Phospholipids in artificially induced muscular dystrophy of calves

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1. Phospholipds were studied in the heart muscles, skeletal muscles and livers of seven Ayrshire calves which received vitamin E-free maize oil (in filled milk) with and without supplementary α-tocopherol from 1 week old for 17 d. The calves that were not given vitamin E developed muscular dystrophy.

2. Decreased amounts of cardiolipin and increased amounts of sphingomyelin were found in the skeletal muscles of vitamin E-deficient calves. There was a significant decrease of phosphatidyl choline in the livers of vitamin E-deficient calves.

3. The decrease in cardiolipin concentration confirmed the electron-microscopical picture (reported elsewhere) of preferential destruction of mitochondria in muscular dystrophy of calves.

Morphological studies on muscular dystrophy in vitamin E-deficient animals have been well described in the literature. Many reports are available on the ultrastructural alterations of the degenerated tissues (Howes, Price & Blumberg, 1964; Cheville, 1966; Van Vleet, Hall & Simon, 1967). These electron-microscopical studies indicate that the initial damage to the tissue occurs in the mitochondrion.

The present study on the phospholipid composition of tissues of calves with experimentally induced muscular dystrophy was carried out simultaneously with light and electron-microscopical studies published elsewhere (Oksanen & Poukka, 1972). Phospholipid analysis of the tissues of calves with enzootic muscular dystrophy had previously revealed certain differences compared with tissues of healthy calves of the same age (Poukka, 1968). However, in the earlier study proper dietary controls were lacking, whereas in the present study both control and test calves were fed with vitamin E-free maize oil (in filled milk), but the controls were given a supplement of vitamin E.

EXPERIMENTAL

Animals and their feeding. Seven Ayrshire calves were used in this experiment. 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 not to be given 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 4 l per d of this filled milk. The milk ration given to the vitamin E-treated calves was supplemented by the

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addition daily of 400 mg DL-α-tocopheryl acetate (F. Hoffmann – La Roche & Co. Ltd, Basle, Switzerland). Both rations were supplemented daily with 2000 μg retinyl acetate (Sigma Chemical Co., Cleveland, Ohio, USA). Antibiotics to prevent diarrhoea were given to all the calves.

After 17 d on the experimental diets the calves were killed. Immediately after slaughter, samples were taken from the heart and liver, and from the following skeletal muscles: musculus serratus ventralis, musculus vastus intermedius, and musculus splenius.

Methods used. Lipids were extracted from the tissues by the method of Bligh & Dyer (1959). Fractionation of phospholipids into the phosphatidyl and phosphatidal compounds was performed by the method of Owens (1966). In this method, lipid classes, resolved by unidimensional thin-layer chromatography, are treated with a mercuric chloride spray reagent and the (2-acyl)lysophospholipids derived from plasmogens are separated from unaffected phospholipids (diacylphospholipids) by development in the second dimension. Silica gel H plates, 250 μm thick, were washed with the mixture chloroform–methanol–water (60:35:8, by volume) before activation. The amount of lipid phosphorus applied to the plate was 24 μg. After spraying with 10·3 M-H_2SO_4, the spots were removed and digested directly by the method of Doizaki & Zieve (1963). Phosphorus was determined by the method of Bartlett (1959). The extinction was read at 830 nm in a Unicam SP 600 spectrophotometer. The mean recovery of phosphorus was 82·1% (SE 1·3) in twenty-one experiments.

RESULTS AND DISCUSSION

The calves that were not given vitamin E developed severe muscular dystrophy. Post-mortem examination of the muscles showed that whereas the muscles of the calves which had received the milk ration that was supplemented with vitamin E were normal, those of the vitamin E-deficient calves were heavily degenerated – the clinical and post-mortem observations have been described by Poukka & Oksanen (1972). There was a significant decrease of phosphatidyl choline concentration in the livers of the vitamin E-deficient calves.

Oksanen & Poukka (1972) found electron-dense formations in the mitochondria of the myocardium and skeletal muscle of vitamin E-deficient calves. In the affected myocardium the changes were slight, whereas in the affected skeletal muscle intact mitochondria were uncommon. In the present study, biochemical analysis of the phospholipids in these tissues did not reveal any significant differences in the phospholipid composition of the heart muscle, whereas in the skeletal muscle the concentration of sphingomyelin increased and that of cardiolipin decreased in the degenerated tissue (Table 1). Cardiolipin is a phospholipid that occurs almost exclusively in mitochondria, whereas sphingomyelin is mainly extramitochondrial (Fleischer & Rouser, 1965). The decrease in the amount of cardiolipin points to preferential destruction of mitochondria, confirming the electron-microscopical studies.

The significant increase in sphingomyelin of affected skeletal muscle in the present experiment is in accord with the results obtained by other investigators of muscular

Table 1. *Effects of vitamin E on the composition (% of the total phospholipid) of phospholipids from the tissues of calves given maize oil*

(Mean values with their standard errors)

Tissue	No. of samples	Lysophosphatidyl choline		Sphingomyelin		Phosphatidyl choline		Phosphatidal choline		Phosphatidyl serine		Phosphatidal serine		Phosphatidyl inositol		Phosphatidyl ethanolamine		Phosphatidal ethanolamine		Cardiolipin	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heart																					
+vitamin E	4	3·7	0·8	7·8	0·5	19·3	2·5	23·4	2·9	1·4	0·2	1·3	0·2	2·9	0·2	15·2	1·2	15·4	0·7	9·1	0·7
−vitamin E	3	2·2	0·3	9·5	1·1	24·1	3·9	21·5	2·9	1·1	0·5	1·1	0·4	2·3	0·6	12·0	1·3	14·8	0·6	9·6	0·5
Liver																					
+vitamin E	4	1·7	0·2	7·9	1·0	59·1	1·0**	1·2	0·2	1·7	0·9		—	2·4	0·5	23·3	1·9	3·7	0·7	1·5	0·8
−vitamin E	3	1·7	0·3	6·8	0·8	52·9	1·2	1·7	0·3	2·1	0·2	0·6	0·1	3·7	0·6	24·4	1·6	3·7	0·4	2·3	0·4
Skeletal muscle																					
+vitamin E	12†	1·7	0·2	8·3	0·4*	35·7	1·8	15·1	1·2	1·6	0·1	0·9	0·2	2·9	0·3	12·3	0·2	15·8	0·2	5·8	0·5**
−vitamin E	9†	1·8	0·3	12·8	0·9	36·3	1·7	14·2	2·1	2·3	0·3		—	2·8	0·5	10·7	0·7	16·7	0·6	3·3	0·4

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

† Three muscles were taken from each animal.

dystrophies in mice and human beings (Hughes & Frais, 1965; Kunze, Olthoff & Schellnack, 1967; Davidenkova, Shvarts & Rosenberg, 1971). Davidenkova *et al.* (1971) also found decreases in the concentrations of phosphatidyl choline and cardiolipin.

Earlier studies on enzootic muscular dystrophy (Poukka, 1968), showed changes in sphingomyelin and cardiolipin concentrations which, although not statistically significant, tended to be the same as those encountered in the present study. However, liver phosphatidyl choline concentration was significantly lower in diseased calves in both enzootic (Poukka, 1968) and experimentally induced muscular dystrophy (Table 1). This decrease may be caused by an increased demand for phospholipid in regenerating skeletal muscle, liver being the main organ for phospholipid synthesis. Another possibility is that vitamin E is involved in the synthesis of phosphatidyl choline in liver.

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