The Nutrition of the Young Ayrshire Calf

8. Muscular Dystrophy in the Growing Calf

By K. L. BLAXTER (in Receipt of a Senior Award of the Agricultural Research Council)
P. S. WATTS and W. A. WOOD
Hannah Dairy Research Institute, Kirkhill, Ayr

(*Received* 27 *March* 1951)

In earlier work on the nitrogen metabolism of the young calf (Blaxter & Wood, 1951*a*, *c*) the sole source of vitamin E in the diets was high-quality dried skim milk that had been packed in sealed tins. At the time it was thought that this source of vitamin E activity would be sufficient to protect the animal during the short-term experiments envisaged, especially in view of the very considerable difficulties that have been met in devising cattle diets containing little vitamin E (Gullickson, Palmer, Boyd, Nelson, Olson, Calverley & Boyer, 1949; Gullickson & Calverley, 1946). One of our early experiments, however, had to be abandoned owing to the onset of a syndrome in which vitamin E appeared to be involved.

Six animals were affected 24–60 days after birth. The animals became very weak and unwilling to stand for more than a few minutes; the pastern joints became straightened; the hind-legs crossed when attempts to walk were made; there was a general ataxia, and the neck was outstretched in a very characteristic way. A tetany, not involving the extremities, was common during the latter stages and was seen only when the severely affected animal was placed on its feet. Relaxation of the musculature of the throat and inability to control movements of the tongue made drinking difficult and, in the terminal stages, the calves choked, owing to liquid food entering the trachea. Loss of hair at the bulbal base of the ear was always a common feature; the coat was staring and the skin thickened, the latter making superficial venesection difficult. In two animals oedema of the brisket and ascites were present. This was of rapid onset as judged by gains in body-weight and seemed to be associated with a slight exophthalmos. In these oedematous calves the pulse was highly erratic and generally increased in frequency. Death occurred suddenly if such signs of cardiac abnormality were present; otherwise the animals died following a period of prostration. At post-mortem examination ascitic fluid was found in the abdominal cavity, the carcass was generally slightly oedematous, and one constant feature was the rapidity of putrefactive change. The liver and kidneys were normal; the lungs were pneumonic and contained milk and blood. The heart was dilated in some animals, and slight white lesions were found in the walls of the left ventricle in one of those animals affected by oedema. The bones were calcified quite normally but some erosion of the articular cartilages was present. This, however, was also found in normal calves. The skeletal muscle was very pale and flabby, and the fat was a light

brown compared with the normal whitish yellow colour. Before death, and when clinical signs were present, blood samples were taken and normal serum values for calcium, inorganic phosphorus and magnesium were found. There was no response to magnesium sulphate given intravenously, suggesting that the tetany and prostration were not the result of hypomagnesaemia, i.e. 'milk' tetany of the calf as described by Duncan, Huffman & Robinson (1935). Urinary excretion of vitamin B_1 was not affected, thus excluding aneurin deficiency. The only biochemical abnormalities found were an elevation of the urinary elimination of creatine from normal values of 200–300 mg/24 h to as much as 1·3 g/24 h, and an elevation of basal oxygen consumption by 20–60 %. This syndrome and the post-mortem findings appeared comparable to those obtained by Goettsch & Pappenheimer (1931) in guinea-pigs given diets deficient in vitamin E. A diagnosis of avitaminosis E was made, and attempts to cure two affected animals followed. In one calf, no response occurred following the daily administration of 25 mg DL-α-tocopherol. An aqueous solution of 100 mg α-tocopheryl phosphate was then given for 5 days. Improvement was rapid; from complete prostration the calf's behaviour changed markedly so that it could jump and frisk in its cage; the balance improved but it still found difficulty in rising. This was undoubtedly due to bruising of the hocks, stifles and knees during the 14 days' prostration. The creatine content of its urine fell from nearly 600 to 270 mg/day, and on slaughter its musculature appeared normal. A further calf, however, when severely affected was given 100 mg α-tocopheryl phosphate daily for 10 days without response. In this animal, there was an acute alimentary disturbance and a pneumonia which did not respond to sulphonamide therapy. The creatine content of a sample of skeletal muscle was very low and the extent of the muscular involvement was so great that a cure could hardly have been expected. Thus in only one of the two calves was a cure effected.

Later, histological examination of the tissues from two of the calves showed a degeneration and inflammatory infiltration of the cardiac and skeletal muscles, and in the spinal cord a degenerative process of the neurones associated with oedema and gliosis (proliferation of the neuroglia). Affected skeletal muscle showed granular degeneration and fragmentation of muscle cells and their replacement by fibroblasts, endothelial cells and plasma foci. Nuclear proliferations were also present. Cardiac muscle from the left ventricle also showed grossly degenerate and granular myocardial and endothelial cells and replacement by fibroblastic tissue. The Purkinje fibres were swollen.

These findings were not completely comparable to those observed in rabbits where muscular dystrophy is associated with replacement of normal muscle cells by fatty tissue (Pappenheimer, 1948) and where nervous tissue does not appear to be affected, but the nature of the whole syndrome suggested a deficiency of vitamin E.

Muscular and other disorders associated with dietary deficiencies of vitamin E have been reviewed by Pappenheimer (1940–1, 1943, 1948), Follis (1948), Parrish (1949) and Loosli (1949). Muscle lesions have been shown in rabbits and guinea-pigs (Goettsch & Pappenheimer, 1931), mice (Pappenheimer, 1942), rats (Knowlton & Hines, 1938; Pappenheimer, 1939), ducks (Victor, 1934; Pappenheimer & Goettsch,

1931), monkeys (Mason & Telford, 1947) and dogs (Anderson, Elvehjem & Gonce, 1939). Even with chicks in which vitamin E deficiency manifests itself primarily as an encephalomalacia, abnormalities of the musculature are nevertheless present (Pappenheimer, 1948).

In cattle, the recent studies of Gullickson and his collaborators (Gullickson & Calverley, 1946; Gullickson, 1949; Gullickson *et al.* 1949) have shown that vitamin E deficiency is associated with sudden death of cattle resulting from cardiac failure. Electrocardiography showed a decreased functional activity of the myocardium. At post-mortem examination no gross pathological changes were found, and a short report on the histology of the heart indicated that the condition resembled human endocarditis. No mention was made by Gullickson of any dystrophic lesions of either heart or skeletal musculature in these animals, although they had subsisted for as long as 5 years on diets very low in vitamin E. In 1947, however, Vawter & Records (1947) discovered a series of farms in Nevada, U.S.A., on which calves on the range died with symptoms of dystrophy closely comparable to those associated with 'stiff lamb disease' (Willman, Asdell, Grams & Hagan, 1931), a disease known to be cured by DL-α-tocopheryl acetate (Willman, Loosli, Asdell, Morrison & Olafson, 1945, 1946). Vawter & Records (1947) were unable to show that this condition was a true muscular myopathy caused by vitamin E deficiency, but they drew attention to the close resemblance of the disease to muscular dystrophy induced in other species by dietary deficiencies of vitamin E.

There are numerous records in the literature of muscle and heart lesions in young calves that appear comparable to those described by Vawter & Records. Most, but not all, of these reports refer to the toxicity of cod-liver oil. One of the earliest descriptions of the disease was by Slagsvold (1925), though Agduhr (1927) claims to have observed it first in 1922. Later, Hjärre & Lilleengen (1936*a*, *b*) traced records of a disease called *weisses Fleisch* to the latter part of the nineteenth century, and one of the earliest reports was that of Pollock (1854). The symptoms are well summarized by Hjärre & Lilleengen. The same authors have attributed the naturally occurring muscular dystrophies of calves to vitamin C deficiency on the basis of the appearance of the disease when heated oxygenated milk was given. Such methods destroy not only vitamin C but vitamin E as well, and in view of the absence of dietary requirement of vitamin C in the ruminant such an explanation does not seem likely.

The many reports of Agduhr incriminating cod-liver oil (Agduhr & Strenström, 1929) stimulated considerable work on other animals. Dystrophy was produced in sheep and goats given cod-liver oil in large amounts (Madsen, McCay & Maynard, 1935) and also in pigs (Wagener, 1936), though attempts to confirm it in pigs were not successful (Scorgie & Miller, 1944). With calves the experiments to confirm the European reports were in all cases unsuccessful. Davis & Maynard (1936, 1938) reared calves on rations containing cod-liver oil, but the only animal that died showed no evidence of dystrophy. Later experiments showed that the electrocardiograms of such calves were normal (Barns, Davis & McCay, 1938). Experiments with seventy-two calves by Gullickson & Fitch (1944) in which 25–35 ml. cod-liver oil were given every day did not reveal any adverse effects. Turner, Meigs & Converse (1936)

briefly reported death of a calf or calves when 2·0 g cod-liver oil/kg body-weight (possibly as much as 100 ml./day) were given, but the general contention has been that muscular dystrophy cannot be produced by giving cod-liver oil to calves. Experiments in other species, however, have confirmed that cod-liver oil can destroy vitamin E (Cummings & Mattill, 1930–1), and with cows Ferrando, Chenavier & Cormier (1949) have shown that administration of cod-liver oil depresses the content of tocopherols in the blood plasma.

In view of these reports and our own preliminary investigations, an experiment was planned to provide further information. The object of the experiment was two-fold. Firstly, to find whether the inclusion of vitamin E in a diet containing minimal quantities of vitamin E would prevent muscular dystrophy; and secondly, to find whether the addition of cod-liver oil to this diet would result in the incidence of a more severe form of the disease.

EXPERIMENTAL

Plan of experiment

Calves. Sixteen Ayrshire bull calves, purchased from the market when 2–3 days old, were used as experimental animals. Eight were purchased in January 1950, and eight in July 1950. They were confined in individual pens and were bedded on straw or peat moss in a room thermostatically controlled between 60 and 65° F. After a short preliminary period to allow the calves to become accustomed to their new home, they were grouped into pairs on the basis of their age and general well-being and the pairs were then allocated at random to one of two diets. The diets were similar in every respect, save that in one vitamins A and D were supplied as cod-liver oil and in the other as the pure vitamins in equivalent quantity dissolved in arachis oil. One animal of each pair was then allocated at random to receive a supplement of α-tocopherol, the other receiving none. There were thus four treatments, the cod-liver oil diet without α-tocopherol (CLO.O.) the cod-liver oil diet with α-tocopherol (CLO.E.), the arachis-oil diet without α-tocopherol (AO.O.) and the arachis-oil diet with α-tocopherol (AO.E.). The calves are referred to by treatment and replication number. Thus calves nos. CLO.E. 1 and CLO.O. 1 were the first pair of animals receiving the cod-liver oil diets, whereas calf no. AO.E. 4 and calf no. AO.O. 4 were the last pair of animals receiving the arachis-oil diets. Pairs of animals were always treated in exactly the same manner, save for the fact that one received α-tocopherol. In each series of eight, two pairs of animals received the cod-liver oil diet and two pairs the arachis-oil diet.

Diets. The basic diets were almost the same in each experiment, the only difference being that the second eight calves received additional glucose. The composition of the diets is given in Table 1. The first group of eight calves were given 3·6 l./day for the first 14 days and 4·6 l. subsequently. The second group of eight were given 4·0 l. until they were 60 days old when the quantity was increased to 4·8 l. In the second group, however, the concentration of arachis oil or cod-liver oil was reduced when the ration was increased so that the calves ingested the same quantities of vitamins A and D as when 4·0 l. of diet were given. With these diets the amount of arachis oil or cod-liver oil taken daily was 15–18 ml. This quantity is considerably less than that

recommended for calf rearing by the Ministry of Agriculture and Fisheries (1937). The dried skim milk was of high quality and high solubility. It had been packed in the factory in sealed cans, and these were opened as required. The lard was household-quality lard. The cod-liver oil was purchased from recognized veterinary suppliers and was veterinary quality refined oil with a guaranteed potency. Both the arachis-oil solution of vitamins A and D and the cod-liver oil were stored in a refrigerator. The α-tocopherol supplement given to the calves in groups CLO.E. and AO.E. was the synthetic racemic α-tocopheryl acetate. It was also refrigerated, and 50 mg dissolved in 0·2 ml. arachis oil were given daily in a small gelatin capsule.

Table 1. *The basic diet of the calves*

Constituent	Quantity/l.
Spray-dried skim-milk powder	82·5 g
Lard	41·2 g
Glucose (second series only)	13·7 g
'Trace'-mineral solution*	2·5 ml.
Cod-liver oil, or arachis-oil solution of vitamins A and D	4·1 ml.

* Containing 1·25 mg $MnSO_4.4H_2O$, 1·25 mg $CuSO_4.5H_2O$, 0·25 mg $CoCl_2$, 50 mg ferric citrate and 150 mg $MgCl_2$.

The diets were made by methods previously described (Blaxter & Wood, 1951*a*) twice weekly at intervals of 3 and 4 days and were stored in an immersion cooler at 5–7°.

Management of the calves. The calves were fed at 7 a.m. and 5 p.m. The α-tocopherol supplement was always given at 2 p.m., between normal feeding times. When the calves became unable to drink they were suckled.

After some time in their pens the calves, especially those that had grown well, began to eat their straw bedding. This was immediately controlled by muzzling them with leather greyhound-muzzles except when they were fed. After a further period, however, the calves learned to eat straw through these muzzles. Peat moss was then substituted for the straw but, even so, the calves managed to consume some of it, and peat moss was commonly found in the rumens of the older calves when they were slaughtered, but the amounts involved were small.

Examination of the calves during life. Notes on the animals' behaviour were made twice daily, and when they were weighed at weekly intervals the calves were exercised on a cement floor and abnormalities of gait noted. The time taken to consume their food was recorded at intervals, and urine specimens (not 24 h samples) were also collected. Animals nos. AO.E. 3, AO.O. 3, AO.E. 4 and AO.O. 4 were subjected to balance experiments for a period of 14 days once signs had become apparent, using the technique of Blaxter & Wood (1951*a*). Oxygen consumption and carbon-dioxide production were determined after an 18 h fast, using the technique of Blaxter & Howells (1951).

Post-mortem examination of the calves. When the symptoms of muscular dystrophy were judged to be sufficiently advanced in any calf it was killed for dissection, patho-

logical examination, and chemical analysis. Its corresponding pair-mate was killed 3–6 days later and the same routine repeated.

Cultures were taken from any organs that appeared abnormal. One fore-leg and one hind-leg from each animal were dissected in detail, and a description was recorded of any lesions present in each of fourteen muscles, representing the main muscles of the limbs. These muscles, dissected free of fat, tendon and muscle sheath, were subsequently used for chemical study. Notes on the abnormalities of other skeletal muscles were also made, and the weights of the liver, brain, kidneys and heart were obtained.

RESULTS

Signs of muscular dystrophy shown by the calves

Group AO.E. Arachis-oil diet with α-tocopherol

All calves in this group behaved normally throughout the experiment. On several occasions no. AO.E. 2 and more especially no. AO.E. 1 were affected by an acute diarrhoea, whereas no. AO.E. 4 was but slightly affected at any time. No abnormalities of gait or balance were noted in the calves, and their coats were sleek, except when affected by diarrhoea. Calves nos. AO.E. 3 and AO.E. 4 had gained over 20 kg by the time they were killed, corresponding to a daily gain of 230 g. Calves nos. AO.E. 1 and AO.E. 2, however, did not gain as much, owing to the incidence of alimentary disturbance. A photograph of a normal calf (no. AO.E. 3) is given in Pl. 1, 1.

Group AO.O. Arachis-oil diet without α-tocopherol

Calf no. AO.O. 1. At the 10th day of treatment this calf was thought to be quite normal. On the 16th day, however, the eyes were sunken, the coat was rough, and on standing the legs flexed at the knees. On walking, the hind-feet were placed in a crosswise fashion and the hock joints were consequently twisted outwards. Breathing was laboured and muscles of the tongue and throat soon became useless. Pneumonia was diagnosed and the calf was killed. It was obvious in this calf that a partial paralysis of throat muscles and interference with swallowing reflexes contributed to its condition. Over the whole period, including the terminal phase, no gain in weight had occurred.

Calf no. AO.O. 2. No abnormalities were observed until the 25th day of treatment when muscular co-ordination seemed abnormal; skin reflexes, however, were unaffected. The condition deteriorated until the 30th day when violent tremors and shivering were observed. The calf stood with an arched back and had to be helped to its feet. The hind-legs appeared weak. During this period the faeces were quite firm. After the 34th day an improvement occurred, but on the 38th day further abnormalities of the gait were seen, consisting of forward flexion of the knees in a manner similar to that observed in calf no. AO.O. 1. Signs then became more pronounced. The coat became rough, the calf dull and lethargic, a slight cough developed on the 44th day. By the 47th day the calf stood with all four feet bunched together, with the back elevated and the head held very low. It could only rise with difficulty and could only drink with considerable effort. The calf was killed on the

51st day having lost weight severely during the last week of life. The overall weight gain was only 100 g/day.

Calf no. AO.O. 3. Nothing abnormal was noted until the 23rd day when the calf was listless and had a discharge from the nose, diagnosed as a slight cold. By the 31st day the cold had gone, but the calf spent very much longer lying down than he had done previously or than his pair-mate no. AO.E. 4 did at the time. This lethargy continued until the 50th–52nd day when the gait appeared slightly stiff. Before this, both gait and balance had been perfectly normal. A week later the shoulders appeared to be abnormal, and on running the animal often stumbled and fell as the result of failure to flex the fetlock joint. This state continued until the 76th day, when coughing after meals was observed and the constant lying down and listlessness appeared again. The claws of the hoof were spread considerably, and by the 83rd day there was difficulty in rising. The calf was slaughtered on the 91st day without further exacerbation of these signs being noted. It had gained 200 g/day throughout.

Calf no. AO.O. 4. Nothing abnormal was noted until the 39th day when the time taken to consume a meal was prolonged. On the 54th day the calf was very quiet, and on the 55th day the shoulders were noted to be abnormal. This abnormality persisted until death, increasing markedly with increase in weight. Slowness in drinking and constant lying down were the only other symptoms observed. Slight coughing after feeding was noted on only one occasion. The calf was killed on the 98th day and had gained 210 g/day.

Group CLO.E. Cod-liver oil diet with α-tocopherol

Calf no. CLO.E. 1. This calf was quite normal until the 17th day when the gait was staggering and the fetlocks flexed. These signs did not get worse, and nothing further was noted until the 36th day when the time taken to consume food was excessively prolonged. The next day the calf appeared stiff, and muscle tremors occurred on standing. It was killed on the 38th day since the other calf of the pair had already succumbed.

Calf no. CLO.E. 2. When the experiment commenced this calf had a tendency to flex the knee joint of the left leg, a sign which in the preliminary experiments had been associated with dystrophy. This grew worse throughout the experiment, but in a periodic fashion. Thus, by the 21st day the calf, on rising, would fall forward and the knees would flex, but by the 31st day it was able to rise unaided and the front legs became bent only after prolonged standing. The signs became worse again on the 40th day when it could straighten the right leg but not the left, and its balance was markedly affected. The calf rarely moved the hind-legs when in its pen, but when exercised moved them stiffly. It could walk quite quickly on its permanently flexed front legs. It was killed on the 45th day. A photograph of this calf is given in Pl. 1, 2.

Calf no. CLO.E. 3. Except for showing bouts of nervousness, this calf remained perfectly normal for the first 44 days. On the 45th day it became rather quiet and very slow in drinking. The habit of standing with its hind-quarters in the corner of its pen was adopted, and on exercise marked ataxia was present. A slight cough

developed on the 48th day and the animal lay down at every opportunity. The shoulders and back were normal but the gait was slightly unsure and the feet were placed farther forward than is normal on walking. It was killed as a pair control on the 55th day without further signs developing.

Calf no. CLO.E. 4. This calf remained normal throughout the experiment save that it was lethargic 2 days before slaughter on the 35th day, the pair-mate having previously succumbed.

Group CLO.O. Cod-liver oil diet without α-tocopherol

Calf no. CLO.O. 1. This calf was normal for the first 14 days. By the 23rd day, however, it was very slow to rise, and found difficulty in drinking. Shivering on standing was noticed on the 27th day and it was not able to suck properly. The front legs became stiff, and flexion of the knee joints was absent. On the 29th day respiration was slightly pneumonic, and when lying the calf adopted unnatural attitudes. On the 33rd day it could stand only for a few minutes and with violent muscle tremors and seemed unable to use the tongue or lips, or to be able to swallow. The calf was killed on the 34th day, and at death its weight had fallen to the initial weight, though, up to the onset of symptoms, growth had been normal.

Calf no. CLO.O. 2. This calf, apart from a slight cold and a disturbance of the alimentary tract, was normal for the first 41 days. On the 41st day a tendency to flex the carpal joint of the right fore-leg was noted. By the 46th day drooping of the head and a rough coat were the predominant signs. On the 65th day just before slaughter both fore-legs were flexed at the carpus, and stiffness of the hind-limbs was noted.

Calf no. CLO.O. 3. This calf was normal for the first 25 days, except for slowness in drinking and loss of hair from the bulbal base of the ear. Slight coughing was noted, which became worse until the 36th day. During this period there was no increase of rectal temperature; the calf was lively and his gait normal save that when pushed he was slow to regain his balance. A rough coat and prolonged drinking time were the next signs observed, and by the 43rd day an elevation of the suprascapular cartilage was evident. On the 45th day the calf was very quiet, the respiratory rate was increased but there was no rise in rectal temperature. The following day it lay down at every opportunity but could rise easily, although the suprascapular cartilage had risen still further. The next day great difficulty in rising was observed, and on the following day (the 48th) the hind-legs were tucked under the body on standing and the fore-legs were straight. Violent rigors occurred on standing but not when lying. The pulse rate and rectal temperature were normal but the respiratory rate was elevated. A million units of penicillin were given without response, and the calf was killed on the 49th day. It had gained 260 g/day during the period, a normal amount as judged by the calorific value of the diet.

Calf no. CLO.O. 4. Nothing abnormal was noted in this calf until the 16th day, when it was sluggish and inclined to lie down a great deal. By the 21st day, though able to rise and stand, it would lie down immediately after each meal. Nothing more specific was noted until the 24th day when the back was arched and the feet were flat,

the latter owing to relaxation of the carpal joint. Violent shivering attacks occurred and the calf appeared breathless. The following day the calf had a great struggle to get to its feet, and on standing shivered violently and was unable to remain standing for long. A slight cough was present. The following day it could rise only if assisted, and was unable to use the tongue for drinking or sucking. It was killed on the 29th day, having gained 130 g daily over the period, this low gain being partly due to failure to consume food towards the end of life.

General observations

From these case reports, it will be seen that the same signs of muscular weakness occurred in several of the calves as occurred previously in preliminary experiments, and the same variation in time of onset of symptoms was apparent. Signs of failing circulation, however, were only observed in one calf, no. CLO.O. 3 (*vide infra*). Table 2 summarizes the severity of the signs of muscular involvement as judged by the daily observations. It will be noted that the most severe signs were observed in the calves given cod-liver oil and deprived of vitamin E.

Table 2. *Summary of treatment and signs* of the calves*

No. of calf in group	Vitamins A and D supplied by: Cod-liver oil — With vitamin E (CLO.E.)	Cod-liver oil — Without vitamin E (CLO.O.)	Solution in arachis oil — With vitamin E (AO.E.)	Solution in arachis oil — Without vitamin E (AO.O.)
1	First signs at 36 days Killed at 38 days Slight	First signs at 27 days Killed at 34 days Severe	Normal Killed at 24 days Nil	First signs at 16 days Killed at 18 days Slight
2	First signs at 21 days, relapse at 40 days Killed at 45 days Severe†	First signs at 41 days Killed at 46 days Severe	Normal Killed at 44 days Nil	First signs at 25 days, relapse at 38 days Killed at 44 days Severe†
3	First signs at 43 days Killed at 55 days Slight	First signs at 43 days Killed at 49 days Very severe†	Normal Killed at 95 days Nil	First signs at 50 days Killed at 91 days Slight†
4	Normal Killed at 35 days Nil	First signs at 24 days Killed at 29 days Very severe†	Normal Killed at 101 days Nil	First signs at 59 days Killed at 98 days Slight†

* Signs are roughly classified as nil, slight, severe and very severe on the basis of the case reports.
† First animal of pair to be killed.

The syndrome in the affected animals always started in the same way: a disinclination to stand, together with non-specific dullness and listlessness. More specific signs appeared later. Forward bending of the knee joints occurred in some animals, and is illustrated in Pl. 1, 2 (calf no. CLO.E. 2). In these calves the feet were placed far forward on the ground, and in calf no. CLO.E. 2 the metacarpal bone made an angle of 30° with the ground. In two calves similar bending of the fetlock joint was noted. Another common symptom was the retraction of the fore-limbs under the body, resulting in the calf standing on its toes. A photograph of a calf in such a position is given in Pl. 1, 3 (calf no. CLO.O. 3). In the second part of the experiment the protrusion of the scapula was noted. Pivoting on the glenoid cavity, the scapula

moved to a more upright position dorsal to the neural spines of the thoracic vertebras. This left a hollow between the two scapulas and the back of the animal. A photograph of an affected calf is given in Pl. 1, 4 (calf no. AO.O. 4). This sign is comparable to winged scapula in human muscular dystrophy of the scapulo-humeral type as illustrated by Bicknell & Prescott (1946). Relaxation of the musculature of the foot, an increase in the slope of the pastern and coronet bones and a splaying of the hoofs were also noted. In the hind-limbs the tibia and metacarpal bones assumed a straight line and were moved stiffly together. A characteristic outward swinging of the hock joints was noted in some calves, but in general the hind-limbs did not appear to be so severely affected as the fore-limbs. A photograph of a normal calf (calf no. AO.E. 3) is given in Pl. 1, 1 for comparative purposes. Each one of these peculiar postures and gaits appeared to be the result of an adjustment to minimize strain on part or parts of the skeletal musculature. This aspect is discussed later. The shivering and tetany appeared to be an 'effort' tetany rather than an irritability phenomenon. Skin reflexes were always present, but attempts to elicit deep tendon reflexes in any of the calves, normal or affected, were not very successful. An involvement of the nervous system could not therefore be discounted. Attempts were made to localize the muscular abnormalities by subjecting the animals to stress. Weakness of the suspensory muscles of the neck could be diagnosed in a comparative fashion by placing the hand on the head of the calf and pushing downwards. Similar methods were adopted for the hind-quarters and fore-quarters. Palpation of the muscles was extremely difficult. In young dairy animals the musculature is not well developed, and the only muscles that could be palpated with ease were the triceps group in the fore-limb and the vastus group in the hind. There was no indication of any enlargement or atrophy of these muscles in any calf, but the normal resilience of the muscular tissue was reduced in badly affected animals such as no. CLO.O. 3 and the muscles felt leathery. A diagnosis of dystrophy made in this way, however, was not infallible.

One common sign was the difficulty the calves experienced in drinking their food. Table 3 summarizes the results of a series of observations on the eight calves of the second series of experiments. It will be noted that affected calves took considerably longer to consume their food. Eventually it became impossible for seriously affected calves to drink at all and they had to be suckled. At no time was it noted that the sphincters of the bladder or bowel were affected.

Nitrogen metabolism of the calves

The mean daily nitrogen balances of calves nos. AO.E. 3, AO.E. 4, AO.O. 3 and AO.O. 4 are given in Table 4. They apply to 10-day experiments made when the calves were 80 days old. Those calves that did not receive vitamin E stored less nitrogen than their respective pair-mates. In Fig. 1, the nitrogen balances are plotted against the mean creatine content of the musculature as determined at post-mortem examination. The amount of nitrogen stored daily was related to the creatine content of the musculature; those calves that stored most nitrogen had most normal muscles as judged by their creatine content.

Table 3. *Time (sec) taken by each of eight calves to consume 2·0 l. of the diet*

	Vitamins A and D supplied as							
	Cod-liver oil				Solution in arachis oil			
	With vitamin E		Without vitamin E		With vitamin E		Without vitamin E	
Day	CLO.E. 3	CLO.E. 4	CLO.O. 3	CLO.O. 4	AO.E. 3	AO.E. 4	AO.O. 3	AO.O. 4
36	40	42	108	125	36	42	64	60
43	89	50	78	135	30	31	45	39
46	95	52	100	—*	30	32	65	64
57	95	—†	97	—*	32	25	35	58
60‡	90	—†	95	—*	40	42	53	96
70‡	72	—†	132	—*	26	30	30	77
Mean	97	(46)	102	(130)	32	34	49	66
Symptoms of dystrophy	Slight	Nil	Very severe	Very severe	Nil	Nil	Slight	Slight

* Killed as severely dystrophic. † Killed as pair-mate to no. CLO.O. 4. ‡ 2·4 l. given.

Table 4. *Mean daily nitrogen balances of the calves*

(The average quantity of nitrogen ingested by each calf was 22·00 g/day)

		Nitrogen excreted		Nitrogen stored
Calf no.	Treatment	Faeces (g)	Urine (g)	(g)
AO.E. 3	Given vitamin E	2·42	9·94	9·64
AO.E. 4		1·44	8·82	11·74
AO.O. 3	Deprived of vitamin E	2·87	10·73	8·40
AO.O. 4		3·16	11·60,	7·24

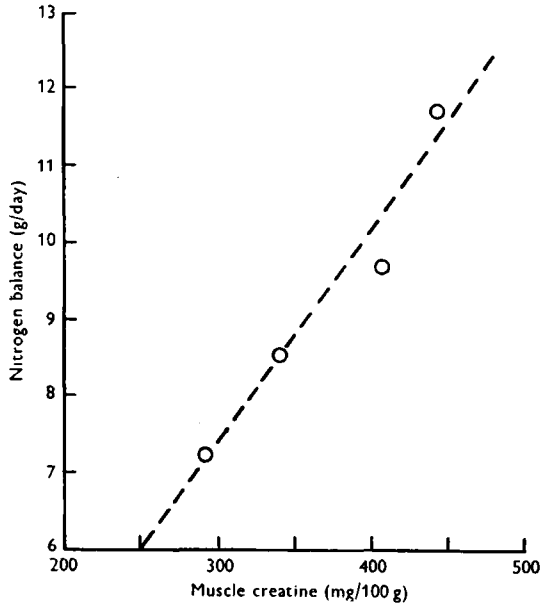


Fig. 1. Relation between the mean muscle creatine of the calves and their nitrogen balances determined immediately before slaughter. The muscle creatine refers to the mean content of fourteen appendicular skeletal muscles.

In Table 5 mean values are given for the excretion of nitrogenous metabolites by these four calves. The greater excretion of nitrogen in the urine of the calves deprived of vitamin E was traced to a greater excretion of the total of urea, ammonia and amino-acid nitrogen. The total excretion of creatinine nitrogen was lower in the vitamin E-deficient animals, suggesting a smaller functional muscular mass. Though dystrophy was present in the carcass of calf no. AO.O. 4, a creatinuria was not observed. A creatinuria in which the ratio creatine nitrogen : creatinine nitrogen was greater than 1·0 was noted, however, in calf no. AO.O. 3. Dystrophy was very advanced in the former animal, and it is possible that a creatinuria is not diagnostic in such advanced cases.

Table 5. *Mean daily excretion of nitrogen (g) in different nitrogenous metabolites by the calves*

	Calves given vitamin E		Calves deprived of vitamin E	
Nitrogenous metabolite	AO.E. 3	AO.E. 4	AO.O. 3	AO.O. 4
Protein	0·38	0·26	0·32	0·33
Urea	4·79	4·53	5·74	7·14
Ammonia	0·81	0·56	1·12	0·91
Amino-N	0·36	0·26	0·31	0·24
Total urea, ammonia and amino-N	5·96	5·53	7·17	8·29
Purine base	0·18	0·16	0·15	0·19
Uric acid	0·05	0·03	0·04	0·06
Allantoin	0·70	0·61	0·76	0·89
Total purine	0·93	0·80	0·95	1·14
Creatinine	0·63	0·60	0·34	0·43
Creatine	0·20	0·29	0·38	0·20
Total creatine	0·83	0·89	0·72	0·63

Energy metabolism of the calves

Six determinations of metabolism were made. In only two instances, however, were the calves badly affected at the time of the determinations. The heat production of the affected calves, nos. CLO.O. 3 and CLO.O. 4, was 63·5 and 59·4 Cal./kg body-weight/24 h compared with normal values of 45–49 obtained with the other calves. An increase in basal heat production would account for an increase in urinary nitrogen excretion limited to the urea, ammonia and amino-nitrogen fractions, as has been shown elsewhere (Blaxter & Wood, 1952). An increase in basal metabolism in vitamin E deficiency has been demonstrated in intact rats by Biddulph & Meyer (1942) and an increase in the metabolic rate of isolated muscle from animals deficient in vitamin E is well known (Mason, 1944).

Post-mortem examination of the calves

Group AO.E. Arachis-oil diet with α-tocopherol

Calf no. AO.E. 1. The internal organs all seemed normal and no growth was obtained from cultures of spleen and bone marrow. The heart showed some sub-endothelial valvular haemorrhage, probably comparable to the haematomas observed

by Florence (1922). Their occurrence in calves can be regarded as normal. The skeletal musculature was pale.

Calf no. AO.E. 2. A small area of pneumonia was detected in the right apical lobe. There was no bronchitis, but very slight gelatinous adhesions. Cultures from the lung showed *Bacterium neapolitanum* to be present. The skeletal musculature was normal.

Calf no. AO.E. 3. This calf was completely normal in every respect, save that the thymus was unusually large. The skeletal musculature was normal.

Calf no. AO.E. 4. Apart from an apparent cavitation of the right adrenal gland all internal organs were normal. The skeletal musculature was normal.

Group AO.O. Arachis-oil diet without α-tocopherol

Calf no. AO.O. 1. On examination, the organs of this animal appeared normal. Subendothelial auriculo-ventricular haemorrhages were present, but the heart and skeletal musculature appeared normal.

Calf no. AO.O. 2. An area of pneumonia was found in the right apical lobe, spreading to the left and right cardiac lobes. Subendothelial haemorrhages were present in the heart, but the muscle appeared firm. The bone marrow was pale. The musculature was generally pale and some muscles showed white areas, more especially the supraspinatus and infraspinatus. The degree to which the muscles were affected, however, was not severe.

Calf no. AO.O. 3. A very slight pneumonia was found in the right apical lobe of the lung. This could not be regarded as serious. The heart showed slight inflammation of the pillars of the left ventricle. Two white areas were found in the rectus femoris, and further areas in the vastus lateralis. Otherwise the muscles, though pale, appeared normal.

Calf no. AO.O. 4. The carcass of this calf had a peculiar and unpleasant sweetish odour. Pneumonia was present in the right apical lobe. The internal organs were otherwise normal. The heart muscle was grossly dystrophic, showing large white areas and two areas of haemorrhage in the wall of the left ventricle. A sketch of these lesions is shown in Fig. 2. The skeletal musculature was also dystrophic. Not all muscles were affected. The neck and facial muscles appeared to be those most affected. The supraspinatus, infraspinatus, and long heads of the triceps appeared to be the most seriously affected parts of the fore-limb. In the hind-leg, the gastrocnemius seemed most affected.

Group CLO.E. Cod-liver oil diet with α-tocopherol

Calf no. CLO.E. 1. A consolidated area of congestion was present in the right apical lobe of the lung and some petechiation of the muscle ridges of the bowel wall was noted. The heart appeared flabby but otherwise normal save for auriculo-ventricular haemorrhages. The skeletal muscles were dystrophic. The biceps femoris was completely white in colour and markedly oedematous. Other muscles were not so severely affected, some appearing normal and others showing a patchy distribution of white areas. The supraspinatus was markedly haemorrhagic, whereas other muscles merely appeared pale.

Calf no. CLO.E. 2. No pneumonia was noted and the internal organs were normal. The heart was normal and apart from paleness of all the muscles and an impression that the musculature appeared wet, no specific areas of dystrophy were apparent except possibly in the fore-limb.

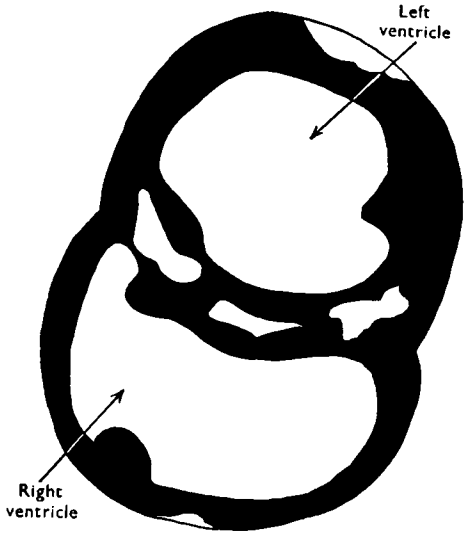


Fig. 2. Cross-section of the heart of calf no. AO.O.4 at the level of the coronary pillars. White areas of wall represent dystrophic areas.

Calf no. CLO.E. 3. No abnormality of the viscera was observed save that both kidneys showed haemorrhagic infarcts. The heart muscles appeared quite normal. The musculature, on the other hand, was severely dystrophic. The diaphragm showed small areas of dystrophy and the adductor and brachial muscles appeared grossly affected throughout. This calf was the most severely affected of the group.

Calf no. CLO.E. 4. This calf, killed as a control for calf no. CLO.O. 4, was quite normal at post-mortem examination.

Group CLO.O. Cod-liver oil diet without α-tocopherol

Calf no. CLO.O. 1. The heart was dark in colour and extremely flabby. Myocardial degeneration could be seen in the internal ventricle walls. The brain showed an area of apparent inflammation in the right frontal lobe. Other internal organs were normal. The muscles were generally very pale and had a fibrous appearance. Obvious dystrophic areas were few.

Calf no. CLO.O. 2. The internal organs appeared quite normal. The heart appeared to be unaffected. The skeletal musculature, however, was grossly dystrophic. The occipital muscles of the neck were completely white, and the leg muscles were dystrophic throughout. Thus the supraspinatus showed intense white mottling, with oedema between the muscle bundles; the coraco-radialis was completely white, whereas the anterior extensor of the metacarpus and the semi-membranosus appeared normal.

Calf no. CLO.O. 3. The carcass was fairly fat and the fat showed a typical brown discoloration. There was a tendency for some gelatinous breakdown, especially at the rib insertions. The internal organs, including the heart, were normal. A gross involvement of the diaphragm was, however, present; this, in the absence of pneumonia, accounted for the severe dyspnoea observed before death. The skeletal muscles were very severely dystrophic; in fact the only groups of muscles not obviously affected were the facial muscles and the muscles running parallel to the trachea.

Calf no. CLO.O. 4. Some increase in pericardial fluid and pneumonia of the right apical lobe of the lung was noted. The heart was normal except for small haemorrhages on the auriculo-ventricular valve. Other internal organs were normal. The skeletal musculature was again grossly dystrophic, though not quite so seriously as in calf no. CLO.O. 3.

General observations

Several features of the above post-mortem examinations are of interest. The smell of the freshly killed carcasses was extremely characteristic. The carcasses of the dystrophic calves had a peculiar, offensive sweet odour, and most calves that had received cod-liver oil had a very slight fishy odour. Browning of the fat was apparent in two dystrophic carcasses only. The dystrophic lesions in the muscles were highly variable. In some muscles the dystrophic areas were highly circumscribed, and in others the areas ran from the origin to the insertion of the muscle, parallel to the line of the muscle bundles. In some muscles only the outer shell remained normal, whereas in others it was this area alone that was dystrophic. A photograph of grossly affected muscles is given in Pl. 2. This is a section through the left femur of one of the calves in the earlier series and shows lesions similar to those later observed.

Attempts were made to classify muscles and muscle groups according to the degree to which they were affected. It was quite obvious that from calf to calf there was no agreement either between the least affected or the most affected muscles. In one calf the supraspinatus was the most involved; in another, it was the least. On the other hand, when one side of the carcass was compared with the other, it was evident that there was a high degree of bilateral symmetry. It could not be concluded, in agreement with Hjärre & Lilleengen's (1936*a*, *b*) description of *weisses Fleisch*, that the lesions were mostly in the infra- and supraspinatus. Fig. 3 illustrates this symmetry, and also shows that there was no close association of the incidence of dystrophy with any particular muscle. The pattern of the visibly dystrophic lesions within each muscle, however, did not conform exactly with bilateral symmetry, but in general where a muscle was partly dystrophic, perhaps with 25 % involvement on the left side, the corresponding muscle was involved to the same extent on the right side.

In some instances it was possible to state that the front limb was more affected than the hind, or the shoulder muscles more than those of the arm or forearm. Pappenheimer (1940–1) has stated that in guinea-pigs and rabbits dystrophic lesions are not usually bilaterally symmetrical. Pamukcu (1948), however, has observed bilaterally symmetrical lesions in the former species. In the *weisses Fleisch* disease of

Hjärre & Lilleengen (1936*a*, *b*) lesions are symmetrical, and in human muscular dystrophy the disease generally affects both legs and both shoulders (Bicknell & Prescott, 1946). Attempts were then made to relate the severity with which a particular group of muscles was affected to the posture adopted by the calf before death. Agreement was good. Incidence of dystrophy in the rhomboideus muscle and to a lesser extent the trapezius was associated with the winged scapula of calf no. CLO.O. 3. The characteristic flexing of the carpus (knee) was associated with involve-

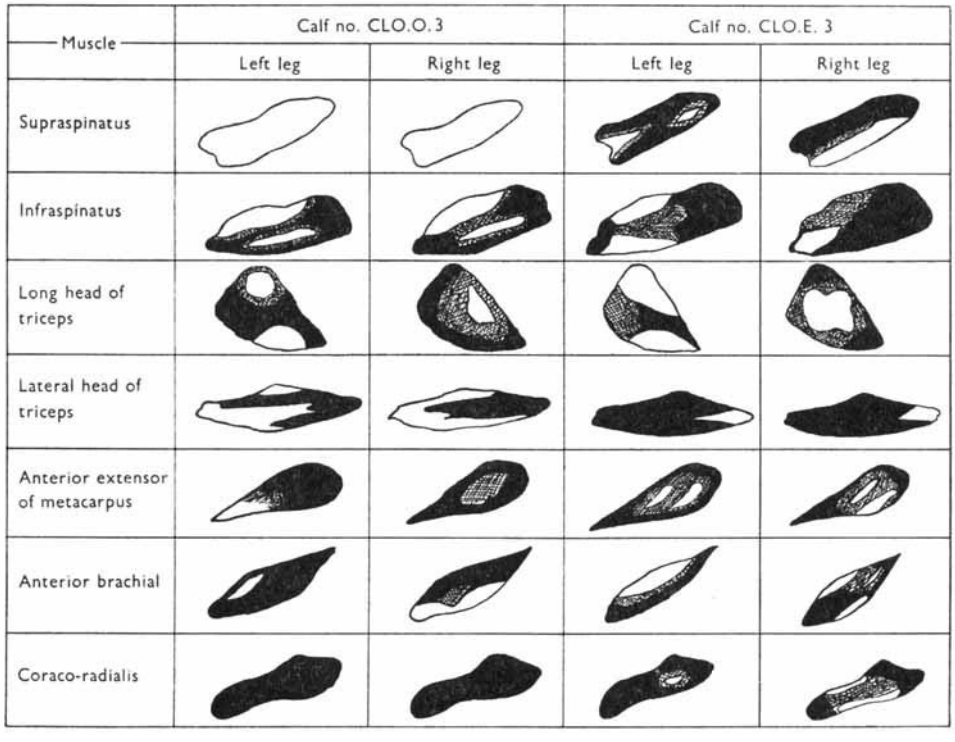


Fig. 3. Lesions of the muscles of the right and left front limbs of calves nos. CLO.O. 3 and CLO.E. 3, illustrating the bilateral symmetry of the dystrophic lesions. White areas are dystrophic; cross-hatched areas are mildly affected; black areas are normal.

ment of the shoulder muscles and those of the humerus group. Where spreading of the hoof claws was noted, the anterior extensor of the metacarpus and the muscles of the lower forearm appeared most affected. The adoption of a peculiar attitude and unnatural gait seemed related to removal of strain from these affected muscles. The calves in which the dystrophy was most marked in the hind-limbs minimized strain on these muscles by protrusion of the head and body and by placing the fore-limbs well under the body. This attitude is shown in calf no. CLO.O. 3 (Pl. 1, 3). Where dystrophy was most marked in the fore-limbs strain on these was minimized by a transference of weight to the hind-limbs and a forward stance, as is shown by the photograph of calf no. CLO.E. 2 (Pl. 1, 2).

The presence of pneumonia in a proportion of the calves is of interest. Davis & Maynard (1938) noted that a high proportion of their calves given cod-liver oil

showed pneumonia-like symptoms, though they did not succumb to any dystrophic disease. Most of the pneumonia encountered in the present study was probably caused by food entering the lungs, and this in turn was related to lack of control of the throat and tongue muscles.

Table 6. *Mean weights of different organs of the calves*

	Vitamins A and D supplied as			
	Cod-liver oil		Solution in arachis oil	
Organ	With vitamin E	Without vitamin E	With vitamin E	Without vitamin E
	(g)			
Brain	290	266	283	283
Liver	827	862	911	896
Heart	232	242	309	273
	(g/kg body-weight)			
Brain	8·47	7·51	6·47	6·80
Liver	24·2	24·3	20·9	21·5
Heart	6·78	6·85	7·07	6·54

Weights of the major organs of the calves were determined and are summarized in Table 6. No differences of a statistical significance were present. If the calves were classified according to the presence or absence of dystrophy (see Table 2), there was no evidence of any hypertrophy or atrophy of these organs in affected animals. The hearts of those animals that had been found abnormally flabby at post-mortem (calves nos. CLO.O. 1 and CLO.E. 1), however, tended to be the heaviest. The testes of the animals appeared to be of normal weight and no difference that could be ascribed to difference in treatment was seen.

DISCUSSION

The calves that were given the arachis-oil diet with a supplement of α-tocopherol appeared normal both before and at post-mortem examination. Those calves that received the same diet but did not receive α-tocopherol, however, were abnormal in behaviour and carriage, and when slaughtered three were found to have grossly dystrophic skeletal muscles and the other a degenerate myocardium. From the data presented it would seem that the lesions observed and the clinical appearance of the animals were in fact due to a dietary deficiency of vitamin E, thus confirming the preliminary observations. The disease appears quite comparable to the 'white muscle disease' of Vawter & Records (1947), the *weisses Fleisch* of Hjärre & Lilleengen (1936*a*, *b*) and the waxy degeneration (*wachsartige Muskeldegeneration*) of earlier authors. These diseases were not experimentally produced but were found in animals on farms. We have found and confirmed two similar field cases in Ayrshire. The lesions that have been described in the calves appear to be quite comparable to those described in other animals. They are bilaterally symmetrical and are not limited to particular muscles; they are sometimes associated with haemorrhages, and render the muscle non-translucent. The signs, too, show considerable agreement—somnolence and unwillingness to place particular muscles under stress, unnatural postures and, finally, complete prostration.

Of the eight calves that were given cod-liver oil with or without vitamin E, only one did not show signs of dystrophy and only one was not dystrophic on post-mortem examination. The signs and post-mortem appearances of these animals were in all respects similar to those of the calves given the vitamin-E-deficient diet in which fat-soluble vitamins were supplied in solution in arachis oil. It may be concluded, therefore, that administration of cod-liver oil results in a severe exacerbation of symptoms of dystrophy and effectively neutralizes the protective action of α-tocopherol. As little as 15–18 ml. cod-liver oil given daily was sufficient to render ineffective the protective action of a daily supplement of 50 mg DL-α-tocopheryl acetate. It may also be concluded, therefore, that the syndrome of cod-liver oil poisoning of calves described by the Scandinavian workers represents a conditioned vitamin E deficiency.

The length of time taken for the condition to develop is of interest. In severe cases the calves were first noted to be abnormal at 21–41 days from the time the experimental diet was first given. Two very severe cases were first noted at 24 and 43 days. Such variation shows that individuals differ considerably in their sensitivity to vitamin E deficiency. Whether this is due to variation in body stores of tocopherols, to differences in daily requirement or to other causes is not known.

SUMMARY

1. Observations on a disease of young calves are given and the reasons for the diagnosis of vitamin E deficiency are presented.

2. An experiment with sixteen Ayrshire calves is described. All were given a basal diet containing dried skim milk, lard and a mineral supplement. Eight were given vitamins A and D as the pure substances dissolved in arachis oil, and eight were given equivalent amounts as cod-liver oil. Four animals in each of these groups received in addition 50 mg DL-α-tocopheryl acetate daily.

3. Signs of dystrophy occurred in all groups other than that receiving both α-tocopherol and the arachis-oil solution. These signs are described in detail.

4. Nitrogen-balance studies with four calves showed that animals that were found dystrophic at post-mortem stored less nitrogen than the others. The high urinary excretion of nitrogen was due to an increase in excretion of urea and ammonia.

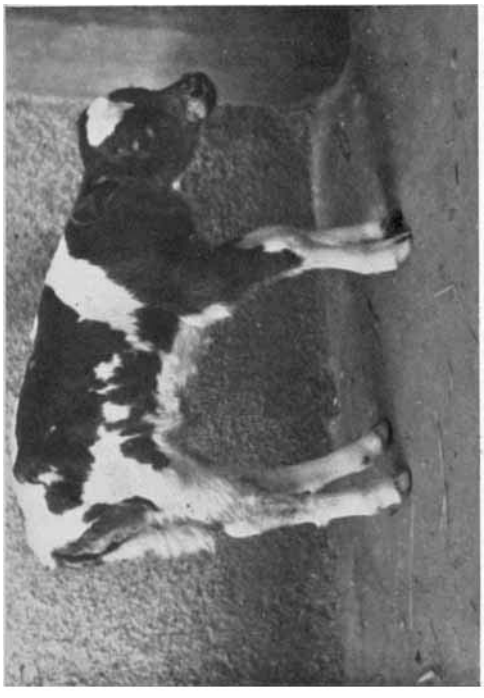
5. The oxygen consumption of dystrophic animals was increased. The excessive nitrogen katabolism and the increased heat production were probably interrelated.

6. Post-mortem examination of the affected calves revealed an extensive dystrophy with oedema, involving both skeletal and cardiac muscles. The dystrophy was bilaterally symmetrical.

7. The characteristic attitudes adopted by the calves before death, flexing of the carpus, straightening of the hind-limbs, spreading of the claws of the foot and winging of the scapulas, were all associated with severe dystrophy of the muscles controlling the movement of the joints concerned.

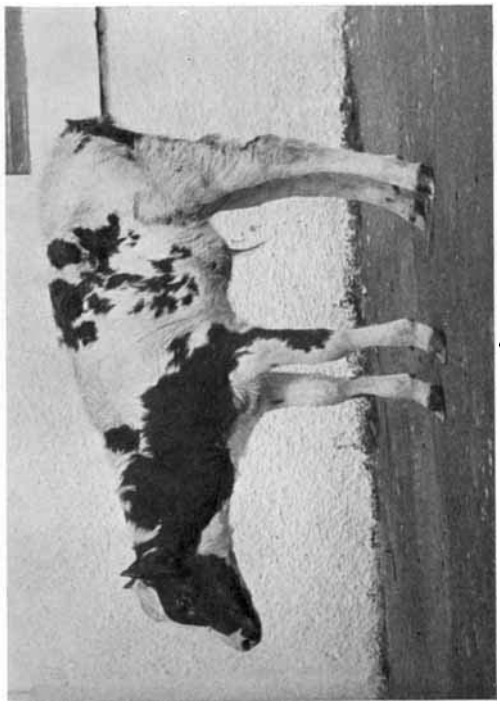
8. The daily ingestion of 15–18 ml. cod-liver oil neutralized the protective action of 50 mg DL-α-tocopheryl acetate daily.

9. The abnormalities found are compared with those described in similar naturally

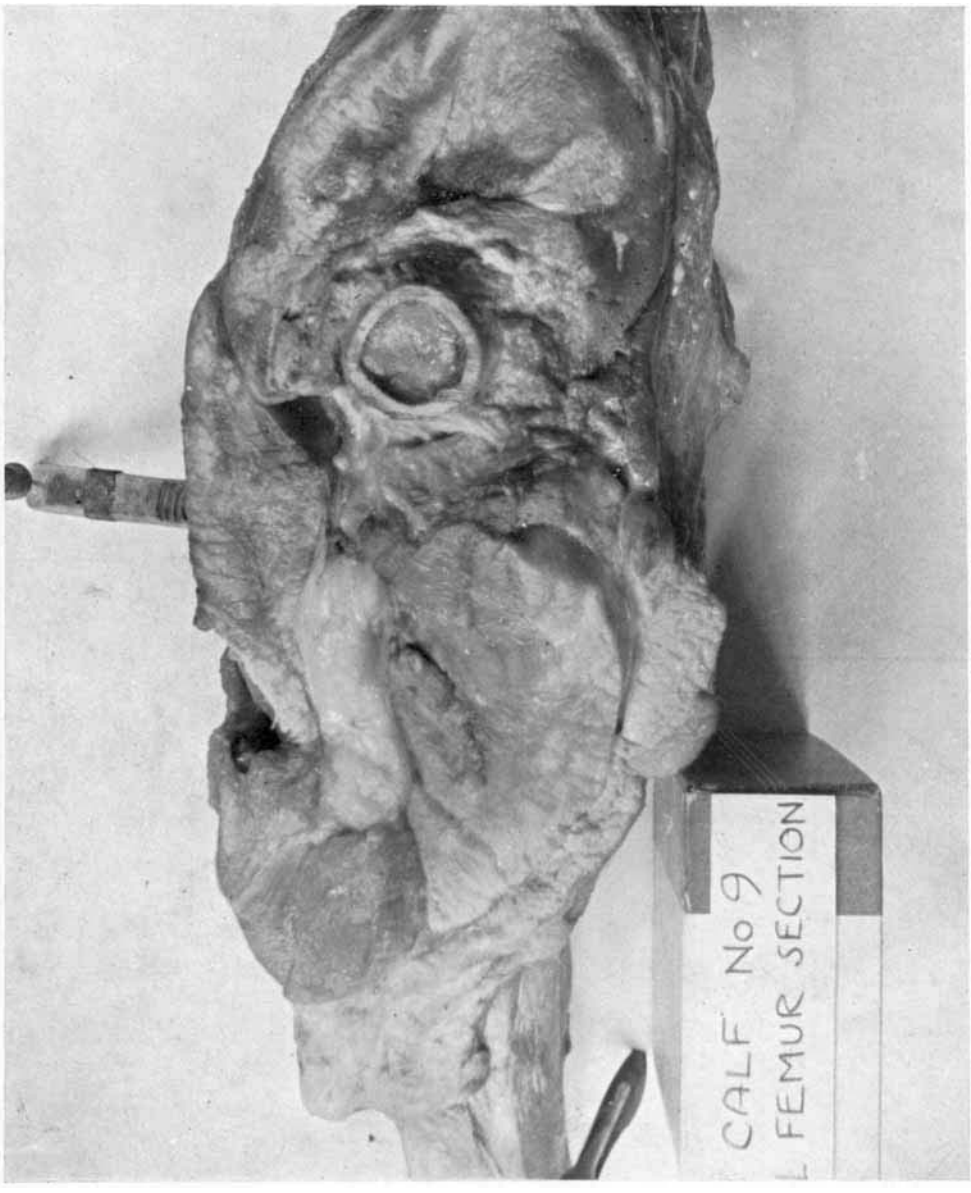


2

1



3



occurring diseases in Europe and America, and it is concluded that all these conditions probably result from vitamin E deficiency.

We are indebted to Dr T. Moore for the gift of α-tocopheryl phosphate for use in the preliminary trials, and to Miss A. M. Goodall for the histological examination of tissues from two of the calves in the earlier series. Our thanks are due to Mr D. Paterson, Mrs A. Arbuckle and Miss E. Semple for their technical assistance.

EXPLANATION OF PLATES

Plate 1

1. Normal calf no. AO.E. 3, which received sufficient vitamin E and showed no signs of dystrophy.
2. Dystrophic calf no. CLO.E. 2. Dystrophy was most evident in the fore-limbs and the strain on these muscles was minimized by transference of weight to the hind-limbs.
3. Dystrophic calf no. CLO.O. 3. Dystrophy was most evident in the hind-limbs, resulting in the abnormal stance adopted.
4. Shoulders of dystrophic calf no. AO.O. 4, showing protrusion of the suprascapula above the level of the spinal processes. This sign is comparable to winged scapula in muscular dystrophy of man.

Plate 2

Cross-section through the femur of a dystrophic calf, showing large white areas of dystrophy interspersed with small areas apparently unaffected.

REFERENCES

Agduhr, E. (1927). *Acta paediatr., Stockh.*, **6**, 165.
Agduhr, E. & Stenström, N. (1929). *Acta paediatr., Stockh.*, **8**, 493.
Anderson, H. D., Elvehjem, C. A. & Gonce, J. E. Jr. (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 750.
Barns, L. L., Davis, G. K. & McCay, C. M. (1938). *Cornell Vet.* **28**, 16.
Bicknell, F. & Prescott, F. (1946). *The Vitamins in Medicine*, 2nd ed. London: William Heinemann Ltd.
Biddulph, C. & Meyer, R. K. (1942). *Endocrinology*, **30**, 551.
Blaxter, K. L. & Howells, A. (1951). *Brit. J. Nutrit.* **5**, 25.
Blaxter, K. L. & Wood, W. A. (1951*a*). *Brit. J. Nutrit.* **5**, 11.
Blaxter, K. L. & Wood, W. A. (1951*b*). *Brit. J. Nutrit.* **5**, 29.
Blaxter, K. L. & Wood, W. A. (1951*c*). *Brit. J. Nutrit.* **5**, 55.
Blaxter, K. L. & Wood, W. A. (1952). *Brit. J. Nutrit.* **6**, 1.
Cummings, M. J. & Mattill, H. A. (1930–1). *J. Nutrit.* **3**, 421.
Davis, G. & Maynard, L. A. (1936). *J. Dairy Sci.* **19**, 442.
Davis, G. K. & Maynard, L. A. (1938). *J. Dairy Sci.* **21**, 143.
Duncan, C. W., Huffman, C. F. & Robinson, C. S. (1935). *J. biol. Chem.* **108**, 35.
Ferrando, R., Chenavier, P. & Cormier, M. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 810.
Florence, A. (1922). *Amer. J. Dis. Child.* **23**, 132.
Follis, R. H. (1948). *The Pathology of Nutritional Disease.* Oxford: Blackwell Scientific Publications Ltd.
Goettsch, M. & Pappenheimer, A. M. (1931). *J. exp. Med.* **54**, 145.
Gullickson, T. W. (1949). *Ann. N.Y. Acad. Sci.* **52**, 256.
Gullickson, T. W. & Calverley, C. E. (1946). *Science*, **104**, 312.
Gullickson, T. W. & Fitch, J. B. (1944). *J. Dairy Sci.* **27**, 331.
Gullickson, T. W., Palmer, L. S., Boyd, W. L., Nelson, J. W., Olson, F. C., Calverley, C. E. & Boyer, P. D. (1949). *J. Dairy Sci.* **32**, 495.
Hjärre, A. & Lilleengen, K. (1936*a*). *Virchows Arch.* **297**, 565.
Hjärre, A. & Lilleengen, K. (1936*b*). *Nord. Med. Tidskr.*, p. 472.
Knowlton, G. C. & Hines, H. M. (1938). *Proc. Soc. exp. Biol., N.Y.*, **38**, 665.
Loosli, J. K. (1949). *Ann. N.Y. Acad. Sci.* **52**, 243.
Madsen, L. L., McCay, C. M. & Maynard, L. A. (1935). *Mem. Cornell agric. Exp. Sta.* no. 178.
Mason, K. E. (1944). *Vitamins & Hormones*, **2**, 107.
Mason, K. E. & Telford, I. R. (1947). *Arch. Path.* **43**, 363.
Ministry of Agriculture and Fisheries (1938). *Bull. Minist. Agric., Lond.*, no. 10, 2nd ed.
Pamukcu, A. M. (1948). *Microfilm Abstr.* **8**, no. 2, p. 20.
Pappenheimer, A. M. (1939). *Amer. J. Path.* **15**, 179.
Pappenheimer, A. M. (1940–1). *J. Mt Sinai Hosp.* **7**, 65.
Pappenheimer, A. M. (1942). *Amer. J. Path.* **18**, 169.
Pappenheimer, A. M. (1943). *Physiol. Rev.* **23**, 37.

Pappenheimer, A. M. (1948). *On Certain Aspects of Vitamin E Deficiency.* Springfield, Ill.: C. C. Thomas.
Pappenheimer, A. M. & Goettsch, M. (1931). *J. exp. Med.* **53**, 11.
Parrish, D. B. (1949). *Ann. N.Y. Acad. Sci.* **52**, 251.
Pollock, A. W. (1854). *Fmrs' Mag.* (3rd Ser.), **6**, 329.
Scorgie, N. J. & Miller, W. C. (1944). *Emp. J. exp. Agric.* **12**, 74.
Slagsvold, L. (1925). *Norsk vet. Tidsskr.* **6**, 161.
Turner, W. A., Meigs, E. B. & Converse, H. T. (1936). *J. biol. Chem.* **114**, civ.
Vawter, L. R. & Records, E. (1947). *J. Amer. vet. med. Ass.* **110**, 152.
Victor, J. (1934). *Amer. J. Physiol.* **108**, 229.
Wagener, K. (1936). *Tierärztl. Rdsch.* **42**, 789.
Willman, J. P., Asdell, S. A., Grams, W. T. & Hagan, W. A. (1931). *Proc. Amer. Soc. Anim. Prod.*, p. 231.
Willman, J. P., Loosli, J. K., Asdell, S. A., Morrison, F. B. & Olafson, P. (1945). *J. Anim. Sci.* **4**, 128.
Willman, J. P., Loosli, J. K., Asdell, S. A., Morrison, F. B. & Olafson, P. (1946). *Cornell Vet.* **36**, 200.

The Nutrition of the Young Ayrshire Calf

9. Composition of the Tissues of Normal and Dystrophic Calves

BY K. L. BLAXTER (IN RECEIPT OF A SENIOR AWARD OF THE AGRICULTURAL RESEARCH COUNCIL)
AND W. A. WOOD
Hannah Dairy Research Institute, Kirkhill, Ayr

(*Received 27 March* 1951)

In a previous paper (Blaxter, Watts & Wood, 1952) data were presented which showed that experimental diets could be prepared which, when given to young calves, caused a very high incidence of muscular dystrophy. The present paper is concerned with the composition of muscles and other tissues of these calves.

EXPERIMENTAL

General

The plan of the experiment, details of experimental animals, their diets and their management were given in the previous paper (Blaxter *et al.* 1952). The same nomenclature is used here, namely:

Group AO.E. Vitamins A and D were supplied in solution in arachis oil; 50 mg α-tocopheryl acetate were given daily.

Group AO.O. Vitamins A and D were supplied in solution in arachis oil; no vitamin E was given.

Group CLO.E. Vitamins A and D were supplied by cod-liver oil; 50 mg α-tocopheryl acetate were given daily.

Group CLO.O. Vitamins A and D were supplied by cod-liver oil; no vitamin E was given.

Individual calves are referred to by treatment and replication number.

Methods

Post-mortem methods and details of dissections have been described (Blaxter *et al.* 1952). The following muscles were analysed chemically: supraspinatus, infraspinatus,