The Nutrition of the Young Ayrshire Calf

13. The Toxicity of the Unsaturated Acids of Cod-liver Oil

By K. L. BLAXTER and F. BROWN
Hannah Dairy Research Institute, Kirkhill, Ayr
and A. M. MacDONALD
Glasgow University and Royal Hospital for Sick Children, Glasgow

(*Received 7 January* 1953)

Previous work with calves has shown that when cod-liver oil is added to a ration of reconstituted dried separated milk the animals die as the result of muscular dystrophy (Blaxter, Wood & MacDonald, 1953). Ingestion of dried separated milk does not cause the disease if vitamins A and D are supplied in a concentrate instead of in cod-liver oil. The present paper deals with the toxicity of various fractions of cod-liver oil when given to calves.

In efforts to elucidate the factor or factors present in cod-liver oil concerned in the production of those diseases of laboratory animals generally ascribed to a dietary deficiency of vitamin E, fractions of cod-liver oil have been given to mice (Agduhr, 1935), guinea-pigs (Madsen, McCay & Maynard, 1935) and young rats (Dam & Granados, 1945*a*). The experiments of Agduhr (1935) and Madsen *et al.* (1935) were equivocal, since pathological changes occurred whatever fraction was used. Nevertheless it appeared probable that the saponifiable fraction of cod-liver oil produced the most severe changes. The experiments of Dam & Granados (1945*a*), however, showed that toxicity was concentrated in an unsaturated acid fraction of the oil having an iodine value of 283. This produced depigmentation of the enamel of the incisors of the rats, a marked discoloration and peroxidation of the body fat, acute anaemia and diarrhoea. The non-saponifiable fraction and two further saponifiable fractions (iodine values 13·4 and 99·4) failed to produce any pathological changes or retardation of growth.

Dam's (1944) studies with chicks receiving fractions of hog-liver fat added to a diet deficient in vitamin E showed that, although the most highly unsaturated fatty-acid fraction (iodine value 241) rapidly produced a severe encephalomalacia, both exudative diathesis and encephalomalacia occurred also when an unsaturated fraction with an iodine value of 104 was given. It appears fairly certain, therefore, that in laboratory species toxicity resides in the saponifiable fraction of these fats. It has, however, been pointed out by Moore & Wang (1945) that when cod-liver oil is given in large amounts there is a danger of hypervitaminosis A, and that in accounting for the toxicity of cod-liver oil hypervitaminosis A must be considered also. A daily dose of 28 g cod-liver oil provides approximately 28,000 i.u. vitamin A and approximately 2800 i.u. vitamin D, far more than the minimal requirements for

a 30 kg calf. The presence of haemorrhagic muscles in a number of our experimental calves given cod-liver oil (Blaxter, Watts & Wood, 1952) might suggest that hypervitaminosis A was involved in the disease (Moore & Wang, 1945). Haemorrhagic lesions have, however, also been noted in field cases of muscular dystrophy, when there was no possibility of any hypervitaminosis A (Stamp & Blaxter, 1951).

EXPERIMENTAL

Animals

Twenty Ayrshire bull calves were used in two experiments. Exp. 1, with ten calves, began in early March 1952, and Exp. 2, also with ten calves, began in late May 1952.

Treatment. The housing, management and feeding routine were as previously described (Blaxter *et al.* 1952; Blaxter, Wood & MacDonald, 1953). The calves in each experiment were allocated by lot to treatment groups, details of which are given in Table 1. The individual numbers of the calves are given in Table 3.

Table 1. *Experimental treatments of the calves*

Treatment group	Daily addition to the ration	No. of calves Exp. 1	No. of calves Exp. 2
Negative controls	Two capsules of halibut-liver oil	2	2
Positive controls	28 g cod-liver oil	2	2
Non-saponifiable residue	0·28 g non-saponifiable matter of cod-liver oil	2	—
Saturated acids	3·2 g saturated acids of cod-liver oil and two capsules of halibut-liver oil	2	2
Total unsaturated acids	23·6 g unsaturated acids of cod-liver oil and two capsules of halibut-liver oil	2	—
Less unsaturated acids	15·0 g less highly unsaturated acids of cod-liver oil and two capsules of halibut-liver oil	—	2
Highly unsaturated acids	8·6 g highly unsaturated acids of cod-liver oil and two capsules of halibut-liver oil	—	2

The calves were examined twice daily for signs of abnormality, weighed once a week and killed either when signs of dystrophy were pronounced or when, after a long period of experimental treatment, it seemed highly unlikely that signs would develop. The carcasses were dissected and examined for evidence of dystrophic degeneration.

Diet. The basal diet was spray-dried skim milk, to which a solution of trace minerals was added twice weekly. The composition of this mineral mixture has been described (Blaxter *et al.* 1952). All animals except one negative control calf, no. 170, were given in addition 2·5 mg α-tocopheryl acetate daily. The dried milk contained approximately 0·5 mg tocopherol, so that the basal ration supplied approximately 3 mg vitamin E daily. Those calves not receiving cod-liver oil or the unsaponifiable fraction of cod-liver oil were given capsules of halibut-liver oil to supply vitamins A and D. The calves were given the cod-liver oil or its fractions twice daily mixed with their allowance of the basal ration.

Chemical analyses

Tocopherols were determined at intervals in the serums of the calves by the method of Emmerie & Engel (1938) as modified by Blaxter, Brown & MacDonald (1953).

Dry matter, ash and creatine were determined in eight muscles of the limbs and in the heart, and total lipid was determined in samples of liver, perinephric fat and muscles of each calf. The tocopherol content of the lipid of these organs was determined by the method previously described (Blaxter, Brown & MacDonald, 1953). The peroxide content of the lipid was determined by the method used by Dam & Granados (1945*b*).

Histological examination

Histological examination was made of samples of the myocardium and the skeletal musculature of most calves and of samples of the kidneys of calves given the saturated acids of cod-liver oil.

Method of fractionating cod-liver oil

The oil, which was a sample complying with British Standard Specification no. 839/1939 was hydrolysed in batches of 100 g and the acids from 500 g were combined for fractionation. The detailed steps were as follows: 100 g of the oil were dissolved in 200 ml. of 95% ethanol containing 28 g KOH and 55 ml. ether (just sufficient to form a homogeneous solution) were added. After allowing the mixture to stand for 24 h in a stoppered bottle at room temperature, 830 ml. water were added and the solution was extracted with 800 ml. ether. The ether extract, after washing free from alkali, was evaporated to give the crystalline, non-saponifiable material. The residual soap solution was made just acid to Congo Red, by the addition of conc. HCl, and then extracted with ether. After washing the ether extract with water until it was free from mineral acid and evaporating the ether, the residual fatty acids were treated as follows:

Experiment 1. The acids from 500 g oil were dissolved in 6 l. 76% (v/v) ethanol; the solution was then held at 0° for 24 h (Ku, 1937). The crystalline acids were removed by filtration, washed with 600 ml. cold 76% ethanol and dried in a vacuum desiccator over calcium chloride. The combined filtrate and washings were evaporated at 50° under reduced pressure in an atmosphere of nitrogen, to yield the unsaturated fraction.

Experiment 2. The highly unsaturated acids were prepared by the method of Hilditch & Maddison (1942); the acids from 500 g oil were dissolved in about 1900 ml. acetone and titrated with saturated aqueous lithium hydroxide solution until just alkaline to phenolphthalein. After adjusting the acetone concentration to 95% the mixture was gently refluxed and allowed to cool slowly. The solid lithium salts were removed by filtration and washed with 95% acetone. The combined filtrate and washings containing the highly unsaturated acids were evaporated in a stream of nitrogen until most of the acetone had been removed, and acidified with conc. HCl. The highly unsaturated acids were then obtained by extraction with ether.

The solid lithium salts were heated with excess dilute HCl, and the mixed saturated and less highly unsaturated acids extracted with ether. After washing the

ether extract with water, the solvent was evaporated and the residual acids were dissolved in 76% (v/v) ethanol and treated as described in Exp. 1 above. The yields of the fractions, their iodine values and the amounts of unsaturated material, expressed in terms of iodine value, supplied to the calves each day are summarized in Table 2.

The fractions were stored in tightly stoppered bottles at 0° until required.

Table 2. *Iodine values of cod-liver oil and cod-liver oil fractions*

Material	Amount of oil accounted for by fractions (%)	Iodine value	Unsaturation of fractions given each day (as g iodine)	Iodine value of oil accounted for by fractions (%)
Cod-liver oil	100·0	158	44·2	100·0
Fractions:				
Non-saponifiable residue	1·0	144	0·4	0·9
Saturated acids (Exp. 1)	11·4	32*	1·0	2·3
Saturated acids (Exp. 2)	11·4	14*	0·4	1·0
Total unsaturated acids	84·3	164	38·7	87·5
Highly unsaturated acids	30·7	270	23·2	52·5
Less unsaturated acids	53·6	110	16·5	37·3

* Owing to the difficulty of complete separation the saturated acid fraction contained some unsaturated acids.

RESULTS

Vitamin E content of the blood serum

The initial concentration of tocopherols in the blood serum fell with continued treatment. After 28 days of experiment there was less than 50 μg/100 ml. total tocopherols in the serum of all the calves except no. 178, which received the non-saponifiable fraction. The results are presented in Table 3 and indicate clearly the

Table 3. *Tocopherol content of blood serum of experimental calves*

Calf no.	Treatment group	Days on treatment: 0	7	14	28	Last value obtained
		Tocopherol content (μg/100 ml.)				
170	Control	123	—	49	—	0 (56 days)
171		148	—	80	—	0 (35 days)
195		356	135	76	24	—
197		190	141	127	30	—
172	Whole cod-liver oil	198	—	91	—	13 (51 days)
173		73	—	22	12	—
198		144	—	105	40	—
199		144	78	26	—	—
178	Non-saponifiable residue	288	—	83	—	97 (35 days)
179		202	—	74	—	20 (57 days)
176	Saturated acids	154	—	60	—	—
177		60	—	64	—	18 (56 days)
204		95	56	50	—	—
205		85	46	52	14	—
174	Total unsaturated acids	—	—	42	—	—
175		104	—	39	—	—
202	Less unsaturated acids	—	111	30	16	—
203		260	163	88	21	—
200	Highly unsaturated acids	151	63	52	26	—
201		232	186	—	45	—

absence of any differences to be attributed to treatment. Even with calf no. 170, from which the daily supplement of 2·5 mg α-tocopherol had been withheld, there was little evidence of a marked acceleration in the rate of fall of serum tocopherol.

Table 4. *Clinical signs shown by calves, and fate of the calves*

Treatment group	Calf no.	Days of treatment	Predominant clinical signs	Fate	Cause of death
Negative controls	170	57	No abnormality observed	Slaughtered	—
	171	36	No abnormality observed	Slaughtered	—
	195	44	No abnormality observed	Slaughtered	—
	197	73	No abnormality observed	Slaughtered	—
Whole cod-liver oil	172	52	Considerable muscular stiffness	Killed *in extremis*	Generalized muscular dystrophy
	173	29	Prostration with failure of appetite	Killed *in extremis*	Infection of umbilicus and generalized septicaemia
	198	36	Abdominal respiration and inability to rise	Killed *in extremis*	Generalized muscular dystrophy
	199	28	Muscular weakness and cardiac signs	Died	Cardiac failure
Non-saponifiable residue	178	36	Loss of appetite	Slaughtered	—
	179	58	No abnormality observed	Slaughtered	—
Saturated acids	176	33	No abnormality noted before death	Died	Severe tympanites
	177	57	No abnormality observed	Slaughtered	—
	204	36	Lumbar pain	Slaughtered	—
	205	58	No abnormality observed	Slaughtered	—
Total unsaturated acids	174	24	Erratic pulse, culminating in a series of heart attacks	Died	Cardiac failure
	175	35	Abdominal respiration, muscular weakness and cardiac abnormalities	Died	Cardiac failure and kidney infection
Less unsaturated acids	202	41	Slight signs of muscular weakness	Slaughtered	—
	203	73	Inappetence early in experiment	Slaughtered	—
Highly unsaturated acids	200	78	Slight signs of muscular weakness (days 30–50) which regressed	Slaughtered	—
	201	38	Prostration with marked weakness	Died	Cardiac failure

Observations before death

The clinical signs shown by the calves are summarized in Table 4. Abnormalities were not observed in the control calves that did not receive cod-liver oil, but the calves receiving cod-liver oil all showed some signs of muscular weakness or cardiac abnormality. In calf no. 173, however, the general weakness was not the result of a dystrophic involvement of the limbs. The body temperature was elevated, there was pneumonia with laboured breathing, and the navel was swollen, tense and hot. It being clear that the calf was suffering from an infection, it was therefore removed from the experiment and killed.

The calves receiving the non-saponifiable material of cod-liver oil both showed

normal behaviour for the first 30 days. Calf no. 179 remained normal in all respects, but calf no. 178 tended to lose appetite and weight. Since both calves that had received the unsaturated acids of cod-liver oil had by this time succumbed, calf no. 178 was killed on the 36th day for examination, and its companion was left for a further 4 weeks. No signs of dystrophy were observed in either.

One of the calves receiving the saturated acids of cod-liver oil, no. 176, died suddenly after an acute attack of bloat. No obvious signs of dystrophy were noted during its lifetime. The cause of the tympanites was not discovered, but it was certainly unlike that previously observed in dystrophic calves (Blaxter, Wood & MacDonald, 1953). Only one, no. 204, of the remaining three calves showed any abnormality. It developed an abnormal stance with spread legs, comparable in fact to the stance adopted by calves with dystrophy of the lumbar muscles. Evident pain was felt from pressure applied to the lumbar region, and the calf was very dull for a period of several days. Its body temperature was slightly elevated for 2–3 days when this sign was present, but soon fell to normal. It was killed without having shown further signs and when its condition was quickly improving.

The calves receiving the total unsaturated acids of cod-liver oil both died. There was little doubt from the clinical signs that calf no. 175 was severely dystrophic. Its heart rate had been erratic for 8 days before death, the gait was ataxic and the respiration was laboured without any rise of body temperature being apparent. Three heart seizures and prostration occurred during the last 4 days of calf no. 174's life and its fore-legs were extremely weak. It fell suddenly during feeding on the 24th day, as the result of a cardiac seizure and died within 90 sec. During this time there was grunting, writhing, and protrusion of the tongue. The pulse was imperceptible.

Calf no. 203, which received the less unsaturated acid fraction of cod-liver oil, showed no particular abnormality other than arching of the back and signs of lumbar pain similar to those noted with calf no. 204, which had received the saturated acid fraction. These signs occurred during the 30th–40th days of treatment. This calf was given the experimental diet for a further 28 days before it was killed. It showed no further signs of abnormality. Its companion, however, calf no. 202, showed slight signs indicative of muscular dystrophy rather than the local pain in the region of the kidneys. Separation of the claws of the feet and a relaxation of the carpal-metacarpal joint took place; some slight abnormalities of the heart were noted. These signs, although not sufficient to warrant an unequivocal diagnosis, were suggestive of muscular dystrophy. It was killed for further examination.

Calf no. 201, which received the highly unsaturated acids of cod-liver oil, rapidly lost condition after the 4th week of treatment. It became weak, had to be fed forcibly and spent much time lying in an unnatural position. It was found dead on the morning of the 38th day of experiment, having kicked violently during the last hours of life. Its heart had been judged abnormal when it was examined on the previous day. Calf no. 200, which also received the highly unsaturated acids of cod-liver oil, showed slight signs of muscular weakness early in the experiment. These did not increase in severity; when the calf was slaughtered on the 78th day of experiment,

nothing, apart from a weakness in the shoulder region, was apparent. It had gained 328 g/day during the experimental period, which was normal.

Observations post mortem

From the observations made before death it was clear that three calves suffered from a circumscribed lumbar pain not due to the muscular weakness associated with dystrophic degeneration. These calves were no. 178, which received the non-saponifiable matter of cod-liver oil, no. 204, which was given the saturated acids, and no. 203, which received the less unsaturated acid fraction. At post-mortem examination it was found that the kidneys were covered with small white spots, a few mm in diameter, extending deep into the cortex. There was no marked inflammatory reaction, but from these areas a *Corynebacterium* was isolated. Calf no. 203 occupied the pen that had been vacated by calf no. 178, and calf no. 204 the pen immediately adjoining. Though the pen had been thoroughly scrubbed before calf no. 203 was admitted, it seems likely that infection had been brought in by calf no. 178 and transmitted to the others.

Table 5 summarizes the results of examining the calves' musculature. Muscles of those receiving the control ration, the saturated acids of the oil or its non-saponifiable residue were normal, in agreement with the clinical observations made before death. Muscles of calves receiving either whole cod-liver oil or the total unsaturated acid fraction were severely dystrophic, as had also been inferred from the clinical signs shown before death.

Muscles of the two calves receiving the less unsaturated acids were judged to be normal. A very small area of pallor was noted on the epicardium of the right ventricle of calf no. 202; on section of the heart a similar small area of pallor was found in the myocardium of the right ventricle. If it was a lesion, it was of very doubtful significance, for clearly the calf would not have died of dystrophy. The relaxation of the front limb of this calf was not associated with any lesion other than the pallor of the muscles concerned.

Examination of the two calves receiving the highly unsaturated acids revealed very mild dystrophy. The disposition of blood in the lungs of calf no. 201 suggested a cardiac failure affecting the right heart. Small areas of the myocardium of the left ventricle were dystrophic also, giving the wall a mottled appearance on section. Similar small areas were found in the muscles of the hind-limb. The heart of calf no. 200 was streaked with a whitish brown discoloration and a similar discoloration was noted in the substance of the myocardium. The skeletal muscle was very pale throughout, and the gastrocnemius of the hind-limb was found to be mildly dystrophic. This calf was slaughtered at the same time as no. 205, which had received the saturated acids and was not dystrophic. By comparison the marked pallor of the musculature of no. 200 was particularly striking.

Creatine contents of muscles

The mean creatine content of muscles and muscle groups is given in Table 6. The amount of creatine was lower in the muscles of calves given cod-liver oil than in those of controls. The muscles of both calves receiving the non-saponifiable material of

Table 5. *Classification of muscles of calves given cod-liver oil or cod-liver oil fractions according to severity of dystrophy*

Treatment group	Calf no.	Fate	Forelimb and forelimb suspension	Hind-limb and hind-limb suspension	Trunk and neck excepting intercostals	Diaphragm and intercostals	Heart
Negative controls	Nos. 170, 171, 195 and 197 were slaughtered and were found to be normal						
Cod-liver oil	172	Killed *in extremis*	Severe	Severe	Very severe	Very severe	Normal
	173*	Killed *in extremis*	Normal	Normal	Normal	Normal	Very slight
	198	Killed *in extremis*	Very severe	Severe	Very severe	Very severe	Very severe
	199	Died	Slight	Slight	Slight	Very slight	Severe
Non-saponifiable residue	Nos. 178 and 179 were slaughtered and were found to be normal						
Saturated acids	176†	Died	? Normal	Normal	Normal	? Normal (pressure)	Deformed by abdominal pressure
	Nos. 177, 204 and 205 were slaughtered and were found to be normal						
Total unsaturated acids	174	Died	Very severe	Severe	Slight	Severe	Severe
	175	Died	Severe	Very severe	Very severe	Normal	Very severe
Less unsaturated acids	202	Slaughtered	Normal	Normal	Normal	Normal	? Normal
	203	Slaughtered	Normal	Normal	Normal	Normal	Normal
Highly unsaturated acids	200	Slaughtered	? Normal	Normal	Normal	? Normal	Very slight
	201	Died	Slight	Normal	Normal	Normal	Severe

* This calf died as the result of an ascending infection from the umbilicus.

† The severe tympanites which caused the death of this calf made it impossible to state whether the muscles were normal. They were certainly not severely dystrophic.

cod-liver oil contained more creatine than did those of the controls. In this respect it is of interest that this fraction of the oil contains appreciable amounts of α-tocopherol (Brown, 1953), suggesting that the negative control ration employed was indeed marginal in meeting vitamin E requirements. The same conclusion may be drawn from comparing the creatine contents of the muscles of these calves with those of calves given adequate amounts of α-tocopherol (Blaxter, Wood & MacDonald, 1953). For convenience these values have been inserted at the foot of Table 6.

Table 6. *The mean creatine content of groups of muscles of the calves*

		Creatine content of fresh muscle				
Treatment group	No. of calves	Shoulder*	Forearm†	Lower arm‡	Rectus femoris	Heart
		(mg/100 g)				
Negative controls	4	329	341	341	412	258
Positive controls (with cod-liver oil)	3	288§	275§	300§	396	201§
Non-saponifiable residue	2	403	422	407	462	266
Saturated acids	3	345	349	344	409	237
Total unsaturated acids	2	260§	295§	324	364§	178§
Less unsaturated acids	2	326	344	372	407	223
Highly unsaturated acids	2	310	300§	294§	377§	212§
Animals given ample α-tocopherol¶	6	373	375	372	429	258

* Infraspinatus and supraspinatus.
† Long and lateral head of triceps.
‡ Coracoradialis, anterior brachial and anterior extensor of metacarpus.
§ Judged to be lower than the corresponding values for the negative-control calves.
¶ Mean values for normal calves given α-tocopheryl acetate and no cod-liver oil in previous experiments (Blaxter, Wood & MacDonald, 1953).

The amount of creatine in muscles of calves receiving the saturated acids of the oil did not differ from that of the control calves. Apart from those of the lower arm, muscles of the two calves receiving the total unsaturated acid fraction contained less creatine than did those of the controls. The marked lowering of the creatine content of the muscles of these two calves confirms the diagnosis of severe dystrophy. The calves receiving the less saturated acids had skeletal muscles that did not depart from normality, though the creatine of both hearts was slightly low. The amounts of creatine of all muscles, except those of the shoulders, of the two calves receiving the highly unsaturated acids were lower than those of the comparable control calves.

Histological examination of the tissues.

Histological examination of the myocardia and certain skeletal muscles of the control calves revealed no abnormalities. Lesions were observed in the myocardia of nos. 198 and 199, which had received cod-liver oil, but, in agreement with the gross pathological examination, not in the myocardium of calf no. 172. Skeletal muscles and the myocardia were not dystrophic in calves given the non-saponifiable material or the saturated acids of cod-liver oil. The myocardium of the left ventricle of no. 204 which had received the saturated acids was, however, abnormal. A diffuse oedema

of the interstitium with slight round cell infiltration was present. This was judged to be an early inflammatory change, probable in view of the corynebacterium infection. It was not a dystrophic lesion. The muscles of the calves receiving the total unsaturated acids of cod-liver oil were clearly dystrophic. A remarkable lesion was observed in the neck muscle of calf no. 175. An extensive hyaline degeneration with necrosis had occurred, together with sarcolemmal proliferation, and this was associated with calcium deposition. We have previously commented (MacDonald, Blaxter, Watts & Wood, 1952) on the conspicuous absence of calcification of the lesion induced under our experimental conditions, whereas calcification commonly occurs under field conditions. The calf showed some evidence of kidney pyaemia; it is possible that calcification of the muscle occurs only when kidney damage has taken place. Tissue necrosis was absent, but there were several cortical foci of interstitial inflammation and casts in related tubules. The skeletal muscles of calves receiving the less unsaturated acids showed no severe hyaline degeneration. Hyaline fibres were, however, found in the skeletal muscle of calf no. 200, which had received the highly unsaturated acid fraction. These results agree with those obtained by visual examination of the carcasses and by estimation of muscle creatine.

Tocopherol content of tissues

Table 7 summarizes the tocopherol content of muscles, liver and perinephric fat.

Table 7. *Tocopherol in tissues of the calves*

Treatment group	Calf no.	Muscle (rectus femoris) mg/kg	Muscle (rectus femoris) μg/g fat	Liver mg/kg	Liver μg/g fat	Perinephric fatty tissue mg/kg	Perinephric fatty tissue μg/g fat
Negative controls	170	9·1	939	6·3	227	25·9	46·6
	171	13·4	1155	6·6	206	15·9	44·7
	195	6·0	504	8·2	252	17·5	25·6
Cod-liver oil	197	7·8	988	6·6	251	11·5	34·6
	172	11·7	976	11·1	318	17·9	46·4
	173	4·8	226	2·5	61	—	—
	198	7·4	607	12·3	322	17·0	28·6
Non-saponifiable residue	178	11·3	802	9·0	254	12·8	23·5
	179	6·8	612	8·8	260	21·6	5·17
Saturated acids	176	6·6	497	8·3	268	9·3	18·9
	177	2·9	227	4·4	135	20·6	—
	204	—	—	15·7	438	15·0	41·4
	205	7·9	724	9·3	256	—	—
Total unsaturated acids	174	6·6	522	6·6	167	21·4	50·3
	175	—	—	8·3	263	15·3	35·7
Less unsaturated acids	202	—	—	10·8	318	18·7	45·8
	203	6·5	644	16·7	292	—	—
Highly unsaturated acids	200	3·2	327	8·6	236	24·3	43·0

The experimental diets had no obvious effect on the concentrations of tocopherols in fresh tissues or tissue fat. Further, dystrophic animals still contained tocopherols, often indeed more than was present in control calves that were not dystrophic. The recti femoris of nos. 172, 198 and 174 were visibly dystrophic, yet still contained

tocopherols. Our previous (unpublished) work has indeed shown that completely degenerate, white, oedematous muscle often contains more tocopherols than the contiguous normal muscle of the same animal. The fact that the tocopherol content tends to be higher in affected muscle is in agreement with previous observations that the lipids of muscle increase in concentration in dystrophy owing to a marked fall in protein content (Blaxter & Wood, 1952). Analysis of single muscles in which bands of dystrophy were present has also shown no evidence that there was less tocopherol in the dystrophic areas. Further proof that the substance present in muscle and reacting as α-tocopherol in the Emmerie-Engel test is indeed α-tocopherol was furnished by paper chromatography of the muscle extracts. The ferric-chloride reacting substance had the same R_F value as α-tocopherol. Attempts to isolate from dystrophic muscle α-tocopherol as the acid succinate were, however, unsuccessful because of the minute quantities present in the muscle. They were equally unsuccessful with normal calf tissues.

Tissue peroxides

Attempts to demonstrate the presence of peroxides in the lipids of tissues failed. If peroxidation of the body fats occurs when cod-liver oil is given, these peroxides must either have an extremely transitory existence or be present in minute concentrations.

DISCUSSION

From the above experiments it is clear that the toxicity of cod-liver oil to the calf resides in the unsaturated fatty-acid fraction. When this was given severe dystrophy occurred, recognizable clinically, chemically, on dissection and histologically as the same entity that occurs when the whole oil is given. There is thus no possibility that the disease is related to hypervitaminosis A or D.

Further fractionation of the total unsaturated acids into less highly and more highly unsaturated groups showed that mild dystrophy was produced by the latter, but, apart from slight clinical signs, a slight reduction in the creatine content of the heart and pallor of the muscles, nothing abnormal was observed in calves receiving the less unsaturated fraction, suggesting that the two fractions combined were more effective in producing the disease than either fraction alone. It has previously been emphasized that there are differences between calves in their resistance to the disease (Blaxter, Wood & MacDonald, 1953), so that a firm conclusion can hardly be drawn. It seems of interest in this connexion, however, that the total unsaturation of the lipids ingested daily by calves consuming a dystrophy-producing diet containing lard (Blaxter *et al.* 1952) was 'equivalent to 70 g iodine'; this is equivalent to about double the total unsaturation of the lipids consumed when the unsaturated acids of the cod-liver oil were given, as may be seen from Table 2. Both produced a comparable degree of dystrophy. These results tend to support those of Dam (1944), in which toxicity of hog-liver fat to chicks was found to reside in a number of unsaturated acid fractions of widely differing iodine values.

SUMMARY

1. Calves were given a basal ration of dried skim-milk powder with small supplements of α-tocopheryl acetate. Cod-liver oil or fractions of cod-liver oil were added to this diet. When vitamins A and D were not supplied by cod-liver oil or its fractions, a concentrate containing them was given.

2. Severe muscular dystrophy developed in the calves given cod-liver oil or the total unsaturated acids of the oil. Slight dystrophy occurred in calves given the highly unsaturated acids of the oil.

3. Calves receiving the non-saponifiable residue of the oil or the saturated fatty-acid fraction did not develop dystrophy. The calves receiving the less unsaturated acids of the oil did not develop dystrophy, but their musculature was pale.

4. Muscles of both dystrophic calves and normal calves contained approximately the same amount of total tocopherols. Dystrophic muscles contained the same amount as contiguous apparently normal muscles of the same animal, or even more.

5. It is concluded that the toxicity of cod-liver oil to calves is due to its content of polyunsaturated fatty acids, and not to any hypervitaminosis A or D, and it is suggested that the toxicity may be a general effect of polyunsaturation rather than of a single polyethenoid acid.

We are indebted to Miss M. K. Hutcheson for the care of the experimental calves and to Miss G. Breckenridge and Miss E. Rennie for analytical assistance.

REFERENCES

Agduhr, E. (1935). *Uppsala LäkFören. Förh.* N.S. **40**, 183.
Blaxter, K. L., Brown, F. & MacDonald, A. M. (1953). *Brit. J. Nutr.* **7**, 105.
Blaxter, K. L., Watts, P. S. & Wood, W. A. (1952). *Brit. J. Nutr.* **6**, 125.
Blaxter, K. L. & Wood, W. A. (1952). *Brit. J. Nutr.* **6**, 144.
Blaxter, K. L., Wood, W. A. & MacDonald, A. M. (1953). *Brit. J. Nutr.* **7**, 34.
Brown, F. (1953). *Nature, Lond.*, **171**, 790.
Dam, H. (1944). *J. Nutr.* **28**, 297.
Dam, H. & Granados, H. (1945*a*). *Science*, **102**, 327.
Dam, H. & Granados, H. (1945*b*). *Acta physiol. scand.* **10**, 162.
Emmerie, A. & Engel, C. (1938). *Rec. Trav. chim. Pays-Bas*, **57**, 1351.
Hilditch, T. P. & Maddison, L. (1942). *J. Soc. chem. Ind., Lond.*, **61**, 169.
Ku, P. S. (1937). *Industr. Engng Chem. (Anal.)*, **9**, 103.
MacDonald, A. M., Blaxter, K. L., Watts, P. S. & Wood, W. A. (1952). *Brit. J. Nutr.* **6**, 164.
Madsen, L. L., McCay, C. M. & Maynard, L. A. (1935). *Mem. Cornell agric. Exp. Sta.* no. 178.
Moore, T. & Wang, Y. L. (1945). *Biochem. J.* **39**, 222.
Stamp, J. T. & Blaxter, K. L. (1951). Unpublished observations.