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AN IMPROVED MICROBATH

BY

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A method is described for the study of the actions of drugs and tissue extracts on small pieces of smooth muscle suspended in a bath with a capacity of 0.027 ml. The small size of the bath is an advantage when the total amount of active substance is small. The contractions are amplified 50- to 500-times and recorded isotonically, or approximately isometrically, with a pen writer. This apparatus has the advantage over earlier models that it is more compact and convenient and that the temperature of the muscle can be controlled. It has been used for the estimation of small quantities of acetylcholine and other substances in tissue extracts.

Most of our knowledge of the physiological importance of acetylcholine and other pharmacologically active substances, which are found in extracts of tissues, is based on bioassays and many of these bioassays depend on the contractions of various isolated muscles. At one time the volume of the bath in which these muscles were suspended was about 100 ml., but this volume has gradually been reduced in order to increase the sensitivity of the test. In the technique known as superfusion (Gaddum, 1953; Adam, Hardwick & Spencer, 1954) the muscle is suspended in air and the solution runs over its surface. The solutions to be tested are then applied, in suitable dilutions, directly to the muscle, while the flow of the solution may be temporarily stopped to give the active substances time to act. When this technique is used, the volume of fluid in which the drug is dissolved is generally about 0.5 ml. A smaller volume will not replace the fluid on the surface of the strips of muscle normally used, and superfusion of smaller pieces of muscle is complicated by effects due to surface tension. These considerations led Gaddum & Stephenson (1958) and Gaddum & Szerb (1961) to construct a microbath with a volume of about 0.025 ml. The muscle was attached to an isotonic lever carrying a mirror which reflected a light on to a photocell, from which an amplified current controlled a pen writer. This apparatus gave satisfactory results at room temperature, but was not satisfactory at higher temperatures, and a series of more complicated baths were made before the simple one described here was devised. The object of the present paper is to describe this bath, which can be used at any temperature. The original lever and amplifying current have been replaced by a compact apparatus with transistors.

METHODS

The microbath. In earlier models the bath was horizontal. In the present model it is vertical (Fig. 1, 1) and is made from a block of Perspex (65 × 30 × 12 mm). It is attached to a brass rod, which is held in a Palmer rack-and-pinion block (2), which gives coarse and

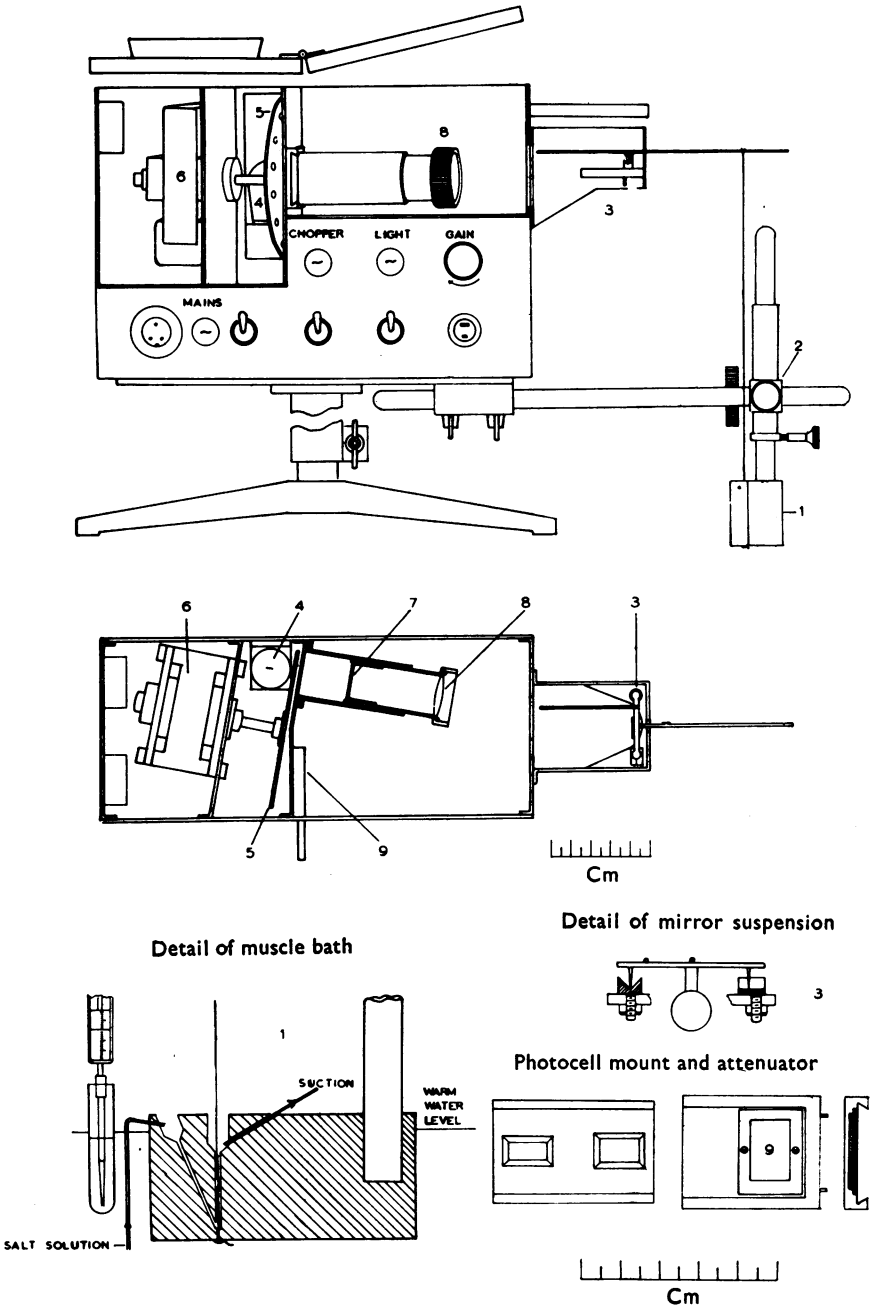

Fig. 1. Above, general view of the apparatus; below, some details on a larger scale. Scales in cm. 1, Muscle bath. 2, Rack and pinion. 3, Mirror suspension. 4, Light. 5, Chopper. 6, Motor. 7, Stop. 8, Lens. 9, Photocell.

fine adjustments in height. The position of the bath can be adjusted by rotating the brass rod and sliding the block along a horizontal rod, which is attached to the case containing the recording apparatus. It is held so that it is almost completely immersed in water of the desired temperature. The strip of muscle is contained in a vertical bath 15 mm long and 1.5 mm in diameter, with a volume of about 0.027 ml. A small hole (0.6 mm) is drilled through the bottom of the bath to take the thread holding the muscle. The bath fluid is contained in a funnel from which it flows to the bottom of the bath at a rate of 1 to 3 ml./min. The rate of flow is controlled by a plastic tap (Townson & Mercer, Ltd.) designed especially for controlling slow flows, and is measured by timing drops. The fluid is warmed in a tube which passes through the warm bath and then runs through a hole in the Perspex into a small well, from the bottom of which it flows to the bottom of the muscle bath. It flows up through the muscle bath into a wider hole (6 mm) from which it is sucked by a tube connected with a water pump.

When drugs are applied the flow is stopped completely by a relay which compresses the inflow tube, and a constant volume (0.05 or 0.1 ml.) of a suitable dilution of the drug in the bath fluid is added to the well, through which the fluid has been running. This rapidly replaces the fluid in the muscle bath. In order to avoid effects due to temperature the drug solutions are warmed in small test tubes immersed in the warm bath. A suitable volume is sucked into a small clean glass tube by withdrawing the appropriate volume of air into a syringe and then transferred rapidly (in about 2 sec) to the well. After a suitable interval (30 to 60 sec) the flow is started again and the drug washed away. The error in the measurement of the volume by this method is unimportant compared with the fact that each active solution comes in contact only with a small glass tube which has been carefully cleaned and dried. The effect on the muscle depends mainly on the concentration of active substance in the solution added and small changes of volume do not affect it. When this system is working properly the fluid in the inflow well falls, when the flow stops, until the well is empty, and no more. This depends on the diameter of the inflow tube (about 0.8 mm) and the difference in height between the bottom of the well and the top of the organ-bath (3 to 4 mm). This height is likely to need some adjustment when a new bath is made.

The thread from the muscle runs vertically to a lever made of fine (0.5 mm) aluminium wire attached to a light cross-bar upon which the mirror is fixed (3). In some experiments the movements were recorded isotonically, with weights attached to a second horizontal aluminium wire. In other experiments the lever was spring loaded and the record was approximately isometric (Szerb, 1961). In still other experiments a comparatively heavy (10 to 20 g) weight of plasticine was suspended on a vertical wire to form a pendulum or auxotonic lever (Paton, 1957).

The cross bar is carried on two sharply pointed hardened steel pins. One of these rests in a circular hardened steel cup angled at 60° inclusive, and the other rests in a small V-block also angled at 60° inclusive; the mounting stud of this block is offset to allow the whole suspension to be rotated so that the light falls on the photocell. In this way 5 degrees of freedom are determined by the suspension, leaving 1 degree of freedom to be determined by the muscle. It was found that there was very little friction even when heavy weights were attached to the suspension. The mirror is plain and its diameter is 1 cm. The contractions of the muscle are amplified 50- to 500-times by this apparatus.

A 4 V, 1 A Miners light bulb (4) (W. G. Pye & Co., catalogue 8565) is mounted on an adjustable holder. The light is interrupted by a chopper (5) which consists of an aluminium disc of 8 cm diameter and 0.7 mm thickness having fourteen holes (diameter 6 mm) on a 3 cm radius. It is rotated by a motor (6) (type SC 9 240 V AC with 0.5 in. stack, from Oliver Pell Controls). The motor speed is 2,400 revs/min giving a frequency of 560 cycles/sec. Because the motor is very lightly loaded, an electrical resistance is inserted in series to promote quieter running and to lessen the tendency to hunt. The value of the resistance is determined by trial and error.

The light then goes through a stop (7), the size of which is adjusted on assembly, so that its focused image just covers the photocell. The condenser lens (8) has a diameter of 1 in.

and a focal length of 1.675 in. (Hummel Optical Co.). It was calculated that this lens would throw an image of the lamp filament on the mirror, and an image of the light stop on the photocell. In practice the position of the lens is adjusted until the image of the light stop is sharply focused on the photocell.

The photocell is a selenium cell (9) (11 × 18 mm ; Evans Electroselenium), which is mounted on an ebonite holder machined to take a slide in which slots have been cut, limiting the effective area of the cell. This prevents overloading of the circuit when the gain is high. With the slot in position and fully illuminated the gain is adjusted until the pen is at the top of the paper.

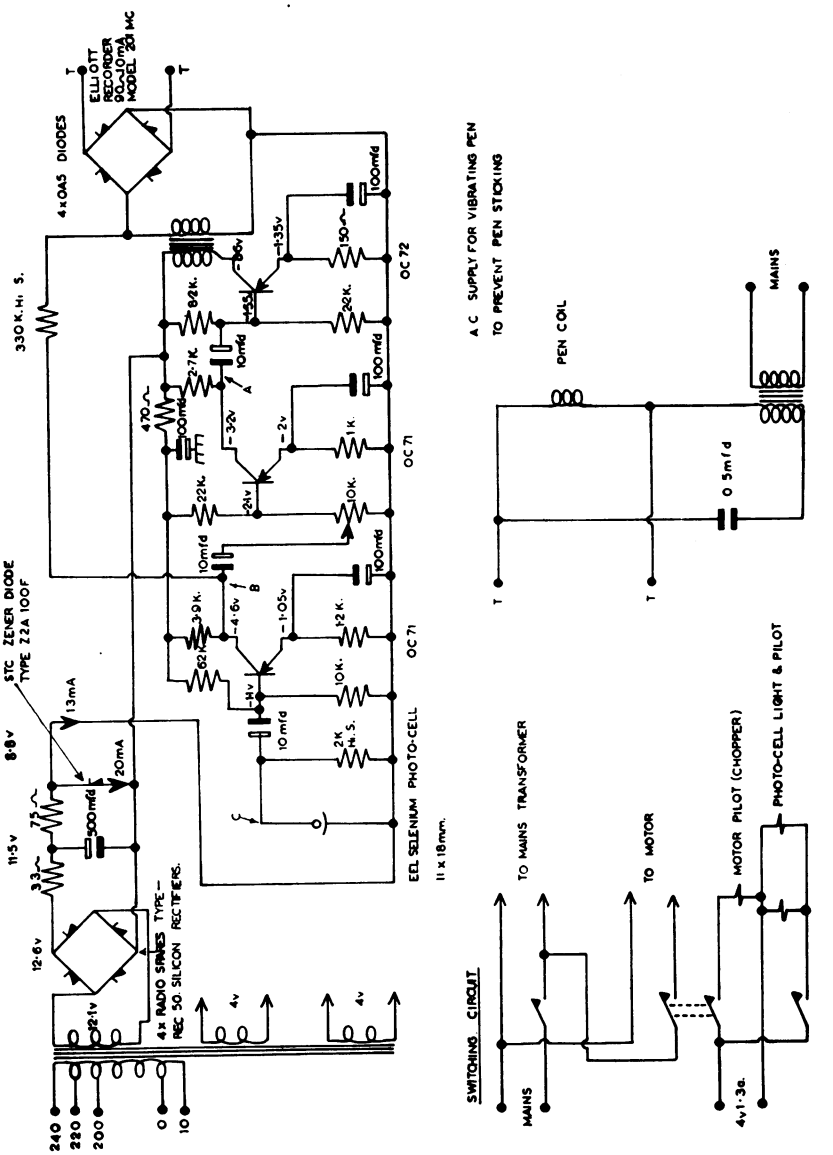

Fig. 2. Electrical circuit.

The electric circuit is shown in Fig. 2. It contains a conventional zener diode stabilized power supply which supplies DC voltage to a three stage transistor a.f. amplifier. The secondary coils in the mains transformer supply 12 V, 40 mA to the amplifier, 4 V, 150 mA to the mains pilot and 4 V, 1.3 A to the photocell lamp and pilot and the motor pilot. Full scale deflection is given by 52 mV at A, 3.1 mV at B and 220 μV at C. The output stage in this case was designed for use with an Elliott Recorder (Model 201 MC, 90 Ω, 10 mA). The output matching transformer was wound to specification (Fortiphone; turns ratio 3:1, primary inductance 1.5 H at 9 V r.m.s. 100 cycles/sec and 11 mA DC; primary resistance 20 Ω; secondary resistance 3.1 Ω).

The chopped light reaching the selenium cell causes a small current to flow through the cell load resistance (2 kΩ) and the AC voltage produced is amplified, rectified and used to deflect the moving coil recorder. A small AC voltage, which causes the pen to vibrate slightly, can also be fed into the recorder in order to combat the tendency of the pen to stick.

The whole of the above apparatus is mounted in a case, attached to a vertical stand, so that it can be adjusted vertically and rotated.

Preparation of the muscle. The strip of muscle is attached to the bottom of the bath and to the lever with threads made by unwinding a fine nylon thread into three. The dissection of goldfish intestine (Gaddum & Szerb, 1961) and of the dorsal muscle of the leech (Szerb, 1961) have already been described. Strips of rat uterus have been prepared as follows. A short length of uterus (3 to 6 mm) is cut off, divided along the mesentery and pinned down on cork. Threads are passed with a needle through the muscle and tied in the middle of each of two opposite sides of the rectangle of muscle. A suitable strip of muscle with a thread tied in the middle of each end is then removed with scissors. One of these threads is then passed through the hole in the bottom of the bath and fixed with plasticine. The other thread is attached to the lever.

RESULTS

Fig. 3 shows an isotonic record of the effects of various doses of 5-hydroxytryptamine from 2 to 8 ng on a strip of rat uterus.

Fig. 4 shows an isotonic record of the contractions of goldfish intestine. A constant dose of an extract of tissue alternates with various doses of uridine diphosphate. It will be seen that the log dose/effect curve was remarkably steep and that the experiment showed that the effect of the extract was equal to that

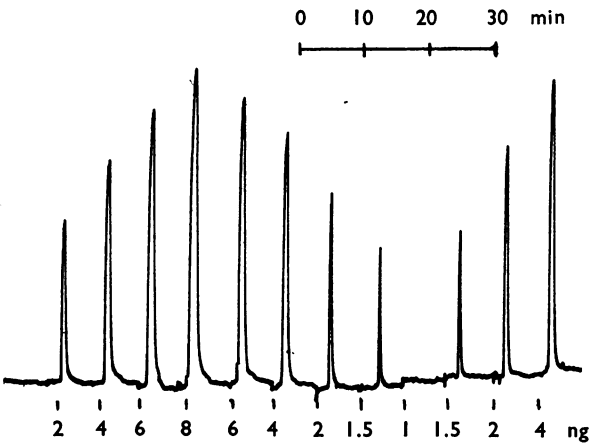

Fig. 3. Isotonic record of the contractions of rat uterus preparation due to 5-hydroxytryptamine. Tension, 200 mg. Time marks, 10 min. Cycle time, 6 min. Total doses in ng.

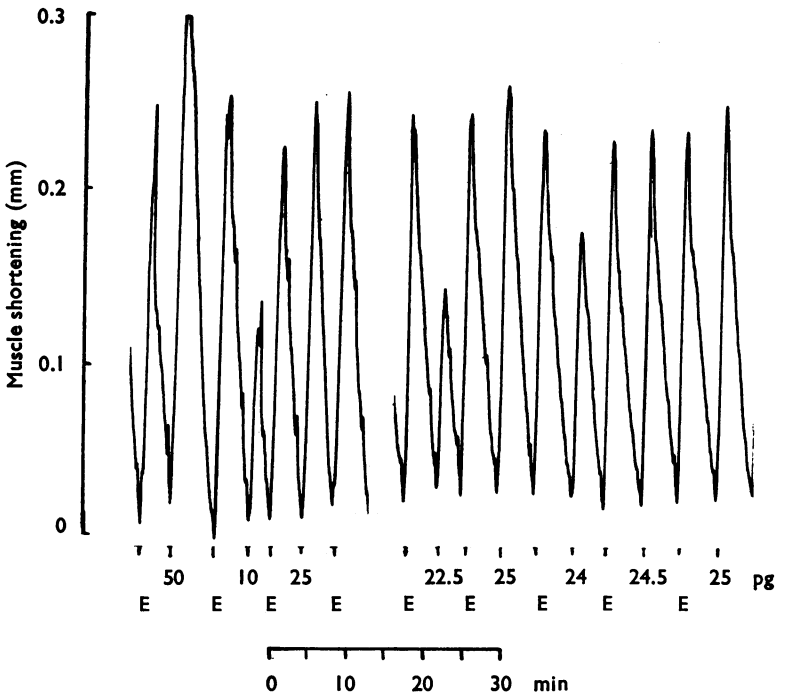

Fig. 4. Isotonic record of the contractions of a goldfish intestine preparation due to a constant dose of tissue extract (E) and various doses of uridine diphosphate. Total doses in pg. Cycle, approximately 4.5 min. Vertical scale, actual shortening of the muscle in mm.

of 24.5 pg and greater than that of 24 pg or less of uridine diphosphate. In this particular experiment the muscle was unusually sensitive and the log dose/effect curve unusually steep, but this kind of result is common in experiments with this apparatus. There is often little difference between the smallest dose to cause a maximal effect and the largest dose to have no effect at all. If the doses are timed and the conditions kept constant the threshold may be constant for long periods and convincing assays may be obtained.

Assays of this type, where the log dose/effect curve is very steep, may give precise and reproducible results, but they are generally more troublesome than assays in which the log dose/effect curve is flatter, and their error cannot easily be calculated. The factors controlling the steepness of the curve are largely unknown. In the assays described by Gaddum, Randić & Smith (1964) a rat uterus and a pendulum lever were used to assay extracts of horse intestine. The log dose/effect was flat and the result depended on (2+2) dose assays, in which the ratio of large to small doses was 2. In other experiments the same technique gave much steeper log dose/effect curves.

DISCUSSION

The apparatus described here has been gradually improved over the course of several years with the object of studying the effects of small volumes of fluid on

plain muscle. The volume of the actual bath is about 0.027 ml., but the drug is generally added in 0.05 ml., which has been found to give consistent, though not necessarily maximal, effects, or in 0.1 ml. The same principle could probably be applied to smaller baths and smaller pieces of muscle dissected out under a microscope, but the present bath has proved small enough for some purposes. The method of suspension of the lever makes it remarkably free of friction, which is negligible compared with the 100 mg or more of tension applied to the pieces of muscle actually used. In good experiments the muscle gives responses to the same concentrations of drug as it would in a larger bath, so that the quantity of extract used in an assay is 0.1 to 0.05 of what it would be in a 1 ml. bath.

The fact that the solution to be tested is applied without dilution means that the result is easily affected by artefacts due to differences in the temperature, tonicity and *p*H of the solutions used and constant care must be taken to avoid this. Frequent tests must be made with control solutions to ensure that they have no action.

In Szerb's (1961) experiments on acetylcholine, the amplification system was that described here, but the bath was horizontal and the lever was spring-loaded and the system approximately isometric. He used it to estimate 25 to 100 pg of acetylcholine with a time cycle of 4 min. This has proved valuable for the detection of acetylcholine in synaptic vesicles (Whittaker, Michaelson & Kirkland, 1964).

Gaddum & Szerb (1961) described a sensitive test for substance P using this apparatus and goldfish intestine, and Gaddum & Smith (1963) showed that the same test is very sensitive to uridine diphosphate.

It seems likely that apparatus of this type will be used with various kinds of smooth muscle to detect active substances of various kinds, but experiments with small pieces of muscle in these small baths have not been uniformly successful. It does not follow that any test on a large piece of muscle can be made more sensitive by using a smaller piece in a small bath.

I am deeply indebted to D. V. Barker, who designed and constructed the compact form of the apparatus described here, and to J. S. Chilvers, who was responsible for the electronic circuit. I am indebted to Dr. M. W. Smith for Fig. 4.

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