Modified plethysmometer for measuring foot volume of unanaesthetized rats

SIR,—We have modified the plethysmometric method of measuring oedema of the rat paw (Buttle, D'Arcy & others, 1957; Harris & Spencer, 1962). Our method differs in that water replaces the two immiscible liquids, anaesthesia is unnecessary and the procedure is carried out by the experimenter.

The apparatus (Fig. 1) consists of limb G made of a 2 ml burette graduated to 0·02 ml and connected through a glass U-tube to another limb which divides into two identical chambers A and B (int. diam. 14 mm; length 4 cm) at its upper end. S(1) and S(2) are two glass stoppers and t and t′ are side-tubes which are connected by polythene tubes through stoppers to a 5 ml syringe for refilling the apparatus with water and for readjustments. Immediately above the stopper S(2) there is an hour-glass constriction.

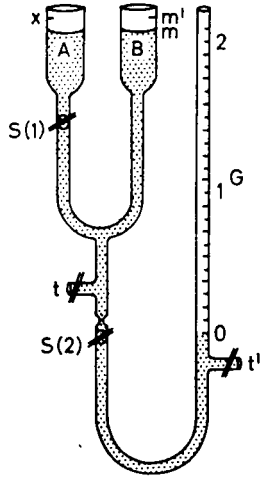

FIG. 1. Diagram of apparatus, see text for explanation.

The apparatus is filled to the mark m of the chamber B with tap water (containing a few drops of Teepol) keeping all the stoppers open and avoiding air bubbles. The side-tube t and the stopper S(2) are then closed and with the syringe the water level in G is brought to 0 and the side-tube t′ closed.

The volume of the rat paw oedema is measured by holding with one hand the unanaesthetized rat wrapped in a towel and by vertically dipping the animal's extended foot, already marked at the ankle-joint with some water-proof ink, into the chamber A till this mark coincides with the mark x. After waiting for a few sec to allow the water level in chamber B to rise (say to m′) the stopper S(1) is closed and the rat's foot withdrawn. The stopper S(2) is then slowly turned allowing the water level in chamber B to return to its initial level at m. This results in a rise in the water level above 0 in limb G. This rise is recorded and the value represents half of the total displacement produced by the animal's foot because of equal distribution of the water in chambers A and B and hence it is multiplied by 2 to give the actual volume.

Values obtained for carrageenan-induced oedema of the rat paw by the method of Harris & Spencer (1962) and by the proposed method were respectively: means 0·60 (7 exp) s.d. 0·23; 0·78 (9 exps) s.d. 0·15; coefficients of variation 38·3 and 19·2%.

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References

Buttle, G. A. H., D'Arcy, P. F., Howard, E. M. & Kellet, D. N. (1957). *Nature, Lond.*, **179**, 629.
Harris, J. M. & Spencer, P. S. J. (1962). *J. Pharm. Pharmac.*, **14**, 464–466.

Antagonism between calcium ions and some myolytic agents on depolarized guinea-pig taenia coli

SIR,—Our previous investigations on the mechanism of action of papaverine and certain derivatives demonstrated that these drugs exert an inhibitory effect on oxidative phosphorylation and that an antagonism occurs between calcium ions and spasmolytic agents, both in polarized and in KCl- or K_2SO_4-depolarized smooth muscle preparations. According to these results, it was suggested that the mechanism of action of papaverine-like drugs could be ascribed to an impairment of the energy supply to the contractile system and to an interference with the essential function of calcium ions in muscular contraction (Santi, Contessa & Ferrari, 1963; Santi, Ferrari & Contessa, 1964; Ferrari, 1964; Ferrari & Gaspa, 1965).

Further investigations demonstrated that the inhibition of oxidative phosphorylation was elicited only by papaverine and other oxy-alkyl-benzylisoquinoline derivatives, whereas the interference with calcium ions was shared by all the spasmolytic agents tested (Tòth, Ferrari & others, 1966). In view of the importance of the latter property as a general mechanism of action of spasmolytic drugs, we have attempted to elucidate whether calcium ions and some myolytic agents behave as competitive or non-competitive antagonists.

The investigations were made with guinea-pig taenia coli depolarized by immersion in calcium free, potassium rich solution, at 35°; contractions were triggered by addition of $CaCl_2$ at concentrations ranging from 0·25 to 100 mM; higher concentrations elicited auto-inhibitory effects. The experiments were made according to Rossum (1963), following the conventional dose-response method and the cumulative dose-response method. In the conventional dose-response experiments, after each contraction, the preparations were bathed in calcium-free Tyrode medium for 5 min and then washed briefly with K_2SO_4-Ringer at room temperature (Ferrari & Gaspa, 1965) to obtain rapid relaxation; finally, K_2SO_4-Ringer was substituted after 3 min by KNO_3-Ringer (Urakawa, Karaki, Ikeda, 1967) to which $CaCl_2$ was added. In this medium $CaCl_2$ induces rapid well-maintained contractions and precipitation of calcium salts is avoided. As antagonists, we employed three myolytic agents with different mechanisms of action: papaverine (hydrochloride) ($1·4$ and $3·2 \times 10^{-5}$M), eupaverin (sulphate) ($1·4$ and $3·2 \times 10^{-5}$M), KCN (2×10^{-4} and 10^{-3}M). These drugs were added to the bath 3 min before $CaCl_2$. In the cumulative dose-response experiments five $CaCl_2$ doses were applied at 3 min intervals in a geometric sequence of increasing doses, to a final $CaCl_2$ concentration giving the maximal response (8–12 mM). Spasmolytic drugs were added 3 min before initiating the cumulative dose-response curve, at the lowest indicated concentrations.

The results obtained both with the conventional dose-response method and with cumulative dose-response procedure demonstrate that all the drugs tested