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Effect of Oral and Parenteral Administration of Vitamin E on Creatinuria and Symptoms of Dystrophic Rabbits.*

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Nutritional muscular dystrophy in the rabbit may be cured or prevented by the oral administration of d,l-α-tocopherol[1] (synthetic vitamin E). Mattill[2] has stated that the acetate of α-tocopherol is much less effective when given parenterally or subcutaneously than by mouth, and Eppstein and Morgulis[3] have reported that the intramuscular injection of the acetate in olive oil at 5 or 10 mg levels failed to cure dystrophic rabbits. According to Knowlton, *et al.*,[4] the injection of the ester was more effective in curing the relatively mild symptoms of dystrophy in older rats than in preventing them in young animals. It was shown by Demole[5] that 20 times the active oral dose of α-tocopherol acetate was ineffective when injected in preventing resorption in E-deficient female rats. On the other hand, the unesterified vitamin when injected showed one-fifth to one-tenth of its oral activity. Goettsch and Pappenheimer[6] have recently reported a somewhat higher activity for injected E in the rat. We wish to report the results of injecting dystrophic rabbits with α-tocopherol† (in a pure form or in peanut oil solution) and with the water-soluble sodium salt of the phosphoric acid ester of α-tocopherol.[7]

Since we have previously shown that an abnormal creatinuria (and extensive muscle lesions) may exist in actively growing chronically E-deficient rabbits in the absence of overt symptoms,[8] urinary

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

2 Mattill, H. A., *J. Nutrition*, 1940, **19**, Proc. Am. Inst. Nutr., p. 13.

3 Eppstein, S. H., and Morgulis, S., *J. Nutrition*, 1941, **22**, 415.

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

5 Demole, V., *Ex. Compt. Rend. de la Société des Physiologistes Suisses*, 1939, July.

6 Goettsch, M., and Pappenheimer, A. M., *J. Nutrition*, 1941, **22**, 463.

† Kindly supplied by Merck and Company, Inc.

7 Karrer, P., and Bussmann, G., *Helv. Chemica Acta*, 1940, **23**, 1137.

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

creatine and creatinine were determined daily, resulting in the amplification and extension of earlier observations on the excretion of these substances in dystrophic and cured animals. Furthermore, the production and symptomatology of an extremely severe dystrophy of several months' duration is reported.

The methods employed in these experiments have been described elsewhere.[1, 8] The terms "creatine" and "creatinine" are used to designate chromogenic substances in urine giving a positive Joffé reaction according to the procedure of Folin.[9] Creatine is expressed as milligrams of creatinine. All of the animals used had been cured of one previous attack of dystrophy by the oral administration of α-tocopherol.

Sixteen young rabbits on diet 13 were allowed to develop a daily creatinuria of 60 to 70 mg. At that time they weighed approximately 1000 g. Eight were injected intramuscularly or subcutaneously with 20 mg of d,l-α-tocopherol in 0.2 cc of peanut oil, 5 received the same dose orally, and 3 were untreated. The untreated animals died in 8, 9, and 10 days after losing 185, 80, and 380 g. One of the injected animals died in 7 days, and was omitted from the group. The other 7 were alive at 12 days, although the physical symptoms of dystrophy had grown progressively worse. Food consumption, however, remained at the preinjection average of 20 g daily, and the sharp decline in weight usually associated with such severe physical symptoms did not occur. The average loss in weight for the 12-day period was 100 g. Although the oral administration of the same dose of α-tocopherol resulted in the disappearance of physical symptoms within 7 days, and a growth response in 2 days (with an average gain of 250 g during 12 days), the results of this experiment suggest that the injected tocopherol was not altogether without effect in prolonging life, and stabilizing food consumption and weight.

A comparison of the creatine and creatinine excretion of these two groups is illustrated in Fig. 1. The creatine excretion of the 3 untreated animals is also included. The consistently high creatine excretion of the injected animals is in contrast to the rapid drop of urinary creatine in the group receiving the same dose of α-tocopherol orally. The creatine excretion curve of the latter group demonstrates the value of daily creatine determinations in measuring the level of vitamin E nutrition. During the last 3 days of the experiment, while the creatine was rising, the average daily gain in weight was 25 g, and there were no physical symptoms. Yet from the

[9] Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

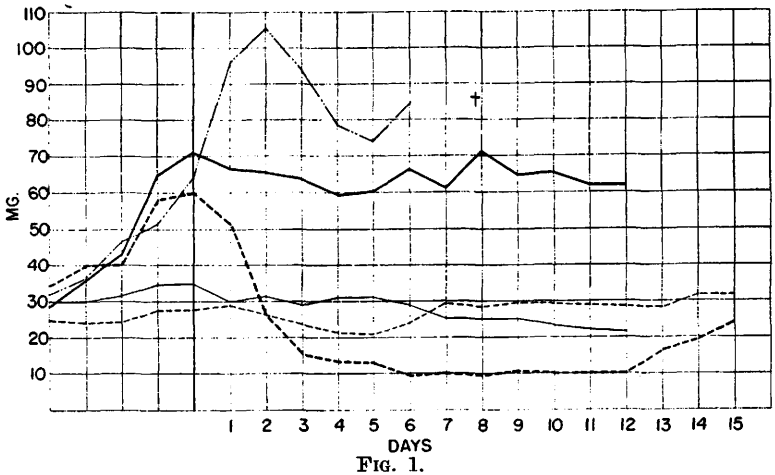

FIG. 1.

Average daily creatine and creatinine excretion of dystrophic rabbits receiving 20 mg of d, l-α-tocopherol in 0.2 cc of peanut oil orally or parenterally.

The time of treatment is indicated by the thickened vertical line at 0 days. The 4 previous days are not numbered. The uppermost line (long dash) represents the creatine excretion of 3 untreated animals, and † indicates the approximate time of their death. The heavy and thin unbroken lines represent, respectively, the average creatine and creatinine excretion of 7 injected rabbits. The heavy and thin broken lines represent, respectively, the average creatine and creatinine excretion of 5 rabbits treated orally.

point of view of adequate nutrition, a deficiency of the vitamin had again set in, and it is likely that once more muscle lesions had developed.[8]

Of interest is the fall in urinary creatinine in the injected animals beginning on the seventh day. This was to be expected on the basis of Bloch and Schoenheimer's[10] observation that urinary creatinine in normal rats represents 2% of the body creatine. Our previous failure to detect an unmistakable decrease in creatinine excretion was probably due to the earlier death of most of our dystrophic rabbits. The temporary fall in creatinine, paralleling the fall in creatine in animals responding to vitamin E, has already been noted.[1]

We were prompted by the results of this experiment to try the effect of injecting larger amounts of d,l-α-tocopherol. Two dystrophic rabbits were injected with 100 mg and 2 with 200 mg of pure α-tocopherol, and 2 with 200 mg of α-tocopherol in peanut oil. One animal of each pair was injected intramuscularly and the other subcutaneously. The site of injection and the form of the preparation were apparently unimportant. Two of the 4 animals receiving 200 mg responded in an uneventful albeit somewhat slow fashion, 12 days being required for the creatine to reach 10 mg as compared with

[10] Bloch, K., and Schoenheimer, R., *J. Biol. Chem.*, 1939, **131**, 111.

5.6 days for the orally treated animals (Fig. 1). However, the other 2 animals receiving 200 mg behaved in a more interesting fashion as did one of those receiving 100 mg. (The other rabbit on the latter level died in 17 days.) These 3 rabbits started to gain weight one week after injection. Simultaneously the urinary creatine (70 to 90 mg) began a gradual decline which terminated at a level of 20 to 40 mg on about the 38th day and then began to rise again. The urinary creatinine began to rise slowly after 20 days. One of the animals was killed at 41 days, with a rising urinary creatine and creatinine. At that time it had gained a total of 550 g and was still growing.

The other 2 rabbits were continued. Urinary creatine rose to 65 mg by the 58th day. It was accompanied by an increase in the creatinine excretion to 45 mg daily. The creatinine then slowly declined from that level to 5 mg by the 75th day. There was a concomitant fall in creatine excretion in both animals. Rabbit No. 311 developed diarrhea, lost weight for 3 days, and died on the 80th day. The daily creatine excretion at this time averaged 15 mg. Rabbit No. 307 developed the same symptoms and died on the 95th day. Its creatine excretion for the last few days of life averaged 25 mg. During the entire experimental period both rabbits exhibited severe symptoms of dystrophy. This was particularly so of No. 307, whose death was predicted as early as the second week of the experiment. From that time on it was practically prostrate. Nevertheless, it gained 300 g between the 30th and 92nd day. Rabbit No. 311 gained 1000 g during the experiment excluding the 3 days before death.

The microscopic picture of the thigh muscles of these growing dystrophic animals was most interesting. There was extreme fatty infiltration, a moderate increase in connective tissue, and severe atrophy of the remaining muscle fibers. Some of these atrophic fibers showed hyalinization and necrosis, indicating that the active degeneration characteristic of all stages of E deficiency in the rabbit had not ceased. Similar changes in guinea pigs have been described by Goettsch and Pappenheimer.[11] The large numbers of fat cells explains in part why these animals were gaining weight while their muscle fibers were undergoing necrosis or atrophy. This picture was in sharp contrast to that found in growing chronically E-deficient rabbits which exhibit creatinuria, but no physical symptoms.[8] Although their muscle fibers showed hyalinization and necrosis, re-

[11] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

pair was apparent, and neither fatty infiltration nor marked atrophy was observed.

In an endeavor to throw some light on the poor and variable response to the injection of massive doses of α-tocopherol, a finding in harmony with Demole's results in rats,[5] we prepared the water-soluble sodium phosphate of α-tocopherol according to the procedure of Karrer and Bussmann.[7] Since the purity of our preparation was not determined, the doses employed represent maximum values only. Aqueous solutions containing the equivalent of 20 and 30 mg of α-tocopherol were injected subcutaneously into 2 dystrophic rabbits. The high urinary creatines of the 2 rabbits fell to 10 mg daily in 8 and 9 days respectively. There was a prompt growth response and the physical symptoms disappeared within a few days. This suggests that the activity of injected vitamin E in rabbit dystrophy, as in rat sterility,[5, 7] is determined largely by its physical state.

Conclusions. The oral administration of 20 mg of α-tocopherol to dystrophic rabbits produces a rapid fall in the urinary creatine. Following the parenteral administration of the same dose, the creatine remains at a high level, and the creatinine decreases. The injection of massive doses of α-tocopherol cures dystrophy in some cases, while in others it extends life and promotes growth for several months without curing the symptoms of the disease.

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Clinical Fetal Electrocardiography. Its Practical Accomplishment.

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The recording of the electrocardiogram of the human fetus *in utero*, although long attempted (Cremer,[1] Foá[2]), has been only indifferently successful even late in pregnancy.[3, 4, 5]

We have developed a technic of definite clinical value by using a

1 Cremer, M., *München med. Wchnschr.*, 1906, **53**, 811.

2 Foá, C., *Giornale della R. Acad. di Med. di Torino*, 1911, **4**, 90.

3 Strassmann, E. O., *Proc. Staff Meet. Mayo Clin.*, 1938, **13**, 251.

4 Bell, G. H., *J. Obst. and Gyn. Brit. Emp.*, 1938, **45**, 802.

5 Mann, H., and Bernstein, P., *Am. Heart J.*, 1941, **22**, 390.