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The Nutrition of the Young Ayrshire Calf

14. Some Effects of Natural and Synthetic Anti-oxidants on the Incidence of Muscular Dystrophy Induced by Cod-liver Oil

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The muscular dystrophy of calves caused by giving cod-liver oil (Blaxter, Wood & MacDonald, 1953), the unsaturated acids of cod-liver oil (Blaxter, Brown & MacDonald, 1953*b*) or rations containing lard (Blaxter, Watts & Wood, 1952) may be prevented by giving α-tocopherol.

The mode of action of α-tocopherol in these diseases is unknown. A reasonable supposition, however, is that it acts simply as an anti-oxidant, possibly protecting the animal against the toxicity of unsaturated fatty acids. If this were so, other anti-oxidants might be equally effective. The efficacies of ascorbic acid, ethyl gallate, methylene blue and biotin have therefore been compared with that of α-tocopheryl acetate in preventing the muscular dystrophy induced by giving cod-liver oil to calves.

It has been claimed (Hjärre & Lilleengen, 1936*a*, *b*) that enzootic muscular dystrophy is due to ascorbic-acid deficiency. This is unlikely, for reasons discussed elsewhere (Blaxter & Brown, 1952–3). It is of interest, however, that ascorbic acid is

partially effective in preventing signs of vitamin E deficiency in chicks given rations containing cod-liver oil (Zacharias, Goldhaber & Kinsey, 1950; Dam, Kruse, Prange & Søndergaard, 1948). Further, a decline in serum ascorbic acid occurs in horses affected by myoglobinuria paralytica, a disease in which a muscle degeneration, similar to that of calves deficient in vitamin E, takes place.

Methylene blue has been shown to be of considerable value in treating the above disease of the horse (Elzén, 1932*a*, *b*), and recent experimental studies by Dam and his colleagues have shown that methylene blue can replace vitamin E in preventing many of the signs of vitamin E deficiency in rats and chicks (Dam, Kruse, Prange & Søndergaard, 1951; Christensen & Dam, 1951; Aaes-Jørgensen, Dam & Granados, 1951; Dam & Granados, 1951, 1952; Dam, Prange & Søndergaard, 1951, 1952*a*, *b*). This work has recently been confirmed and extended by Moore and his colleagues working with rats; they have found that some, but not all, manifestations of vitamin E deficiency are prevented by methylene blue, and that the effect is specific, not being shared by a range of redox dyestuffs and anti-oxidants (Moore, Sharman & Ward, 1953*a*, *b*).

Biotin deficiency in the calf (Wiese, Johnson & Nevens, 1946) has been associated with spasticity of gait and prostration, signs similar to those observed in our calves given cod-liver oil. Further, Sullivan, Kolb & Nicholls (1942) have observed muscle changes in biotin deficiency of rats similar to those in vitamin E deficiency, and in vitro studies (Pavcek & Shull, 1942) have indicated that biotin is inactivated by rancid fats. Experiments with calves by Kon & Porter (1951), however, have failed to confirm the observations of Wiese *et al.* (1946).

EXPERIMENTAL

Animals. Forty-two bull calves, purchased when a few days old, were used as experimental animals. Their housing and management were as previously described, save that sawdust was used as bedding instead of peat moss, since the latter was found to contain a substance reacting as a tocopherol in the Emmerie & Engel (1938) test for tocopherol.

Diets. The diet of dried skim milk was prepared and given throughout the experiment in the amounts described by Blaxter, Wood & MacDonald (1953). Each calf received 1 fluid ounce of cod-liver oil daily. This basal ration may be regarded as a standard dystrophy-producing diet. It contained approximately 7·5 mg α-tocopherol, of which 0·5 mg was supplied in the dried skim-milk powder and 7 mg in the cod-liver oil (Brown, 1953). Each calf received a supplement of iron, copper, manganese, cobalt and magnesium. Ten ml. of the solution used in previous experiments (Blaxter, Watts & Wood, 1952) were given once weekly.

Experimental treatment. The effects of α-tocopheryl acetate, ascorbic acid, biotin, ethyl gallate and methylene blue were studied according to the scheme of treatment shown in Table 1. Two other calves, not mentioned in Table 1, were used in studies of urinary excretion and serum concentration of ascorbic acid.

Methods. The calves listed in Table 1 were first grouped according to the concentration of tocopherols found in their blood serum, and then the animals within

each group were allocated at random to treatment groups. The calves were examined twice daily and were weighed and exercised once weekly, observations being recorded as previously described (Blaxter, Wood & MacDonald, 1953). Blood samples were taken weekly for the first 4 weeks and at longer intervals thereafter. The first sample taken on arrival of the calf was examined for the presence of colostral globulins by

Table 1. *Experimental calves (laboratory numbers) and their treatment*

Date of purchase	Basal ration only (controls)	α-Tocopheryl acetate	Ascorbic acid	Biotin	Ethyl gallate	Methylene blue
27. ii. 51	79	—	—	87*	—	—
	80	—	—	88*	—	—
15. v. 51	105	107†	109‡	—	—	—
	106	108†	110‡	—	—	—
1. vi. 51	112	114§	116‖	—	—	—
	113	115§	117‖	—	—	—
7. viii. 51	122	—	125¶	—	—	—
	123	—	126¶	—	—	—
	124	—	127¶	—	—	—
16. x. 51	143	—	—	—	140**	139††
	146	—	—	—	144**	141††
	147	—	—	—	145**	142††
8. i. 52	159	—	—	—	160**	158††
	161	—	—	—	162**	163††
	166	—	—	—	164**	165††
Total no. of calves	15	4	7	2	6	6

* 0·4 mg/day biotin by subcutaneous injection.
† 200 mg/day DL-α-tocopheryl acetate by mouth in a single dose.
‡ 1·6 g/day ascorbic acid by mouth in a single dose.
§ 400 mg/2 days DL-α-tocopheryl acetate by intramuscular injection.
‖ 3·2 g/2 days ascorbic acid by intravenous injection.
¶ 1·6 g/day ascorbic acid by mouth in two doses.
** 2·0 g/day ethyl gallate by mouth in a single dose.
†† 1·0 g/day methylene blue by mouth in a single dose.

the quantitative method of Aschaffenburg (1949), using the Hilger Spekker photo-electric absorptiometer, as well as for tocopherols. In the blood serum of calves receiving ascorbic acid and that of their corresponding controls ascorbic acid was determined by the method of King (1946). Tocopherol in blood serum was determined by the method of Emmerie & Engel (1938), as modified and briefly described by Blaxter, Brown & MacDonald (1953*a*).

Calves that developed gross and easily recognized signs of muscular dystrophy were slaughtered. Those calves that did not continued on experiment until it appeared unlikely that such signs would develop, when they also were killed. The carcasses of all calves, whether they died, were killed *in extremis* or were killed without showing gross signs of dystrophy, were dissected and examined, particular attention being paid to the musculature. The chemical methods employed for determining creatine and tocopherol contents of muscles and other tissues and the histological methods employed have been described by Blaxter, Brown & MacDonald (1953*b*).

RESULTS

The details for each calf are not given, results being summarized in Tables 2 and 3.

The effect of α-tocopheryl acetate. Fig. 1 shows the mean concentrations of tocopherols in the blood serum of control calves and of those given α-tocopheryl acetate. Although 200 mg α-tocopheryl acetate by mouth increased the blood concentration, when 400 mg α-tocopheryl acetate were injected intramuscularly every 2 days, the

Table 2. *Mean creatine content of groups of skeletal muscles and the hearts of experimental calves expressed as mg/100 g fresh muscle*

	Muscle or muscle groups					
Treatment group	Shoulder	Forearm	Lower arm	Rectus femoris	Heart	Conclusion
α-Tocopheryl acetate by mouth	355·2	350·8	382·9	408·2	236·7	Marked difference
Controls	297·9*	312·0*	334·0	326·4*	179·0*	
α-Tocopheryl acetate by injection	378·1	407·1	378·1	417·6	245·7	Marked difference
Controls	229·9*	283·4*	349·1	384·5	179·2*	
Ascorbic acid	302·7*	329·5*	328·9*	403·7	189·9*	No difference
Controls	283·2*	308·9*	319·5*	302·1*	198·8*	
Ethyl gallate	299·1*	319·0*	312·7*	355·4*	212·2*	No difference
Controls	292·9*	308·9*	319·5*	403·4	210·6*	
Methylene blue	373·6	382·8	378·9	440·4	255·0	Marked difference
Controls	292·9*	308·9*	319·5*	403·4	210·6*	
Biotin	229·3*	325·1*	368·5	413·0	207·2*	No difference
Controls	299·4*	331·4*	333·4*	339·1*	149·7*	
Normal†	373	375	372	429	258	—

Shoulder muscles = infraspinatus and supraspinatus.
Forearm muscle = long and lateral head of triceps.
Lower-arm muscles = coracoradialis, anterior brachial and anterior extensor of metacarpus.

* These values are judged to be lower than the normal (Blaxter, Wood & MacDonald, 1953).

† Mean values for normal calves given α-tocopheryl acetate and no cod-liver oil in previous experiments (Blaxter, Wood & MacDonald, 1953).

concentration of tocopherols in the serum did not differ from that observed in control calves, nor did analysis of the blood reveal the presence of the acetate ester, suggesting very slow or negligible absorption of the vitamin from the injection sites. At post-mortem examination of the two calves dissection of the injection sites revealed the presence of oily droplets of the ester within the muscles. Histological examination of the sites showed fine droplets of the vitamin between muscle bundles and a marked reaction on the part of fibrous tissue, effectively sealing off each droplet. There was a distinct eosinophilic infiltration with some polymorphonuclear reaction at the periphery of each droplet.

Clinical and post-mortem examination of the calves showed no dystrophy in those given α-tocopheryl acetate by mouth. In one of the calves given the vitamin by injection, however, histological examination revealed typical hyaline lesions of the muscle and sarcolemmal proliferation in the region where the vitamin had been injected. No other lesions were found, and chemical analysis showed that the

Table 3. *Summary of incidence of muscular dystrophy in the calves*

Treatment group	No. of calves*	No. that died or were killed *in extremis*	Clinical signs observed in	Mean age at death (days)	Mean score indicating severity of dystrophy†	Conclusion
α-Tocopheryl acetate by mouth	2	0	0	—	0·5	Complete prevention of muscular dystrophy
Controls	2	2	2	23	24·0	
α-Tocopheryl acetate by injection	2	0	0	—	8·5	Considerable prevention of muscular dystrophy
Controls	2	2	2	32	24·5	
Ascorbic acid	6	2	3	36	12·5	Slight prevention of muscular dystrophy
Controls	7	6	6	33	22·1	
Ethyl gallate	6	3	3	44	15·8	Ineffective in preventing muscular dystrophy
Controls	4	3	4	50	20·5	
Methylene blue	5	1	1	38	1·6	Complete prevention of muscular dystrophy
Controls	4	3	4	50	20·5	
Biotin	2	2	2	33	21·5	Ineffective in preventing muscular dystrophy
Controls	2	2	2	28	27·0	

* The calves that died as the result of causes outwith the experiment were not included.

† The scoring system was based on the classification of the muscle groups at post-mortem. The following numerical values were used:

Normal = 0	Slight = 4
? Normal = 1	Severe = 6
Very slight = 2	Very severe = 8

and the values given represent the mean of the sums of scores for each of five groups of muscles. A completely normal animal would thus have a score of 0·0 and a completely affected animal a score of 40·0. Errors of differences in scores are approximately ±8·0 units.

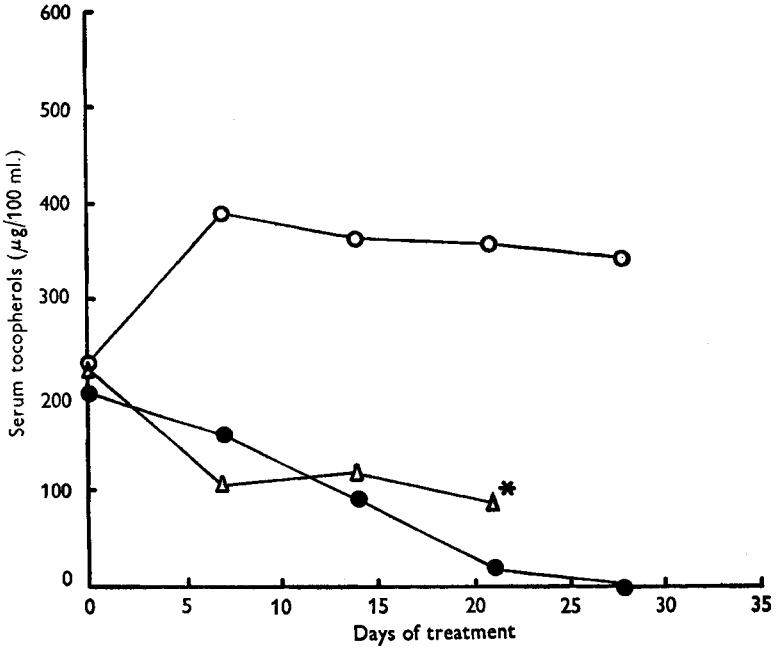

Fig. 1. Tocopherol content of blood serum of calves receiving cod-liver oil and vitamin E. △, cod-liver oil alone; ○, vitamin E by mouth and cod-liver oil; ●, vitamin E by injection and cod-liver oil.

* Both animals dead.

muscles of both groups of calves given tocopheryl acetate could be regarded as normal, unlike those of the controls.

The effect of ascorbic acid. Ascorbic acid given by mouth or by injection had no effect on the rate of tocopherol disappearance from the blood. A high concentration of ascorbic acid in the blood serum was not, however, attained by the methods of administration employed, nor did α-tocopherol administration result in any elevation

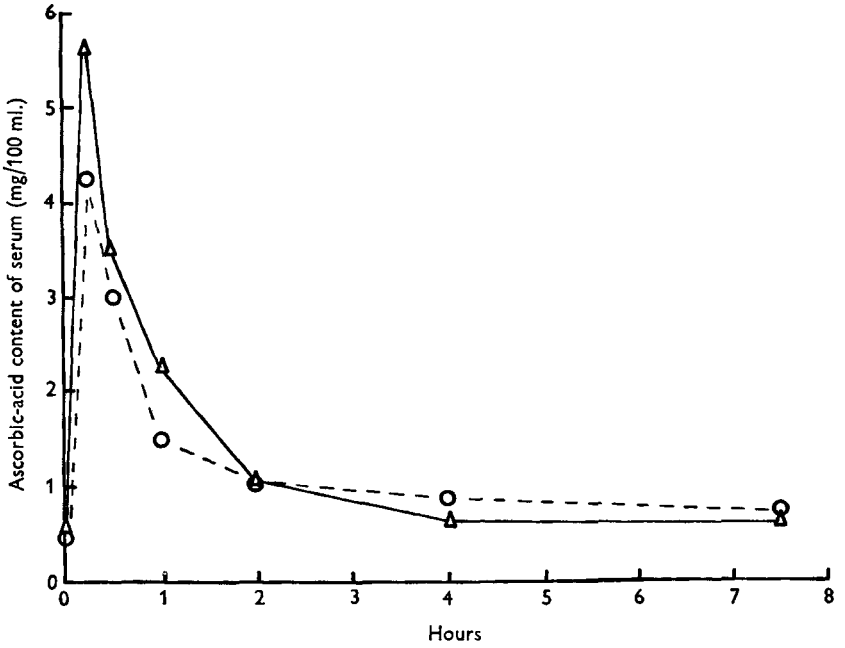

Fig. 2. Effect of an intravenous injection of 1·6 g ascorbic acid on the ascorbic-acid content of the blood serum of each of two calves.

of the ascorbic-acid concentration. Serum ascorbic acid was determined 24 h or sometimes 12 h after dosage or injection. Rapid excretion of ascorbic acid and its rapid destruction in the gut or tissues were possible reasons for the low levels of ascorbic acid found.

When 1·6 g ascorbic acid were given intravenously to each of two calves maintained on a diet not containing cod-liver oil, it had all left the blood stream within 2–4 h, as shown in Fig. 2. The urine concentration was extremely high during the initial period. When 1·6 g ascorbic acid were given by mouth, the highest serum concentration occurred during the 1st hour in one calf and at the 4th hour in the other. The concentration was still elevated at the 8th hour, but had returned to normal by the 24th hour. Again the urine concentration of ascorbic acid was elevated. These results with the calf differ from those obtained with the adult cow in which oral administration of large doses of ascorbic acid does not increase the blood or urine concentration (Knight, Dutcher, Guerrant & Bechdel, 1941), owing to rapid destruction of the vitamin in the rumen. The present results in the calf suggest, however, that high concentrations of ascorbic acid in the blood serum, and presumably the tissues, were

not maintained in any of the experiments owing to the rapid excretion of it in the urine rather than destruction in the gut. It can therefore be assumed that the tissues of the calves were maintained at a peak of ascorbic-acid saturation throughout the experiment. One of the calves given ascorbic acid was killed after severe diarrhoea and was found to have suffered from severe enteritis. *Bacterium coli* was isolated from the heart blood. This animal has been excluded from the results given in Tables 2 and 3. It will be noted that muscular dystrophy occurred despite massive dosage

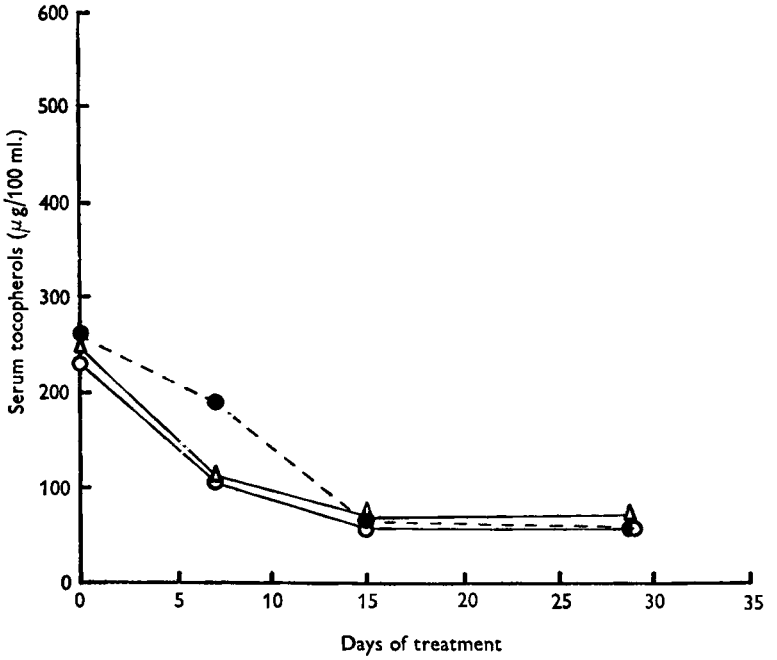

Fig. 3. Effect of ethyl gallate and methylene blue on the tocopherols of the blood serum of calves given cod-liver oil. △, cod-liver oil alone; ○, ethyl gallate and cod-liver oil; ●, methylene blue and cod-liver oil.

with ascorbic acid. In two of the calves, however, no gross lesions were observed, but the carcasses were distinguished by their marked pallor. Histological examination and determinations of creatine in the muscles confirmed that ascorbic acid had little effect in preventing the disease.

The effect of ethyl gallate. The calves given ethyl gallate did not thrive as well as calves given other supplements or even given no supplements. They developed an intense dislike for their food. Since the ethyl gallate was given by capsule and was not mixed with the basal diet, a systemic disturbance of appetite was responsible rather than a reaction to the presence of an abnormal taste in the daily allowance of food. The breath of two of the calves had an abnormal soapy, semiputrid smell, but whether or not this was related to the abnormality of appetite was not ascertained. Food refusals were few, since the calves could generally be coaxed to consume the whole of their food.

Fig. 3 shows the mean tocopherol contents of the blood serum of calves given ethyl

gallate and those of similar control calves during the course of the experiment. There were no differences to be ascribed to treatment. Two of the calves acting as controls to those receiving methylene blue and ethyl gallate died of infectious enteritis. The remainder were, with one exception, dystrophic. As shown in Tables 2 and 3, ethyl gallate was ineffective in preventing the muscular dystrophy induced by cod-liver oil. In one of the calves given ethyl gallate the auricles of the heart were involved in the cardiac dystrophy. This has been the sole case so far observed of dystrophy involving the auricles.

The effect of methylene blue. The dose of 1 g daily of methylene blue to the calves caused some alimentary disturbance with consequent dehydration. The faeces they passed were colourless, turning dark blue on exposure to the air. Their urine was always dark blue. The urine and faeces were collected quantitatively from three calves that received the drug. They showed, of the 1 g of methylene blue ingested, 182, 226 and 295 mg excreted in the urine and 274, 356 and 168 mg in the faeces. An average of approximately 500 mg was not accounted for and was presumably destroyed.

As shown in Fig. 3, between the mean tocopherol contents of the blood serum of the control calves and of those receiving methylene blue there was no difference to be ascribed to treatment.

Two calves succumbed, one to an acute diarrhoea, which did not respond to phthalylsulphathiazole, and the other to an inhalation pneumonia, also associated with diarrhoea. Examination of the muscles of these calves showed them to be normal. Apart from occasional alimentary disturbance, the remaining calves appeared quite healthy. After slaughter the musculature was conspicuously deep red in colour and in one calf only was any evidence of muscular dystrophy found. Histological examination of the affected supraspinatus muscle of this calf showed some hyaline fibres, an early sarcolemmal reaction and some interstitial oedema. Examination of the remaining calves of this group revealed no gross histological abnormality. Since the control calves in this series and the ethyl-gallate calves had shown considerable evidence of muscular dystrophy, it was evident that methylene blue had afforded protection. As shown in Table 2, the creatine contents of the muscles and muscle groups of these calves were normal, indistinguishable in fact from those of calves given no cod-liver oil and adequate vitamin E in previous experiments and decidedly greater than those of the negative control calves.

The effect of biotin. The injection of biotin intramuscularly in two calves did not counteract the effect of cod-liver oil. Both calves died, as did their contemporary controls, and all were severely dystrophic on post-mortem examination. As shown in Table 2, the creatine contents of their muscles were very low. Though the controls had more severe cardiac lesions, it was obvious that those given biotin were in general as severely dystrophic.

Tocopherol content of the tissues. Table 4 presents the amounts of tocopherol found in the tissues of the calves at slaughter. Tissues were taken only where it was certain that less than 60 min had elapsed between death and the excision of the tissue. It will be noted there were no differences in the tocopherol contents of the tissues

ascribable to an effect of the supplements. Even the supplement of α-tocopherol by mouth had no effect on the amounts found in the muscles. The livers of those calves given α-tocopherol were not examined.

Table 4. *Tocopherol content of tissues*

Treatment group	Values for individual calves	Mean
	Muscle tocopherol (mg/kg fresh weight)	
Controls	9·9; 11·5; 5·6; 10·4; 9·8; 12·2; 9·0	9·8
α-Tocopheryl acetate by mouth	12·3; 7·8	10·1
α-Tocopheryl acetate by injection	18·4*; 8·7*	13·6
Ascorbic acid	8·8; 8·9; 8·4; 3·6; 8·2	7·6
Ethyl gallate	11·0; 6·4; 11·0; 3·7; 8·8	8·2
Methylene blue	11·0; 14·3; 7·7; 4·2	9·3
	Liver tocopherol (mg/kg fresh weight)	
Controls	7·7; 12·8; 11·9; 14·0; 14·9; 17·0	13·1
α-Tocopheryl acetate by injection	10·9; 11·8	11·4
Ascorbic acid	13·4; 14·5; 13·1	13·7
Ethyl gallate	15·9; 10·7; 9·9	12·2
Methylene blue	8·4; 12·3; 11·6; 12·4	11·2
	Perinephric-fat tocopherol (mg/kg extracted lipid)	
Controls	44·0; 101·8	—
Ethyl gallate	33·0; 49·4; 55·9; 58·7	49·2
Methylene blue	56·0; 45·8; 50·8	50·9

* Triceps muscle, others rectus femoris.

The wide variation in the tocopherol content of the tissues of the calves making up each group was not associated with variation in the severity of dystrophy in individuals; in fact, the worst affected muscles tended to contain a greater concentration. Nor was it closely related to the length of time the calves had been subject to experiment. There was, however, a slight correlation between the concentration of tocopherols in the blood serum when the calves were purchased and the concentration in the muscles at death. The regression was significant ($P = 0{\cdot}05$) and suggests that initial reserves of tocopherols are of importance in maintaining muscle concentration of tocopherols, even after several months' subsistence on a diet of low vitamin E activity and containing unsaturated fat.

The relation of the concentrations of tocopherols and of colostral globulins in the blood serum with the season of the year in which the calves were born. Previous work suggested that the incidence of experimental dystrophy was greatest in calves born in the spring months (Blaxter, Wood & MacDonald, 1953), and it was later found that even apparently normal calves born in the spring had muscles lower in creatine than those of calves born in the autumn (Blaxter, Brown & MacDonald, 1953*a*). The calves used in these experiments were purchased at different times of the year, and figures for concentrations of tocopherols in their blood serum at purchase are presented in Table 5. Results obtained on calves used as experimental animals in studies of the toxicity of cod-liver oil fractions (Blaxter, Brown & MacDonald, 1953*b*) are also included.

Statistical analysis of the data of Table 5 showed that there were highly significant differences among the concentrations of tocopherols in the serum of calves purchased at different times, values obtained during the winter months being decidedly lower. It has been shown, however (Blaxter, Brown & MacDonald, 1953*a*), that the serum level of tocopherols reflects the recent intake of tocopherols rather more than it does tissue reserves. The first food of the calf is colostrum, the ingestion of which can be detected by analysis of the blood for globulins, since colostral globulins are absorbed intact through the mucosa of its gut. In Table 5 estimates of the serum concentration

Table 5. *Concentration of tocopherols and of colostral globulins in the blood serum of calves when purchased*

	Date of purchase	No. of calves	Serum tocopherol. Value with its standard error (μg/100 ml.)	Colostral globulins in serum. Values with their standard errors. Spekker absorptiometer reading of turbidity (arbitrary units)
	15 May	6	334·7 ± 33·0	—
Summer	12 June	5	259·0 ± 36·2	—
Summer	7 August	6	257·3 ± 33·0	—
	16 October	9	285·6 ± 27·0	0·621 ± 0·081
	30 October	5	174·0 ± 36·2	0·392 ± 0·130
Winter	8 January	9	182·4 ± 27·0	0·141 ± 0·081
Winter	4 March	10	148·6 ± 25·6	0·086 ± 0·076
	25 May	10	183·5 ± 25·6	0·420 ± 0·076
	All calves	60	220·8 ± 10·4	—

of colostral globulins are also given, and it will be seen that groups of calves with low serum concentrations of tocopherols also had low values of colostral globulins. Analysis of covariance showed that the regression of tocopherol concentration on globulin concentration was significant ($0{\cdot}05 > P > 0{\cdot}01$); when the tocopherol means were adjusted to take into account variation in globulin content, no seasonal differences of statistical significance were observed. This suggests that the total intake of colostrum by calves born on commercial farms in winter is less than that of those born in summer. The reason for this may be economic rather than physiological, since the price the farmer receives for milk in winter is nearly twice that obtaining in summer.

DISCUSSION

The main conclusions of the experiments are presented in Table 3. It was evident that α-tocopheryl acetate when given by mouth prevented muscular dystrophy. The same was not true of α-tocopheryl acetate given by injection, since negligible absorption took place from the injection sites, and evidence was found that encapsulation of the ester within the muscle was beginning. With ascorbic acid, chemical analysis of selected muscles showed no evidence of any protection; clinical appraisal and pathological examination of the animals suggested that the severity of the disease was only slightly less than in the control calves. Whatever protection was afforded, however, must have been extremely slight, so that the conclusions of Hjärre &

Lilleengen (1936*a*, *b*), that muscular dystrophy is due to vitamin C deficiency, are wrong. Ethyl gallate was completely ineffective in preventing muscular dystrophy, despite the fact that dosage was sufficient to cause obvious signs of a systemic disturbance reflected in failure of appetite. Biotin given by injection was also ineffective. Methylene blue, however, afforded considerable protection of the calves against the toxicity of cod-liver oil. This result supports those obtained by Dam and his colleagues in the study of similar 'vitamin E deficiency states' in rats and chicks, as previously outlined.

These results are extremely difficult to explain. One explanation is that in some unknown way the effect of tocopherols in preventing muscular dystrophy is due to their action in the tissues as 'anti-oxidants', that is, they are more readily oxidized than the substrate they protect. Possibly they prevent the formation from dietary unsaturated acids of epoxides and other peroxides that might cause tissue damage. There are several facts to suggest that this is not the explanation. The natural tissue constituents, ascorbic acid and biotin, which could also compete at the site of fat peroxidation, have no effect in preventing the disease, and ethyl gallate, a fat-soluble anti-oxidant, given in large doses also had no effect. Differences in the redox potential of the compounds might explain these findings. Blood and tissue analysis, affords no evidence that these compounds prevent the loss of tissue tocopherols. It is true that methylene blue might protect in the manner outlined, since some is presumably present in the tissues as the leuco compound. It certainly does not prevent muscular dystrophy by protection of tocopherols in the tissues, because the blood concentration of tocopherol declined at similar rates whether methylene blue was given or not, and at slaughter tissue concentrations of tocopherol were the same in both groups.

No metabolic role has yet been assigned to tocopherols. The normal levels of tocopherol in muscles of dystrophic animals might suggest that tocopherol is not intimately concerned with normal muscular metabolism as coenzyme or prosthetic group. It is clear, however, that dietary unsaturated acids in the absence of large amounts of either tocopherol or methylene blue in the diet cause in muscle a sequence of events culminating in dystrophic degeneration. These events all appear contingent upon a primary uncoupling of phosphorylation from respiration, in fact a role in oxidative phosphorylation was originally suggested by Weil-Malherbe (1948) as a possible mode of action of tocopherol. Thus, the increased heat production of the intact calf, the decreased excretion of creatinine and the increased excretion of creatine in the urine (Blaxter, Watts & Wood, 1952), together with the loss of myosin, potassium and creatine from the muscle cell and the infiltration of sodium and water (Blaxter & Wood, 1952), all suggest a decrease in the rate of formation of high-energy phosphate bonds. The simplest explanation of the value of tocopherols and of methylene blue is that they can form a part of some system that either prevents the formation of toxic products from unsaturated fats capable of uncoupling phosphorylation from respiration or alternatively facilitates their dissimilation or deposition as triglycerides.

SUMMARY

1. Experiments with forty-two calves were carried out to determine the value of α-tocopheryl acetate, ascorbic acid, biotin, ethyl gallate and methylene blue in preventing the muscular dystrophy resulting when a diet of dried skim-milk powder and cod-liver oil is given. This diet produced clinically recognizable muscular dystrophy in 87% of animals given no supplement. Protection was judged by clinical signs, post-mortem appearance, chemical analysis of the muscles and histological examination.

2. DL-α-tocopheryl acetate given by mouth increased the concentration of tocopherols in the blood serum and protected the animal against the toxic action of the unsaturated acids of the cod-liver oil. Intramuscular injection of the same amount of DL-α-tocopheryl acetate caused no increase in the tocopherol concentration of the serum and resulted in only slight protection of the animals, since it was poorly absorbed from the injection sites.

3. Ascorbic acid in massive doses by mouth or by intravenous injection had no effect on blood or tissue concentrations of tocopherols and did not prevent muscular dystrophy.

4. Large daily doses of ethyl gallate by mouth caused a systemic disturbance in the calves, but had no effect in preventing dystrophy.

5. Biotin given by intramuscular injection had no effect on the development of muscular dystrophy.

6. Daily administration of 1 g methylene blue resulted in protection of the calves against the toxicity of cod-liver oil. This was not associated with higher concentrations of tocopherols in serum, muscle, liver or perinephric fat than in control calves which succumbed.

7. The concentration of tocopherols in the blood serum of calves at the beginning of the experiments was highly correlated with the amount of colostrum they had been given, as judged by the concentration of colostral globulins in the blood.

8. The results are discussed in relation to explanations of the effect of α-tocopherol in terms of anti-oxidant activity.

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Absence of Correlation between Fatty Changes in the Liver and Impairment of Water Diuresis in Protein-deficient Mice

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It has been shown repeatedly that in rats the diuretic response to water administration can be decreased by dietary measures. Such decreases have been shown to occur in rats kept on low-protein (0·5%) diets containing 7·5–15·5% fat (Dicker, Heller & Hewer, 1946; Heller & Dicker, 1946–7; Dicker, 1950). Similar findings in the same species were made by Leslie & Ralli (1947), who used a diet containing 8% protein and 38% fat. Shay, Kolm & Fels (1945), whose rats received a diet containing 28% protein and 60% fat, reported likewise an impairment of water diuresis. Fatty changes in the liver can be produced either by protein-deficient or by high-fat diets; they may be presumed, or in some cases have actually been shown (Heller & Hewer, unpublished results; György, 1944; Shay *et al.* 1945) to occur with the diets discussed. Are these fatty changes or rather the biochemical or circulatory (Himsworth, 1947; Hartroft, 1949) events in the liver associated with them the common factor in the alterations of the water metabolism obtained with such widely different nutritional régimes? In view of this possibility and in view of current hypotheses which try to link changes of water metabolism with impairment of liver function, it