Inhibition of Microbial Multiplication by Hypocholesteraeic Compounds

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SUMMARY

The hypocholesteraeic compounds benzmalecene and triparanol inhibited multiplication of certain bacteria (especially Gram-positive organisms), protozoa, algae and ascomycetes but not other higher fungi. Oleic acid (or methyl oleate) annulled the inhibition in bacteria, algae, protozoa and a yeast.

INTRODUCTION

Certain hypocholesteraeic compounds, e.g. benzmalecene and triparanol, which interfere with sterol synthesis in mammals (Holmes & Di Tullio, 1962) have been observed to inhibit protozoan multiplication (Aaronson, Bensky, Shifrine & Baker, 1962) and respiration (Aaronson, 1964*a*), and *Rhodopseudomonas palustris* multiplication (Aaronson, 1964*b*). The inhibitions of microbial multiplication were annulled best by unsaturated fatty acids; sterols and sterol precursors were less effective or were ineffective. Presumably the hypocholesteraeic compounds interfere with unsaturated fatty acid metabolism in the micro-organisms, rather than with sterol metabolism as in mammals.

METHODS

Micro-organisms from the collection of Haskins Laboratories and the Biology Department, Queens College, New York, were used. For disc assays, filter-paper discs (12·7 mm. diam.) were soaked in solutions of the test compound, dried and autoclaved. Various agar media appropriate for the growth of the micro-organisms, e.g. nutrient agar for the bacteria, and Sabouraud's glucose agar for the fungi, were seeded with test micro-organisms. Cultures were incubated at 25° for 5–10 days. In some experiments chemically defined liquid media were used appropriate to the protozoan or alga. Benzmalecene (BM; *N*-[1-methyl-2,3-di-(*p*-chlorophenyl)-propyl]maleamic acid) was supplied by Dr D. Hendlin (Merck, Sharp and Dohme Laboratories, Rahway, N.J.). Triparanol (TR; 1-[*p*-(β -diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-*p*-chlorophenyl ethanol) was supplied by Dr F. J. Murray (W. S. Merrell, Cincinnati, Ohio). Benzmalecene was dissolved in dilute alkali, and triparanol in 95 % (v/v) ethanol in water. Oleic acid (99 % pure by gas-liquid chromatography) was bought from the Hormel Institute, Austin, Minnesota. The other chemicals were of a commercial grade.

Table 1. *Inhibition of micro-organisms by hypocholesteræmic compounds*

Key:

¹ Bacteria showed zone of inhibition around discs soaked in 0.1 % or 1.0 % (w/v) concentration of inhibitor.

* Inhibited on nutrient agar plates but not in defined liquid medium.

† Inhibited on nutrient agar plates and in defined liquid medium.

‡ Results of M. Shifrine.

§ Results of G. G. Holz, Jr.

Schizomycetes	Benz- malecene ¹	Tri- paranol ¹		Benz- malecene ¹	Tri- paranol ¹
Gram-positive			Ascomycetes		
<i>Bacillus cereus</i>	+	+	<i>Dipodascus uninucleatus</i>	.	+‡
<i>B. megatherium</i>	+	+	<i>Endomycopsis javanensis</i>	.	+‡
<i>B. mesentericus</i>	+	+	<i>Lipomyces starkey</i>	.	+‡
<i>B. mycoides</i>	+	+	<i>Penicillium chrysogenum</i>	.	+§
<i>Brevibacterium linens</i>	+	+	<i>Pichia fermentans</i>	.	+‡
<i>Corynebacterium linens</i>	+	+	<i>Rhodotorula marina</i>	.	+‡
<i>C. pointsettiae</i>	+	+	<i>Saccharomyces cerevisiae</i>	+	+
<i>C. pseudodiphtheriticum</i>	+	+	<i>S. rosei</i>	.	+‡
<i>C. xerosis</i>	+	+	<i>Saccharomycopsis guttulata</i>	.	+‡
<i>C. bovis</i>	+	+	<i>Schizosaccharomyces pombe</i>	.	+‡
<i>Lactobacillus casei</i>	—	—	<i>Sordaria</i> sp.	+	+
<i>L. plantarum</i>	.	+§	<i>Torulopsis utilis</i>	.	+§
<i>Micrococcus luteus</i>	+	+	Phycomycetes		
<i>M. lysodeikticus</i>	+	+	<i>Phycomyces</i> sp.	—	—
<i>M. roseus</i>	+	+	<i>Rhizopus nigricans</i>	—	—
<i>M. sodonensis</i>	+	+	Basidiomycetes		
<i>Sarcina flava</i>	+	+	<i>Sporobolomyces</i> sp.	.	—‡
<i>S. lutea</i>	+	+	Algae		
<i>Staphylococcus albus</i>	+	+	<i>Coccochloris elabens</i>	+	+
<i>S. aureus</i>	+	+	(Cyanophyta)		
<i>S. citreus</i>	+	+	<i>Chlorella pyrenoidosa</i>	.	+§
<i>Mycobacterium lacticola</i>	+	+	(Chlorophyta)		
<i>M. smegmatis</i>	+	+	Protozoa		
<i>M. phlei</i>	.	+§	<i>Euglena gracilis</i>	+	+
Gram-negative			<i>Ochromonas danica</i>	+	+
<i>Aerobacter aerogenes</i>	—*	—*	<i>Tetrahymena pyriformis</i>	+	+
<i>Agrobacterium tumefaciens</i>	.	+§	<i>T. corlissi</i>	.	+§
<i>Escherichia coli</i>	—†	—†			
<i>Klebsiella pneumoniae</i>	—	—			
<i>Paracolobacterium intermedium</i>	—	—			
<i>Proteus mirabilis</i>	—	—			
<i>P. vulgaris</i>	—	—			
<i>Pseudomonas aeruginosa</i>	—	—			
<i>Salmonella gallinarum</i>	—	—			
<i>Serratia marcescens</i>	—	—			
<i>Rhodopseudomonas palustris</i>	+	+			

RESULTS

Almost all the Gram-positive bacteria and almost none of the Gram-negative bacteria tested were inhibited by BM and TR (Table 1). Multiplication of several algae, protozoa and ascomycetes but not phycomycetes or a basidiomycete was also inhibited (Table 1). The Gram-positive mycobacteria were most sensitive to BM and TR by plate assay (Table 2).

Inhibition by BM and TR of the multiplication of a bacterium *Corynebacterium bovis*, a blue-green alga *Coccochloris elabens*, and a yeast *Saccharomyces cerevisiae*,

Table 2. Comparison of the antimicrobial activity of benzmalecene and triparanol on several bacteria

The bacteria were grown on nutrient agar plates.

Organism	Concentration of disc (%)					
	0.01	0.1	1.0	0.01	0.1	1.0
	Diameter of inhibition zone (cm.)					
	Benzmalecene			Triparanol		
<i>Mycobacterium lacticola</i>	0	4	3	3	4	9
<i>M. smegmatis</i>	0	3	5	4	8	9
<i>Bacillus mycoides</i>	0	1	5	0	0	4
<i>B. mesentericus</i>	0	0	3	0	0	3
<i>Staphylococcus albus</i>	0	0	3	0	0	2
<i>S. citreus</i>	0	0	2	0	0	2
<i>Micrococcus luteus</i>	0	1	2	0	5	5
<i>M. roseus</i>	0	0	3	0	1	4
<i>Sarcina flava</i>	0	0	2	0	0	4
<i>S. lutea</i>	0	2	3	0	0	4
<i>Corynebacterium poinsettiae</i>	0	0	2	0	0	3
<i>C. xerosis</i>	0	0	3	0	0	2
<i>Escherichia coli</i>	0	0	0	0	0	0
<i>Klebsiella pneumoniae</i>	0	0	0	0	0	0
<i>Proteus vulgaris</i>	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0
<i>Serratia marcescens</i>	0	0	0	0	0	0

Table 3. Annulment of benzmalecene and triparanol inhibition of growth by lipids

	Inhibition of multiplication by benzmalecene and triparanol annulled by		
	Oleic acid	Ergosterol	Squalene
Sterols not synthesized			
Bacteria			
<i>Corynebacterium bovis</i>	+++	++	+
<i>Rhodopseudomonas palustris</i>	+++	++	+
Blue-green alga			
<i>Coccochloris elabens</i>	+++	++	+
Protozoon			
<i>Tetrahymena pyriformis</i>	+	+	—
Sterols synthesized			
Ascomycete			
<i>Saccharomyces cerevisiae</i>	+++	++	+
Protozoon			
<i>Ochromonas danica</i>	+++	—	—

Key: + + +, most active; + +, less active; +, least active; —, not active.

was annulled by oleic acid (or methyl oleate), ergosterol, and squalene in that order, as was also inhibition of the bacterium *Rhodopseudomonas palustris* (Aaronson, 1964*b*). Saturated fatty acids were ineffective. Inhibition induced by BM and TR in micro-organisms was annulled best by oleic acid (Table 3).

DISCUSSION

The difference in sensitivity between the Gram-positive and the Gram-negative bacteria examined may be associated with the difference in lipid content of bacteria:

0.3% in Gram-positive bacteria and 11–22% in Gram-negative (Salton, 1960). The sensitivity of the ascomycetes and the insensitivity of the other fungi tested draw attention to the mounting evidence for deep-seated differences between the ascomycetes and eumycophyta. Bergh, Webb & McArthur (1964) suggested that the Gram reaction may be associated with the resistance of the lipids of Gram-positive organisms to extraction by polar solvents. Ascomycetes are Gram-positive and like the Gram-positive bacteria were inhibited by benzmalecene and triparanol, both compounds known to affect lipid metabolism in animals. The annulment of BM and TR inhibition of microbial multiplication by oleic acid, rather than by saturated fatty acids, sterols or sterol precursors, suggests that lipid metabolism in the microorganisms tested differs from lipid metabolism in mammals. In mammals BM and TR inhibit primarily sterol synthesis (Holmes & Di Tullio, 1962) and fatty acid synthesis is only secondarily affected (Garattini *et al.* 1961). The hypocholesteremic compound triparanol has a low toxicity for humans even in high concentrations (Furman & Robinson, 1961) although side-effects have been noted (Steinberg, 1962; Achor, Christensen, Berge & Mason, 1963; Roe, 1964). The sensitivity of the Gram-positive bacteria tested, notably the *Mycobacterium* species, to triparanol and benzmalecene may prove useful in the design of a new group of chemotherapeutic agents.

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Note added in proof

Since writing this paper, the author's attention has been drawn to Smith, R.F., Shay, D. E. and Doorenbas, N. J. (1963) 'Antimicrobiol action of nitrogen-containing steroids' *J. Bact.* **85**, 1295, who state that nitrogen-containing steroids, compounds resembling certain hypocholesteremic compounds i.e. azacholesterol, inhibited the multiplication of certain yeasts, moulds, actinomycetes, and Gram-positive bacteria but not most Gram-negative bacteria tested.