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Effect of Blood of Myasthenia Gravis Patients on Acetylcholine Sensitivity of Prostigminized Frog and Leech Muscle.*

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The idea of a circulating toxic factor in the blood of patients with myasthenia gravis as set forth by Bramwell and Campbell,[1] and Oppenheim[5] received confirmation in a clinical experiment by Walker.[7] She reported a case of myasthenia gravis in which, by cutting off the circulation in both arms and maximally exercising the arms, she was able to induce drooping of the eyelids and then generalized weakness by merely releasing the tourniquets. This observation suggested to us the possibility of demonstrating in the blood of patients with myasthenia gravis a factor which would depress the acetylcholine sensitivity of prostigminized leech dorsal and frog rectus muscles. In a search of the literature we could find no such previous attempt.

Methods. The dorsal muscle of the leech and the rectus of the frog were each set up in a test tube and the response to acetylcholine measured according to the method of Chang and Gaddum,[2] with slight modifications. The muscle was immersed in 25 cc of frog Ringer's

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1 Bramwell, H. and Campbell, E., *Brain*, 1900, **23**, 277.

2 Chang, H. C. and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

3 Harvey, A. M., and Lilienthal, J. L., Jr., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 566.

4 McEachern, D., *Medicine*, 1943, **22**, 1.

5 Oppenheim, H., *die myasthenische Paralyse*, Berlin, S. Karger, 1901.

6 Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 224.

7 Walker, M. B., *Proc. Roy. Soc. Med.*, 1938, **31**, 722.

solution, prostigmine added to a concentration of 2.5×10^{-5}, and then acetylcholine in concentration varying from 1×10^{-7} to 2.5×10^{-7} depending on the reactivity of the individual muscle. This was in each case the control contraction, to which we compared the contraction obtained under the same concentrations of prostigmine and acetylcholine in the presence of 1 cc of different blood samples. Nine normal subjects and 3 patients with myasthenia gravis were the blood donors. In all cases the blood was drawn from a deep antecubital vein, with the arm at rest and again after the forearm muscles had been exercised to a state of fatigue with the tourniquet applied just above the antecubital fossa. In 4 experiments the blood was drawn after the tourniquet had been loosened. Blood was allowed to stand for 6 hours at 0°C before being used; it was then centrifuged and the serum used. In all experiments save one the serum was clear; in this experiment the blood was purposely hemolyzed by drawing with a wet syringe, and the serum used contained many hemolyzed red blood cells. The Ringer's solution was always brought to a pH of 7, and air constantly bubbled through the solution. All materials were at room temperature when used.

Results. In the presence of clear serum. Neither the normal serum nor the serum from patients with myasthenia gravis had any effect on either the prostigminized frog or leech muscle before the addition of acetylcholine. The height and the slope of the contraction to acetylcholine did not appreciably differ from that obtained in the control (a) whether there was serum from normal subjects or patients with myasthenia gravis present, (b) whether the serum was obtained before exercise or after exercise, or (c) whether the blood was drawn with the tourniquet applied or with the tourniquet loosened.

In the presence of serum containing hemolyzed red blood cells. Both the height and the slope of contraction obtained in the presence of serum containing hemolyzed red blood cells were greater than the control, but there was no difference between that obtained with normal blood and that with blood from patients with myasthenia gravis.

Discussion. Although the literature has abundant references to the possible presence of a circulating toxic factor in myasthenia gravis, laboratory confirmation has been lacking. By injecting the freshly drawn venous blood of myasthenic patients, McEachern[4] attempted unsuccessfully to demonstrate a "curariform" effect in cats. Other authors such as Harvey and Lilienthal[3] spoke of a curare-like substance in patients with myasthenia gravis. Our work shows that in the six-hour-old sera of myasthenic patients there is no curare-like substance, as this type of blood did not interfere with the acetylcholine sensitivity of prostigminized leech dorsal or frog rectus muscle. Any possibility of demonstrating the presence of this factor in red cells was obscured by the stimulating effects of potassium and lecithin released from hemolyzed red cells. One is not justified, however, in denying the presence of a toxic factor outright, for the following three reasons. First, the methods may have been inadequate to isolate, preserve, and demonstrate this substance. Second, is the fact that we used frog and leech muscle, presumably from healthy animals. There is a difference between the presumably normal frog and leech muscles and the easily-fatigued muscles of a myasthenia gravis patient. Moreover, as Walker showed in her experiment, the muscles of a myasthenia gravis patient are not all affected to the same extent necessarily. These facts suggest that if there is a circulating factor, it can produce its effects only upon a susceptible muscle. Finally our experiment was limited to demonstrating the effect of myasthenia gravis serum upon the action of previously synthesized acetyl choline. It is therefore possible that the circulating factor affects not the action but the synthesis of acetyl choline. Torda and Wolff[6] have recently shown that frog brain incubated in the presence of serum from patients with myasthenia gravis synthesizes less acetylcholine than it does in the presence of serum from normal people or patients with other debilitating diseases. They suggest that the defect in myasthenia gravis is in the synthesis of adequate amounts of acetylcholine to maintain muscular contraction, and that the faulty synthesis may be due to several factors, one of

which is the presence of inhibitor substances.

Summary. Attempts were made to experimentally demonstrate a circulating factor in the blood of normal people and of patients with myasthenia gravis before and after exercise to a fatigued state, which would depress the response of prostigminized frog and leech muscle to acetylcholine. Before the addition of acetylcholine to the perfusion fluid it was found that neither normal serum nor serum from myasthenic donors had any effect upon the prostigminized leech or frog muscle. Serum, whether from a normal or myasthenic donor, had no significant effect upon the acetylcholine sensitivity of either frog or leech muscle. Although hemolyzed blood from both normal and myasthenic donors increased the acetylcholine sensitivity of prostigminized leech and frog muscle, there was no quantitative difference in the effect of the two types of hemolyzed blood. A circulating factor depressing the acetylcholine sensitivity of frog and leech muscle could not be demonstrated with our methods in myasthenic patients. Some reasons for the non-detection of this hypothetical factor and some theories as to the etiology of myasthenia gravis were discussed.

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Penicillin: Miscellaneous Pharmacological Results.*

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Penicillin has surprised all investigators in its non-toxicity. Various phases of acute and chronic toxicity, and *in vitro* activity, are reported in this paper. The results corroborate those of van Dyke[1] in the paucity of adverse pharmacodynamic effects.

1. *Effect on Excised Organs.* The contractions of strips of pregnant guinea pig uterus in an excised-organ bath were not affected by 1, 2 or 10 cc of a solution containing 1500 u of penicillin per cc. Similarly, several strips of rabbit abdominal aorta were not influenced by from 1 to 5 cc of the same penicillin, while 1 cc of a 1:5 million dilution of epinephrine produced contraction.

2. *Vomiting in Pigeons.* Two pigeons were given penicillin intravenously, 4000 and 7500 u respectively in 5 cc of water, without the production of vomiting, or other symptoms, in 30 minutes.

3. *Anticatalytic Effect.* Two hundred and fifty individual tests were made of the *in vitro* effect of penicillin on the liberation of oxygen from hydrogen peroxide by a catalyst.[2] The results were similar, irrespective of whether Fuller's earth or collargol served as the catalytic agent. Penicillin from 6 different sources, in high concentrations, invariably inhibited the liberation of oxygen, while in low concentrations (50 units or less in the system), it produced no effect. It was at first hoped that

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[1] van Dyke, H. B., PROC. SOC. EXP. BIOL. MED., 1944, **56**, 212.

[2] Hanzlik, P. J., and Cutting, W. C., *Science*, 1943, **98**, 389.