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## An improved method for obtaining twitch responses to acetylcholine in the rat diaphragm

SIR,—Retrograde injection into the venous drainage of the right hemidiaphragm was originally developed by Burgen, Dickens & Zatman (1949) as a method for obtaining twitch responses to added acetylcholine. The method has more recently been described in detail by Paterson (1965) in studies on the pharmacology of denervated and innervated rat muscle.

In our quantitative investigations we experienced some difficulty in obtaining repeatable results using the preparation as described. Since the response of this preparation to retrograde injection is dependent not only upon the dose of drug, but also on the speed of delivery and volume of the injection, we have devised a method of mechanically delivering drug solutions to the diaphragm. The preparation was made as described by Paterson (1965) with the exception that the dead space of the injection cannula was reduced to 0.07 ml by using fine bore polythene tubing (Arnold Howell Ltd., ARH/3, hand drawn to 1 mm o.d.) as the cannula, and tied as low down into the thoracic vena cava as possible to minimise "ballooning" from the pressure of injection. After filling the cannula

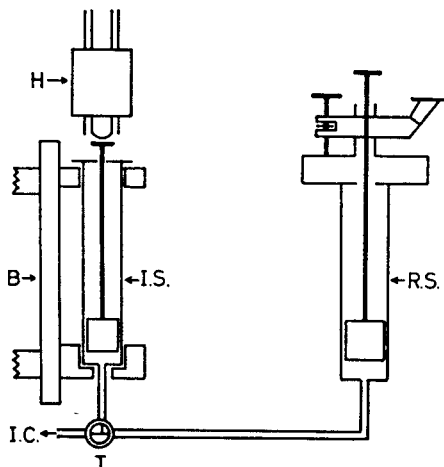


FIG. 1. Diagram of apparatus for the mechanical retrograde intravenous injection of acetylcholine into the isolated rat diaphragm. B. Brass holder for injection syringe. H. Knee jerk hammer. I.C. Injection cannula. I.S. Injection syringe. R.S. Reservoir syringe. T. Three way tap.

with the appropriate concentration of drug solution, 0.1 ml of the solution was injected from a 1 ml record type tuberculin syringe with a metal plunger and nozzle (injection syringe) held rigidly in a brass holder, and connected to a three way tap (C. F. Thackray Ltd., I.T. 4126) (Fig. 1). The plunger was mechanically actuated by a knee jerk hammer (Wright & Schweizer, 1937) supplied by C. F. Palmer, London, Ltd., mounted vertically above the syringe plunger. On connecting the coil to a 12V DC source, the hammer struck the plunger of the injection syringe, and the drug solution was injected. Accurate measurement of the drug solution volume was obtained by filling the injection syringe from a reservoir syringe capable of metering small volumes of fluid (Chance Repette syringe 2 ml).

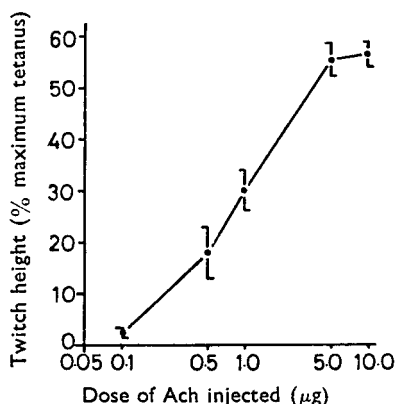


FIG. 2. Log dose-response curve of isolated rat diaphragm to retrograde intravenous injection of acetylcholine, showing standard errors of means of six diaphragm preparations. Abscissa: dose of Ach injected in 0.1 ml saline.

The advantages of these modifications to the described method may be judged from the results shown in Fig. 2, taken from a series of experiments in which responses to graded doses of acetylcholine were obtained.

The preparation was the right hemidiaphragm of the rat, suspended in Krebs solution at 27°, stimulated indirectly at a rate of 2 shocks/min. Retrograde injections of acetylcholine 0.1–10.0 µg were given at 5 min intervals, the indirect stimuli being suspended for 1 min periods before and after injection.

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