The influence of maize oil on the fatty acid composition of tissues of calves with and without vitamin E

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1. Fatty acid levels were studied in the tissues of 1-week-old calves receiving maize oil (in filled milk) with and without supplementary α-tocopherol. The calves that were not given vitamin E developed muscular dystrophy.

2. Decreased amounts of linoleic acid and increased amounts of arachidonic acid were found in nearly all the lipid fractions of skeletal muscles, hearts, livers and kidneys of vitamin E-deficient calves. The concentration of the polyunsaturated fatty acids beyond arachidonic acid remained about the same in both groups. There was a significant decrease of 20:2ω6 fatty acid in the phosphatidyl choline, phosphatidyl ethanolamine and free fatty acid fractions in the livers and kidneys of vitamin E-deficient calves.

3. It is suggested that vitamin E has an inhibitory effect on the desaturating but not on the chain-elongation enzymes of microsomes in the liver and kidney.

4. Maize-oil feeding had only a slight effect on erythrocyte fatty acid composition, and the erythrocyte haemolysis test was negative even in the vitamin E-deficient animals.

The protective effect of vitamin E and selenium against the enzootic muscular dystrophy of calves has been clearly demonstrated. In order to produce muscular dystrophy experimentally not only is a deficiency of vitamin E necessary but also dietary supplementation with unsaturated fat has been shown to be essential (Adams, Gullickson, Sautter & Gander, 1954; Blaxter & McGill, 1955). The possibility exists that, in enzootic muscular dystrophy also, unsaturated fatty acids may be involved; this is supported by the finding that, in the tissues of calves with enzootic muscular dystrophy, the concentration of linoleic acid is higher than normal (Poukka, 1966, 1968).

Because the special role of linoleic acid in inducing the muscular dystrophy has been demonstrated by many investigators (Lindberg & Orstadius, 1961; Hutcheson, Hill & Jenkins, 1963; Calvert, Desai & Scott, 1964) it was of interest to investigate the effect of high levels (27% of calorie intake) of dietary linoleic acid (in the form of stripped maize oil) on the fatty acid composition of tissues of calves with and without a vitamin E supplement.

EXPERIMENTAL

Animals and their feeding. Seven calves of Ayrshire breed were used in this experiment. They came from different farms where they were fed with colostrum and whole

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milk during their 1st week of life. At the age of 1 week the calves were divided into two groups: a group of four calves to receive vitamin E, and a group of three calves without vitamin E. Milk fat was replaced in their diet by vitamin E-free maize oil (Eastman Stripped Corn Oil; Eastman Kodak Company, Rochester, NY, USA). The oil was homogenized with skim milk in a Waring Blendor about 5 min before feeding. The calves received this filled milk, 4 l/d. The milk ration given to the vitamin E-treated calves contained, in addition, 400 mg DL-α-tocopheryl acetate (F. Hoffmann-La Roche & Co. Ltd, Basle, Switzerland), added daily. To the rations of both groups, 2000 μg retinyl acetate (Sigma Chemical Company) were added. Antibiotics to prevent diarrhoea were given to all calves.

After 17 d on experimental diets the animals were killed. Immediately after slaughter, samples for chemical and histological examination were taken from the heart, liver and kidney and from the following skeletal muscles: musculus serratus ventralis, musculus vastus intermedius (affected lower part), musculus vastus intermedius (healthy upper part), musculus splenius.

Methods used. Blood was taken from the calves at the beginning and at the end of the experiment and serum was analysed for aspartate transaminase and for α-tocopherol. Lipids were extracted from serum and tissue by the method of Bligh & Dyer (1959). Lipids were extracted from the red blood cells and the haemolysis test was carried out as described by Bieri & Poukka (1970). Phospholipids and neutral lipids were analysed by thin-layer chromatography on Kieselgel G (E. Merck AG, Darmstadt) using, as solvents, a mixture of chloroform–methanol–water (75:25:4, v/v) for phospholipids (Vikrot, 1964) and light petroleum (b.p. 40–60)–diethyl ether–acetic acid (90:10:1, v/v) for triglycerides (Bowyer, Leat, Howard & Gresham, 1963). The plates were sprayed with 0·2% dichlorofluorescein in ethanol to make the bands visible under ultraviolet light. The fractions of phosphatidyl choline, phosphatidyl ethanolamine, triglycerides and free fatty acids were eluted from the plates, using chloroform for triglycerides and free fatty acids and a mixture of chloroform and methanol (1:3) for phospholipids. The lipids of red blood cells and serum were not fractionated by thin-layer chromatography.

The boron trifluoride method of Bieri & Poukka (1970) was used to transesterify the lipids for gas–liquid chromatography. Methyl esters of the fatty acids were separated from the dimethyl acetals of the fatty aldehydes by thin-layer chromatography according to the method of Mahadevan, Viswanathan & Lundberg (1966).

Gas–liquid chromatography of fatty acid methyl esters was performed as described by Poukka (1968) except that 15% (w/w) ethylene glycol succinate on Chromosorb W was used. For fatty acids beyond linoleic acid temperature programming from 170° to 195° was used.

For identification of fatty acids the equivalent chain lengths were calculated and compared with those given by Hofstetter, Sen & Holman (1965). Hydrogenation of methyl esters, according to Poukka, Vasenius & Turpeinen (1962), was used for identification. For further identification methyl esters of fatty acids were fractionated on Kieselgel G–$AgNO_3$, where they separate according to the number of double bonds. The method was that given by Witting & Horwitt (1967). Gas–liquid chromato-

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graphy was performed on the methyl esters of each fraction and the retention times were calculated and compared with those found on gas-chromatograms obtained under identical conditions from the total lipid extract.

RESULTS

Clinical observations

Despite the prophylactic use of antibiotics, all the calves had diarrhoea, especially at the beginning of the experiment. One of the vitamin E-deficient calves showed the first clinical signs of muscular dystrophy on the 8th day of experiment, and the other two calves showed the same signs 2 or 3 d later. Toward the end of the experiment these calves had diarrhoea with yellowish ill-smelling faeces, as is characteristic of this type of muscular dystrophy. During the experiment, abnormalities of the skin were found in calves from both groups. The hair became loose, and hairless areas could be found around the eyes and ears and under the lower jaw.

Post-mortem examination

The vitamin E-deficient calves had symmetrical bilateral lesions in many of the muscles of the back and shoulder areas. These were most severe in the lower part of the m. vastus intermedius. Microscopically, some slightly degenerated fibres in this muscle were found in calves nos. 2 and 4, which were on the vitamin E-supplemented diet.

The heart muscle of all the calves appeared macroscopically to be healthy but in two of the vitamin E-deficient calves (nos. 3 and 5) degenerative changes were found on microscopical examination. Liver and kidney seemed to be unaffected both macroscopically and microscopically.

Biochemical investigation

Table 1 shows the aspartate transaminase values of plasma at the beginning and end of the experiment. There was a marked increase in the values for all three vitamin E-deficient calves. In addition, there was an increase in three of the four calves in the vitamin E-treated group. However, for calf no. 7 the increase was slight. Somewhat increased aspartate transaminase values in the serum of calves nos. 2 and 4 and a slight affection of the lower part of m. vastus intermedius showed the inability of vitamin E (in the dosage used) to protect completely against muscular dystrophy.

The concentrations of vitamin E in the serum of the calves were low at the beginning of the experiment and remained low in calves not given vitamin E (Table 1). The high linoleic acid concentrations found in serum of all the calves (Table 2) could be attributed to the maize oil in the diet. However, in calves without vitamin E lower concentrations of linoleic acid were found in serum, compared with those found in the vitamin E-treated animals.

The concentration of linoleic acid in the erythrocytes (Table 2) remained low, compared with that in the serum. In the red blood cells of the vitamin E-treated calves both linoleic and arachidonic acid concentrations were higher than in those of the

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Table 1. *Aspartate transaminase values and α-tocopherol concentrations in the serum of calves at the beginning and at the end of the experiment*

Aspartate transaminase (Wroblewski units*/ml)						α-Tocopherol (μg/100 ml)					
Vitamin E-treated			Vitamin E-deficient			Vitamin E-treated			Vitamin E-deficient		
Calf no.	Beginning	End	Calf no.	Beginning	End	Calf no.	Beginning	End	Calf no.	Beginning	End
2	33	81	1	69	500	2	64	561	1	15	49
4	35	169	3	47	321	4	69	364	3	27	37
6	42	33	5	37	696	6	49	113	5	20	45
7	27	38				7	192	965			

* One Wroblewski unit is the amount of enzyme present in 1·0 ml serum that causes an absorbance decrease of 0·001 at wavelength 340 nm in 1 min under the test conditions.

Table 2. *Major fatty acids in the serum and in the erythrocytes of vitamin E-deficient and vitamin E-treated calves (% of total fatty acids)*

(Mean values with their standard errors)

	No. of animals	16:0	16:1	18:0	18:1	18:2ω6	20:4ω6
Plasma							
+Vitamin E	4	13·1 ± 0·4	1·2 ± 0·3	9·8 ± 0·2**	12·6 ± 0·6	57·6 ± 0·7**	1·3 ± 0·2
−Vitamin E	3	13·4 ± 1·7	1·7 ± 0·1	12·7 ± 0·6	15·0 ± 1·0	50·3 ± 1·5	2·4 ± 0·9
Erythrocytes							
+Vitamin E	4	16·9 ± 5·2	3·4 ± 0·3	17·4 ± 3·2	29·3 ± 3·5	8·1 ± 1·3**	3·7 ± 0·6*
−Vitamin E	3	33·6 ± 4·0	3·7 ± 1·4	20·4 ± 4·3	19·6 ± 3·3	3·2 ± 0·2	1·6 ± 0·6

Significance of differences between deficient and treated animals: * $P < 0·05$; ** $P < 0·01$.

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Table 3. *Mean values (% of total fatty acids by weight) with their standard errors for the proportions of fatty acids present in the phosphatidyl choline from the tissues of the vitamin-E deficient and vitamin E-treated calves*

Tissue	No. of samples	16:0	16:1	18:0	18:1	18:2ω6	20:0 20:1	20:2ω6	20:3ω6	20:4ω6
Skeletal muscle:										
+Vitamin E	16†	22·5±1·2	1·2±0·1	10·7±0·6	16·0±0·6	41·9±1·2***	0·6±0·1	0·8±0·1	1·0±0·1***	3·5±0·2***
−Vitamin E	12†	23·1±1·7	2·0±0·3	11·7±0·6	17·6±0·7	28·3±2·3	0·7±0·1	1·0±0·3	2·1±0·3	6·8±0·6
Heart:										
+Vitamin E	4	20·4±1·1	0·6±0·2	7·9±0·3	8·6±0·5	56·6±1·4	0·5±0·0	0·5±0·0	0·5±0·1	3·3±0·6
−Vitamin E	3	16·6±3·0	1·0±0·1	10·9±1·9	11·2±0·8	53·1±0·7	0·6±0·0	0·6±0·1	1·0±0·0	4·3±0·7
Liver:										
+Vitamin E	4	11·9±1·2	0·4±0·1	13·6±0·9***	13·1±1·5	43·0±1·8**	1·5±0·1	10·1±0·5***	1·3±0·7	2·8±0·2
−Vitamin E	3	10·3±0·8	0·5±0·1	32·4±1·0	12·4±0·8	34·7±1·3	0·7±0·3	1·8±0·3	1·4±0·4	3·4±1·0
Kidney:										
+Vitamin E	4	34·2±2·9	0·8±0·0	10·9±1·1	11·6±0·2	31·9±1·8	0·8±0·0	2·3±0·2**	3·0±0·1***	5·5±0·6
−Vitamin E	3	30·4±3·1	1·1±0·2	12·7±0·8	19·3±1·7	25·8±0·5	0·5±0·1	1·0±0·1	0·4±0·1	6·5±0·7

Significance of differences between deficient and treated animals: ** $P < 0·01$; *** $P < 0·001$.

† Because no significant differences in the fatty acid composition were found between the different muscles of vitamin E-treated and vitamin E-deficient calves, the results for four muscles studied from four calves were pooled.

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Table 4. *Mean values (% of total fatty acids by weight) with their standard errors for the proportions of fatty acids in the phosphatidyl ethanolamine from the tissues of vitamin E-deficient and vitamin E-treated calves*

Tissue	No. of samples	16:0	16:1	18:0	18:1	18:2ω6	20:0 20:1	20:2ω6	20:3ω6	20:4ω6	Unknown	22:4ω6	22:5ω6	22:6ω3
Skeletal muscle:														
+Vitamin E	16	2·1 ± 0·2	0·4 ± 0·3	15·6 ± 0·8	14·9 ± 0·5	24·4 ± 0·1***	0·4 ± 0·1	0·8 ± 0·2	3·2 ± 0·3	24·0 ± 0·8**	2·2 ± 0·3	2·7 ± 0·3	6·4 ± 0·3	1·5 ± 0·2
−Vitamin E	12	3·4 ± 0·5	0·6 ± 0·1	15·9 ± 0·6	15·2 ± 0·7	17·9 ± 0·8	0·4 ± 0·1	0·5 ± 0·1	2·8 ± 0·3	27·2 ± 0·9	2·9 ± 0·4	3·7 ± 0·7	7·2 ± 0·5	1·8 ± 0·3
Heart:														
+Vitamin E	4	1·8 ± 0·2	0·2 ± 0·1	24·9 ± 1·8	5·4 ± 0·2	40·3 ± 2·7*	0·4 ± 0·1	0·5 ± 0·1	0·9 ± 0·1	19·0 ± 1·8	1·7 ± 0·3	0·7 ± 0·1	2·9 ± 0·1	0·7 ± 0·3
−Vitamin E	3	2·3 ± 0·2	0·5 ± 0·0	26·7 ± 1·1	7·3 ± 1·4	31·9 ± 0·5	0·3 ± 0·1	0·5 ± 0·2	1·1 ± 0·0	22·6 ± 1·5	1·5 ± 0·3	0·8 ± 0·2	4·2 ± 0·6	1·4 ± 0·5
Liver:														
+Vitamin E	4	5·9 ± 0·6	—	16·8 ± 1·3***	17·5 ± 1·5	32·8 ± 1·7*	1·3 ± 0·1	7·3 ± 0·5***	1·3 ± 0·4	12·0 ± 0·8	0·5 ± 0·1	1·0 ± 0·2	2·9 ± 0·2	2·9 ± 0·2
−Vitamin E	3	4·6 ± 0·6	—	32·2 ± 1·5	11·5 ± 1·8	23·3 ± 3·4	0·6 ± 0·3	1·0 ± 0·3	1·3 ± 0·1	13·4 ± 2·2	0·5 ± 0·2	1·5 ± 0·3	4·0 ± 0·9	3·3 ± 0·6
Kidney:														
+Vitamin E	4	3·8 ± 0·5	—	16·4 ± 0·6	20·7 ± 0·5	22·5 ± 0·5	0·7 ± 0·0	1·3 ± 0·1***	1·1 ± 0·4	25·8 ± 2·0	0·7 ± 0·0	1·2 ± 0·2	2·7 ± 0·1	2·1 ± 0·3
−Vitamin E	3	2·2 ± 0·9	—	18·0 ± 2·4	25·2 ± 1·7	18·8 ± 2·1	0·4 ± 0·1	0·3 ± 0·1	0·9 ± 0·2	28·7 ± 1·9	0·9 ± 0·2	0·6 ± 0·3	2·6 ± 0·2	1·1 ± 0·4

Significance of differences between deficient and treated animals: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Table 5. *Mean values (% of total fatty acids by weight) with their standard errors for the proportions of fatty acids in the free fatty acid fraction from the tissues of vitamin E-deficient and vitamin E-treated calves*

Tissue	No. of samples	16:0	16:1	18:0	18:1	18:2ω6	20:0 20:1	20:2ω6	20:3ω6	20:4ω6
Skeletal muscle:										
+Vitamin E	10	9·5 ± 1·1**	1·6 ± 0·1	9·1 ± 0·8***	23·6 ± 0·6***	45·9 ± 2·5***	0·9 ± 0·1	1·5 ± 0·2	0·9 ± 0·1	3·6 ± 1·0***
−Vitamin E	12	13·1 ± 0·6	2·5 ± 0·2	13·4 ± 0·8	27·6 ± 0·6	26·9 ± 1·3	1·4 ± 0·3	1·3 ± 0·2	1·1 ± 0·2	8·5 ± 0·4
Heart:										
+Vitamin E	4	4·0 ± 0·6	0·5 ± 0·0	2·4 ± 0·3**	18·3 ± 0·9	69·4 ± 0·9***	1·2 ± 0·2	0·7 ± 0·3	0·7 ± 0·3	1·3 ± 0·2*
−Vitamin E	3	10·7 ± 1·2	1·4 ± 0·3	12·3 ± 1·9	20·7 ± 3·5	42·2 ± 3·3	0·9 ± 0·0	0·9 ± 0·4	0·8 ± 0·0	8·3 ± 2·8
Liver:										
+Vitamin E	4	6·2 ± 0·4**	1·8 ± 0·2	3·3 ± 0·4**	12·2 ± 0·2	64·2 ± 0·9***	1·6 ± 0·9	5·4 ± 0·3**	1·0 ± 0·3	1·4 ± 0·2**
−Vitamin E	3	13·3 ± 1·3	2·1 ± 0·1	16·8 ± 3·9	17·3 ± 1·8	38·9 ± 2·2	0·9 ± 0·3	2·0 ± 0·7	1·0 ± 0·2	4·6 ± 0·8
Kidney:										
+Vitamin E	4	11·9 ± 1·4	1·1 ± 0·1	6·2 ± 0·7	18·5 ± 0·8**	48·2 ± 2·1**	1·0 ± 0·1	2·8 ± 0·3**	0·4 ± 0·1	8·2 ± 0·4
−Vitamin E	3	15·1 ± 1·6	1·9 ± 0·2	10·7 ± 1·7	26·4 ± 1·7	30·8 ± 1·6	1·1 ± 0·1	1·2 ± 0·2	0·5 ± 0·2	10·5 ± 1·4

Significance of differences between deficient and treated animals: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

vitamin-E deficient calves. When the red blood cells were tested for haemolysis in vitro, the test was negative for both groups.

Table 3 shows the fatty acid composition of the phosphatidyl choline fraction of the tissues. In skeletal muscle the concentration of linoleic acid was significantly lower and that of 20:3ω6 and 20:4ω6 higher in vitamin E-deficient calves than in calves treated with vitamin E. In heart muscle no differences were found, whereas in the liver the concentration of 18:2ω6 was low and that of 18:0 high in the vitamin E-deficient calves. The most striking difference in the liver was the much higher concentration of 20:2ω6 fatty acid in the vitamin E-treated group as compared with the untreated group. The same was true for the kidney. In this tissue also, 20:3ω6 was present at higher concentration in vitamin E-treated calves.

In the phosphatidyl ethanolamine fraction (Table 4) the differences in the fatty acid composition were about the same as in the phosphatidyl choline fraction; 18:2ω6 in the muscle, heart and liver and 20:2ω6 in the liver and kidney were significantly decreased, whereas 20:4ω5 in the muscle and 18:0 in the liver were significantly increased in the tissues of vitamin E-deficient animals as compared with those of vitamin E-treated animals.

In the free fatty acid fraction (Table 5) the concentration of 18:2ω6 was decreased and that of 20:4ω6 increased in all the tissues of vitamin E-deficient animals when compared with those of vitamin E-treated animals. In the liver and kidney the decrease of 20:2ω6 was again significant. The decrease of lineolic acid was compensated for by an increase of saturated fatty acids.

Table 6. *Mean values (% of total fatty acids by weight) with their standard errors for the proportions of fatty acids in the triglycerides from the tissues of vitamin E-deficient and vitamin E-treated calves*

Tissue	No. of samples	16:0	16:1	18:0	18:1	18:2ω6
Skeletal muscle:						
+Vitamin E	16	20·3±1·0**	2·9±0·2	10·3±0·8***	33·2±1·1	29·0±2·2***
−Vitamin E	12	23·9±0·8	3·0±0·2	17·2±1·4	35·6±1·3	12·7±1·4
Heart:						
+Vitamin E	4	29·1±3·0	2·6±0·4	16·7±0·3	33·1±2·1	16·7±1·3***
−Vitamin E	3	30·7±0·2	3·1±0·1	21·3±1·9	33·7±0·4	4·5±1·0
Liver:						
+Vitamin E	4	24·1±6·2	2·0±0·3	8·9±4·8	19·7±5·4	41·4±12·3
−Vitamin E	3	35·7±3·5	1·7±1·0	9·1±2·7	15·7±1·7	29·1±2·8
Kidney:						
+Vitamin E	4	19·1±0·9	2·3±0·3	12·9±2·2	38·1±1·6	22·0±3·8*
−Vitamin E	3	16·0±1·7	2·5±0·8	16·8±3·2	46·0±3·7	8·4±4·5

Significance of differences between deficient and treated animals: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In the triglyceride fraction (Table 6) the concentration of linoleic acid was lower in all the tissues of vitamin E-deficient calves than in those of vitamin E-treated calves, but in the liver the variation was especially high. In the skeletal muscle the loss of 18:2ω6 was again compensated for by an increase of 16:0 and 18:0 fatty acids.

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DISCUSSION

This study showed that, when calves are given a diet containing a high level of stripped maize oil without supplementary vitamin E, there is a severe loss of linoleic acid from many tissues. Decreased amounts of linoleic acid in the serum and in the free fatty acid fraction of all the tissues of vitamin E-deficient calves may indicate either peroxidation in the intestine or poor intestinal absorption. However, the lowered concentration of 18:2ω6 seems to have been due in part to its conversion into 20:4ω6, as arachidonic acid was present in higher concentrations in the free fatty acid fraction of all the tissues of vitamin E-deficient animals than in that of the tissues of vitamin E-treated animals, irrespective of whether or not the tissues were histologically affected. In the skeletal muscle the increase in arachidonic acid concentration was also significant in the phosphatidyl choline and phosphatidyl ethanolamine fractions of the vitamin E-deficient calves. A decrease of 18:2ω6 and an increase of 20:4ω6 have previously been shown to occur in the vitamin E-deficient rat (Bernhard, Leisinger & Pedersen, 1963; Bieri, 1964; Witting & Horwitt, 1967).

The metabolic conversion of linoleic acid in liver and kidney of vitamin E-deficient animals thus seems to differ from that in vitamin E-treated animals. The chain elongation and desaturation reactions whereby linoleic acid is converted into arachidonic acid are known to proceed by two pathways: either 18:2ω6 $\rightarrow$ 18:3ω6 $\rightarrow$ 20:3ω6 $\rightarrow$ 20:4ω6, or via 20:2ω6. According to Marcel, Christiansen & Holman (1968), the first pathway is favoured over the second by a factor of 16 in the microsomes of a normal rat. However, in essential fatty acid deficiency the preference for this pathway is reduced to 4:1. Vitamin E seems to have an inhibitory effect on the desaturating enzymes of the liver and kidney microsomes, whereas chain elongation from 18:2ω6 to 20:2ω6 freely continues.

In two previous studies (Poukka, 1966, 1968) an increased concentration of linoleic acid was found in enzootic muscular dystrophy of calves as compared with normal healthy calves. This effect was reversed in the present experiment on fat-induced dystrophy. However, an increased arachidonic acid concentration was found in muscles of calves with enzootic muscular dystrophy as compared with the healthy calves (Poukka, 1966, 1968). This led to the conclusion that in enzootic muscular dystrophy the metabolism of fatty acids of the linoleic acid family was disturbed because of possible vitamin E deficiency, and this deficiency was reflected in a higher content of arachidonic acid.

In the present study the fatty acid composition of the healthy part of m. vastus intermedius did not differ from that of the affected parts of the same muscle. The changes in the fatty acid composition in the muscles did not exceed those found in the liver and kidney, which were not affected by the tissue lesions. The arachidonic acid concentration was indeed increased also in phosphatidyl choline and phosphatidyl ethanolamine fractions of the affected skeletal muscle. In vitamin E deficiency the polyunsaturated fatty acids beyond arachidonic acid were present in the same concentration as in the tissues of vitamin E-supplemented calves.

Witting and his colleagues found highly significant decreases in linoleate, doco-

sapentaenoate and docosahexaenoate and an equally significant increase in arachidonate in skeletal muscle, liver and testes of vitamin E-deficient rats (Witting & Horwitt, 1967; Witting, Likhite & Horwitt, 1967; Witting, Theron & Horwitt, 1967; Witting, 1967). Using [1-^{14}C]acetate, they also showed an increased synthesis not only of arachidonate but also of the pentaenoic acids. They suggested that fatty acids disappeared from the tissue phospholipids according to the kinetics of lipid peroxidation in vitro, but they considered the process to be resisted by a homoeostatic mechanism that increases the conversion of lower polyunsaturated fatty acids into the higher members of the series. The haemolysis test for vitamin E deficiency, which in the rat measures erythrocyte fragility and correlates well with the concentration of vitamin E in the plasma, gave no positive results in any of the calves, irrespective of their vitamin E status. The haemolysis test has been shown to be positive with erythrocytes obtained from sheep, after maize-oil feeding (Boyd, 1968). It would seem that the test may be unsuitable for use with calf corpuscles. The present investigation shows that the concentration of linoleic acid expressed as a proportion of total fatty acids is low in the red blood cells, as compared with that in the serum.

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