curvature was noticed by Solandt, who was unable to account for this divergence from theory.

Comparison of the electrotonic effects which have been described with those obtained by Nivet[4] for chronaxie and rheobase indicates a possible relationship between λ and k which is contrary to the suggestion of their independence made by Hill and Solandt but which is not the simple direct one insisted upon by the Lapicques.[5]

Confirmation of the effect of electrotonus on λ is seen (1) in the observation by Parrack[6] that accommodation at the anode is smaller than at the cathode, (2) in the decline of excitability following the initial rise during the passage of a linearly increasing current (Fabre[7]) instead of a rise in excitability to a maximum which should theoretically be maintained, and (3) in the decrease of "Einschleichzeit" (which Hill has shown is related to λ) obtained by Schriever[8] with catelectrotonus.

11366

Occurrence of Tremors and Incoördination in Vitamin E-Deficient Adult Rats.

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Paralysis in adult rats grown and maintained on a vitamin E-deficient diet was first described in detail by Ringsted[1] and later by Burr, Brown and Moseley.[2] Einarson and Ringsted[3] reported degenerative changes in the central nervous system and voluntary muscles, that were prevented but not cured by wheat germ oil. The

4 Nivet, M., *C. R. Soc. Biol.*, 1934, **116**, 1013; *Ibid.*, 1939, **131**, 262.

5 Lapicque, L., and M., *C. R. Soc. Biol.*, 1937, **125**, 260; *Ibid.*, 1938, **129**, 724.

6 Parrack, H. O., *Am. J. Physiol.*, 1939, **126**, 597; *Proc. Am. Physiol. Soc.*, 52nd Annual Meeting, 1940, p. 142.

7 Fabre, P., *C. R. Soc. Biol.*, 1934, **116**, 1065.

8 Schriever, H., *Zeitschr. f. Biol.*, 1932, **93**, 123.

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

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

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.

muscle pathology has also been described by Evans, Emerson and Telford.[4] We have previously reported[5] the prevention of gross symptoms with a vitamin E concentrate, and Knowlton, Hines and Brinkhous[6] have demonstrated that alpha-tocopherol acetate will prevent or cure the muscle changes occurring prior to the appearance of gross symptoms.

This paper concerns the production of paralysis within 8 to 10 months in rats obtained from a stock receiving ample amounts of vitamin E, the symptomatology of these animals, and their response to a vitamin E concentrate.

Four male and 4 female rats weighing 35 g were placed on a highly purified vitamin E-deficient diet containing but 0.0056% of non-vitamin lipids.[5] One female died at 15 weeks and one at 29 weeks of unknown causes. At 32 to 40 weeks the remaining animals developed the first stage of paralysis as manifested by a spreading of the hind legs and a marked lowering of the posterior abdominal region while walking. The weekly administration of 40 mg of a vitamin E concentrate,[7] possessing antisterility activity in a single 3 mg dose, to one of the female rats showing the first signs of paralysis prevented the development of further symptoms during the remaining 37 weeks of the experiment.

By 45 to 50 weeks the untreated animals showed the second stage of paralysis characterized by extreme abduction of the hind legs, which were now practically useless for locomotion. In another 10 to 12 weeks the disease had become so severe that in walking the hind quarters were dragged along the floor with both legs swinging from side to side. This, the third stage of paralysis, had not developed in the untreated female by the 73rd week of the experiment.

Several weeks after the second stage of paralysis the untreated animals, particularly the males, developed tremors and incoördination of the forelegs and head. So severe did these symptoms become that when eating, the rats were unable to maintain their fore feet in one position in the low food pans. The continuous jerking of the head rendered unsuccessful many of the attempts to obtain a mouthful of food. The tremors, which were most marked when the

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

[5] Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *Biochem. J.*, 1939, **33**, 935.

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

[7] Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *Pub. Health Rep.*, U. S. P. H. S., 1938, **53**, 1779.

animals moved, were intensified by a shrill note from an air hose. This stimulus caused 3 of the animals with stage 2 paralysis to run frantically around the cage for 20 to 30 seconds. The rear legs were used during this remarkable outburst of activity which terminated in collapse without convulsions or loss of consciousness.

From 3 to 10 weeks after the appearance of the first stage of paralysis the weights of the male rats (330 to 380 g) began to decline. At the end of 16 to 20 weeks they had lost 60 to 100 g. A representative weight curve is shown in Fig. 1.

Attempts to cure the paralysis in 2 of the males through the administration of 40 mg of the vitamin E concentrate per week for 16 and 20 week periods were unsuccessful. However, the progress of the neuro-muscular symptoms was definitely arrested and a growth response was elicited (Fig. 1). Doubling the carotene or methyl linolate intake or supplementing with 0.6 g of ether-extracted yeast daily failed to retard the development of symptoms or the decline in weight.

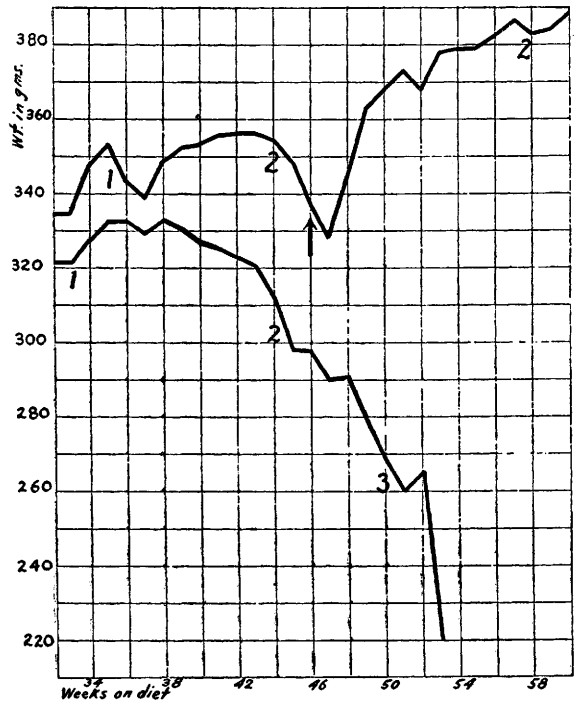

FIG. 1.

Weight curves of adult male rats reared and maintained on a low-fat vitamin E-deficient diet. Numerals indicate stage of paralysis. ↑ indicates the addition to the diet of a vitamin E concentrate.

Microscopic examination of the thigh muscles revealed lesions similar to those described by other workers.[3, 4, 6] These lesions were not so extensive as those occurring in young rabbits with severe nutritional muscular dystrophy, a condition cured by vitamin E.[8]

Summary. Rats grown and maintained on a highly purified vitamin E-deficient diet developed paralysis of the rear legs accompanied by tremors and incoördination of the fore legs and head. Although cures could not be obtained, the administration of a vitamin E concentrate arrested the development of these symptoms and stimulated growth.

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A Test Proposed to Measure Vitamin B_1 Saturation in Humans.

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A test designed to measure the individual patient's saturation with respect to Vitamin B_1 would have a wide clinical application. Experiments have been carried out in an attempt to develop such a procedure. The validity of such a test depends upon, among other things, the accuracy of the method of assay for the Vitamin B_1. The assay methods fall into two categories—chemical and biological. The chemical methods on the whole are specifically for pure thiamin. Among the biological methods the Schultz, Atkins and Fry technic which employs the rate of fermentation of glucose by a yeast is the most suitable for clinical investigation. This method measures not only the thiamin but the pyrimidines as well. The pyrimidines present in the urine may be considered for practical purposes as originating from the members of the B complex. The actual test in its present form employed in this laboratory is summarized as follows:

Patients were injected with 1 mg of thiamin hydrochloride intramuscularly in the fasting state. The urine was collected for a 4-hour period following the injection. The Vitamin B_1 activity of this collected urine was assayed by means of the Schultz, Atkins and Fry yeast fermentation method. Fig. 1 shows the tabulation of the

8 Mackenzie, C. G., and McCollum, E. V., *J. Nutr.*, 1940, **19**, 345.