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A METHOD FOR MEASUREMENT OF ANALGESIC ACTIVITY ON INFLAMED TISSUE

BY

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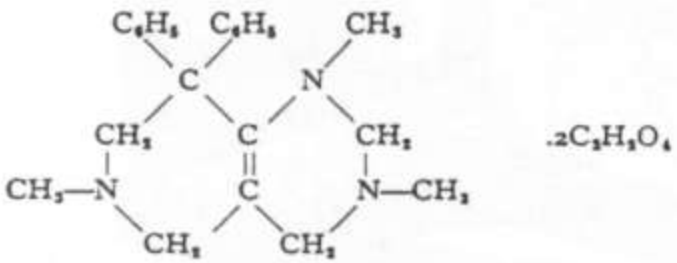
A new method for measuring analgesic activity has been developed based on the principle that inflammation increases the sensitivity to pain and that this increased sensitivity is susceptible to modification by analgesics. It has been found that inflammation decreases the pain reaction threshold and this low pain reaction threshold is readily elevated by non-narcotic analgesics of the salicylate-aminopyrine type as well as by the narcotic analgesics. The various methods heretofore available have readily measured narcotic analgesics but usually failed to measure salicylate.

The radiant heat method of HARDY & WOLFF as modified by ERCOLI and LEWIS (1945) was capable of measuring aminopyrine and codeine but was not sufficiently sensitive to measure salicylate activity. However, WINDER, PFEIFFER and MAISON (1946) believed that salicylate activity could be measured in guinea pigs by a radiant heat method. The pressure analgesimeter of GREEN, YOUNG and GODFREY (1951) was satisfactory for measuring meperidine and stronger analgesics (MILLAR and STEPHENSON, 1956; GREEN and WARD, 1956). It has also been applied to the measurement of codeine, salicylamide and hydroxyisophthalic acid, but it was not adequate for measuring salicylate (COLLIER and CHESHER, 1956). The activity of salicylamide was measured by a similar method by WAY, TAKEMORI, SMITH, ANDERSON and BRODIE (1953), but this method also was inadequate for salicylate. DOMENJOZ (1952) described the analgesic effects of phenylbutazone in rabbits and dogs as measured on the response to electrical stimulation of a tooth by the method of KOLL and REFFERT (1938). In rabbits,

phenylbutazone in doses of 50-100 mgm/kgm I.V. had less effect than 12 mgm/kgm of morphine but was said to have the same analgesic activity as salicylate, pyrazoles, and phenacetine.

It has long been recognized that inflammation causes pain, edema, increased temperature and redness and that salicylate reduces these reactions. During the course of our studies of the edema produced by yeast when injected into the rat's foot (SELITTO and RANDALL, 1954, 1956) it was observed that the inflamed foot was very sensitive to touch. A pressure device was developed which measured quantitatively the increased sensitivity in the inflamed foot and it was found that salicylate, aminopyrine and all narcotic analgesics readily increased the pain reaction threshold.

A new compound, Ro 2-5383, was found to be similar to salicylate in analgesic properties. Ro 2-5383 (1,3,6-Trimethyl-8,8-diphenyl-1,2,3,4,5,6,7,8-octahydropyrido [4,3-*d*] pyrimidine oxalate) ($C_{22}H_{27}N_3 \cdot 2C_2H_2O_4$) was prepared by Drs. PLATI and WENNER.



METHOD

Inflammation was produced by the injection of 0.1 ml. of a 20 per cent suspension of Brewer's yeast into the plantar surface of the rat's foot. The pain threshold was measured by a pressure device which is illustrated in Figure 1. The pain threshold was measured as the amount of pressure in mm.Hg. required to induce the flight reaction (struggle) when applied to the foot. Air pressure from an air line was admitted through a needle valve to a 10 ml. syringe and to a pressure gauge which was connected by a T-tube. The syringe was mounted with the plunger downward to which was connected a short bullet-shaped wooden peg. The pressure was applied through the wooden tip to the plantar surface of the rat's foot at the rate of 20 mm. Hg. per second. The end point was reached when the rat struggled.

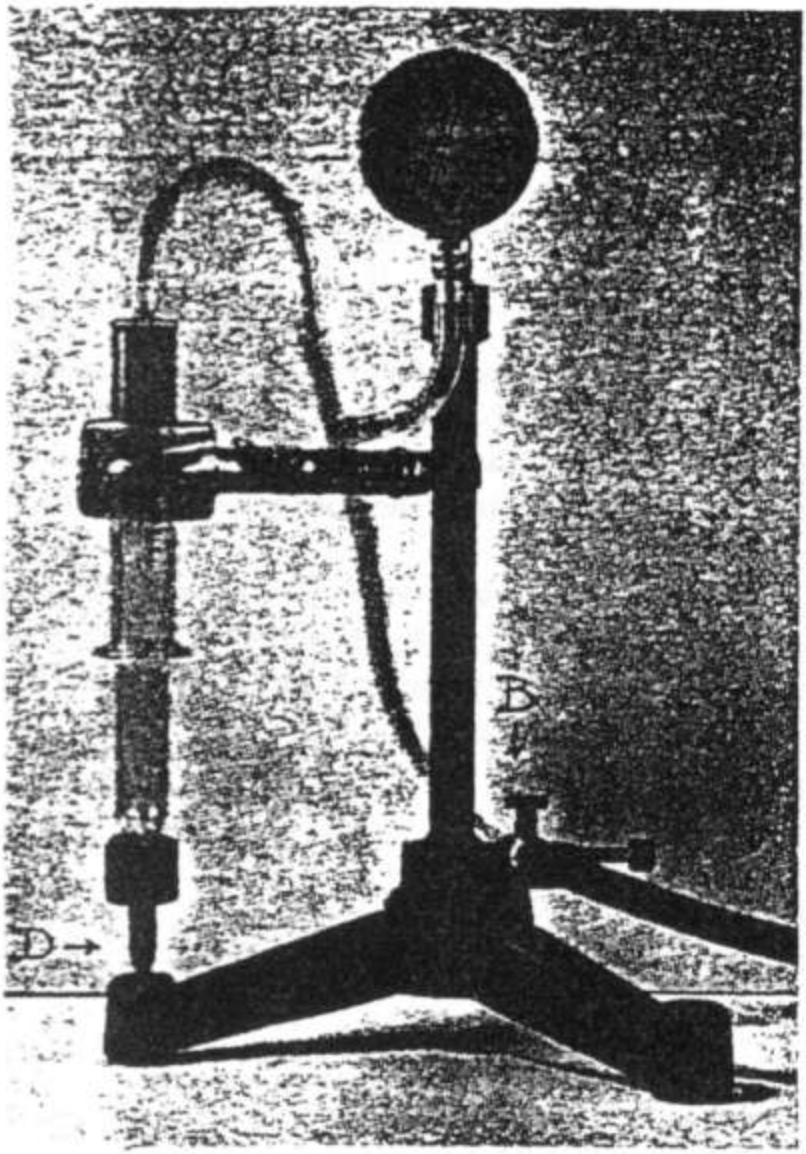


FIG. 1

Photograph of Pressure Device

A. Air line.
B. Air release valve.
C. Air pressure gauge.
D. Wooden peg through which pressure is applied to rat's foot.

RESULTS

The pain threshold of the inflamed foot was compared with that of the control foot on up to 85 rats weighing 101-134 grams in groups of 5 rats in each experiment. The mean and standard errors are given in Table I.

In measuring the effects of drugs, the thresholds were measured at 1, 2, and 4 hours after subcutaneous injection. Five rats per dose

TABLE I

Control pain thresholds

Hours after Yeast Injection	# of Rats	Inflamed Foot mm. Hg. ± s.e.	Control Foot mm. Hg. ± s.e.
1	85	73 ± 1.7	161 ± 0.4
2	70	60 ± 1.8	156 ± 1.8
4	85	55 ± 0.7	157 ± 0.4
24	75	92 ± 2.8	156 ± 2.6
48	10	163 ± 2.3	173 ± 1.7
72	10	160 ± 1.7	159 ± 1.5
96	5	159 ± 1.0	164 ± 0.8

and 3 dose levels spaced at log intervals were employed. The degree of analgesia was calculated as the per cent increase in threshold of treated rats over the average threshold of 5 control rats.

The inflamed foot was slightly more than twice as sensitive to pressure-induced pain as the control foot. The threshold of the inflamed foot was slightly lower at the intervals of 2 and 4 hours than after the first

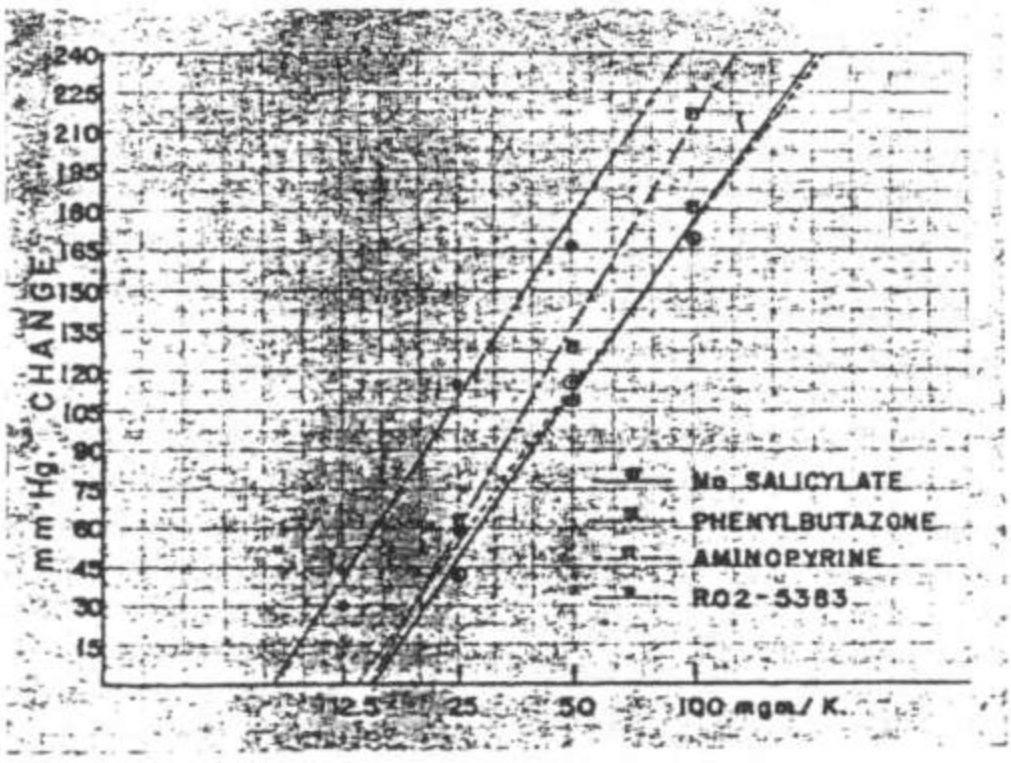


FIG. 2

Log dose response curves illustrating the analgesic effect of salicylate, phenylbutazone, aminopyrine and Ro 2-5383 on the pain threshold of inflamed foot of the rat.

hour and returned to normal in 48 hours. The threshold of the control foot remained reasonably constant.

Figure 2 shows that phenylbutazone has the same analgesic effects as salicylate while Ro 2-5383 is stronger.

In Figure 3, the log dose response curves are plotted for 7 standard analgesic agents. In the graph, the increase in pain threshold of the

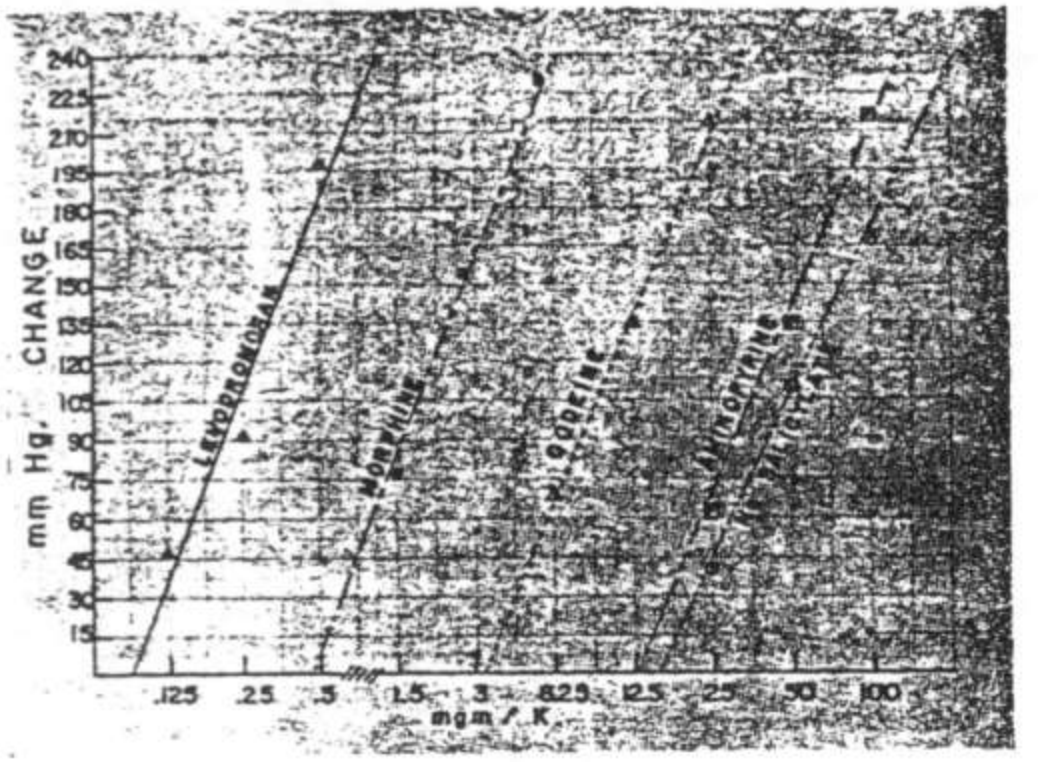


FIG. 3

Log dose response curves illustrating the analgesic effect of salicylate, aminopyrine, codeine, meperidine, alphaprodine, morphine and levorphan on the pain threshold of the inflamed foot of the rat.

inflamed foot, measured in mm. Hg. pressure, is plotted against the log of the doses. The results show a straight line relationship for all the drugs and the lines are parallel for the various drugs. The method is capable of measuring all degrees of analgesic potency from that of salicylate to that of levorphan.

The relative potencies and the limits of error were calculated by the method of BLISS and MARKS (1939). An example of the calculations, comparing salicylate with Ro 2-5383, is given in Table II.

From the calculated potencies and limits of errors, the following relationships were obtained :

Aminopyrine = 1.2 (1.0-1.4) times salicylate.

Phenylbutazone = 1.3 (1.1-1.5) times salicylate.

Ro 2-5383 = 1.8 (1.6-2.1) times salicylate.

Morphine = 25 (21-30) times salicylate.
Morphine = 4.1 (3.6-4.7) times codeine.
Morphine = 4.1 (3.6-4.7) times meperidine.
Alphaprodine = 1.1 (1.0-1.2) times morphine.
Levorphan = 8.5 (7.4-9.3) times morphine.

TABLE II

Calculation of Potency and Limits of Error

	Salicylate			Ro 2-5383				
Dose, mgm/kgm	25	50	100	12.5	25	50		
	0	75	185	45	80	165		
	35	95	145	20	90	160		
	10	135	225	40	30	170		
	60	70	250	15	75	170		
	55	95	140	25	45	180		
Sum =	160	470	945	145	320	845	= 2885	1976975
Sum of squares =	795	46800	187975	4875	23050	143025	= 406520	

$$\text{Correction term} = \frac{(\text{sum of resp.})^2}{(\text{no. of resp.})} = \frac{(2885)^2}{30} = 277441$$

$$\text{Sum of squares} = 406520 - 277441 = 129079$$

$$\text{Between doses} = \frac{1976975}{5} = 395395 - 277441 = 117954$$

Analysis of Variance

	Degrees of Freedom	Sum of Squares	Mean Square	s
Total	29	129079	4451.0	
Between doses	5	117954	23590.8	
Within doses	24	11125	463.541	21.3

Factorial Analysis

	S_1	S_2	S_3	U_1	U_2	U_3	$NS(x)^2$	$S(xyp)$	$\frac{S(xyp)^2}{NS(x)^2}$	$\sqrt{\quad}$
Sample	−1	−1	−1	+1	+1	+1	30	−265	−2340.833	−48.382 = D
Slope	−1	0	+1	−1	0	+1	20	1485	110261.2	332.055 = B
Parallel	+1	0	−1	−1	0	+1	20	85	36125	
Curvature	+1	−2	+1	+1	−2	−1	60	515	442041	

$$M = Xs - Xu + \frac{KID}{B} = 1.6990 - 1.3979 + \frac{1.633 \times .301 \times (-48.382)}{332.055} =$$

$$.3011 - 0.3526 = .2658$$

$M = \log \frac{\text{pot. of unknown}}{\text{pot. of standard}}$ $I = \log$ dose interval $= .3010$

$K = 1.6330$ for 3 dose assay D & B = from table of factorial analysis

$$\text{Potency} = \text{antilog } 2 + .2658 = \text{antilog } 2.2658 = 184\ \%$$

$$\text{Limits of Error} = Sm = \frac{sKI\sqrt{D^2 + B^2}}{B^2} = \frac{21.53 \times 1.633 \times .301\ \sqrt{2340.833 + 110261.2}}{110261.2} =$$

. 0319

Upper limit = antilog $1.96 \times .0319$ = antilog $.0638 = 116$ $116 \times 184 = 213\ \%$
Lower limit = recip. antilog $= 1.9362 = 86$ $86 \times 184 = 158\ \%$

Potency $= 184\ \%$ *Fiducial Limits* $= 158\ \%$ - $213\ \%$

The results recorded in Table III show that salicylate readily increases the pain threshold of the inflamed foot but it has no effect on the pain threshold of the control foot. In these experiments, 5 rats per dose of each compound were used. The inflamed foot was sufficiently sensitive so that doses of 25-100 mgm/kgm S.C. raised the pain threshold. Doses of 100 mgm/kgm had no effect on the threshold of the normal foot.

In a similar manner, Ro 2-5383 and phenylbutazone raised the threshold of the inflamed foot while they did not affect the threshold of the normal foot.

Whereas the analgesic effects of salicylate, phenylbutazone and Ro 2-5383 could be measured only on the inflamed foot, aminopyrine and the narcotic analgesics raise the pain threshold of both the inflamed and control feet. For the latter compounds the relative order of potency as measured on the pain threshold of control and inflamed feet is similar. The order of increasing potency is aminopyrine, codeine, meperidine, morphine, alphaprodine and levorphan.

The pain threshold of the inflamed foot is more sensitive to the effects of aminopyrine and the narcotic analgesics than that of the control foot. The increased threshold is always greater when measured on the inflamed foot than on the control foot at any given dose level of the analgesics and smaller doses can be measured on the threshold of the inflamed foot than on that of the control foot.

TABLE III

Increase in pain threshold in mm. Hg.

Compound	Dose mgm/kgm S.C.	Inflamed Foot			Control Foot		
		1 hr.	2 hr.	4 hr.	1 hr.	2 hr.	4 hr.
Na Salicylate	25	30	0	0	−2	−4	0
	50	92	42	4	−7	−2	−2
	100	187	109	92	−9	−6	7
Phenylbutazone	25	23	58	−8	−7	0	3
	50	83	108	46	−6	0	0
	100	168	236	127	7	11	15
Ro 2-5383	12.5	36	22	3	−3	8	0
	25	61	48	35	4	7	6
	50	174	153	70	−2	7	9
Aminopyrine	25	59	17	6	19	2	0
	50	94	86	6	73	29	0
	100	212	188	34	129	78	19
Morphine	1.5	72	18	15	17	16	12
	3	146	94	68	85	51	32
	6	240	174	134	137	80	18
Codeine	6.25	81	0	0	0	9	0
	12.5	135	112	43	105	49	0
	25	240	170	101	140	90	0
Alphaprodine	1.5	59	44	7	35	19	0
	3	151	132	44	122	68	29
	6	227	225	60	141	127	21
Meperidine	6.25	75	70	3	48	28	4
	12.5	157	120	59	104	63	12
	25	235	209	93	143	136	44
Levorphan	0.125	51	54	13	38	38	16
	0.25	137	91	26	110	90	18
	0.5	223	190	89	137	134	55

DISCUSSION

The new method of measuring activity of analgesics on inflamed tissue is sufficiently sensitive to make practical the assay of weak anal-

gesics of the salicylate type. Salicylate gives quite reproducible dose response curves when the log of the dose is plotted against the increase in threshold to pain. The lack of demonstrable activity of salicylate on the threshold of normal tissue confirms numerous reports of the failure to measure salicylate activity (ERCOLI and LEWIS, 1945; COLLIER and CHESHER, 1956; WAY et al., 1953; LA BELLE and TORNABEN, 1952).

The activity of phenylbutazone is slightly greater than that of salicylate in intensity while the duration of action and specificity of action on inflamed tissue are similar. This confirms the analgesic activity of phenylbutazone that was reported by DOMENJOZ (1952) who used the tooth pulp stimulation method in rabbits and dogs.

The new compound, Ro 2-5383, also has the specific analgesic activity of salicylate on inflamed tissue with an intensity nearly twice that of salicylate and a duration of action similar to salicylate. Besides the analgesic activity, this compound was reported to have antipyretic and anti-edema characteristics similar to those of phenylbutazone and salicylate (SELITTO and RANDALL, 1956).

It is believed that the elevation of pain threshold induced by salicylate, phenylbutazone and Ro 2-5383 on the inflamed foot is related to the simultaneous reduction in swelling, edema and temperature. By preventing the accumulation of edema fluid in the rat's foot, they prevent the increase in sensitivity to pain which is recorded as an increase on the threshold to pain. However, the prevention of the accumulation of edema fluid in the inflamed foot is probably not the whole explanation of the analgesic effect since the threshold to pain in this foot is increased to levels above the pain threshold of the control foot. Even though the salicylate-like compounds have no effect on the threshold of the normal foot, they still increase the pain threshold of the inflamed foot above the normal pressure threshold of the control foot. It is possible that salicylate compounds accumulate in the edema fluid in the foot and exert a local effect on the pain threshold of the inflamed foot. This process probably does not occur in the normal foot. WILHELMI and PULVER (1955) have shown that phenylbutazone accumulates in the edema fluid in higher concentration than in normal tissue juice. If salicylate and Ro 2-5383 should accumulate in a manner similar to that of phenylbutazone, this fact would help explain the local analgesic effect of these compounds.

The fact that aminopyrine-type antipyretics and the narcotic analgesics affect the pain threshold of the normal foot as well as that of the inflamed foot suggests a central mechanism for the increase of the pain threshold by these compounds. The new method of measuring anal-

gesia on inflamed tissue serves to differentiate the analgesics of the salicylate type whose action may be partially local from the centrally-acting antipyretics of the aminopyrine type and the narcotic analgesics of the morphine type.

Summary

A new method for measuring analgesic activity has been developed which is based on the principle that inflammation increases the sensitivity to pain.

Salicylate, phenylbutazone and Ro 2-5383 raise the pain threshold of the inflamed tissue but not normal tissue.

Aminopyrine and narcotic analgesics raise the threshold of both normal and inflamed tissue.

The procedure, therefore, differentiates analgesics of the salicylate type from the central antipyretics of the aminopyrine type and the central narcotics.

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