the other shock theories, it has the advantage of being compatible with all experimental observations which have so far been made on the subject of shock.

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Muscular Dystrophy in the Absence of Testicular Degeneration in Vitamin E Deficiency.*

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As a result of the symptoms leading to its discovery, vitamin E has been defined as a fat-soluble essential preventing a characteristic and specific sterility in the male and female rat. Evans and Burr[1] described paralysis in the nursing young of female rats which had received minimal amounts of vitamin E. Olcott,[2] and later others, observed lesions of the skeletal muscles in the deficient young. However, the appearance of young rats weaned from stock females to a vitamin E-deficient diet is normal except for a relatively slight (compared to most deficiency states) impairment in the rate of growth until they develop paralysis of the rear limbs after many months on the diet. These paralyzed adult animals, according to Einarson and Ringsted,[3] exhibit first microscopic lesions in the central nervous system and later lesions in the voluntary muscles. Recently Knowlton, *et al.*,[4] have reported that in the long period elapsing between the appearance of gross neuro-muscular symptoms in suckling and adult E-deficient rats, chemical and functional changes and occasional necrotic fibers can be detected in the skeletal muscles in the absence of overt symptoms. Nevertheless, for many months of the rat's life, sterility remains the most prominent symptom produced thus far by a dietary deficiency of vitamin E.

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

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

3 Einarson, L., and Ringsted, A., 1938, *Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats*, Oxford University Press.

4 Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 453; 1939, **42**, 804.

In contrast to the rat, young rabbits placed on a vitamin E-deficient diet pass through the following sequence in 3 to 6 weeks: abnormal creatinuria, decreased rate of growth, loss of weight, muscular weakness, inanition, and death.[5] Hyalinization and necrosis of the skeletal muscle fibers is extreme. If a partial deficiency is produced by administering subminimal doses of alpha-tocopherol the animals grow for months, eat well and show no gross symptoms.[6] However, during this time the urinary creatine is elevated and on histological examination extensive microscopic lesions are found in the skeletal muscles.

In view of the specific and irreversible nature of the testicular degeneration found in vitamin E-deficient rats,[7] experiments were undertaken to determine whether the testes in adult rabbits suffering from a complete or partial deprivation of E are more or less susceptible to damage than the skeletal muscles.

Seven adult bucks were unilaterally castrated under ether anesthesia. The testes were weighed, fixed and sectioned. All were normal. Five days after the operation the animals were placed on vitamin E-deficient diet No. 13.[5] Three of the rabbits, serving as controls, received 5 mg of alpha-tocopherol† in ethyl laurate orally 6 days a week. After a week on the diet one of the controls, No. 120, persistently refused food.

All of the deficient animals eventually lost weight and developed symptoms of dystrophy. No. 124 showed gross symptoms at 50 days. A single 15 mg dose of alpha-tocopherol administered on the 56th day resulted in the disappearance of symptoms and a rapid gain in weight. On the 98th day the animal succumbed to its second attack of the disease. The other vitamin E-deficient animals were not supplemented and either died or were killed when moribund after 49 to 79 days on the diet. Throughout the last month of the experiment the food consumption of controls Nos. 123 and 127 was paired with E-deficient rabbits Nos. 122 and 126. At autopsy the remaining testis was removed and weighed, a smear of the epididymis examined for motile sperm, and sections prepared of the testis, epididymis and thigh muscles. The weight changes and degree of muscle damage are given in Table I. Although extensive, the muscle lesions did not quantitatively equal those generally found in young dystrophic rabbits.[5, 6]

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5 Mackenzie, C. G., and McCollum, E. V., *J. Nutrition*, 1940, **19**, 345.

6 Mackenzie, C. G., Levine, M. D., and McCollum, E. V., *J. Nutrition*, 1940, **20**, 399.

7 Mason, K. E., *Am. J. Anat.*, 1933, **52**, 153.

† Kindly supplied by Merck & Company, Inc.

TABLE I.
Weight Changes and Muscle Lesions in Male Rabbits Maintained on a Vitamin E-deficient Diet with and without the Addition of Various Amounts of Alpha-tocopherol.

Rabbit No.	Alpha-tocopherol* mg	Initial wt, g	Max. wt, g	Time of max. wt, days,	Final wt, g	Duration of exper., days	Microscopic muscle lesions
120	5	2380	2390	7	1570	22	—
121		1990	2050	41	1720	49	+±
123	5	2400	2450	70	2250	75	—
122		2130	2220	69	1940	73	++
127	5	2240	2400	77	2280	79	—
126		2280	2320	66	1830	79	+±
124		2080	2200	83	1890	98	++
50	3	400	2540	168	2540	168	—
22B	1	470	2310	126	2310	126	±
72	0.5	420	3170	140	3170	140	+±
74	†	370	2720	194	2220	234	+±

*Alpha-tocopherol given 6 days a week.
†See text for levels of alpha-tocopherol administered.

In addition to this group of adult animals, 4 immature rabbits were fed diet 13 plus varying levels of alpha-tocopherol (administered 6 days a week). Rabbit No. 50, receiving a 3 mg level of the vitamin, showed no symptoms or muscle lesions after 168 days on the diet.

Rabbit No. 22B was fed alpha-tocopherol at a 1 mg level. Daily creatine determinations initiated at the eightieth day revealed a slight but definitely abnormal creatinuria. When the animal was sacrificed at 126 days the creatinuria had subsided, and only 1 or 2 muscle lesions were present in each section of thigh muscle. The vitamin E intake was evidently extremely near the minimum anti-dystrophy level for this animal.

The daily creatine excretion of No. 72 (0.5 mg level of alpha-tocopherol), determined from the 88th to the 105th day, was found to average 45 mg as compared to the normal level of 10 mg or less.

Rabbit No. 74 (0.5 mg level of alpha-tocopherol) excreted an average of 30 mg of creatine daily from the 50th to the 82nd day, at which time the daily dose of alpha-tocopherol was increased to 3 mg. On the 95th day the dose was dropped to 0.3 mg and on the 180th day it was discontinued altogether. The maximum weight was attained on the 194th day; thereafter the animal gradually lost weight until the 233rd day, when it developed physical symptoms of dystrophy and was killed. Animal No. 74, therefore, represents a chronic case of dystrophy terminating in a more acute phase of the disease.

The duration of the experiment, weight changes and muscle lesions for these animals are recorded in Table I.

The testes from both the chronic and acute deficiency groups, with the exception of those from 3 animals, were normal. The second testis of rabbit No. 121 showed some indication of disorganization. The epididymis, on the other hand, contained but a few scattered germ cells, mixed with an abundance of spermatozoa. There is a possibility of mild injury in this case. The second testis of rabbit No. 124 showed sloughing and degeneration of germ cells. The ducts of the epididymis, particularly the proximal ones, contained germ cells of all types mixed with spermatozoa. In the testes of rabbit No. 72 a small number of germ cells were either being sloughed or degenerating *in situ*. Only a few germ cells were found in the epididymides. In all 3 of these cases many tubules showed normal spermatogenesis, and in none of them did the alterations closely resemble those occurring in the testes of vitamin E-deficient rats.

The testes and the contents of the epididymides of the remaining animals of both groups were normal. In all animals, including the 3 discussed above, motile sperm were found in the epididymides at autopsy. The ratio of gram testis weight to kilogram body weight determined at the beginning and end of the experiment on the group of unilaterally castrated animals showed no significant change, the average ratios being 1.12 and 1.23 respectively.

A specific effect of vitamin E on the testes of the adult rabbit is not excluded by these experiments. Prolonging either the chronic or acute deficiency might reveal such an effect. In the latter case inanition and respiratory infections would have to be eliminated as contributing factors. The results do indicate, however, that on the E-deficient diet employed the rabbit's skeletal muscles are more susceptible to pathological alterations than the testes. Muscle necrosis can precede testicular damage and be prevalent in its absence. This is in sharp contrast to the early development of testicular degeneration in rats reared on E-deficient diets. Whether or not there is always a concomitant necrosis of occasional muscle fibres in this species is not known.

Normal testis structure (and probably function) in the rabbit is no more a criterion of adequate E intake than are continued growth, good appetite, and normal appearance.[6] Urinary creatine determinations and histological examination of the skeletal muscles may reveal the existence of an unsuspected chronic vitamin E deficiency in apparently normal animals.

Conclusions. In contradistinction to the rat, the skeletal muscles of the male rabbit with a partial or complete dietary deficiency of

vitamin E are, under the conditions employed, more sensitive to morphological alterations than the testes. The absence of testicular degeneration in the rabbit does not preclude the existence of vitamin E deficiency and necrosis of the skeletal muscles.

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Tocopherol Level in Serum of Normals and Patients with Amyotrophic Lateral Sclerosis.*

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Synthetic vitamin E (dl-α-tocopherol) has recently been introduced in the treatment of amyotrophic lateral sclerosis.[1] It seemed of interest to study the level of this vitamin in untreated and treated patients with this condition, and to compare it with the serum level of normal individuals. Analytical methods for the determination of tocopherol have been developed for the control of preparation and concentration of this substance from its natural sources, such as wheat germ oil. The dipyridyl method, based on the reduction of trivalent iron by tocopherol to the bivalent state and subsequent colorimetric determination of the ferrous iron as α,α′-dipyridyl complex, has been developed for the assay of tocopherol concentrates. It has been used for the study of the tocopherol level in the serum of tocopherol-deficient and tocopherol-treated rats.[2]

We have adapted this method to the analysis of human serum by means of a photoelectric colorimeter. The employment of a photoelectric method is advantageous because the pink color of the iron-dipyridyl complex is not very suitable for visual comparison.† Ten ml of serum from a fasting blood specimen are sufficient for the determination.

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1 Wechsler, I. S., *J. A. M. A.*, 1940, **114**, 948; *Am. J. Med. Sci.*, 1940, **200**, 765; *Arch. Neurol. Psych.*, 1941, in press.

2 Emmerie, A., and Engel, C., *Rec. Trav. Chim. des Pays-Bas*, 1938, **57**, 1351; 1939, **58**, 283, 895; *Vitamin E, A Symposium*, London, 1939, p. 14.

† The details of this method will be reported elsewhere.