D_2 or D_3.

The toxicities of the 3 preparations do not completely correlate with their ability to elevate serum calcium. In the case of vitamins D_2 and D_3 the correlation is as satisfactory as can be expected from the experimental procedures: an equivalent toxic dose of vitamin D_2 is 57% greater, and an equivalent hypercalcemic dose 54% greater than that of vitamin D_3. However, if the toxicity of dihydrotachysterol depended on its hypercalcemic effect alone, one would expect the equivalent toxic dose (as determined experimentally) to be about 1.6 mg/kg/day instead of 1 mg/kg/day. This might have been anticipated since dihydrotachysterol as marketed is a mixture and its physiological action may be the summation of the effects of several substances.[10] It is difficult to conceive of crystalline materials such as vitamins D_2 and D_3 containing a hypercalcemic and an antirachitic principle distinct from each other. Correll and Wise,[11] using vitamin D from cod liver oil, were able to produce toxic effects in chicks without raising the blood calcium. On the other hand, toxic doses of irradiated ergosterol and of dihydrotachysterol did elevate the serum calcium.

The survival times reported must be considered as approximate since individual animals or groups of animals are evidently rather variable in their responses from time to time, due to factors beyond control. For example, in 2 experiments with vitamin D_2 the average survival times were 33 and 42 days although in the latter case the daily dose was 20% larger. Attention is also called to the fact that a small decrease in dose level may result in a disproportionate lengthening of the survival time. Thus, in Experiment B a decrease of 25% in the daily dose more than doubled the survival time. If the dose is not sufficiently large to kill the animals within the first three weeks, they appear to develop a tolerance which may permit them to survive for a considerable period of time. For this reason it is felt that the median survival times may be more significant than the averages.

Summary. For the albino rat, medicated daily until death, crystalline vitamin D_3 is about 55%, and commercial dihydrotachysterol about 260% more toxic than crystalline vitamin D_2 (based on the actual weights of the active principles). So far as vitamins D_2 and D_3 are concerned, the toxicities correlate very well with the hypercalcemic effects of the preparations. However, in the case of dihydrotachysterol the toxicity and ability to elevate serum calcium are not in that same proportion. In view of the difference in response of different species, these considerations do not necessarily apply to any species other than the albino rat.

[10] Werder, F. von, *Z. physiol. Chem.*, 1939, **260**, 119.

[11] Correll, J. T., and Wise, E. C., *J. Nutrition*, 1943, **26**, 641.

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Neuromuscular Transmission in Parathyroid Tetany.*

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Calcium is known to be an important factor in the transmission of nerve impulses. It has been shown that in its absence there is a failure of the liberation of acetylcholine at the synapse which otherwise occurs on stimulation of the nerve.[1] In view of this it was considered interesting to study neuromuscular transmission during hypocalcemia produced

* This work was performed with the aid of a grant from the Rockefeller Foundation.

[1] Harvey, A. M., and MacIntosh, F. C., *J. Physiol.*, 1939-40, **97**, 408.

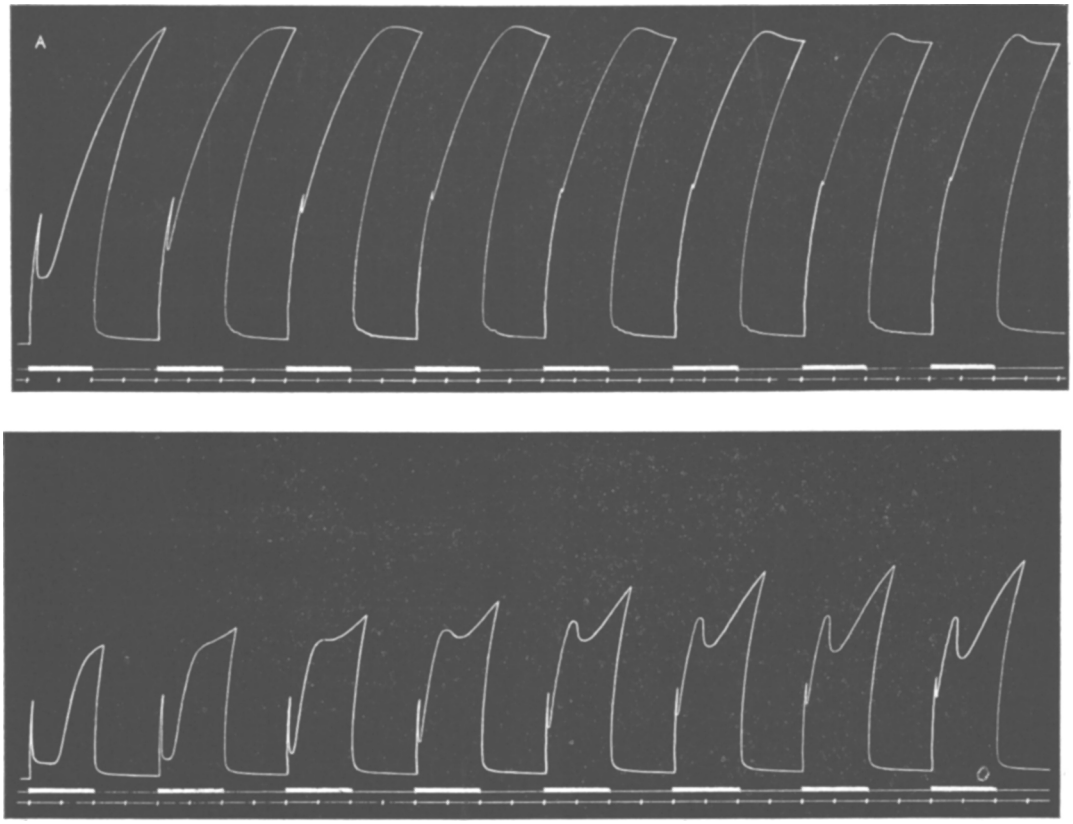

FIG. 1.

Effects of thyroparathyroidectomy in the stages of neuromuscular transmission to repeated stimuli at high frequency, and its normalization after $CaCl_2$ injection.

Cat of 2.5 kg. Anesthesia with nembutal. Thyroparathyroidectomy performed 4 days before. Just before the experiment the animal showed symptoms of tetany. Soleus. Indirect stimulation at 500 per sec. The record is continuous, the stimuli being applied repeatedly for 10 sec., with rest periods of 10 sec.

A. (Top) Before $CaCl_2$ injection. Blood calcium: 6.7 mg%.

B. (Bottom) 30 minutes after 5 cc of 2% $CaCl_2$ injection. Blood calcium: 12.5 mg%.

by removal of the parathyroids.

Method. The thyroids and parathyroids were removed aseptically in 31 cats. Two to 4 days later, when the animals presented clear signs of tetany, they were anesthetized with dial or nembutal. The bones of the legs were fixed by drills, records were taken from the soleus muscle, and the nerve was stimulated with condenser discharges whose frequency was controlled electronically. In 16 animals the blood calcium was determined by the classical method of Kramer and Tisdall. The level of blood calcium shifted between 4.6 mg % and 7.6 mg % (the value of normal cats was about 12 mg %). The effects of thyroidectomy were prevented by giving, in 6 out of the 31 animals, 0.10 g of thyroid extract daily, beginning on the day of operation. In 8 out of the 31 animals, after being tested the neuromuscular transmission and the blood calcium value .2 cc per kilo of a 2% solution of $CaCl_2$ was injected intravenously. And a new test of the neuromuscular transmission was done. The blood calcium 30 minutes after the injection was found to a level between 9.4 mg % and 12.6 mg %.

Results. Indirect stimulation of the muscle produces contractions whose characteristics vary according to the frequency of stimulation. We have fully confirmed the stages of neuromuscular transmission which have been described,[2,3,4] namely, stages 1, 2, 3a, 3b, 3c, 4,

and 5. These stages change during parathyroid tetany. Not all of them were uniformly altered in 31 animals studied. Rosenblueth and Cannon[4] showed that repeated periods of 10 to 15 sec. indirect stimulation of the gastrocnemius-soleus at a frequency of 500 per sec., with pauses of 10 to 15 sec., made increasingly clear the series of stages, 1, 2, 3a, and 3b, while the tension developed increased. This phenomenon, confirmed many times in this department, is regularly changed in hypocalcemic animals. Stages 3b and 3c do not appear; the stages do not become more distinct, and the tension does not increase. On the contrary, the muscle shows a marked tendency to fatigue. This always occurred, even when the first period of stimulation produced a response with stages 1, 2, 3a, and 3b.

In order of importance, the other changes are as follows: loss of the characteristic responses at frequencies of stimulation of 500, 400, 300, 200, 100, and 60 per sec. Frequently stage 3c as normally seen with 300 or 150 per sec. does not appear. Marked neuromuscular fatigue often occurs even at a frequency of 60 per sec.

In the group of animals in which the blood calcium was increased to normal values by injecting $CaCl_2$ the synaptic behavior resembles very much that of the normal cats.

Comment. These changes cannot be attributed to the removal of the thyroid glands, since the operated animal which received thyroid extract showed the same changes.

The abnormal synaptic reaction of the parathyroidectomized animals is probably due to the low value of blood calcium during tetany as it is normalized by increasing the blood calcium to normal values.

It is accepted that the chemical transmitter of the neuromuscular synapse is acetylcholine. Harvey and MacIntosh[1] showed that the calcium ion is fundamental for cholinergic transmission, since without this ion, acetylcholine is not released.

The marked fatigability of the neuromuscular synapse in animals with parathyroid tetany may be explained by the work of these authors. The decrease of calcium ion would hinder the release of acetylcholine and thus lead to the failure of transmission.

Conclusions and Summary. The responses of the soleus muscle of the cat on stimulation of its nerve at varying frequencies were studied in animals rendered hypocalcemic by parathyroidectomy. The responses at certain frequencies were markedly altered. These alterations were normalized by calcium administration.

[2] Rosenblueth, A., and Morison, R. S., *Am. J. Physiol.*, 1937, **119**, 236.

[3] Rosenblueth, A., and Luco, J. B., *Am. J. Physiol.*, 1939, **126**, 39.

[4] Rosenblueth, A., and Cannon, W. B., *Am. J. Physiol.*, 1940, **130**, 205.

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Strain Differences in the Choline Requirement of Rats.*

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In earlier work on choline in this laboratory, 2 strains of rats were used. Rats from these 2 strains appeared to differ in their requirement for choline. In order to determine whether these differences were genetic in character, females from the 2 strains were selected and bred to their respective littermates. The progeny were designated as the F_2 generation. It was previously reported[1] that the F_2 generation of the 2 strains showed significant dif-

* Published with the approval of the Director of the Alabama Agricultural Experiment Station.

[1] Engel, R. W., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 281.