Brit. J. Pharmacol. (1950), **5**, 381.

AN AUTOMATIC ASSAY APPARATUS

BY

E. I. GODFREY, G. A. MOGEY, AND D. L. TAYLOR

From the Wellcome Research Laboratories, Beckenham, Kent

(Received March 9, 1950)

The biological assay of all new samples of *d*-tubocurarine salts for clinical use is now obligatory (T.S.A., 1948). The most accurate method for this purpose so far described is that by Mogey, Trevan, and Young (1949). Briefly, it depends upon the comparison of two doses (high and low) of the test sample with similar doses of the standard *d*-tubocurarine. The four doses are allotted at random to the elements of a 4×4 Latin square. A rat-diaphragm preparation is exposed to the action of each of the sixteen doses, in an order determined by the square, for precisely five minutes. Washes out are so arranged that three washes plus recovery time occupy a similar time interval to the action of the drug so that ten minutes elapse between the beginning of the action of one dose and the next.

The assay therefore entails emptying and filling the isolated organ bath nearly 100 times at precisely determined intervals. Accuracy demands that the bath be filled to the same mark on each occasion and that the correct dose of drug be measured accurately each time it is added. The whole test occupies nearly three hours and becomes rather monotonous when frequently repeated.

To obviate the tedium and increase the accuracy of such routine testing we have designed the automatic apparatus described below.

APPARATUS AND METHOD

A rat diaphragm-phrenic nerve preparation is set up in the usual way and a few stabilizing doses of *d*-tubocurarine added. These preliminary doses are necessary because the sensitivity of the preparation increases greatly for the first three or four doses and after this the sensitivity usually goes on increasing gradually and linearly for the remainder of the test as shown in Fig. 1. In any case the sensitivity of individual

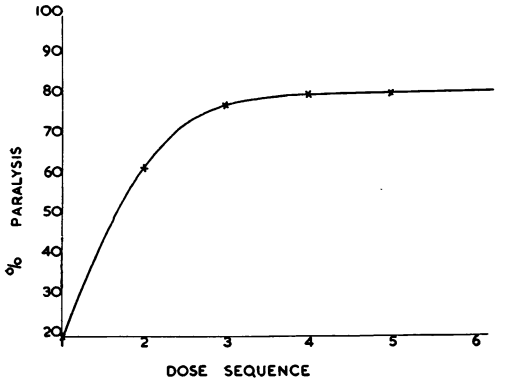

FIG. 1.—Response of rat diaphragm to successive equal doses of *d*-tubocurarine chloride (mean of several experiments). Concentration of tubocurarine chloride was 2×10^{-6}.

rat diaphragms, although fairly constant, varies slightly and it is necessary to determine suitable doses of *d*-tubocurarine for each preparation. The preliminary stabilizing doses also serve to determine the concentrations of drug required for the main assay.

Suitable concentrations having been established, about a litre of each is made up in Ringer-Locke solution and put into one of the drug containers shown in Fig. 2.

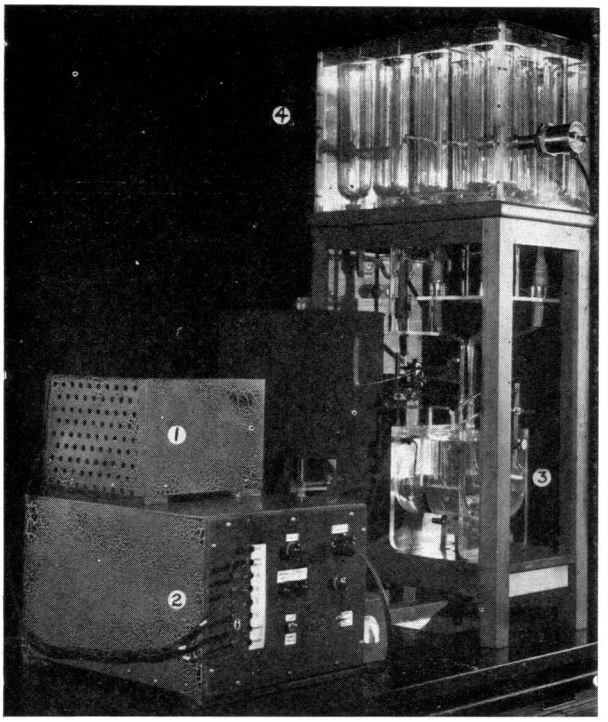

FIG. 2.—Assay apparatus. (1) Power pack, housing transformer and rectifier (2) Master controller containing timing motor and uniselectors. (3) Isolated organ bath assembly. (4) Reservoir bath. The four cylinders at the nearer end of the bath are for the drug solutions: each has its own outlet valve. The other four cylinders are for drug-free Ringer: these have a common outlet valve.

Ringer-Locke containing no drug is kept in the other four cylinders. These eight containers are in a water bath thermostatically controlled at 40° C. and well stirred by an air bubbler. They were made from broken 2-litre and 1-litre graduated cylinders drawn out into a neck at one end. Four containers have been used for the Ringer because it is difficult to obtain a cylindrical glass tube sufficiently large to contain enough Ringer for a complete test; broken 2-litre cylinders are suitable as regards size and the heating arrangements are simplified. The required volumes of solution vary with the size of the isolated organ bath being used. The flow of the solutions from these reservoirs is controlled by a series of valves, one for each concentration of drug and one for the drug-free Ringer. The sequence in which the valves operate and the times of their opening and closing are all controlled by a mechanism described later.

Each valve was cut from a $3\frac{1}{2}$ in. length of "perspex" cylindrical rod of 1 in. diameter (Fig. 3). A cylindrical hole was bored along the centre of the rod, the top two thirds being of greater diameter than the lower third: the junction between these two portions must be carefully made, for it must form a true seat for the valve-plug. A small stopper is arranged to occlude the lumen at the constriction. Attached to the stopper is a stainless steel rod around which is a movable stainless steel sleeve.

At the top and bottom of the rod are flanges past which the sleeve cannot go. The sleeve is of pearlitic type steel ; the rod may or may not be magnetic. It is important to choose non-copper-containing steel and yet to have high resistance to corrosion. The outer surface of the sleeve is not quite circular in cross section so that there is space to allow the flow of fluid past it. Rates of flow may vary slightly according to the hydrostatic pressure. We have not found it necessary to compensate for this

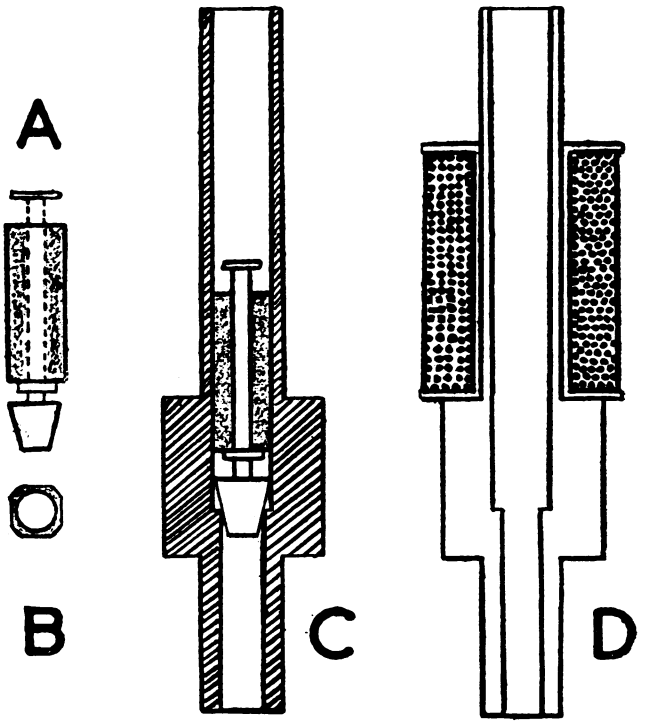

FIG. 3.—Magnetic valve. A. Rubber stopper mounted on central rod having flanges at top and bottom. A loose sleeve moves on this central rod. B. Cross section of sleeve. C. Plug in position in perspex valve. D. Position of solenoid.

since the actual volume of solution reaching the organ bath is of little importance, provided the preparation is bathed in it, as the concentration of the active drug is not affected. When the diaphragm is suspended fairly low in the bath great latitude in the rate of the flow of the solutions is permissible.

The outer surface of the " perspex " rod is shaped so that there is a shoulder on it just above the level of the internal constriction. This ledge supports a solenoid wound from 36 SWG enamelled copper wire and of 180 Ω resistance, with the upper end of the " perspex " valve occupying the core of the coil. When the solenoid is activated the loose stainless steel sleeve is pulled upwards without having to unseat the stopper as it begins its movement. When it impinges against the upper flange it has sufficient momentum to lift the stopper, which is held up until the solenoid is de-activated. The solutions flow past the valve and are conducted via glass tubing to the organ bath situated directly below. A screw clip on a rubber connexion here may be used to control rates of flow, but again we have had no trouble since the outlet from the glass conducting tube was fixed at a suitable size.

The organ bath is of the usual type except that it has a reception chamber built on to it (Fig. 4). It is into this cup that the solutions pour from the valves so that

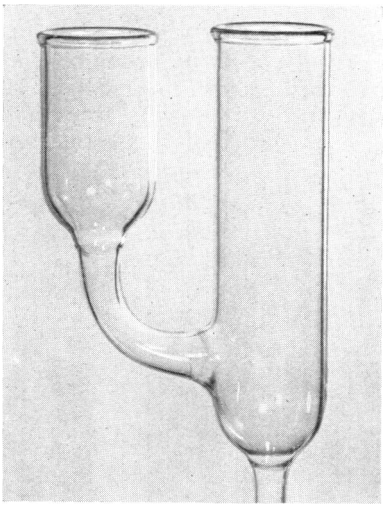

FIG. 4.—Isolated organ bath.

the organ bath proper is filled gently from below. This prevents any injury to the preparation and also serves to mix cold solution from the connexion tubes with warm solution from the reservoirs before contact is made with the diaphragm. The organ bath is fixed in a small thermostatically controlled water bath and its outlet is controlled by a valve of the type described above.

The mechanism which controls the operation of the valves is an extension of the principle already described by Schild (1946) and Stephenson (1949). A standard Post Office uniselector is used. The terminals are connected to the solenoids described above so as to produce the sequence of events shown in Table I. This cycle of events is then repeated sixteen times, the drug solution varying each time according to the Latin square chosen for the wiring system (see Table II).

The timing arrangements for any one cycle are controlled by a mains driven "synclock" motor, geared down on to a brass disc of the type illustrated in Fig. 5. The disc, revolving at the rate of one turn in ten minutes, operates a relay spring-set switch of the make-before-break type, as illustrated in Fig. 6, so that current flows only while both contacts are made—i.e., for a period of about half a second while the wheel descends into and ascends from the grooves cut in the circumference of the disc. The impulse thus set up is transmitted to the driving magnet of the uniselector. (We used two uniselectors, with the smaller of which, of 80 Ω

TABLE I

SEQUENCE OF EVENTS, DURING ONE TURN OF TIMING DISC, RELATED TO UNISELECTOR TERMINALS

Terminal on uniselector	Event	Duration of event
		min. sec.
1	Starting position	
2	Drug in	0 17
3	Stimulator on (drug action)	5 0
4	Drug out (wash)	0 11
5	Blank*	0 1
6	Ringer in	0 17
7	Stimulator on (recovery)	1 46
8	Ringer out (wash)	0 11
9	Blank*	0 1
10	Ringer in	0 17
11	Stimulator on (recovery)	1 46
12	Ringer out (wash)	0 11
13	Blank*	0 2
	Total duration of cycle	10 0

10 min. = 1 revolution of timing disc (Fig. 5). 1 revolution of disc = 1 complete cycle of events (above). 16 cycles (as above but 1 is not repeated) = 1 complete assay. The drug solution varies with each cycle—see Table II.

* These blanks are not necessary: they were inserted to ensure seating of the drain valve before the filling valve opened.

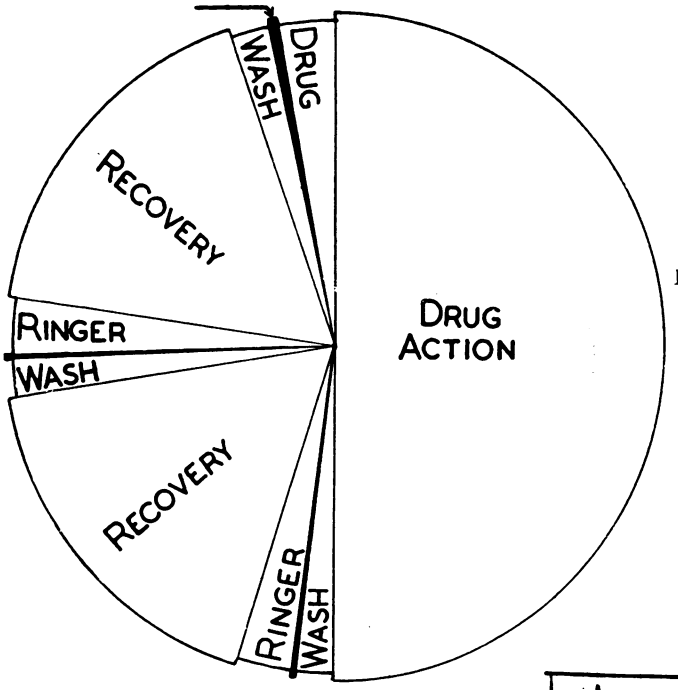

FIG. 5.—Timing disc. Revolves anticlockwise once in 10 min. and operates switch (Fig. 6) which controls uniselector. The arrow indicates position of switch when disc is at starting position. The stimulus is applied to the phrenic nerve only during the drug action and the two recovery periods.

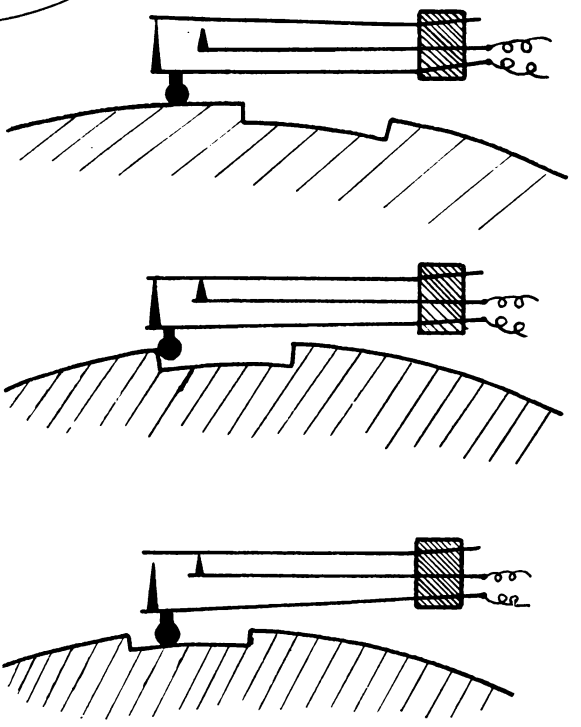

FIG. 6.—Make-before-break spring-set switch. A short impulse is transmitted to the uniselector each time the switch wheel falls into or rises out of a notch in the circumference of the revolving timing disc (Fig. 5).

TABLE II

CORRELATION BETWEEN UNISELECTOR TERMINALS, DRUG SOLUTIONS SUPPLIED TO PREPARATION, AND CYCLE ORDER

No. of cycle in assay	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Drug supplied to preparation	A	B	C	D	C	A	D	B	D	C	B	A	B	D	A	C
Uniselector terminal No. ..	2	14	26	38	51	63	75	87	100	112	124	136	149	161	173	185

A, B, C, and D are allotted randomly to high and low concentrations of the test and standard materials.

resistance, we found that the current flowing was sufficient to burn the spring set contacts and make the uniselector respond repetitively so we interposed a relay operating a micro-switch between the spring set and the uniselector.)

Two uniselectors, one with eight and one with four banks each of twenty-five terminals, were used to obtain a large number of points. In Fig. 7 the terminals are shown at the top arranged in banks. The banks are placed in order of functioning, but are numbered above in Roman numerals according to their actual position in the uniselector. The index 8 refers to the larger 8-bank uniselector and index 4 to the smaller 4-bank switch. The sequence in which the active terminals are contacted by " wipers " (w) is shown by the small Arabic numerals on the first and last terminals of the banks and where changes from one bank to another occur. The peculiar route from terminal 49 (penultimate terminal of bank V^8) to 50 (penultimate terminal of bank VI^8) to 52 (top of II^8) and at other places in the sequence is explained as follows. Each bank has twenty-five terminals equally spaced along the circumference of a semicircle: the banks are arranged side by side and so lie on half the surface of a cylinder. The wipers, one for each bank, revolve around an axle which lies at the centre of the cylinder and are arranged in two groups in each selector. These two groups of wipers are diametrically opposite each other and each group moves one step forward when the uniselector magnet works. Wipers I^8, II^8, III^8, and IV^8 form group 1 and V^8, VI^8, VII^8, and $VIII^8$ form group 2. While group 1 wipers are sweeping round the corresponding banks of terminals group 2 wipers are moving freely in air. When group 1 wipers have completed their sweep and move off their last terminals group 2 move on to their first terminals. At the beginning of a test, during the first double sweep of the wipers, only banks I^8 and V^8 of terminals are alive. When wiper V^8 reaches terminal 49 relay R1 operates and so cuts out banks I^8 and V^8 and switches in II^8 and VI^8. Now when wiper V^8 is on the penultimate terminal of bank V^8 wiper VI^8 is on the corresponding terminal of bank VI^8. This means that when relay R1 operates activity is transferred from the penultimate terminal of bank V^8 (terminal 49) to the penultimate terminal of bank VI^8 (terminal 50). Then when group 2 wipers move on two steps group 1 wipers commence their second circuit. This time bank II^8 is alive, i.e., terminal 52 of the assay programme in the first of bank II^8. Similarly when wiper VI^8 reaches terminal 98 relay R2 operates, cuts out banks II^8 and VI^8 and activates banks III^8 and VII^8. So terminal 99 is on bank VII^8 at a position equivalent to terminal 98 on VI^8. Terminal 102 is the last of VII^8 and 103 is the first of III^8. When wiper VII^8 reaches terminal 147 relay R4 transfers activity from the driving magnet of the large to that of the small uniselector and relay R3 activates banks I^4 and III^4. The other details of the circuit are clear in the diagram. For the sake of simplicity, resistances, values of components, etc., are omitted.

A full wave rectifier and step down transformer are incorporated between the A.C. mains and the uniselector circuit so that the selector has a 75 volt D.C. supply.

Although the Latin square picked out each time by the uniselector is fixed, the sequence of valve operation may be altered at will because the connexions between

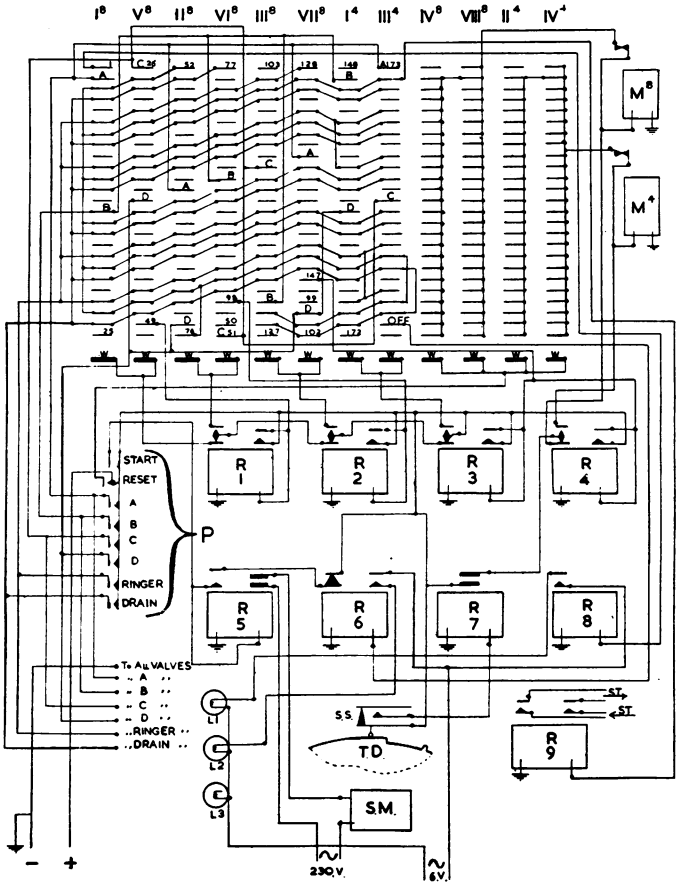

FIG. 7.—Simplified wiring diagram. I^8, II^8, I^4, etc., banks of uniselector terminals · the indices 8 and 4 refer respectively to the 8-bank and 4-bank uniselectors. W. " wiper " which sweeps over the terminals ; one for each bank. 99, uniselector terminals ; small Arabic numerals indicate the operative sequence (for details see text) ; those terminals which supply the drug solutions to the diaphragm are indicated by one of the letters A, B, C, or D. See Table I for sequence of events between drugs. M^8, M^4, magnets operating uniselectors. T.D., timing disc (Fig. 5). S.M., " synclock " motor driving T.D. S.S., make-before-break switch (Fig. 6) operated by T.D. L1, lamp indicating that uniselectors are at starting position. L2, lamp indicating that assay is complete. L3, lamp indicating that T.D. is at starting position (L3 is not connected up because circuit for resetting T.D. has been omitted from diagram). R1, relay switching from I^8 and V^8 to II^8 and VI^8. R2, relay switching from II^8 and VI^8 to III^8 and VII^8. R3, relay switching from III^8 and VII^8 to I^4 and III^4. R4, relay switching from M^8 to M^4. R5, relay controlling start. R6, relay operating L2. R7, relay controlling impulse to M^8 or M^4. R8, relay operating L1. R9, relay controlling stimulation. ST., stimulus from stimulator and to preparation. P, push buttons. The holding contacts to the right of R1, R2, R3, and R4 must make before the other contacts change.

the uniselector and the solenoids are made via a set of " banana " plugs which may be arranged in any desired order ; so any desired sequence of doses can be obtained. Provision is also made for operating each valve manually by the insertion of a series of push buttons which by-pass the uniselector and activate the solenoids directly.

To begin a test the uniselector and timing disc must be in unison. For this purpose a resetting device, operated by a push button, is incorporated into the uniselector to return it to the starting position. Two complete banks of terminals on each uniselector are used for this purpose (IV^8, $VIII^8$, II^4 and IV^4) and when starting position (terminal 1) is reached an indicator lamp is lit. A separate circuit is also included to return the timing disc to its starting point. This is so arranged that while the disc is being reset no other part of the circuit functions and the driving motor switches itself off and lights an indicator lamp when it reaches the correct starting position. This circuit is not included in Fig. 7.

At the end of a complete test an indicator lamp is lit and the machine switches itself off.

Discussion

Although the apparatus is described for a rigid form of assay it is quite flexible. The principle could be applied to almost any routine pharmacological assay on isolated organs. The method of altering the sequence of events by using the banana plugs has already been described: in addition, variations in the order of doses of drugs can be obtained by altering the reservoir into which any drug is placed. The timing of events is also easily changed by substituting other timing discs to suit particular needs.

The principles involved are well known, but the valve is new and may have many uses in a pharmacological laboratory.

One great advantage is that all the apparatus is easily and cheaply obtainable. Most of the electrical equipment used by us was obtained from second-hand stores.

Summary

A previously described principle using P.O. uniselectors is employed to operate new-type valves supplying and draining solutions to and from an isolated organ bath.

The method has been applied to the biological assay of *d*-tubocurarine, but is flexible enough to have a very much wider use. The resulting assay is completely automatic.

We should like to thank Mr. B. Attwood for his help with the construction of this apparatus.

References

Mogey, G. A., Trevan, J. W., and Young, P. A. (1949). *Analyst*, **74**, 577.
Schild, H. O. (1946). *Brit. J. Pharmacol.*, **1**, 135.
Stephenson, R. P. (1949). Demonstration to the British Pharmacological Society. Bristol: July, 1949.
Therapeutic Substances Amendment Regulations, 1948. London: H.M. Stationery Office.