THE ANALGESIC AND ANTIPYRETIC PROPERTIES OF SOME DERIVATIVES OF SALICYLAMIDE

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Introduction

The increase in the use of salicylamide (2-hydroxy benzamide) as an analgesic in recent years and its introduction into clinical use in this country prompted us to investigate the pharmacology of the substance and some of its derivatives. Other workers[1,2] have reported that salicylamide is a more powerful and less toxic analgesic than acetylsalicylic acid. As a part of a study of the chemistry of salicylamide, a series of alkyl ethers was prepared, and the analgesic and antipyretic activities of some of these compounds has been compared with those of salicylamide.

Experimental

(i) *Chemistry*

The preparation of Alkyl Ethers of Salicylamide. The ethers were prepared by treatment of salicylamide (1 mole) with the corresponding alkyl bromide (1 mole) in boiling ethanol in the presence of sodium ethoxide (1 mole). In the case of the methyl and amyl ethers, the iodide was found to work as satisfactorily as the bromide. The mixture, after refluxing for 6 hours, was freed of ethanol by distillation and the alkyl ether of salicylamide precipitated with water. The 2-*n*-dodecyl ether (compound 221) was recrystallised from ethanol and the hexadecyl (compound No. 268) from light petroleum (b.pt. 80° to 100° C.). All the other compounds were recrystallised from aqueous ethanol (50 per cent.). The yields in most cases were between 70 and 80 per cent. of a theoretical yield. With the exception of methyl,[(a)] ethyl[(b)] and *iso*propyl ethers,[(c)] all the compounds synthesised have not previously been reported.

(ii) *Pharmacology*

(*a*) *Toxicity Tests.* The acute intraperitoneal LD50's to mice were approximately determined, using up to 5 mice in groups separated by logarithmic dosage intervals. Animals which died on the same day were examined for unabsorbed drug and for any abnormalities due to the injection. The following day, and at the end of the 4-day test period, survivors were sacrificed and examined for unabsorbed drug. This gave an idea of the absorption of these compounds.

The acute oral LD50's to mice of the first 5 compounds in the series were determined on 10 to 20 mice in groups separated by logarithmic dosage intervals. The drugs were administered by stomach tube in the form of suspensions in 10 per cent. mucilage of acacia. Owing to poor absorption, the toxicities of the higher members were only roughly

TABLE I

Compound number	Name	Melting point °C.	Analysis		
			C per cent.	H per cent.	N per cent.
H.P. 209	2-Methoxybenzamide	127	—	—	—
,, 208	2-Ethoxybenzamide	130	—	—	—
,, 206	2-*n*-Propoxybenzamide	98 to 99	Found: 67·00	7·35	7·90
			Required: 67·00	7·30	7·80
,, 215	2-*iso*Propoxybenzamide	67 to 68	Found: 67·35	7·50	7·60
			Required: 67·00	7·30	7·80
,, 165	2-*n*-Butoxybenzamide	75 to 76	Found: 68·30	7·80	7·35
			Required: 68·35	7·80	7·25
,, 211	2-*n*-Amyloxybenzamide	86	Found: 69·15	8·10	6·95
			Required: 69·50	8·30	6·75
,, 216	2-*n*-Hexyloxybenzamide	71	Found: 70·60	8·50	6·45
			Required: 70·50	8·65	6·35
,, 217	2-*n*-Heptyloxybenzamide	56 to 57	Found: 71·40	8·80	5·70
			Required: 71·40	9·00	5·95
,, 218	2-*n*-Octyloxybenzamide	58 to 59	Found: 72·15	9·30	5·80
			Required: 72·25	9·30	5·60
,, 219	2 (3′-5′-5′-trimethylhexyloxy) benzamide	87 to 88	Found: 72·80	9·45	5·40
			Required: 72·95	9·60	5·30
,, 221	2-*n*-Dodecyloxybenzamide	83 to 84	Found: 74·80	10·25	4·75
			Required: 74·70	10·25	4·60
,, 268	2-*n*-Hexadecyloxybenzamide	79 to 80	Found: 76·00	11·15	4·10
			Required: 76·40	10·90	3·90

Analyses by Drs. Weiler and Strauss. m.pts. uncorrected.

(*a*) Sachs and Harold (*Ber. dtsch. chem. Ges.*, 1907, **40**, 2724) report m.pt. 127°
(*b*) Remsen and Reid (*J. Amer. chem. Soc.*, 1899, **21**, 290) report m.pt. 132·1°
(*c*) Kraut (*Liebigs Ann.*, 1869, **150**, 8) prepared 2-*iso*propoxybenzamide, but no melting point is reported.

determined. In each test, salicylamide was administered to 3 groups of mice as a standard and the figure for this compound is expressed as the mean and standard error of these 5 results.

White male mice weighing from 18 to 25 g. were used for both types of test and for both they were starved for 18 hours prior to injection. The experimental period was 4 days. The LD50's with their standard errors were calculated by the graphical method of Miller and Tainter.[3]

The chronic oral toxicity of salicylamide was determined on female white rats. 30 rats weighing from 115 to 130 g. were divided into 3 groups of 10 to receive daily:—

(*a*) 0·005 ml./g. of 10 per cent. of gum acacia in normal saline solution.
(*b*) 0·005 ml./g. of 5 per cent. of salicylamide in 10 per cent. mucilage of gum acacia = 0·25 mg./g.
(*c*) 0·005 ml./g. of 10 per cent. of salicylamide in 10 per cent. mucilage of gum acacia = 0·5 mg./g.

Food was removed from the cages in the morning until doses had been administered at the end of the afternoon. The animals were weighed twice weekly. The experiment was continued for 19 weeks during which time 4 animals were killed by the catheter entering the trachea instead of the œsophagus. Total red and white cell counts were made at intervals, and at the end of the experiment. Also at the end of the experimental period, plasma prothrombin times were determined on samples of blood, using the technique of Quick.[4] The animals were examined for macroscopic abnormalities, especially gastric ulcers.

(*b*) *Antipyretic Effect.* Rats were used for this work. Pyrexia was produced by injecting a dose of 1·0 ml. of 20 per cent. suspension of dried yeast B.P.C. subcutaneously at 5.0 p.m. on the afternoon previous to the actual test.[5,6] The next morning, using clinical thermometers, 3 readings of rectal temperature were made at 20-minute intervals. The rats were sorted into groups of 4 with the same average temperature. The drugs were usually administered in 10 per cent. mucilage of acacia intraperitoneally to facilitate rapid administration and absorption. A few experiments were performed to compare the effect of intraperitoneal and oral injections of the butyl ether (2-*n*-butoxybenzamide H.P. 165) the ethyl ether (2-ethoxybenzamide, H.P. 208) and acetylsalicylic acid. In every experiment two control groups were used, one was injected with 0·01 ml./g. of 10 per cent. gum acacia in normal saline solution and the other with 0·05 mg./g. of acetylsalicylic acid.

(*c*) *Analgesic Effect.* A modification of the Hardy-Wolff-Goodell apparatus was used, as described by Thorp,[7] in which the tail of the rat is exposed to the heat from a strong beam of light. We have measured the time in seconds for the tail to be withdrawn, using a constant intensity of light. The average normal response was about 5 seconds. The rats were kept in tubular cages for the duration of the experiment. The tails were blackened in order that they should absorb the heat and that the reflected light should not dazzle the operator. To avoid any risk of actual burning, the tails were exposed for no longer than 12 seconds. When no response was obtained, it was recorded as 12 seconds. Rats of both sexes, weighing about 200 g., were used.

The drugs were administered intraperitoneally in the same form as used in the antipyretic tests with the exception of acetylsalicylic acid which, on account of the high toxicity of the free acid at the dose required, was dissolved in the minimum amount of sodium hydroxide to give a soluble sodium salt.

In agreement with other workers,[7,8] we found that the rats require a training period before they react regularly to the stimulus. Readings on each rat were taken at 20-minute intervals throughout the experiments, i.e., at 20, 40, 60, 80, 100 and 120 minutes after the administration of the drug. 3 readings were taken before the administration of the drugs to provide normal reaction times. The increase in seconds of the reaction time of a group after the administration of a drug over the normal reaction time is called the response. This was estimated at each time interval. When these responses were plotted against the logarithm of the dose, we found that the effect after 20 minutes gave the best discrimination.

Our rats are obtained from dealers, and we find that, while the normal reaction times of the animals are remarkably constant, the sensitivity of the rats to analgesic drugs varies to a considerable extent from batch to batch. For instance, the analgesic activity of salicylamide could be detected on one batch of rats in a dose of 0·05 mg./g. but at the other extreme, another batch of animals was not sensitive to 0·2 mg./g. For this reason, we did not think it desirable to express our results, as the AD50 of Ross Hart,[1] but to give them in comparison with a compound

of recognised analgesic value. Salicylamide was used as this standard and a further check was made by including some tests on the widely used acetylsalicylic acid. In screening tests 2 doses of salicylamide and 2 doses of the new compound were used with 4 rats in each group. For more detailed studies latin square tests were devised where the same 16 rats were used for each point.

RESULTS

Toxicity Test

All these compounds except the higher members of the series exhibited a sedative effect similar to that of salicylamide. Although an efficient analgesic, the sedative effect of this compound is very weak in man, and it might be supposed that its derivatives should behave similarly. The results of the acute toxicity tests to mice are expressed in Table II. The decrease in toxicity above the amyl derivative may be due to poor absorption and this view is confirmed by the finding of appreciable quantities of the nonyl and dodecyl derivatives in the intraperitoneal cavity twenty four hours after administration.

TABLE II

ACUTE TOXICITY TO MICE G./KG.

Compound number	Compound	Oral LD50	Oral S.E.	Intra-peritoneal LD50	Intra-peritoneal S.E.	Analgesic ratio compound salicylamide
H.P. 34	2-Hydroxybenzamide (Salicylamide)	1·59	± 0·05	0·89	± 0·029	1
„ 208	2-Methoxybenzamide	1·2	± 0·116	0·9		1 – 2
„ 209	2-Ethoxybenzamide	1·76	± 0·256	0·4		2 – 4 *2·3*
„ 206	2-*n*-Propoxybenzamide	1·26	± 0·01	0·51		2 – 4
„ 165	2-*n*-Butoxybenzamide	1·3	± 0·086	0·36	± 0·019	2 – 4 *3*
„ 211	2-*n*-Amyloxybenzamide	>4·7		0·9		2 – 4
„ 216	2-*n*-Hexyloxybenzamide	>4·7		1·0		2
„ 217	2-*n*-Heptyloxybenzamide	>4·7		1·7		1 – 2
„ 218	2-*n*-Octyloxybenzamide	>4·7		>4·7		<1
„ 219	2-, (3'-5'-5'-Trimethylhexyloxy) benzamide	>4·7		>4·7		<1
„ 221	2-*n*-Dodecyloxybenzamide	>4·7		>4·7		<1
	Acetylsalicylic Acid	1·3	± 0·06	0·28	± 0·015	*0·3*

In the chronic toxicity test on salicylamide no rats died due to daily doses of 0·5 mg./g. and 0·25 mg./g. and all the 3 groups gained weight evenly. No difference between the treated and control groups was observed in total red and white cell counts, nor in prothrombin times. On post-mortem no macroscopic abnormalities were observed, in particular, no ulcers were detected in the stomachs.

Antipyretic Effect. The results are shown in Figure 1 where all drugs were administered intraperitoneally in doses of 0·05 g./kg. These results were subjected to a "t" test. At 20 minutes salicylamide is not significantly different from acetylsalicylic acid, but its effect wears off rapidly. The effect of the methyl ether (2-methoxy benzamide H.P. 209) lasts much longer. There is then a remarkable rise in activity and the ethyl derivative has a very powerful and enduring effect. Above this, however, the activity decreases so that the propyl and butyl derivatives are not significantly different from each other, but are more active than

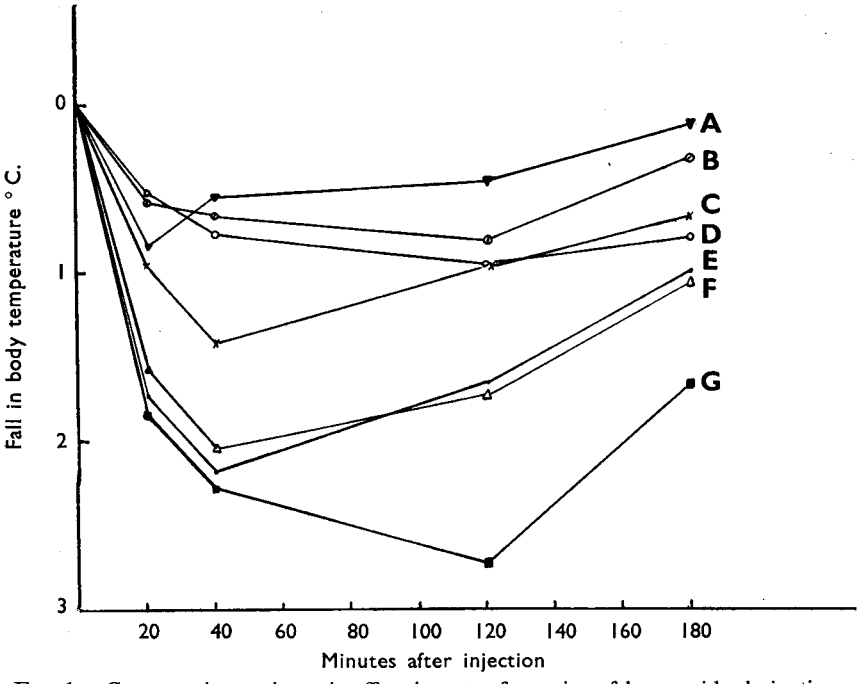

FIG. 1. Comparative antipyretic effect in rats of a series of benzamide derivatives. Dose for each compound, 0·05 g./kg.

A. 2-Hydroxybenzamide (salicylamide). B. 2-Methoxybenzamide.
C. 2-*n*-Amyloxybenzamide. D. Acetylsalicylic acid.
E. 2-*n*-Butoxybenzamide. F. 2-*n*-Propoxybenzamide.
G. 2-Ethoxybenzamide.

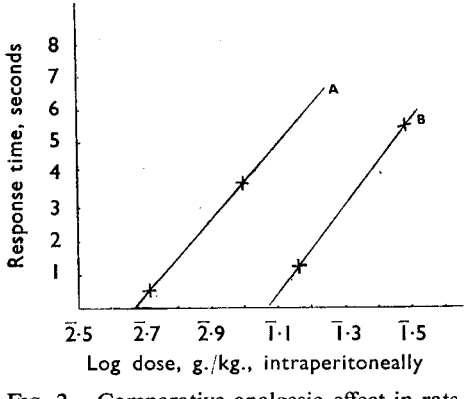

FIG. 2. Comparative analgesic effect in rats of 2-ethoxybenzamide and 2-hydroxybenzamide (salicylamide).

A. 2-Ethoxybenzamide.
B. 2-Hydroxybenzamide.

the amyl ether (2-*n*-amyloxybenzamide H.P. 211). The amyl ether only maintains its superiority to acetylsalicyclic acid for forty minutes.

When oral and intraperitoneal doses of acetylsalicylic acid, ethyl and propyl ethers were compared, it was found that the oral doses were about half as effective as the intraperitoneal. The ethyl, propyl and butyl derivatives exert a remarkable antipyretic effect and experiments have shown that they are capable of lowering the normal temperature of the rat without any permanent toxic symptoms.

Analgesic Effect. These results are expressed in Table II, those in italics representing results from latin square tests. The results for a typical latin square test are given in Figure 2, from which it can be calculated graphically that ethoxybenzamide possesses 2·3 times the analgesic activity of salicylamide. Statistical analysis of the results confirms that the two dose response lines may be regarded as parallel and provides an estimate of 2·25 (fiducial limits 80 per cent. to 133 per cent. P = 0·95) for the ratio of analgesic activity of ethoxybenzamide to salicylamide. We are indebted to Mr. E. C. Feiller for the statistical examination of these results. It can be seen that the propyl, butyl and amyl ethers are better analgesics than salicylamide and a considerable improvement on acetylsalicylic acid.

Discussion

We have confirmed that salicylamide is a considerably better analgesic but a poorer antipyretic than acetylsalicylic acid. We have confirmed that it is less toxic than the latter, especially on repeated doses, as the rats used throughout our chronic toxicity test showed remarkable tolerance to the drug and a complete absence of macroscopic signs of damage to the stomach.

Contrary to Litter *et al*[9] who claim that salicylamide shortens the prothrombin time in human subjects, but in agreement with Ichniowski and Heuper,[10] we did not find the drug to have any significant effect on the prothrombin time of rats fed on a normal diet.

Some of the derivatives made in these Laboratories are, without seriously increasing the toxicity, a considerable improvement on salicylamide. The best of these, for example, may have 10 to 12 times the analgesic activity of acetylsalicylic acid. Some, notably the ethyl derivative, have remarkable antipyretic properties, including the ability to lower the normal temperature without permanently damaging the animal. The activities of the higher members are probably limited by poor absorption.

Summary

1. The toxicity and analgesic and antipyretic properties of salicylamide have been studied. A long term toxicity test using rats has shown it to be remarkably well tolerated.

2. A series of salicylamide derivatives has been investigated, and some members have shown better analgesic activity than salicylamide and better antipyretic activity than acetylsalicylic acid.

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DISCUSSION

The paper was presented by Mrs. P. D. WATERHOUSE.

DR. F. HARTLEY (London) said it was stated that at post-mortem on rats no macroscopic damage was found. He was not certain how long it would take for an ulcer to develop in a rat's stomach, and asked whether any histological examination had been made. It might have been wiser to use a larger animal than the rat in the investigation of possible gastric damage.

MR. D. N. GORE (Dorking) asked whether there was any clinical evidence of the comparative value of salicylamide and salicylates as antirheumatic agents.

MRS. P. D. WATERHOUSE, in reply, said that as far as the histological examination of the stomach was concerned there were no obvious signs of tissue damage. It might perhaps be worth while carrying out small scale toxicity tests on larger animals. Salicylamide had been used on the Continent for many years and there were a number of reports on its use in the treatment of rheumatism. It had been reported that there were fewer side effects with salicylamide than with salicylates.