Inhibition of Yeast Multiplication by Hypocholesteremic Compounds and its Annulment by Lipids[1] (35192)

S. AARONSON

Biology Department, Queens College City University of New York, Flushing, New York 11367; and Haskins Laboratories, New York, New York 10017

Recently Gallo and Zygmunt (1) pointed out that while the pathways of sterol synthesis in mammalian systems and yeasts are similar, it is dangerous to assume that inhibitors of sterol biosynthesis in mammals would act similarly in microbial systems. Evidence is presented here that verifies and extends their conclusion that it is dangerous to assume that inhibitors of sterol synthesis in higher eucaryotic organisms must necessarily function the same way in sterol biosynthesis in lower eucaryotic microorganisms. Evidence is also presented that comparative studies of this kind, may, however, reveal metabolic targets not easily uncovered in mammals and may thus be used to predict unusual side-effects of drugs in mammals (2, 3).

The hypocholesteremic compounds, benzmalecene (BM) and triparanol (TR) inhibited cholesterol synthesis in mammals (4). BM and TR also inhibited the multiplication of procaryotic (5, 6) and eucaryotic (2, 6–8) microorganisms. Here the inhibition of yeast multiplication by BM and TR and the annulment of this inhibition by lipids is described and its implications are discussed.

Methods. *Saccharomyces cerevisiae* diploid strain LK 2G12 was grown in a chemically-defined medium modified from Phaff *et al.* (9) and lacking folic acid and amino acids at 30° in an incubator for 1 week. Multiplication was measured in optical density units with a Welch Densichron equipped with a red-sensitive light probe and a cuvette with a 1-cm light path. All experiments were repeated at least 3 times with identical results. The fatty acids (99% pure by gas liquid chromatography) were purchased from the Hormel Institute, Austin, Minn. Benzmalecene was supplied by Dr. D. Hendlin (Merck, Sharpe and Dohme Research Laboratories, Rahway, N.J.) and triparanol (MER-29) was obtained from Dr. F. J. Murray, (W. S. Merrell Co., Cincinnati, Ohio).

Results. The inhibition of *S. cerevisiae* multiplication by BM and TR (Table I) was annulled by lecithin and by the following defined lipids in order of effectiveness: oleic acid, lauric acid, ergosterol, squalene. The following lipids were inactive: mevalonic acid lactone, geraniol, farnesol, lanosterol, cholesterol, β-sitosterol, stigmasterol; and myristic, palmitic, and stearic acids.

Discussion. In mammals, BM and TR inhibited primarily sterol synthesis (4). In procaryotic microorganisms (5) which synthesize little, if any, steroids and eucaryotic microorganisms (2, 5) including, here, *S. cerevisiae* which synthesize steroids, the inhibition of multiplication by BM and TR is annulled most effectively by the unsaturated fatty acid, oleic acid. This implied that the prime metabolic target of BM and TR involved either the synthesis or function of an unsaturated fatty acid. Earlier work from this laboratory (3, 10) indicated that aerobic respiration in the eucaryotic phytoflagellate *Ochromonas danica* was inhibited by BM and TR (10) and that this inhibition of aerobic respiration was annulled most effectively by unsaturated fatty acids and not at all by saturated fatty acids, cholesterol, or squalene (3). The annulment of the TR inhibition of aerobic respiration of *O. danica* seemed to be a physical phenomenon in which the TR and unsaturated fatty acid formed a non-covalent complex which prevented the TR from acting as an inhibitor (3). It seems then that the main metabolic target of BM and TR in

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TABLE I. Inhibition of Multiplication of *S. cerevisiae* by BM and TR and Its Annulment by Lipids.

		Optical density; conc (m*M*)									
		BM					TR				
Compound	Conc	0	0.03	0.08	0.16	0.30	0.007	0.01	0.02	0.07	0.14
	(m*M*)										
0		0.98	1.00	0	0	0	0.95	0.62	0	0	0
Oleic acid	0.04	0.96	1.18	0	0	0	0.95	0.96	0.97	0	0
	0.35	0.98	1.24	1.32	0.22	0.46	0.94	0.96	0.98	0.99	0
	0.70	1.00	1.20	1.32	1.34	1.31	0.98	0.99	0.98	0.99	1.27
Lauric acid	0.04	0.94	0.99	0	0	0	0.94	0	0	0	0
	0.35	0.86	0.91	0.77	0.14	0.21	0.90	0.89	0.89	0	0
Squalene	0.04	0.97	1.00	0	0	0	0.95	0.93	0	0	0
	0.35	1.12	1.17	0.16	0.32	0.32	0.95	0.98	0.82	0	0
Ergosterol	0.04	0.99	0.99	0.06	0	0	0.97	0	0	0	0
	0.35	1.16	1.18	0.30	0.41	0.53	1.16	1.16	0.66	0	0
	(mg/100 ml)										
Lecithin	1.0	0.97	1.12	0.03	0	0	0.93	0.95	0.96	0.04	0
	3.0	0.97	1.12	0.03	0	0	0.91	0.96	0.97	0.02	0
	10.0	1.12	0.99	0.81	0.03	0	0.98	0.99	0.97	0.96	0.04
	20.0	0.99	1.00	0.96	0.06	0	0.96	0.97	0.98	0.93	0.03
	30.0	1.12	1.12	0.99	0.21	0	0.98	1.12	0.99	0.92	0.95

microorganisms was an unsaturated fatty acid perhaps in the more accessible respiratory organelle, probably the membrane of the mitochondrion of eucaryotes or the cell membrane of procaryotes. This is also supported by the greater annulment ability of the phospholipid, lecithin.

That hypocholesteremic compounds may exercise an important inhibitory role on ATP synthesis is also borne out by the following observations. BM and TR uncoupled oxidative phosphorylation in rat liver mitochondria (11, 12) and at higher concentrations BM and TR inhibited mitochondrial electron flow (12). Other hypocholesteremic compounds, *i.e.*, an azasteroid, inhibited oxidation of reduced NAD in bacterial membranes (13) and respiration in heart submitochondrial particles (14). Conversely, inhibitors of oxidative phosphorylation inhibited the synthesis of nonsaponifiable material and cholesterol in rat liver homogenates (15).

It may also be pertinent to note that while most observers have found that hypocholesteremic compounds inhibited sterol biosynthesis in mammals there are several observations that suggest that fatty acids synthesis may also be a target, *i.e.*, TR inhibited fatty acid synthesis in the ciliate, *Tetrahymena* (16), and in rats (17). The inhibition of multiplication in an ascomycete, *Sordaria*, by several hypocholesteremic compounds was annulled by unsaturated fatty acids but not saturated fatty acids or sterols (18). It is thus useful to study the inhibition of drugs on a variety of biological systems for these studies may yield important clues to potential side-effects in humans. The annulment of the BM and TR inhibition by the saturated 12-carbon fatty acid, lauric acid, remains unexplained.

Summary. The hypocholesteremic compounds, benzmalecene and triparanol, inhibited the multiplication of the yeast, *Saccharomyces cerevisiae*, and this inhibition was annulled by lecithin, oleic acid, lauric acid, ergosterol, and squalene in that order. Saturated fatty acids, other sterols, and sterol precursors were ineffective. The implications of these results for uncovering unsuspected metabolic targets and side-effects of drugs in humans is discussed.

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