A SIMPLE TECHNIQUE TO MEASURE PAW VOLUME IN ANTI-INFLAMMATORY STUDIES

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Summary : A simple technique using common laboratory materials based on overflow-refill principle is described. The technique provides a retrievable permanent record for re-check, ease of single handed operation, and accurate and dependable volume measurements. The use of mercury and observer's bias have been completely eliminated. Trustworthiness of the technique was confirmed by comparing the estimated and known volume (0.3 to 2.4 *ml*) of different objects. Further comparison of volume estimation of rat paw by this technique with that by another method, showed the new method to be more accurate with coefficient of variation of 8.98% as opposed to 15.60% for the other method.

Key words: anti-inflammatory studies   carrageenan oedema   overflow-refill principle
  simple technique   paw volume

INTRODUCTION

The rat paw oedema test has been one of the most popular anti-inflammatory screening tests and methods to measure the paw volume are many. Most of the methods in vogue rely on the vertical displacement of a pool of mercury which is directly read or is later estimated by pressure change or alteration in electrical resistance of a graphite rod dipped in the mercury. Recent methods involve the use of pressure and volume transducers. Apart from certain disadvantages like high cost, complicated instruments, use of mercury etc., all these systems leave no permanent record of the volume measured which could be rechecked later and are highly subjected to operator's bias as the volume has to be read when the paw is immersed in the appropriate fluid. The present paper describes a simple technique for measuring the volume of rodent paw, without the use of any complicated electronic instruments. The technique involves the over-flow refill principle based on the method of Ferreira (2).

MATERIALS AND METHODS

A schematic diagram of the apparatus is shown in Fig. 1. It consists of an immersion chamber (I) about 2.5 *cm* in diameter, made of glass, connected by means of a pressure rubber tubing to an overflow chamber (Y) about 1 *cm* in diameter with an overflow arrangement, connected to a drop recorder. The pointer of the recording magnet records on a slow moving kymograph, the speed of which can be varied. The inflow is through capillary

tube dipped into the overflow chamber such that its tip is about 4 *cm* below the initial level of the fluid.   The other end of the capillary tube is connected to a slow injector (pump). The rate of inflow can be varied by adjusting the speed of the injector.   Before the start of the experiment the apparatus is filled with normal saline (containing lauryl sulphate 2 *mg ml*$^{-1}$ and ethanol 50 *ml litre*$^{-1}$ to reduce surface tension) to the level (AA) in the immersion chamber such that when the inflow is started, there is constant overflow and this is recorded as vertical lines on the kymograph (inset Fig 1); each vertical marking corresponds to a single drop.
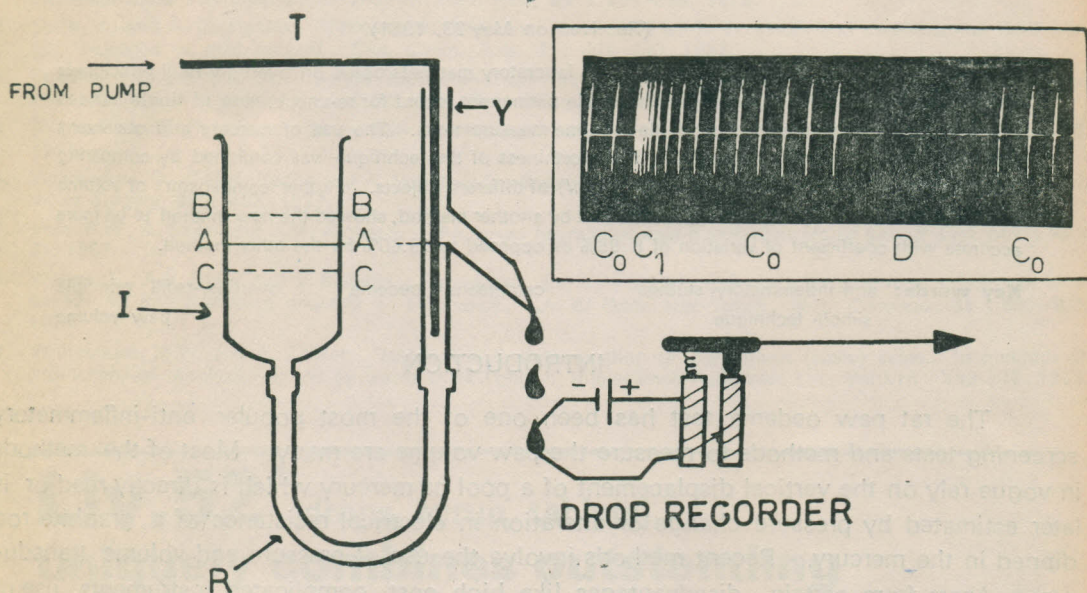

Fig. 1 : *Diagrammatic representation of the technique.*

    I   = Immersion chamber.
    Y   = Overflow chamber.
    R   = Rubber tube connecting I to Y
    T   = Capillary inflow tube from the pump.
    AA = Initial fluid level.
    BB = Raised fluid level on paw immersion.
    CC = Decreased fluid level on paw removal.

    Inset : Sample kymograph record.
    Co = Control drop markings corresponding to AA.
    C$_1$ = Drop markings on paw immersion corresponding to BB
    D   = Absence of drop markings on paw removal.

For measurement, the rat paw is immersed in the Chamber (**I**) upto the tibio-tarsal articulation, when the initial level of the fluid (AA) in the immersion chamber rises to the level (BB) (above the control level AA) with the result, there is a momentary increase in the outflow as seen by the crowded markings on the drum.   This lasts till the level returns

to (AA) and the overflow comes back to control level. At this stage, the paw is removed, with the result, the level in the immersion chamber falls to the level (CC) (below the level AA) and the outflow stops, resulting in a gap in the markings shown as a horizontal line on the kymograph.   The outflow starts when the displaced volume is replaced by the inflow and the level in the immersion chamber comes back to (AA).   Volume of the paw can be calculated by using the formula :

$$v = \frac{dy}{x}$$

where,

v = volume of the paw
d = Gap in recording expressed in mm
y = rate of inflow in ml/sec
x = speed of the drum expressed mm/sec

RESULTS

The accuracy and dependability of the technique was tested by comparing the estimated volume of objects with known volume ranging from 0.3 ml to 2.4 ml.   From the readings (Fig. 2) it could be observed that the estimated volumes (mean of six estimates $\pm$SE) are comparable to the corresponding known volumes, indicating the reliability and accuracy of the technique.

Further to test the accuracy and reliability of the technique, the zero hr volume after the intraplanter injection of 0.05 ml of 1% carrageenan was measured in 24 rats using our technique and that of Bhatt et al. (1). The mean volume by our technique was 1.49$\pm$0.03 ml and by the technique of Bhatt et al. 1.40$\pm$0.05 ml.   The volumes estimated by both the techniques did not differ significantly.   The coefficient of variation calculated by our technique with mean 1.49$\pm$0.13 (SD) was 8.98% while for the technique of Bhatt et al. with mean 1.40$\pm$0.22 (SD) was 15.60%; thus showing the greater accuracy of the technique.

The inhibitory effect of the varying doses of acetylsalicylic acid viz 200 mg, 400 mg and 800 mg/kg orally on carrageenan oedema was 22.39$\pm$2.10, 28.51$\pm$1.68 and 34.54$\pm$1.79 percent respectively when estimated by our technique.   Thus the inhibitory effect was found to be dose-dependent; this again shows that the new technique is trustworthy.

COMMENTS

The obvious advantages of this method include :
(a)   its easy fabricability with common laboratory materials.
(b)   its ease for single-handed operation.

(c) operator bias has been eliminated.

(d) permanent and retrievable recordings available for recheck.
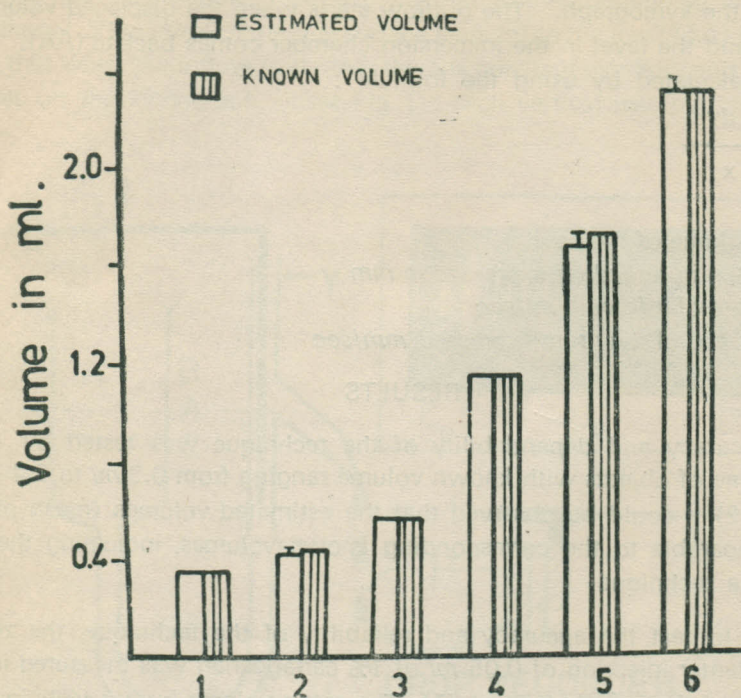
(e) it gives accurate and dependable volume measurements.

Fig. 2 : *Bargraph comparing the estimated volume and known volume :*
Each pair represents estimated volume.
(mean of six estimates ± SE) and known volume of a test object.

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REFERENCES

1. Bhatt K.R., R.K. Mehta and P.N. Shrivastava. A simple method of recording anti-inflammatory effects on the rat paw oedema. *Ind. J. Physiol. Pharmac.*, **21** : 399-400, 1977.

2. Ferreira, S.H. A new method for measuring variations of rat paw volume. *J, Pharm, Pharmac.*, **31**: 648, 1979.