Assay of Acetylcholine on the Rat Blood Pressure

SIR,—The rat blood-pressure preparation does not seem to have been fully exploited for the assay of depressor substances, despite its use for the assay of pressor substances by Crawford and Outschoorn[1] and Dekanski[2]. By using sodium pentobarbitone with urethane as the anaesthetic and allowing the body-temperature to fall to about 28° it has proved possible to get a long-surviving, stable sensitive preparation.

Method. The method is a modification of that described by Dekanski. Male rats of about 250 g. are preferred. Anaesthesia is obtained by the intraperitoneal injection of a mixture of urethane (40 mg./100 g.) and sodium pentobarbitone (3 mg./100 g.) and the animal is placed in a supine position on an unwarmed table. The depth of anaesthesia can be increased by injecting more pentobarbitone (1-1·5 mg./100 g.). A short glass cannula is tied in the trachea and the animal is allowed to breathe naturally without artificial respiration. The right carotid artery is dissected carefully from the surrounding structures and tied high in the neck.

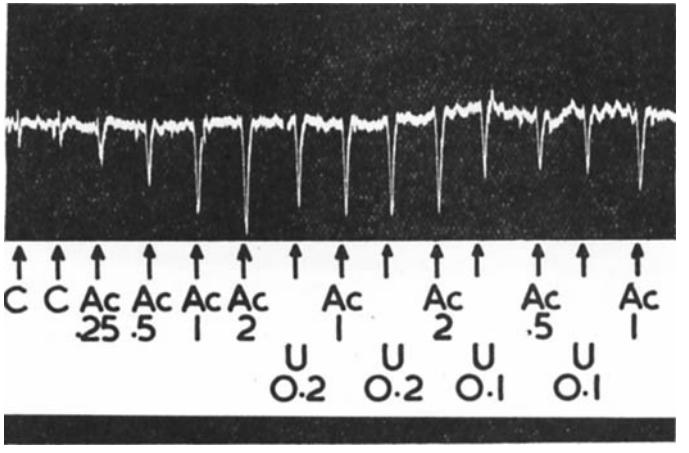

FIG. 1. Dose response of acetylcholine (Ac) in ng. of base. Volumes of unknown solution (U) in ml. (C) Injection artifact of 0·3 ml. Kreb's fluid with neostigmine 1×10^{-6}.

On the left side the femoral nerves are cut. The femoral vein is dissected from the artery up to the inguinal ligament and cannulated with a short fine bore polythene cannula connected by rubber tubing to a 1 ml. tuberculin syringe. The syringe is refilled with washing-in fluid through a side arm from a separating funnel. The venous cannula and a short section of the tubing just distal to it (into which injections are made) are held rigidly in a grooved perspex block clamped to the bench. This device facilitates the injection of fluid into the cannula and reduces injection artifacts. The dead space of the system is 0·03 ml. and thus only small volumes of fluid are needed for washing in injections. Before the anticoagulant (2000 units of dextran sulphate) is injected a check is made to see that there are no bleeding points. Ice cold saline swabs may be used if necessary to promote haemostasis. The artery is now cannulated and connected through polythene tubing to a Condon type manometer. It is more

satisfactory to inject several ml. of the washing-in fluid (which contains neostigmine methyl sulphate 1×10^{-6}), at intervals before beginning the assay, than to sensitise the preparation with a single injection of neostigmine.

The animal is left for 20 minutes and then the assay is begun, injections being made at 2 minute intervals for many hours.

The preparation shows a depressor response to as little as 0·5 ng. of acetylcholine, and the threshold may be as low as 0·25 ng. in the winter months (Fig. 1). Irregularities occuring in the course of an assay if not due to tracheal obstruction, may be reduced or abolished by the intravenous injection of 1 or 2 mg. of sodium pentobarbitone.

The successful use of this method for many months now suggests that it can replace the more conventional cat blood-pressure preparation, particularly in the estimation of the acetylcholine released by nerve stimulation from various nerve-muscle and ganglion preparations.

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October 28, 1958.

REFERENCES

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2. Dekanski., *ibid.*, 1952, **7**, 567.