# CHANGES IN ACTIVITY OF SOME ACID HYDROLASES IN MUSCLE TISSUE OF RABBITS WITH AVITAMINOSIS E

B. F. Korovkin and V. V. Budnyakov

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Activity of acid ribonuclease, acid phosphatase, and cathepsins was investigated in the skeletal muscles of rabbits with avitaminosis E. The results showed that in vitamin E deficiency the total and free acid hydrolase activity in the skeletal muscles is sharply increased. The increase in acid hydrolase activity in the skeletal muscle precedes the morphological changes which arise in muscle tissue in avitaminosis E.

It is now 50 years since Evans and Bishop published their investigations, but many aspects of metabolism in the muscle tissue of animals with vitamin E deficiency remain unexplained, and the available evidence is contradictory.

The object of the present investigation was to study the activity of certain enzymes of the lysosomes of rabbit muscle tissue in avitaminosis E. The special character of the lysosomal enzyme spectrum and the diversity of their functions suggest that lysosomes participate in the development of many pathological processes, including the muscular dystrophy of avitaminosis E.

TABLE 1. Changes in Acid RNase, Acid Phosphatase, and Cathepsin Activity in Muscle Tissue Homogenates of Rabbits with Avitaminosis E (enzyme activity in $\mu$moles substrate per gram tissue per minute)

| Enzyme | Group of animals | Day of experiment | Activity of enzyme | | Free activity as % of control |
|---|---|---|---|---|---|
| | | | total | free | |
| Acid RNase | Control | 10—15-й | 30,7±10,6 | 7,7±2,5 | 25 |
| | | 20—25-й | 32,1±6,3 | 8,1±0,4 | 25 |
| | | 30—35-й | 32,5±7,0 | 8,4±0,4 | 26 |
| | Experimental | 10—15-й | 188,1±11,6 | 87,5±6,3 | 46 |
| | | 20—25-й | 215,4±26,6 | 117,6±9,3 | 54 |
| | | 30—35-й | 250,2±16,9 | 142,0±16,9 | 57 |
| Acid phosphatase | Control | 10—15-й | 22,5±3,5 | 8,2±0,4 | 36 |
| | | 20—25-й | 23,1±3,6 | 8,9±0,6 | 38 |
| | | 30—35-й | 24,0±3,6 | 9,3±0,6 | 38 |
| | Experimental | 10—15-й | 148,2±7,9 | 73,8±6,7 | 50 |
| | | 20—25-й | 231,2±8,3 | 181,2±11,4 | 78 |
| | | 30—35-й | 208,2±12,7 | 180,1±8,1 | 91 |
| Cathepsins | Control | 30—35-й | 21,7±4,3 | 4,2±0,5 | 19 |
| | Experimental | 30—35-й | 215,9±12,6 | 179,1±21,6 | 82 |

Note. $P < 0.001$; value of P calculated relative to control group of animals investigated at the same times as the experimental rabbits.

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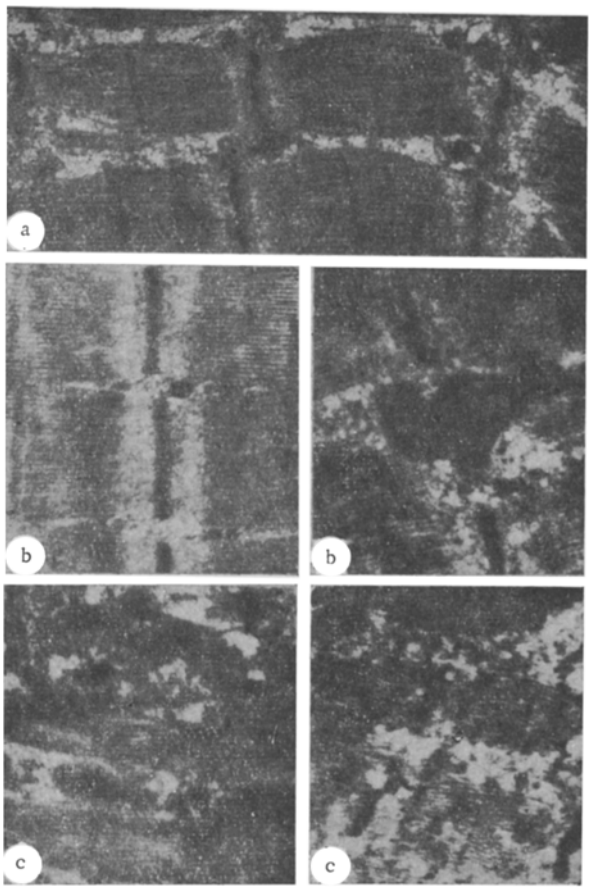


Fig. 1. Electron micrograph of striped muscle: a) normal rabbit muscle; b) muscle of rabbit with avitaminosis E (10th day of experiment); c) muscle of rabbit with avitaminosis E (30th day of experiment), 60,000 ×.

## EXPERIMENTAL METHOD

Experiments were carried out on growing rabbits kept on a special diet [3]. The control group consisted of rabbits of the same age kept on a normal diet. Each group included 12-15 animals. In the course of the experiment urine was collected from the experimental and control animals (the rabbits were kept in special cages) and the creatine concentration in it was determined. At the stipulated times (10-15, 20-25, 30-35 days) the animals were decapitated.

Activity of acid ribonuclease (2.7.7.16), acid phosphatase (3.1.3.2), and cathepsins (3.4.4) was investigated in muscle homogenates. Two forms of activity of the enzymes were determined: total and free. Total activity of the enzymes was investigated in homogenates previously treated with a 0.2% solution of the detergent Triton X-100. No detergent was added to homogenates in which the free activity of the enzymes was determined. The homogenates were centrifuged for 60 min at 50,000 g and the activity of the lysosomal enzymes was estimated in the supernatant.

Acid phosphatase activity was determined by means of the special kits supplied by the firm of Bochringer (West Germany), while acid ribonuclease (RNase) and cathepsin activity was determined by Pokrovskii's method [2] with slight modification. Parallel electron-microscopic investigations were made of the tissue of the experimental and control rabbits.

## EXPERIMENTAL RESULTS AND DISCUSSION

On the 10th-15th day of the special diet the total and free acid RNase and acid phosphatase activities in skeletal muscle homogenates of the rabbits with avitaminosis E were raised. As the experiment proceeded, the activity of the enzymes continued to rise (Table 1).

On the 20th-25th day of the experiment the total acid RNase activity was increased by seven times and the free activity by 14 times. At these same times the total acid phosphatase activity was increased by 10 times and the free activity by 20 times compared with the rabbits of the control group.

Cathepsin activity was investigated only on the 30th-35th day of the experiment. The total cathepsin activity in the skeletal muscle homogenates of the experimental rabbits was 10 times higher, and the free activity 40 times higher, than in the control.

On the 10th-15th day of the experiment (Fig. 1) electron microscopy of the muscle tissue of the rabbits with avitaminosis E revealed some thickening of the Z disks. The number of mitochondria between the myofibrils was increased, and often they were irregular in shape. Otherwise the ultrastructure of the muscle cells showed no significant change. Later during the experiment the ultrastructural changes in the muscle cells were more marked (the Z disks were considerably thickened, their electron density was higher, and their course was uneven and twisting; in the region of some sarcomeres they were broken up into fragments; the mitochondria were still larger and more numerous than before).

The increase in activity of the acid hydrolases investigated in the skeletal muscle homogenates was presumably connected either with an increase in the catalytic activity of the existing enzymes or with their

increased biosynthesis. However, the second hypothesis is unlikely to be true, for investigations have shown that the protein content in the skeletal muscles of animals with avitaminosis E is lowered [1, 6]. It is also known that the content of high-energy compounds, so essential for synthesis, in the tissues also is reduced in avitaminosis E [4].

The possibility of activating the enzymes was studied by the method of addition of crystalline enzymes in experiments in vitro.

However, these experiments showed that the degree of activation was slight and the increase amounted to only 10-15% of the activity added.

Consequently, this method cannot be used to obtain evidence of activation of lysosomal enzymes in avitaminosis E. The mechanism of activation of the acid hydrolases in this pathological state is very complex. One possible cause of activation of the lysosomal enzymes in avitaminosis E is the deficiency of vitamin E itself, for under normal conditions it somehow inhibits the action of acid hydrolases in the tissues (a similar state of affairs is known for vitamin P and hyaluronidase).

There is reason to suppose that the activation of lysosomal enzymes and the sharp increase in their free activity in the skeletal muscle precede the morphological changes which are so characteristic of the muscle dystrophy in avitaminosis E [7].

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