J. Pharm. Pharmacol., 1965, 17, 176–182 Received January 15, 1965

An automatic apparatus for repeated stimulation of isolated organs by agonist drugs

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A simple commutator for attachment to a standard laboratory kymograph has been designed to operate automatically an apparatus for producing repeated contractions of isolated organs by agonist drugs.

THE advantages of an automatic apparatus are now well-established for quantitative and repetitive experiments on isolated organs. For example, in estimates of drug antagonisms (Schild, 1947), or in biological assays (Gaddum & Lembeck, 1949; Godfrey, Mogey & Taylor, 1950; Boura, Mongar & Schild, 1954), an increase in accuracy is achieved because of the greater uniformity of time intervals and fluid volumes; also the experimenter is able to work two or more organ baths concurrently.

This paper describes an automatic apparatus for producing uniform contractions of isolated organs by the repeated injection of agonist drugs. The apparatus has been used in experiments to investigate the inhibitory actions of spasmolytic drugs, using the uniform contractions of the organs as a reference level of excitatory activity. The apparatus is an improved design of that demonstrated to the British Physiological Society (Wilson, 1957), and has proved to be reliable during several years' use in this department. It differs in two ways from the apparatus described by other workers (Schild, 1946, 1947; Gaddum & Lembeck, 1949; Godfrey, Mogey & Taylor, 1950; Boura, Mongar & Schild, 1954). Firstly, it uses a simple mechanical switching system for operating a series of the electromagnetic valves described by Schild (1947). Secondly, instead of producing contractions by replacing the organ bath contents with the final dilution of an agonist drug (Gaddum & Lembeck, 1949; Boura, Mongar & Schild, 1954), it uses an automatic syringe for injecting small volumes of a concentrated solution of the drug. Lock (1961) has described some of the benefits of an automatic syringe; in the present experiments the method has the additional advantage that unlike the apparatus used by Gaddum & Lembeck (1949), an inhibitory drug added to the organ bath during the rest period of the preparation is not drained out before the subsequent challenge with the agonist solution.

EXPERIMENTAL METHOD

This is shown in Fig. 1 which is a kymograph record of a typical experiment on a preparation of guinea-pig small intestine. The automatic apparatus was used to produce a series of uniform contractions at each of two dose levels of histamine. The contractions caused by the larger dose of histamine were used as a reference level of excitatory activity from which was plotted a dose-response relation of the inhibition of histamine contractions by methylamphetamine.

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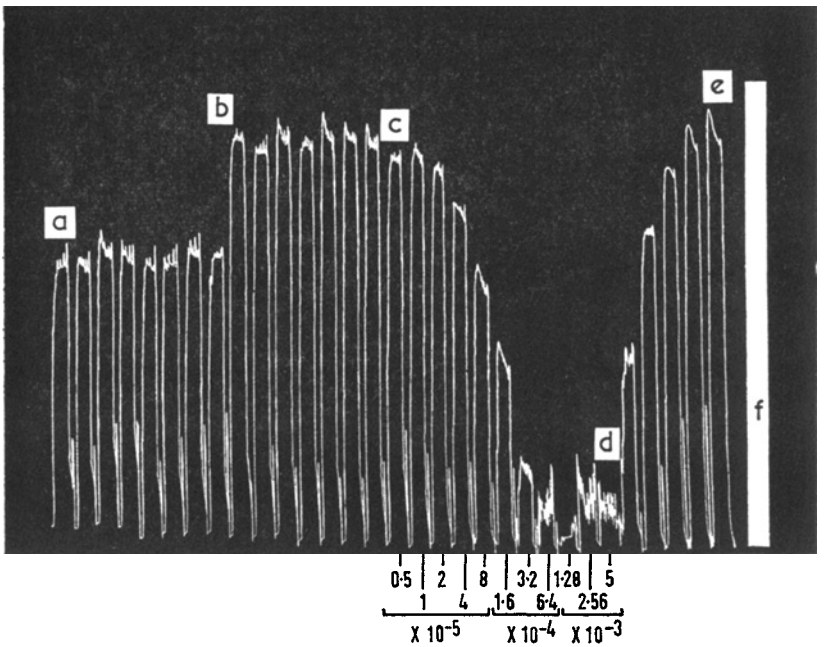

FIG. 1. Uniform longitudinal contractions of the guinea-pig isolated ileum to repeated injections of histamine at each of two dose levels, and a sequential dose-response record of the inhibition of the contractions by methylamphetamine. Doses of histamine were injected automatically at 4 min intervals and allowed to act for 1 min; the ileum was then washed twice with fresh Krebs solution. a–b = final bath concentrations of 4×10^{-8} histamine; b–e = final bath concentrations of 8×10^{-8} histamine. c–d = inhibition caused by graded doses of methylamphetamine, added to the organ bath by hand 2 min before a subsequent automatic injection of histamine; final bath concentrations of methylamphetamine are given on the molar scale. d–e = recovery during 4×10^{-8} histamine. f = maximal contraction of the preparation to histamine.

The spasmolytic drugs used in the experiments are not injected automatically, but are manually injected into the organ bath directly. This method allows the dose-inhibitory response relations of the spasmolytic drugs to be established by randomised or sequential consecutive graded doses (Fig. 1). It also avoids adding the spasmolytic drugs to the reservoir of physiological saline solution, a technique which gives satisfactory results with competitive antagonists (Schild, 1947; Godfrey, Mogey & Taylor, 1950) but which can cause errors in experiments using low concentrations of unstable drugs, such as the sympathomimetic amines.

AUTOMATIC OPERATION OF THE ORGAN BATH

For the automatic working of one organ bath, five electromagnetic valves (Schild, 1947) are used to compress polyvinyl or latex tubing; they control the measurement of Krebs solution, the filling and emptying of the organ bath, and the measurement and injection of the agonist drug solution (Fig. 2). Table 1, first column, lists the sequence of operations which, in the experiment shown in Fig. 1, were performed automatically

to elicit and record each histamine contraction and to return the preparation to rest; the sequence was repeated every 4 min. As judged by the uniform contractions to repeated doses of the agonist drugs (Fig. 1), the precision of the automatic measurements is high.

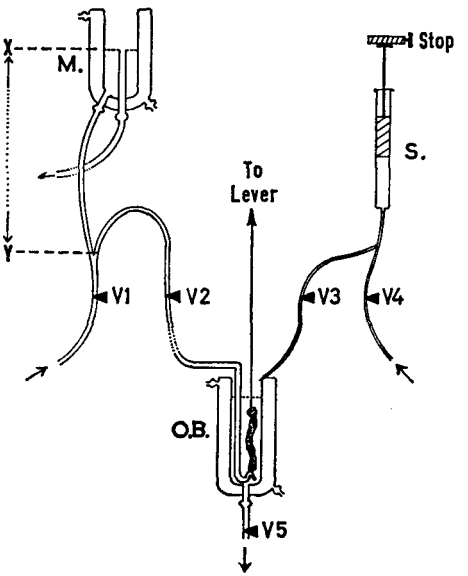

FIG. 2. The arrangement of the apparatus and electromagnetic valves (V1 to V5) used for the automatic operation of one organ bath (O.B.). V4 controls the filling of an automatic syringe (S) from a reservoir of agonist drug solution pressurised to 180 mm Hg; with V3 alone open, the weight of the syringe piston forces the measured dose of agonist into the organ bath. Sequential operation of V1 and V2 controls the filling of an overflow measuring chamber (M) with pre-warmed Krebs solution from a reservoir above the apparatus, and the flow of the measured volume of solution (X—Y) into the organ bath. V5 empties the organ bath to waste. Polyvinyl tubing of 3 mm outside diameter and 2 mm inside diameter was used for V3 and V4. Latex thyroid drain tubing, ¼ inch diameter was used for V1, V2 and V5.

SWITCHING SYSTEM AND TIMER

The switching system is a commutator of 100 separate stud contacts, which can energise the electromagnetic valves with current taken from a moving contact wiper. The timer is a standard laboratory kymograph, the main spindle being used to drive the contact wiper (Fig. 3).

The commutator has a flat circular base-plate on which there are one continuous ring contact and two outer concentric circles each of 50 separate stud contacts (Fig. 3). The base-plate is rigidly mounted on the body of the timing kymograph; the contact wiper clamps to the spindle and has three downward-facing brushes which rotate on the upward-facing ring or stud contacts of the base-plate (Fig. 5). The single brush of the wiper receives direct current from the ring, and the other two brushes transfer the current, in sequence, to each stud in the two circles; the backs of the studs are wired to an equal number of separate terminals on a wiring panel.

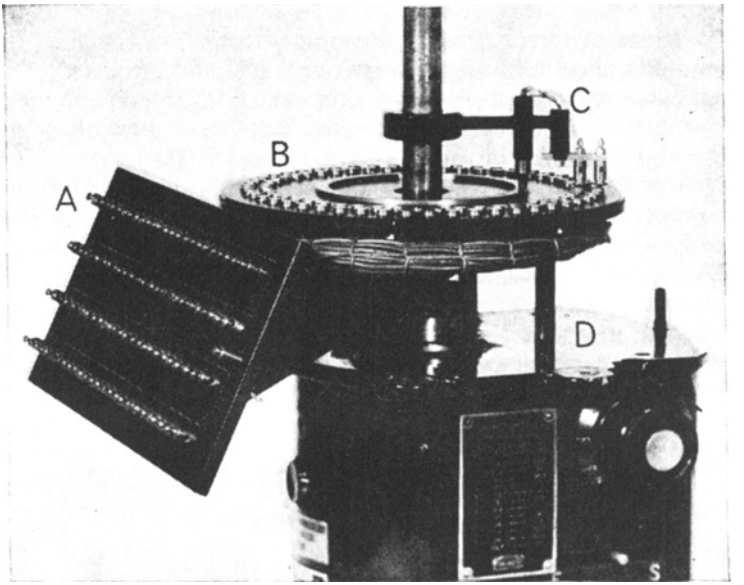

FIG. 3. The switching system and timer. A = wiring panel. B = commutator base-plate. C = contact wiper. D = timing kymograph.

The timer drives the contact wiper and its speed governs the frequency of operation of the electromagnetic valves. A Palmer "Electric Twelve" laboratory recording kymograph has been used, and makes a readily available timing unit with a speed range to suit all types of isolated organs: the spindle speed (and hence contact wiper speed and valve cycle) is almost continuously variable from one revolution in 0·8 sec to one revolution in 13 hr.

TABLE 1. CONNECTIONS OF THE ELECTROMAGNETIC VALVES (V1 TO V5), TWIN-CONTACT RELAYS (RK AND R1 TO R5) AND COMMUTATOR STUD CONTACTS (1 TO 100) USED FOR THE AUTOMATIC WORKING OF THE ORGAN BATH IN THE EXPERIMENT SHOWN IN FIG. 1.

Sequence of automatic operations	Electromagnetic valve	Twin contact relay	Commutator contacts
Fill automatic syringe with agonist drug solution ..	V4	R4	1–4
Recording kymograph on	—	RK	5–7
Inject agonist drug solution; recording kymograph running	V3	R3	8–12
Recording kymograph running	—	RK	13–27
Measure Krebs solution; recording kymograph running	V1	R1	28–31
Recording kymograph running	—	RK	32–34
Empty Organ Bath	V5	R5	35–37
Fill organ bath with Krebs solution	V2	R2	38–41
Measure Krebs solution	V1	R1	46–49
Empty Organ bath	V5	R5	53–55
Fill organ bath with Krebs solution	V2	R2	56–59

Column one gives the sequence of operations which was repeated at intervals of 4 min. to stimulate and record one contraction of the ileum and to return the preparation to rest.

WIRING CIRCUIT

Connections of the electromagnetic valves to the stud contacts of the commutator are made through the wiring panel and are determined by the particular requirements of each experiment. Table 1, columns two and four, lists the connections used in the experiment shown in Fig. 1.

The contact wiper can be provided with current (100 V, D.C.) to energise the electromagnetic valves directly, but because heavy arcing burns the commutator contacts, an indirect system is preferred. In the indirect system the commutator is used in a low voltage circuit (9 V, D.C.) to operate a series of 100 ohm Post Office telephone relays (Table 1, column 3): the twin contact sets of these relays carry 100 V, D.C. to the electromagnetic valves, or 240 V, A.C. to the kymograph on which the contractions of the preparation are recorded.

Fig. 4 gives the circuit for the simultaneous automatic operation of two organ baths; in this instance the apparatus shown in Fig. 2 is duplicated.

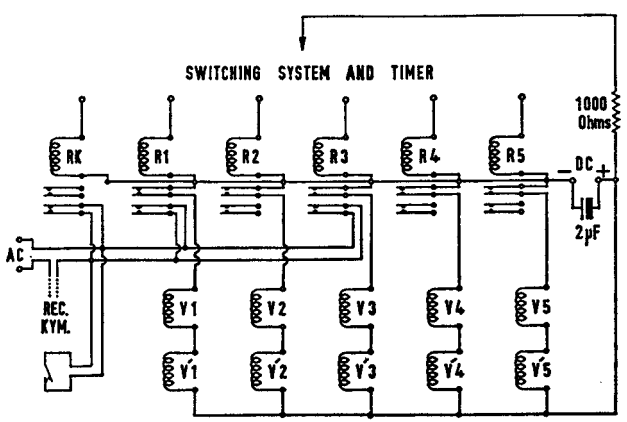

FIG. 4. The circuit for the simultaneous automatic operation of two organ baths. V1 to V5 are the electromagnetic valves whose use is illustrated in Fig. 2; V'1 to V'5 are the equivalent electromagnetic valves of a duplicate apparatus. R1 to R5, and RK are relays whose twin contact sets carry 100 V, D.C. to the electromagnetic valves, or 240 V, A.C. to the recording kymograph (REC.KYM.) on which the contractions of the preparations are recorded. The resistance of each relay and electromagnetic valve is 100 ohms.

CONSTRUCTION OF COMMUTATOR

The base-plate of the commutator is an 8-inch diameter disc of $\frac{1}{4}$-inch "Ebonite" or similar material. One hundred 4 BA hexagonal-head brass screws are turned to form the separate stud contacts and are mounted in two concentric circles of fifty each, 3 inches and $3\frac{3}{8}$ inches respectively from the centre of the base-plate; the stud contacts in the outer circle alternate with those in the inner at intervals of 3° 36′. A brass ring (4 inches outside diameter, $\frac{1}{4}$ inch wide and $\frac{3}{16}$ inch thick), slotted into the base-plate concentrically with the studs, forms the continuous ring contact. All the contacts are turned smooth to a level about $\frac{1}{8}$ inch proud of the upper surface of the base-plate.

A lip on the circumference of the base-plate takes a push fit dust cover with a perspex top, the centre of which, like that of the base-plate itself, is drilled to pass and to clear the $\frac{3}{4}$-inch diameter main spindle of the timing kymograph. The base-plate is rigidly mounted on three brass rods which are screwed into the top of the timing kymograph.

The wiring panel is a sheet of $\frac{1}{4}$-inch "Ebonite", 11 inches by $4\frac{1}{2}$ inches. This is fixed to the under surface of the commutator base-plate, at an angle of about 45 degrees, by two "Ebonite" arms each approximately 8 inches long. The panel is drilled to take four rows of 25 separate screw terminals; the back of each terminal is permanently wired to the back of a stud contact on the commutator base-plate, in the sequence in which the moving wiper makes contact with the studs (that is, adjacent screw terminals are wired to adjacent stud contacts on the inner and outer circles alternately). An additional screw terminal on the panel is wired to the ring contact on the base-plate to carry current to the contact wiper.

The contact wiper has a split collar of brass which clamps to the main spindle of the timing kymograph (Fig. 3), and an arm of $\frac{1}{4}$-inch diameter brass rod which carries three spring-loaded brushes (Fig. 5). The wiper

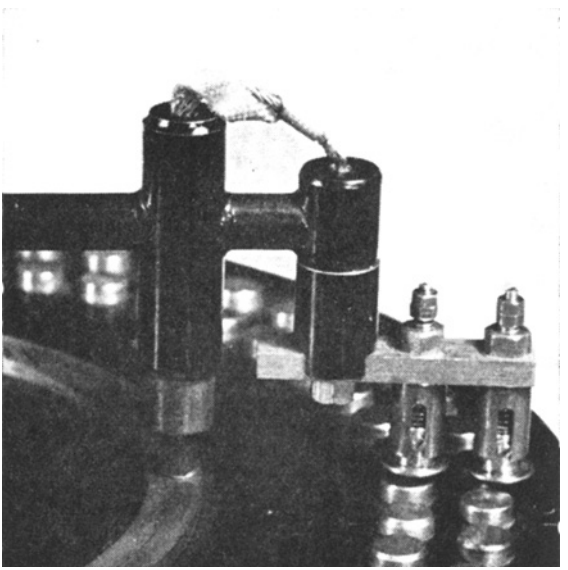

FIG. 5. The three downward-facing brushes of the contact wiper, which rotate on the ring or stud contacts of the commutator base-plate.

is insulated from the spindle of the timing kymograph by a "Tufnol" insert in the split collar. The inner brush on the wiper arm is a spring-loaded copper rod which transfers current from the ring contact on the commutator to the two outer brushes: each of these has a domed foot of brass which runs on one of the two circles of stud contacts. The feet must offer minimal mechanical resistance to the motor of the timing

kymograph, and to avoid intermittent function of the electromagnetic valves, must provide electrical continuity between successive stud contacts in the switching sequence. These requirements are met, firstly, by having the angle of the domed feet greater at the leading than at the trailing edges, and secondly, by making the domes of a size and shape (overall diameter about $\frac{5}{16}$ inch, with short trailing edges) which prevents them from bridging adjacent contacts in their own circles, whilst at the same time maintaining continuity between adjacent contacts of the inner and outer circles. The orientation of the domed feet is maintained by a locating pin on their stems.

USE OF APPARATUS

The apparatus was designed for use in experiments to investigate the spasmolytic actions of physiological or "independent" (Gaddum, 1957) antagonists of stimulant drugs, but it is also suitable for use in experiments with competitive antagonists, for example, in estimating pA_2 (Schild, 1947) by the method of Lockett & Bartlet (1956). The commutator switching system and the kymograph timing system may also find application in other automatic procedures, as a flexible alternative to the more usual combination of telephone uniselectors and a timing device (Schild, 1946, 1947; Gaddum & Lembeck, 1949; Godfrey, Mogey & Taylor, 1950; Boura, Mongar & Schild, 1954).

Acknowledgement. I am grateful to all my colleagues who have helped in the design and construction of the apparatus, and in the preparation of this paper.

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