# THE POSITIVE INOTROPIC ACTION OF CYCLOPROPANE ON HUMAN INTERCOSTAL MUSCLE *IN VITRO*, AND ITS MODIFICATION BY *d*-TUBOCURARINE

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IN A RECENT paper by Watland *et al.* (1), it was noted that cyclopropane anesthesia produced a considerable increase in the height of contraction of the gastrocnemius of the intact rabbit in response to single shock stimulation of the sciatic nerve. It was also remarked that, when *d*-tubocurarine was administered during this period, a completely altered picture appeared.

Adriani, Martin, and Rovenstine (2) described the production of chromodacryorrhea ("red tears") in eserinized albino rats under cyclopropane anesthesia. This led them to the conclusion that cyclopropane induces the liberation of acetylcholine in mammalian tissues.

The experiment to be described was designed to investigate whether the positive inotropic action of cyclopropane would also be seen in strips of human intercostal muscle and whether its site of action could be established.

## METHODS

A strip of human intercostal muscle was obtained and attached to a platinum electrode holder as described previously (3). The preparation was then immersed in a bath containing 50 ml. of physiological medium modified from that suggested by Krebs and Henseleit (4). Its composition in m*M* was: $Na^+$, 145.0; $K^+$, 5.0; $Ca^{++}$, 1.3; $Mg^{++}$, 1.2; $Cl^-$, 126.0; $HCO_3^-$, 25.0, and $SO_4^{--}$, 1.2, with 200 mg. per cent glucose. The medium was equilibrated by bubbling a mixture of 5 per cent carbon dioxide and 95 per cent oxygen through it for half an hour. The contents of the bath were warmed to 37 C. and kept at that temperature by circulating warm water through a jacket surrounding the bath. The muscle was stimulated alternately, directly and indirectly through the nerve, at the rate of one stimulus every ten seconds. A clipped condenser discharge stimulator (3) provided the stimuli which were adjusted to a slightly supermaximal value. The muscle twitches that resulted from this electrical stimulation were recorded by a Statham strain gauge on an Offner dynograph. An anesthesia machine provided the means whereby mixtures of varying concentrations of cyclopropane in carbogen could be administered at any given time to the preparation in the bath. For the first part of this study, the nerve muscle preparation was gassed with 25 and 50 per cent

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cyclopropane. The second part was concerned with the modification of the positive inotropic action of cyclopropane by *d*-tubocurarine. The technique employed during this experiment is described with the results.

## Results

The results of the first part of this study are shown in figure 1 A and B, representing a concentration of cyclopropane in the gassing mixture of 25 and 50 per cent respectively. Figure 1A shows the dramatic rise in the twitch tension developed as a response to both direct and indirect stimulation (the smaller twitch is that evoked by indirect stimulation). The rise in tension begins immediately after the cyclopropane is switched into the gassing circuit and reaches a maximum of 2.5 times the control level in thirty minutes. The recovery from the effects of cyclopropane are equally rapid, the fall in

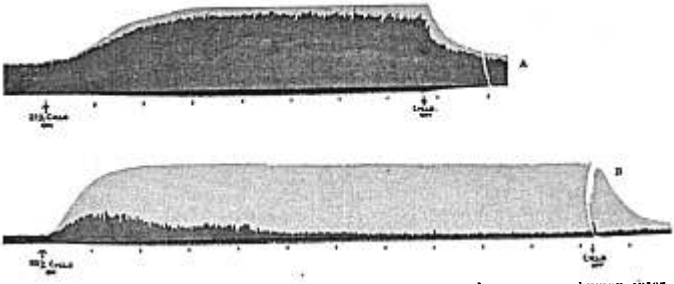

Fig. 1. (A) The positive inotropic effect of 25 per cent cyclopropane on human intercostal muscle. Time markings at 10 minute intervals. (B) Effect of 50 per cent cyclopropane in gassing mixture showing block to indirect stimulation which appears to be permanent. Time markings at 10 minute intervals.

the height of the twitch beginning as soon as the cyclopropane is switched off and the control level being reached in ten minutes.

Figure 1B, which is the record of the effect of 50 per cent cyclopropane on the twitch response, presents a more complex picture. The amplification in this record has been cut down to one-fourth that of the record in figure 1A, so that a deflection of 0.5 cm. of the recorder pen represents a tension of 2.5 Gm. on the arm of the strain gauge. As soon as the cyclopropane is switched on, the height of the response to direct stimulation is seen to rapidly attain a peak tension, nine times that of the control within twenty minutes, at which high level it remains for the duration of the experiment. The response to indirect stimulation also increases at first, reaching a peak tension about four times that of the control level in fifteen minutes. From this point on, however, the response to indirect stimulation steadily falls, reaches control

level at the end of one hour, and is half the height of the control level at one and one half hours. When the cyclopropane is switched off, the direct response falls abruptly, reaches control height in ten minutes, but the indirect response never recovers to reach its control height, probably because of some irreversible change due to gassing with large doses of cyclopropane over long periods of time.

The results of the second part of this study, which is concerned with the modification of the effect of the cyclopropane by *d*-tubocurarine, are shown in figure 2, A and B. Figure 2A represents the effect of introducing 30 per cent cyclopropane in the gassing circuit. The resulting record is seen to be similar to that shown in figure 1A. To obtain the record shown in figure 2B, the following procedure was employed:

After a short control record was obtained, 5 $\mu g$ *d*-tubocurarine chloride were added to the bathing medium. At the end of one hour, the record shows that a steady 20 per cent curare block had been achieved. These steady states of paralyses have been shown to occur

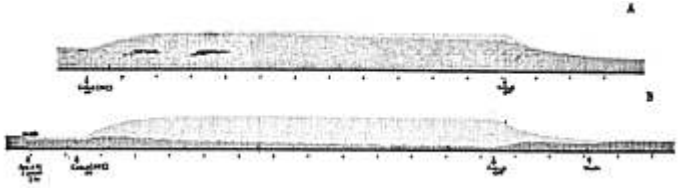

FIG. 2. (A) Positive inotropic effect of 30 per cent cyclopropane in gassing mixture. Time marked at 5 minute intervals. (B) Modification of the action of cyclopropane by *d*-tubocurarine. Time marked at 5 minute intervals.

after about 45 minutes of equilibration (5). Then, at the point shown, 30 per cent cyclopropane was switched into the gassing circuit. The response to direct stimulation began rising immediately and reached a high level, 2.7 times the control height in fifteen minutes. The response to indirect stimulation rose slightly during the first 10 minutes after the cyclopropane was started, but then gradually fell and at the end of one hour reached a low level, only 37.5 per cent of its height before cyclopropane was introduced into the gassing mixture. At this point, the cyclopropane was switched off. It is now seen that the height of the response to direct stimulation fell, while that due to indirect stimulation rose, to return to "precyclopropane, postcurare" levels in fifteen minutes. At this point, the *d*-tubocurarine was washed out of the bath with fresh Krebs saline, and within five minutes both responses have returned to pre-experimental levels, showing that all the changes that had taken place during this experiment were completely and rapidly reversible.

Attention is drawn to the remarkable similarity between the records

shown as figures 1B and 2B, the very important difference being that when prolonged gassing with a large dose of cyclopropane was employed, the changes were irreversible.

## Discussion

It has been shown by Watland *et al.* (1) that cyclopropane produces a marked increase in the strength of the muscle twitch in the rabbit and has been confirmed by the present study for the human. The fact that cyclopropane causes the liberation of acetylcholine from mammalian tissues (2) is too simple an explanation for this phenomenon, as it still cannot explain the peculiar results obtained by combining a "competitive blocking agent," such as *d*-tubocurarine, with an agent possessing positive inotropic properties.

It has been known for a long time that certain abnormal anions ($NO_3^-$, $Br^-$ and $I^-$) act upon frog muscle to cause an increase in the strength of contraction due to a variety of stimulating agents (6–12). That these anions increased the contractile response of skeletal muscle by a "sensitizing" action upon the excitatory membrane of the individual muscle fibers (11) appears to be a generally accepted theory.

Following Hill's concept of the active state (13, 14), the tension developed during a simple muscle twitch is much less than that developed during a tetanus because the active state during a twitch lasts for such a short time and decays so rapidly that it is impossible for full tension to be developed. This explains the finding of Watland *et al.* (1) that the height of tetanic contraction remains unaltered during cyclopropane anesthesia. We propose to look for some correlation between the peculiar action of cyclopropane on skeletal muscle and the similar properties exhibited by the abnormal anions mentioned. It may follow that if the similarity is great, the arguments advanced to explain the site and mechanism of one would apply to the other.

## Summary

The positive inotropic action of cyclopropane on human intercostal muscle has been demonstrated and described.

Cyclopropane in moderate doses greatly increases the magnitude of contractions of *in vitro* human skeletal muscle preparations. In large doses over long periods of time, or in the presence of very small doses of *d*-tubocurarine the positive inotropic action of cyclopropane is much modified. This modification consists of a gradually increasing depression of the contractions evoked by indirect stimulation even though the contractions evoked by directly stimulating the muscle remain markedly increased in size.

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