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# An accurate and simple method for measurement of paw edema

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**Abstract**

Several methods for measuring inflammation are available that rely on the parameters changing during inflammation. The most commonly used methods estimate the volume of edema formed. In this study, we present a novel method for measuring the volume of pathologically or artificially induced edema. In this model, a liquid column is placed on a balance. When an object is immersed, the liquid applies a force $F$ to attempt its expulsion. Physically, $F$ is the weight ($W$) of the volume of liquid displaced by that part of the object inserted into the liquid. A balance is used to measure this force ($F = W$). Therefore, the partial or entire volume of any object, for example, the inflamed hind paw of a rat, can be calculated thus, using the specific gravity of the immersion liquid, at equilibrium mass/specific gravity = volume ($V$). The extent of edema at time $t$ (measured as $V$) will be $V_t - V_o$. This method is easy to use, materials are of low cost and readily available. It is important that the rat paw (or any object whose volume is being measured) is kept from contacting the wall of the column containing the fluid whilst the value on the balance is read. 

*Keywords:* Plethysmometer; Inflammation; Paw edema; Rat

## 1. Introduction

There are various methods for measuring inflammation that usually have some restraints or difficulties in their use. For example, the cotton-pellet-induced granuloma (see for example: Meier, Sehuler, & Desaulles, 1950; Niemegeers, Vanbruggen, Awouter, & Janseen, 1975; Sehiatti et al., 1989; Winter & Porter, 1957) is used frequently in inflammation research. Pellets of sterilized surgical cotton are usually implanted bilaterally under the animal's skin and after several days, they are removed and the pellet weights recorded. The difference between the initial and final weights of the pellets are taken to represent the extent of the granuloma tissue, and may be expressed as milligrams of granuloma tissue formed per 100 g body weight. The weights of the adrenals, spleen, liver and changes in body weight may also be noted. Various biochemical parameters such as alanine and aspartate transaminase activities (Reitman & Frankel, 1957), and acid phosphatase activity (Kind & King, 1954) can also be estimated.

Measurements of increased vascular permeability may also be performed, using Evans blue vital dye (Basile, Hanada, Sertie, & Oga, 1984; Wilhelm, Mill, Sparrow, Mackay, & Miles, 1958). The dye is introduced into the peritoneal cavity and the dye concentration estimated by spectrophotometer following the induction of inflammation. Leukocyte counts and/or determining the amount of prostaglandins in the exudate collected from pleural cavity, following induction of inflammation by substances such as carrageenan are other methods (Panthong, Tassaneegakul, Kanjanapothi, Tantiwachwuttikul, & Reutrakul, 1989; Vinegar, Traux, & Selph, 1973).

Amongst the different methods applied for measuring inflammation, there have been estimates of the volume of edema by measuring the dorso-ventral diameter of rat hind paw pads (Murayama, Mori, Bando, & Amiya, 1991; Tsurufuji, Ohuchi, Ishigura, & Miura, 1979) or comparing the weights of excised limbs (first introduced by Winter, Risley, & Nuss, 1963, and thereafter improved by other investigators, Bhatt, Mehta, & Shrivastava, 1977; Chattapadhyay, Chattapadhyay, Roy, & Maitra, 1986; Andreadou, Rekka, Demopoulos, & Kourounaks, 1992). Inserting the inflamed paw in a tube of fluid elevates the fluid level, and test and control levels can be compared. The method offers a

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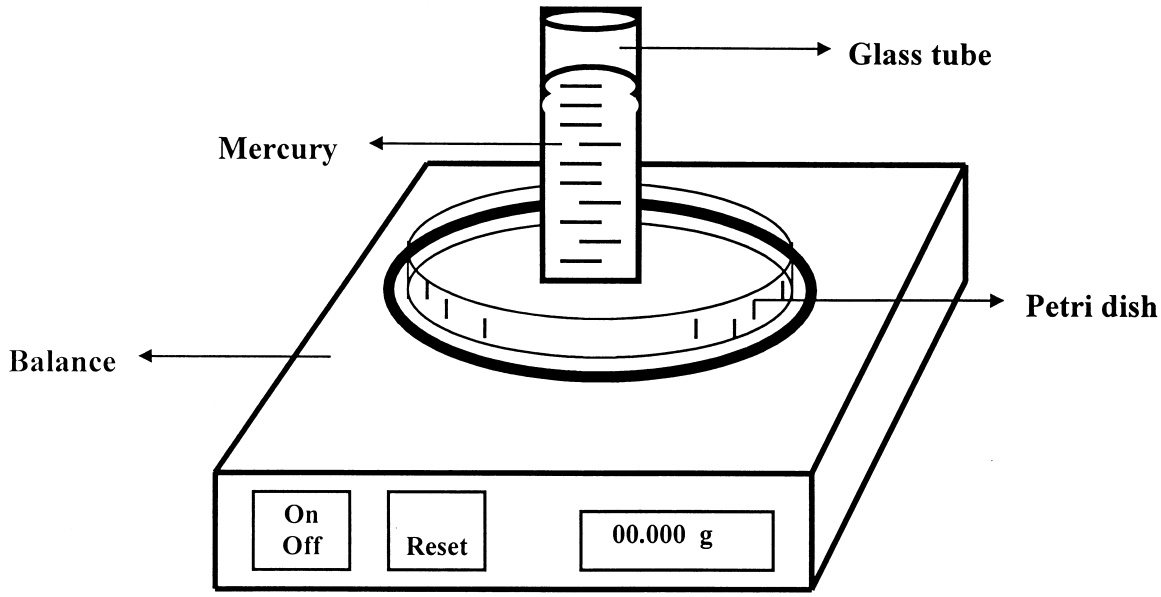


Fig. 1. A line diagram of the apparatus used for determining edema. A 5-cm glass cylinder with 4 cm mercury content is secured to the center of a petri dish and placed on an electronic balance. After immersing the animal paw into the mercury to a predetermined depth, a weight will appear on the balance. The paw volume will be this value divided by the specific gravity of mercury (13.6).

precise and efficient technique when using a hydroplethysmometer (Milanino, 1988), particularly if two platinum electrodes immersed in an electrolyte are used to determine fluid level changes.

In the present study, a novel method is offered for measuring the precise volume of pathologically or artificially induced inflammation. Briefly, a cylinder of fluid cylinder is placed on a sensitive digital balance and the forces necessary to insert the paw into the fluid column, before and after induction of edema, are compared.

## 2. Methods

### 2.1. Measurement instrument

For this purpose, a glass open-top cylinder with an internal diameter of 2 cm and height of 5 cm was attached to the center of a 10-cm diameter petri dish. Four centimeters of the cylinder was filled with mercury, and the cylinder covered with a cap to avoid spilling and evaporation. The petri dish was placed on a digital balance with a suitable sensitivity (Fig. 1). Suitable sensitivity for the balance is proportional to the gravity of the fluid (e.g., 0.1 and 0.01g if mercury and/or water are used, respectively). After removing the cap and resetting, the investigator would be able to begin the experiment.

### 2.2. Standardization tests

To evaluate the accuracy of the method, objects with bizarre shapes and different volumes (1 to 6 ml) were dipped into the mercury using a mechanical driver. The values on the digital balance were recorded. Thereafter, according to the mercury gravity the expected measures were calculated and compared with the observed value. The formula used for this measurement is $V = W/p$, in which $V$ stands for volume, $W$ for weight and $p$ for gravity. The procedure was repeated twice.

#### 2.2.1. Regrading

Objects with pregraded volumes were inserted in the mercury until the balance showed 13.6. Then the volume of inserted part of object was recorded and compared with the real amount. Regrading was continued until readings of 27.2, 40.8, 54.4, 68 and 81.6 were shown on the balance. Each test was repeated twice.

### 2.3. Experimental test

Formalin induced paw edema model and NMRI rats (220–270 g) were used. Formalin 2.5% (0.05 ml) was

Table 1
Comparison between the balance values read following the insertion of different volumes into the mercury column and the corresponding calculated values, did not show any significant difference.

| | Volume (ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | $\chi^2$ | $K$ |
| Expected value (g) | 13.6 | 37.2 | 40.8 | 54.4 | 68 | 81.6 | – | – |
| Observation 1 (g) | 13.9 | 37.1 | 40.4 | 53.7 | 67 | 80.7 | 0.044 | 5 |
| Observation 2 (g) | 13.6 | 37.8 | 41 | 54.1 | 67.2 | 81.1 | 0.024 | 5 |

$K$ = degrees of freedom.

Table 2
Effect of sodium salicylate on formalin-induced paw edema

| Group | *n* | Dose (mg/kg) | Volume of edema (ml) |
|---|---|---|---|
| Distilled water | 8 | nil | 236 ± 10 |
| Sodium salicylate | 7 | 300 | 166 ± 12 * |

Volume of edema: mean ± SEM.
* $p < 0.01$ evaluated by Student *t*-test.

injected subcutaneously in the plantar surface of the rat's left hind paw 30 min after intraperitoneal (i.p.) administration of vehicle (distilled water 0.1 ml/100 g body weight). The paw volume was measured using the above mentioned method prior to, and 1 h after the formalin injection. In other groups of rats, sodium salicylate (300 mg/kg, i.p.) was administered 30 min before the formalin injection. Measurement of the paw volume was carried out before, and 1 h after, formalin injection.

### 2.4. Statistics

The results of standardization tests were compared using $\chi^2$ test. Unpaired Student *t*-tests were used for comparing experimental tests.

## 3. Results

To evaluate the accuracy of the system, different volumes were inserted in the mercury column. Table 1 shows the corresponding values read on the balance. The expected weights are calculated and compared with the observed values by means of $\chi^2$. There were no significant differences between the expected and observed numbers. The results show a high accuracy for the method. The resulting volumes in the regrading procedure were coincident to the real volumes.

Table 2 shows the mean ± S.E.M. of edema volume induced by formalin in the presence and absence of sodium salicylate (300 mg/kg, i.p.). The model obviously clearly confirms the anti-inflammatory activity of sodium salicylate ($p < 0.01$), as manifested by a reduction in edema volume.

## 4. Discussion

When a liquid column is placed on a balance, and an object inserted into it by a force equal to *F*, the displacement of liquid results in an opposing force that 'pushes' against the object to expel it. Physically, *F* is equal to the weight (*W*) of the displaced volume of liquid, and this in turn is equal to the inserted volume of the object into the liquid column, and the balance shows this force ($F = W$).

At equilibrium, $V = W/p$ because at sea level, each kilogram of mass has the same kilogram force weight. *p* for each liquid is predetermined, thus the volume of the inserted part of the object can be calculated, which is the basis of this measurement instrument for edema volume.

The result of the standardization test shows that the method is accurate and the result of the experimental tests shows that the method is qualified for exact measurement of edema volume. Sodium salicylate (300 mg/kg, i.p.) produced anti-inflammatory activity ($p < 0.01$).

The cotton-pellet-induced granuloma method, (Meier et al., 1950; Niemegeers et al., 1975; Sehiatti et al., 1989; Winter & Porter, 1957) is limited to a measurement of subacute or chronic inflammation, and is time-consuming. Furthermore, the present test will also permit the repeated measurement of paw volume as the edema develops. The inflammatory agent bioassay requires expensive instruments and its performance is difficult. In the measurement of increased vascular permeability, estimation of the dye concentration is made by a spectrophotometer and the method is restricted to acute inflammatory tests. Carrageenan-induced pleurisy method is available for acute inflammatory tests. In this test, leukocyte count or the amount of prostaglandins in the pleural liquid before examination is undetermined. In the manual dorsoventral measurement of paw diameter (as a score of edema), the precision might be insufficient and necessitate the use of a larger number of animals and/or repetitive measurements. Hydroplethysmography is a fast and exact method using a similar principle to the new test described here, but during a course of experiments fluid or electrolyte properties can change, necessitating repeated calibration.

A plethysmometer and digital balance are available in most laboratories. This method is exact and easy to perform, but the paw must be kept from contact with the wall of the plethysmometer cylinder. Other investigators can make the hydroplethysmometer by use of high-sensitivity balance, and by adding suitable ITI hard- and software the data can be recorded and statistically evaluated electronically.

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