sulin injection. The latter was related to an unexplained increase in insulin sensitivity in the reactors. No change in sensitivity occurred in the non-reactors.

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Received September 4, 1953. P.S.E.B.M., 1953, v84.

Botulinum Toxin and the Motor End Plate.* (20570)

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(Introduced by J. B. Bateman.)
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The extreme toxicity of botulinum toxin has long been known to be due to its paralytic action on skeletal muscle. Guyton and MacDonald(1) confirmed and extended the observation that peripheral nerve conduction in toxin-paralyzed rabbits and guinea pigs was unimpaired. They also pointed out that the skeletal muscles of such organisms could contract if stimulated directly by electric shock. It seemed evident, therefore, that the toxin exerts its effect upon the neuromuscular junction by blocking conduction (a) in the terminal fibers or (b) in the motor end plate (perhaps by inhibiting the release of acetyl choline from the end plate). Burgen, Dickens, and Zatman(2) demonstrated that the amount of acetyl choline released by a nearly toxin-paralyzed muscle was greatly decreased; it was less than 15% of the original output. Brooks(3), using cat and guinea pig muscle, has presented preliminary evidence in favor of the blocking hypothesis (a). The present investigation was undertaken in the hope of adducing more evidence for the site of action of the toxin.

Materials and methods. The preparation used was the sartorius-sartorius nerve of the frog, *Rana pipiens*. The muscle was mounted in a lucite chamber and bathed with Frog Ringers. A water jacket maintained the temperature at 25°C. The muscle was stimulated indirectly via the sartorius nerve with short (0.1-0.3 msec) pulses of 1.5 V amplitude obtained from a Grass S4A stimulator. The electric reactions of the muscle were detected with a platinum-iridium, glass-coated microelectrode, led off through a single stage cathode follower amplifier mounted directly on the electrode. The signal was then carried through a differential preamplifier with voltage gain of 1000 and pass band of 0.2-40 KC and thence to a DC coupled amplifier with pass band of 0-100 KC. The signal was displayed on one gun of a 3-gun CRO. Time signals were injected on the trace of another gun, operated from a common sweep generator. The time signal generator was calibrated against a crystal controlled pulse generator. End plate potentials were located by probing when the muscle was nearly paralyzed with toxin or by poisoning the muscle with a 1 μM solution of curare

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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and moving the microelectrode until an end plate was found. After the muscle recovered from curare, type A botulinum toxin was applied, the most convenient concentration being 1.25 x 10^6 mouse LD_{50}/ml; this concentration poisoned the muscle in 3-4 hours. In several experiments check assays of the diluted stock toxin solution were made on mice.

Results. For a period of about 15 minutes after botulinum poisoning becomes evident (failure of a previously supramaximal stimulus to elicit a muscle action potential) 2 such stimuli delivered in close succession will cause a muscle spike (and contraction). This is the familiar summation of subliminal stimuli. If the toxin is paralyzing by blocking conduction in the terminal nerve fibers, then summation may be explained by the first stimulus electrotonically increasing the excitability of the nerve on the distal side of the block and the second stimulus "jumping" the block, which results in a contraction of the muscle(4). It is obvious that only the stimulus artifact would be recorded in response to the first stimulus and in response to the second, a stimulus artifact followed by a muscle action potential. If, however, the action of the toxin is to depress the end plate mechanism, one might expect to observe a small end plate potential (insufficient to trigger the muscle spike) after the first stimulus. Summation could then be explained in terms of the acetyl choline theory. The first stimulus causes release of a subthreshold quantity of acetyl choline which adds to that released by the second stimulus and contraction follows.

Our observations lend support to the second hypothesis. In 6 experiments we consistently observed end plate potentials following the first "unsuccessful" stimulus (Fig. 1a). If a second identical stimulus is applied within 4-18 msec, summation occurs and a muscle action potential is observed (Fig. 1b). If the 2 stimuli are of the order of 20 msec apart, no summation occurs and 2 end plate potentials are recorded (Fig. 1c). It is possible of course that the terminal fibrils are poisoned secondarily, after the end plates have been poisoned.

Burgen, Dickens, and Zatman(2) also have demonstrated that botulinum toxin affects

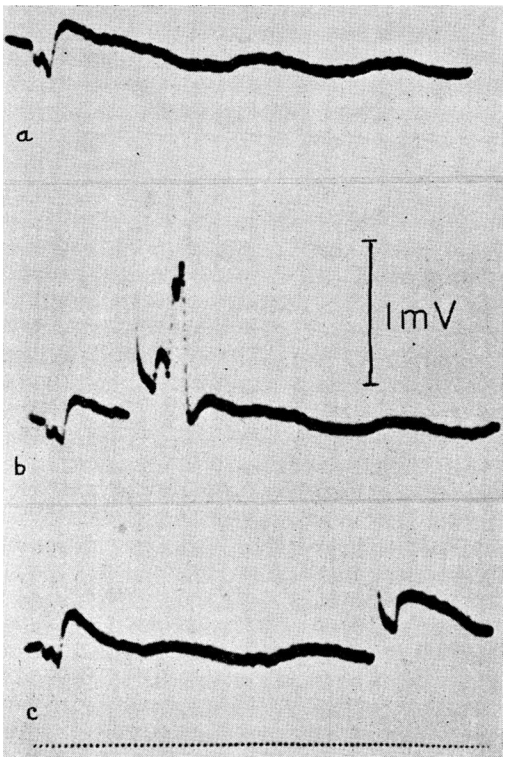

FIG. 1. Response of a partially paralyzed muscle to electric stimuli. (a) An end plate potential in response to a single stimulus. (b) Summation of end plate potentials in response to 2 stimuli 11 msec apart. (c) Two end plate potentials recorded in response to 2 stimuli 32 msec apart. Time: 0.28 msec marks.

neither the enzymes acetylating choline nor choline esterase and that added acetyl choline will cause a toxin-paralyzed muscle to contract. It is most probable, therefore, that the paralytic effect of the toxin is due to poisoning of the mechanism which releases acetyl choline.

Summary. The paralytic action of botulinum toxin is due primarily to a depression of the motor end plate mechanism and not to a blocking of the terminal nerve fibers. The mechanism, whereby acetyl choline is released, is probably inhibited.

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Received September 8, 1953. P.S.E.B.M., 1953, v84.