THE EFFECT OF NICOTINE ON THE GASTROCNEMIUS MUSCLE OF THE FROG.*

BY RUPERT A. CHITTICK.[1]

INTRODUCTION.

The value of nicotine as a means for the scientific study of muscle irritability and the relationship to nervous control was first established by Langley and Dickinson (1). At that time they believed that "the effect of nicotine upon the skeletal muscles of mammals appears to be entirely due to a stimulation of the central nervous system." Later work, however, carried out by Langley (2) demonstrated that the characteristic muscle twitching produced by nicotine was still in evidence when the nerves had been allowed to degenerate and that the muscle fibers assumed a characteristic "spindle form" when a solution of nicotine was applied directly to the muscle. This characteristic spindle formation, when dilute solutions of the alkaloid were used, was most in evidence at the myoneural junction but as the strength was increased the reaction gradually spread to more remote portions. For this reason Langley designated the action of nicotine as being upon certain "receptive substances" interposed between the nerve endings and the contractile portion of the muscle.

Further light was thrown upon the nature of these receptive substances by Lucas (3) by the use of optimal electric stimuli. He was able to show that the number of receptive substances varied in different muscles and those containing two or more varied in their irritability to the stimulus, depending upon the strength of the stimulus. In other words the receptive substances were scaled as to their sensitiveness to stimuli.

It has been shown (4) that the non-user of tobacco doing voluntary work has, during the morning, an increase in his ability to do work and a fall in power during the evening. It has been further demonstrated that "moderate smoking, although it may influence in diminishing the power to do muscular work, neither stops the morning rise nor, when done early in the evening, hinders the evening fall."

If the skeletal muscles were the only points of action of nicotine the hygienic as well as the moral problem of smoking would be greatly decreased. But it has been repeatedly demonstrated (1), (5), (6), that a more marked action occurs on the higher centers of the central nervous system and the ganglia of both the sympathetic and para-sympathetic systems. It is reasonable to suppose, then, the diminishing effect upon muscular work might be caused as a secondary effect of the primary nicotine nervous action.

It is desirable, then, to study the action of skeletal muscle under the influence of nicotine without the use of a stimulus that has been altered by passing through a depressed central nervous system.

METHOD.

The frog was chosen as the animal best suited for experimentation along this line because of its suceptibility to the action of nicotine and because of the ease

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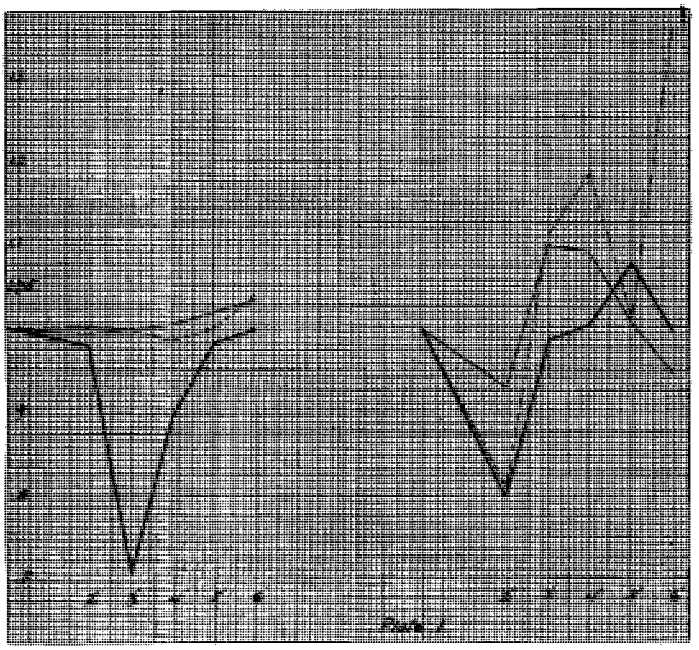
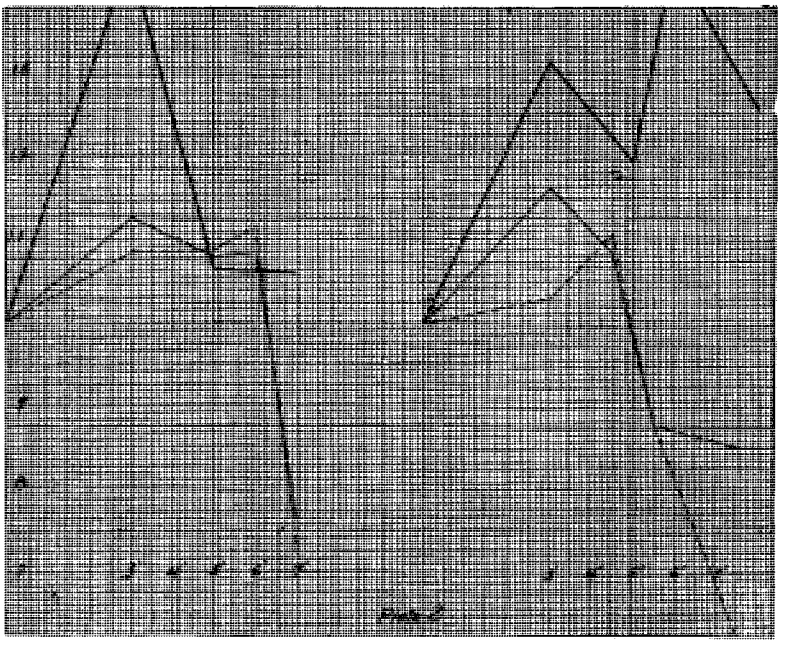
[1] College of Pharmacy of the University of Nebraska.



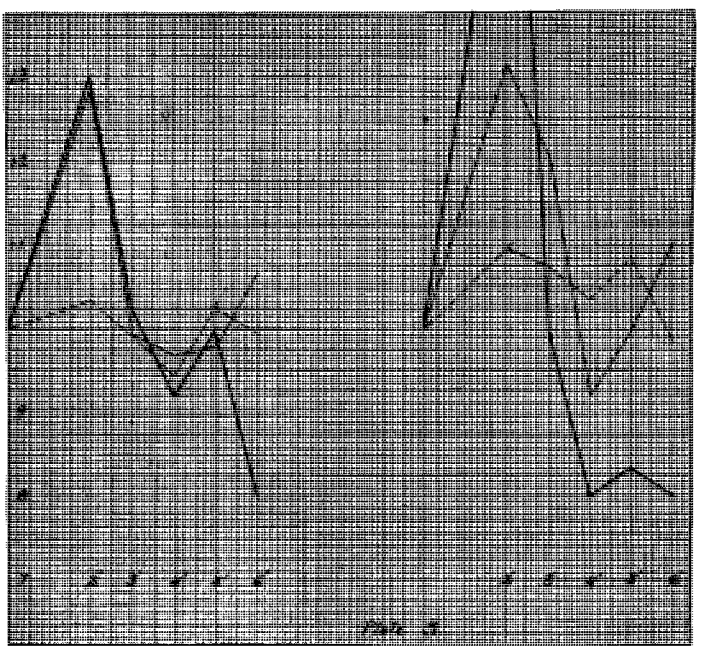
with which it can be handled. As for the method of procedure, two main objects were sought. First, a method by which each animal could be made its own control, and second, one that would keep the muscle under as nearly normal conditions as possible. In order to accomplish this, the following method of procedure was adopted:

For each experiment a healthy frog was selected. On being brought into the laboratory it was carefully weighed to the tenth of a gram and the brain and cord pithed. It was then placed on its back and an incision of one-half to three-fourths



of an inch in length made through the skin on the ventral surface of the thigh. The adductor magnus and sartorius muscles were then spread and the femur bone

exposed. This was cut far enough above the knee so as to be easily fastened in a muscle clamp. Care was taken, however, to avoid cutting main blood vessels and in case any marked hemorrhage occurred the animal was discarded from use in this experiment. A circle of skin was then cut from over the ankle and the tendon of Achilles freed. By inserting a bent pin through this tendon the classical nerve-muscle preparation was obtained with but slight injury to the normal circulation. The muscle was then fastened in the clamp and attached to a light muscle lever arranged to mark upon the smoked paper of a kymograph. In stimulating the muscle, a special electrode and boot were prepared so that the stimulus could be applied directly to the sciatic nerve. Single "make" and "break" induction shocks were used and a signal magnet included in the primary circuit. The stimuli were at all times submaximal, at times the "make" stimulus failed to cause a contraction. The magnification of the contraction remained constant throughout the study, as did the load lifted. The speed of the rotating cylinder was always taken with a tuning fork vibrating one hundred times a second. A series of normal make and break contractions were first taken from the right leg and then from the left. One one-hundredth of a cubic centimeter, per gram body weight, or its equivalent, of the nicotine solution was injected into the anterior lymph sac of the frog and allowed to act for two or three minutes after which the muscle was again placed in the clamps and a second series of contractions taken from each leg. The time of injection was recorded as well as the time at which the contraction of each leg was taken.



RESULTS.

For the sake of brevity the results are shown graphically. The abscissa shows the ratio of the nicotinized to the normal muscle while the ordinate refers to the number of minutes between the time at which the nicotine was injected and the time at which the contraction was recorded. In each plate the left-hand series refers to the make contractions, and the right-hand series to the break contractions. The broad line refers to the latent period, the fine line to the period of contraction (the

phase of shortening and of relaxation together), while the broken line refers to the height of the contraction. Plate 1 shows the results obtained from a solution of 0.00001% nicotine. Plate 2 refers to the 0.0001% solution and Plate 3 to the 0.001% solution.

When injected into the normal unpithed frog, the 0.00001% solution caused no visible outward effects save a short period of increased excitability. The two higher strengths did not show the stage of increased excitability but were markedly depressing from the first and maintained that condition for two or three hours.

The Latent Period.—Studying the graphs of the latent period, it will be noticed that for the weakest solution the latent period is first shortened and finally comes back to normal or longer than normal. In the next weakest solution the period is lengthened from the first though it finally returns to nearly normal. The next solution causes a similar lengthening of the latent period but the duration is much less and finally a second period of shortening occurs.

The Period of Contraction.—The time required for the shortening and relaxation of the muscle shows an almost constant tendency to be lengthened. In only one case (Plate 2) does it show any marked shortening.

The Height of Contraction.—The first effects of the nicotine seem to cause a heightened contraction followed by a depression of the contractility and then a second increase. It does not vary as greatly as the latent period but more than the contraction period.

INTERPRETATION OF RESULTS.

Following up Langley's idea of the action of nicotine upon the receptive substances of the muscle, we would expect a change in the susceptibility to external stimuli. As the receptive substance is stimulated by the alkaloid, the contractile portion responds by a quicker action. As the receptive substance is depressed, the contractile portion is correspondingly slower in coming into action. But since there are clearly two curves of stimulation and depression as shown by the latent period and the height of contraction, it would seem that there are at least two receptive substances in the gastrocnemius muscle and that they are affected in order of their sensitiveness. That is, the more sensitive substance is first stimulated and depressed and then the next lower receptive substance. As was stated in the introduction, Keith Lucas demonstrated the presence of receptive substances of varying sensitiveness by the use of optimal electric stimuli. It seems reasonable that although all may be acted upon simultaneously, the most sensitive would show its action first and as it lost in activity or the second gained in activity its action would gradually be displaced by that of the less sensitive receptive substance.

SUMMARY.

1. Nicotine causes at least two phases of shortening and lengthening of the latent period. 2. The time required for a muscular contraction is increased under nicotine. 3. The height of contraction shows two stages of increase and decrease under nicotine.

BIBLIOGRAPHY.

(1) Langley and Dickinson, *J. Physiol.*, VII, 265. (2) Langley, *Ibid.*, XLVII, 159. (3) Keith Lucas, *Ibid.*, XXXV, 103. (4) Vaughan Harley, *Ibid.*, XVI, 122. (5) Eggleston and Hatcher, *J. Pharm. Exp. Ther.*, VII, 225. (6) Pilcher and Sollman, *Ibid.*, VI, 325.