Antimetabolites of Mevalonic Acid Utilization in *Lactobacillus acidophilus* 4963.* (23478)

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Mevalonic acid refers to a new compound, beta - methyl - beta, delta - dihydroxy valeric acid (MVA) discovered by Skeggs *et al.*(1) as a growth factor for certain lactobacilli in a medium devoid of acetate, isolated by Wright *et al.*(2) from a large quantity of distillers' solubles, and characterized as described above by Wolf *et al.*(3). The compound has been shown by Tavormina *et al.*(4) to be converted readily to cholesterol by an enzyme system of rat liver. Subsequent studies from several laboratories have confirmed the high order of activity of MVA as an intermediate in cholesterol or squalene biosynthesis and have supplied information on the mechanism of the incorporation(5,6,7,8,9). During the course of some current studies on MVA metabolism various compounds that have been implicated with a relationship to cholesterol biosynthesis in mammalian tissue have been examined for their effect on mevalonic acid utilization by *Lactobacillus acidophilus* 4963, an organism that may be grown on a synthetic medium containing relatively small amounts of MVA or relatively large amounts of acetate. The microbiological results may be of interest for comparative purposes.

Methods and materials. Microbiological determinations were carried out with *Lactobacillus acidophilus* ATCC 4963 essentially as described by Skeggs *et al.*(1). MVA was used in the form of the DL N, N¹-dibenzylethylenediammonium salt.† The extent of bacterial growth was determined with a Klett-Summerson photoelectric colorimeter and the results given are in terms of scale units corrected for the reading of the blanks (no added MVA). Hydroxy methyl glutaric acid (HMG) and dimethyl acrylic acid (DMA, methyl crotonic acid, senecioic acid) were commercial products. Farnesinic acid (FA) #1‡ was synthesized by the Reformatsky re-

TABLE I. Influence of Farnesinic Acid (FA) on Response of *Lactobacillus acidophilus* 4963 to Mevalonic Acid (MVA).

Exp. No.	MVA, γ/10 ml	FA, γ/10 ml	Turbidity
1	0		0
	.1		29
	.2		44
	.3		53
	.4		56
	.6		65
	1.0		75
	.1	100, #1	8
	"	200	0
	"	300	0
	"	500	0
	"	1000	0
	1	100	73
	1	200	65
	1	300	58
	1	500	42
	1	1000	0
	10	100	82
	"	200	80
	"	300	71
	"	500	51
	"	1000	7
2	0		0
	.1		19
	.2		32
	.3		40
	.4		51
	.6		69
	1.0		88
	.1	10, #1	19
	"	20	21
	"	30	19
	"	50	23
	"	100	13
	"	200	0
	"	300	0
	"	500	0
	.1	10, #2	20
	"	20	18
	"	30	22
	"	50	21
	"	100	22
	"	200	13
	"	300	4
	"	500	0

* Supported in part by research grant from National Science Fn.

† Kindly supplied by Dr. Karl Folkers, Merck Sharp and Dohme Laboratories, Rahway, N. J.

‡ Kindly supplied by Dr. James M. Sprague, Merck Sharp and Dohme Laboratories, West Point, Pa.

TABLE I (continued).

Exp. No.	MVA, γ/10 ml	FA, γ/10 ml	Turbidity
3	0		0
	.1		28
	.2		36
	.3		40
	.4		50
	.6		64
	1.0		79
	.1	200, #1	6
	"	400	0
	"	600	0
	.1	200, #2	19
	"	400	4
	"	600	0
	.1	200, #3	18
	"	400	3
	"	600	0

action from geranyl acetone, samples #2§ and #3‖ were prepared from nerolidol through farnesal. α-Phenyl butyric acid (PBA) and α-p-biphenylyl butyric acid (BBA) were synthesized by accepted methods.‡

Results. As indicated by the data of Tables I and II farnesinic acid (FA), α-p-biphenylyl butyric acid (BBA), β-hydroxy-β-methyl glutaric acid (HMG), α-phenyl butyric acid (PBA), and possibly dimethyl acrylic acid (DMA) are all antimeabolites of MVA in decreasing order of activity as listed in the growth of *Lactobacillus acidophilus* 4963. There is some suggestion that FA sample #1 prepared by the Reformatsky reaction and likely to contain a monocyclic byproduct impurity is more active than the other 2 samples. Dituri *et al.*(10) have presented evidence that farnesinic acid is an intermediate between acetate and squalene. Sandermann and Stockmann, on the other hand, have reported(11) that C^{14}-labeled FA prepared by the Reformatsky reaction from geranyl acetone is not converted to C^{14}-cholesterol by rat liver, but sufficient experimental data are not given for a critical evaluation. Evidence that FA is not an intermediate of squalene synthesis in yeast is provided by the results of Amdur *et al.*(6) obtained with doubly-labeled (carbon(C^{14}) and hydrogen(tritium, T)) MVA. This group found that 2-C^{14}-5-di-T-MVA is incorporated into squalene by a particle-free

§ Kindly supplied by Dr. Gilbert Stork, Columbia University, N. Y. City.

‖ Kindly supplied by Drs. Frank Dituri and Samuel Gurin, University of Pennsylvania, Philadelphia.

TABLE II. Influence of Various Compounds on Response of *Lactobacillus acidophilus* 4963 to Mevalonic Acid (MVA).

Exp. No.	MVA, γ/10 ml	Analog, γ/10 ml	Turbidity
1	0		0
	.1		52
	.2		55
	.3		59
	.4		63
	.6		70
	1.0		80
	.1	100 HMG	43
	"	200	38
	"	300	30
	"	400	22
	"	1000	9
	"	2000	4
	"	3000	4
	"	4000	6
	.2	100	56
	"	200	53
	"	300	55
	"	400	55
	"	1000	42
	"	2000	25
	"	3000	19
	"	4000	16
2	0		0
	.1		34
	.2		50
	.3		57
	.4		59
	.6		73
	1.0		84
	.1	100, FA #1	3
	"	200	2
	"	300	0
	"	500	0
	.1	100 HMG	20
	"	200	17
	"	300	12
	"	500	3
	.1	100 DMA	21
	"	200	23
	"	300	20
	"	500	13
	.1	100 PBA	24
	"	200	23
	"	300	24
	"	500	15
	.1	100 BBA	16
	"	200	13
	"	300	11
	"	500	9

FA = Farnesinic acid. HMG = Hydroxy methyl glutaric acid. DMA = Dimethyl acrylic acid. PBA = Phenyl butyric acid. BBA = Biphenylyl butyric acid.

system of yeast with no change in the T:C^{14} ratio. If FA were an intermediate a decrease in the T:C^{14} ratio of 33⅓% would be required since in the formation of FA from MVA the primary alcohol of 1 MVA in 3 is oxidized to a carboxyl group with corresponding loss of all T or H. Wright and Cleland have reported(9) that the conversion of C^{14}-MVA to cholesterol by rat liver homogenates is markedly reduced by the presence of FA. Such an effect would be expected of a compound that is either a precursor or an antimetabolite of MVA. The present microbiological results favor the interpretation that FA is an antimetabolite of MVA utilization. It must be admitted, however, that the fate of MVA in the lactobacillus is unknown and it is conceivable that FA is a metabolite of MVA in the liver system and an antimetabolite in the microbiological system.

PBA and BBA have found some utility as hypocholesterolemic agents, particularly in Europe. Tavormina and Gibbs have studied (12) these compounds as antimetabolites of acetate and mevalonate in the rat liver homogenate system that synthesizes cholesterol and have reported that BBA suppresses acetate and mevalonate incorporation 99 and 93% respectively whereas PBA has no significant effect on acetate or mevalonate incorporation. It is of interest, therefore, that the present microbiological procedure evaluates these 2 compounds in the same order as antimetabolites of mevalonic acid utilization. It is suggested that this microbiological system may have utility in the search for other hypocholesterolemic compounds.

The finding that HMG is an antimetabolite of MVA was not expected. The compound is known to occur in liver but it is no longer considered to be a metabolite in cholesterol synthesis(13). The compound was not found to have significant antimetabolite activity in the liver system(9). DMA, a compound that is incorporated into cholesterol by mammalian tissue at such a low rate as to be considered off the main path of biosynthesis(4), has low to questionable antimetabolite activity.

Summary. Farnesinic acid, α-p-biphenylyl butyric acid, β-hydroxy-β-methyl glutaric acid, α-phenyl butyric acid and dimethyl acrylic acid were found to be antimetabolites of mevalonic acid in this order of decreasing activity for the growth of *Lactobacillus acidophilus* 4963. Implications of these findings are discussed.

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Received July 29, 1957. P.S.E.B.M., 1957, v96.