ALLOMETRIC METHOD FOR THE LONG TERM OBSERVATION OF ADJUVANT ARTHRITIS IN RATS

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Accepted January 13, 1976

Abstract—An allometric method for estimating the volume change in inflamed paw of rats is described. The technique is effective and advantageous for long term observation of adjuvant arthritis which lacks a suitable reference criterion due to the simultaneous swelling of the control paw. In the present method, the inflammatory intensity (IF) of paw edema is estimated by means of the formula:

$$\text{IF} = \frac{2V_t cX^d(W_t cX^c - W_t)}{W_t} \cdot 100$$

where $V_t$ is the paw volume, $W_t$ is the body weight and $X$ is the tail length of the inflamed rat. The constants $a$, $b$, $c$ and $d$ are obtained from the normal rats using the relative growth law, $W = aX^b$ and $V = cX^d$ (where $W$ is the body weight, $V$ is the paw volume and $X$ is the tail length).

The differential volume of paws between untreated and treated legs of the same experimental animal has been employed as a convenient parameter of the inflammatory intensity of the paw edema in rats (1, 2). This method is effective in assessing the short-term experiment, but is not adequate for the long term observation of a delayed type inflammatory reaction such as adjuvant arthritis. The reason is that the control paw usually swells as a consequence of systemic immune response. In order to overcome this difficulty, a modified experimental design is usually employed, where a population of rats is randomly divided into two groups, one as control and the other as the experimental set. The volume of the paws is measured in both sets at decided intervals and the difference between the two groups is analyzed statistically. However, this method has the drawback that during prolonged experimentation, rats in the experimental set lose not only body weight but also the volume of paw due to the physiological changes caused by chemical treatments. This feature makes direct comparison between two groups of rats ambiguous.

There is to date no satisfactory method for assessing long term observation of the delayed type of inflammation. We devised an allometric method based on the "Relative Growth Law" of Huxley and Teissier (3) which allows for a quantitative estimation of the volume of inflamed paws. This new method involves a two-step compensation to obtain control values depending on the length of tail and body weight. The derived equations have proven to give correct estimates in the inflammatory reaction in rats.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats, weighing 250–270 g were housed in metal net cages and
sustained on a diet of CE-2 (CLEA Japan Inc., Tokyo) and water *ad libitum*. Temperature (24±2°C) and humidity (50 to 70%) were maintained at constant levels.

**Inflammatory reagent**

Complete adjuvant (10 mg dead *Mycobacterium tuberculosis*, Aoyama B strain in 1 ml liquid paraflin) was used to produce adjuvant-arthritis.

**Mathematical consideration**

By application of the "Relative Growth Law" (3), body weight (W) and paw volume (V) of growing rats can be expressed as a function of tail length (X) as follows,

\[ W = aX^b \]
\[ V = cX^d \]

where a, b, c and d are presumed to be allometric constants of rats under identical living conditions.

The experimental treatments, such as inflammatory irritation and drug administration inevitably result in changes in the body weight and the paw volume of each animal, but not in the length of the tail. Therefore, the above equations including tail length as a function will give rise to the body weight and the paw volume of individual rats equivalent to the values when they are not treated. In order to determine actual reference volumes of paw volume for the experimental set, a second derivatization is necessary. The systemic effect of drugs and/or irritant is reflected by the change in the body weight and the paw volume. As demonstrated in the results, the following relationship was found between the body weight and the paw volume when the body weight of rats is deliberately reduced by fasting.

\[ \frac{JV}{cX^d} = \frac{JW}{2aX^b} \quad \text{------------------(A)} \]

where \( JV \) and \( JW \) are changes in the paw volume and the body weight respectively induced by fasting.

It is assumed that the above relationship (A) holds true also for animals losing body weight by the systemic effect of drugs and/or irritant. The adjusted volume of paw, \( cX^d - (cX^d/2) \left[ 1 - \frac{aX^b \cdot JW}{aX^b} \right] \), is regarded as the reference for estimating the true change in paw volume induced by inflammation. Thus, inflammatory intensity (IF) is estimated by means of the formula:

\[ \text{IF}(\% ) = \left( \frac{V_t}{cX^d \cdot JV} - 1 \right) \times 100 \]

\[ = \left( \frac{2V_t}{cX^d \left[ 1 - \frac{aX^b \cdot JW}{aX^b} \right]} - 1 \right) \times 100 \quad \text{--------------(B)} \]

where \( V_t \) is the paw volume and \( W_t \) is the body weight of the treated rat. The body weight \( (W_t) \) is theoretically equal to \( aX^b \cdot JW \). The tail length(X) of the treated rat is almost
On the other hand, relative changes in body weight, which are assumed to reflect the systemic effect of drug/or irritant, are estimated as follows:

\[
\frac{W}{W_0} \times 100 = \left( \frac{W}{aX^b} \right)^{100}
\]

Procedure

Using a sterile syringe, the rats were injected subcutaneously with 0.05 ml of complete adjuvant into the plantar portion of one of the hind paws. Body weight (g), paw volume (ml) and tail length (cm) were measured at various intervals after the injection. The volume of hind paw was measured plethysmographically with a modified apparatus described by Van Arman et al. (1) and Winter et al. (2). For the measurement, the rat paw (below Malleolus lateralis) was immersed into a mercury reservoir connected with a pressure transducer (Nippon Koden Co., Model MP-4) and a recorder (Toa Denpa Co., Model EPR-2T).

RESULTS

Allometric constants

The relationships among tail length (X, cm), body weight (W, g) and hind paw volume (V, ml) of normally growing rats (8 to 13 weeks old) were analyzed to determine the allometric constants a, b, c and d. It was ascertained that both the relations between log X and log W, and between log X and log V were almost linear. There was no significant discrepancy among each constant in the experiments as shown in Table 1.

Consequently, deviations from the above standard correlations in body weight and paw volume were evaluated when the rats were fasted for one or two

<table>
<thead>
<tr>
<th>Exp. Date</th>
<th>log W</th>
<th>log (a\times b\times X)</th>
<th>log V</th>
<th>log (c\times d\times X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May, 12, 1971</td>
<td>1.36 (10^{-2})</td>
<td>3.56</td>
<td>3.72 (10^{-4})</td>
<td>3.09</td>
</tr>
<tr>
<td>Aug., 15, 1971</td>
<td>1.26 (10^{-2})</td>
<td>3.61</td>
<td>2.14 (10^{-4})</td>
<td>3.34</td>
</tr>
<tr>
<td>Nov., 11, 1971</td>
<td>1.38 (10^{-2})</td>
<td>3.53</td>
<td>3.45 (10^{-4})</td>
<td>3.01</td>
</tr>
</tbody>
</table>

Each observation was done for 5 weeks from the date mentioned.
Constants a, b, c and d were obtained by the least square method.
days. It was found, as shown in Fig. 1, that $JW/cX^d$ was almost equivalent to $JW/2aX^b$.
This relation can be also applied to the cases of body weight reduction induced by the
systemic effect of drugs and/or irritant. Equating these expressions gives the formulae (A)
and (B) described in the method.

Observations of adjuvant arthritis

Rats were injected subcutaneously with 0.05 ml of complete adjuvant into the plantar
side of the right hind paw. Untreated (control) rats were also prepared to determine the con-
stants $a$, $b$, $c$ and $d$ for this experiment. The representative time courses of volume increase
in the inflamed paw shown in Fig. 2 were calculated by the equation (B). Changes in body
weight calculated by the equation (C) are also given.

In another series of experiments, body weight in some rats with inflamed paws was
reduced by excessive administration of anti-inflammatory drugs given to determine the
efficacy of the present method as shown in Fig. 3. The data are summarized in Table 2.
The inflammatory intensities (IF) of Rat C and Rat D in the figure were compared with
those which were calculated, irrespectively of body weight reduction, from the volume
difference between the left paw without secondary inflammation and other inflamed paw of
the same animal (Rat C), or between the mean volume of normal paws of untreated rats
(normal group) and the inflamed paw of Rat D. Relative changes in body weight ($JW/ aX^b$),
which were calculated by the present method (Equation C), are also shown. In the
case of Rat C, there was no discrepancy in the inflammatory intensity between the present

![Inflammatory intensity (IF)]

**Fig. 2.** Representative time course of volume change in inflamed paw and of body
weight reduction.
Rat A: one case with secondary inflammation.
Rat B: one case without secondary inflammation.
rp: a right paw with adjuvant injection,
lp: a left paw without adjuvant injection
Fig. 3. Model cases of adjuvant arthritis for the evaluation of the present method.

Rat C: A case with an excessive administration of hydrocortisone acetate (25 mg/kg/day s.c., from the 6th day after adjuvant injection). Right hind paw (●) was inflamed at time 0 day. The left paw (●) did not show any secondary inflammation.

Rat D: A case with an excessive administration of indomethacin (5 mg/kg/day p.o.). Administration of the drug was started from the 16th day after irritation, where the secondary inflammation had appeared in the left paw (●) as well as in the right paw (●).

Table 2. Comparison of the inflammatory intensity between the present method and others (cf. Fig. 3)

<table>
<thead>
<tr>
<th></th>
<th>Rat C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>8</td>
<td>10 (days)</td>
<td></td>
</tr>
<tr>
<td>I. Body weight change (%)(^1)</td>
<td>1.9</td>
<td>9.1</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>II. Inflammatory intensity (%(^2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) by the present method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right paw*</td>
<td>137.1</td>
<td>126.3</td>
<td>72.2</td>
<td></td>
</tr>
<tr>
<td>left paw</td>
<td>0.1</td>
<td>0.7</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>ii) by others(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right paw*</td>
<td>136.2</td>
<td>109.1</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>left paw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>18</td>
<td>20 (days)</td>
<td></td>
</tr>
<tr>
<td>I. Body weight change (%)(^1)</td>
<td>8.7</td>
<td>14.1</td>
<td>-16.8</td>
<td></td>
</tr>
<tr>
<td>II. Inflammatory intensity (%(^2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) by the present method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right paw*</td>
<td>208.6</td>
<td>91.8</td>
<td>58.0</td>
<td></td>
</tr>
<tr>
<td>left paw</td>
<td>118.6</td>
<td>42.2</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>ii) by others(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right paw*</td>
<td>187.0</td>
<td>69.2</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>left paw</td>
<td>100.0</td>
<td>23.8</td>
<td>-17.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) inflamed by adjuvant injection, \(^1\) body weight change was calculated by the equation (C), \(^2\) \((\text{right paw volume} - \text{left paw volume}) \times 100, 3) \((\text{each paw volume of inflamed} \times \text{mean paw volume of normal group}) - 1) \times 100
method and the others. This is reasonable as there is an actual reference volume of the paw in Rat C. Rat D presents a good example of a single calculation which neglected to consider body weight reduction and incorrect estimates were deduced.

**DISCUSSION**

The present technique appears to be effective and advantageous for long term observation of adjuvant arthritis which lacks suitable reference criteria due, for example, to the simultaneous swelling of both paws. The present method can be also applied for "individual diagnosis or prognosis".

Our six months experience applying this technique showed that there is no remarkable difference in the allometric constants among tail length, body weight and paw volume when the same strain of rats is used under constant environmental conditions. The constants a, b, c and d, however, are better determined for each experiment, as changes in rearing environment easily affect body weight.

Since there are many allometries among length or weight of living body parts (4–5), the present thesis can be applied to other types of experiments such as chronic toxicity tests to determine changes in weight of tissues or teratologic tests to assess changes in length of body parts. When applying the present technique, it is of importance to determine how the systemic effect of treatment, such as body weight reduction, affects standard correlations.

Acknowledgements: Sincere gratitude is due to Prof. S. Inoue of Gunma University for going over the manuscript. The authors are also indebted to Mrs. E. Suzuki for skillful technical assistance.

**REFERENCES**

3) **Huxley, J.S. and Teissier, G.**: *Nature*, **137**, 780 (1939)