(3) The similar orders of activity of the isomeric analogues of the promedols that lack a 5-methyl substituent (α- and β-III) shows that the orientation of the 2-methyl group has little influence upon the activity of 4-phenylpiperidine analgesics.

In view of recent studies of brain levels of α- and β-II in mice (Abdel-Monem, Larson & others, 1970), it is probable that potency differences between isomeric promedols are due to differences in their affinities for the receptors rather than distribution and metabolism.

Details of evidence establishing the stereochemistry of the promedol isomers and their 2-methyl analogues will be given elsewhere.

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Lipid depletion of bacteria induced by biotin deficiency and its relation to resistance to antibacterial agents

Previous communications from this laboratory have shown that Gram-positive bacteria grown in the presence of glycerol increase in lipid content and that this is accompanied by an increase in resistance to penicillins (Hugo & Stretton, 1966a, b), and phenols (Hugo & Franklin, 1968). We now report that bacteria grown under conditions of biotin deficiency are depleted in their lipid content and this in turn is accompanied by a decrease in resistance to a variety of antibacterial agents.

The organisms used were *Staphylococcus aureus* (Oxford) NCTC 6571 which includes biotin amongst its growth requirements, and *Escherichia coli* T94A (strain 58–278 M), obtained from Professor W. W. Umbreit, which requires biotin and phenylalanine for growth. The biotin requirement of the *E. coli* can be alleviated by the presence of aspartate in the medium. The Oxford staphylococcus was grown in nutrient broth and the cells contained 68·4 μg/mg dry weight lipid in agreement with previous findings (Hugo & Stretton, 1966a, b; Hugo & Franklin, 1968). Growth in Difco biotin assay medium at half strength and supplemented with 2 μg/litre of biotin (optimum 10 μg/litre) produced cells in which the lipid content had fallen to 55·4 μg/mg dry weight, significantly less (19%) than the nutrient broth grown cells.

E. coli was grown in the synthetic medium of Gavin & Umbreit (1965), biotin deficient cells were obtained by growth in this medium from which biotin had been omitted and biotin adequate cells in the same medium containing 10 μg/ml of biotin. Biotin-adequate cells had a lipid content of 179 μg/mg dry weight and biotin deficient cells, 109 μg/mg dry weight, a decrease of 39%.

The biotin adequate and biotin deficient cells of both species were challenged with a series of antibacterial agents. Table 1 gives the minimum inhibitory concentrations.

Table 1. *Minimum inhibitory concentrations, μg/ml, for organisms grown in (a) a nutrient broth, (b) Difco biotin assay medium + 2 μg/litre biotin, (c) a synthetic medium, biotin adequate, (d) a synthetic medium, biotin-deficient*

Agent	*S. aureus*			*E. coli*		
	a	b	$\frac{b}{a}$	c	d	$\frac{d}{c}$
Phenol	2200	1550	0·70	1060	1000	0·95
4-Methylphenol	1390	1160	0·84	540	510	0·95
4-Ethylphenol	640	520	0·82	260	240	0·89
4-n-Propylphenol	390	220	0·79	155	145	0·94
$C_{12}H_{25}^-$ +	13·6	1·5	0·11	26·5	25·0	0·95
$C_{14}H_{29}^-$ +	7·5	1·2	0·16	14·5	12·0	0·83
$C_{16}H_{33}^-$ $-N(Me)_3\bar{B}r$	5·7	0·6	0·11	4·4	4·2	0·96
$C_{18}H_{37}^-$ +	10·5	1·2	0·11	4·0	3·6	0·90
Tetracycline	0·12	0·012	0·10	—	—	—
Oxytetracycline	0·12	0·016	0·13	—	—	—
Chlortetracycline	0·12	0·012	0·100	—	—	—
Benzylpenicillin	0·05	0·012	0·24	27·0	13·0	0·48
Chloramphenicol	—	—	—	10·0	9·0	0·90
Polymyxin B	—	—	—	0·53	0·50	0·95
Proflavine	—	—	—	31·0	27·0	0·88
Actinomycin D	25	1	0·04	>200	>200	—

A statistical analysis of the results with cetrimide (C_{18}) and phenol against the *E. coli* strains, using the method of paired comparisons, showed a significant difference, the former at the 5% level, the latter at the 2·5% level.

With *E. coli* the minimum inhibitory concentration ratio is almost a constant irrespective of the drug used, although benzylpenicillin is an exception. We also note that despite the change in lipid content induced in *E. coli*, the increased susceptibility is generally less than that induced by EDTA treatment (Leive, 1965, 1968; Hamilton-Miller, 1966) which removes lipopolysaccharide and we were unable to induce susceptibility to actinomycin D (Leive, 1968).

With *S. aureus* an increase in sensitivity was found with all the antibacterial agents used, the largest occurring with actinomycin D. The ratios of the other antibacterial agents tested fell into two distinct groups, the phenols showing a smaller increase than the rest (Table 1).

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