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Cure of Repeated Attacks of Nutritional Muscular Dystrophy in the Rabbit by Alpha-Tocopherol.*

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The extreme degeneration of the skeletal muscles in rabbits originally described in detail by Goettsch and Pappenheimer[1] was sub-

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1 Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

sequently shown by us to be the result of vitamin E deficiency.[2] At that time we reported the absence of microscopic abnormalities in the muscles of rabbits cured of severe dystrophy with vitamin E. In a later experiment 3 successive attacks of the disease were produced and cured.[3] Goettsch and Pappenheimer[4] have recently described the very rapid restoration of normal muscle tissue in dystrophic guinea pigs following vitamin E therapy.

The present investigation was undertaken to determine whether or not the remarkable reparative process elicited by α-tocopherol would persist after a long series of attacks, and to throw some light, if possible, on the rather discouraging results obtained by many workers in the treatment of human dystrophies with α-tocopherol. Undoubtedly chronic and intermittent deficiencies in the human diet are much more common than persistent complete deficiencies.

Seven young male and female rabbits weighing from 340 to 640 g each were placed on dystrophy-producing diet 13.[1] The criteria employed to diagnose dystrophy; namely, creatinuria, loss of weight, reduced food consumption, and physical symptoms, have been previously discussed in detail.[2] After stage II dystrophy (rabbits easily laid on their sides) or, in the great majority of cases, stage III dystrophy (rabbits readily pushed off their feet) had persisted for several days, the animals were cured with a single dose of d, l-α-tocopherol† in ethyl laurate solution. This procedure was repeated during each attack of the disease. Five to 15 mg of α-tocopherol were used to cure the first 3 attacks, and 20 to 40 mg of the vitamin for subsequent attacks.

From 4 to 6 attacks of severe dystrophy were thus produced and cured in experimental periods of from 16 to 32 weeks. As the number of attacks increased, the disease tended to run a somewhat longer course before reaching a dangerously acute stage. Two typical growth curves are shown in Fig. 1. The response to α-tocopherol was typical[2, 5] in all cases with the exception of the final attack in one animal to be described below. The high urinary creatine promptly fell to a normal level of 10 mg or less per day,

[2] Mackenzie, C. G., and McCollum, E. V., *J. Nutrition*, 1940, **19**, 345.

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

[4] Goettsch, M., and Pappenheimer, A. M., *J. Nutrition*, 1941, **21**, Proc. Am. Inst. Nutr., p. 7.

† Supplied by Merck and Company, Inc.

[5] Mackenzie, C. G., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 642.

growth was resumed, appetite restored, and the physical symptoms disappeared. There was no decline in the rapidity of response to vitamin E with successive attacks, and the duration of the growth response following each treatment was roughly proportional to the dose of α-tocopherol administered.

Rabbits Nos. 218 and 220 were allowed to succumb to their 4th and 5th attacks of dystrophy at 23 and 19 weeks respectively. Rabbit No. 222 died with diarrhea during its 5th attack 2 days after receiving α-tocopherol. The remaining 4 animals were subjected to 4, 5, or 6 attacks of the disease, and were then given 10 mg of α-tocopherol daily until they were sacrificed 2 to 4 weeks later. The response to this comparatively high level of α-tocopherol was excellent with one exception. Although vitamin E therapy produced a prompt drop in the creatine excretion of rabbit No. 225 from a level of 45 to 20 mg daily, and an immediate growth response as shown in Fig. 1, the daily creatine excretion still averaged 20 mg after 3 weeks of treatment. The α-tocopherol was then increased to 20 mg daily, and the creatine fell to 10 mg in 3 days. In all of the other attacks the daily creatine excretion fell to 10 mg or less 4 or 5 days after therapy was initiated.

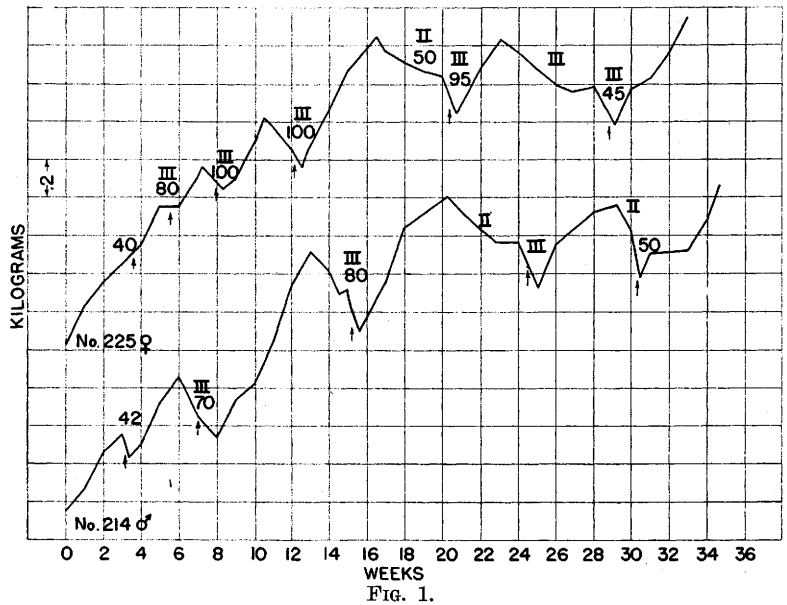

FIG. 1.

Growth curves of young rabbits subjected to repeated attacks of dystrophy. The administration of α-tocopherol is indicated by arrows. The daily creatine excretion and the stages of dystrophy observed during the attacks are shown by the Arabic and Roman numerals above the growth curves.

Sections were prepared from the vastus lateralis, biceps femoris, and ventricles of the hearts of all of the animals, and in the case of the males from the epididymides and testes. All sections were stained with hematoxylin and eosin, and in addition those from the thigh muscles were stained with Van Gieson's connective tissue stain. The thigh muscles from the 3 rabbits dying with dystrophy showed severe hyalinization and necrosis, and an increase in cellular elements as described by Goettsch and Pappenheimer.[1] Connective tissue and fat were definitely increased in the muscle sections taken from these animals. On the other hand, the thigh muscles of the 4 rabbits treated with α-tocopherol for from 2 to 4 weeks (following their last attack of dystrophy) were devoid of hyalinization and necrosis, and contained no accumulations of cellular elements. While the amounts of connective tissue and fat were apparently less than those observed in the muscles of the 3 dystrophic rabbits, this may have been due entirely to a relatively greater volume of muscle tissue in the cured animals, for the former elements were still readily discernible. The number of attacks of dystrophy, the final treatment, and the degree of muscle hyalinization and necrosis are given in Table I.

Histological examination of the ventricles revealed no myocardial lesions with the exception of several accumulations of cells with large, pale, elliptical or irregular nuclei in the left ventricle of 2 animals (Nos. 218 and 220) dying with dystrophy. Morphologically these lesions resemble those described by Miller[6] in normal rabbits.

Five of the 7 animals, including the 3 dying with dystrophy, were males. The epididymides of these 5 rabbits contained an abundance of sperm, and very few germ cells (no more than are commonly

TABLE I.

Effect of Alpha-tocopherol on Muscle Lesions in Dystrophic Rabbits Previously Subjected to Repeated Attacks of the Disease.

Rabbit No.	Sex	Initial wt, kg	No. of attacks	Final attack, wks	Final wt, kg	Final treatment	Microscopic muscle lesions
218	♂	.34	4	23	1.5	Died	+++
220	♂	.60	5	19	1.7	"	+++
222	♂	.62	5	16	2.2	"	+++
227	♂	.56	6	32	2.4	α-t* 2 wks	—
215	♀	.36	5	31	2.3	α-t 3 "	—
214	♂	.35	5	31	2.1	α-t 4 "	—
225	♀	.64	6	29	2.4	α-t 4 "	—

*d,l,-α-tocopherol.

[6] Miller, C. P., *J. Exp. Med.*, 1924, **40**, 543.

found in our normal animals). Although not abundant, sperm were found in many tubules of the testes of all 5 rabbits. They were more plentiful in the cured animals than in those dying with dystrophy. Aside from a slight sloughing of germ cells in the testes of rabbit No. 218, which died with dystrophy, and in the testes of rabbit No. 227, which was cured of the disease, the germinal epithelium of all animals was normal. This confirms and extends our recent finding[7] that widespread muscle lesions occur in vitamin E-deficient rabbits in the absence of testicular degeneration.

In this experiment the dystrophic rabbits developed no symptoms other than those referable to changes in the skeletal muscles. Furthermore, the remarkable stimulus given to muscle repair by α-tocopherol therapy was not lost even after 6 attacks of the disease.

Conclusions. As many as 6 successive attacks of nutritional muscular dystrophy have been produced and cured in rabbits. Continued α-tocopherol therapy following the last attack resulted in the complete repair of hyalinization and necrosis of the thigh muscles. Testicular degeneration was not observed.

13550 P

Production of Subcutaneous Sarcoma by Azo Dye and the Influence Thereon of Liver Feeding.

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Interest in the liver tumors produced by azo dyes has recently been directed chiefly along two lines, to wit: (a) studies of the inhibition of carcinogenesis by accessory food substances, particularly the demonstration that extracts of liver or yeast will protect rats against the butter-yellow liver cancer,[1] and (b) the apparent difference in biological activity between the azo compounds and the polynuclear hydrocarbons which might be dependent upon the presence

[7] Mackenzie, C. G., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 148.

* This work was carried out in part under a fellowship grant from the Commonwealth Fund.

[1] Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 3.