Inactivity of Dihydrouracil and β-Ureidopropionic Acid as Pyrimidine Precursors for Lactic Acid Bacteria. (21053)

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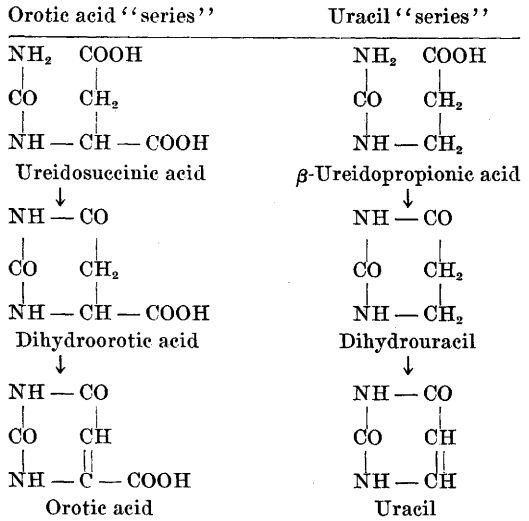
A recent study(1) showed that certain "pyrimidineless" lactobacilli may be classified into 3 groups (a) those (*Lactobacillus arabinosus* ATCC 8014 as an example) for which uracil rather than orotic acid is the "preferred" pyrimidine, (b) one (*Lactobacillus bulgaricus* 09) that utilizes orotic acid but not uracil, and (c) those (*Lactobacillus bulgaricus* ATCC 8001 as an example) for which uracil and orotic acid are equally active and essentially indistinguishable. These results at least suggest the possibility that there are 2 independent mechanisms for pyrimidine nucleotide or nucleic acid pyrimidine synthesis. One would appear to involve orotic acid while the other would appear to involve uracil. A number of intermediates in the biosynthesis of orotic acid have been established and include dihydroorotic acid(1,2) and ureidosuccinic acid(4-6). These compounds are active in supporting growth of *L. bulgaricus* 09 which utilizes orotic acid but not uracil. The corresponding compounds in a similar hypothetical biosynthesis of uracil are dihydrouracil and β-ureidopropionic acid.



This paper presents data to show that dihydrouracil and β-ureidopropionic acid are inactive in promoting growth of "pyrimidineless" lactobacilli that utilize uracil instead of orotic acid or utilize uracil and orotic acid equally well. The obvious conclusion from these studies is that orotic acid and uracil do not originate by "related" biosynthetic pathways.

Experimental. β-Ureidopropionic acid was synthesized by treatment of β-alanine methyl ester with KCNO according to the procedure of Lengfeld and Stieglitz(7). Dihydrouracil was synthesized from β-ureidopropionic acid by cyclization in acid solution(7). Both compounds as used were analytically correct. Dihydrouracil and β-ureidopropionic acid were examined for activity in promoting growth of *L. leichmannii* (ATCC 4797), *L. brevis* (ATCC 8287), *L. arabinosus* (ATCC 8014), *L. helveticus* (ATCC 335), *L. bulgaricus* 09, *L. bulgaricus* Sarles, *L. bulgaricus* (ATCC 8001), and *L. bifidus* (ATCC 4963) on media and under conditions described previously in detail(1). For *L. leichmannii, L. arabinosus,* and *L. helveticus* a pyrimidine source is only *stimulatory*. With these strains it was necessary to employ an incubation period of about 18 hours. For *L. brevis, L. bulgaricus* 09, *L. bulgaricus* Sarles, *L. bulgaricus* 8001, and *L. bifidus* a utilizable pyrimidine source is *essential*. With these strains an incubation period of 72-120 hours was used. The response to a pyrimidine source was determined by turbidimetric measurement with a Klett-Summerson photoelectric colorimeter.

Results. Both dihydrouracil and β-ureidopropionic acid are completely inactive in satisfying the pyrimidine requirements of the 8 strains of lactobacilli studied. Typical data demonstrating this point with *Lactobacillus bulgaricus* (ATCC 8001) are given in Table

TABLE I. Response of *Lactobacillus bulgaricus* (ATCC 8001) to Uracil, Dihydrouracil and β-Ureidopropionic Acid.

Compound (γ/10 ml)	Turbidity (Klett-Summerson units)
0	42
5 γ uracil	51
10	74
15	78
20	88
50	178
100	315
500	387
10 to 500 γ dihydrouracil	43
10 to 500 γ β-ureidopropionic acid	43

I. It is interesting to note that while this strain, for example, can utilize dihydroorotic acid presumably by unsaturation to orotic acid and can utilize uracil as well as orotic acid(1) it does not accomplish the unsaturation of dihydrouracil. In this connection Lieberman and Kornberg recently have shown(3) that dihydroorotic dehydrogenase from a bacterial source that is concerned with the reversible reaction: Orotic acid + DPNH + H^+ ⇌ Dihydroorotic acid + DPN is inert towards uracil, cytosine, 5-methyleytosine, or thymine.

Summary. Dihydrouracil and β-ureidopropionic acid, the "uracil counterparts" of dihydroorotic acid and ureidosuccinic acid, intermediates in the biosynthesis of orotic acid, are inactive in promoting growth of 8 pyrimidineless lactobacilli.

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Effect of Diacetyl on Microorganisms.* (21054)

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It is not clear whether diacetyl enjoys a major or relatively minor role in the metabolism of plants and animals. This compound occurs in a large variety of natural products such as tobacco, butter, coffee, cocoa, beer, and honey. The citric acid-fermenting streptococci produce diacetyl and cultures of these organisms are widely used for the production of desirable flavors in dairy products(1). This compound is metabolized by bacteria, bacterial extracts and mammalian tissue through

TABLE I. Inhibitory Concentration of Diacetyl for Microorganisms.

Organism	Diacetyl (μg/ml)	Organism	Diacetyl (μg/ml)
E. floccusum	200	*A. aerogenes*	400
A. niger	"	*P. vulgaris*	"
S. carlsbergensis	"	*B. mycoides*	"
S. griseus	"	*B. subtilis*	"
E. coli	300	*B. anthracis*	"
P. pyocyaneus	"	*Cl. tetani*	"
S. paradysenteriae	"	*S. viridans*	"
S. schottmuelleri	"	*M. pyogenes*	600
S. typhosa	"	*S. hemolyticus*	800
M. tuberculosis (607)	"	*S. fecalis*	"
M. phlei	"	*S. lactis* (8043)	1200
N. catarrhalis	"	*L. casei* (7469)	1600

Laboratory strains except where indicated.

* A portion of this work was done in the Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.