Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs. (27849)

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Among the many methods used for screening and evaluation of antiinflammatory drugs, one of the most commonly employed technics is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat by injection of a phlogistic agent. The most frequently used phlogistic materials include brewer's yeast, formalin, dextran and egg albumin. Edema produced by these agents is not specifically influenced by antiinflammatory compounds, but responds even better to a variety of other drugs. For example, dextran edema is inhibited by chlorpromazine, antihistaminics and by some adrenergic agents, but not by cortisone, and only slightly(1) or not at all(2) by very large doses of phenylbutazone.

The doses of antiinflammatory drugs which have been employed to demonstrate inhibition of edema have been almost without exception within the toxic range. The dose of phenylbutazone administered by intraperitoneal or subcutaneous route is usually 200 mg/kg(1,3-7). None of these papers present data on a dose-response relationship, or statistical analyses which enable one to judge with known degrees of precision the potency of an unknown compound in terms of a standard.

Such considerations led Lorenz(8) to regard the effect of phenylbutazone on edema induced by egg albumin and formalin to be wholly nonspecific. He reported that edema induced by injection of kaolin suspension, unlike that of other phlogistic agents, responded to oral doses of phenylbutazone within the known therapeutic range. The effect of the antiphlogistic drug was said to be dose dependent up to 50 mg/kg, although dose-response data were not given.

After testing a variety of edema producing materials, we have concluded that the phlogistic agent of choice for testing antiinflammatory drugs is carrageenin, a mucopolysaccharide derived from Irish sea moss, *Chondrus*. Gardner(9) employed carrageenin to produce experimental arthritis in rabbits and guinea pigs. He expressed the view that the response to this material depends entirely upon local stimulus to an inflammatory process, it is not known to be antigenic, there are no systemic effects, and there is a high degree of reproducibility. Inflammatory responses to single subcutaneous injections of carrageenin have been described by Robertson and Schwartz(10), Benitz and Hall(11) and McCandless(12).

Materials and methods. Young adult male Holtzman rats of 125 to 165 g body weight were maintained in air conditioned quarters with water and food (Rockland Mouse Diet) *ad libitum*. Drugs in aqueous suspension were administered by gastric gavage in a volume of 1 ml per 100 g body weight, followed immediately by tap water to a total of 5 ml per rat. Controls received only the tap water. This treatment was given 1 hour before injection of the phlogistic agent into the foot. It was found that by thus insuring uniform hydration of all rats, variability of edematous response in the paw was minimized.

The drugs employed were hydrocortisone (free alcohol), phenylbutazone and acetylsalicylic acid. The latter was ground in a mortar and passed through a 100-mesh screen after preliminary tests indicated that such treatment reduced the variability of response to the drug.

The phlogistic agent was carrageenin, an extract of *Chondrus* obtained from Algin Corp. of America, prepared as 1% suspension in sterile 0.9% NaCl. A volume of 0.05 ml was injected through a 26-gauge needle into the plantar tissue of the right hind paw. Immediately thereafter, the volume of the injected foot was measured. Swelling of the paw reached a peak in 3 to 5 hours, then retained about the same degree of edema for several hours. For routine drug testing, in-

crease in foot volume 3 hours after phlogistic agent was adopted as a measure of effect.

The method of measuring foot volume was an adaptation of that employed by Van Arman (personal communication). The paw of the unanesthetized rat was immersed in mercury exactly to an ink mark on the skin over the lateral malleolus. The mercury was contained in a glass cylinder 25 mm diameter and 60 mm deep. The mercury column was connected with a Statham pressure transducer model P23BB range 0-5 cm Hg. The output from the transducer was led, through a Statham control unit powered by a 12-volt constant battery eliminator, to a galvanometer, Leeds and Northrup model 2430, 0.005 microamp. per scale division. Galvanometer readings were calibrated in terms of ml displacement of mercury; immersion in the mercury of an object with volume of 1 ml produced a deflection of 35 scale divisions on the galvanometer. The method is reproducible and very rapid. With one person holding the rat and another reading the galvanometer, 50 animals can be read in 10 minutes.

Results. In Experiment 1, 60 rats were divided into 10 groups of 6 each, and received oral doses of drug as indicated in Table I. The entire experiment was replicated on a second day and the results of the 2 experiments were pooled. The steroid and the 2 non-steroid drugs were effective antiphlogistic agents when single oral doses were given. Response was clearly dose related. Phenylbutazone and acetylsalicylic acid were effective in much lower doses than those generally employed when testing effects on rat paw edema(1-7).

The value of any assay procedure depends largely upon the precision with which relative potencies of drugs can be estimated. Such estimates cannot be obtained when a testing procedure is used which requires near lethal doses of drug to produce relatively small effects. Hence, it is appropriate to present a statistical analysis of the results obtained in the present experiment (Table II). Such an analysis seems not to have been presented for any previous assay of antiphlogistic drugs in the foot edema test.

The analysis in Table II may be summarized as follows: Absolute values differed on the 2 experimental days (Days, $P < 0.001$), but there was no change from one day to another in relative activities; all interactions with days were non-significant. The log dose response curves for all 3 drugs did not deviate significantly from linearity and they were parallel within the limits of experimental error. From these data, the potencies with 95% confidence limits were, relative to acetylsalicylic acid: phenylbutazone 2.0 (1.3, 2.9); hydrocortisone 16.0 (10.7, 24.0). These values were obtained using Fieller's theorem(13) and Dunnett's t (for comparing several test preparations against a standard)(14).

TABLE I. Inhibition of Carrageenin-Induced Edema in Rat Paw by Antiinflammatory Drugs. Each figure is average of 2 experiments, 6 rats on each dose level each day.

Drug	Oral dose, mg/kg	Edema, ml ± S.E.	Inhibition of edema, %
Exp 1			
Controls	—	.82 ± .01	—
Acetylsalicylic acid	33.3	.58 ± .02	29
	100	.50 ± .03	39
	300	.37 ± .04	55
Hydrocortisone	2	.61 ± .09	26
	6	.53 ± .03	35
	18	.32 ± .02	61
Phenylbutazone	10	.68 ± .03	17
	30	.53 ± .04	35
	90	.43 ± .03	48
Exp 2			
Controls	—	.91 ± .02	—
Cyproheptadine	3	.86 ± .02	—

In an additional experiment cyproheptadine, a potent antagonist of both histamine and serotonin(15) was ineffective against carrageenin edema, at a dose several times that required to antagonize edema induced by serotonin.

Discussion. Carrageenin appears to possess distinct advantages over the phlogistic agents heretofore used in studying the effect of antiinflammatory agents on foot edema in the rat. An important advantage is that single oral doses of drugs at non-toxic levels are effective. Variability is relatively low, as shown by the small standard errors in Table I. The linear log dose response yields bioassay results with a reasonable degree of precision. A bioassay of antiphlogistic drugs in

TABLE II. Analysis of Variance for All Animals in Experiment 1 Treated with Drug.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	Significance (P)
Among subclasses	17	1.7034			
Doses	8	1.2932			
Drugs	2	.0820	.0410	5.26	<.01
Common slope	1	1.1577	1.1577	148.42	<.001
Lack of parallelism	2	.0173	.0087	1.12	NS
Curvature	1	.0077	.0077	<1	"
Opposed curvature	2	.0285	.0143	1.83	"
Days	1	.3245	.3245	41.60	<.001
Interactions:					
Days × doses	8	.0857	.0107	1.37	NS
Days × drugs	2	.0088	.0044	<1	"
" × common slope	1	.0190	.0190	2.44	"
" × lack of parallelism	2	.0146	.0073	<1	"
" × curvature	1	.0292	.0292	3.74	"
" × opposed curvature	2	.0142	.0071	<1	"
Within subclasses (error)	90	.7019	.0078		
Total	107	2.4053			

Mean of all determinations = .505. Standard deviation for single determination = .09.
Common slope = −.2658. g = .034.

the foot edema test seems not to have been presented heretofore. The near-toxic doses commonly used to inhibit most phlogistic agents make it difficult to obtain reliable log dose-response effects. In numerous trials in this laboratory, we were never able to assay antiphlogistic drugs in edema produced by such agents as formalin and mustard (unpublished results) with a degree of reliability approaching that described herein. We also attempted to duplicate the results of Lorenz (*loc. cit.*) using kaolin as a phlogistic agent, but in our hands carrageenin gave much more consistent results. When kaolin was suspended in a sterile medium, very little edema was produced. Similar results have been obtained in another laboratory (C. G. Van Arman, personal communication).

The mechanism of edema production by carrageenin is not known, but this substance probably does not release histamine or serotonin, since relatively large doses of a potent antagonist of serotonin and histamine were ineffective with carrageenin. The effects of 3 known antiinflammatory agents are presented, one of them a steroid. It is somewhat surprising that the log dose response curve for hydrocortisone was parallel with that for aspirin and phenylbutazone. It is interesting, though possibly fortuitous, that the relative potencies of the 3 compounds in the carrageenin assay are within the range of the ratios of their respective daily clinical doses in rheumatic diseases.

Summary. A method is presented for measuring the edema induced by injection of 0.05 ml of 1% solution of carrageenin, an extract of *Chondrus,* into the plantar tissues of the hind paw of the rat. Peak edema develops within the first 3 to 4 hours, and is inhibited by pretreatment of the animals by single oral doses of antiinflammatory agents, steroid or non-steroid. Log dose responses to drugs are linear and parallel, and yield potency ratios with relatively narrow confidence limits. The potency ratios obtained for aspirin, phenylbutazone and hydrocortisone are fairly close to the ratios of their respective daily doses in the treatment of rheumatic disease. A potent antihistaminic-antiserotonin compound, cyproheptadine, is without effect on carrageenin-induced edema.

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Inactivation of Methicillin, Oxacillin and Ancillin by *Staphylococcus aureus.**† (27850)

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The effect of penicillin G (benzyl penicillin) on penicillinase-producing staphylococci (1-3) and on some gram-negative penicillinase-producing organisms(3) has been shown to depend in part on the size of the inoculum. The individual cell, or small concentrations of cells of different strains of *Staphylococcus aureus*–both penicillinase-producers and non-penicillinase-producers–have been found to be about equally susceptible to small concentrations of penicillin G(1). Moderate concentrations of some alpha phenoxyalkyl penicillins (*e.g.,* phenethicillin and propicillin) appear to be active *in vitro* against somewhat larger inocula of penicillinase-producing staphylococci than the same concentrations of penicillin G but are equally ineffective against larger inocula of the same strains(4, 5). Only a few of the semisynthetic penicillins, such as methicillin(4,5), oxacillin(6) and ancillin(7) have *in vitro* activity that is virtually independent of inoculum size. The latter penicillins, which are active against large inocula of penicillinase-producing staphylococci have been generally considered to be resistant to that enzyme(8).

In the course of attempts in this laboratory to develop (select) staphylococcal strains resistant to some of the semisynthetic penicillins, it was found that a drop of a fully grown culture added to 0.5 ml of broth containing 100 or 200 μg of ancillin per ml and incubated at 37°C resulted in growth of the staphylococci although the minimum inhibiting concentration (MIC) of this penicillin for the same culture in the conventional test was only 0.4-0.8 μg/ml. The inoculum in the latter test provides about 10^5 viable units per ml, whereas about 1000 times that number was provided in the former. The MIC of ancillin for the staphylococcus used was the same when the starting inoculum of 10^5 viable units was provided from a culture that had never been exposed to the antibiotic or when it was obtained from a culture previously grown in the presence of 100 μg/ml from the larger inoculum.

Because these findings suggested that the drug may have been inactivated by the larger inoculum of staphylococci, experiments were performed in which simultaneous determinations of residual antibiotic activity and of the

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