

Comparison of Circumferimetry with Observation in Quantitation of Serotonin-Induced Edema in Rat Paw. (23993)

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Selitto and Randall(6) described a method for evaluating anti-inflammatory drugs by their ability to suppress edema resulting from injection of yeast under the plantar aponeurosis of the rat hind foot. This swelling reached its peak in 2 to 4 hours, and lasted to 48 hours. Inhibitory action of the drug was measured by circumferimeter. Later Benditt and Rowley(1) showed that serotonin in minute dosage was capable of producing edema which reached its peak in 2 hours, with total duration about 4 hours. Kelemen (4) demonstrated ability of salicylates to protect against serotonin induced edema. The magnitude of this action was estimated visually.

The present communication reports results of study designed to compare accuracy of circumferimetric measurement with that of visual scoring in the rat paw made edematous by serotonin injection.

Methods. Albino rats ranging from 150-175 g were distributed by random assortment into 4 groups of 10 rats each. A hydrating dose of 25 cc/kg of water was fed to each rat to insure fairly consistent hydration. One hour later, aminopyrine was injected subcutaneously at logarithmically spaced doses of 50, 100, and 200 mg/kg. These treatments were assigned at random. This injection was followed immediately by plantar injection of serotonin (2.5 μ g in 0.1 cc) in both hind feet. One group served as a control. The hind feet of each rat were measured by a circumferimeter, made by affixing a loop of silk suture to barrel of a 10 ml Luer-Lok® syringe. A line was marked on the foot prior to measurement that all readings be taken at the same site. Measurements were taken initially and 2 hours after drug administration. Logs of increments in diameter were averaged. After each measurement the edema of each animal was scored by 3 scorers using a 0-4 scale. The scorers had no knowledge of the treatment of

the animals. Each cage of rats could contain animals treated with any dose level of aminopyrine and controls, assorted by random assignment of drug and dose. Since no statistically significant difference was observed among the scorers, the 3 scores on each animal were combined into a single average.

Results. Table I presents Y_1 , the mean scores obtained by 3 scorers observing 4 groups of animals 2 hours after treatment with aminopyrine, and Y_2 , the circumferimetric data obtained on same animals. The circumferimetric data are expressed in terms of the response metameter,

$$Y_2 = 100 \log \frac{2 \text{ hr post treatment reading}}{\text{Zero hr control reading}}.$$

The metameter Y_2 was adopted because it adequately allows for inequalities in zero hour readings and its variability is independent of its mean value as required in a bioassay (Finney, 3).

Fig. 1 shows dose response curves obtained with these 2 types of response.

To reach a decision concerning which of the 2 available technics was better suited for quantitative work, the index of precision (Ott, (5)); Bliss and Marks(2) was computed for each procedure. As discussed in the cited references, λ is a dimensionless quantity which specifically characterizes the inherent precision of the assay in question. The smaller the value of λ the greater the inherent precision of the assay. The values obtained were as follows:

For scoring technic $\lambda = .952$

For circumferimetric assay $\lambda = .568$

Thus, the inherent precision of the assay using the scoring procedure may be expected to be much less than that of an assay based on circumferimetric assay expressed as metameter defined in above equation. To assay the potency of a sample with an error of $\pm 25\%$ would require a total of 308 animals using the

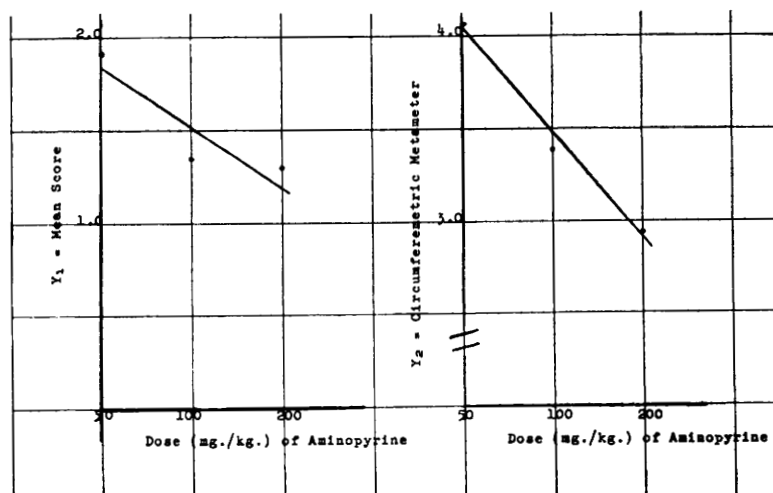


FIG. 1a (left). Dose response curve obtained using scoring technic.

FIG. 1b (right). Dose response curve obtained using circumferimetric data.

scoring procedure and 100 animals using the circumferimetric technic. Thus, on the basis of assay precision alone, the circumferimetric technic is the better. An assay based on this technic, while certainly not of high inherent precision is feasible, being about as precise as the well-known assay for Vit. A in the depleted female rat.

Discriminant function analysis (Finney, 3) indicated that no additional information was contributed by use of the combination Y_1 and Y_2 over that obtained from use of Y_2 alone.

Summary. 1. The edema of the rat foot produced by serotonin injection as influenced

by aminopyrine has been quantitated circumferimetrically and observationally. 2. The data obtained by circumferimetry have been analyzed by means of response metameter,

$$Y_2 = 100 \log \frac{2 \text{ hr reading}}{\text{Zero hr reading}}$$

3. Results obtained by circumferimeter can serve as basis of quantitative anti-edema assay with inherent precision $\lambda = .57$. 4. Subjective scores either alone or in combination with circumferimetrically derived data are less satisfactory for use in this assay.

TABLE I. Y_1 and Y_2 and Varying Aminopyrine Dosage.

Doses of aminopyrine (mg/kg)	Response		N	
	Y_1	Y_2	Y_1^*	$Y_2^\dagger$
0	$2.23 \pm .17$	$5.37 \pm .38$	30	10
50	$1.90 \pm .17$	$4.10 \pm .38$	"	"
100	$1.37 \pm .17$	$3.47 \pm .38$	"	"
200	$1.33 \pm .17$	$2.98 \pm .38$	"	"

* No. of scores.

† No. of measurements.

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