was still above the initial levels a month after the injection of gum acacia; we consider this a manifestation of the reaction of the liver to injury. In absence of criteria which would enable us to identify in the serum the "alkaline" phosphatases of various origins, we regard the rise of serum phosphatase in liver involvements—particularly when there is no evidence of obstruction of the biliary passages—as an indication that the liver is a source of serum phosphatase. We assume that the "alkaline" phosphatase in the serum represents the sum total of contributions from various organs and tissues capable of producing "alkaline" phosphatase, the contribution of each being increased by an injury to which it is able to react.

This study, including histological investigations, is being continued.

11058

Cure and Prevention of Vitamin E-Deficient Muscular Dystrophy with Synthetic α-Tocopherol Acetate.

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It is now well established that adult rats reared from birth or weaning on a vitamin E-deficient diet eventually develop a muscular dystrophy.[1-3] It has also been shown that muscle changes, characteristic of dystrophy, can be detected before overt symptoms appear.[4] The criteria offered were (1) increase in the concentration of water and (2) of chlorides of the muscle, (3) decrease in the maximum strength, (4) focal hyaline necroses of muscle fibers, and (5) decrease in the creatine concentration of the muscle. Using the first 4 criteria it has been shown that wheat germ oil would prevent the development of the dystrophy.[4] It was later shown that wheat germ oil or its vitamin E-active concentrate would induce recovery from

1 Blumberg, Harold, *J. Biol. Chem.*, 1935, **108**, 227.

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

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

4 Knowlton, G. C., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 655.

all the symptoms, except the loss of muscle strength, when fed to animals with an established dystrophy.[5]

The above experiments do not eliminate the possibility that the effect of wheat germ oil may be due, not to vitamin E itself, but to some other component of the wheat germ oil. The present experiments show, however, that synthetic vitamin E (d-l α-tocopherol acetate, *i. e.*, Ephynal*) is effective in the prevention and cure of the muscular dystrophy.

Methods. Female rats, when obviously pregnant (16-18th day of gestation), were placed on a vitamin E-deficient diet[6] and maintained on this diet throughout lactation. Males only were used, and these were weaned at 24 days of age and continued on the vitamin E-deficient diet throughout the experiment. The development and course of this dystrophy were studied in rats at ages of 1, 2, 3, 4, 5 and 8 months.

The curative effects of α-tocopherol were tested on a group of E-deficient dystrophic male rats which, beginning at an age of 5 months, were given weekly subcutaneous injections of 3 mg of α-tocopherol acetate in 0.1 cc olive oil. These weekly supplements were continued until the animals reached an age of 8 months, at which time the muscles were studied. A group of litter mate controls were given weekly subcutaneous injections of 0.1 cc of olive oil over the same period of time.

In the experiments testing the preventive effects of α-tocopherol, the weekly supplements were given in the same amount and manner as described above. The supplements were started at 30 days of age, at which time the characteristic dystrophic changes had not yet appeared, and were continued until 150 days of age, when the muscles were studied for evidences of dystrophy.

The gastrocnemii of each animal were studied as to (1) maximum strength, (2) water concentration, (3) chloride concentration, and (4) histologic appearance. The methods used have been described previously.[5]

Results. Muscle strength at 1 month of age is normally about 55% of the adult value. Water and chloride concentrations are higher in young animals than in the adult.[7] In the present paper we

[5] Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 453.

* Ephynal was furnished through the courtesy of Dr. E. D. Shaner of the Hoffmann-LaRoche Laboratories, Inc.

[6] Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

[7] Hines, H. M., and Knowlton, G. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 133.

TABLE I.
Development of Muscular Dystrophy on a Vitamin E-Deficient Diet.

Age in months	No. of animals	Muscle			Necrotizing lesions
		Max. strength, % of normal	Water, % of normal	Chloride, % of normal	
1	3	59.	98.9	98.	0
2	4	73.	100.1	103.	+++
3	4	74.	100.9	112.	++
4	4	86.	100.8	115.	++
5	6	69.	100.4	117.	++
8	5	70.	101.1	147.	++

have compared deficient animals with normal animals of the same age and strain, and the values given in the tables are expressed in percentage of the normal levels at that same age. In addition to these data, the tables also show the approximate extent of the focal necrotizing lesions in each group. In most animals, even with well-developed dystrophic changes, only a few fibers in any one section showed degenerative changes. Associated with the hyaline necrosis

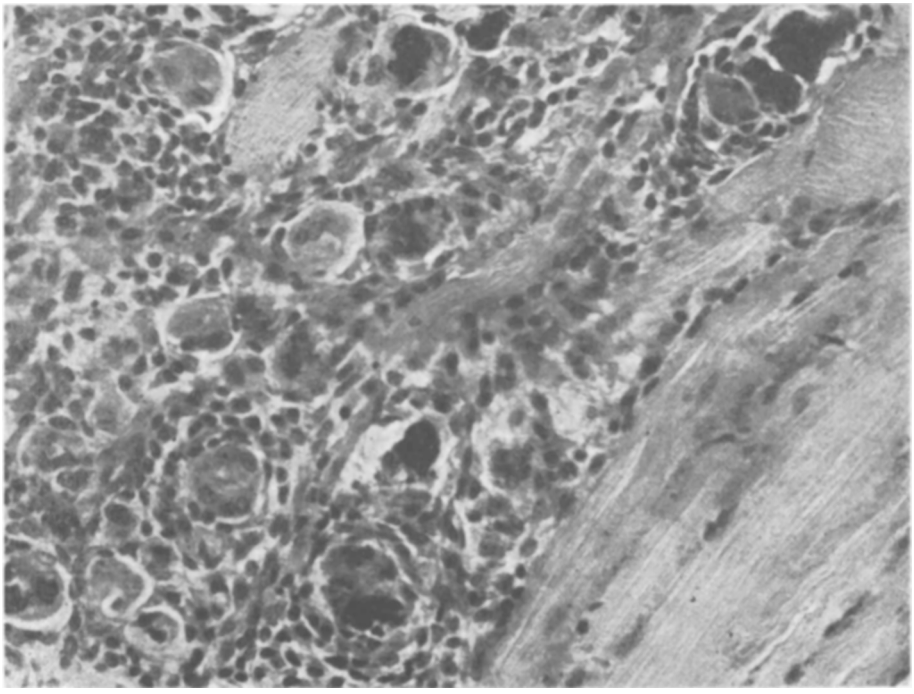

FIG. 1.
Gastrocnemius muscle from a vitamin E-deficient rat, 2 months of age. Marked proliferation of myocytes. Partial calcification of necrotic muscle substance. × 350.

there were often reactive inflammatory changes and beginning regeneration of muscle fibers. In addition to these lesions, many intact fibers in the dystrophic muscles showed an increase in the number of subsarcolemma nuclei (see also Evans, Emerson, and Telford,[8] Knowlton, Hines, and Brinkhous[5]). These proliferative changes are interpreted as a late stage of regeneration or as a reaction to a mild injury not sufficient to cause necrosis.

From Table I it is seen that dystrophic changes are clearly evident by the time the deficient animals reach the age of 2 months. At the age of one month there is some evidence of muscle weakness. However, the number of animals used was small and the individual variations are so great in animals of this age that the value obtained is not significantly different from normal. The other criteria of dystrophy were entirely absent at this age.

Beginning with the second month the muscle strength improved somewhat up to the fourth month of age, but thereafter it suffered

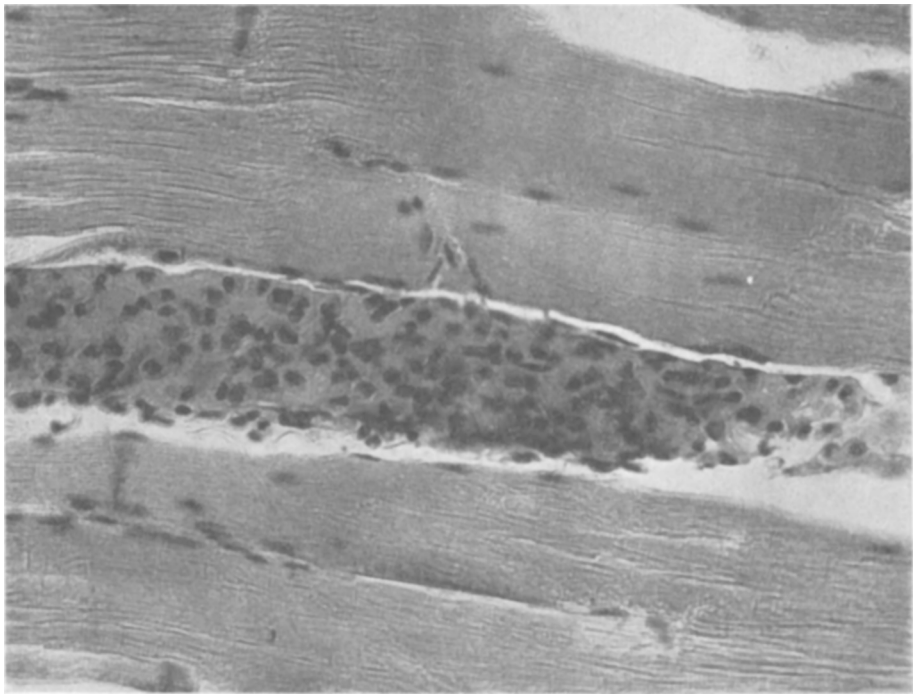

FIG. 2.
Gastrocnemius muscle from a vitamin E-deficient rat, 8 months of age. Isolated necrotic muscle fibre. × 350.

[8] Evans, H. M., Emerson, G. A., and Telford, I. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 625.

considerable impairment. The concentrations of water and chlorides increased steadily from the very beginning.

The histologic lesions, although very patchy in distribution, were most severe at the end of the second month. One muscle at this age which showed very marked changes histologically (Fig. 1) also showed gross lesions. After the second month the necrotizing lesions were much less extensive (Fig. 2). Proliferative muscle lesions were observed in all deficient animals after the first month of age.

TABLE II.
Cure of Nutritional Muscular Dystrophy with α-Tocopherol (Rats 8 Months of Age).

		Muscle			
No. of animals	Supplement	Max. strength, % of normal	Water, % of normal	Chloride, % of normal	Necrotizing lesions
6	α-tocopherol	96.	99.6	111.	0
5	olive oil	73.	100.8	150.	++
5	none	70.	101.1	147.	++

FIG. 3.
Gastrocnemius muscle from an 8-months-old rat on a vitamin E-deficient diet. Treated with α-tocopherol acetate since age of 5 months. Practically complete recovery. × 350.

TABLE III.
Prevention of Nutritional Muscular Dystrophy by Use of α-tocopherol (Rats 5 Months of Age).

No. of animals	Supplement	Muscle: Max. strength, % of normal	Muscle: Water, % of normal	Muscle: Chloride, % of normal	Necrotizing lesions
12	α-tocopherol	78.	99.4	95.	0
11	olive oil	66.	100.4	120.	++
6	none	69.	100.4	117.	++

α-tocopherol acetate caused complete recovery of an established dystrophy in the deficient rats (Table II). In those dystrophic rats which were started on the supplement at 5 months of age and continued on this regimen to 8 months of age, the characteristic dystrophic changes were absent. The maximal strength and water and chloride concentrations had returned to a normal or nearly normal level, and necrotizing muscle lesions were absent. The proliferative muscle lesions, not recorded in the table, had practically disappeared. Only a very few muscle fibers showed increase in the number of subsarcolemma nuclei. Fig. 3 shows an area of muscle which is fairly representative of this group of rats cured of the dystrophy.

Table III shows the effect of α-tocopherol in preventing lesions in animals maintained on the deficient diet. Injections of α-tocopherol were given weekly, beginning at one month of age. At the end of 5 months, the muscle strength was somewhat subnormal, but was definitely greater than in the control groups. The water and chloride concentrations were normal and necrotizing lesions were entirely absent. Proliferative muscle lesions did develop, however.

From these data, it appears that α-tocopherol acetate in the dosage used, is even more effective in promoting a cure in the older animals than in preventing symptoms in the younger animals. This may depend upon: (1) a greater requirement during the period of rapid growth, and (2) a less efficient utilization of the injected α-tocopherol ester by the younger animals.

Conclusion. From these results it may be concluded that subcutaneous injections of synthetic α-tocopherol acetate are effective in both the cure of and the prevention of the nutritional muscular dystrophy seen in rats maintained from an early age on a vitamin E-deficient diet.