees of severity, which were found in deficient and control lambs.

The macroscopic observations about the relation of the lesions to the circulatory system showed that areas of degeneration were usually located in the vicinity of important heart vessels (Fig. 4). The histological study of heart tissues perfused with gelatin carmine is being carried out at the present time and the results will be reported in the near future.

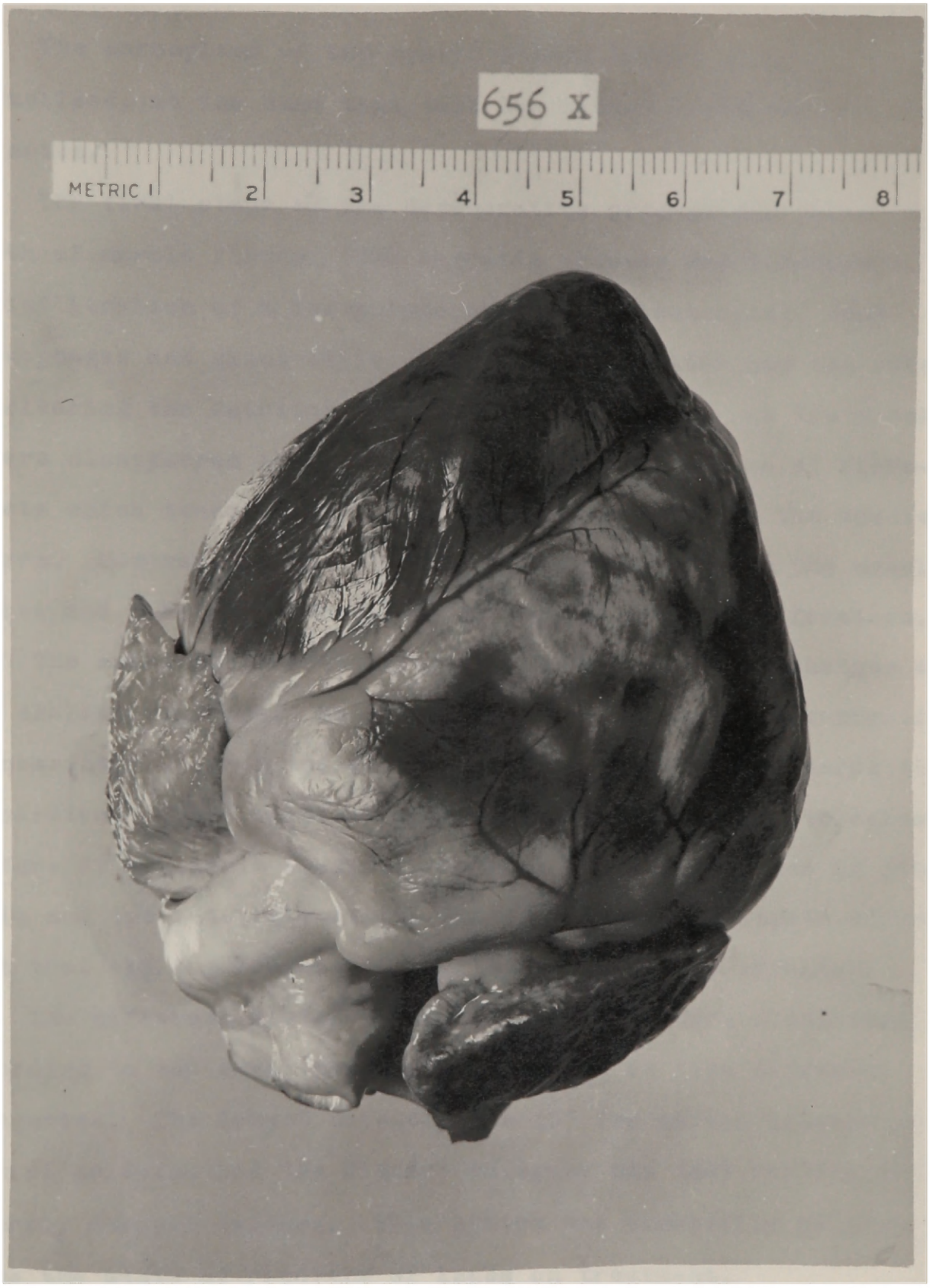
Results of the Histological Studies

The changes produced by the vitamin E deficiency in skeletal muscles of the lamb can be summarized as a process involving hyaline degeneration of the sarcoplasm and a coagulation necrosis in the presence of cellular infiltration.

The muscle fibers first became swollen, shrunk and fragmented while the cross and longitudinal striations disappeared. These fibers stained deeper with the hematoxylin-eosin.

At the same time that the degenerative process was taking place the reverse process of regeneration was also noticed. This was characterized by an increase proliferation of muscular fiber nuclei, which induced formation of new fibers. The new fibers stained fainter with the hematoxylin-eosin and showed the multiple nuclei arranged in rows at the center of the fiber.

Figure 4. Heart of vitamin E-deficient lamb No. 12, showing degeneration and calcification of the right ventricle. Note the apparent relation of the whitish patchy areas to the coronary vessels.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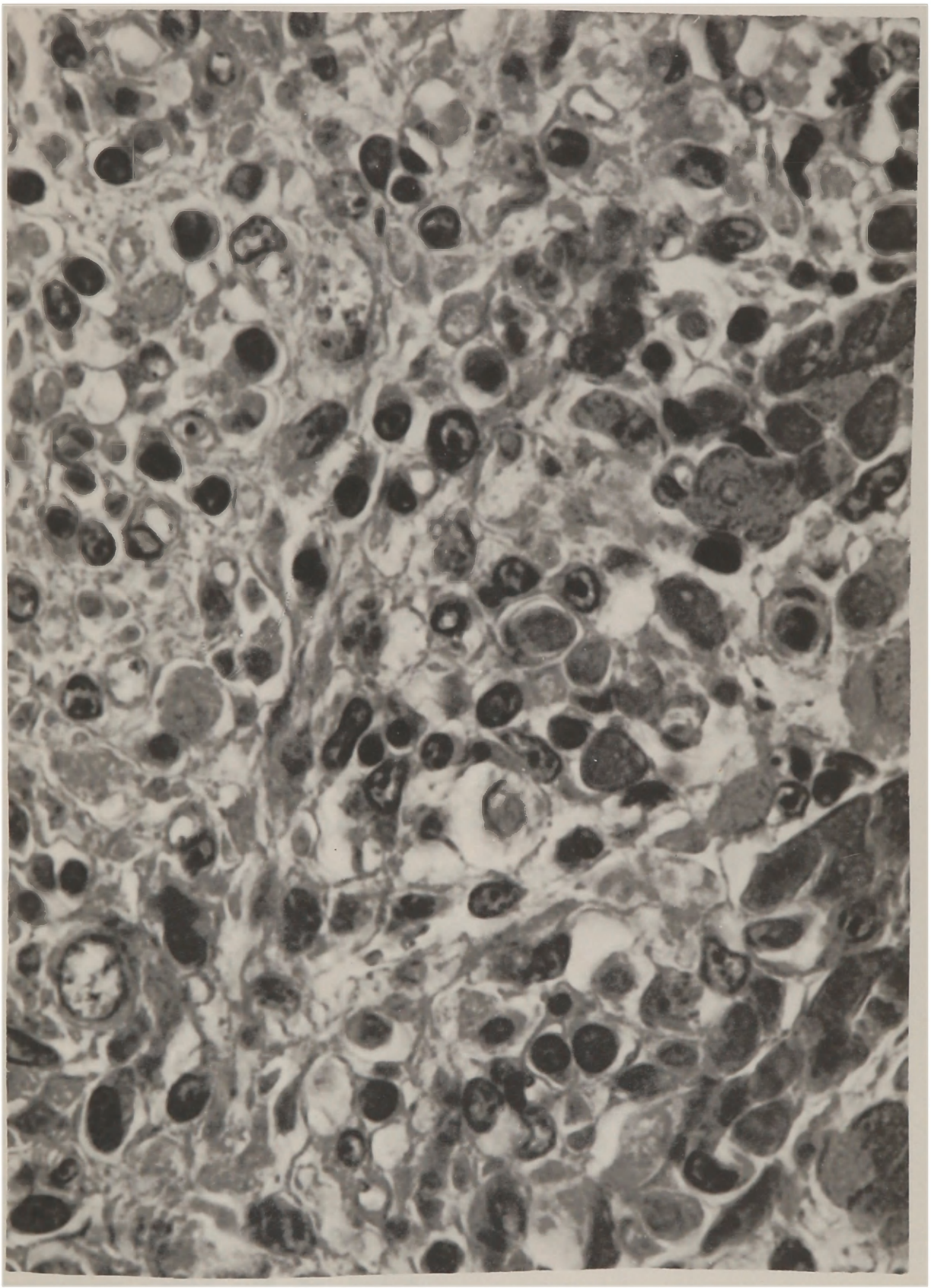
The sarcoplasm of the muscle fibers became granular then vacuolized, at the same time that the nuclei ordinarily became pyknotic.

The final stage of the degenerative process was marked by death of muscle fibers. The necrotic process was accompanied by infiltration of a few polymorphonuclear leukocytes, many marcophages and giant cells (Fig. 5). The latter had the role of clearing the detritus from the necrotic area. As the muscle fibers disappeared there was an active proliferation of fibroblasts which tended to fill the empty space left by the muscle fibers. However, at the time of muscle reganeration the muscle nuclei and fibers overshadowed the fibroflastic proliferation.

The heart muscle essentially underwent the same changes as the skeletal muscles. But it can be added that the degenerative process started from the endocardium and progressed towards the epicardium. Other relevant characteristics of the histological picture of a vitamin E-deficient heart were the absence of giant cells and the integrity of the Purkinje fibers, in spite of the fact that all the surrounding cells were severely affected.

The different histopathological symptoms were classified according to the severeness of the disease in five different categories. The lowest category was (0) for normal tissues of control animals, and the highest category was (4+) for the most severely damaged tissues. This system was especially helpful to judge the state of recovery of lambs on treatment.

Figure 5. A microphotograph of a cross-section of skeletal muscle from vitamin E-deficient lamb No. 14, which shows necrosis and histiocytic infiltration. Hematoxylin and Eosin stain x750



The recovery of deficient animals treated with alpha tocopherol was apparent through the increased muscular fiber nuclei proliferation, as well as the number of macrophages and giant cells. The origin of the regenerating fibers was thought to be in the sarcolemma or in the actively proliferating nuclei. It seemed that the most active part of the regenerating fibers was the rounded end of the new fibers. It was observed that when the time of therapy was longer there were lesser histological abnormalities which were classified as (1+). The therapy aforementioned, therefore, seemed to have been satisfactory. Nevertheless, it cannot be said that the tissues of lambs treated with vitamin E for 40 days were perfectly normal, since there was an increased number of cell nuclei. Probably a more prolonged treatment would have lowered the number of cell nuclei to a normal value.

The success of the cortisone and prostigmine treatments was very difficult to establish because previous tissue samples were not taken before the beginning of the therapy, to indicate the initial state of the animal.

Despite this limiting factor, it could be said that the lesions found in prostigmine and cortisone treated lambs were mild (2+) as compared to (3+) and (4+) of deficient lambs. These findings indicated the possibility of beneficial effects derived from cortisone and prostigmine treatment, though a definite conclusion could not be drawn.

Field cases showed similar histological signs of deficiency to those found in the present experiment. The only noticeable difference between them was a more pronounced damage in the case of field animals; the lesions were always graded as (44).

Results of Blood Studies

a) Tocopherol content:

The tocopherol levels of the lambs fed the diet A in the first experimental trial are summarized in Table VI, and those fed diet B (II and III trials) in Table VII.

From the study of these tables a wide variation can be observed in the initial quantity of total tocopherol of the blood plasma. The values ranged from 0.059 to 0.372 mg./100 ml. of blood plasma.

The tocopherol content of control lambs in the three trials shows a gradual increase of levels from the beginning to the end of the experiment. In the first trial, the total tocopherol content increased from 0.059 to an average of 0.246 mg./100 ml., and in the II and III trials from 0.105 to 0.261 mg./100 ml. of blood plasma.

The reverse process of decrease of the levels of vitamin E in blood plasma values was apparent in deficient lambs fed diets A and B.

Animals of the deficient group in trial I, fed diet A, had an average initial value of 0.226 which diminished to 0.094

TABLE VI.

TOCOPHEROL CONTENT OF BLOOD PLASMA OF LAMBS FED A COMMERCIAL LARD DIET (DIET A) AND THE EFFECT OF COD LIVER OIL TREATMENT ON THE LEVEL OF PLASMA TOCOPHEROLS

(All values expressed in mg. per 100 ml. of blood plasma)

Diet	Lamb No.	Days on experiment 0-10	20-30	30-40	40-50	50-60	60-70
A	6	0.244	-----	0.075			
	8	0.216	0.085	0.097	0.109	0.112*	0.069
	11	0.372	0.275	0.147	0.097	0.399*	0.119
	12	-----	0.165	0.141			
	14	-----	0.147	-----	cod liver oil		
	17	0.072	0.041	-----	cod liver oil		
	18	-----	0.056	0.106			
	19			0.141	cod liver oil		
	Av.	0.226	0.128	0.118	0.103	-----	0.094
A plus α-Tocopherol	7		0.485		0.281		
	10	0.059	0.162		0.212		
	13		0.428		cod liver oil		
	15		0.200		cod liver oil		
	Av.	0.059	0.319		0.246		
A plus cod liver oil	14				0.063	0.147*	0.072
	17				0.088	0.141*	0.047
	19				0.081	0.563*	0.072
	Av.				0.077	-----	0.063
A plus α-Tocopherol and cod liver	13			0.409	0.266	0.109	
	15			0.175	0.266	0.122	0.072
	Av.			0.292	0.266	0.115	0.072

*Unexpected values. They were not considered for the calculation of the averages value.

TABLE VII.

TOCOPHEROL CONTENT OF BLOOD PLASMA IN LAMBS FED A MOLECULAR DISTILLED LARD DIET (DIET B) AND THE EFFECT OF α-TOCOPHEROL THERAPY ON THE LEVEL OF PLASMA TOCOPHEROL

(All values expressed in mg. per 100 ml. of plasma)

Diet	Lamb No.	Days on experiment 0-10	20-30	30-40	40-50	50-60	60-70
B	22	0.144	0.178				
	23	0.115	0.266*				
	24	0.187	0.350*	0.097	0.040		
	27	0.131	0.054	0.134*			
	29	0.159	0.109				
	34		0.228	0.050	0.187*	0.116	
	38	0.162	0.084	0.103	0.069	0.131*	0.069
	39	0.040	0.097	0.091	0.097		
	42	0.127	0.156	0.078	E-therapy		
	43	0.116	0.097	0.091	0.069	0.200*	0.153
	44	0.097	0.134	0.128	0.250*	0.069	0.063
	45		0.050	0.069	0.200*		0.081
	46	0.125	0.250*	0.097	0.119	0.069	0.050
	47	0.097	0.103	0.097	0.806*	0.100	0*E-therapy
	48	0.084	0.050	0.069			
	49	0.103	0.150	0.131	0.084	E-therapy	
	50	0.147	0.069	E-therapy			
	Av	0.122	0.111	0.092	0.080	0.088	0.083
B plus α-Tocopherol (controls)	20	0.159	0.153	0.150			
	21	0.070	0.081	0.309	0.072*	0.131	0.148
	37	0.159	0.228	0.162	0.299	0.406	0.428
	40	0.059	0.112	0.091	0.187	0.234	0.175
	41	0.078	0.172	0.332	0.159	0.366	0.294
	Av	0.105	0.149	0.209	0.215	0.284	0.261
B plus α-Tocopherol (recovery)	42				0.084	0.384	
	49					0.313	
	50			0.128	0.069	0.175	
	Av			0.128	0.076	0.290	

*Unexpected values.
They were not considered for the calculation of the averages values.

mg./100 ml. after 60 days of feeding. Deficient lambs fed diet B in trials II and III only required 30-40 days to diminish their blood plasma tocopherol value from 0.122 to 0.092 mg./100 ml. This value however, changed very little until the end of the experiment when it was 0.083 mg./100 ml.

The cod liver oil supplementation lowered the tocopherol level of the blood plasma not only of the deficient but also of the control lambs. In fact, even the control lambs that were given 100 mg. of alpha tocopherol every other day experienced in 30 days a drop of total tocopherol in the blood plasma from 0.292 to 0.072 mg/100 ml. The depleted animals did not suffer such a drastic change, the level dropped only 0.014 mg./100 ml. from 0.077 to 0.063 mg./100 ml.

The effect of the alpha tocopherol therapy on the level of total tocopherols in the blood was noticeable after 10-20 days of treatment. At this time their tocopherol content was equal or larger than the values found in control animals.

It should be pointed out that the tocopherol picture of the blood of deficient lambs was not always one of a progressive decrease. In many instances, lambs having low values for 20-30 days showed a sudden and extraordinary increase of total tocopherols in the blood plasma. This phenomenum did not last more than 10 days, after which the tocopherol content fell to the original low levels.

b) Blood Hemoglobin:

The results of the hemoglobin analysis are condensed in Tables VIII and IX. As it can be seen, the tocopherol supplementation of diets A and B did not affect in any way the hemoglobin values of the blood, since the control animals showed a similar hemoglobin picture to that of the control lambs.

Likewise, the supplementation of cod liver oil did not modify the hemoglobin content of either control or deficient lambs.

However, in five lambs (lamb Nos. 12, 34, 44 39 and 50) fed vitamin E-deficient diets, the content of hemoglobin dropped to very low values, ranging from 6.5 to 8.4 grams per 100 ml. of blood. These low levels were observed only after the lambs were on the diet for 50-60 days, and they could not be modified by the tocopherol therapy (Table IX, lamb No. 50).

c) Differential White Count:

The changes of white count only occurred along with the appearance of pneumonia. No change of white blood cells could be associated with the deficiency of vitamin E.

d) Blood Pressure:

A total of 24 blood pressure readings were taken from control and deficient lambs on diet B (diet containing the molecularly distilled lard). The results are shown in Table X. Since a certain correlation was found, in deficient lambs, between the

TABLE VIII

HEMOGLOBIN CONTENT OF THE BLOOD OF LAMBS FED A COMMERCIAL LARD DIET (DIET A) AND THE EFFECT OF α-TOCOPHEROL THERAPY ON THE LEVEL OF HEMOGLOBIN IN THE BLOOD

(All values expressed in mg. per 100 ml. of blood)

Diet	Lamb No.	Days on experiment 0-10	20-30	30-40	40-50	50-60	60-70
A	6	11.4	10.1	10.1			
	8	13.3	13.3	11.1	10.8	11.6	12.5
	11	13.3	11.8	10.8	10.4	12.1	10.4
	12	12.9	13.7	11.4	----	----	6.5
	14	12.5	11.6	11.6	cod liver oil		
	17	11.8	12.7	11.4	cod liver oil		
	18	12.9	11.6	10.8			
	19	11.1	12.5	12.1	cod liver oil		
	Av	12.4	12.1	11.1	10.6	11.8	9.8
A plus α-tocopherol	7	12.7	10.8	11.1	12.0		
	10	12.5	10.8	10.8	12.2		
	13	12.7	11.4	12.5	cod liver oil		
	15	11.8	12.3	12.3	cod liver oil		
	Av	12.4	11.3	11.6	12.1		
A plus cod liver oil	14				12.1	11.6	11.6
	17				11.4	11.6	12.5
	19				11.1	10.2	11.1
	Av				11.5	11.1	11.7
A plus α-tocopherol and cod liver oil	13				12.3	12.1	12.9
	15				10.9	11.8	12.7
	Av				11.6	11.9	12.8

TABLE IX

HEMOGLOBIN CONTENT OF THE BLOOD OF LAMBS FED THE DIET CONTAINING MOLECULAR DISTILLED LARD (DIET B) AND THE EFFECT OF α-TOCOPHEROL THERAPY ON THE LEVEL OF HEMOGLOBIN IN THE BLOOD

(All values expressed in mg. per 100 ml. of blood)

Diet	Lamb No.	Days on experiment							
		0-10	20-30	30-40	40-50	50-60	60-70	70-80	80-90
B	22	11.1	10.4						
	23	13.3	11.1	10.6					
	24	11.1	12.1	12.3	12.5				
	27	15.0	12.0	11.4					
	29	14.3	13.2	11.4					
	34	14.8	13.7	12.7	12.3	9.8	10.4	10.4	6.5
	38	13.7	12.5	12.5	11.8	10.8	10.4	11.8	11.1
	39	13.5	10.3	8.1	5.2	8.3			
	42	13.1	13.7	14.1	E-therapy				
	43	10.8	11.8	11.4	12.3	11.4	11.1		
	44	12.5	11.1	10.8	11.4	10.4	11.4	8.1	
	45	11.6	11.8	12.5	10.8	12.1	9.8	11.4	
	46	12.0	11.6	11.8	12.5	10.4	9.5		
	47	12.0	11.1	11.6	12.9	12.1	10.1	E-therapy	
	48	10.4	9.8	10.4					
	49	11.8	11.4	11.8	9.5				
	50	13.3	12.9	E-therapy					
	Av	12.6	11.8	11.6	11.1	10.7	10.4	10.4	8.8

TABLE IX (continued)

Diet	Lamb No.	Days on experiment 0-10	20-30	30-40	40-50	50-60	60-70	70-80	80-90
B plus	20	12.5	10.8	10.8	12.2				
α-Toco-	21	13.7	13.7	12.0					
pherol	37	13.1	11.4	12.5	11.1	11.4	10.1	10.4	
(controls)	40	11.8	12.3	10.4	11.1	10.1	10.8	11.1	
	41	13.3	11.6	10.8	11.1	11.4	9.5	10.1	
	Av	12.8	11.9	11.3	11.3	10.9	10.1	10.5	
B plus	42				11.8	10.4			
	49					11.8	11.8	11.8	
	50			12.6	12.3	9.8	8.1	8.4	
	Av			12.6	12.1	10.6	9.9	10.1	

TABLE X

THE INFLUENCE OF VITAMIN E DEFICIENCY ON THE ARTERIAL BLOOD PRESSURE OF THE LAMBS

A. Lambs Fed Diet B plus α-Tocopherol

Lamb No.	Age (days)	Heart Beats per minute	Blood pressure (mm of Hg) Diastolic	Systolic	Average
			Controls		
37	80	240	112	137	125
40	75	202	75	94	85
41	55	199	90	110	100
	60	205	105	122	113
	75	174	94	119	107
Av.	69	204	95	116	106
			Recovered Animals		
49	50	112	85	118	101
	65	122	91	122	107
	80	102	88	119	103
Av.	65	112	88	120	104

TABLE X (continued)

B. Lambs Fed Diet B

Lamb No.	Age (days)	Heart Beats per minute	Blood pressure (mm of Hg) Diastolic	Systolic	Average
		Not showing symptoms of deficiency			
44	65	202	130	180	155
47	50	146	75	113	94
	55	149	95	132	113
	60	230	130	175	152
Av.	57	182	108	150	128
		Showing symptoms of deficiency			
34	75	242	115	125	120
	85	224	87	122	106
38	60	180	75	90	82
	75	219	84	100	92
	90	153	78	100	89
43	50	132	72	100	86
44	70	137	75	116	95
45	55	140	65	90	77
	70	162	69	94	81
46	55	127	87	120	103
	70	160	31	63	47
47	70	171	77	112	94
Av.	69	171	76	103	89

blood pressures and the symptomatology of the disease, the group was subdivided in two. One, for those not showing the symptoms and another, for those showing the symptoms of the vitamin E deficiency.

Due to the small number of recordings and the complex variations of blood pressures in individual animals, the statistical analysis was not carried out.

Taking the blood pressures of the control and recovered lambs as a level of reference, it can be said that lambs on a diet deficient of vitamin E tended to have higher pressures when they did not show any symptom of vitamin E deficiency, and that lambs showing symptoms of the deficiency of vitamin E generally had lower blood pressures than control animals. Good illustrations of this phenomenum are given by the blood pressures of the deficient lambs Nos. 44 and 47, taken first when they did not show signs of deficiency, and after, when the symptoms of the disease were evident.

e) The Level of Pantothenic Acid in the Blood:

The results of the pantothenic acid analysis of blood plasma of the experimental lambs are summarized in Table XI.

TABLE XI

THE EFFECT OF VITAMIN E DEFICIENCY ON THE LEVEL OF PANTOTHENIC ACID IN THE BLOOD

Lamb No.	Diet	Symptoms	P.A. gamma/ml.
37	Complete	none	0.44
40	"	"	0.42
41	"	"	0.34
44	E-deficient	Goose-stepping	0.38
45	" "	" "	0.56
34	" "	Vitamin E deficiency	0.45
38	" "	" " "	0.46
46	" "	" " "	0.37
47	" "	" " "	0.46
49	" "	" " "	0.43
50	" "	" " "	0.36

There was not a consistent difference in the blood content of pantothenic acid between lambs showing goose-stepping and any of the other deficient or control lambs.

Results of the Urine Studies

a) Effect of Vitamin E on the Urinary Excretion of Creatine and Creatinine:

The results of these studies are tabulated in Table XII.

Creatine: In general it can be stated that the level of creatine

TABLE XII

THE INFLUENCE OF VITAMIN E ON THE EXCRETION OF CREATINE AND CREATININE IN THE URINE, AND THE RATIO OF TOTAL CREATININE (CREATINE PLUS PREFORMED CREATININE) TO PREFORMED CREATININE

Diet	Lamb No.	Days on experiment											
		0-10			20-30			40-50			60-70		
		C[1]	Cn[2]	C.R.[3]	C	Cn	C.R.	C	Cn	C.R.	C	Cn	C.R.
A	12										188	93	2.75
A plus α-tocopherol and cod liver oil	13							68	163	1.36	35	140	1.22
	15							74	377	1.17	0	310	1.00
A plus cod liver oil	14							399	227	2.50			
	17							134	51	3.24			
	19							18	117	1.14	126	151	1.73

[1] C, creatine in mg. per day.

[2] Cn, creatinine in mg. per day.

[3] C.R., creatinuria ratio - the ratio of total creatinine (creatine plus creatinine) to preformed creatinine.

TABLE XII (continued)

Diet	Lamb No.	Days on experiment 0-10 C	0-10 Cn	0-10 C.R.	20-30 C	20-30 Cn	20-30 C.R.	40-50 C	40-50 Cn	40-50 C.R.	60-70 C	60-70 Cn	60-70 C.R.
B	24				103	89	2.00						
	27				243	91	3.31						
	29	97	38	3.20									
	32	104	90	2.00	237	54	4.77						
	39	43	82	1.46	111	107	1.89	144	91	2.06			
	42	58	144	1.34	177	170	1.90	O(-tocopherol					
	43	16	122	1.11	216	168	2.11	89	183	1.42			
	44	60	100	1.52	48	72	1.57	132	119	1.96	66	66	2.92
	45	5	89	1.04	54	115	1.40	31	94	1.29	50	157	1.27
	46	74	152	1.42	49	185	1.23	157	174	1.78	76	88	1.75
	47	121	116	1.90	6	136	1.04				412	135	3.63
	48	122	102	2.03	29	147	1.17						
	49				92	138	1.57	444	170	3.25	O(-tocopherol		
	50	122	127	1.83	O(-tocopherol								
	Controls												
B plus O(- Toco-	20				56	100	1.48	6	97	1.05			
pherol	21				30	93	1.27	17	119	1.12			
	37	86	171	1.43	14	110	1.11	83	170	1.42			
	40	18	86	1.18	84	126	1.57	51	147	1.30	185	214	1.75
	41	56	84	1.57	45	136	1.29	52	215	1.21	29	207	1.12
	Recovered												
	42							17	163	1.09			
	49										44	108	1.35
	50				306	167	2.58	42	96	1.38	182	83	2.89

of E-deficient animals increased above normal as the E deficiency progressed. Some of the lambs did not show gradual increase of creatine excretion in the urine and did not maintain high levels of creatine for a prolonged time.

Common values of creatine excretion in control animals ranged from 18 to 89 mg. per day; only in one instance, lamb No. 40, the creatine excretion was 185 mg. per day. In deficient animals starting to show the symptoms of E deficiency, the creatine values ranged from 177 to 412 mg. per day.

Alpha tocopherol therapy decreased the creatinuria of vitamin E-deficient lambs. After 10 days of treatment the creatine urinary excretion of recovered lambs was equal to that of controls. Lamb No. 50 was an exception. This animal remained in poor condition after treatment, even though it did not show any symptom of vitamin E deficiency. The creatine level, instead of decreasing with the alpha tocopherol treatment, increased to a new height of 306 mg./day, then during the following 10 days decreased to 42 and, 10 days later, increased again to 182 mg. per day.

Cod liver oil proved to be effective in increasing the creatinuria of deficient lambs; however, its action was neutralized by the feeding of alpha tocopherol as can be noticed in Table XII.

Creatinine: The analysis of creatinine indicated that the vitamin E does not affect the urinary excretion of this compound.

The amount of creatinine excreted in the urine varied from animal to animal, but it remained about the same during all the experimental period.

The creatinuria ratio: The creatinuria ratio has been calculated in order to condense in one expression the relative changes of creatine and creatinine produced by the absence of vitamin E in the diet. It is calculated by dividing the total creatinine (creatine plus preformed creatinine) by preformed creatinine. Absence of vitamin E in the diet influenced the creatinuria ratio and the creatine level in a similar manner. They increased as the E deficiency became more acute.

In control animals the creatinuria ratio ranged from 1.05 to 1.75 with an average of 1.32. In deficient animals the creatinuria ratio varied from 1.00 to 4.77 with an average of 1.96. In each deficient lamb the ratio was maximum or close to maximum at the time the lambs showed signs of vitamin E deficiency.

b) Effect of Vitamin E on the Urinary Excretion of Sodium and Potassium.

The analytical results are presented in Table XIII. The daily excretion of sodium and potassium did not reflect the increasing severity of the vitamin E deficiency in lambs fed either diet A or B, or even diet A supplemented with cod liver oil. Therefore, in spite of the fact that the vitamin E affected the content of sodium and potassium in the skeletal muscles and the

TABLE XIII

THE INFLUENCE OF VITAMIN E ON THE URINARY EXCRETION OF SODIUM AND POTASSIUM

Diet	Lamb No.	Days on experiment 0-10 Na mg/day	0-10 K mg/day	20-30 Na mg/day	20-30 K mg/day	40-50 Na mg/day	40-50 K mg/day	60-70 Na mg/day	60-70 K mg/day
A	12							55	992
A plus α-tocopherol and cod liver oil	13					296	467	955	259
	15					506	655	226	310
A plus cod liver oil	14					767	462		
	17					57	117		
	19					703	529	68	512
B	24			701	480				
	27			360	572				
	29	157	380						
	32	277	395	330	524				
	39	133	316	170	340	652	520		
	42	558	405	187	476	α-tocopherol			
	43	459	328	405	510	553			
	44	137	367	91	292	644	404	407	242
	45	362	401	325	288	284	328	320	630
	46	276	319	286	603	364	434	723	365
	47	68	145	389	446			451	231
	48	219	286	257	419				
	49			339	322	247	270	α-tocopherol	
	50	281	443	α-tocopherol					

TABLE XIII (continued)

Diet	Lamb No.	Days on experiment 0-10 Na mg/day	0-10 K mg/day	20-30 Na mg/day	20-30 K mg/day	40-50 Na mg/day	40-50 K mg/day	60-70 Na mg/day	60-70 K mg/day
	Controls								
B plus	20			179	408	767	298		
α-toco-	21			490	357	445	498		
pherol	37	303	384	86	424	276	426		
	40	396	405	158	375	248	276	283	268
	41	657	410	347	396	660	518	475	516
	Recovered								
	42					753	527		
	49							833	441
	50			141	377	375	368	286	243

heart muscle, as will be reported in the next pages, these changes were not apparent through the determination of sodium and potassium in the urine.

Results of the Electrocardiographic Studies

As will be seen in the following pages, deficiency of vitamin E lambs was manifested, in the great majority of cases, by definite changes in the electrocardiogram.

To facilitate the exposition, the results have been arranged under the following headings:

a) The Electrocardiogram of Lambs Fed Diet B Supplemented with Alpha Tocopherol (controls).

b) The Electrocardiogram of Vitamin E Deficient Lambs.

   1) Lambs Showing Acute Cardiac Involvement.

   2) Lambs Showing Chronic Cardiac Involvement.

   3) Lambs not Showing Any Symptom of Cardiac Involvement.

c) The Electrocardiogram of Lambs Fed Diet A Supplemented with Cod Liver Oil and Alpha Tocopherol.

d) The Electrocardiogram of Lambs Fed Diet B, which Received Injections of Cortisone and Prostigmine.

a) The Electrocardiogram of Lambs Fed Diet B Supplemented with Alpha Tocopherol:

The Heart Rate:

The heart rate in the three control lambs (Nos. 37, 40 and 41) ranged from 107 to 250 beats per minute (Table XIV). However, the average rate of individual animals, taken during the

TABLE XIV

THE AVERAGE HEART RATES, PR AND QT INTERVALS AND THE K CONSTANT (BAZETT'S) OF VITAMIN E DEFICIENT AND CONTROL ALMBS

Types of ECG	Lamb No.	Heart Rate*	Intervals PR*	Intervals QT*	Bazett's Constant K*
Controls	37	166	0.08	0.22	0.37
	40	151	0.08	0.23	0.36
	41	155	0.08	0.24	0.37
	Av.	157	0.08	0.23	0.37
Lambs showing Acute Cardiac Involvement	14	232	0.06	0.18	0.35
	19	150	0.10	0.22	0.35
	24	175	0.10	0.22	0.38
	39	149	0.10	0.22	0.35
	48	200	0.08	0.19	0.34
	Av.	181	0.09	0.21	0.35
Lambs showing Chronic Cardiac Involvement	34	155	0.10	0.24	0.37
	38	176	0.10	0.22	0.36
	42	138	0.12	0.26	0.39
	43	139	0.12	0.25	0.38
	44	137	0.10	0.26	0.39
	45	131	0.12	0.25	0.37
	46	115	0.09	0.27	0.37
	47	132	0.11	0.26	0.38
	49	103	0.10	0.29	0.38
	Av.	136	0.11	0.25	0.38
Lambs not Showing any Symptom of Cardiac Involvement	12	150	0.10	0.24	0.38
	32	158	----	0.20	0.33
	50	140	0.08	0.24	0.36
	Av.	149	0.09	0.23	0.36

* Average values.

course of the entire experiment, was very similar. Lamb No. 37 had an average of 166, lamb No. 40, 151, lamb No. 41, 155 and the average for the group was 157 beats per minute.

In every lamb the heart rate was very regular and did not show any correlation with the increasing age of the young lamb.

The PR Interval:

The duration of the PR interval was 0.08 second in each electrocardiogram of the control lambs (Table XIV). The P wave was not always present in every ECG* of the lambs and in all the leads of the same record. Thus, it was impossible to measure the PR interval in all instances, as can be noticed in Table XXIV (Appendix). The measurements of the PR interval as well as the other intervals were made usually in lead II because it was the most favorable.

The variation of the PR intervals among the three leads of the same record never exceeded 0.01 second. Extremes in heart rates ranging from 107 to 250 beats per minute did not influence the duration of the PR interval.

The QRS Interval:

The duration of the QRS interval in the lambs fed diet B plus alpha tocopherol was invariably 0.06 second in lead II (Table XXIV, Appendix). The difference in the values for the duration of QRS among the three leads was rarely larger than 0.01 second and variations in heart rates did not affect the duration of this complex.

* Electrocardiogram.

The QT Interval:

The duration of the QT interval ranged from 0.22 to 0.24 second in the three control lamb Nos. 37, 40 and 41. The average for the group was 0.23 second (Table XIV). The accuracy of this measurement was smaller (0.02 second) than in the measurement of the two previous intervals because it was difficult to exactly determine the end point of the T wave, since it usually joined the TP segment by means of a smooth curve.

The duration of the QT interval varied inversely with the heart rate. For instance, in lamb No. 41 the QT value was 0.18 second when the heart rate was 250 beats per minute and 0.30 second when the rate was 107 beats per minute (Table XXIV, Appendix).

The Systolic Index:

In 28 out of 32 control calculations, Bazett's K values fell in the range of 0.35 to 0.39, whereas for the 32 records the K values ranged from 0.32 to 0.43 (Table XXIV, Appendix). The average K value for the whole group was 0.37 (Table XIV).

The P Wave:

In general the potential of the P wave was low and in some instances isoelectric. When the P waves were measurable, their potential ranged from 0.05 to 0.15 millivolt.

The shape of the P waves were variable. Sharp, rounded, and peaked waves were found, and in some instances P waves changed shape from beat to beat of the heart. Thus, an ECG of

lamb No. 37 showed extra P waves which appeared at successively increasing intervals after each heart beat.

In the same lamb, No. 37, diphasic and inverted P waves were also noticed.

The QRS Complex:

The voltage of the QRS complex was variable and was not influenced by age of the lambs. In the majority of the cases the QRS complex was monophasic but in a few cases it became diphasic in one of the leads but never in two leads at the same time.

When the QRS complex was monophasic the potential ranged, in lead I, from +0.50 to -0.80 millivolt; in lead II, from -0.30 to -1.20 millivolts; and in lead III, from +0.40 to -1.40 millivolts.

When the QRS complex was diphasic the voltage ranged from 0.35 to 1.20 in lead I, from 0.25 to 0.35 millivolt in lead III, and was 0.50 millivolt in lead II.

The arithmetical total of the QRS potentials had a minimum value of 0.95 millivolt and a maximum of 4.35 millivolts.

Slurring of the QRS complex was noticed quite frequently in lead I of the control animals, but not in any of the other leads.

The T Wave:

The voltage of the T waves was positive most of the time. However in certain cases it became isoelectric and in two ECGs,

taken within five to eight days after the experiment was started, inverted T waves were found in lead II. No diphasic T waves were observed in any of the records.

The potential of the T waves varied from -0.05 to 0.45 millivolt in lead I, from 0 to 0.50 millivolt in lead II and from -0.15 to 0.35 millivolt in lead III.

The shape of the T wave was not the same in all records. Some of the more typical ones are reproduced in Figure 6.

The ST Segment:

In a very few instances it was noticed that the ST segments appeared lower or higher than the base line.

The Electrical Axis:

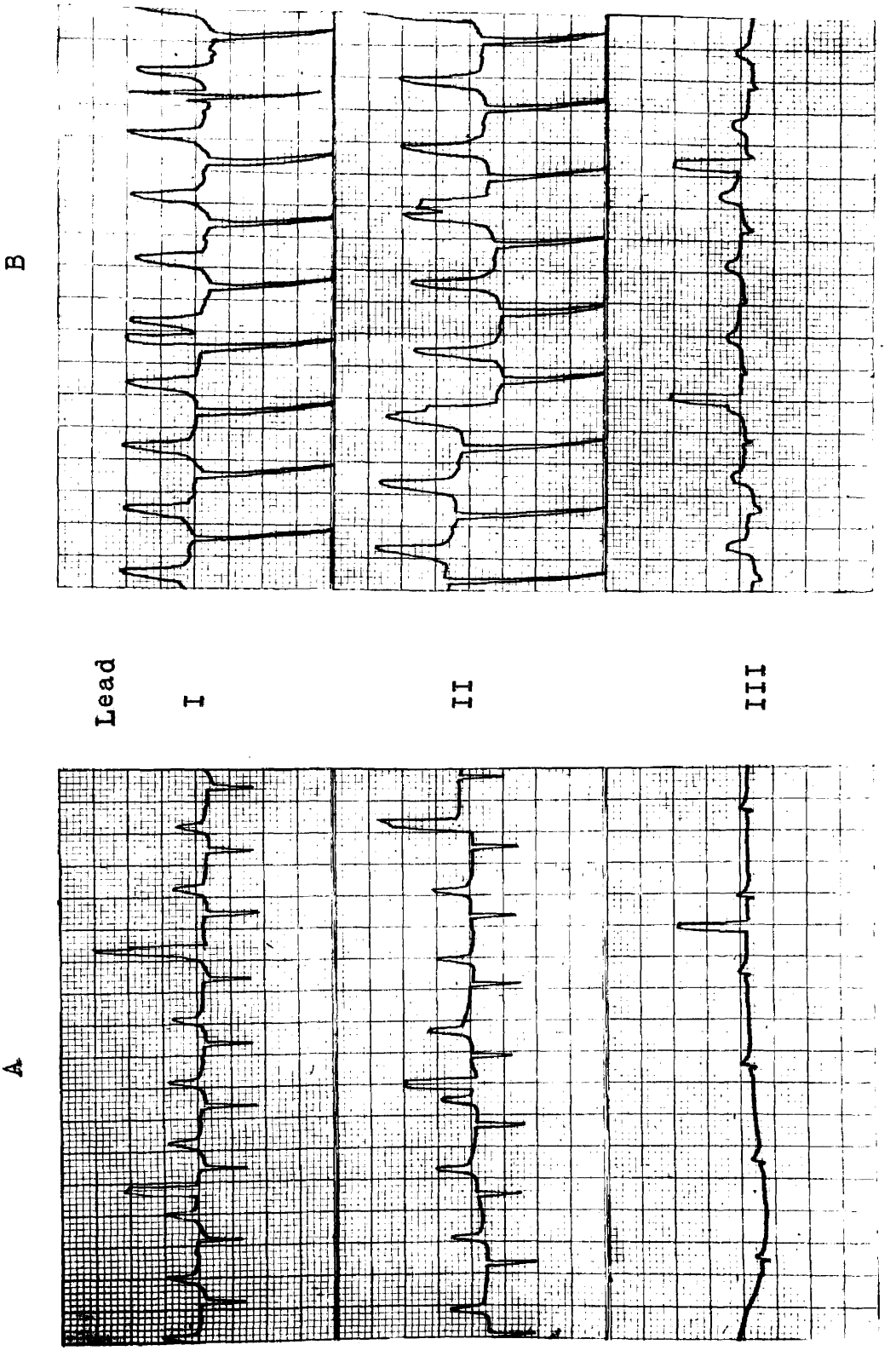
The electrical axes as measured by Wilson's method were quite different from lamb to lamb. Thus, in lamb No. 37 the electrical axis changed from -90° to -60° and then again to -90° during the 81 days of the experiment. In lamb Nos. 40 and 41 the electrical axis fluctuated between -120 to -180° (Table XXIV, Appendix).

b) The Electrocardiogram of Vitamin E Deficient Lambs:

All the lambs considered under this group showed macro and micropathological changes of vitamin E deficiency, high creatinuria ratio, low blood tocopherol content with mineral and amino acid change in the muscles.

The group was subdivided into three categories. The first one was composed of the lambs that died of heart failure, shown

Figure 6. Records from two lambs illustrating the influence of cod liver oil on the electrocardiogram. Record A (lamb No. 40) is a typical electrocardiogram of control animals fed diet B supplemented with dl-α-tocopherol. Record B shown the changes in T waves produced in lamb No. 13 fed diet B supplemented with dl-α- tocopherol and cod liver oil.


B
Lead
I
II
III
A

by the parallelism between the lambs' sudden death and the immediate changes in the electrocardiogram. This type of deficiency was called "acute cardiac involvement" as compared to the "chronic cardiac involvement" described in the next category. In this second category electrocardiographic data of lambs showing progressive alterations of the ECG have been gathered. Finally, in the third category were placed all the lambs which showed pathological and chemical changes characteristic of vitamin E deficiency, although little or no change was observed in the ECG.

1) ECGs of Vitamin E Deficient Lambs Showing Acute Cardiac Involvement:

Before the electrocardiograph was used for the study of the vitamin E deficiency three cases of acute heart failure had already appeared. Thereafter, five more cases of acute heart involvement were observed, which were detected by the changes taking place in the ECGs prior to the lamb's death. Two of the lambs (Nos. 14 and 19) had been fed diet A supplemented with cod liver oil and the other three lambs (Nos. 24, 39 and 48) had been fed diet B.

Up to time of the occurrence of sudden changes in the ECGs, the electrical phenomena accompanying the heart beat did not appear to be very different from animals receiving alpha tocopherol in their diets. The most outstanding ECG change in the lambs of this group was an increase of the heart rate with age (Table XXIV, Appendix). These rates have been summarized in

Table XIV where the average of this group was 181, whereas that of the control was 157 beats per minute.

A lengthening of the average for the PR interval can be noticed in the same Table, besides a slight decrease in the duration of the QT interval and Bazett's constant K.

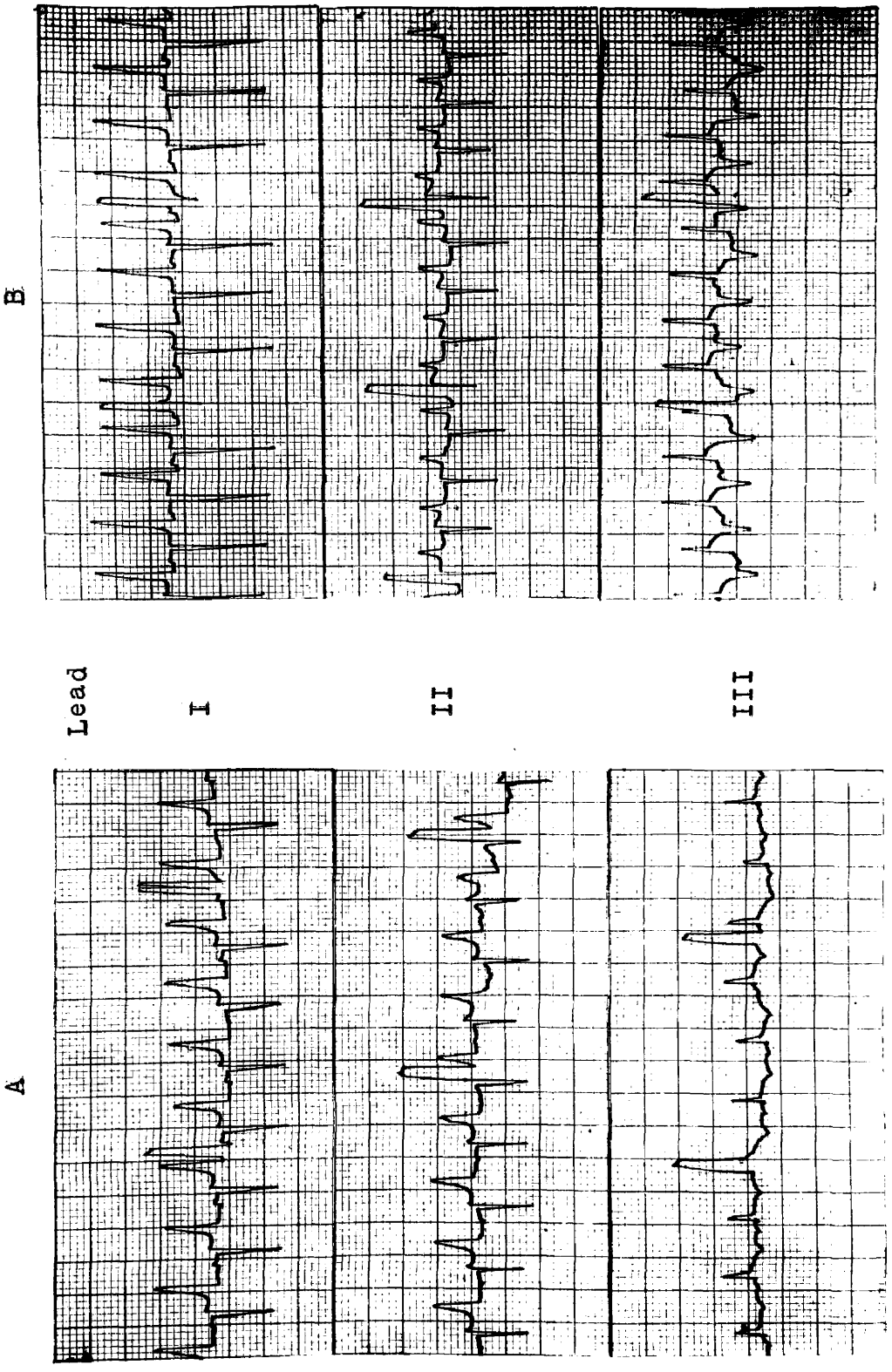
The appearance of abrupt electrocardiographic changes were not identical in the five lambs and did not take place at any fixed interval before death. In lamb Nos. 14, 24 and 48, the sudden electrical changes consisted of very large voltage increases of the T and QRS waves, particularly the former. The T waves consequently assumed a very typical sharp form resembling that of the R waves, as can be observed in Figure 7. The increments of the T wave voltages have been summarized in Table XV which shows that these potentials were much larger than those found in the control lambs.

TABLE XV

THE T WAVE POTENTIALS IN ACUTE CARDIAC INVOLVEMENT PRODUCED BY VITAMIN E DEFICIENCY IN THE LAMB

Lamb No.	Days before death	T potentials (millivolts) L_1	L_2	L_3	Total T potentials (millivolts)
14	0.2	+0.50	+0.60	+0.35	1.45
24	11	0	+0.70	+0.85	1.55
	1	0	+0.80	+0.65	1.45
48	8	+0.85	+0.60	-0.15	1.60
	1	+1.05	+0.40	-0.60	2.05

Figure 7. Electrocardiograms of lamb No. 48 showing the sudden T wave changes produced by vitamin E deficiency. Records A and B were taken eight and one days before death, respectively. Note that during the last seven days there was an increase in the potential of T. Also, the inversion and increase in potential of T in lead III.


B
Lead
I
II
III
A

It is interesting to note that there were inverted T waves in lead III of lamb 48, which did not appear in the records of the control lambs.

Tachycardia, changes in the direction of the various waves and evidence of disorganization of the heart beat, constituted another finding in the cases showing acute heart involvement. This can be seen in Figures 8 and 11 corresponding to lamb Nos. 14 and 39, respectively. The ECG showed extremely large QRS deflections which were followed immediately by large inverted T waves in all three leads. Figure 8B shows another ECG of lamb No. 14 but this time in a prostrate position. This record was taken immediately after that of Figure 8A was recorded, when the lamb was moribund.

In lamb No. 19 electrocardiographic changes were found which differed from any previously seen. These were marked depression of the ST segment (-0.15 millivolt) in leads II and III, variations in the potential and form of the P waves with every heart contration, and diphasic T waves in lead III (Fig. 10).

2) ECGs of Vitamin E Deficient Lambs Showing Chronic Cardiac Involvement:

Another type of ECG was found in nine of the vitamin E-deficient lambs (Nos. 34, 38, 42, 43, 44, 45, 46, 47 and 49). For purposes of differentiation it was named ECG in chronic heart disease since it did not fit into the pattern of acute heart failure.

Figure 8. Electrocardiograms of lamb No. 14 fed vitamin E-deficient diet B, showing tachycardia and changes in direction of the various waves. Record A also shows short episodes of disorganization of the cardiac contraction. These records were taken a few hours before death of the animal. Record A in the standing position and Record B in the prostrate position, when the lamb was moribund.

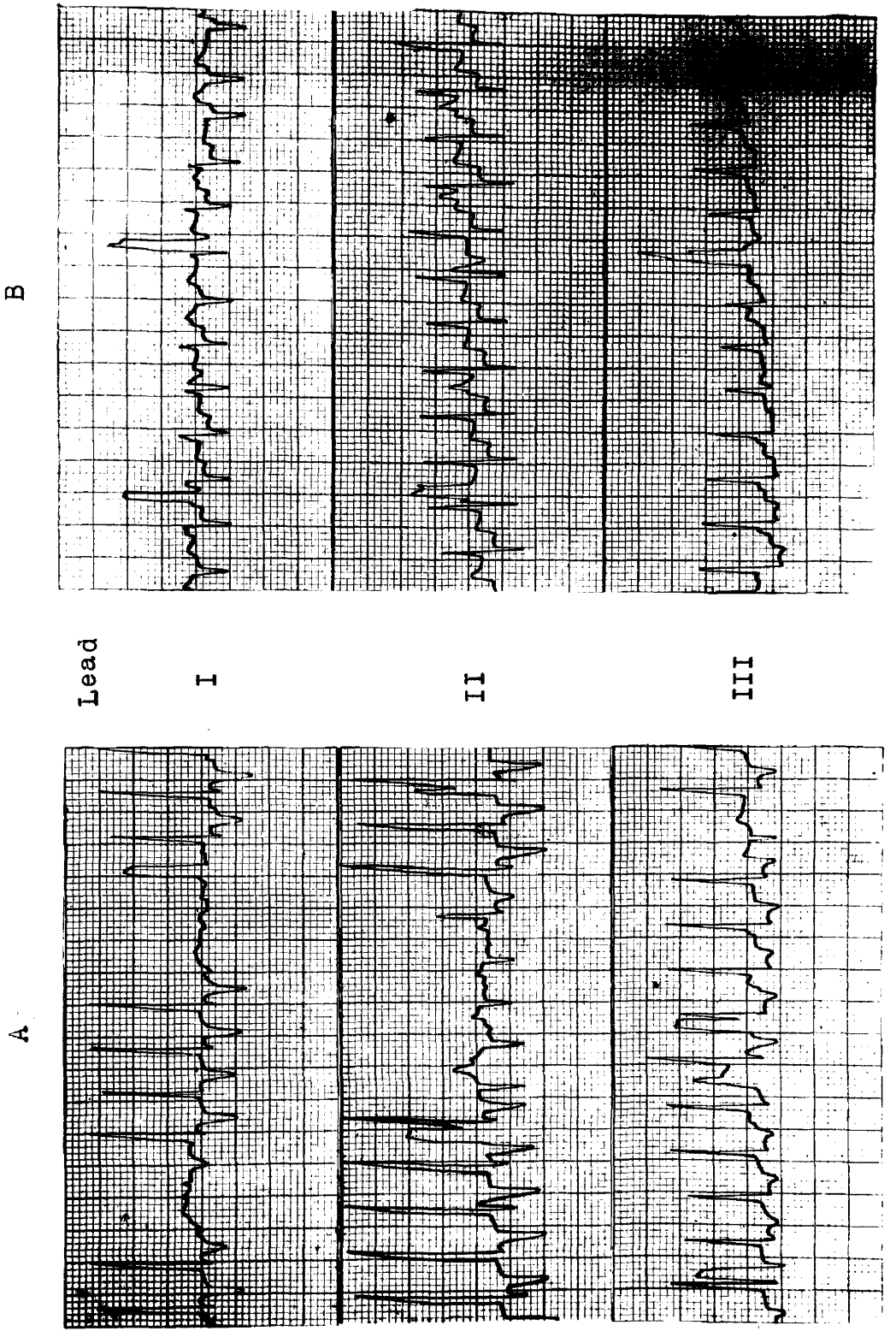

B
Lead
I
II
III
A

Figure 9. Records from three different vitamin E-deficient lambs (Nos. 49, 45 and 43) showing the lengthened PR interval.

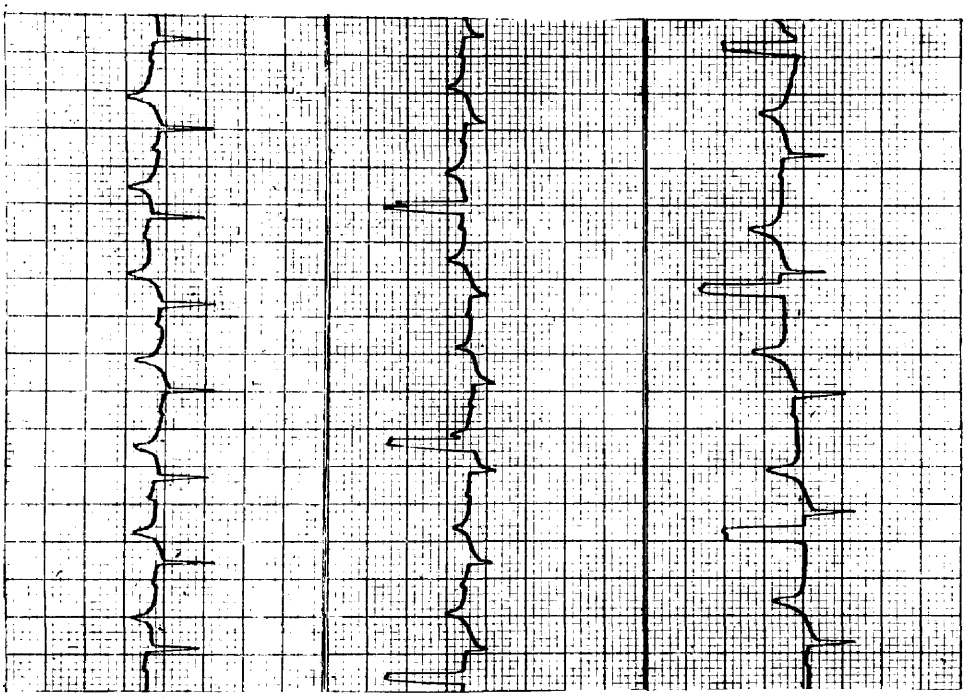

Lead
II
II
I

Figure 10. Electrocardiogram of vitamin E-deficient lamb No. 19 showing marked depression of the ST segment in leads II and III.

Figure 11. Single leads from three different vitamin E-deficient lambs showing various ECG changes.

Record A from lead II of lamb No. 39 illustrated the periods of disorganization of the heart beat interspersed with complexes of variable voltage.

Record B obtained from lead I of lamb No. 50 shows especially during the first four cycles, vibration of the electrocardiographic deflections. This probably was caused by contraction of voluntary muscle.

Record C from lead I of lamb No. 45 shows the occasional appearance of a ventricular extra systole.

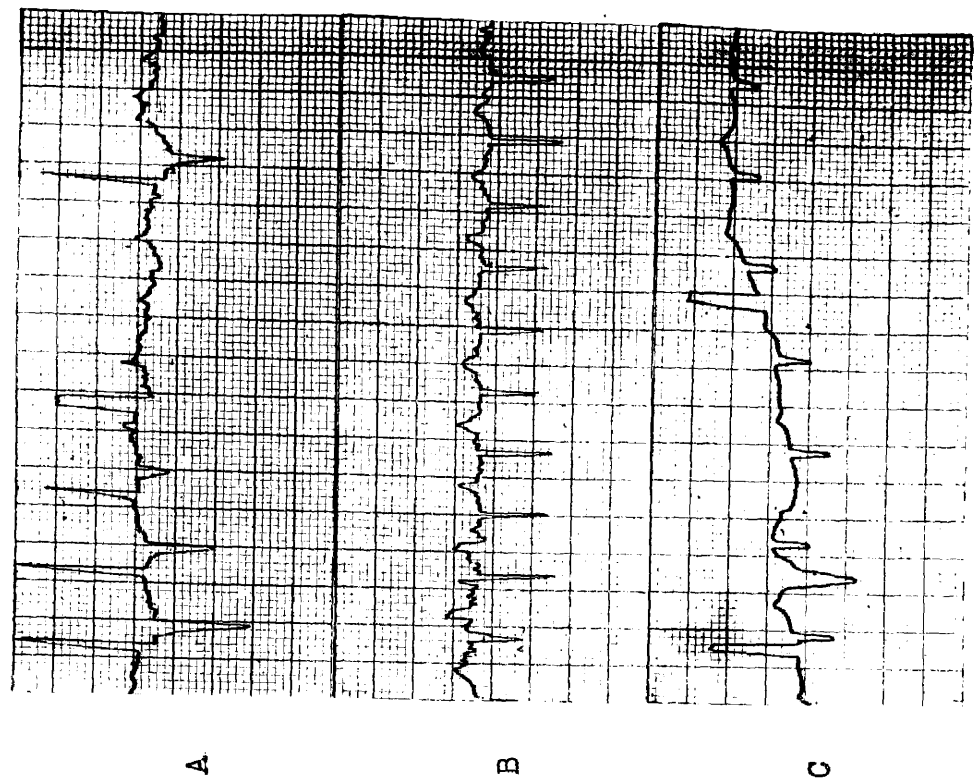


Figure 11

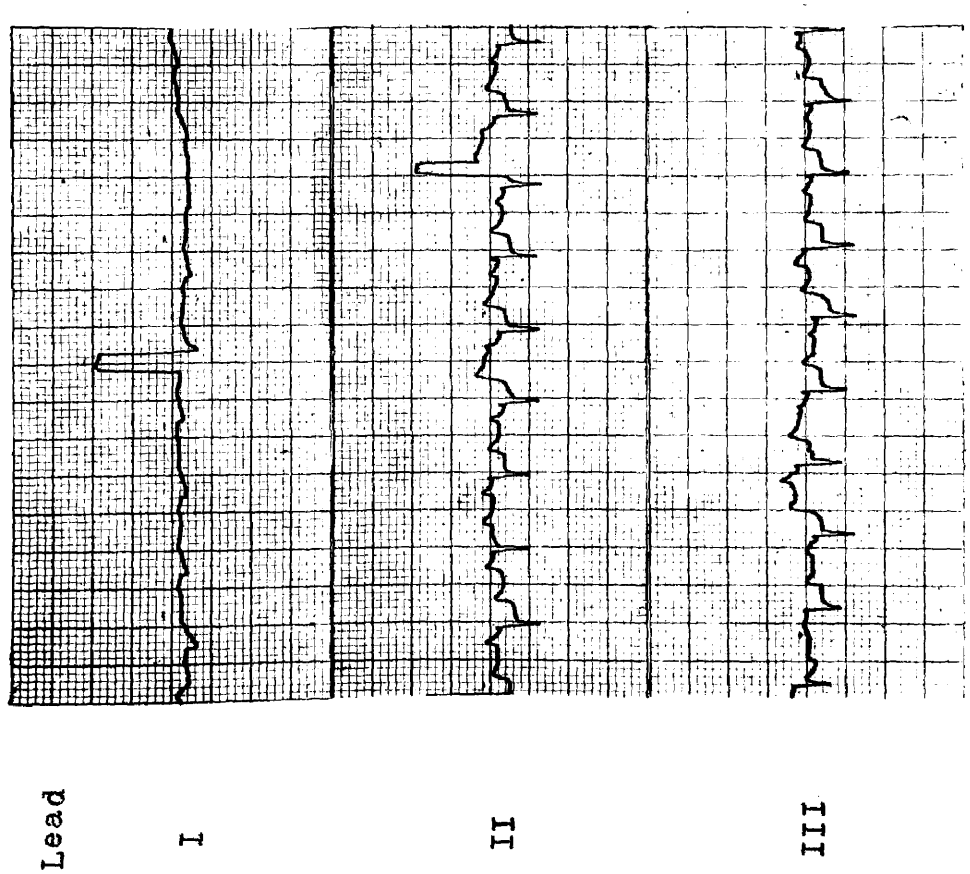


Figure 10

These changes consisted mainly in a decrease in heart rate and an increase in duration of the PR interval. In all instances it was found that, in contrast to the first group, the heart rate decreased with increasing age. The average of the group was 136 beats per minute. This was considerably lower than the control group.

The PR intervals consistently increased in all the ECGs. The maximum value was 0.12 second and the average for the whole group was 0.11 second. This value was larger than the averages seen in the control group (Fig. 9).

The QT interval increased from its original average value of 0.23 second to a final average duration of 0.25 second, but this change was chiefly due to the heart rate, since the K values of this group and the control animals were almost equal.

3) ECGs of Vitamin E Deficient Lambs not Showing Any Symptoms of Cardiac Involvement:

Vitamin E deficiency was also produced in lamb Nos. 12, 32 and 50, as can be proven by the data already presented on the chemical analyses of blood urine and muscles, and the macro and micropathological examination. In spite of the fact that extensive lesions were found in the heart of each one of these animals (Fig. 4 and 14), no electrocardiographic changes were noticed at any time (Table XIV).

In the case of lamb No. 12 the last electrocardiogram was taken 20 days before the death of the animal. At this time the record was similar to that of control lambs.

In the case of lamb Nos. 32 and 50 the ECGs were taken up to the point when the death of the lamb was eminent. Even then, the ECG appeared similar to those of control lambs.

c) The Electrocardiogram of Lambs Fed Diet A Supplemented with Alpha Tocopherol and Cod Liver Oil:

Lamb Nos. 13 and 15 receiving diet A supplemented with alpha tocopherol and cod liver oil showed remarkably low heart rates. The average was 111 beats per minute (Table XVI). The PR intervals were also different from control lambs and averaged 0.11 second. The QT interval too, increased up to the very high average value of 0.33 second which, as was shown by the high K values, was not caused by the decreased heart rate of the lamb.

Very high voltages of T and QRS were observed in lamb No. 13, but the reverse was observed in lamb No. 15, so that it can not be considered as an effect of the feeding regimen. Nevertheless, some of the T waves of lamb No. 13 had a very peculiar form which had never been seen before in any of the ECG of either deficient or control lambs. They have been reproduced in lead II of Figure 2.

d) The Electrocardiogram of Lambs Fed Diet B Supplemented with Alpha Tocopherol (Recovery), Prostigmine and Cortisone:

The administration of alpha tocopherol to vitamin E deficient lamb Nos. 42, 29 and 50 did not modify the ECG of the lambs (Tables XIV and XVI). In only one case (lamb No. 42), was there an increase in the voltage of the ECG deflections.

TABLE XVI

THE AVERAGE HEART RATES, PR AND QT INTERVALS AND THE K CONSTANT (BAZETT'S) OF LAMBS FED THE VARIOUS EXPERIMENTAL DIETS

Group	Lamb No.	Heart rate*	Intervals PR*	Intervals QT*	Bazett's constant K*
Lambs fed diet A plus α-tocopherol and cod liver oil	13	128	0.10	0.30	0.44
	15	95	0.12	0.35	0.42
	Av.	111	0.11	0.33	0.43
Lambs fed diet B plus α-tocopherol (recovery)	42	139	0.12	0.25	0.39
	49	73	----	0.30	0.38
	50	143	0.10	0.24	0.37
	Av.	118	0.11	0.26	0.38
Lambs fed diet B receiving injections of prostigmine	34	161	0.11	0.21	0.34
	38	186	0.08	0.22	0.39
	Av.	174	0.09	0.21	0.36
Lambs fed diet B receiving injections of cortisone	45	107	0.11	0.25	0.33
	46	125	0.10	0.25	0.37
	Av.	116	0.10	0.25	0.35

* Average values

Likewise, the prostigmine and cortisone injections did not influence the ECG of vitamin E-deficient lambs (Table XVI).

Occasionally, other ECG alterations were noticed in most of the animals fed a vitamin E-deficient diet. They were dropped beats, arrythmias of different types, tremors (probably due to voluntary muscle effect), auricular fibrillations and auricular or ventricular extrasystoles. Some of these are illustrated in Figure 11.

Studies of the Mineral Changes of the Skeletal and Heart Muscles due to Vitamin E Deficiency

The results of the quantative analysis of sodium, potassium, calcium, magnesium and phosphorus of the skeletal and heart muscles are summarized in Tables XVII, XVIII, XIX and XX. The values are given on an ash basis as mg. per 100 mg. and on a fresh tissue (muscle) basis as mg. per 100 gm.

Statistical analyses of the data have not been carried out. There were two main reasons for not doing so. First, because of the small number of animals in each group and second, because of the variable degree of deficiency obtained in the lambs, which can be verified by the analysis of alpha tocopherol in the blood and creatine in the urine.

Due to these reasons another system of differentiation was set up to distinguish the magnitude of the chemical changes. An arbitrary scale was made in which a change was called moderate when it did not become larger than 5 or smaller than 1.5 times

the value observed in the control animals. And a change was deemed large when these values exceeded five times that of the control lambs.

From the study of these tables the following information has been obtained in regard to the influence of vitamin E on the ash and each one of the cited elements. Data referring to fresh tissue basis were used to evaluate the alterations in the chemical composition of the muscles.

Sulfated ash: The percent of sulfated ash of the muscles of experimentally E-deficient animals was only slightly increased. The skeletal muscles of lamb No. 12 were the only ones that showed marked mineralization. In spite of this, the heart of the lamb did not show any increase of sulfated ash. The reverse happened in the case of lamb No. 43, which showed mineralization of the heart but not of the skeletal muscles (Table XVII).

Field cases of "stiff-lamb" disease were studied and it was found that the skeletal muscles contained three times as much sulfated ash as the experimental control animals. The ash content of the heart, however, was only slightly higher than the controls.

Sodium: The sodium content of the lambs' thigh muscles increased when the vitamin E deficiency was produced by feeding a diet containing commercial or molecular distilled lard, or in the field cases (Table XVII).

TABLE XVII

INFLUENCE OF VITAMIN E ON THE PERCENT OF SULFATED ASH AND THE SODIUM AND POTASSIUM COMPOSITION OF THE SKELETAL MUSCLES (THIGH)

Diet	Lamb No.	% of sulfated ash	Na ash mg/100mg	Na fresh tissue mg/100gm	K ash mg/100mg	K fresh tissue mg/100gm
A	4	1.26	12.06	151	15.08	189
	6	1.20	10.77	128	15.61	186
	8	1.35	9.43	126	17.76	239
	12	3.11	5.49	170	4.09	127
	18	----	----	172	----	225
	Av.	1.73	9.44	149	13.13	193
A plus α-tocopherol	5	1.49	6.71	100	20.14	300
	7	1.27	4.59	66	18.35	233
	Av.	1.38	5.65	83	19.25	266
A plus cod liver oil	14	1.23	9.96	122	16.06	197
	17	1.34	8.99	120	20.28	271
	19	1.14	14.60	166	15.97	182
	Av.	1.24	11.18	136	17.44	216
A plus α-tocopherol and cod liver oil	13	1.25	7.83	98	22.13	278
	15	1.50	5.02	75	26.68	399
	Av.	1.37	6.43	86	24.41	338
B	24	1.32	13.23	174	17.66	233
	32	1.59	12.18	193	9.76	155
	39	1.37	13.42	184	14.79	202
	43	1.45	13.04	188	12.50	181
	44	1.23	12.63	155	16.95	208
	47	1.31	8.58	112	18.66	243
	Av.	1.38	12.18	168	15.05	203
B plus α-tocopherol (controls)	20	1.43	8.49	121	15.82	225
	21	1.39	7.75	107	23.20	322
	37	1.52	----	---	-----	---
	40	1.42	4.96	70	23.88	338
	41	1.32	6.37	84	23.11	305
	Av.	1.41	6.89	95	21.50	297

TABLE XVII (continued)

Diet	Lamb No.	% of sulfated ash	Na ash mg/100mg	Na fresh tissue mg/100gm	K ash mg/100mg	K fresh tissue mg/100gm
B plus α-tocopherol (recovery)	42	1.31	8.55	112	21.03	275
	49	1.51	7.21	118	20.58	310
	50	1.44	10.68	153	14.08	202
	Av.	1.42	8.81	127	18.56	262
B and prostigmine injection	34	1.45	8.18	118	15.46	224
	38	1.34	8.78	117	19.94	266
	Av.	1.39	8.48	117	17.70	245
B and cortisone injection	45	1.44	8.71	125	16.80	241
	46	1.31	11.49	151	17.97	236
	Av.	1.38	10.10	138	17.38	238
Natural pasture (stiff lambs)	713	1.85	11.57	214	5.94	110
	716	4.08	6.32	258	4.18	170
	721	7.41	3.40	251	3.50	259
	Av.	4.45	7.09	241	4.54	179

Cod liver oil supplementation did not produce any further increase in the level of sodium in the muscles, since lambs receiving diet A and diet A plus cod liver oil had, approximately, an equal content of sodium.

Of the several therapies used for treatment of vitamin E deficiency, alpha tocopherol and prostigmine were effective in returning the sodium levels within the normal values. Cortisone was unable to produce similar effects.

In deficient animals fed purified diets A and B or in the field cases, the sodium content of the heart was not disturbed by the vitamin E deficiency. Nevertheless, it was remarkable to find in heart muscle of recovered animal a high sodium content averaging 225*, which is about double of the found in control animals (Table XIX).

Potassium: Vitamin E deficiency also affected the potassium content of skeletal and heart muscles.

The content of potassium in the thigh muscles of control lambs in diet A was 266* and in diet B 297*, whereas deficient animals on the diets A or B and the natural ration had a low potassium content, averaging 193, 203 and 179* respectively (Table XVII).

Lambs treated with alpha tocopherol, prostigmine, cortisone or cod liver oil had levels of potassium intermediate between the controls and deficient animals. This perhaps indicated that the mentioned supplements protected the skeletal

* mg. per 100 gm. of fresh tissue (muscle)

TABLE XVIII

INFLUENCE OF VITAMIN E ON THE PERCENT OF CALCIUM, MAGNESIUM AND PHOSPHORUS COMPOSITION OF THE SKELETAL MUSCLES (THIGH)

Diet	Lamb No.	Ca		Mg		P	
		ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm
A	4	3.46	43	2.05	26	28.92	363
	6	1.48	18	2.16	26	-----	---
	8	0.60	8	1.29	17	18.96	255
	12	19.26	599	0.73	23	6.69	208
	18	-----	194	----	32	----	295
	Av.	6.20	172	1.55	25	18.19	280
A plus α-tocopherol	5	0.57	8	1.67	25	18.99	283
	7	0.52	6	2.33	30	20.44	260
	Av.	0.54	7	2.00	27	19.72	271
A plus cod liver oil	14	0.64	8	1.87	23	23.04	283
	17	1.78	24	1.99	27	17.91	240
	19	0.77	9	1.70	19	17.94	205
	Av.	1.07	14	1.86	23	19.63	242
A plus α-tocopherol and cod liver oil	13	0.61	8	3.74	47	22.21	278
	15	0.80	12	3.07	46	18.60	278
	Av.	0.70	10	3.40	46	20.40	278
B	24	0.71	9	1.34	18	17.25	228
	32	6.48	103	1.92	30	18.99	263
	39	1.52	21	1.33	18	-----	---
	43	2.31	33	0.55	8	14.02	203
	44	0.64	8	1.04	13	16.29	200
	47	0.40	5	2.32	30	16.08	210
	Av.	2.01	30	1.42	20	16.53	220

TABLE XVIII (continued)

Diet	Lamb No.	Ca		Mg		P	
		ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm
B plus α-tocopherol (controls)	20	0.44	6	1.62	23	17.73	253
	21	0.71	10	2.14	30	17.13	238
	37	----	--	----	--	17.41	265
	40	0.50	7	2.85	40	20.80	295
	41	0.38	5	3.02	40	21.42	283
	Av.	0.50	7	2.40	33	18.89	266
B plus α-tocopherol (recovery)	42	0.73	10	1.13	15	17.53	230
	49	0.59	9	1.69	25	17.44	263
	50	0.64	9	1.23	20	21.42	308
	Av.	0.65	9	1.35	20	18.80	267
B and prostigmine injection	34	0.47	7	0.57	8	16.91	245
	38	0.79	10	2.13	28	25.73	344
	Av.	0.63	9	1.35	18	21.32	294
B and cortisone injection	45	0.64	9	0.93	13	17.04	245
	46	1.54	20	0.85	11	18.65	245
	Av.	1.09	15	0.89	12	17.84	245
Natural pasture (stiff lamb)	713	33.23	616	0.97	18	12.94	240
	716	47.00	871	2.88	38	22.04	900
	721	16.20	1199	0.70	52	9.45	700
	Av.	32.14	596	1.52	36	14.81	613

TABLE XIX

INFLUENCE OF VITAMIN E ON THE PERCENT OF SULFATED ASH AND THE SODIUM AND POTASSIUM COMPOSITION OF THE HEART MUSCLE

Diet	Lamb No.	% of sulfated ash	Na		K	
			ash mg/100mg	fresh tissue mg/100gm	ash mg/100mg	fresh tissue mg/100gm
A	4	1.22	12.30	149	10.39	126
	6	1.20	10.47	125	15.54	186
	12	1.27	10.12	128	17.02	215
	Av.	1.23	10.96	134	14.31	175
A plus α-tocopherol	7	1.29			18.49	238
A plus cod liver oil	14	1.34	6.13	81	14.19	189
	19	1.14	10.09	118	10.18	116
	Av.	1.24	8.11	99	12.18	152
A plus α-tocopherol and cod liver oil	13	1.31	8.47	110	15.89	207
	15	1.36	7.92	107	15.19	206
	Av.	1.33	8.20	108	15.54	206
B	24	1.41	6.92	95	15.54	219
	32	1.29	12.72	163	9.89	127
	39	1.49	8.24	123	17.25	257
	43	2.16	11.26	242	6.59	142
	Av.	1.58	9.79	155	12.32	186
B plus α-tocopherol (controls)	20	1.18	10.57	124	17.24	203
	21	1.32	8.83	116	17.95	237
	Av.	1.25	9.70	120	17.59	220
B plus α-tocopherol (recovery)	50	1.65	13.72	225	17.93	295
Natural pasture (stiff lambs)	713	1.37	11.97	164	13.79	189
	716	1.97	6.77	133	10.86	214
	Av.	1.67	9.37	148	12.33	201

muscles from changing their potassium content when lambs were fed an E-deficient diet.

The level of potassium of the heart muscle was only affected by the diet A alone or supplemented with cod liver oil. It lowered the potassium content of the heart muscle to 175 and 152* respectively. Neither diet B nor natural rations modified the normal potassium content of the heart muscle (Table XIX).

Calcium: When diet A containing the commercial lard was fed to the lambs, calcification took place in the thigh muscles but not in the heart. This can be observed in Table XVIII, showing that practically all the lambs, with the exception of lamb No. 8, showed an increased calcium content of the muscles from 2 to 25 times over the control lambs. Table XX shows that the level of calcium in the heart of E-deficient lambs on diet A ranged from 7 to 10* (average 8*), values which are equal to those of the control lamb (Av. 8.0*).

Cod liver oil favored the calcification of the heart muscle to a great extent (average 50*) even when animals were fed alpha tocopherol as in the case of lamb Nos. 13 and 15 (Av. 18*).

The molecular distilled lard diet produced calcification in the skeletal and heart muscles. It was equally pronounced in both types of muscles and was about seven times greater than in the controls.

Treatment with alpha tocopherol caused a full recovery of the heart and skeletal muscles of deficient lambs from the cal-

* mg. per 100 gm. of fresh tissue (muscle)

TABLE XX

INFLUENCE OF VITAMIN E ON THE CALCIUM, MAGNESIUM AND PHOSPHORUS COMPOSITION OF THE HEART MUSCLE

Diet	Lamb No.	Ca		Mg		P	
		ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm	ash mg/100 mg	fresh tissue mg/100 gm
A	4	0.81	10	2.10	26		
	6	0.63	7	1.73	20		
	12	0.52	7	1.70	22	27.88	353
	Av.	0.65	8	1.84	23	27.88	353
A plus α-tocopherol	7	0.63	8	3.40	44	20.20	260
A plus cod liver oil	14	5.39	66	1.36	18	22.32	298
	19	2.93	33	1.88	21	23.67	239
	Av.	4.16	50	1.62	20	21.63	268
A plus α-tocopherol and cod liver oil	13	2.33	30	2.26	30	26.83	351
	15	0.46	6	1.74	23	23.67	321
	Av.	1.39	18	2.00	26	25.25	336
B	24	2.34	33	0.88	12	23.33	329
	32	0.58	7	0.67	9		
	39	2.29	34	4.92	25		
	43	3.00	65	0.84	18	16.13	348
	Av.	2.05	35	1.83	16	19.73	338
B plus α-tocopherol (controls)	20	0.50	6	2.13	25	22.29	263
	21	0.47	6	1.07	14		
	Av.	0.48	6	1.60	20	22.29	263
B plus α-tocopherol (recovery)	50	0.61	9	1.73	25	21.26	350
Natural pasture (stiff lambs)	713	4.47	61	1.85	25		
	716	6.68	132	0.60	12	16.49	325
	Av.	5.57	97	1.23	19	16.49	325

cification produced by the absence of vitamin E. Prostigmine also appeared effective in the two animals treated for cure of muscular calcification. The action of cortisone was uncertain since in one lamb the level of calcium was not altered whereas the other one showed an increase of 10 mg. per 100 grams of fresh tissue.

Calcification of the skeletal and heart muscles was very extensive in the field cases of "stiff-lamb" disease. The skeletal muscle had 596 mg. of calcium per 100 grams of fresh tissue and the heart muscle 97 mg. of calcium per 100 grams of fresh tissue (Tables XVIII and XX).

Magnesium: Lambs fed diet A alone, diet A supplemented with cod liver oil and a natural feed ration that produced vitamin E deficiency had skeletal muscles with normal content of magnesium, which is 27%. But lambs fed diet B showed low content of magnesium (20%) even when they were supplemented with prostigmine (18%), cortisone (12.2%) or alpha tocopherol (20%) (Table XVIII).

It was surprising to find that skeletal muscles of lambs receiving diet A supplemented with alpha tocopherol and cod liver oil had 46%, which was twice as large as the level of magnesium in skeletal muscles of lambs on diet A alone or diet A plus alpha tocopherol, or diet A plus cod liver oil.

Nothing definite can be concluded about the magnesium changes produced in the lambs' heart by the E-deficient diet A,

% mg. per 100 gm. of fresh tissue (muscle)

because the high value of magnesium (44*) of the control animal No. 7 does not agree with the average level of magnesium (20*) of the control lambs Nos. 20 and 21 fed diet B plus alpha tocopherol (Table XX).

Lambs fed the basal diet B alone or with the several supplementations showed little difference in their magnesium content in the chemical analysis.

Phosphorus: Experimentally produced vitamin E deficiency affected the amount of phosphorus in the skeletal and heart muscle of E-deficient animals very little.

There was a small (about 15% average) but consistent increase of phosphorus in the heart muscle of lambs made E deficient by feeding diets A or B. However, changes in the phosphorus content of the skeletal muscles did not occur at all in lambs deficient in vitamin E.

In field cases of vitamin E deficiency, there were more marked changes in the content of phosphorus of the skeletal muscles. They contained an average 613* (Table XVIII). The changes occurring in the heart muscle of stiff lambs (field cases) and E-deficient experimental animals were of the same magnitude.

Mineral ratios: Mineral ratios were calculated between each one of the elements analyzed (Tables XXI and XXII). The greatest ratio change occurring in E-deficient lambs was in the Ca/Mg and then in the ratio of Ca to P. But, in the case of

* mg. per 100 gm. of fresh tissue (muscle)

TABLE XXI

INFLUENCE OF VITAMIN E ON THE MINERAL RATIOS OF SODIUM, POTASSIUM, MAGNESIUM AND PHOSPHORUS IN THE HEART MUSCLE

Diet	Ca/P	Ca/Mg	Ca/Na	Ca/K	Mg/Na	Mg/K	Na/K	P/Mg
A	0.018 (-2)	0.35 (2)	0.06	0.05 (2)	0.17	0.13 (2)	0.82	16.34
A plus α-tocopherol	0.031	0.18	----	----	----	----	----	5.93
A plus cod liver oil	0.181 (6)	2.61 (15)	0.50 (8)	0.33 (11)	0.20 (2)	0.13 (2)	0.72	13.81 (2)
A plus α-tocopherol & cod liver oil	0.053 (2)	0.65 (4)	0.17 (3)	0.09 (3)	0.25 (3)	0.13 (2)	0.52 (-2)	12.72 (2)
B	0.143 (5)	1.94 (14)	0.21 (4)	0.18 (6)	0.10	0.08	0.92	24.70
B plus α-tocopherol (controls)	0.026	0.28	0.06	0.03	0.23 (2)	0.12 (2)	0.55 (-2)	8.18 (-3)
B plus α-tocopherol (recovery)	0.025	0.36 (2)	0.04	0.03	0.11	0.08	0.76	14.00 (-2)
Natural pasture (stiff lambs)	0.405 (13)	6.78 (40)	0.65 (11)	0.48 (16)	0.13	0.09	0.74	27.54

TABLE XXII

INFLUENCE OF VITAMIN E ON THE MINERAL RATIOS OF SODIUM, POTASSIUM, MAGNESIUM AND PHOSPHORUS IN THE SKELETAL MUSCLES (THIGH)

Diet	Ca/P	Ca/Mg	Ca/Na	Ca/K	Mg/Na	Mg/K	Na/K	P/Mg
A	0.92 (40)*	7.08 (40)	1.16 (12)	0.89 (40)	0.17 (-2)	0.13	0.62 (2)	11.79
A plus α-tocopherol	0.03	0.28	0.09	0.02	0.33	0.10	0.31	10.07
A plus cod liver oil	0.06 (2)	0.56 (2)	0.99 (10)	0.06 (3)	0.17 (-2)	0.11	0.66 (2)	10.59
A plus α-tocopherol and cod liver oil	0.04	0.21	0.11	0.03	0.54 (2)	0.14	0.27	5.98 (-2)
B	0.06 (2)	1.74 (8)	0.21 (3)	0.18 (9)	0.12 (-3)	0.10	0.89 (2)	14.42
B plus α-tocopherol (controls)	0.03	0.22	0.07	0.02	0.37	0.11	0.34	8.32
B plus α-tocopherol (recovery)	0.04	0.48 (2)	0.07	0.04 (2)	0.16 (-2)	0.08	0.51 (2)	13.69 (2)
B and prostigmine injection	0.03	0.60 (3)	0.07	0.04 (2)	0.16 (-2)	0.07	0.48	20.97 (2)
B and cortisone injection	0.06 (2)	1.25 (6)	0.11	0.06 (3)	0.09 (-4)	0.05 (-2)	0.58	20.13 (2)
Natural pasture (stiff lambs)	1.75 (80)	26.75 (100)	3.72 (40)	5.00 (25)	0.15 (-2)	0.20 (-2)	1.48 (5)	16.82

* The numbers within parenthesis indicate the approximate ratio of the experimental to the control ratios. When there is no number the ratio is less than 1.5. A negative sign indicates decrease.

lambs fed cod liver oil, the largest alteration was in the ratio Ca/Na, which was third in importance in the other experimentally E-deficient lambs. The ratio of Ca to K was also modified.

In the hearts, the order of ratio disturbance was as follows: Ca/Mg, Ca/K, Ca/P and Ca/Na.

In summary the chemical changes of the muscles can be characterized in the following manner.

Thigh muscles showed, in general, an increase in the content of calcium and sodium and a decrease in potassium. When diet B, containing a molecularly distilled lard, was fed to the animals a decrease in magnesium was also noticed. Prostigmine was found protective to calcium, sodium and potassium. Cortisone was protective to potassium, whereas cod liver oil protected potassium and magnesium. Alpha tocopherol therapy reversed to normal the mineral composition of the skeletal muscles with magnesium as the only exception.

The changes in the heart muscle of E-deficient animals were different, according to the type of diet (A or B) fed. When diet A, containing commercial lard, was given the calcium content of the heart muscle was normal, but the potassium level was low and the phosphorus level slightly high. The supplementation of cod liver oil largely increased the calcium content of the heart.

Diet B, containing the molecularly distilled lard, greatly increased the calcium and lowered the potassium content of the

heart. Lambs recovered from E deficiency had a mineral composition similar to the control animals with the exception of the sodium content, which was rather high.

Studies on the Amino Acid Changes in the Skeletal and Heart Muscles due to Vitamin E Deficiency

The results of the microbiological analysis of the muscles for the amino acids proline, glycine, leucine and isoleucine are presented in Table XXIII. The quantities of amino acids have been calculated in mg. per 100 mg. of muscle.

Again, in this case, it is not possible to apply statistical analysis because the small number of samples per group and the heterogeniety within each group, due to the different degrees of vitamin E deficiency. In spite of this, the figures do throw some light on the nature of the muscular changes occurring in the absence of vitamin E in the diet.

l-Proline:

The comparison between the proline values of thigh and heart muscles of controls and E-deficient animals fed diets A or B showed that vitamin E deficiency did not have a direct or indirect relationship on the proline content of the muscles. Skeletal muscles of field cases of vitamin E deficiency showed a low value of proline, 0.59% as compared to the average values of control lambs, 0.80% in diets A and B.

% mg. per 100 mg. of muscle

TABLE XXIII

INFLUENCE OF VITAMIN E ON THE PROLINE, GLYCINE, LEUCINE AND ISOLEUCINE COMPOSITION OF THE SKELETAL (THIGH) AND HEART MUSCLES

Diet	Lamb No.	Skeletal muscles				Heart muscles			
		l-proline*	l-glycine*	l-leucine*	l-isoleucine*	l-proline*	l-glycine*	l-leucine*	l-isoleucine*
A	4	0.71	1.48	1.69	1.04				
	8	1.09	1.60	1.24	0.77				
	9	0.68	1.24	1.28	0.88	1.35	1.17	1.32	1.00
	12	0.72	0.80	0.80	0.49	0.72	0.75	1.21	0.81
	18	0.80	1.11	1.09	0.65				
	Av.	0.80	1.25	1.22	0.77	1.03	0.96	1.26	0.90
A plus α-tocopherol	5	0.75	1.01	1.76	0.81				
	7	0.85	1.11	1.17	0.92	0.87	0.92	1.36	0.91
	Av.	0.80	1.06	1.46	0.87	0.87	0.92	1.36	0.91
A plus cod liver oil	14	0.77	0.81	0.97	0.05	1.33	1.00	1.17	0.66
	17		0.76	0.93	0.61				
	19	0.67	0.90	0.95	0.51	0.79	0.89	1.35	0.80
	Av.	0.72	0.82	0.95	0.59	1.06	0.94	1.26	0.73
A plus α-tocopherol and cod liver oil	13	0.75	0.99	1.46	1.00	0.81	1.27	1.48	1.02
	15	0.78	0.96	0.77	0.50	1.29	1.14	1.43	0.96
	Av.	0.77	0.98	1.12	0.75	1.05	1.20	1.45	0.99

* Mg. of amino acid per 100 mg. of muscle

TABLE XXIII (continued)

Diet	Lamb No.	Skeletal muscles				Heart muscles			
		l-proline*	l-glycine*	l-leucine*	l-isoleucine*	l-proline*	l-glycine*	l-leucine*	l-isoleucine*
B	23	1.29	1.26	1.25	0.66				
	24	0.89	0.92	0.99	0.55	1.44	1.18	1.47	1.04
	27	0.73	0.84	1.17	0.77				
	43	0.59	0.56	0.96	0.65	1.54	1.44	1.72	1.16
	44	0.61	0.57	1.06	0.76	0.84	0.88	0.77	0.84
	47	0.64	0.71	0.98	0.72	0.43	0.49	0.70	0.46
	Av.	0.80	0.81	1.07	0.68	1.06	1.00	1.16	0.88
B plus α-tocopherol (controls)	37	0.63	0.76	1.29	0.97	1.08	1.39	2.09	1.31
	40	0.78	0.81	1.44	1.16	1.02	0.96	1.85	1.14
	41	0.52	1.02	1.33	0.97	1.33	0.98	1.29	0.88
	Av.	0.64	0.86	1.34	1.03	1.14	1.11	1.75	1.11
B plus α-tocopherol (recovery)	42	0.44	0.51	0.95	0.61	1.32	1.10	1.36	0.90
	49	0.78	0.75	1.47	1.03	0.94	1.25	1.32	0.88
	50	0.84	0.99	1.57	1.11	1.29	1.11	1.43	1.01
	Av.	0.68	0.75	1.33	0.92	1.18	1.15	1.37	0.93
B and protigmine injection	34	0.68	0.83	1.27	0.73	1.17	1.10	1.15	0.53
	38	0.69	0.73	1.16	0.82	0.91	0.84	1.15	0.66
	Av.	0.68	0.78	1.22	0.77	1.04	0.97	1.15	0.60

* Mg. of amino acid per 100 mg. of muscle

TABLE XXIII (continued)

Diet	Lamb No.	Skeletal muscles				Heart muscles			
		l-proline*	l-glycine*	l-leucine*	l-isoleucine*	l-proline*	l-glycine*	l-leucine*	l-isoleucine*
B and cortisone injection	45	1.17	0.99	1.20	0.83	0.99	1.04	1.11	0.88
	46	1.01	0.97	1.14	0.74	1.05	1.20	1.29	1.03
	Av.	1.09	0.98	1.17	0.78	1.02	1.12	1.20	0.95
Natural pasture (stiff lambs)	713	0.72	0.71	1.30	0.83				
	716	0.42	1.08	1.80	1.09	1.11	0.97	0.91	0.81
	721	0.62	0.82	1.17	0.66				
	Av.	0.59	0.87	1.42	0.86	1.11	0.97	0.91	0.81

* Mg. of amino acid per 100 mg. of muscle

Animals treated with cortisone had higher (double) proline content in the thigh muscles than control animals; 1.09* as compared to 0.80*. Yet, the heart muscle of the same animals showed no change in proline content. It was 0.99 and 1.05* (Av. 1.02*) whereas those of the control animals were 1.08*, 1.02*, and 1.33* (Av. 1.14*).

l-Glycine:

The glycine values of the skeletal and heart muscles of vitamin E-deficient animals fed either diets A or B or of the field cases were not different from those of control lambs. Notwithstanding, the combined action of vitamin E deficiency and cod liver oil lowered the glycine content of the skeletal muscles but their action was largely counteracted by alpha tocopherol feeding. The glycine value averaged 1.06* and 0.86* in control animals, 0.82* in lambs fed diet A supplemented with cod liver oil and 0.98* in lambs fed diet A supplemented with cod liver oil and alpha tocopherol. However, the glycine content of heart muscle was the same in deficient and control animals.

l-Leucine:

The leucine content of skeletal and heart muscles of E-deficient animals was found lower than control lambs. The difference was not very distinct when diet A was fed, but it became well marked when the diet B was given. Thus, in Table XXIII the leucine values of lambs fed diet B averaged 1.07* in skeletal

* mg. per 100 mg. of muscle

muscles and 1.16* in heart muscle. The leucine values of the control lambs for diet B were 1.34* and 1.75* respectively for skeletal and heart muscles.

Cod liver oil supplementation accentuated the lowering effect of vitamin E deficiency on the leucine level of the skeletal muscles (Av. leucine content 0.95*). But, its action perhaps was partly neutralized by the feeding of alpha tocopherol as demonstrated by the value 1.12* of the group receiving diet A plus alpha tocopherol and cod liver oil.

Prostigmine and cortisone seemed to exert a protective action on the leucine level in the skeletal muscles, since the quantities of leucine were intermediate between the values of control and deficient animals. Similar effects could not be observed in the case of heart muscle.

In the case of lambs deficient on a natural feed ration, the leucine values of skeletal muscles were not different from those of control lambs, but the heart content of leucine seemed to be lower than normal.

l-Isoleucine:

Lambs fed diet A showed a lowering of the isoleucine content of the skeletal muscles from 0.87* (control animals) to 0.77* (deficient lambs). This effect was enhanced by the feeding of cod liver oil, which lowered the isoleucine content to an average of 0.59*. Alpha tocopherol, however, again seemed to

* mg. per 100 mg. of muscle

offer protection to the detrimental effect of cod liver oil, as evidenced by the average content of isoleucine (0.75*) of the group fed diet A plus alpha tocopherol and cod liver oil.

The isoleucine changes of the heart muscle of lambs fed diet A were less extensive than in skeletal muscles. The average content of deficient muscles was 0.90* in the control group and 0.91* in the group fed diet A plus alpha tocopherol.

The changes in isoleucine levels produced by the diet B were more marked than those produced by diet A. Thus, the average isoleucine content of skeletal muscles of animals fed diet B was 0.68*, of the control lambs 1.03* and those recovered 0.92*. In the heart muscle similar changes were noticed; thus the average content of the lambs fed diet B was 0.88*, in controls 1.11* and 0.93* in the recovery group. These figures showed that vitamin E deficiency lowered the content of isoleucine of both skeletal and heart muscles and that the recovery from the deficiency is also reflected in the content of isoleucine.

The effect of prostigmine and cortisone on the content of isoleucine could not be found in the skeletal muscles but, in the case of heart muscle, prostigmine injections in animals deficient in vitamin E lowered the isoleucine content even more (Av. 0.60*). On the other hand, cortisone injections to E-deficient lambs did not produce any further modification of the isoleucine content.

* mg. per 100 mg. of muscle.

Field cases of E deficiency did not show isoleucine values different from those of control animals in either diet A or B.

In summary, the deficiency of vitamin E decreased the leucine and isoleucine content of the skeletal muscles, and the isoleucine content of the heart. Neither the proline or glycine content were altered by the E deficiency.

The severity of the E deficiency was most pronouneed when diet B was fed.

Cod liver oil had an antagonistic effect on vitamin E shown by the decrease in the content of glycine and by the larger decrease in the leucine and isoleucine content of the muscles. The lowering effect of cod liver oil in the absence of vitamin E on the glycine content of skeletal muscles was counteracted by alpha tocopherol feeding. Alpha tocopherol, however, only partly reversed the action of cod liver oil on the leucine and isoleucine content of the lamb's muscle.

Prostigmine and cortisone had a protective effect on the leucine and isoleucine level of the skeletal muscles. In addition, cortisone caused the increase of the proline level of skeletal muscles.

DISCUSSION

Growth:

In the present work the weight curves of the lambs clearly indicated that vitamin E deficiency retarded growth. These findings do not confirm the observation of Willman et al. (1934) who reported that the best developed lambs were affected more often by vitamin E deficiency.

It was also found, in regard to the influence of vitamin E on the growth of young lambs, that the type of lard in the diet was a very important factor. This, no doubt, was due to the chemical difference between the two lards. A high peroxide number, before and after four hours of aereation at approximately 98°C., was the most important difference between the commercial and molecular distilled lard. Peroxides seemed to have produced a detrimental effect on growth because rancid fat, a product having a high concentration of peroxides, not only had been shown destructive to vitamin E but, also, to have an intrinsic toxic action on the animal (Fitzhugh et al. 1944; Quackenbush 1945).

However, Dam (1949) has reported that chickens fed diets containing thoroughly rancid cod liver oil did not contract exudative diathesis or encephalomalacia, diseases constantly produced by diets containing highly unsaturated fatty acids. He also found that if the unsaturated fatty acids were oxidized before they were consumed (peroxide formation), they did not produce either disease.

In spite of the fact that peroxides were considered secondary in the developing of vitamin E deficiency in the chicken, there is no doubt that they were important in the production of vitamin E deficiency of the lamb. The unsaturated fatty acids may also play a similar role, but in the present experiment they could not have influenced the severity of the deficiency because both lards had very similar iodine values and, even more, the acid value (free fatty acids) was lower in the molecular distilled lard than in the commercial lard.

A question has been raised as to whether or not the amount of alpha tocopherol given to the control lambs was able to counteract the effects of the peroxides. This question cannot be definitely answered on the basis of the present data. It can be stated, however, that the alpha tocopherol given to the control lambs prevented the symptoms of vitamin E deficiency, the appearance of macro and microscopical lesions typical of vitamin E deficiency, and kept all the analyzed chemical components of the blood, urine and muscles within the normal range reported in healthy young lambs. Probably the success of the alpha tocopherol supplementation was due to the fact that it was given sometime after the milk feeding, which saved it from destruction in the gastrointestinal tract (Matill 1938).

Yet, the deleterious action of the peroxides on the lamb's growth was evident by comparing the growth rate of control animals fed the diet containing the commercial lard with controls

fed the diet containing the molecular distilled lard. The first ones grew faster than the second. Consequently, it must be recognized that the low growth rate of lambs in this experiment was due to three reasons: the action of vitamin E, the growth depressive effect of peroxides in the molecular distilled lard and the four times a day feeding regimen.

Symptoms:

The fact that the vitamin E-deficient lambs of this experiment developed muscular weakness rather than stiffness suggested the existence of an anti-stiffness factor associated with vitamin E deficiency as seen in field cases of "stiff-lamb" disease. On the basis of our present knowledge, several hypotheses on the nature of this anti-stiffness factor can be made. Among them, the effect of steroid and of certain mineral elements were the most attractive.

Extensive investigation has been carried out by van Wagtendonk and Wulzen (1950) in regard to the stiffness syndrome shown by guinea pigs when they were fed skim milk diets containing ample amounts of vitamin E and ascorbic acid. In spite of the composition of the diets, some of the lesions produced were similar to those occurring in ascorbic acid deficiency and others to vitamin E depletion. The effect of several extracts from sugar cane and raw cream on deficient guinea pigs suggested that the compound was of a steroid nature. Studies on tissue metabolism indicated that the steroid substances played an important

role in the energy metabolism through its influence on the adenylic system.

Excessive calcification of the muscles was always present in stiff guinea pigs deficient in steroids. This has been explained by van Wagtendonk and Wulzen (1950) who considered it dependent on two factors, the low concentration of albumin in the blood and the increased concentration of inorganic phosphate in the tissues. These workers felt that the latter factor was influenced by the anti-stiffness factor and the unbalance of calcium and phosphorus (low Ca/P ratio) in the diet.

Van Wagtendonk and Wulzen (1950) proposed the following mechanism for the process of calcification: due to the lack of the anti-stiffness factor, large proportions of the protein bound calcium were liberated which, coupled with the higher concentration of inorganic phosphate, favored the formation of a colloidal calcium phosphate complex.

There is a great similarity between the findings related to the steroid deficiency in guinea pigs and "stiff-lamb" disease of sheep fed a natural ration. For this reason the requirement of such factor (s) for the nutrition of the lamb is appealing and suggests further investigation. Van Wagendonk's work also points to the need for research concerning the effect of minerals in the ration and blood protein components on the process of calcification occurring in vitamin E deficient lambs.

Marsh (1932) suggested that the ratio Ca/P could affect the "stiff-lamb" disease, but it was not until 1939 that Willman and co-workers set up experimental conditions for the study of this problem. They found that balancing the ratio Ca/P of the "stiff-lamb ration" with dibasic sodium phosphate did not significantly influence the occurrence of lamb stiffness.

Their experiment can not be considered conclusive since it was carried out with such a small number of animals and it was difficult to control the influence of other factors in natural rations. In addition, investigation carried out on other animals by Hogan and Regan (1946) have shown that localized deposits of calcium phosphate appeared in bone and muscles of animals fed an excess of phosphorus.

The independent influence of the factors concerned in "stiff-lamb" disease would be an interesting problem to study under strictly controllable experimental conditions. For instance the use of purified diets would be very valuable to investigate the influence of calcium and phosphorus on the development of vitamin E deficiency. Magnesium would be significant to study under these conditions because it is known to be an important factor in abnormal muscular and bone calcification as has been demonstrated by Orent, Kruse and McCollum (1934), Tufts and Greenberg (1936) and Moore et al. (1938). Potassium also would be of interest because its depletion has been shown to bring along muscular paralysis (Ruegamer et al. 1946; Berlin 1946).

One of the most interesting findings of this work was the regular appearance of heart injuries in vitamin E-deficient lambs, since all previous reports had indicated that heart damage was only an occasional finding in "stiff-lamb" disease. It is difficult to explain all of the different manifestations of the so-called vitamin E deficiency in the lamb. However, it must be stated that the older work on this problem, using mixtures of natural feed ingredients, did not allow for strict control of variations in other constituents of the ration.

It is quite possible that in the present study conditions were more favorable for the production of heart injury; the vitamin E deficiency effect could have been modified by the interaction of other factors present in the natural ration. The work of Dam (1944b) gives support to this explanation since it was demonstrated that the symptoms of vitamin E deficiency in the chicken depended on the iodine number of the fat contained in the diet.

This reasoning logically leads to a consideration of cardiovascular diseases in the human, some of which may be related to vitamin E.

Due to technical limitation in experiments carried out on humans, it has been extremely difficult to get the two different schools of thought to concur concerning the importance of vitamin E on the cure of cardiovascular disease. Shute and collaborators found vitamin E a very useful tool in the treatment

of certain heart diseases[1]; whereas other workers have shown that vitamin E does not have a beneficial action in the same cardiovascular diseases reported by Shute[2].

Many animal species have been found to require vitamin E for the prevention of a number of disorders related to the circulatory, muscular, reproductive, nervous, digestive and excretory system. Therefore, it is very unlikely that, in this respect, the human could be an exception.

In the light of this and other recent investigations of the physiological importance of vitamin E, one is inclined to support the thesis that vitamin E deficiency, or a condition associated with vitamin E deficiency, may occur in the human; and that one of its manifestations could involve the heart. In such cases, the vitamin E deficiency no doubt might be alleviated by treatment with an active form of vitamin E which the

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[1] Vogelsang, A. B., E. V. Shute and W. E. Shute (1948) proposed that vitamin E may play a helpful role wherever better capillary circulation or oxygen utilization can improve any pathological condition like: indolent ulcers, arteriosclerotic gangrene, thrombophlebitis, phlebothrombitis, thromboangitis obliterans, mazoplasia, cerebral thrombosis, neutocirculatory asthenia, nephritis, purpura, and heart disease. In this last disease he reported improvement in 80% of the cases.

[2] Discussion of E. V. Shute's paper: "Notes on the Use of Alpha-Tocopherol in the Management of Acute and Subacute Vascular Obstruction as well as in Burns", presented at the 1949 International Convention on vitamin E. (Shute 1949).

human organism could readily metabolize[1].

Since it seems doubtful that vitamin E deficiency alone could be the cause of the heart diseases in question, it follows that vitamin E therapy can not always be an effective treatment. Therefore, until vitamin E deficiency can be identified unequivocally as the cause of some heart ailments, we must agree with Vogelsang et al. (1947) that "vitamin E is a mode of treatment but not a cure for heart disease".

At the beginning of the experiment it was interesting to observe the constant appearance of lesions in the right ventricle of the heart in the vitamin E-deficient lambs. The same findings had been mentioned in the report of Willman et al (1945). However, no explanation has been previously given to the phenomenum. One might postulate that the presence of macroscopic lesions only in the right ventricle was due to the difference of muscular mass between the two ventricles. Perhaps a thinner muscular wall could be affected easier than a thick one.

But there were other differences between the right and left ventricles which should be considered such as the differences in both mineral composition and vascularization of both ventricles.

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[1] Alpha tocopherol may not always be the most active vitamin E compound for the cure of vitamin E deficiency. Milhorat and co-workers (1949) found that alpha tocopherol or its esters were ineffective in patients with progressive muscular dystrophy, whereas dl-alpha-tocopherylhydroquinone reduced creatinuria in patients with similar disease and in rabbits deficient in vitamin E.

Myers (1942) reported that the left ventricle contained higher concentration of creatine, phosphorus, potassium and adenine than the right ventricle. Moe and Visscher (1940) found that the Thebesian veins are relatively insignificant factors in the nutrition of the left heart, whereas they were very important in the nutrition of the right heart. These veins empty into the heart chamber and therefore are subjected to the changes of heart loads. Thus, the nutrition of the right ventricle is dependent on factors affecting the right intraventricular pressure like mitral stenosis, pulmonary embolism, pulmonary fibrosis.

Autopsy of more vitamin E-deficient lambs showed the indiscriminate appearance of lesions in both sides of the heart. Consequently, it was reasoned that perhaps pneumonia was a factor producing more marked symptoms of vitamin E deficiency in the right side of the heart. However, the simultaneous study of the total relative size of injured areas in both sides of the heart and in the lungs did not disclose any relationship of pneumonia to the localization of injuries in the right ventricle of the heart.

In spite of these negative findings the relationship of vitamin E to the circulatory system still seems to be a possibility since vitamin E deficiency produces vascular disturbances in the chicken (Pappenheimer 1939) in the dog (Holman 1947 and 1949) and in the rat (Telford *et al.* 1945 and Mason

1943) and in the guinea pig (Farmer et al. 1950). Moreover Slagsvold and Lund-Larsen (1934) observed the presence of capillary bleeding and edema at autopsy of stiff lambs, which was also found in some of the vitamin E-deficient lambs of this experiment. Furthermore Vogelsang et al. (1947), Vogelsang (1950) concluded that vitamin E brings about clinical improvement of patients suffering from cardiovascular diseases. They proposed that vitamin E acted by: proliferating or reopening capillaries, direct normalizing effect on the myocardium, influence on the pacemaker of the heart and by direct action on thrombi.

The data on the arterial blood pressure gives additional support to the importance of the circulatory system in the development of the vitamin E deficiency. Lambs showing the symptoms of vitamin E deficiency had low diastolic and systolic blood pressure after a short period of high blood pressure. This previous increase of blood pressure, before the appearance of the vitamin E-deficiency symptoms, does not bear any reasonable explanation at the moment, since none of the changes occurring in the vitamin E deficiency was synchronous with or related to the blood pressure changes. The low blood pressure, recorded when the deficiency was well advanced, coincided with the findings of Telford et al. (1945) who, working with vitamin E-deficient rats, found that after one year they showed a reduction of 29.4% of systolic blood pressure as compared to normal

rats of similar age. In this work the average blood pressure of the lambs dropped 16% in only 69 days on a vitamin E-deficient diet.

According to the present knowledge, there are two possible ways by which the deficiency of vitamin E may alter the blood pressure. Ruppel (1949) believed that the decrease in blood pressure was due to the degenerative changes taking place in the muscles of the vessels, which would become as affected by the deficiency of vitamin E as the skeletal and white muscles. A second explanation could be derived from the work of Croxatto and Mois (1950). They showed in in vitro studies that alpha tocopheryl phosphate inhibited the enzyme hypertensinase which was responsible for the restraint of hypertensin. The larger the amount of alpha tocopheryl phosphate, the greater was the inhibition of hypertension by hypertinsinase. Therefore, it could be possible that such in vitro action of vitamin E could be duplicated in the living organism. This would provide another way of explaining the lowering of blood pressure in vitamin E deficiency, provided hypertension could be definitely shown to be essential to circulatory homeostasis, which has not been proven yet (Braun-Menendez et al. 1946).

Pathology:

The microscopic examination of the skeletal and heart muscles revealed that the histopathological alterations experimentally produced by the deficiency of vitamin E were similar to

those found in field cases of "stiff-lamb" disease and to the findings of Metzger and Hagan (1927), Willman et al. (1934) and Slagsvold and Lund-Larsen (1934). This, once more, confirmed the identity of the "stiff-lamb" disease.

The contribution of this study to the better knowledge of the pathological changes occurring in vitamin E deficiency can be summarized as: constant appearance of heart lesions progressing from the endocardium to the epicardium without damaging the purkinje fibers and the absence of giant cells in the degenerated cardiac muscle.

Blood Tocopherol:

The great variation in the initial values of blood plasma tocopherol of lambs pronosticated that lambs having high levels of tocopherol in the blood plasma would require a longer time to become vitamin E deficient than those having a small content of tocopherols. This was not proven to be true in the present experiment. There was not a consistent difference between lambs with high and low values of tocopherol, neither in the time required to show vitamin E insufficiency in the severity of the disease nor in the localization of injuries.

The total tocopherol content of lambs fed diet B decreased more rapidly than those fed diet A, which apparently was due not only to the lower tocopherol content of diet B, but also the high amount of peroxides, since the tocopherol value of control animals fed diet B was also lower than those fed diet A.

The low tocopherol content of E-depleted animals did not always mean appearance of external vitamin E symptoms. But lambs showing outward signs of vitamin E deficiency invariably had low values of tocopherol ranging from 0.047 to 0.081 micrograms per 100 ml. of blood plasma.

The effect of cod liver oil on the vitamin E-depleted lambs was clearly noticed in the tocopherol content of the blood plasma of animals fed E-deficient and E-containing diets. Cod liver oil decreased the tocopherol level of the blood plasma which confirmed the results obtained in cows by Ferrando and co-workers (1949).

Creatine and Creatinine Excretion:

The well known increase of creatine excretion in the urine again was reproduced in this experiment on vitamin E deficiency. The creatinuria ratio increased as well, but the creatinine excretion remained relatively unchanged during the whole experimental period.

Frequently the creatinuria was higher at the time the lambs started to show external manifestations of vitamin E deficiency. In spite of the fact that similar observation had been made about the blood plasma tocopherols, the high creatine excretion did not coincide with the low blood plasma content of tocopherols.

The creatinuria ratios calculated for the vitamin E-depleted lambs of this experiment were somewhat similar to those found by Whiting, Willman and Loosli (1949) in sheep fed natural feeds.

They considered a numerical creatinuria ratio larger than 1.3 as indicative of active muscular dystrophy. In this study the average creatinuria ratio of control animals was 1.32 while those of vitamin E-deficient lambs was 1.96.

The alpha tocopherol therapy of E-deficient lambs reduced the creatinuria of most of the animals except in one advanced case of vitamin E deficiency. Apparently the damage produced by the vitamin E insufficiency was largely irreversible because neither the creatinuria ratio nor the emaciation of the animal improved with the vitamin E treatment. This finding is not novel since Mason (1933) and Butturini (1949) have already reported that the degeneration produced by vitamin E is reversible with vitamin E therapy up to a certain point, but it becomes irreversible when connective tissue is substituted for the functioning parenchyma.

Neither cortisone or prostigmine corrected the abnormal creatinuria or creatinuria ratio of vitamin E-deficient lambs. Since only two lambs per group were treated with the drugs, it is not possible to draw a definite conclusion. It must be said however, that Martin (1946) found prostigmine effective in reducing the abnormal creatine - creatinine ratio of vitamin E-deficient rats and rabbits.

Mineral Changes in the Muscles:

Mineral changes observed in the present experiment are in almost complete agreement with the results obtained by Fenn and

Goettsch (1937) in vitamin E-deficient rabbits.

It was difficult to discern whether or not the vitamin E depletion directly affected the metabolism of some of the analyzed minerals because the antagonisms and synergisms existing between each other (Meltzer and Auer 1908; Orent, Kruse and McCollum 1934, Tufts and Greenberg 1936; Eppright and Smith 1937; Smith 1949 and Gillis 1950).

The most pronounced mineral disturbance of dystrophic muscles was the large Ca/Mg ratio, which was the result of a simultaneous increase in calcium and a decrease in magnesium. The investigation of Snyder and Tweedy (1942) showed that low serum magnesium diminished the serum phosphatase, and that this change was sufficient to increase calcification. Whether this was also the mechanism involved in the vitamin E deficiency remained to be studied.

The increase of muscular calcium could not be blamed as the agent causing paralysis of the skeletal muscles (Lu, Emerson and Evans 1941); nevertheless, excess of muscular calcium or magnesium could be factors influencing the muscular weakness of the lambs (Engel et al. 1949 and Bird 1949).

In spite of the importance of vitamin E in phosphorus metabolism, little or no effect on the phosphorus content of dystrophic muscles has been reported by Weissberger and Harris (1943). Basically this agrees with the slight difference in the phosphorus content of normal and dystrophic muscles found in this

study. Yet other workers (Lu, Emerson and Evans 1941) described marked changes in the total acid soluble phosphorus and in the pyro and organic ester of phosphorus.

A direct relationship between the vitamin E and potassium has been observed by Cutillo (1944). This can be indirectly supported by a series of additional investigations; thus, the following potassium deficiency symptoms reminded us of those occurring in vitamin E deficiency: muscular weakness (Gillis 1948), muscular paralysis (Ruegamer et al. 1946) and heart necrosis (Thomas et al. 1940; Schrader et al. 1937; Follis et al. 1942; Kornberg and Endicott 1946; Follis 1948; and Smith et al. 1950).

Also, other similarities have been found between the action of potassium and vitamin E on the important compound, acetylcholine. In fact, the liberation of acetylcholine from the nerve endings depended on the potassium ion (Brown and Feldberg 1935; Harvey and McIntosh 1939; and Welsh and Hyde 1944) and the deficiency of vitamin E produced a decrease of the cholinesterase enzyme (Bloch 1942, Hess and Biollier 1948).

Therefore, if potassium and vitamin E are related to acetylcholine, and acetylcholine is an essential compound for the transmission of electrical impulses, then potassium and vitamin E should play an important role in the muscular activity. Such seemed to be the case of potassium because Fenn (1940) reported that muscular activity leads to a loss of potassium in exchange for sodium, and Tower (1939) found that denervation of muscles

produced a decrease in the content of potassium and an increase in the levels of phosphorus and calcium of the muscles. Likewise, the close connection between muscular activity and vitamin E was evident through the experiments of Pappenheimer and Goettsch (1940) who found that denervated muscles of young rats did not develop signs of vitamin E deficiency when fed diets lacking vitamin E.

From this information it was reasonable to infer that acetylcholine and potassium should have a predominant role in the vitamin E deficiency. Hence, it was extremely interesting to investigate the influence of acetylcholine on the development of the vitamin E deficiency.

Prostigmine was the drug selected for the study of the influence of acetylcholine on the vitamin E deficiency, because of its availability and easiness of handling. Prostigmine preserves acetylcholine from destruction by cholinesterase (Goodman and Gilman 1949). Accordingly, prostigmine injections to vitamin E-deficient lambs should increase the severity of the disease in the same manner that prostigmine aggravated myotonia congenita (Goodman and Gilman 1949).

The definite effect of prostigmine on vitamin E deficiency in lambs could not be determined because of insufficient experimental animals. The results of the present experiment performed on two lambs seemed to indicate, in opposition to the proposed theory, that prostigmine injections attenuated the effects of vitamin E deficiency. This was evident through the histological

studies and the mineral and amino acid analyses of muscles. Slices of lamb's tissues showed degeneration but not as advanced as in the case of lambs not having any treatment, and the prostigmine therapy protected the levels of calcium, sodium, potassium, leucine and isoleucine of the muscles.

Before any effort can be made to explain these results it would be advisable to repeat the experiment with a larger number of lambs.

Increase in the sodium content of the muscles was another feature of vitamin E deficiency. In spite of this augment of sodium, no potassium diuresis was noticed at any time, which disagrees with the findings of Leaf et al. (1949). Otherwise, the increase of sodium in the muscles was foreseen because sodium is known to enter the tissues to replace potassium when the organism is depleted of the latter element (Orent-Keiler and McCollum 1941).

Amino Acids:

The work of Steinberg (1949) on the relation of vitamin E to collagen in rheumatic fever indicated that there might be a change in the amino acid composition of the muscles caused by the lack of vitamin E.

If the collagens were affected during the deficiency of vitamin E, then probably the muscles should contain different levels of those amino acids which mark the difference between the collagen and muscle protein. According to the Tables of

Block and Bolling (1951) those differential amino acids were: arginine, lysine, leucine, isoleucine, glutamic acid, glycine and proline.

This selection criteria on the amino acids most likely to be disturbed by vitamin E deficiency was supported by the hypotheses of Mason (1944) and Herschel (1946) and the work of Roderuck (1949). Mason (1944) pointed out the possibility that a defective production or utilization of arginine may take place in the metabolic dysfunction of dystrophic muscles. Herschel (1946) theoretically considered the relationship between alpha tocopherol and the metabolism of the amino acids, and concluded that cystine and isoleucine especially should be involved in vitamin E-deficient organisms. Roderuck (1949) found that the glutamine content of dystrophic guinea pig's muscles was only about one-third as much of the glutamine content of the muscles from control animals.

In the present work, the leucine and isoleucine levels of dystrophic muscles were found to be lower than those in the muscles of control animals, which partly confirmed Herschel's hypothesis. Leucine has been shown as essential for the formation of plasma protein and hemoglobin (Madden et al. 1945). Isoleucine however seemed to be less important in the synthesis of the same blood constituents, since only small amounts of isoleucine were required for the regeneration of human plasma protein and hemoglobin (Albanese 1945). Hence, it could have been possible that vitamin E deficiency may have altered the

normal content of blood plasma proteins and hemoglobin. Most of the lambs in the present experiment had levels of hemoglobin comparable to those of control lambs. But in general, severe cases of vitamin E deficiency had hemoglobin values of almost half of control levels. Therefore, it must be concluded that, in the majority of the lambs, changes in the leucine levels of skeletal muscle occurring in vitamin E deficiency could not be verified by the determination of blood hemoglobin.

Regarding the proline and glycine content of the dystrophic muscles in this work, no change was noticed in vitamin E deficiency. It was rather surprising that the glycine content of the muscles was not modified by the lack of vitamin E in the diet, because glycine has been shown to be one of the creatine specific precursors (Bloch and Shoenheimer 1941, Almquist et al. 1941) and creatine has been found to diminish in dystrophic muscles (Houchin and Matill 1942).

Recent research work on cortisone has demonstrated the beneficial value of this drug for the treatment of collagen disease and other disorders (Engleman 1950). Similarly successful has been the use of cortisone in several disorders of muscular functions like, Myotonia congenita (McEachern 1951), menopausal muscular myopathy (Shy et al. 1950 and McEachern 1951) and postoperative stiffness of the hand (Baxter et al. 1950).

Vitamin E deficiency in the lamb is essentially a disease affecting the muscular and connective tissues of skeletal mus-

cles, therefore cortisone therapy could be useful for its cure. The experiment proved that cortisone therapy produced some relief from the vitamin E deficiency. During the initial part of the cortisone treatment the lambs' appearance was bettered, though later they looked the same as vitamin E-deficient lambs. Still, the slices of muscular tissue showed that there had been some improvement as a consequence of the cortisone therapy. The chemical analyses revealed that cortisone had a protective action on the levels of potassium, leucine and isoleucine of the muscles.

The proline content of skeletal muscles was high. This was in a certain degree unexpected because the previous work of Umbreit and Tonhazy (1951) pointed out that the rate of proline oxidation in kidney homogenates of adrenalectomized rats was related to the amount of cortisone supplied.

There is a great deal of evidence proving that vitamin E enters in the metabolism of proteins (Hove and Harris 1947a and b; Moore 1948; Kemeneny et al. 1949; Lindan and Himsworth 1950), which is indirectly supported by the effect of vitamin E deficiency on the amino acid composition of the muscles.

Since the action of vitamin E is so widely spread in most of the systems of the organism, as has been pointed out in the review of literature, then perhaps the most important effect of vitamin E can be exerted on the metabolism of proteins, which subsequently could induce alterations in other metabolic processes. For instance, if vitamin E would influence a basic

metabolic reaction common to certain proteins, likewise, it could induce changes in determined enzymes and hormones in charge of other nutrient's metabolism since they themselves are proteins.

On the other hand, there are many facts pertaining to vitamin E deficiency that seem to have some connection with the mechanisms of the muscular contraction (Knowlton and Hines 1938). Furthermore the intensive studies of Szent-Gyorgi (1947 and 1949) on the muscle chemistry point toward the same direction. For example, stiffness of muscles occurring in vitamin E deficiency could be produced by the decrease of adenosine triphosphate (ATP) since it was found that rigor mortis was caused by the lack of ATP. The striking effect of the ions sodium, potassium and magnesium on the physical state, charge and solubility of myosin would suggest that the disturbance in the mineral content of vitamin E-deficient dystrophic muscles would lead to the modification of the myosin properties and, subsequently, to the alteration of the muscle's physical properties.

Actin has also been shown to have the amazing property (Szent-Gyorgi 1949) of forming thin molecular threads which are able to develop cross striations. The lack of striation in the muscle fibers of dystrophic muscles produced by the deficiency of vitamin E has been described in this and many other investigations. Does this mean a disturbance in the nature of actin caused by the vitamin E deficiency? Certainly

it would be most interesting to find out whether these important proteins, actin and myosin, or ATP are influenced by vitamin E.

Electrocardiogram:

The scarcity of information on the sheep's normal electrocardiogram and absence of any concerning the lamb's ECG were some of the original limitations in this investigation. For this reason it is probably unfortunate that only a few animals could be used as controls.

Notwithstanding, it was remarkable to find that the ECG of control lambs did not change considerably during the first 81 days of life, while they were still fed a liquid diet. Mullick et al. (1948) working with older sheep, however, have reported definite variations in serial ECGs on their control animals.

The most consistent change in serial ECGs of vitamin E deficient lambs was an increase in the duration of the PR interval, whereas this interval in the control lambs was identical in all serial records. Those becoming deficient in vitamin E showed a gradual increase until the maximum value of 0.12 millivolt was attained.

The increment of the PR interval found in the E-deficient lambs reported in the present work was in agreement with the results of Gullickson and Calverley (1946) who observed that cattle fed a vitamin E-deficient ration for one and one-half years showed augmentation of the PR interval, in some instances

as high as 0.08 and 0.12 millivolt. The lengthening of the PR interval in vitamin E-deficient lambs of this experiment was less marked than in cattle (0.02 to 0.04 millivolt) but it was consistent in the majority of the records, and appeared after only 81 days.

A remarkable feature of the ECGs of some of the vitamin E-deficient lambs was the sudden appearance of pronounced disorders in the character, voltage and sequence of the various deflections. In certain cases, there were large increments in the T and QRS voltages along with typical sharp T waves. In other instances, the changes were inversion of the T waves in lead III and depression of the ST segments. Similar changes have been reported by Bragdon and Levine (1949) who found that the ECG of vitamin E-deficient rabbits developed elevations of the ST segments and inversion of the T waves in lead II during the latter part of the observation period.

The findings in neither the macro and micropathological study nor the chemical analyses of the heart muscle of vitamin E-deficient lambs showing "acute" and "chronic" heart involvement could be correlated with changes in the ECG. It was amazing to find that even though hearts appeared seriously injured by the deficiency of vitamin E, yet the ECG of the lambs did not show any abnormality whatsoever. Such was the case of lamb No. 12 (Fig. 4) whose ECG, taken 20 days before the death of the animal, did not differ at all from the ECG of control lambs, but the microscopic observation showed extensive degeneration

of the cardiac muscle (Fig. 12). Could these lesions be produced in only twenty days? Our opinion is that it is not likely, since ECGs of other lambs taken a few days before their sacrifice did not show any abnormality although the macro and micropathological examination revealed lesions of the cardiac muscle.

It is possible that the pattern of distribution of the lesions throughout the cardiac muscle might afford an explanation for these differences. For example, this supposition is rendered more tenable when the distribution of the heart lesions in lambs showing "acute" heart involvement (Fig. 13) were compared to that of an E-deficient lamb not showing abnormal ECG (Fig. 14). It could be observed that the necrotic areas of the latter were evenly distributed and covered all the ventricular wall, while those of the "acute" heart involvement had lesions localized in a defined area (Fig. 13).

Lambs fed diet A and supplemented with alpha tocopherol and cod liver oil resulted in an increase in the duration of the PR and QT intervals. These findings did not coincide with the work of Barnes et al. (1938) who not only found that the ECG of calves was not influenced by cod liver oil feeding but neither were cardiac lesions found as a result of this treatment. In the present work it was quite apparent, through the tocopherol content of the blood plasma and increased severeness of vitamin E deficiency in lambs supplemented with cod liver oil, that it was definitely an antagonistic factor to vitamin E.

Figure 12. A microphotograph of a longitudinal section of cardiac muscle from vitamin E-deficient lamb No. 12. The tissue shows degeneration, necrosis, and absence of muscle fibers, whereas the Purkinje fibers seem to be normal. Hematoxylin and Eosin stain x 170

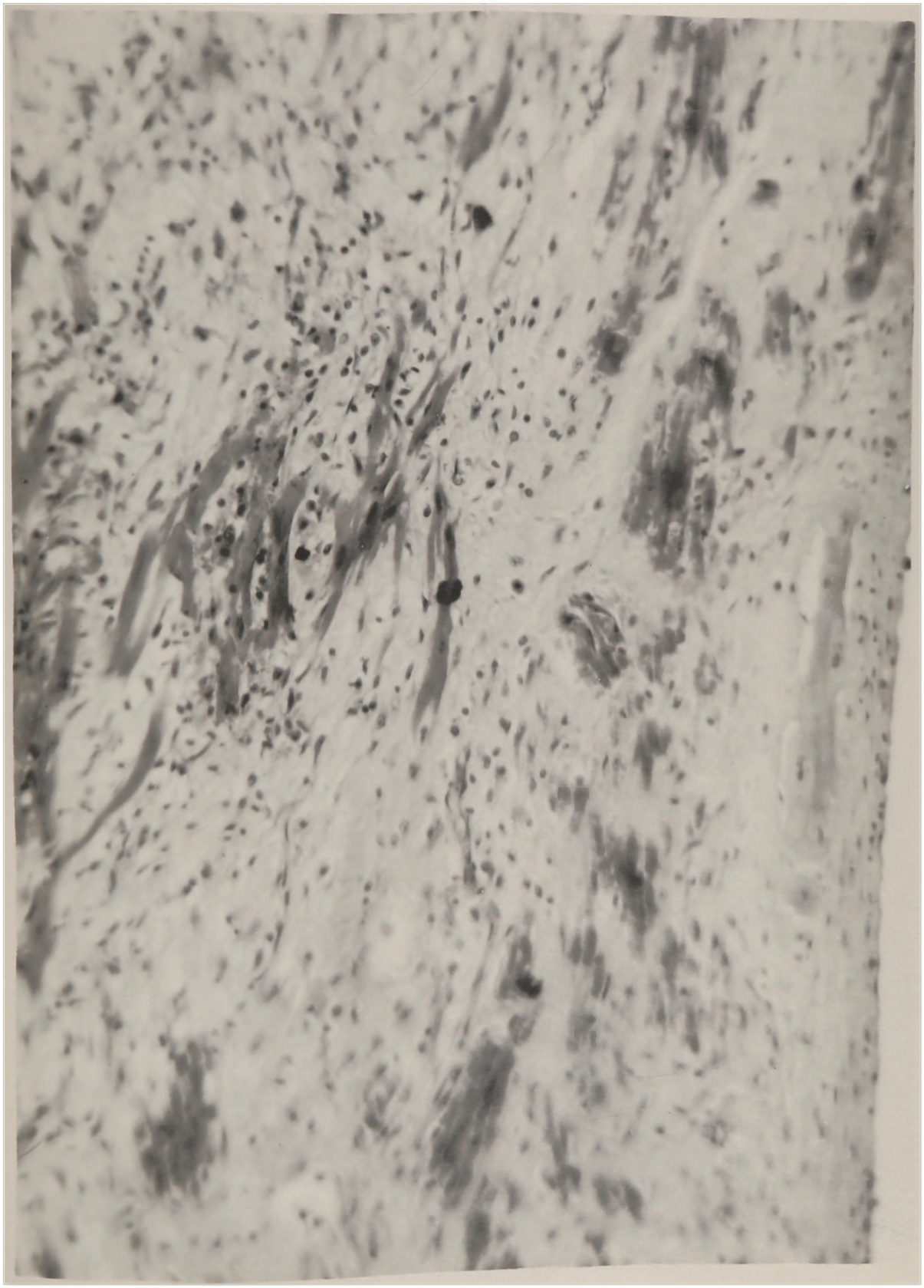


Figure 13. Localized degeneration and calcification of the endocardium occurring in lamb No. 24 caused by the vitamin E deficiency.

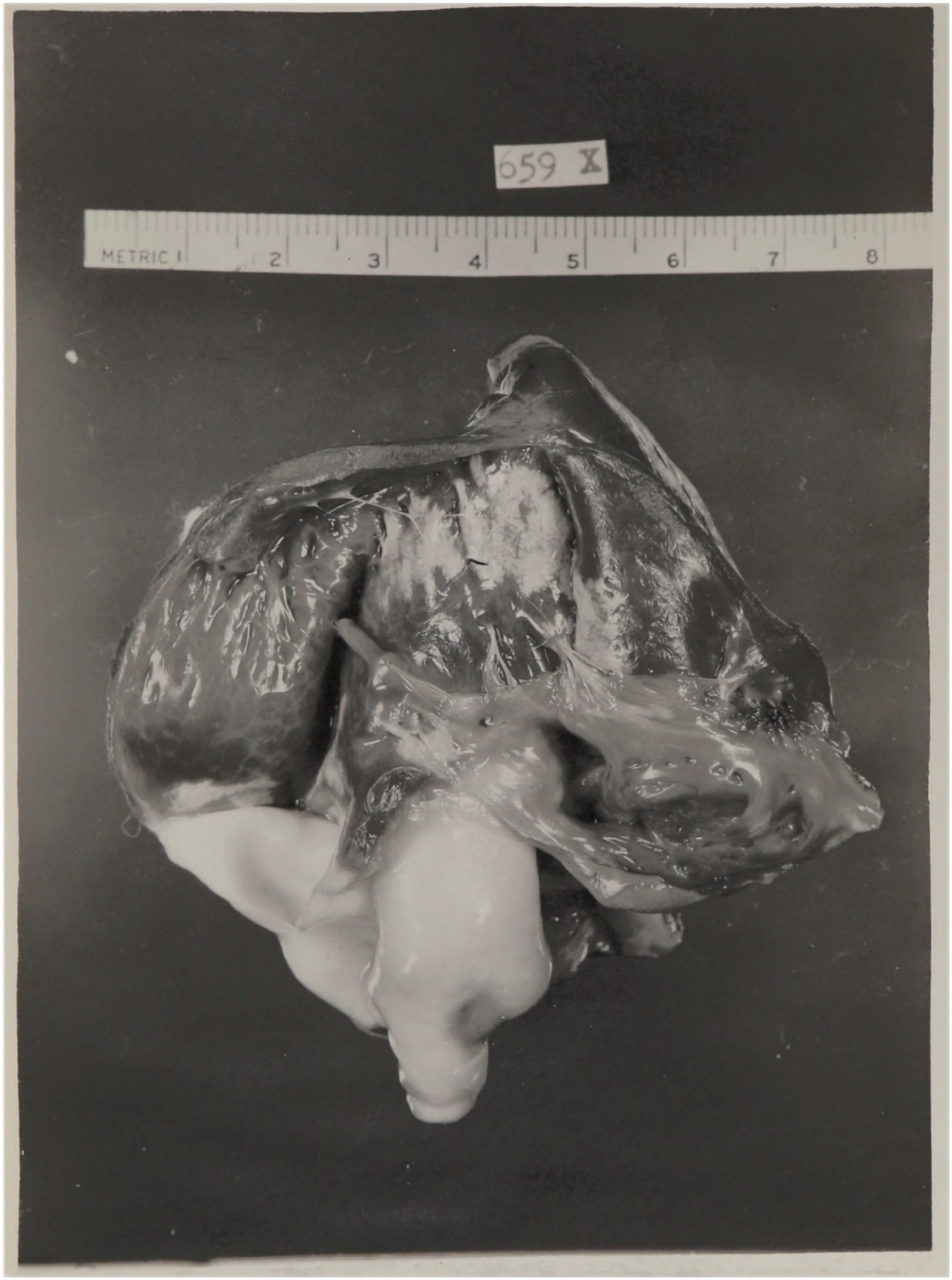
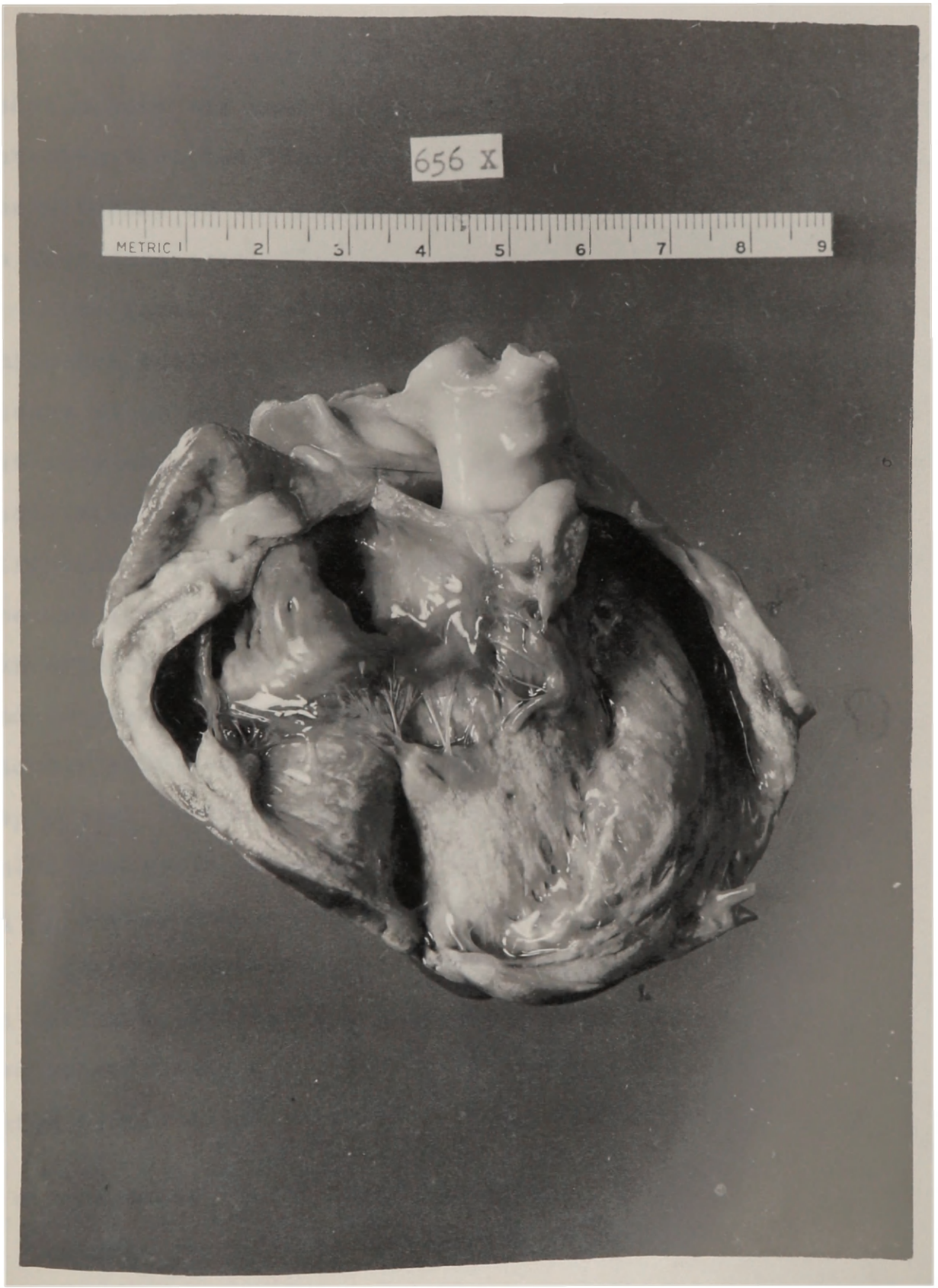

659 X
METRIC 1
2
3
4
5
6
7
8

Figure 14. Extensive and scattered degeneration and calcification on the endocardium of lamb No. 12 produced by the deficiency of vitamin E.


656 X
METRIC 1 2 3 4 5 6 7 8 9

It has been shown above that the mineral composition of the heart muscle was modified by a deficiency of vitamin E. Since investigation has been carried out on the influence of some of the minerals on the ECG of several animals, it is worthwhile to look into some of those findings.

The level of potassium in the organism has an outstanding influence on the ECG of mammals and birds. In the dog, a deficiency of potassium increased the PR interval (Spealman 1942) but an excess of potassium produced sharp T waves of high amplitude (Nicholson 1937; Thomson 1939). In cattle, Sykes and Alfredson (1940) reported that low potassium rations prolonged the QRS intervals of the calves' ECG. The conductive system was affected by the deficiency of potassium in humans, cattle and chickens (Brown et al. 1944; Sykes and Alfredson 1940; and Sturkie 1950). Besides, in humans, potassium deficiency produced depression of the ST segments, voltage lowering or inversion of the T waves and increase in duration of the QT intervals (Brown et al. 1944; Holler 1946; Engel et al. 1949).

These observations show great resemblance between the ECGs in potassium and in vitamin E deficiency which should not be overlooked because, as discussed above, there is some evidence which points to an interaction between vitamin E and potassium.

Field Cases of "Stiff-Lamb" Disease:

Except for certain differences in the amino acid content of muscles, the equality of vitamin E deficiency and "stiff-

lamb" disease has been proven by various chemical and pathological studies.

The symptom of bilaterallity of the muscular dystrophy was more noticeable in stiff lambs due to the more marked calcification of their muscles. At this time it should be mentioned that the only three possible ways muscular dystrophy can appear bilaterally in the muscular system is by affecting the circulatory system, the nervous system or the mechanism of the muscular activity, since the latter is also evenly distributed on both sides of the body. Therefore, it would seem that vitamin E must play a role in either system or on the muscular activity.

The histological studies revealed that field cases of "stiff-lamb" disease were more severely affected than the experimental vitamin E-deficient lambs. This probably was due to the additional effect of several factors, like the absence of vitamin E in the diet of the dams, low tocopherol content of colostrum and milk, the physiological changes produced by the rumen development, and other factors like minerals and specific antagonists to vitamin E that may sensitize the lamb to the attack of the vitamin E deficiency.

Whiting et al. (1949) showed that lambs became stiff when the dams had been fed "stiff-lamb ration" which produced lowering of the blood plasma tocopherol of newborn lambs and of the colostrum and milk of the ewes.

The rumen development of young animals has been shown to bring along several physiological changes, outstanding among

which was that young ruminants had a higher glucose tolerance test than adult ruminants (McCancless et al. 1950). Whether the glucose tolerance or other factors related to the beginning of the ruminal activity are critical in the development of lamb's stiffness will have to be determined by further research. But there is no doubt that the rumen must have a certain importance in the deficiency since the report of many investigators correlated the appearance of "stiff-lamb" disease with the first day of grazing.

The observations of Metzger (1927), Slagsvold and Lund-Larsen (1934), Cheng (1945) and Willman et al. (1945) pointed out that the appearance of heart lesions in cases of "stiff-lamb" disease was only occasional. Field cases of "stiff-lamb" disease were examined while this work was carried out, but extensive heart injuries were observed in all of them. Nevertheless, it could be possible that some lambs may be more susceptible to the heart lesions produced by the insufficiency of vitamin E than others, since in the experimental group, even though all the lambs consistently showed heart injury, the extent and severity of the cardiac damage was variable. Perhaps the high susceptibility of certain lambs was due to a particular anatomic pattern of the coronary vessels, as has been found in coronary arteriosclerosis of human hearts (Schlesinger 1940).

Concluding Remarks:

The present investigation has demonstrated for the first time that vitamin E is required for the adequate nutrition of the lamb. The vitamin E deficiency was found similar to the malady known as "stiff-lamb" disease.

Histological and chemical changes of various degrees have been observed in dystrophic muscles produced by vitamin E depletion. The chemical analyses showed many different possibilities of interrelation between vitamin E and the metabolism of amino acids and minerals.

Also, it was proven that the sudden death of lambs deficient in vitamin E was caused by heart failure.

SUMMARY AND CONCLUSIONS

Vitamin E deficiency has been studied in a group of 51 lambs by feeding liquid purified diets which differed only in the nature of the fat components. Diet A contained commercial lard, and diet B a molecular distilled lard having a high concentration of peroxides.

Findings of the present investigation can be summarized as follows:

Growth:

1) Vitamin E deficiency was shown to influence the growth of lambs especially when peroxides were present in the diet.

Symptoms:

1) The main symptoms of vitamin E deficiency were weakness of the skeletal muscles which appeared to be accelerated by the presence of peroxides in the diet.
2) Oral feeding of 100 mg. of dl-alpha tocopherol prevented and cured the symptoms of vitamin E deficiency.

Pathological Studies:

1) Microscopic examination of field cases of "stiff-lamb" disease and vitamin E deficient lambs showed the equality of the two disorders.

2) The heart was consistently found affected by the vitamin E deficiency.

Blood Studies:

1) Vitamin E deficiency lowered the alpha tocopherol content of the blood plasma.
2) The initial values of alpha tocopherol in the lamb's blood plasma did not influence the time required to become deficient, the severity of the disease or the localization of the heart lesions.
3) All lambs showing symptoms of lack of vitamin E had low blood plasma tocopherol content.
4) When diet B containing a high amount of peroxides or diet A plus cod liver oil were fed to the lambs, the alpha tocopherol content of the lamb's blood plasma was lowered faster than in those fed diet A alone.
5) In general, lambs deficient in vitamin E had lower blood pressure than control animals.

Creatine-Creatinine Studies:

1) Creatine and creatinuria ratios of the urine were increased in lambs deprived of vitamin E.
2) Alpha tocopherol therapy reduced the creatine and creatinuria ratio in lambs that were not severely affected with vitamin E deficiency.

Mineral Studies:

1) An increase in the concentration of calcium and sodium and a decrease in the content of potassium was found in dystrophic thigh muscles.
2) Heart muscles of E-deficient lambs had a low content of potassium and a slightly high content of phosphorus.
3) The diet B containing the high amount of peroxides produced a slight increase of calcium in the heart muscles and decrease of magnesium in the thigh muscles.
4) An interaction between vitamin E and potassium seems possible.

Amino Acid Studies:

1) The vitamin E insufficiency produced decrease of the leucine and isoleucine content of skeletal muscles and also of the isoleucine content of heart muscles.
2) The proline and glycine levels of dystrophic muscles of lambs were similar to those of control animals.

Drug therapy:

1) In general, it can be stated that vitamin E-deficient lambs treated with prostigmine and cortisone showed a certain improvement.

Electrocardiographic Studies:

The electrocardiograms of lambs depleted in vitamin E were divided into three categories:

1) ECG of vitamin E-deficient lmabs showing acute cardiac involvement, which had increased PR intervals, increased heart rate, and sudden changes in the ECG of tachycardia, disorganization of the heart beat, sharp T waves of high voltage and depression of the ST intervals.
2) ECG of vitamin E-deficient lambs showing chronic cardiac involvement, which had increased PR intervals and decreased heart rates.
3) ECG of vitamin E-deficient lambs not showing any electrocardiographic disorder, even though they had degenerative lesions in the heart muscle.

Concluding Statements:

1) Vitamin E is an essential vitamin for the adequate nutrition of the lamb.
2) Vitamin E deficiency retards the growth of young lambs.
3) Vitamin E deficiency produces degeneration of the heart and skeletal muscles of the lamb.
4) Vitamin E-deficient lambs excrete large amount of creatine in the urine.

5) Dystrophic skeletal muscles of vitamin E-deficient lambs have an abnormally high content of calcium and sodium and low content of potassium.

6) Vitamin E deficiency and "stiff-lamb" disease (field cases) produce similar histological disturbances and changes in the content of calcium, sodium and potassium of the skeletal muscles.

7) Injury of the heart muscle produced by vitamin E deficiency is reflected, in the majority of cases, by increase of the PR interval in the electrocardiogram of lambs.

APPENDIX

TABLE XXIV

THE HEART RATE, BAZETT'S CONSTANT K, DURATION OF THE INTERVALS, QRS POTENTIALS AND ELECTRICAL AXIS OF THE ELECTROCARDIOGRAM OF LAMBS FED VITAMIN E DEFICIENT DIETS

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds)			K (Bazett's formula)	QRS potentials (millivolts)				Electrical Axis (degrees)
		PR	QRS	QT		L_1	L_2	L_3	total	
Lamb 37 (Diet B plus α-tocopherol)										
8	150	0.08	0.06	----	----	D 0.35	-0.50	-0.80	1.65	-90
18	214	0.08	0.06	0.20	0.38	D 0.40	-1.00	-1.25	1.65	-90
25	188	0.08	0.06	0.18	0.32	D 0.75	-0.90	-1.40	3.05	-90
32	167	0.08	0.06	0.22	0.37	D 0.40	-1.75	-2.20	4.35	-90
39	167	0.08	0.06	0.20	0.33	D 0.70	-1.10	-1.40	3.20	-90
46	167	0.08	0.06	0.22	0.37	↓ 0.50	-1.20	-1.50	3.20	-60
53	214	0.08	0.06	0.20	0.38	D 0.80	-1.50	-1.60	3.90	-90
60	158	0.08	0.06	0.24	0.39	↓ 0.80	-0.70	-0.95	2.45	-60
67	150	----	0.06	0.24	0.38	D 1.20	-0.75	-1.25	3.20	-90
74	125	----	0.06	0.26	0.38	D 0.70	-0.60	-1.20	2.50	-90
81	125	0.08	0.06	0.26	0.38	D 0.55	-0.45	-0.75	1.75	-90
Lamb 40 (Diet B plus α- tocopherol)										
5	188	----	0.06	----	----	-0.30	-0.60	D 0.35	1.25	-120
15	188	----	0.06	0.22	0.39	-0.40	-0.60	D 0.35	1.35	-150
22	125	----	0.06	0.24	0.35	-0.55	-0.65	D 0.35	1.55	-150
29	150	0.08	0.06	0.22	0.35	-0.70	-0.60	D 0.20	1.50	-150
36	136	----	0.06	0.24	0.36	-0.75	-0.75	D 0.25	1.75	-150
43	150	----	0.06	0.22	0.35	-0.70	-0.70	↓ 0.40	1.80	-180
50	167	0.08	0.06	0.22	0.37	-0.70	-0.60	D 0.30	1.60	-120
57	167	----	0.06	0.22	0.37	-0.30	-0.45	- 0.20	0.95	-170
64	143	----	0.06	0.24	0.37	-0.80	-0.75	↓ 0.25	1.80	-150
71	136	----	0.06	0.24	0.36	-0.65	-0.60	↓ 0.25	1.50	-180
78	111	----	0.06	0.26	0.35	-0.50	-0.30	↓ 0.25	1.05	-120

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 41 (Diet B plus α-tocopherol)						
5	250	----	0.06	0.18	0.37	-0.10	D0.50	D0.25	0.85	-120
15	167	0.08	0.06	0.22	0.37	-0.05	-0.30	-0.20	0.55	-120
22	107	0.08	0.06	0.30	0.37	-0.10	D0.50	-0.35	0.95	-120
29	125	0.08	0.06	0.26	0.38	-0.15	-0.30	-0.20	0.65	-120
36	150	----	0.06	0.22	0.35	-0.15	-0.45	-0.20	0.80	-120
43	150	0.08	0.06	0.22	0.35	-0.15	-0.45	-0.35	0.95	-120
50	136	----	0.06	0.24	0.36	-0.15	-0.45	D0.30	0.85	-150
57	130	----	0.06	0.26	0.38	-0.10	-0.35	-0.25	0.70	-120
64	167	----	0.06	0.22	0.37	-0.35	-0.40	+0.30	1.05	-180
71	120	0.08	0.06	0.26	0.37	-0.20	-0.55	D0.40	1.15	-150
78	115	----	0.06	0.28	0.43	-0.25	-0.35	D0.15	0.75	-150
				Lamb 13 (Diet A plus α-tocopherol and cod liver oil)						
39	107	0.08	0.06	0.30	0.40	-1.70	-1.50	-0.10	3.30	-150
50	150	0.10	0.06	0.30	0.47	-2.20	-2.00	-0.10	4.30	-150
				Lamb 15 (Diet A plus α-tocopherol and cod liver oil)						
39	100	0.10	0.06	0.32	0.41	-0.40	-0.30	+0.20	0.90	-180
50	107	0.12	0.06	0.32	0.40	-0.40	-0.30	+0.10	0.80	-180
60	79	0.12	0.08	0.42	0.46	-0.20	-0.20	+0.10	0.50	-180
				Lamb 12 (Diet A)						
69	150	0.10	0.06	0.24	0.38	-1.05	-1.20	-0.45	2.70	-180
				Lamb 14 (Diet A)						
69	232	0.06	0.04	0.18	0.35	+1.50	+2.00	+1.50	5.00	+ 60

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 19 (Diet A)						
67	150	0.10	0.04	0.22	0.35	-0.10	-0.50	-0.50	1.10	-90
				Lamb 24 (Diet B)						
34	167	0.10	0.06	0.22	0.37	-1.20	+0.25	-0.60	2.05	-180
45	201	0.10	0.06	0.22	0.40	+0.20	-1.70	-1.50	3.40	-90
55	158	0.10	0.06	0.22	0.36	+0.50	-0.80	-0.70	2.00	-60
				Lamb 32 (Diet B)						
16	150	----	0.04	----	----	-0.70	-0.65	-0.45	1.80	+150
27	167	0.08	0.04	0.20	0.33	-1.30	-0.80	-0.60	2.70	+180
				Lamb 34 (Diet B)						
12	158	0.08	0.06	0.24	0.39	-0.10	D0.50	D0.40	1.00	+150
22	136	----	0.06	0.26	0.39	-0.10	D0.35	-0.20	0.65	+150
29	188	0.08	0.06	0.22	0.39	-0.10	D0.20	+0.20	0.50	+150
36	167	----	----	0.24	0.40	-0.10	-0.10	+0.20	0.40	+ 30
43	167	0.10	0.04	0.22	0.37	+0.20	-0.10	0	0.30	+ 30
50	188	----	0.04	0.20	0.35	-0.10	-0.10	+0.20	0.40	+150
57	125	----	----	0.24	0.35	-0.10	0	0	0	+150
64	107	0.12	0.04	0.26	0.35	-0.60	-0.30	+0.20	1.10	+180
				Lamb 34 (Diet B and prostigmine injection)						
71	167	0.12	0.04	0.20	0.33	-0.40	-0.30	+0.10	0.80	+180
78	150	----	0.04	0.22	0.35	-0.30	-0.10	0	0.40	-150
85	167	0.10	0.04	0.20	0.33	-0.10	-0.10	0	0.20	-170

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 38 (Diet B)						
5	232	0.08	0.04	0.16	0.31	-0.50	-0.65	+0.10	1.25	-120
15	232	0.08	0.04	0.18	0.35	-0.60	-0.45	+0.15	1.20	-180
22	177	0.06	0.06	0.22	0.38	-0.60	-0.50	+0.15	1.25	-150
29	150	0.08	0.06	0.24	0.38	-0.70	-0.55	+0.30	1.55	-150
36	188	0.08	0.04	0.20	0.35	-0.70	-0.55	+0.10	1.35	-150
43	158	0.10	0.04	0.22	0.36	-0.60	-0.50	+0.10	1.20	-150
50	136	0.10	0.04	0.24	0.36	-0.45	-0.45	+0.15	1.05	-180
57	136	0.10	0.04	0.28	0.42	-0.20	-0.30	+0.10	0.60	-120
				Lamb 38 (Diet B and prostigmine injection)						
64	214	0.08	0.04	0.20	0.38	0	-0.40	-0.10	0.50	-120
71	177	0.08	0.04	0.22	0.38	-0.45	-0.40	+0.20	1.05	-150
78	167	0.08	0.04	0.24	0.40	-0.50	-0.50	+0.10	1.10	-150
				Lamb 45 (Diet B)						
7	150	0.08	0.06	----	----	D0.30	D0.30	+0.10	0.70	+ 30
14	150	0.08	0.04	0.22	0.35	D0.10	-0.20	+0.05	0.35	-150
21	136	0.10	0.06	0.22	0.33	-0.30	-0.30	+0.15	0.75	-180
28	130	0.12	0.06	0.26	0.38	-0.35	-0.30	+0.15	0.80	-180
35	125	0.12	0.04	0.26	0.38	0	-0.30	-0.20	0.50	-120
42	120	0.12	0.04	0.26	0.37	-0.30	-0.60	+0.30	1.20	-150
49	115	0.12	0.06	0.26	0.36	-0.30	-0.25	+0.40	0.95	-180
56	125	0.10	0.06	0.28	0.41	+0.60	+0.20	-0.55	1.35	-180
				Lamb 45 (Diet B and cortisone injection)						
63	107	0.12	0.06	0.24	0.32	-0.10	-0.15	-0.10	0.35	- 30
70	107	0.10	0.04	0.26	0.35	0	-0.20	+0.15	0.35	-180

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 46 (Diet B)						
7	136	0.08	0.06	0.26	0.39	-0.70	-0.70	+0.20	1.60	-170
14	88	0.10	0.06	0.28	0.34	-0.40	-0.50	+0.20	1.10	-170
21	91	0.08	0.06	0.30	0.37	-0.40	-0.50	+0.20	1.10	-170
28	143	0.10	0.06	0.24	0.37	-2.10	-1.60	+0.15	3.85	-180
35	120	0.08	0.06	0.24	0.34	-0.80	-0.90	+0.05	1.75	-150
42	115	0.09	0.04	0.26	0.36	-1.00	-0.80	+0.05	1.85	-150
49	111	0.09	0.04	0.32	0.44	-0.80	-0.70	+0.10	1.60	-150
				Lamb 46 (Diet B and cortisone injection)						
56	115	0.10	0.06	0.24	0.33	-0.90	-0.80	+0.25	1.95	-180
63	150	0.11	0.04	0.28	0.44	-0.60	-0.60	+0.15	1.35	-150
70	115	0.09	0.06	0.24	0.33	-0.50	-0.20	+0.35	1.05	-180
				Lamb 47 (Diet B)						
7	167	0.08	0.06	0.22	0.37	-0.60	-0.35	+0.20	1.15	+180
14	150	0.08	0.06	0.22	0.35	-0.75	-0.70	+0.30	1.75	+180
21	158	0.08	0.06	0.22	0.36	-1.15	-0.90	+0.20	2.25	+180
28	150	0.08	0.06	0.24	0.38	-1.50	-0.75	+0.15	2.40	+180
35	115	0.08	0.06	0.28	0.39	-0.65	-0.55	+0.20	1.40	+180
42	107	0.08	0.06	0.30	0.40	-0.70	-0.50	+0.30	1.50	+180
49	94	0.10	0.06	0.30	0.38	-0.65	-0.45	+0.35	1.45	+180
56	125	0.12	0.06	0.28	0.40	-0.50	-0.50	0	1.00	+180
63	115	0.11	0.06	0.28	0.39	-0.65	-0.35	0	1.00	+180
70	136	----	0.06	0.26	0.39	-0.75	-0.50	0	1.25	+180

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 48 (Diet B)						
7	250	0.08	0.04	0.16	0.33	-0.80	-1.05	+0.35	1.20	+180
14	167	0.08	0.04	0.22	0.37	-0.75	-0.50	+0.45	1.70	+180
21	167	0.08	0.04	0.20	0.33	-1.00	-0.70	+0.45	2.15	+180
28	214	0.08	0.04	0.18	0.34	-1.45	-0.85	+0.80	3.10	+180
				Lamb 42 (Diet B)						
1	125	0.10	0.05	0.26	0.38	-0.30	D0.45	D0.10	0.85	-150
11	136	0.08	0.06	0.28	0.42	-0.30	-0.25	0	0.55	-150
18	150	0.08	0.06	0.24	0.38	-0.35	-0.35	0	0.70	-150
25	143	0.12	0.06	0.24	0.37	-0.10	-0.30	+0.15	0.55	-120
				Lamb 42 (Diet B plus α-tocopherol)						
32	130	0.10	0.06	0.24	0.35	-0.25	-0.15	0	0.40	-150
39	75	0.10	0.04	0.34	0.38	-0.20	-0.45	-0.75	1.40	- 60
46	107	0.10	0.06	0.30	0.40	-1.10	-0.50	-1.10	2.70	- 30
				Lamb 49 (Diet B)						
4	125	0.08	0.02	0.24	0.35	-0.05	-0.30	D0.20	0.55	- 90
11	125	0.08	0.06	0.28	0.40	-0.15	-0.30	D0.15	0.60	-150
18	115	0.08	0.06	0.28	0.39	-0.15	-0.35	D0.15	0.65	-150
25	94	0.08	0.06	0.34	0.42	-0.10	-0.30	D0.20	0.60	-150
32	72	0.10	0.04	0.34	0.37	-0.55	-0.55	+0.15	1.25	-150
39	94	0.10	0.04	0.32	0.40	-0.45	-0.50	+0.25	1.20	-150
46	72	----	0.08	0.32	0.34	-0.30	-0.65	-0.35	1.30	-150
53	125	0.10	0.10	0.24	0.35	-0.45	-0.70	-0.45	1.60	-120

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 49 (Diet B plus α-tocopherol)						
60	68	----	0.06	0.32	0.34	-0.30	-0.40	-0.30	1.00	+150
67	79	----	0.06	----	----	-0.10	-0.15	-0.10	0.35	+150
				Lamb 50 (Diet B)						
4	107	0.08	0.06	0.26	0.35	-0.65	-0.20	-0.30	1.15	+150
11	115	----	0.06	0.24	0.33	-0.70	-0.50	-0.20	1.40	+150
18	143	0.08	0.06	0.22	0.34	-1.10	-0.70	-0.30	2.10	+180
25	136	----	0.06	0.26	0.39	-1.45	-0.70	-0.45	2.60	+180
32	201	0.08	0.06	0.20	0.37	-0.90	-0.40	-0.70	2.00	+180
				Lamb 50 (Diet B plus α-tocopherol)						
39	150	0.06	0.04	0.24	0.38	-0.90	-0.40	-0.65	1.95	+180
46	150	0.08	0.06	0.26	0.41	-1.20	-1.10	-0.60	2.90	+180
53	150	0.10	0.06	0.22	0.35	-1.00	-1.00	-0.40	2.40	+180
60	150	0.10	0.06	0.22	0.35	-1.00	-0.95	-0.60	2.55	+180
67	115	----	0.08	0.26	0.36	-0.25	-0.25	-0.55	1.05	-120
				Lamb 39 (Diet B)						
5	100	0.08	0.06	0.26	0.34	D0.15	D0.65	D0.55	1.35	-150
15	150	0.08	0.06	0.22	0.35	-0.40	D0.45	D0.35	1.20	-150
22	136	0.10	0.06	0.22	0.33	-0.35	-0.35	+0.35	1.05	+150
29	150	0.10	0.06	0.22	0.35	-0.40	-0.55	D0.40	1.35	-150
36	167	0.10	0.04	0.18	0.30	-0.35	+0.20	+0.45	1.00	+120
43	188	----	0.06	0.22	0.39	-0.50	+0.20	+0.30	1.00	+ 90

TABLE XXIV (continued)

Age (days)	Heart Rate (Beats per Minute)	Duration of intervals (seconds) PR	QRS	QT	K (Bazett's formula)	QRS potentials (millivolts) L_1	L_2	L_3	total	Electrical Axis (degrees)
				Lamb 43 (Diet B)						
1	232	0.06	0.04	0.22	0.43	-0.50	+0.45	+0.35	1.30	+120
11	150	0.08	0.03	0.24	0.38	-0.50	-0.15	+0.35	1.40	+150
18	150	0.08	0.03	0.24	0.38	-0.35	D0.15	+0.35	0.85	+150
25	136	0.10	0.04	0.24	0.36	-0.45	-0.15	+0.35	0.95	+150
32	107	0.12	0.04	0.28	0.37	-0.80	-0.30	+0.35	1.45	+180
39	94	0.12	0.04	0.30	0.38	-0.60	-0.10	+0.35	1.05	+180
46	115	0.12	0.04	0.26	0.36	-1.00	-0.30	+0.55	1.85	+180
53	125	0.12	0.02	0.26	0.38	-0.10	-0.10	+0.10	0.30	+180
				Lamb 44 (Diet B)						
1	177	0.08	0.06	0.22	0.38	D0.30	-0.20	-0.30	0.80	-150
11	167	0.08	0.06	0.24	0.40	-0.30	-0.70	-0.50	1.50	-150
18	115	0.08	0.06	0.28	0.39	-0.45	-0.25	+0.30	1.00	+180
25	177	0.08	0.06	0.22	0.38	-1.50	-1.30	+0.45	3.25	+180
32	150	0.08	0.06	0.24	0.38	-1.20	-1.10	+0.30	2.60	-150
39	150	0.10	0.06	0.26	0.41	-0.55	-0.65	+0.20	1.35	+150
46	136	0.10	0.06	0.26	0.39	-0.60	-0.80	+0.20	1.60	+180
53	115	0.10	0.06	0.28	0.39	-0.65	-0.60	0	1.25	+180
60	136	0.08	0.06	0.26	0.39	-0.65	-0.40	+0.15	1.20	+150
67	100	0.10	0.06	0.28	0.36	-0.20	-0.10	+0.10	0.40	+180
74	88	0.10	0.06	0.32	0.39	-0.30	-0.45	+0.10	0.85	+150

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