NEW APPARATUS

A MODIFIED PLETHYSMOGRAPHIC APPARATUS FOR RECORDING VOLUME CHANGES IN THE RAT PAW

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OEDEMA of the paws is one of the most easily recognised and characteristic outward signs of the anaphylactoid reaction in the rat, and many workers have used the degree of swelling as a measure of the severity of the reaction. Examples of such a measurement include the increase in weight of the paws (Halpern and Briot, 1950), the silhouette area of the paw recorded photographically (Bergel, Parkes and Wrigley, 1951), and the increase in water content of the paws (Rowley and Benditt, 1956). A

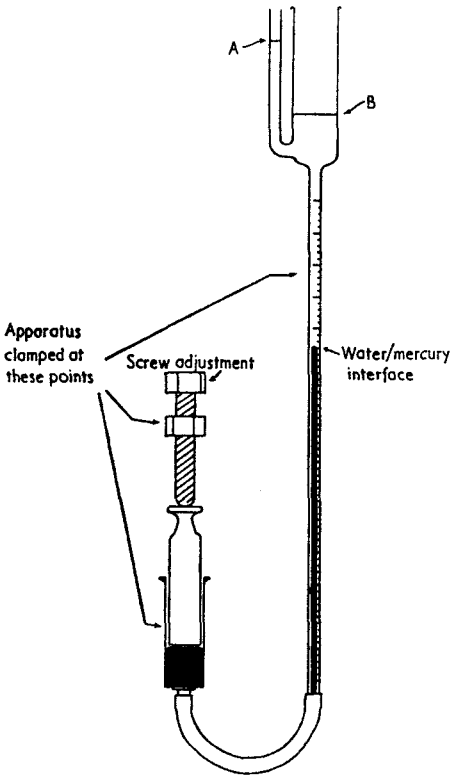

FIG. 1. The modified apparatus for measuring paw volume (see text for explanation).

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more rapid measurement of oedema in a rat paw may be made by a plethysmographic method (Wilhelmi and Domenjoz, 1951; Cerletti and Rothlin, 1955; Adamkiewicz, Rice and McColl, 1955). Buttle, D'Arcy, Howard and Kellett (1957) described a simple apparatus to measure paw volume, in which the paw of an anaesthetised animal was immersed to a pre-determined depth in water, the volume of water so displaced being the volume of that part of the paw immersed.

Two important modifications to this apparatus are now proposed: a side-tube has been added to the mouth of the apparatus, and mercury has replaced the carbon tetrachloride solution of sudan III in the lower part of the apparatus (see Fig. 1).

The modified apparatus consists of a 3 ml. microburette with a side-tube of approximately 4 mm. internal diameter fused to the reservoir above the stem. Thick-walled rubber tubing connects the base of the burette to a 5 ml. syringe. Mercury fills the syringe and lower part of the burette, whilst the upper part contains water to which a little surface-active agent (for example, Teepol) has been added. This ensures complete wetting of the paw during measurement. There are two marks on the upper part of the burette: A, in the side-tube, is approximately 1·5 cm. below the top of the burette, and B, in the burette reservoir, about 5 cm. below the top.

The animal is lightly anaesthetised with ether, and the hind-limb is shaved 24 hr. before the first measurement is made, care being taken to avoid injury to the skin. Before each measurement, the animal is re-anaesthetised with ether just sufficiently deeply to ensure that the limbs are flaccid. The level of the water meniscus is adjusted to mark A by means of the syringe and screw attachment, and the burette reading of the water: mercury interface recorded. The rat's paw is now placed into the mouth of the burette until the tip of the third toe coincides with mark B, thereby causing the water level to rise in the burette and side-tube. The syringe plunger is then withdrawn to bring the water meniscus back to mark A, the new reading of the water: mercury interface being recorded. It has been found in practice to be more accurate if, both before and after immersion of the paw, the syringe is first withdrawn a little too far and then the water level brought to the mark A from below. The difference between the two readings represents the volume of the immersed part of the limb.

The two modifications have been made to the original apparatus for the following reasons. With a side-tube fused on to the burette reservoir and the mark A transferred from the reservoir to this, the water meniscus remains clearly visible when the animal's paw is present in the burette reservoir, and this aids accurate adjustment of the water level. Mercury has a number of advantages over carbon tetrachloride. The carbon tetrachloride solution used in the original apparatus emulsified in the presence of the wetting agent, and made accurate measurement impossible. Also, when making a large number of readings, continuous evaporation of the carbon tetrachloride necessitated its frequent replacement.

Repeated measurements may be made on individual rats. However, we have found that prolonged anaesthesia with ether, urethane or the

barbiturates inhibits the anaphylactoid reaction to egg-white or dextran in rats. For this reason, severe ether intoxication must be avoided and measurements on individual rats should be made at not less than 10 to 15 min. intervals.

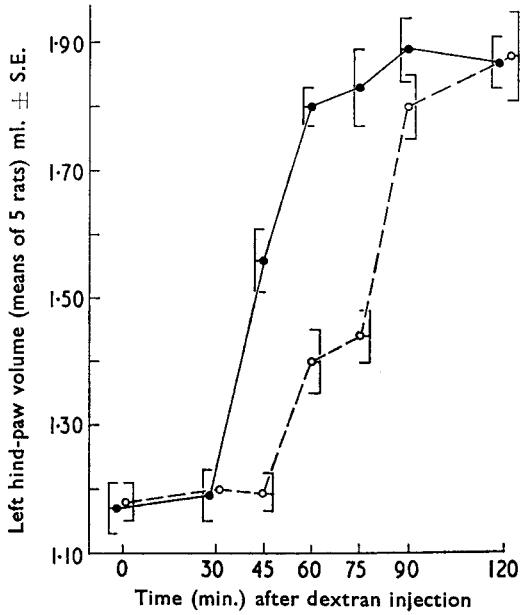

FIG. 2. Effect of intraperitoneal dextran (300 mg./kg.) on left hind-paw volume in untreated male rats (○- - -○), and rats pretreated with soluble insulin (20 units/kg.) 60 min. before challenge (●——●).

Fig. 2 shows a typical record of hind-paw volume changes after the intraperitoneal injection of dextran (300 mg./kg.) into untreated male rats and male rats pretreated with soluble insulin (20 units/kg.) 1 hr. before challenge.

Measurement of paw volume in these animals has revealed that insulin pretreatment hastens the onset of oedema, but does not affect the final degree of oedema after dextran injection.

This apparatus was first demonstrated at the Winter Meeting 1960, of the British Pharmacological Society.

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