Brit. J. Pharmacol. (1958), **13**, 493.

A MICROBATH

BY

J. H. GADDUM* AND R. P. STEPHENSON

From the Pharmacological Laboratory, Edinburgh University

(RECEIVED SEPTEMBER 22, 1958)

A microbath, with a volume of less than 50 cubic millimetres, for testing the effects of drugs on small pieces of smooth muscle, is described. Drugs are applied automatically in any desired order and the record of their effects is amplified electrically. In this apparatus the response of small pieces of rat uterus to drugs is apt to be nearly all-or-none. Contractions of the muscle from a single ring of guinea-pig trachea were recorded. A fall of *p*H was found to reduce the effect of 5-hydroxytryptamine on this muscle while leaving the effects of tryptamine and acetylcholine unchanged.

Those who wish to detect small amounts of drug by their effects on isolated smooth muscle generally use small baths in order to increase the concentration produced by a given total dose of drug. However, it is generally not convenient, with the usual type of bath, to diminish the volume below about 2 ml. If the muscle is suspended in air, drugs may be applied to the surface of the muscle, as in the method known as superfusion (Gaddum, 1953) where the salt solution normally flows continuously over the muscle and is stopped when drugs are applied. This system has been found satisfactory for certain purposes but has various disadvantages. The volume of salt solution is probably about as small as is possible with large pieces of muscle, but the technique does not lend itself to the use of small pieces of muscle. The mechanical effects of each drop then become important. If the suspended muscle is fixed horizontally the fluid may be drained away through a tapered tube fixed under the muscle without forming drops, but it is then difficult to adjust the apparatus satisfactorily. Attempts to overcome this problem have led to the use of the small baths through which fluid flows continuously. These had a volume of 25 to 35 c.mm. which is equal to that of a small drop, but smaller baths on the same principle could probably be made.

METHODS

The apparatus is shown in Fig. 1. The bath is a horizontal one. It consists of a hole (diameter 1.7 mm.) drilled parallel with the axis through a piece of Perspex rod (diameter 2 cm., length 1.5 cm.) so that it just does not reach the opposite face. It is completed by a smaller hole just large enough to take the smallest available sewing needle. Near this small hole a series of radial holes are drilled (diameter 0.8 mm.) through which various solutions may be made to flow. At the far end of the bath the fluid runs vertically down a narrow groove on a flat Perspex surface without forming drops. The fluid is collected on a shelf so that it runs back through a larger hole in the Perspex rod over the bulb of a thermometer and then out through a hole in the side of a small rod of Perspex fixed below the bath, so that the fluid again runs over a vertical Perspex surface before eventually forming drops.

One bath was made with annular platinum electrodes near each end so that the muscle could be stimulated electrically when desired.

A brass rod is screwed into the side of the bath to hold it in position. Before reaching the bath the fluids may be warmed in glass tubes fixed to a Perspex plate. This plate and the bath are both attached to a horizontal brass rod which can be moved in the direction of its own length by a rack and pinion. The fluids run from the glass tubes to the bath through fine polythene tubing pulled to a point and inserted directly into the radial holes in the bath.

The temperature is maintained by an infra-red electric lamp above the apparatus the position of which is adjusted to give the desired temperature on the thermometer in the bath and the smallest possible changes when the fluids are changed; this was found to mean that the lamp was nearer the bath than the warming coils. In some early experiments, bubbles formed in the bath; this complication was avoided when the solutions were equilibrated with air at a temperature of about 40 to 45° and then cooled before the experiment started.

* Present address: The Institute of Animal Physiology, Babraham, Cambridge.

Drugs can be applied to the muscle with a small syringe through one of the radial holes in the Perspex rod, after stopping the flow of saline. A volume of 0.25 ml. is sufficient to replace all the fluid in the bath 7 to 10 times, and smaller volumes can be used.

The drug solution is left in the bath for a fixed time and then washed out by starting the flow of saline again. The syringe needle is left in the hole until this stage, in order to avoid sucking drug back into the hole when the syringe is removed.

Drugs can also be applied mechanically according to a prearranged schedule. Four different drug solutions are held in glass tubes fixed in a stand about 20 cm. above the bath. The flow from these is controlled by relays similar to those used by Schild (1942). The solutions then run through glass tubes on the Perspex plate by the side of the tube which warms the main solution. They then flow through fine polythene tubing to four different radial holes in the Perspex bath.

The relays are controlled by a timing device similar to that described by Austin (see Adam, Hardwick, and Spencer, 1954). The first timing circuit controls the flow of drug at the bath which lasts a few seconds. The second circuit controls the duration of its stay in the bath. The third is not normally used. The fourth and fifth control the flow of salt solution during the interval between contractions. This cycle of events is timed on a stopwatch which is automatically started from zero when each dose of drug is applied.

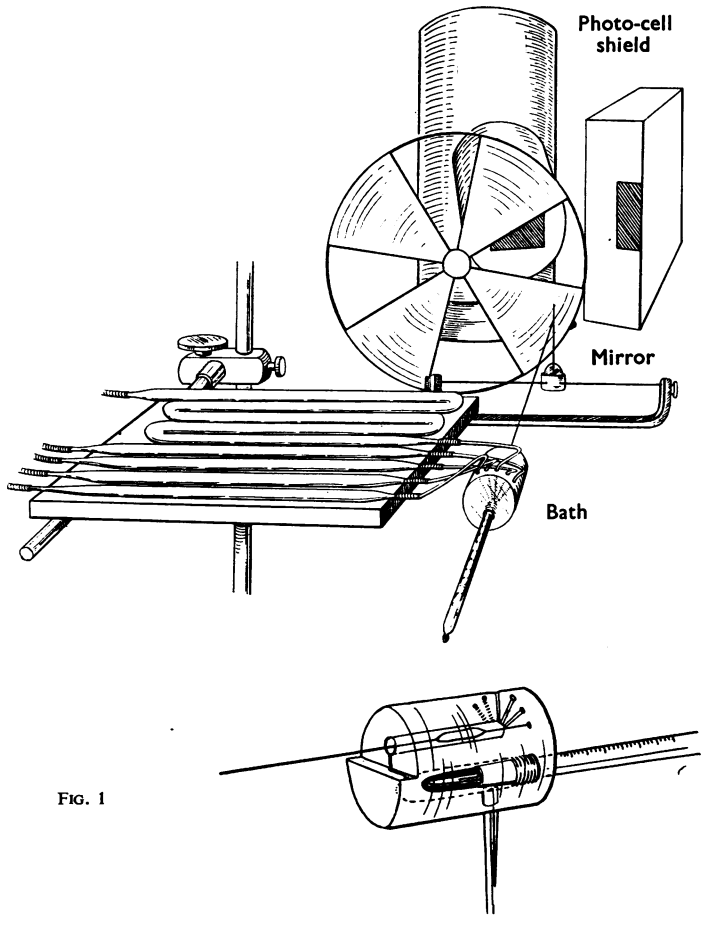

FIG. 1

The order in which the drugs are given is controlled by a set of 24 six-way switches which are connected with 24 positions on a post office uniselector, so that each switch is used in turn. The first four positions of each switch are connected with the four relays which control the solutions. The fifth is connected with nothing at all, so that drugs may be omitted, and the sixth position is connected so that the selector jumps past that position. The apparatus thus allows up to 4 drug solutions to be automatically applied in any order to produce a repeating series of up to 24 doses.

Fine threads are obtained by untwisting terylene gossamer thread and warming to remove the twist. These are attached to each end of a small piece of muscle (5 to 10 mm. in length); one of these is threaded with a needle through the small hole at the end of the bath; the muscle is pulled into position in the bath and the thread fixed with plasticine; the other end is attached to a lever. The most satisfactory results have been obtained with a torsion lever. Isotonic levers tend to be unreliable since a very small frictional resistance is enough to restrict the free movement of these small pieces of muscle. The torsion lever is similar to that described by Condon (1957) and consists of 11 cm. of thin steel wire in a holder which allows the tension in the wire, and the resting position of the lever to be independently adjusted. The thread from the muscle can be attached at different distances from the wire; sometimes the muscle contractions have been nearly isometric, but usually the muscle has been allowed to shorten appreciably. A concave mirror is fixed to the torsion wire to record its movements by altering the amount of light falling on a photocell (G.E.C. gas-filled potassium cell). The filament of a lamp (24 watts, 24 v. D.C.) is focused on to the concave mirror which in turn focuses the image of a square diaphragm on to the photocell. A 108 v. battery is connected across the photocell with a resistance of

2 megohms in series; the voltage changes across the resistance are led, through a capacitance, to an A.C. amplifier. The light beam is interrupted by a rotating sector disc so that the output of the photocell fluctuates and provides a suitable input for the A.C. amplifier. The photocell is screened as much as possible, but some stray light inevitably falls on it. When the rotating disk is placed immediately in front of the photocell this stray light is interrupted and contributes to the record. If, however, the light beam is interrupted between the lamp and the mirror, stray light falls on the photocell without interruption and, provided the photocell is not saturated, the A.C. component of its output is not much affected. The amplified current is rectified and fed to a pen recorder writing on slow-moving kymograph paper. A small 50 cycle A.C. voltage is added to the rectified amplifier output; this imparts a small vibration to the pen which diminishes the effects of friction. When too much vibration was applied a curious artefact appeared in which the pen wandered with a slow frequency over the paper producing a record which seemed, at first, to be due to spontaneous movements of the muscle. This artefact was partly due to the use of a steel pen whose end was not quite smooth and was reduced when the actual writing point was polythene.

Flexibility in operation is obtained by using a short optical lever and relying on electrical amplification to provide most of the magnification of small contractions. A long optical lever provides additional sensitivity, but larger contractions then move the light right off the photocell. The length of the optical lever is largely determined by the radius of the concave mirror. We use a mirror with a radius of 15 cm. and a condenser lens with a focal length of 5.5 cm. The mirror is about 10 cm. from the square diaphragm and about 30 cm. from the photocell. With the thread attached 3 cm. from the torsion wire the optical magnification is 10. The photocell is 3 cm. long so that a 3 mm. contraction of the muscle can be recorded. With maximum amplification, however, the full range of the recorder (about 10 cm.) is covered by a contraction of 0.03 mm.

Results

When the continuous flow of fluid was interrupted the temperature recorded on the thermometer was liable to alter by about 1° and this sometimes affected the behaviour of the muscle. In many cases, for example, there was a temporary increase of temperature when the flow was resumed, which sometimes caused a contraction of the uterus and a relaxation of the trachea. In order to detect errors due to this cause drug-free solutions were often tested and, when the automatic apparatus was used, were usually alternated with each application of drug. It was generally possible by adjusting the position of the heating lamp to avoid serious interference due to this effect.

On several occasions loss of activity of dilute 5-hydroxytryptamine solutions was observed. This was first seen when polythene tubes were used to warm the solutions and disappeared when these were replaced by glass tubes. Something similar happened later and was shown to be due to destruction of 5-hydroxytryptamine by micro-organisms growing in the small diameter rubber tubing connecting the drug reservoir to the warming tubes.

Rat Uterus.—Small pieces of longitudinal muscle of the uterus of rats have been used. They were generally cut from the surface by hand with a fine pair of scissors in one cut so that they were slightly tapered at the ends. These were about 5 mm. long and 0.5 mm. wide. They were suspended in de Jalon solution (Gaddum, Peart, and Vogt, 1949) which had been equilibrated with air at 45°. They contracted in the presence of 5-hydroxytryptamine and carbachol in concentrations similar to those used in larger baths and, in good conditions, gave regular results. If doses were given too frequently tachyphylaxis to both carbachol and 5-hydroxytryptamine developed. The preparations were particularly liable to give all-or-none results, so that one dose had no apparent effect and a dose 50% larger had a nearly maximum effect (Fig. 2).

When the solutions were equilibrated with a mixture of 5% CO_2 in oxygen the effects of 5-hydroxytryptamine and carbachol were reduced (Fig. 3).

Guinea-pig Trachea.—Castillo and de Beer (1947), who introduced the use of chains made by tying pieces of guinea-pig trachea together, give some references to earlier work with tracheal muscle.

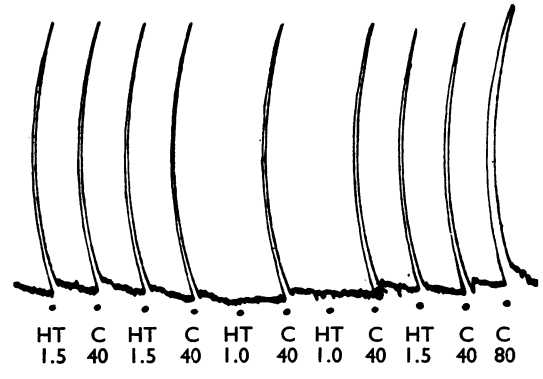

FIG. 2.—Rat uterus, 30°. Drug cycle, 3½ min. HT, 5-hydroxytryptamine; C, carbachol. Concentrations are given in mg./100 l. Blank control between each dose. A steep dose-effect curve is seen with 5-hydroxytryptamine.

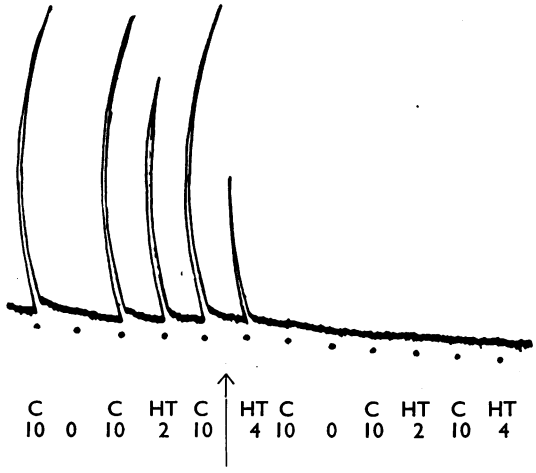

FIG. 3.—Rat uterus, 25°. Drug cycle, 3½ min. HT, 5-hydroxytryptamine; C, carbachol. Concentrations, mg./100 l. de Jalon solution equilibrated with air; from arrow the solution was equilibrated with 5% CO_2 and 95% O_2. Effects depressed.

In the present experiments, preparations were made from a single ring of guinea-pig trachea. The ring was opened by cutting through the cartilage at a point directly opposite the strip of muscle; thread was tied round each piece of cartilage about half-way between the muscle and the cut end. As much as possible of the cartilage was then cut off, but some cartilage remained between the ligatures so that the preparation was not quite flat. When the thread was tied closer to the muscle the preparation was flatter but was less sensitive to drugs. In some later experiments, the diameter of the bath was increased to accommodate the cartilage.

In the early experiments the bathing fluid was Tyrode solution, but in later experiments more satisfactory results were obtained with Locke solution containing 0.1% (instead of 0.015%) $NaHCO_3$ and 0.8% NaCl.

In experiments with Tyrode solution equilibrated with air at 40 to 45° sensitivity was slow to develop and the preparations were often set up in the evening for use the next day. Fig. 4 shows that histamine caused a contraction in a concentration of 10 mg./100 l.

When air is bubbled through such solutions the *p*H is commonly greater than 8. In these conditions the tone of the preparation was apt to increase. This increase could sometimes be stopped by lowering the temperature, and was found to be depressed when the bathing fluid was made more acid either by adding ascorbic acid or by bubbling a mixture of 5% CO_2 in oxygen

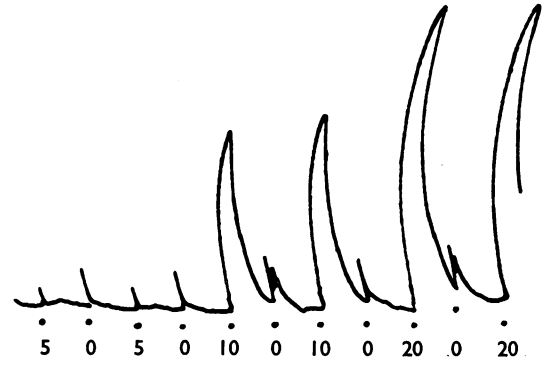

FIG. 4.—Guinea-pig trachea. Tyrode, 26°. Drug cycle, 8½ min. with controls between. Drug contact, 104 sec. Histamine concentrations in mg./100 l. are shown on the abscissa.

through the fluid at 40 to 45° before use. The Locke solution used in the later experiments was therefore generally treated with CO_2 in this way. In these conditions the muscle responded to drugs even in the early stages of the experiment though it was still generally more sensitive on the second day.

Making the solution more acid had the effect of reducing the response to 5-hydroxytryptamine, but it had comparatively little effect on the responses to tryptamine and acetylcholine (Fig. 5).

Preparations made from one particular guinea-pig showed regular bursts of rhythmic activity which were inhibited by exposure to a small dose of adrenaline for a short time (Fig. 6). Atropine had no effect, except in high concentrations (4×10^{-7}) perfused continuously, which reduced the frequency of the bursts.

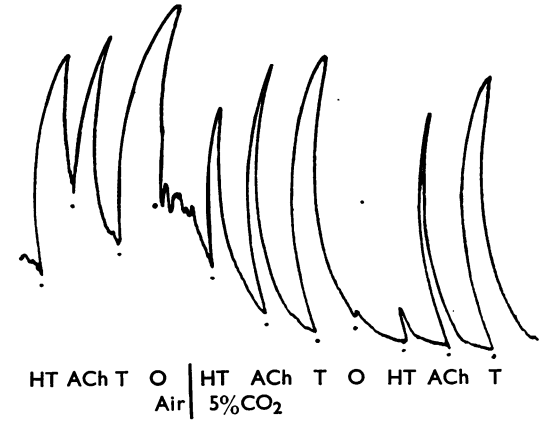

FIG. 5.—Guinea-pig trachea. Tyrode, 30°. Drug cycle, 4¾ min. HT, 5-hydroxytryptamine, 0.1 μg./ml.; ACh, acetylcholine, 0.1 μg./ml.; T, tryptamine, 40 μg./ml.

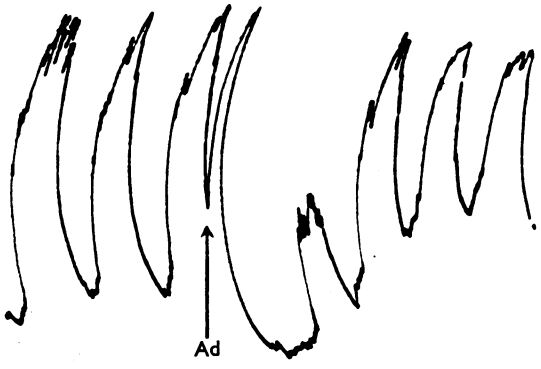

FIG. 6.—Guinea-pig trachea. Tyrode, 26°. Spontaneous movements depressed by adrenaline (Ad) 100 mg./l. for 30 sec.

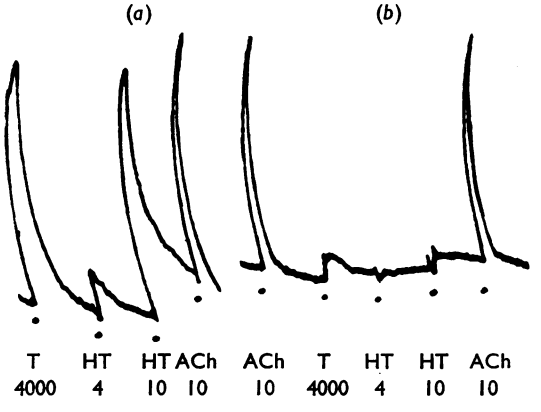

FIG. 7.—Guinea-pig trachea. Tyrode. Drug cycle, 4 min. Drug contact, 35 sec. T, Tryptamine; HT, 5-hydroxytryptamine; ACh, Acetylcholine. Concentrations are shown in mg./100 l. Between (*a*) and (*b*) the muscle was exposed to 2-bromolysergic acid diethylamide (BOL) for 45 min. Effects of T and HT were depressed.

Fig. 7 shows that the response to 5-hydroxytryptamine and tryptamine was abolished by 2-bromolysergic acid diethylamide while the response to acetylcholine was unchanged. The effect of acetylcholine (2×10^{-7}) on another preparation was abolished by atropine (10^{-8}).

DISCUSSION

This apparatus is suitable for studying the responses of small pieces of plain muscle which might otherwise be difficult to use. It might perhaps be suitable for the hearts of molluscs which are sometimes liable to make records on smoked paper which cannot easily be reproduced (Gaddum and Paasonen, 1955). The pen-writer produces clear records of almost any desired size.

It might perhaps be used in experiments such as those of Gaddum, Jang, and Kwiatkowski (1939) where the fluid from a perfused tissue runs continuously over a piece of plain muscle and substances liberated by stimulating a nerve in the tissue are detected as they flow over the muscle.

We are much indebted to Dr. D. C. Simpson for his advice and help in making the recording apparatus.

REFERENCES

Adam, H. M., Hardwick. D. C., and Spencer, K. E. V. (1954). *Brit. J. Pharmacol.*, **9**, 360.

Castillo, J. C., and de Beer, E. J. (1947). *J. Pharmacol. exp. Ther.*, **90**, 104.

Condon, N. E. (1957). *J. Inst. Science Technol.*, **3**, 16.

Gaddum, J. H. (1953). *Brit. J. Pharmacol.*, **8**, 321.

—— and Paasonen, M. K. (1955). Ibid., **10**, 474.

—— Peart, W. S., and Vogt, M. (1949). *J. Physiol. (Lond.)*, **108**, 467.

—— Jang, C. S., and Kwiatkowski, H. (1939). Ibid., **96**, 104.

Schild, H. (1942). Ibid., **101**, 115.