LETTER TO THE EDITOR

A SIMPLE DEVICE FOR RAPID MEASUREMENT OF RAT PAW OEDEMA FOR EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

(Received on December 4, 1979)

Sir,

During our studies on new anti-inflammatory compounds we devised a simple method for rapid and accurate measurement of rat paw volumes by the mercury displacement technique with the help of a travelling microscope. The method can be applied equally successfully to other laboratory animals like mouse and guineapig. The advantage of this method over other reported methods (1-6) is the unique combination of simplicity, rapidity, accuracy and reproducibility. The accuracy of this method was confirmed by plotting a standard curve which was essentially linear with minimum deviations (Fig. 2).

The apparatus (Fig. 1) consists of a right cylindrical glass tube (A) (size, 8.0 cm x 2.2 cm) connected to a narrow side-arm (B) (size, 10.0 cm x 0.72 cm). The walls of both tubes were of uniform cross-section and both had open upper end. The apparatus is filled with mercury so that mercury meniscus in the wide arm remains approximately 1.5 cm below the rim when it is clamped vertically rigidly to a stand. A travelling microscope (vertical and horizontal movement type) with vernier constant 0.01 mm is set with its body

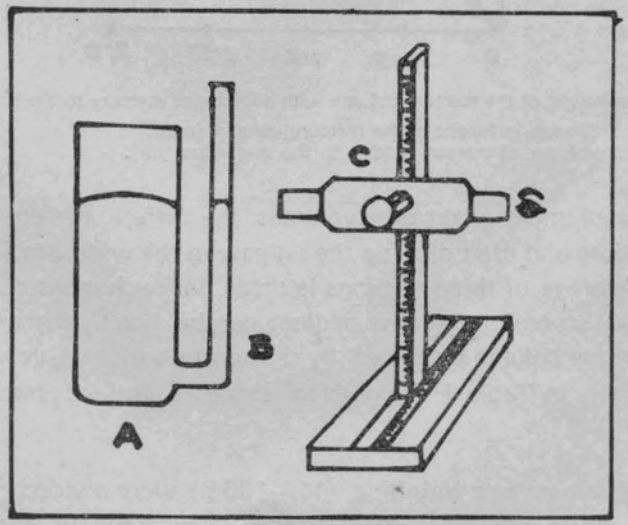

Fig. 1 : A schematic diagram of paw volume measuring apparatus.

tube (C) levelled perpendicular to the mercury column in the narrow arm. The mercury is focused clearly by adjusting the distance of the objective using the adjusting knob.

The mercury meniscus in the narrow arm is coincided with the fixed mark of the eye-piece of the travelling microscope and the reading is made from the vertical scale (initial reading). For each addition of mercury to the wide arm, the new mercury meniscus in the narrow arm is coincided with the fixed mark of the eye-piece and the reading is made as described earlier. The difference in reading gives the change in height of the mercury column. A standard curve (Fig. 2) is prepared using the change in height (mm) of the mercury column and the corresponding volume (ml) of mercury added.

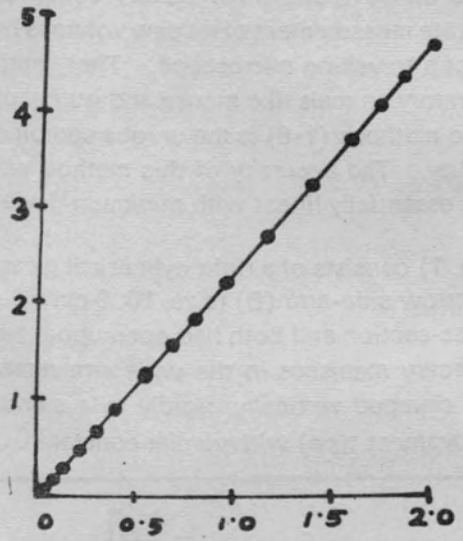

Fig. 2 : Change in height of the mercury column with addition of mercury to the wide arm of the apparatus.
Ordinate : Change in height of the mercury column (*mm*).
Abscissa : Volume of mercury added to the apparatus (*ml*).

For the measurement of rat paw volumes, the differential change in height of the mercury column before and after dipping the rat paw in the wide arm is interpolated in the standard curve. Average of three readings is made for each measurement. Alternatively, for anti-inflammatory screening, rat paw oedema can be directly compared with the change in height of the mercury column produced by the control and drug-treated group of animals. An example is shown in Table I using three standard anti-inflammatory drugs at some selected doses.

Healthy male albino rats weighing (110-130 *g*) were divided into groups of six animals each. Each group received orally either a 10% aqueous suspension of carboxy

methyl cellulose (control group) or a particular drug in the same suspension (test group). After 1 hr 0.05 *ml* of 1.0% carageenin in 0.9% normal saline was injected intradermally in

TABLE I : Percent inhibition of carageenin–induced rat paw oedema by anti-inflammatory drugs.

Drug treatment	*Oral dose*[a] *mg/kg*	*Oedema volume (ml) mean ± S.E.*	*Percent inhibition of oedema*	*Change in height of mercury column (mm) mean ± S.E.*	*Percent reduction in height*[b]
Control (1.0% CMC suspension)	—	0.736±0.032	—	1.75±0.08	—
Aspirin	100	0.484±0.023 P[c]<.005	34.24	1.15±0.06 P<.005	34.30
Phenyl-butazone	30	0.555±0.037 P<.025	24.60	1.32±0.09 P<.025	24.58
Indomethacin	9	0.378±0.042 P>.001	48.64	0.90±0.10 P>.001	48.57

[a] six rats were used per dose.
[b] Percent reduction in height of mercury column is equivalent to percent inhibition of oedema.
[c] Probability values were calculated against control using Student's t-test.

the plantar surface of right hind paw of each rat and the paw volume upto the level of lateral malleolus was measured immediately (7). After 3 hr the paw volume was again measured. The percent inhibition of oedema formation by individual compounds was calculated as–

$$\text{P.C. inhibition} = \frac{Vc - Vt}{Vc} \times 100 \quad \text{or} \quad \frac{Hc - Ht}{Hc} \times 100$$

where, Vc and Hc are *average paw volume* in control group and the corresponding average change in height of mercury column respectively.

and V^t and H^t are average paw volume in drug-treated group and the corresponding average change in height of mercury column respectively.

The results obtained are summarized in Table I which could be reproduced within limits of biological variation.

The salient features of the method which contribute to accuracy and reproducibility of the measurements are as follows : a) the mercury level need not be adjusted to a fixed mark before each measurement; b) the use of the narrow arm facilitates to maintain a clear mercury meniscus and c) the use of travelling microscope enables accurate measurement upto

0.01 mm (which is equivalent to 0.0042 *ml* of volume in our apparatus but can be further decreased by reducing the diameter of the arms). Above all, the mercury displacement technique can avoid the error due to wetting of rat paws.

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