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"CONSTANT FLOW" ORGAN-BATH TECHNIQUES

BY

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It has remained the common practice, since Magnus (1904) first showed that an excised strip of intestine would continue its rhythmic contractions if suspended in warm oxygenated Ringer-Locke solution, to change the contents of an organ bath by a *series* of washings. A drug is injected to give a known final concentration, rapid mixing being brought about by the stream of oxygen bubbles passing up through the bath; then, when the response of the muscle has been recorded, the solution is flushed out and replaced with fresh Ringer-Locke solution. Good techniques of this kind have been described recently by Chen, Ensor, and Clarke (1948) and by Miller, Becker, and Tainter (1948).

That it might be advantageous for some purposes to change the contents of an organ bath gradually instead of intermittently occurred to us in the course of experiments in which blood vessels were perfused at a constant rate. These studies (Fastier, 1948; Fastier and Reid, 1948) suggested that the duration of pharmacological effects observed under such conditions might be no less significant than their initial magnitude; for when a common site of action is indicated, the more active of two stable drugs is likely to be the one whose structural features, apart from providing the necessary "active groups," permit stronger adsorption at the site of action (Albert, 1944; Pfeiffer, 1948). It seemed important to check the idea that at least some of the drugs which have lasting effects upon preparations of this type are ones which are retained tenaciously by the muscle in the face of constant washing with the perfusing salt solution. Accordingly we sought a technique which would enable the outflowing Ringer's solution to be collected for analysis at the same time as the pharmacological effects of an added drug were being recorded.

The first type of apparatus devised for the purpose utilized a constant output pump (Messrs. Palmers' model F31), and the most compact form is illustrated in Fig. 1B. Well-oxygenated Ringer's solution is pumped from the reservoir *R* into the thermostatically controlled vessel *V*, which in turn maintains a constant temperature in the small organ bath *B*. Ringer's solution is forced into the bath *B* at a constant rate via the rubber tubing *I*, which also provides a convenient site for the injection of drug solutions. We have found that even when the pendulum movement of a muscle strip is quite vigorous, reasonably constant changes in tonus can be obtained by injecting such drugs as adrenaline, histamine, and acetylcholine in fixed small doses (Fig. 2). A good base-line is provided meanwhile by the general level of tonus. The Ringer's solution finally escapes through the overflow *O*, where successive portions may be collected for analysis.

The arrangement shown in Fig. 1A does not require a mechanical pump, a steady flow of Ringer's solution

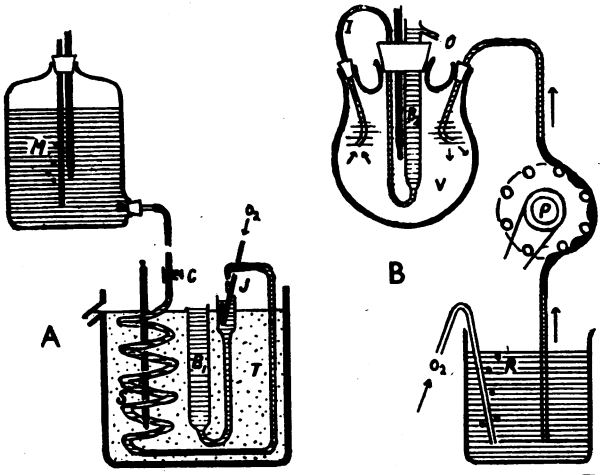

FIG. 1 (schematic).—Two types of apparatus permitting a slow, steady flow of Ringer's solution through organ baths (B_1 and B_2).

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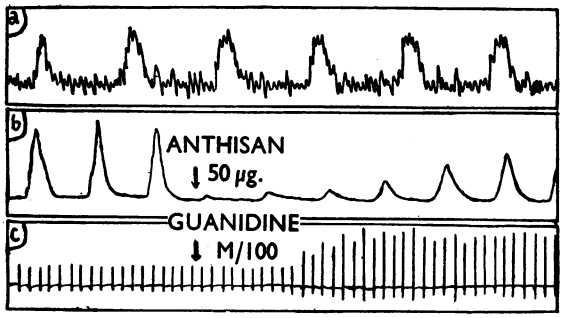

FIG. 2.—Facsimiles of kymograph records obtained by means of constant flow organ-bath techniques.
(*a*) Rabbit ileum washed with Ringer-Locke solution. The increases in tonus were produced with 0.2 μg. doses of acetylcholine injected at 3-min intervals. Pumping rate 30 c.c. per min.
(*b*) Guinea-pig ileum washed with Krebs-Henseleit solution. Histamine (5 μg.) injected at 4-min. intervals. "Anthisan" (50 μg.) injected at the arrow 1 min. before the fourth injection of histamine.
(*c*) Frog sartorius stimulated electrically at 10-sec. intervals. Guanidine hydrochloride (*M*/100) added to the perfusing Ringer-Harvey solution at the arrow. The effect of the stimulus is soon potentiated.

being secured by means of the Mariotte bottle *M*. The screw-clamp *C* adjusts the output. After passing through the spiral *S* in the thermostatic bath *T*, the Ringer's solution drips into the receiving chamber *J*, where it may be further oxygenated before it reaches the preparation. Drugs are injected here. In order to decide upon the best shape for the bath it has been found helpful to watch how a small quantity of injected dyestuff streams past the preparation; this shows whether a drug solution will be distributed satisfactorily in transit.

To what extent the various principles followed—constant flow of Ringer's solution, "external" oxygenation and addition of drugs, the strip filling up most of the bath and always immersed in Ringer's solution—have been used previously, has proved almost impossible to ascertain. This is due not only to the widespread use of organ-bath technique but also to the relative infrequency with which it has been described on its own account. In a paper on the assay of substances liberated from adrenergic nerves, Gaddum, Jang, and Kwiatkowski (1939) have shown that it is very convenient to drip the perfusate from the organ under investigation on to a strip of hen's rectal caecum. Later Kwiatkowski (1941) successfully modified this dripping technique for the assay of histamine. More recently Schild (1947) has wellnigh perfected a method based upon the removal by suction of the excess of Ringer's solution entering a bath from a reservoir; he has also demonstrated the advantages of adding an antagonist drug to the bath fluid before it reaches the gut.

SUMMARY

It has been found advantageous for some purposes to change the contents of an organ bath gradually instead of intermittently. Two types of apparatus based upon this principle are described.

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