## 15501

# Effects of Ether and Curare on Neuromuscular Transmission.

WILLIAM SCHALLEK. (Introduced by Geo. H. Bishop.)
*From the Laboratory of Neurophysiology, Washington University Medical School, St. Louis, Mo.*

Anesthetists have noted that the amount of curare necessary to cause muscular relaxation is less in the presence of ether than of other anesthetics. Gross and Cullen[1] found that the contraction of the gastrocnemius muscle in response to electrical stimulation of its nerve is less in dogs anesthetized with ether than with other agents. They concluded that ether exerts a curariform action on neuromuscular transmission.

However these authors first determined the minimal strength of stimulus to which the muscle would respond, and then showed that after ether the muscle no longer responded to that strength of stimulus. Under these circumstances a failure of muscular contraction might be caused by a rise in the nerve threshold rather than by block of the neuromuscular junction. The present study avoids this difficulty by using maximal stimuli throughout. Each determination records the maximal response obtainable at that time.

In addition to the mechanical contraction, the electrical response of the muscle was also measured. This permits differentiation of the effect of ether on the contractile mechanism from its effect on the muscle action potential.

*Method.* The following studies were made on rats. The animals were anesthetized through a tracheal cannula attached to an ether bottle. The bottle was provided with a valve which controlled the amount of ether in the inspired air. Animals were kept lightly etherized throughout an experiment; when the effect of ether was to be studied the valve was turned to permit the maximal amount of ether to be drawn into the lungs. If natural respiration stopped, artificial respiration was applied by attaching an intermittent air pump to the side arm of the cannula. Natural breathing was usually resumed after a few seconds' artificial respiration.

In the experiments on curare Intocostrin (Squibb) was used. One cc of this preparation is described as containing the equivalent of 20 units of standard drug. It was injected into the femoral vein. The mechanical response was recorded through a lever attached to the tendon of the gastrocnemius, while the electrical responses were led off by needle electrodes inserted into the muscle. The height of potential was measured on an oscilloscope.

*Results.* The maximal etherization possible with the apparatus used blocks respiration before neuromuscular transmission. After 12 minutes respiration stops, while the electrical response of the muscle to maximal stimulation of its nerve shows an average depression of 12% (8 to 15) in 5 experiments. It is possible that the greater depression obtained by Gross and Cullen is due to their recording the mechanical contraction of the muscle. However a record of the mechanical response to maximal stimulation showed a depression of only 9% after 20 minutes of deep etherization. More probably the different results of Gross and Cullen are assignable to their use of minimal stimuli.

Since ether causes respiratory depression, the decreased muscular response might be due to anoxia rather than to direct action of ether. This possibility was tested by having the lightly etherized animal rebreathe into a balloon attached to the tracheal cannula. This kept the ether concentration reasonably constant, but permitted the oxygen concentration to drop rapidly. Two experiments showed an average depression of the muscle action current of 5% in 12 minutes. As a further check, after recovery the same animals were heavily etherized and oxygen was then blown in through the ether bottle. This increase in oxygen with little if any change in ether concentration caused an average recovery of 5% in 9 minutes. It appears

[1] Gross, E. G., and Cullen, S. C., *J. Pharm. and Exp. Therap.*, 1943, **78**, 358.

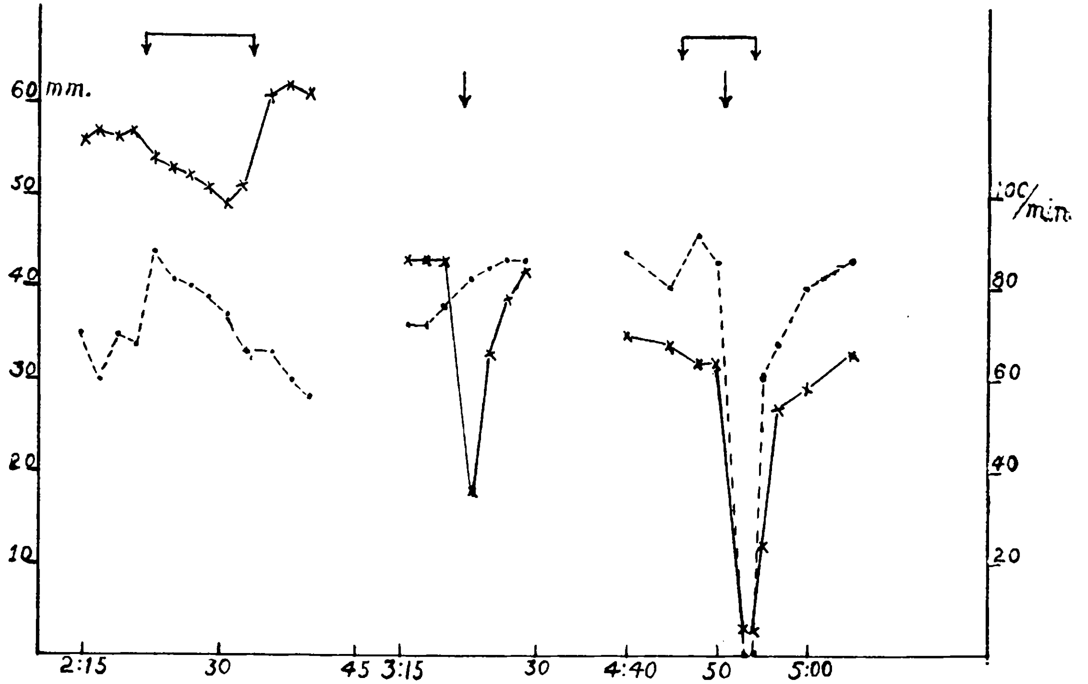


FIG. 1.

Effect of ether and curare on muscle action potential and respiratory rate in the rat. A progressive decline in the height of the action potential occurs during a long experiment, presumably due to deterioration of the muscle substance around the needle electrodes. Other experiments in which the order of the tests was varied show that this decline does not affect the conclusions. Intocostrin administered 0.1 unit per kg. Dash curves, rate of respiration, scale per minute at right margin. Full curves, height of muscle spike, scale at left margin in mm. Time on base line. Light ether throughout except between double arrows, deep ether. At single arrows, curare administered.

then that of the 12% depression following etherization, 5% may be caused by asphyxia.

The remaining depression is not enough to account for the observed increase of the action of curare in the presence of ether. That there is a synergism between the two agents is seen in Fig. 1. The maximal dose of ether alone caused a 14% depression of muscle action potential in this experiment, while 0.1 unit curare/kg caused an average depression of 46%, with minimal ether. Although the sum of the depressions caused by these agents given separately is 60%, the same dose of curare given 3 to 5 minutes after deep etherization has begun caused a depression of 91%. When the same dose of curare was given one minute after deep etherization began, the depression was only 72%, indicating that the ether had not yet reached the optimum concentration at the site of action.

In addition to this effect, a synergic action on respiration is shown by the same experiment. Ether alone stops respiration in 12 minutes, while this dose of curare used alone has no significant effect on respiration. But if this dose is given 3 to 5 minutes after deep etherization has begun, respiration stops 2 minutes after curare is injected.

*Discussion.* Although Gross and Cullen reported complete neuromuscular block under ether as tested by minimal stimuli, only a slight decrease of amplitude is found in the present study when maximal stimuli are used. This suggests that the depression which the above workers obtained may have been due to increase in the nerve threshold. The present paper does not permit localization of the peripheral action of ether. Since the depression obtained in the mechanical response was no greater than that obtained in the electrical response, ether evidently acts proximal to the contractile process of the muscle. The synergism shown between ether and curare might suggest that they act at the same site, but this does not necessarily follow. Since we do not yet know the exact point at which curare acts, further speculation as to the peripheral action of ether does not seem warranted.

As noted above, curare has been reported to cause greater "relaxation" of muscles during anesthesia with ether than during the same degree of anesthesia with other agents. While this suggests that there is a greater synergism of curare with ether than with these other agents, another possibility exists. Anesthetists judge the degree of anesthesia to a large extent by the respiration, and amounts of ether and other agents which depress the respiratory center to the same degree may have different effects at other centers, as well as on the neuromuscular junction. A detailed examination of the actions of various anesthetics would be necessary to settle this point.

*Summary.* The response of the gastrocnemius to maximal stimulation through its nerve was studied in the rat. During deep etherization there is a depression of 12% in the muscle action potential. At the same time the respiration is depressed; anoxia alone due to this may account for 5% depression. Hence a muscular depression of only 7% can be attributed to the action of ether, not necessarily involving neuromuscular block. Nevertheless there is a synergism between ether and curare, since the effects of these agents given together is one-third greater than the sum of their separate effects.

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## 15502

## Effect of Enzyme Inhibitors on Transformation of Enzymes in the Living Cell.

JOHN M. REINER. (Introduced by M. B. Visscher.)

*From the Department of Physiology, The Medical School, University of Minnesota.*

Transformations in the enzymatic constitution of the living cell may occur as a result of gene mutation or as a physiological transformation in the presence of a constant genome. Changes of the latter type are most easily detected and studied in the cells of microorganisms, which are less specialized and more flexible than the cells of mature multicellular organisms, and present fewer complicating factors than the closely interrelated complex of cells found in the embryo. These changes have long been familiar to microbiologists; but they have until recently been the subject of much controversy, centering around the question whether they are only apparent transformations resulting from the action of natural selection upon pre-existing mutants in microbiological populations. Recent developments in the genetics of yeast[1] have made it possible to settle the controversy at least for this group of organisms; and it has been demonstrated by Spiegelman and coworkers[2,3] that modifications of enzyme constitution take place in genetically stable yeast strains, without the intervention of natural selection and without cell multiplication.

Most domestic strains of yeast readily ferment glucose, but do not ferment galactose. Some of them can, however, acquire the ability to ferment galactose after

1 Lindegren, C. C., *Bact. Rev.*, 1945, **9**, 111.

2 Spiegelman, S., Lindegren, C. C., and Hedgecock, L., *Proc. Nat. Acad. Sci.*, 1944, **30**, 13.

3 Spiegelman, S., and Lindegren, C. C., *Ann. Mo. Bot. Gard.*, 1944, **31**, 219.