A device for the rapid measurement of rat foot volume

SIR,—One of the most commonly used methods for studying experimentally induced inflammation in animals is that of measuring the swelling after injection of an irritant substance into the foot of the rat. Our plethysmographic technique is based on that of Kopf & Møller Nielsen (1958), who measured the amount of fluid required to replace that displaced by immersion of the rat paw into a suitable fluid filled vessel.

The fundamental components of the apparatus are an automatic microburette (Technico Automatic 5 ml, B.W. 644, A Gallenkamp & Co.) and a glass displacement chamber (Fig. 1), the outlet tube of the burette being connected by polythene tubing to the inlet tube A of the displacement chamber. The whole is filled with water which serves as the displacement fluid. An electrically rotated tap is used to control the flow of water from the burette to the displacement chamber. The tap (P.T.F.E., "Interkey", G. Springham & Co. Ltd., Harlow New Town) is connected by a flexible spindle coupling (Eddystone Radio) to the armature shaft of a rotary solenoid (Ledex, type LX/511/16357/DHZ, N.S.F. Ltd., London) and is rotated through 90° when the solenoid is energized. The tap is turned by the solenoid either by manual operation of a biased double-pole change-over switch or automatically, depending upon the stage of the measuring procedure.

The dimensions of the components of the glass displacement chamber are fairly critical. Chamber A is 4 cm long and has an inside diameter of 1·3 cm. The inlet tube to A and the tube connecting A to B are of 0·6 cm internal diameter. The inside diameter of B is 1·7 cm and tube C (0·6 cm), is fused centrally into it extending to the same level as the brim of A. Chamber B is 1 cm longer than chamber A. The inlet tube to A and the outlet tube C pass through a Perspex base, and the whole is cemented firmly to this with Araldite [AY 103, Ciba (A.R.L.) Ltd.] moulded into the form of a block. The electrodes E1 and E2 consist of 22 S.W.G. platinum wire soldered to miniature sockets fixed firmly in a strip of Perspex. The electrodes are positioned in B on either side of C so that E2 projects at least 1 cm below E1. The electrode assembly is

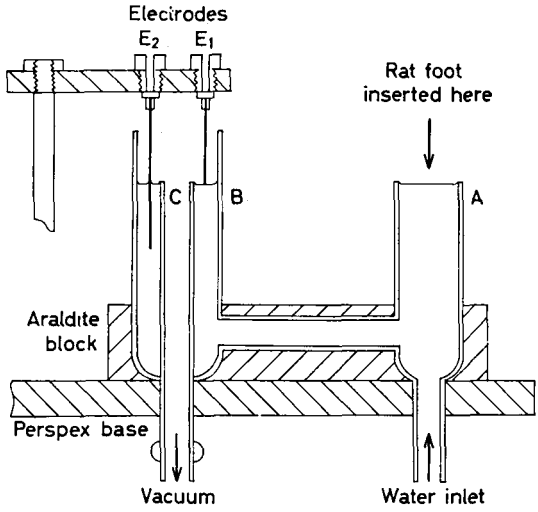

FIG. 1. Glass displacement chamber and electrode assembly.

fixed into the sleeve of a Palmer rack-work 'X' block (D36) attached to the Perspex base so that the electrodes are positioned in B and the level of the tip of E1 can be adjusted to coincide with the brim of tube C.

The glass displacement chamber contains water to the brim of tubes A and C. The rat foot is immersed into tube A to a constant anatomical level, this being indicated by a pad on the plantar surface of the hind foot. Water is displaced into tube B, and an equal volume immediately removed via tube C attached to a vacuum line through a water trap. The volume displaced by the foot is that needed to refill the displacement chamber to the original level. This is found by manually operating the double-pole change-over switch which causes the rotary solenoid to open the tap. Water is thus allowed to flow from the micro-burette into tube A until the level reaches the tip of electrode E1. A circuit is then completed by the water between E1 and E2, which causes the rotary solenoid to turn a further 90° and so cut off the water flow. The volume of water required to fill the chamber is read from the burette which is then refilled to the zero mark. The usual method of refilling this type of automatic burette is to increase the pressure in the reservoir by a hand operated rubber bulb but for convenience we use the pressure from a 5 lb/inch^2 compressed air line controlled by an electromagnetic valve.

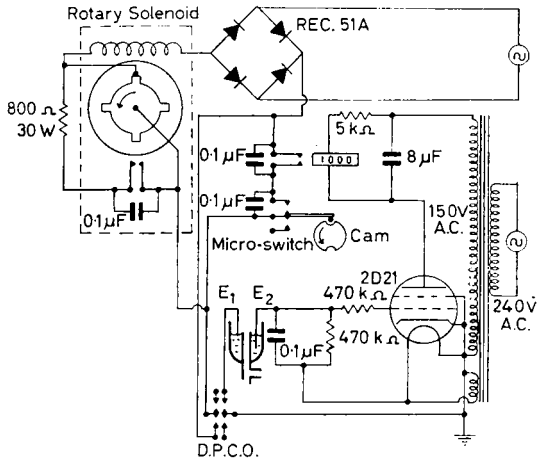

FIG. 2. Circuit diagram.

The electrical circuit (Fig. 2) uses a Thyratron 2D21 valve which is non-conducting until the grid circuit is completed by water touching the foot bath electrodes. The Thyratron then conducts and the relay in the anode circuit becomes energized. The relay contacts allow 240 V DC to pass to the rotary solenoid which rotates through 90° turning the tap to the closed position. A micro-switch, operated by a cam attached to the armature shaft of the rotary solenoid, breaks the current flow to the solenoid when it has rotated the tap to the closed position. This prevents over-heating of the solenoid coil, due to prolonged energization during the period that both electrodes are in contact with the water. The use of a biased double-pole change-over switch to operate the tap also avoids the possibility of permanent energization of the solenoid coil. One pole of this switch energizes the rotary solenoid, whilst the other opens the electrode circuit. When the switch returns to its biased position the electrode

circuit is again completed, causing the rotary solenoid to be re-energized and the water tap closed.

To allow efficient operation of the apparatus the vacuum source, assisting the removal of liquid from tube B, should be maintained at a constant level (−10 cm water) during a series of measurements. The time for immersion of the rat foot should be standardized, 5 sec is optimal. The rate of passage of water from the burette to the foot bath should be adjusted to just less than that causing an overshoot into chamber B.

The error of the measurements when the same foot of each of ten rats was measured five times in random sequence was calculated to be 3·6%.

The advantage of this apparatus over others in common use is that speed of repetitive measurement is achieved without loss of accuracy. The components cost approximately £20 and this compares favourably with commercially available equipment.

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Reference

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The distribution of small concentrations of active ingredients in tablet granules

SIR,—It is common practice when preparing tablets containing small quantities of potent materials to add the active ingredient in solution to an inert basis granulate to obtain even distribution. After drying, coarse aggregates are broken down by sifting and the resultant granulate is tabletted.

During the preparation of a small batch of tablets containing [4-^{14}C]-lynoestrenol, an investigation into the distribution of the lynoestrenol indicated that this was uneven when the drug was applied to the tablet granulate in ethanolic solution and after drying under an infrared lamp. All assays were made using liquid scintillation counting in a Packard Tricarb Spectrometer Model 3003, with and without an internal standard for correcting quenching, on the granulate extracted quantitatively with benzene and ether. Because of the small size of the sample, it was not possible to make a particle size evaluation, therefore samples of the coarser and very fine fractions of the granule were taken and compared with the original. The results are in Table 1. A second sample of basis granulate was prepared and reduced to a fine powder of uniform appearance before the labelled lynoestrenol was added. The distribution found is in Table 2.

TABLE 1. INFLUENCE OF PARTICLE SIZE ON LYNOESTRENOL DISTRIBUTION IN GRANULES. Added amount of labelled lynoestrenol: 2·53 mg (corresponding to 21,800 disintegrations/min) per 98·0 mg granulate.

Type of granule sample	Weight of granulate (mg)	Measured radio activity (d/min)	Calculated mg drug per 98·0 mg granulate
Coarse particles	98·0	33,400	3·89
Mixed	98·0	26,800	3·11
Mixed	98·7	26,300	3·06
Mixed	98·4	24,200	2·82
Fine	98·4	14,500	1·69