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Study of Ureidosuccinic Acid and Related Compounds in Pyrimidine Synthesis by *Lactobacillus bulgaricus* 09. (19455)

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Lactobacillus bulgaricus 09 has been found to respond to orotic acid as a growth factor (1-4). Several intermediates in the chemical synthesis of orotic acid have been tested and ureidosuccinic acid was shown to have from 10-20% of the activity of orotic acid(2). In a study of certain natural products it was found that human urine gave a response equivalent to about 100 γ of orotic acid per milliliter(2). Isolation studies in these laboratories have revealed that the microbiological activity of urine is due to urea.

The utilization of urea by *L. bulgaricus* raises the question as to whether or not the organism utilizes only the urea portion of the ureidosuccinic acid molecule in the synthesis of pyrimidines. Both orotic acid and ureidosuccinic acid are utilized for the biosynthesis of pyrimidines as shown by tracer studies(5). In these studies it was demonstrated only that the ureide carbon of ureidosuccinic acid is incorporated into pyrimidines of nucleic acids. Supporting evidence is presented at this time to show that in all probability the entire molecule of ureidosuccinic acid is utilized by *L. bulgaricus* 09 in pyrimidine synthesis.

Experimental. The compounds were tested for orotic acid-like activity by the method of Wright *et al.*(2), with one exception namely that the basal medium contained no added uracil. All compounds were tested alone, but in order to obtain information as to any possible antagonistic or enhancing activity, some were tested in 2 additional ways, with orotic acid at levels of 20 γ per tube and with ureidosuccinic acid at levels of 100 γ per tube. Control tubes of orotic acid were run at levels from 0 to 100 γ per tube and ureidosuccinic acid from 100 γ to 500 γ per tube. Ureidosuccinic acid also was tested using aseptic addition to detect the possibility of structural alteration during autoclaving. No difference was found between the results obtained by the two methods. The compounds tested and the results obtained are shown in Table I. The ureides were prepared by the usual method(6) of treating the appropriate amino acid with an equivalent amount of potassium cyanate in a concentrated aqueous solution, evaporating to a syrup, or in some cases, to dryness on a steam bath, redissolving in water, acidifying with concentrated HCl with cooling and recrystallizing the product from boiling water. The succinyl thiourea was prepared by the dry fusion of succinic anhydride and thiourea (7). The 3-acetyl 2-thiohydantoin was prepared from glycine and potassium thiocyanate(8).

Results. Of the compounds tested, ureidosuccinic acid was the only compound with activity comparable to orotic acid. Urea showed a greater activity than most compounds tested. No significant enhancing effects were noted, and only thiourea showed any antagonism.

Discussion. A comparison of the various ureide structures with that of orotic acid readily shows that ureidosuccinic acid most easily meets the structural requirement as a precursor of orotic acid. That urea itself is

TABLE I. Response of *L. bulgaricus* 09 to Various Ureides and Related Compounds.

Compound	Range tested (mg/tube)	Activity (% orotic acid)	Antagonism
Orotic acid	.02—10	100	
Urea	.2 —10	.25	
Ureidoacetic acid	.2 —10	<.1	
α Ureidopropionic acid	.2 —10	<.1	
β " "	.2 —10	.2	None
α Ureidobutyric "	.2 —10	<.1	
Ureidosuccinic "	.1 — .5	10–20	
α Ureidoglutaric "	.2 —10	<.2	
α Ureido-B methyl valeric acid	.2 —10	.0	
Fumaric acid diamide	.2 —10	<.2	
Thiourea	.2 —10	<.2	Slight at high level*
Succinyl thiourea	.2 —10	.0	"
3-Acetyl 2-thiohydantoin	.2 — 1	.0	None
Dihydro-orotic acid	— 5	.5	"

* Against ureidosuccinic acid and orotic acid at the given levels of these compounds.

relatively inactive is strong evidence alone for the requirement of a more specific structure. The fact that orotic acid has from 5 to 10 times the activity of ureidosuccinic acid and that both of these compounds are incorporated into the same 2 nucleotides(4) is evidence that orotic acid is a probable intermediate in the conversion of ureidosuccinic acid to nucleic acids. The postulated steps in the formation of orotic acid from ureidosuccinic acid are shown in Fig. 1.

Ureidofumaric acid is shown in Fig. 1 as a possible intermediate. That the double bond is formed before ring closure is evidenced by the fact that dihydro-orotic acid exhibits only 0.5% the activity of orotic acid. Attempts to prepare ureidofumaric acid in these laboratories have failed up to the present.

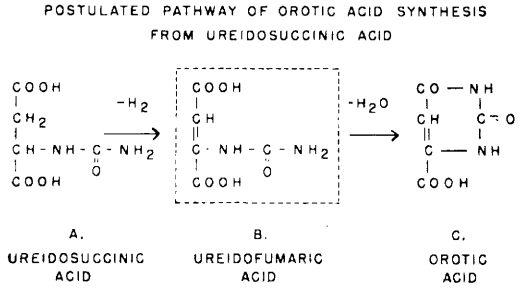

FIG. 1.

Summary. A number of ureides were tested for orotic acid-like activity using *L. bulgaricus* 09. Ureidosuccinic acid, the only compound that could be visualized to yield a substituted pyrimidine ring by cyclization, was the only ureide with significant activity.

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