LETTERS TO THE EDITORS

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Analgesic and Antipyretic Activities of 4-Hydroxy*iso*phthalic Acid

4-Hydroxy*iso*phthalic acid is a by-product of the manufacture of salicylic acid by the Kolbe–Schmitt reaction and has been found to be a major constituent of the 'brown dust' residues from the sublimation process for the purification of salicylic acid[1]. Analysis of a typical sample of 'brown dust' showed it to contain 10 per cent of salicylic, 82 per cent of 4-hydroxy-*iso*phthalic and 3 per cent of 2-hydroxy*iso*phthalic acids, together with some inorganic material. From this source there is thus potentially available a considerable quantity of 4-hydroxy*iso*phthalic acid, the chemistry of which is being investigated as part of a research programme on the Kolbe–Schmitt reaction at the Chemical Research Laboratory[2]. From a consideration of its structural similarity to salicylic acid, it was suggested at the Chemical Research Laboratory that 4-hydroxy*iso*phthalic acid might also possess similar pharmacological properties. Accordingly, in February 1954, a pharmacological investigation was initiated at Ware and, since the findings were favourable, biochemical studies were also undertaken.

In the initial experiments, drugs were administered by the intraperitoneal route, aspirin and phthalic acid derivatives being suspended in 5 per cent gum acacia. In analgesic tests, a simplified form of the analgesiometer of Green, Young and Godfrey[3] was used, which measures responses in terms of the pressure, applied to the tip of the tail, required to elicit a squeak from a young rat. This method showed that 4-hydroxy*iso*phthalic acid undoubtedly possesses analgesic properties, its median effective dose being 303 (limits 261–353) mgm. per kgm. In comparative tests it exhibited 4·1 (limits 3·2–5·5) per cent of the activity of codeine. The median lethal dose (*LD*50) for young rats was 1,071 (limits 968–1,185) mgm. per kgm., and a dose of 600 mgm. per kgm. was tolerated by all eighty-seven rats which received it. Comparison of the *LD*50 of 4-hydroxy-*iso*phthalic acid with the corresponding published figures for codeine[4] indicates that it possesses about 10 per cent of the toxicity of codeine. Compared with 4-hydroxy*iso*phthalic acid, aspirin was both more toxic and less effective. The *LD*50 was 541 (limits 485–603) mgm. per kgm., and a dose of 300 mgm. per kgm., which was tolerated by all rats, failed to show any analgesic effect, although higher doses appeared to do so.

In comparative tests of antipyretic activity in rabbits, it was found that 4-hydroxy*iso*phthalic acid was about as effective as aspirin in counteracting fevers caused by a preparation of pyrogen from *Proteus vulgaris*.

Since 4-hydroxy*iso*phthalic acid promised to have clinical value of a type similar to, but greater than, aspirin, a chronic toxicity test was carried out by administering the drug at 0·5 and 1·0 per cent in the diet to mice for a period of fourteen weeks. In this experiment, it exhibited very low toxicity, of the same order as that of aspirin, which was used for reference.

Analgesic tests were also performed with 2-hydroxy-*iso*phthalic, phthalic, *tere*phthalic and *iso*phthalic acids. The 2-hydroxy acid was about equipotent to the 4-hydroxy, while the unsubstituted acids showed slight activity.

Preliminary investigations on the excretion of 4-hydroxy*iso*phthalic acid by rats showed that within 24 hr. about 40 per cent of a 10-mgm. dose given by stomach tube was excreted unchanged in the urine and about 25 per cent in the fæces. The method of estimation was based on the reddish-purple colour formed when the acid is allowed to react with ferric nitrate. Acid hydrolysis failed to increase the amount of free 4-hydroxy*iso*phthalic acid, suggesting the absence of certain conjugated forms from the urine, while the absence of other possible metabolites, such as salicylic, *p*-hydroxybenzoic and gentisic acids, was demonstrated by means of paper electrophoresis.

Clinical trials to investigate the possible therapeutic applications of these findings are in progress.

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Imidazole Complexes of Myoglobin and the Position of the Hæm Group

We have prepared complexes of imidazole, 1-methyl imidazole and 4-methyl imidazole with various species of myoglobin, and crystallized them in forms previously described[1]; the X-ray diffraction patterns of the crystals were then compared with those obtained from corresponding forms of met-myoglobin crystals. The results, which were similar for all three imidazoles, are summarized in Table 1. (In each case a large excess of imidazole was used: without this precaution combination is always incomplete.)

Table 1

Species	Form	*a*	*b*	*c*	*β*	Intensities
Sperm-whale (ammon. sulphate)	Met- *A*	64·6 A.	31·1 A.	34·8 A.	105·5°	Scarcely changed
	Imid- *A*	64·4	30·7	34·7	105·5°	
Sperm-whale (phosphate)	Met- *B*	48·9	40·2	79·3		Considerable changes
	Imid- *B*	48·8	40·3	78·5		
Gentoo penguin (ammon. sulphate)	Met-	(no crystals formed)				
	Imid- *I*	94·5	38·3	43·5		
Gentoo penguin (phosphate)	Met- *G*	48·3	80·2	78·5		
	Imid- *H*	106·4	39·1	45·3		