Enhancement of Mouse-virulence of Group A Streptococci by Bactericidal Resorcinols

It seems to be generally agreed that agents capable of enhancing the virulence of bacteria, particularly mucins, do so by interfering with the defences of the host¹⁻³. Recently, it was shown⁴ that broth or peptone injected intraperitoneally into *W*-Swiss mice even 1 hr. after a non-lethal saline suspension of group A streptococci converts the non-lethal dose into an LD100, the time-lag pointing again to an action on the host. The present communication brings further evidence in this respect; it also shows that substances bactericidal *in vitro* can act as virulence-enhancing agents *in vivo*.

The resorcinols were the 4-*n* substituted alkyl derivatives, ethyl, propyl, butyl, amyl and hexyl resorcinol⁵. The intraperitoneal injection of 0.5 ml. of a 0.1 per cent saline solution of these compounds is without marked effect on mice.

Two strains of group A streptococci were used, one type 12, the other type 22; the LD100 was 0.5 ml. of a 10⁻² saline dilution of culture, the culture itself containing about 2 × 10⁷ viable cells per ml. The organisms were grown in broth consisting of 2 per cent 'Oxoid' peptone, 0.8 per cent 'Oxoid Lab. Lemco' broth powder, 0.2 per cent glucose and 0.2 per cent sodium bicarbonate made up in distilled water, sterilized by Seitz filtration and tubed in 4-ml. lots in $\frac{1}{2}$ oz. screw-capped Bijou bottles.

Two types of experiment were carried out. First, 18-hr. old cultures of streptococci were diluted 1 in 10 in 0.1 per cent solutions of the various resorcinols and kept, with occasional shaking, at room temperature, 24°, for 30 min., a 10⁻¹ saline-dilution of culture serving as control; thereafter *W*-Swiss mice were injected intraperitoneally with 0.5 ml. of the suspensions from which viable counts were made⁶; all control animals and all animals injected with the ethyl and propyl resorcinol suspensions of organisms died. There were no deaths in the groups injected with the organisms exposed to butyl, amyl and hexyl resorcinol. The butyl, amyl and hexyl suspensions were found sterile whereas the ethyl, propyl and saline suspensions still contained approximately the same number of viable organisms.

In the second group of experiments the organisms were not exposed *in vitro* to the action of the resor-

cinols: instead, mice were first injected intraperitoneally with 0.5 ml. of 10⁻²–10⁻⁶ saline dilutions of culture and immediately afterwards with 0.5 ml. of a 0.1 per cent saline solution of the resorcinols; control animals were given intraperitoneally 0.5 ml. of the saline dilutions of culture followed by 0.5 ml. of saline. Under these conditions the resorcinols proved to be not disinfectants but virulence-enhancing agents and a 100- to 1,000-fold decrease of the LD100 was observed (Table 1).

Thus it has been shown that the same substance can act both as a bactericide and as a virulence-enhancing agent, depending on the conditions of the experiment, and that in the alkyl resorcinols examined virulence-enhancing activity parallels bactericidal activity. The experiments rule out a true enhancement of virulence of the infecting organism and demonstrate that the action of the resorcinols is on the host.

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³ Smith, H., *J. Inf. Dis.*, **88**, 207 (1951).

⁴ Cameron, J., *Nature*, **180**, 1136 (1957).

⁵ Frobisher, M., *J. Bact.*, **13**, 163 (1927).

⁶ Miles, A. A., and Misra, S. S., *J. Hyg. (Camb.)*, **38**, 732 (1938).

A Possible Source of Error in the Chemical Detection of Indolyl Acetic Acid in Plants

DURING investigations on the growth substances of fruits of cacao and bananas (*Musa* spp.), it was of interest to find that a substance was present in seeded bananas which gave a pink colour with ferric chloride/perchloric acid or *p*-dimethyl amino-benzaldehyde/hydrochloric acid, corresponding to the *R_F* of indolyl acetic acid and to the zone of bioassay using the wheat coleoptile straight-growth test. Subsequent experimentation showed that although the straight-growth response was probably due to indolyl acetic acid, the colour reaction was not. The technique of extraction and bioassay was essentially similar to that of Bennet-Clark and Kefford¹ with some minor modifications. Duplicate samples of the acid fraction from seeded banana fruit extracts were developed by the descending method on Whatman's 3 MM or No. 1 chromatography paper in isopropanol/ammonia/water (10:1:1). Synthetic indolyl acetic acid was developed as a marker. One sample was bio-assayed by the wheat straight-growth test and the other sprayed with ferric chloride/perchloric acid (1 ml. 0.05 *M* ferric chloride + 50 ml. 5 per cent perchloric acid).

The ferric chloride/perchloric acid spray, on heating, developed a pink or pinkish-purple spot on the chromatogram corresponding to both the zone of growth activity after bio-assay and to the indolyl acetic acid marker. The top half of the chromatogram (*R_F* < 0.5) was usually streaked with oxidized polyphenolic substances which could be detected visually without treatment by the brown colour and confirmed by diazotized *p*-nitraniline. It was found that if synthetic indolyl acetic acid was spotted into

Table 1. EFFECT OF 4-*n* ALKYL-SUBSTITUTED RESORCINOLS ON MOUSE-VIRULENCE OF GROUP A STREPTOCOCCI

Strain	Alkyl resorcinol	Deaths in groups of 16 mice inoculated with the following culture dilutions*				
		10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
Cumming Type 22	None-saline control	16	8	0	0	0
	Ethyl	—	8	3	0	0
	Propyl	—	9	5	0	0
	Butyl	—	—	16	13	0
	Amyl	—	—	16	16	11
	Hexyl	—	—	16	16	9
1965 Type 12	None-saline control	16	10	1	0	0
	Ethyl	—	9	7	0	0
	Propyl	—	13	5	0	0
	Butyl	—	—	16	14	0
	Amyl	—	—	16	16	11
	Hexyl	—	—	16	16	12

* Mice injected intraperitoneally, first with 0.5 ml. of the stated saline dilutions of culture, then with 0.5 ml. of saline or of a 0.1 per cent saline solution of an alkyl resorcinol.