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Effect of Vitamin E Deficient Diet Upon Skeletal Muscle.

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It has been shown by Evans and Burr[1] that muscular weakness, paralysis, and death might occur in young rats which suckled mothers receiving a diet deficient in vitamin E. Symptoms became obvious when the animals were about 3 weeks of age. The muscles of animals so afflicted show marked histologic changes (Olcott[2]). However, all of the animals are not affected in this way. Many survive and live to maturity even though continued on a vitamin E deficient diet after weaning.

The object of these experiments was to study the functional capacity of the gastrocnemii muscles of adult rats which had been reared on a vitamin E-deficient diet. Through the coöperation of the Biochemistry Department such rats were made available to us for study. The prospective mothers were given a vitamin E-free diet (Olcott[2]) with just enough wheat germ oil to produce almost continuous fertility. Those of the young which survived to the 28th day were weaned and placed on the vitamin E-deficient diet.[2] A part of them which received a wheat germ supplement served as the controls.

The animals were continued on this diet to an age of from 4 to 6 months. At that time the maximum isometric tension developed by the intact gastrocnemius in response to condensor discharges, at the rate of 50 per second, was determined. In addition the creatine, chloride and water concentrations of the gastrocnemius were determined. Some of the muscles were reserved for histological examination. In certain instances the muscles were tested for sensitivity to remote acetylcholine injection.

The results of the tension experiments and chemical analysis are summarized in Table I. The tension developed by the muscles of the experimental males is definitely less than that found for the control animals whether the muscles were stimulated directly or through the peripheral motor nerve. Furthermore, the tension developed by the muscles of the animals on the vitamin E-free diet in response to nerve stimulation was considerably below the tension developed upon direct stimulation. Thus it appears that the vita-

1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

2 Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

min E-deficient diet has resulted in a failure of the muscle to exhibit normal contractile power. It may be noted here that the tensions developed by the muscles of animals receiving the vitamin E-free diet plus wheat germ oil are of precisely the same order as for rats of a similar age reared on our stock diet.

TABLE I.

Sex	♂	♂	♀	♀
Diet	E-free	E-free + wheat germ oil	E-free	E-free + wheat germ oil
No. animals	12	8	14	9
Body wt. (g)	235	307	188	206
Muscle tension, g per g muscle				
(a) Nerve stim.	802	1680	1264	1790
(b) Muscle stim.	1188	1773	1412	1774
$\frac{\text{Muscle wt.}}{\text{Body wt.}} \times 100$	0.58	0.53	0.58	0.60
Creatine, mg/100 g muscle	368	451	442	477
% water in muscle	76.6	75.4	—	—
Cl in mg/100 g muscle	71.0	45.0	—	—

The changes in creatine and chloride concentrations are also indicative of muscle dystrophy. The constancy of the muscle weight to body weight ratio in the experimental and control series indicates that there was no gross atrophy.

Histologic examination* showed a focal hyaline necrosis in only a few fibers and an occasional instance of proliferation of sarcolemma nuclei. This alteration in contractile ability of the muscles in the absence of characteristic histologic change is reminiscent of certain types of myopathy in humans where the clinician is able to elicit signs of impaired muscle function while very little pathologic change is present in the biopsy material.

Ringsted[3] reported the development of paresis in rats after 4-5 months on a vitamin E-free diet. This was based on direct observation of the animals and no more intimate studies were made of the musculature. The animals used in our experiments gave no such outward signs of muscular weakness, even though the maximum power of the muscles was considerably reduced.

Burr, Brown and Mosely[4] have described paralysis in rats after 22 months on a vitamin E-free diet. Their remark that the muscles

* We are indebted to Dr. K. M. Brinkhous for the preparation and interpretation of the tissue sections.

3 Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

4 Burr, G. O., Brown, W. R., and Mosely, R. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 780.

are unaffected is difficult to evaluate as it is not clear whether any quantitative measure of the response of the muscle to stimulation was made. Certainly our animals have given unmistakable functional and chemical evidence of dystrophic changes in the muscle after 5 months.

In view of the consistent differences in tension found between the responses to direct and motor nerve stimulation the sensitivity of the muscles to remote acetylcholine injection was tested in a group of experimental and control animals. In no instance was there any indication of a response to 400 gamma of acetylcholine bromide injected into the jugular vein of atropinized, eserinized control males while in the majority of experimental males a slight but definite response was elicited. The exact significance of this is not clear. So far as we are aware, the only procedure which will definitely sensitize skeletal muscle to acetylcholine is motor denervation. On that basis the acetylcholine test would indicate that the peripheral motor nerve is only slightly affected.

The data concerning the experimental and control females show the same changes as those of the males. Quantitatively the changes are less. It appears that there is a definite sex difference in the effect of deficient vitamin E intake upon the skeletal musculature. Whether this marked difference in the effect upon males and females is due to a gonadal or somatic effect of the deficiency is not known. It may be noted that the effect upon size is more pronounced in the males than in the females. The growth-promoting effect of vitamin E has been shown by Evans[5] to be independent of any effect upon the gonads.

Conclusions. Rats reared from birth on a vitamin E-deficient diet show dystrophic changes of the skeletal musculature within 6 months. This is evidenced by a decreased maximal contractile power, and decreased creatine and increased chloride concentration of the gastrocnemius muscle. The male is affected to a greater extent than is the female. These changes become manifest in the presence of only minor histologic change in the muscle, and without the appearance of gross symptoms. It is apparent that wheat germ oil will protect completely against these changes.

[5] Evans, H. M., *J. Nutrition*, 1928, **1**, 23.