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Received March 11, 1958. P.S.E.B.M., 1958, v98.

Etiology of Chemically Induced Writhing in Mouse and Rat. (23984)

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Since it is generally agreed that analgesic compounds aside from opiates have little effect when tested by technics involving infliction of thermal or mechanical pain in normal experimental animals, many investigators have searched for methods which assess activity of "low potency" analgesics. Among these are Vander Wende and Margolin(9), and Siegmund *et al.*(7). The former group employed sodium iodomethamate intraperitoneally to produce "writhing" (a syndrome consisting of intermittent rubbing of the belly on the cage floor) in rats and arrived at oral PD_{50} values for opiates and antipyretic analgesics. This group blocked writhing by previous injection of procaine intraperitoneally. Siegmund *et al.* employed mice in a similar study using phenylquinone as inciting agent for writhing. 5-Hydroxytryptamine (5-HT) produces severe pain on intradermal injection (Armstrong *et al.*(1)) and has been reported (Lu, personal communication) to produce writhing when injected intraperitoneally in mice.

We studied effects of morphine, aminopyrine, lysergic acid diethylamide (LSD) and chlorpheniramine in prevention of writhing by phenylquinone, 5-HT and other substances, to arrive at the mechanism whereby writhing is produced and to ascertain optimal inciting agent of those mentioned above for efficient testing of new compounds.

Methods. Male Carworth mice weighing 18-22 g were distributed into groups of 5 animals each. Compounds under consideration as writhing producing agents were injected intraperitoneally, in aqueous solutions. Compounds investigated as blockers of writhing were administered subcutaneously, one-half hour prior to injection of the writhing agent. One group received only the writhing agent and served as control in each experiment. The usual period of observation for presence or absence of writhing was 10 minutes. If no response was observed the material was considered ineffective. If writhing was observed after this period, it was classified as "delayed." Such results are marked with an asterisk in the Table which gives number of animals which writhed, divided by total number in the group. Rats were also employed, weighing 150-200 g and treated in the same manner. Phenylquinone, histamine, which is commonly believed to play a role in inflammation, serotonin, heparin, and various releasers of these substances (Table I) such as L-1935, 48/80, and reserpine were used as writhing-inciters. NaOH and HCl were employed to produce pain, possibly without specific release of other substances. Distilled water and 0.9% NaCl solution were also tried. Writhing inciters were given in total volume of 0.25 ml/mouse or 1 ml/rat. Since our primary object was to attempt to elucidate the mechanism of action, we chose 4 drugs with different pharmacological activities, as possible blockers of the writhing effect: Morphine (a narcotic analgesic), aminopyrine (a non-narcotic analgesic), lysergic acid diethylamide (a blocker of serotonin), and chlorphe-

TABLE I. Substances Released by Various Compounds.

Compound	Substance released	Reference
48/80	Histamine-partial (mouse)	Riley and West(6)
"	5-HT and histamine (rat)	Bhattacharya and Lewis(2)
5-Hydroxytryptamine	Histamine (rat)	Sparrow and Wilhelm(8)
Histamine	No release of 5-HT in rat	Bhattacharya and Lewis(2)
L-1935	Histamine (rat)	Feldberg and Lecomte(3)
Reserpine	5-Hydroxytryptamine (rat)	Parratt and West(4)
48/80	Heparin (rat)	Riley, Shepherd, West, Stroud(5)

niramine (a potent anti-histamine). The results are summarized in Table II.

Results. Phenylquinone, as well as 5-HT, L-1935 and dilute hydrochloric acid produced immediate writhing in the mouse, differing only in duration. Writhing persisted for more than one hour after phenylquinone, but only 20-25 minutes after other inciters. Such agents as heparin, reserpine, water and saline produced delayed writhing whereas dilute sodium hydroxide and 48/80 gave no response. Histamine and bradykinin (not tabulated) were variable in effect.

In the rat, phenylquinone and HCl were the only substances of this group which induced writhing.

Among the various possibilities as to etiology of writhing appeared the following: 1. Direct irritation; 2. Release of serotonin; 3. Histamine release.

The pH values of solutions producing writhing, ranged between 4 and 5. If direct irritation by acidity of drug were causative in writhing, any non-acid substance should be inactive. To test this theory, we first injected a solution of serotonin at pH 4.7 which gave a 100% response, then neutralized the solution to pH 7 and tested it again on another set of mice. All of these mice also writhed. This result appears to rule out acidity of inciter as the only causative agent. Sodium hydroxide did not produce writhing in either species so that an elevated pH appears not to be concerned in the phenomenon. Reserpine, a serotonin releaser in the rat, produced only delayed writhing in the mouse and was inactive in the rat. Compound 48/80, known to release serotonin in the rat, did not produce writhing in either species at doses from 1 to 2.5 mg/kg. Serotonin itself produced writhing only in the mouse. These findings suggest that serotonin is not primarily etiologic.

Although serotonin releases histamine in the rat the reverse is not the case. Histamine itself was only variably active as a writhing inciter in the mouse and not at all in the rat. The anti-histamine chlorpheniramine produced no decrease in percentage of mice which

TABLE II. Blockade of Irritant Induced Writhing.

		—		Aminopyrine, 100 mg/kg		Morphine, 1.0 mg/kg		LSD, 5.0 mg/kg		Chlorpheniramine, 5 mg/kg	
Irritant	Dosage, mg/kg	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat
Phenylquinone	2.5	50/50	5/5	0/5	4/5	0/5	4/5	5/5	2/5	5/5	4/5*
Serotonin	"	32/35	0/5	"		"		1/5		3/5*	
Histamine	"	15/25*	"	"		"		"		8/15*	
Hydrochl'c acid	10.0	15/15	5/5	"	2/7	"	0/5	4/5	0/5	5/5	3/5
L 1935	2.5	10/10	0/5	"		"		0/5		2/5*	
Reserpine	"	1/15*	"							3/5*	
Sodium hydroxide	"	0/5	"								
48/80	"	0/10	"								
Heparin	"	0/5*									
Water		"	"								
Saline		"	"								

* Plus some animals which showed delayed writhing.

Above figures signify $\frac{\text{No. of animals writhing}}{\text{No. of animals tested}}$.

writhed after histamine. The histamine releaser I-1935 produced immediate writhing in the mouse but its action was only partially blocked by chlorpheniramine during 10 minutes of observation. Compound 48/80, also a histamine releaser in the rat was inactive in the mouse. These observations are not consistent with the hypothesis that histamine release (as judged by data on such release in rats to be sure) may be an inciting cause of writhing in the mouse. However, it is apparently unknown whether L-1935 can release substances other than histamine. Compound 48/80 was only partially effective as histamine releaser in the mouse(6).

Since phenylquinone and hydrochloric acid were the only effective writhing producers in the rat, it would seem that release of serotonin or histamine is not to be considered in causation in this species.

Writhing in the mouse was blocked in all cases by morphine, LSD, and aminopyrine except that LSD did not block writhing produced by phenylquinone or hydrochloric acid. Chlorpheniramine partially blocked writhing produced by histamine, serotonin, or L-1935. It did not block writhing produced by phenylquinone or hydrochloric acid.

Summary. 1. Histamine is probably not a basic cause of writhing in the mouse. 2. It is possible that 5-HT may be a basic etiologic agent but, if true, the usual releasers of 5-HT do not so function in the mouse. 3. Acidity of injected solution *per se* is probably not etiologic. 4. It is worth considering that phenylquinone, HCl, serotonin, and L-1935 may release some substance other than histamine, serotonin, heparin, or bradykinin which in turn may incite writhing. 5. Direct irritation of nerve endings remains as a distinct causative possibility. 6. In screening for analgesics, both opiate and non-opiate types, phenylquinone and HCl are most useful of writhing agents tried, in eliminating the possibility of false positives. Phenylquinone may be preferable because of its greater duration of action.

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Received March 18, 1958. P.S.E.B.M., 1958, v98.

Determination of Cell Viability. (23985)

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The difficulties which arise from attempts to assess viability of tissue cells solely in terms of reproductive capacity include: suitability of substrates and circumstance; necessity of employing population data to deduce what has happened to individuals; ultimate impossibility of distinguishing "static" from "cidal"; and the fact that certain types of tissue cells have not been induced to proliferate *in vitro*. Ideally, viability should be determined by methods which are simple, rapid, and applicable to the non-proliferating individual as well as to cultivable populations. If diverse factors required for reproduction are set aside, the capacity to control internal ionic environment is a universal characteristic of living beings. Many observations concerning exclusion of dyes by living yeasts(1,2), bac-