BACTERIOLOGICAL REVIEWS, June 1976, p. 403-468
Vol. 40, No. 2

Printed in U.S.A.

Degradation of Purines and Pyrimidines by Microorganisms

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INTRODUCTION

Dedicated to H. A. Barker

In spite of the long history of studies on the biological conversions of purines and pyrimidines, only a few reviews on the microbial degradation of these compounds have been published (147, 167, 207). Some of the degradation products, i.e., uric acid, allantoin, and urea, were described in the eighteenth century (47, 83, 457); in 1853 Wöhler (598) first studied the degradation of allantoin by yeast, and a few years later Stokvis (489) demonstrated the conversion of uric acid to allantoin in biological materials.

Purines and pyrimidines, the building stones of nucleic acids, are degraded in animals to waste nitrogenous substances; some of these products appear to perform special functions in plants. The compounds occur in large quantities in nature and are subject to further degradation by various microorganisms. This review will deal mainly with the degradation of purines which, as a result of their complexity, offer a larger versatility of degradative routes and have been the subject of a larger number of investigations than have the pyrimidines.

Purine Degradation in Animals

The products formed from purines by various animals are given in Table 1. The phylogenetic significance of the predominant nitrogenous waste products of animals, namely, uric acid, urea, and ammonia, was first discussed by Needham (361). It is related to the water economy of the animal during both embryonic as well as adult life. The waste product of an animal is ammonia, if ample water is available for the rapid removal of this highly toxic substance. When water is not readily available, urea may serve as a water-conserving device and is temporarily retained as a less toxic excretory product. Such a situation occurs during aestivation of the African lungfish, in which urea accumulates to values as high as 0.5 g/100 g of body weight in 1 year (160).

Needham (362) generalizes that animals whose eggs are protected against water loss (cleidoic) tend to excrete relatively nontoxic and insoluble compounds such as uric acid. A number of animals store uric acid, i.e., ascidians, insects, terrestial gastropods, and land crabs. Insects accumulate uric acid in special organs, i.e., a fat body or rectal sac, which in certain lepidopterans consists of 55% uric acid (414). The amount of uric acid formed depends on the quantities of endogenous or exogenous proteins metabolized.

Variable amounts of white particulate matter occur in the homocoel of the land crab *Cardisoma guanhumi*. This material, mainly uric acid, accounts for 0.2 to 15.9% of the total weight of a number of crabs and appears to increase during intermolt. In one instance 55% of the nonshell solids was uric acid (187).

Uricase is absent in humans, anthropoid apes, and several South American monkeys (109, 358). The enzyme of Old World Monkeys has been found to be highly unstable (109). However, small amounts of uric acid produced in humans may be degraded in three possible ways: by peroxidases, which have been re-

TABLE 1. *Products of purine degradation in animals*

Product excreted or stored	Animals	Reference
Guanine and/or xanthine	Molluscs (e.g., *Octopus*, gastropods)	145, 238, 386, 387
Uric acid	Primates	109, 358
	Dalmation dog	212, 275, 293
	Birds	
	Reptiles	128, 342, 426
	Molluscs	145, 387
	Ascidians	191
	Some terrestial crustaceans	187
	Various insects	268, 389, 414
Allantoin	Other mammals	
	Various insects	51, 268, 389, 414
Allantoic acid	Various insects	414
Urea	Some aquatic reptiles	426
	Terrestial amphibians	23
	Teleost fishes	190
	Dipnoans (during aestivation)	75, 190
	Elasmobranchii	75, 510
Ammonia	Aquatic amphibians	23
	Crustaceans	474
	Dipnoans	190
	Echinoids	309
	Some insects	268

(203), or by the action of intestinal flora (184, 319, 320, 501).

The feeding of yeast or other single cells to humans can increase urinary uric acid excretion, so that in individuals with a genetic tendency to primary overproduction of uric acid there may be precipitation of uric acid crystals in joints (gout), in soft tissues (tophi), or in the formation of stones in the urinary tract. The effect of feeding yeast (*Torulopsis utilis*) or yeast ribonucleic acid on the serum levels of uric acid and the excretion of uric acid was tested by Edozien et al. (149) and Waslien et al. (581). The results indicate that an amount of 2 g of single-cell nucleic acid (equivalent to about 30 g of food yeast) is probably a safe limit for most normal subjects, whereas 3 g of single-cell nucleic acid per day doubles the daily uric acid excretion (0.4 to 0.6 g) to an undesirably high level (149). The excretion of nitrogenous waste includes several billion kilograms of uric acid and allantoin per year which are recycled by microorganisms.

Purine Metabolism in Plants

Uric acid, allantoin, and allantoic acid are present in a large number of plants (61, 418, 514). Allantoin and allantoinase, in particular, are common components of plants, but the presence of allantoicase could be established only in very few higher plants (479, 514), whereas *Hepaticae* showed a highly efficient hydrolysis of allantoic acid (418).

In a number of plants, allantoin and allantoic acid play an important role in the storage and translocation of nitrogen. In the bleeding sap of maple, allantoin and allantoic acid account for as much as 70 to 100% of the total soluble nitrogen. In spring these compounds ascend chiefly in the xylem, providing nitrogen for protein systhesis, and in the fall the reciprocal process takes place (418).

The amount of allantoin present in various *Leguminosae* is reported to be as high as 3.3 g/kg of plant material (514).

Tracey (514) concluded in 1955 that the importance of allantoin and allantoate seems to have been insufficiently appreciated so far. Twenty years later we want to stress this statement.

Degradation of Purines and Pyrimidines by Microorganisms

Purines are degraded to the level of xanthine and uric acid along pathways that are not strongly influenced by the presence of oxygen. Microorganisms which use purines under aerobic conditions convert uric acid to allantoin,

ported by Canellakis et al. (97) to attack uric acid in a manner qualitatively similar to that of uricase, by the cytochrome oxidase system whereas under anaerobic conditions xanthine (and perhaps other purines) is converted along pathways, avoiding the involvement of uricase. Allantoin can be degraded both under aerobic and anaerobic conditions. Pyrimidines are degraded along pathways that involve either a oxidative or a reductive step.

AEROBIC DEGRADATION OF PURINES: ENZYMATIC STEPS

Methylpurines

Caffeine and related methylated xanthines are widely distributed in nature and are especially produced in high concentration in the tissues of a number of well-known beverage plants. Caffeine has been reported to bring about an immediate, although reversible, inhibition of both the synthesis of ribonucleic acid and of protein in sensitive bacteria (392), and it acts as a potent mutagen by inhibition of repair processes (204, 563).

Recent studies of Woolfolk (603) have demonstrated that some strains of *Pseudomonas putida* and *Pseudomonas fluorescens* are able to grow in media containing caffeine (1,3,7-trimethylxanthine) as the sole source of carbon and nitrogen. *P. putida* will grow on any *N*-methyl derivative of xanthine containing one or more methyl groups at the 1, 3, or 7 positions; the methyl groups are hydrolytically removed, and methanol is formed (Fig. 1). It will not grow on methanol as a sole source of carbon, but glyoxylate that is formed by degradation of uric acid is used in this way. Urea is not hydrolyzed.

Cells grown on any one of the *N*-methylpurines given in Fig. 1 displayed activity toward all these compounds, but cells grown on xanthine were active only toward xanthine or uric acid. Cells grown on succinate and ammonia were not active with any of the compounds.

1-Methyluric acid is not degraded by caffeine-grown cells, but xanthine dehydrogenase from the cells is active against xanthine (100%), 1-methylxanthine (19%), and 3-methylxanthine (41%) when tested in a ferricyanide-linked reaction (603). The specificity of this enzyme against methylxanthines will be discussed in a later section.

Methanol is converted to formaldehyde by methanol dehydrogenase. This enzyme is inactivated by its substrate. The demethylation reactions, except for 7-methylxanthine, seem to depend on the activity of methanol dehydrogenase and, as a consequence, degradation of caffeine and the other methylated compounds is

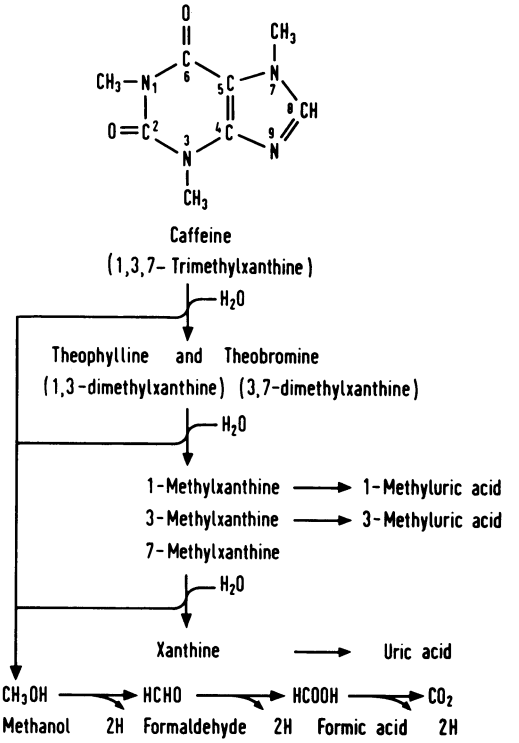


FIG. 1. *Degradation of caffeine by Pseudomonas putida (603).*

inhibited under conditions of methanol accumulation (603).

Caffeine, theobromine, and xanthine can be used as the sole source of either carbon or nitrogen for growth by *Penicillium roqueforti* and a species of *Stemphylium* that was isolated by enrichment on caffeine-containing agar. A strain of *Bacillus coagulans* isolated from soil utilizes caffeine in this way, too (292). A complete degradation of the substrates was observed, but no intermediates were detected (292). Theophylline formation from caffeine was observed in studies with *P. roqueforti* (468).

Adenine

Adenine deaminase (adenine aminohydrolase, EC 3.5.4.2), which converts adenine to hypoxanthine and ammonia (Fig. 2), is commonly found among microorganisms but appears to be absent in animals in which the aminohydrolase reaction involves mostly the respective nucleoside and nucleotide.

Adenine deaminase exhibits no activity toward adenosine (434, 436) and catalyzes a reaction that is virtually irreversible (229). The enzyme of *Azotobacter vinelandii, Candida utilis* (211), and *Schizosaccharomyces pombe*

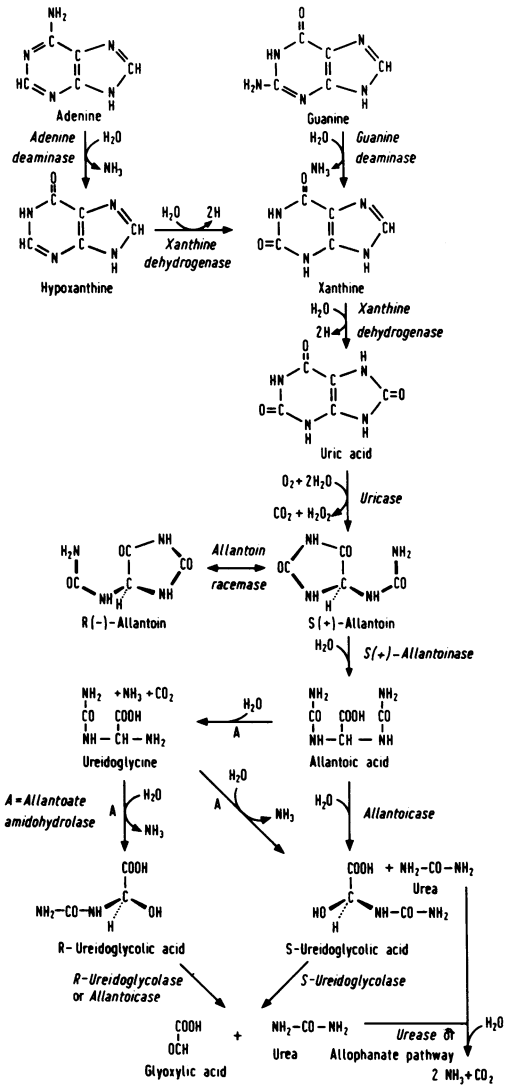


FIG. 2. *Pathways of purine degradation under aerobic conditions. The various enzymatic steps are discussed in the text. Organisms possessing allantoin racemase are given in Table 3. The presence of RS-allantoinase in bakers' yeast (307) is not accounted for in the figure. The occurrence of allantoicase and allantoate amidohydrolase in various microorganisms is given in Table 4. R-ureidoglycolase is demonstrated only in Pseudomonas acidovorans.*

(1) acts also upon 6-chloro-, 6-iodo-, and 6-hydrazinopurines and replaces these groups by a hydroxyl group.

Some microorganisms which lack adenine deaminase are able to convert adenine into hypoxanthine or xanthine by the use of bypasses. Adenosine and inosine are intermediates in the conversion of adenine to hypoxanthine by *Salmonella typhimurium* (225), and the same bypass appears to be an alternative route to *S. pombe* (1). Adenosine 5'-monophosphate (AMP), inosinic acid, and xanthylic acid are involved in the conversion of adenine to xanthine by *Candida guilliermondii* (462, 463, 475).

Adenine, adenosine, and 2'-deoxyadenosine exert an inhibiting effect on the growth of a number of organisms. The inhibiting effect, observed in studies with *Aerobacter aerogenes*, was reversed by thiamine or its pyrimidine moiety 4-amino-5-hydroxymethyl 2-methylpyrimidine and also by histidine and succinate (72, 354). The inhibitory effect was explained as feedback inhibition of purine nucleotide synthesis (354). Growth inhibition by adenine and adenosine was reported for *Escherichia coli* (228, 363, 422) and is not overcome by thiamine (228, 350, 422). Consequently, this inhibition must be caused in a manner different from that proposed for *A. aerogenes*. The bacteriostatic effect of adenine on *E. coli* appeared to be due to inhibition of de novo synthesis of pyrimidine nucleotides. However, the mechanism of the inhibition remains obscure and was attributed either to an inhibition of the reaction catalyzed by orotidine-5'-phosphate pyrophosphorylase (EC 2.4.2.10) or orotidine-5'-phosphate decarboxylase (EC 4.1.1.23) or to an effect on the level of 5-phosphoryl-ribose-1-pyrophosphate (228).

Inhibition was also reported for *Agmenellum quadruplicatum* (234), *Corynebacterium sepedonicum* (64), *Staphylococcus aureus* (134), *Pseudomonas acidovorans* (263), and *Myxococcus virescens* (155). Adenine inhibits growth of *Brevibacterium vitarumen* var. *uricum* when urate served as the sole nitrogen source but not when NH_4^+ salts were used as such (265).

Xanthine Dehydrogenase

Enzymes that catalyze the oxidation of hypoxanthine and xanthine have been obtained from a wide variety of biological sources (166, 291). Some are readily autoxidizable in oxygen, whereas others autoxidize relatively slowly compared with their dehydrogenation by electron acceptors, such as nicotinamide adenine dinucleotide (NAD), ferredoxin, or various dyes.

Oxygen is readily used as hydrogen acceptor by the mammalian species, among which is the intensively studied milk xanthine oxidase (xanthine:oxygen oxidoreductase [EC 1.2.3.2]). This enzyme was first described by Spitzer (484) and Wiener (597) in 1899 and later by Schittenhelm (461). The Schardinger (456) enzyme, which was found in 1902 in milk and catalyzes the oxidation of formaldehyde in the presence of

methylene blue, is identical to it (347). The xanthine dehydrogenases of mammalian liver and milk can use also NAD as hydrogen acceptor (37, 130).

Cofactors. Xanthine dehydrogenase of *Veillonella alcalescens (Micrococcus lactilyticus)* was purified 550-fold by Smith et al. (482) and exhibits a molecular weight of about 250,000. It contains nonheme iron, sulfide, flavin, and molybdenum in a molar ratio of 8:8:2:>1.5. These values are quite similar to those found for milk xanthine oxidase and chicken liver xanthine dehydrogenase. The same cofactors are present in the 26-fold purified enzyme from *Clostridium cylindrosporum*. Molybdenum is also required in the activity of xanthine dehydrogenase of *P. aeruginosa* (3, 344), and it has been suggested that a molybdenum-containing cofactor is common to dissimilatory and assimilatory nitrate reductase and xanthine dehydrogenase for *Aspergillus nidulans* (19), *Neurospora crassa* (359), and *P. aeruginosa* (554).

The reaction of milk xanthine oxidase can be described by the following formulation:

$$E_{ox} + X \rightleftarrows E_{ox} \cdot X \rightarrow E_{red} + U$$

$$E_{red} + O_2 \rightleftarrows E_{red} \cdot O_2 \rightarrow E_{ox} + H_2O_2$$

where E_{ox} and E_{red} represent oxidized and reduced enzyme and X and U represent xanthine and uric acid, respectively (373). The two half-reactions are physically separate steps. If flavine adenine dinucleotide (FAD) is removed from the enzyme, the enzyme exhibits no oxidase activity but is still reduced by xanthine to the same extent and at an even higher rate than that of the native xanthine oxidase. In contrast to the native enzyme which exhibits $NADH_2$ oxidase activity, the deflavoenzyme can no longer react with $NADH_2$, which donates electrons via oxidized FAD. Xanthine reacts directly with molybdenum, and electrons are donated through this group to Fe/S centers and FAD (373). Xanthine is probably bound to a persulfide of the enzyme during the reaction (148), and the major rate-limiting step in the oxidation of the substrate is the breakage of the persulfide bond (374).

Specificity. Various electron donors and acceptors may be used by these enzymes. A number of the substrates that are oxidized by xanthine dehydrogenase of *C. cylindrosporum* and the relative velocities of the oxidations are represented in Fig. 3. It is evident that the substituents present in the molecule exert a directing effect upon the point of oxidative attack. The enzyme of *V. alcalescens* oxidizes xanthine (100%), hypoxanthine (94%), purine (87%), and salicylaldehyde (13%), whereas 9-methylhypo-

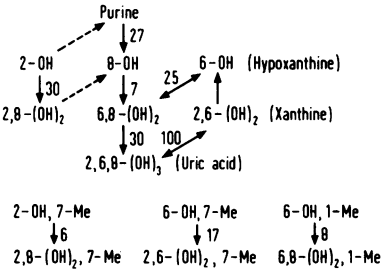


FIG. 3. *Oxidation (downwards arrows) and reduction (upwards arrows) reactions catalyzed by xanthine dehydrogenase from Clostridium cylindrosporum (66). The various purine derivatives are indicated by use of symbols for the location of the hydroxyl (OH) and methyl (Me) groups at the purine skeleton. Solid arrows: known reactions. Broken arrows: possible reactions. Numbers adjacent to the arrows give relative V_{max} for the oxidation reactions.*

xanthine, 6,8-dihydroxypurine, benzaldehyde, and acetaldehyde are oxidized at values less than 13% (482). The numbers within parentheses refer to the relative velocities of the oxidations. The enzyme of *P. aeruginosa* is most active with xanthine, 3-methylxanthine, hypoxanthine, 6-mercaptopurine, 2-thioxanthine, and 3-methyl-2-thioxanthine (50, 139). 2-Aminopurine, its mono- and dimethylamino derivatives, and 6-mercaptopurine are attacked at position C2, in contrast to the milk enzyme which oxidizes at position C8 (48, 50). The enzyme of *P. putida* oxidizes both 1- and 3-methylxanthine (603), but *P. aeruginosa* attacks 3-methylxanthine only (50). The enzyme of *P. acidovorans* oxidizes xanthine (100%), hypoxanthine (65%), and purine (4%) (478).

Hypoxanthine can be converted to uric acid via xanthine or 6,8-dihydroxypurine. The intermediary state of the latter compound was demonstrated in the metabolism of *Proteus rettgeri* and *Serratia marcescens* (150, 341) and of various *Streptomyces* species (584). Adenine and guanine react only very slowly or not at all with the xanthine dehydrogenases.

Some of the compounds given in Fig. 3 have been shown to serve also as electron acceptors in the enzymic reaction. Reduction of uric acid by xanthine dehydrogenase constitutes the first step in the degradation of this compound under anaerobic conditions, as will be discussed below. The enzymes of *C. cylindrosporum* and *V. alcalescens* catalyze some reversible dismutation reactions in which the same compound, e.g., xanthine or 6,8-dihydroxypurine, serves as electron donor and acceptor (66, 482). Catalytic amounts of a viologen dye enhance this dismutation reaction in *C. cylindrosporum*

(66). Smith et al. (482) tested the dismutation reaction with the purified enzyme of *V. alcalescens* and observed that an equimolar mixture of hypoxanthine and uric acid is converted under anaerobic conditions to an equilibrium mixture consisting of 64.0% xanthine, 17.3% uric acid, 10.5% hypoxanthine, and 8.2% 6,8-dihydroxypurine. The reduction of uric acid occurs also in the presence of sodium hydrosulfite, 2-hydroxypurine (66), or salicylaldehyde (482).

The xanthine-oxidizing enzymes utilize various nonphysiological compounds as electron acceptors; ferricyanide, 2,6-dichlorophenolindophenol, methylene blue, and tetrazolium derivatives are used by the enzymes of *C. cylindrosporum* and *V. alcalescens* (66, 482), but the enzyme of *P. acidovorans* did not use the latter dye (478).

NAD is used by the avian enzymes (6) and the enzyme of *P. acidovorans* (478). The latter enzyme exposes a 10-fold lower activity with NADP. NAD and NADP are not used by the enzymes of *C. cylindrosporum* and *V. alcalescens* (6, 66, 482). As a consequence, the enzyme of *C. cylindrosporum* exhibits little or no $NADH_2$ oxidase activity (66). The natural acceptor in these anaerobic bacteria is ferredoxin (6, 67, 534).

The various enzymes react also with cytochrome *c* and oxygen but generally at much lower but apparently significant rates (66, 139, 478, 482). This rate is greatly enhanced by the addition of ferredoxin to the enzyme of *V. alcalescens* (482).

The enzymes differ considerably as to the relative specificity to substrates as well as to electron donors and electron acceptors. Moreover, the sensitivity to various inhibitors varies strongly; e.g., the enzyme of *V. alcalescens* is rather insensitive to cyanide, arsenite, and borate and very sensitive to methanol, in contrast to milk xanthine oxidase (482). However, the similarities in the catalyzed reactions and enzyme composition warrant consideration of them all as a multifunctional xanthine dehydrogenase.

Other enzymes oxidizing purines. Scazzocchio et al. (125, 454, 455) presented immunological and genetic evidence indicating the presence of two distinct xanthine dehydrogenases in *A. nidulans*. The wild-type enzyme (XDH I) is induced by uric acid, and a constitutive enzyme (XDH II) is present in allopurinol-resistant mutants. Both enzymes share one or more common components.

In *Peptococcus aerogenes (Micrococcus aerogenes)* two enzymes are present that catalyze the oxidation of 2-oxypurine (605). One has the broad substrate specificity typical of xanthine dehydrogenase; the other is very specific with regard to the substrates oxidized and will oxidize only 2-oxypurine and closely related compounds. It is named 2-oxypurine dehydrogenase and was purified some 1,000-fold. 2-Oxypurine, 2-oxy-8-azaxanthine, 2,8-dioxypurine, 2-oxypyrimidine, and 2-oxypteridine are oxidized exclusively at position 6. A wide variety of artificial electron acceptors is used, but molecular oxygen is very poorly utilized. The enzyme contains nonheme iron but not flavin or molybdenum (605).

A separate enzyme catalyzing the oxidation of 6,8-dihydroxypurine was proposed (584) to occur in *Streptomyces* species on the basis of the sensitivity of this reaction to chloramphenicol inhibition, but further studies are desirable to warrant this conclusion.

Uricase

In contrast to the foregoing enzyme, uricase (urate:oxygen oxidoreductase [EC 1.7.3.3]) exhibits a stringent specificity regarding both the electron donor and acceptor. Only oxygen is used in the latter way and, therefore, no organism is known to pass the uric acid barrier under anaerobic conditions.

Uric acid is one of the oldest organic compounds known. It was isolated from human urine in 1776 by Scheele (457) and Bergman (47). The structure of uric acid was elucidated in 1889 by Behrend and Roosen (43), and the first studies on uricase were performed by Schittenhelm (461), Wiechowski (596), and Battelli and Stern (38) in the first decade of this century.

Microbial uricases have been recently applied in the treatment of children suffering from hyperuricemia (146) and patients with primary gout (71, 269). Intramuscularly or intravenously administered uricase of *Aspergillus flavus* caused a rapid reduction in the serum and urinary levels of uric acid; allantoin was excreted.

Properties. Table 2 summarizes the properties of purified uricases of various species.

The uricases of animal origin (129, 218), the protozoan uricases of *Acanthamoeba terricola* (357), *Hartmanella culbertsoni* (105), *Polytomella caeca* (185), *Ochromonas malhamensis* (322), and presumably also of *Chaos chaos* and *Amoeba proteus* (356), the algal uricase of *Chlorogonium elongatum* (485), and some yeast uricases (378) were found to be associated with the peroxisomes. Uricase of *Alcaligenes eutrophus* (*Hydrogenomonas* H16), *Micrococcus denitrificans*, and *P. aeruginosa* appear to be firmly bound to structural components, since

TABLE 2. *Properties of purified uricases of various sources*

Source	Times purified	Sp act of purified material (U/mg of protein)[a]	Homogeneity of purified material	Molecular mass (kdaltons)	Isoelectric point (pH)	Metal content (atoms/mol of enzyme): Cu^{2+}	Metal content (atoms/mol of enzyme): Fe^{3+}	Flavoprotein	pH optimum
Swine liver	5,000	10.2	75%	125[c]	6.3	1			8.5–9.3
Rat liver	300	9.3							
Ox kidney	30			104					
Acanthamoeba terricola	2.5								
Neurospora crassa	400								
Alternaria tenuis									7
Aspergillus flavus			+	93	6.2				8.5
Candida utilis	130	10.5	+	120	5.4	<0.12	0.7–1.0	–	8.5
Arthrobacter pascens	4,100	20.5	+	±100		<0.2	<0.2	–	9.2
Micrococcus varians	5	20	–						8.5–8.8
Alcaligenes eutrophus									8.5–9.0
Bacillus fastidiosus	3[e]	37[e]		145				–[e]	9.0

Source	K_m (10^{-6} M)	Inhibition by CN at: 1 mM	Inhibition by CN at: 1 μM	Inhibition by: PCMB[b]	EDTA	Hg^{2+}	Cu^{2+} (1 mM)	Cu^{2+} (1 μM)	Fe^{3+}	SH enzyme	References
Swine liver	20	+	+	–	–		–	–		–	230, 328, 381, 382
Rat liver		+	–				+				513
Ox kidney	17.5			+			–	–		+	237, 515
Acanthamoeba terricola		+	+								357
Neurospora crassa											201
Alternaria tenuis	3,000	(–)	–								172
Aspergillus flavus	60	+		+	–	+			(–)		295–297
Candida utilis	6	+		+	–	+	+		–	+	235, 438
Arthrobacter pascens	200						+	+		[d]	18, 369
Micrococcus varians		+		+	–		+				267
Alcaligenes eutrophus	1,100	+	+								248, 250
Bacillus fastidiosus	250[e]	+[e]			–[e]	–[e]	+[e]	–[e]			329

[a] Micromoles of uric acid oxidized per minute per milligram of protein.
[b] PCMB, *p*-chloromercuribenzoate.
[c] Composed of four subunits with a molecular weight of 32,000.
[d] Stimulation by reducing substances.
[e] G. Bongaerts, unpublished data.

they sediment from ultrasonic preparations at 100,000 × *g* (250). Also, the enzyme of *Histoplasma capsulatum* is particle bound (316), but the uricases of *Bacillus fastidiosus* (253), *Arthrobacter pascens* (18, 369), and *Fusarium oxysporum* (330) are soluble.

Uricase is commonly reported to be a metalloenzyme containing either Cu^{2+} (swine liver)

or Fe^{3+} (*C. utilis*). However, the data are not conclusive. The enzyme of *C. utilis* was reported to be a copper enzyme by Roush and Shieh (438), but Itaya et al. (235) demonstrated the presence of only trace amounts of copper and 0.7 to 1.0 atoms of Fe^{3+} per mol of enzyme. The presence of functional Cu^{2+} in the enzyme of swine liver is questioned by Truscoe and Williams (515). The assumed presence of copper is partly based on the following observations: (i) Urate oxidation to allantoin can be mediated solely by Cu^{2+} or by Cu^{2+} plus H_2O_2 at pH 6.0 to 8.2 (459) or at higher pH values in the presence of polyvalent anions or cyanide (40). Fe^{3+} was without effect (459). (ii) Cyanide exerts an inhibiting effect. The presence of functional metal ions needs further investigation for all uricases.

The effects exerted by *p*-chloromercuribenzoate and heavy metal ions indicate that most uricases contain an essential SH group and, as far as tested (180, 235), uricase appears to be no flavoprotein. The pH optima of the enzymes are all located aroung pH 9, except for the enzyme of *Alternaria tenuis* which has a pH optimum at pH 7 (172) and that of a *Streptomyces* for which a pH optimum in the range pH 6.0 to 8.5 was reported (583). The K_m values of the enzymes differ strongly. However, K_m values measured for uricase are only reliable when measured with freshly prepared solutions, since uric acid is readily oxidized in alkaline solution to oxonate, a very potent inhibitor of the enzyme. Fridovich (175) obtained for hog liver uricase a K_i value for oxonate and a K_m value for urate of 1.1×10^{-7} M and 0.5×10^{-5} M, respectively. A very low K_m value (3×10^{-8} to 6×10^{-8} M) was reported for liver uricase from freshwater teleosts (121). In various instances uricase exhibits substrate inhibition when tested at higher substrate concentrations; this inhibition may be due to the accumulation of an intermediate (380). The uricase activity of *A. pascens* is strongly influenced by the ionic strength of the test medium (18) in contrast to the enzyme of swine liver (39). Fitzpatrick et al. (158) compared the immunological properties of uricase of *B. fastidiosus*, *C. utilis*, and *Aspergillus niger* and observed a complete antigenic independence.

Specificity. The physiological reaction catalyzed by uricase is shown in Fig. 2. Uricase exhibits a stringent specificity. A large number of purine bases and related compounds have been examined; a number of them inhibit the reaction, but none appears to be a substrate, except perhaps 8-aminoxanthine (39, 327, 329), which is oxidized at about 10^{-3}-fold the rate of uric acid (327). On the other hand, oxygen seems to be the only known electron acceptor (172, 357, 381). Due to this stringent specificity, uricase plays a role in the degradation of purines by aerobic organisms only; anaerobic microorganisms have evolved degradative routes of purines evading this reaction. H_2O_2 is formed in stoichiometric amounts during the reaction (357), and on this basis a quantitative determination of uric acid can be performed (140, 161). Smaller amounts of H_2O_2 (70%) (172) or no H_2O_2 (250) is formed in a number of bacteria or fungi due to the presence of catalase (250). On the other hand, the accumulation of H_2O_2 may reduce the amount of allantoin formed; catalase, when added to incubation mixtures lacking this enzyme, enhanced substantially the amount of allantoin formed with the uricase of *A. flavus* (297).

The stoichiometric amount of allantoin is formed only when uricase is tested in buffers unlike borate (172, 583). In the presence of borate, a stoichiometric amount of oxygen is consumed, but a large part (70%) of urate is not converted to allantoin and CO_2 but to equimolar amounts of alloxanic acid and urea (Fig. 4). In all studies performed with various uricases in borate buffer (pH, about 9), a fairly constant amount of allantoin (30%) was observed among the products of the reaction (96, 230, 297).

Mechanism of action. The mechanism of the uricase reaction has been studied extensively during the past 50 years, but no clear-cut picture is yet available. Since the uricases of *A. flavus* (296), *C. utilis* (235), and *A. pascens* (18) are obtained in a homogeneous form, the uricase action must be due to one enzyme. The physiological product formed by this enzyme is allantoin, which occurs in nature in the optically active S(+) form. Moreover, a number of natural allantoinases are specific for this optical isomer. Therefore, it is possible that uricase forms S(+)-allantoin (173).

The reaction mechanism must explain a one-enzyme reaction yielding S(+)-allantoin via a number of intermediates, as well as an oxidative and a decarboxylative step. Figure 4 summarizes the results obtained. Bentley and Neuberger (45) demonstrated in experiments with $^{18}O_2$ and $H_2{}^{18}O$ that the oxygen of H_2O_2 originated exclusively from gaseous oxygen. Therefore, the oxidation of uric acid consists in the transfer of two electrons or two H atoms from each uric acid molecule to molecular oxygen. No superoxide radical is produced during this reaction (381). The electron transfer would probably result in the formation of a carbonium ion, the rearrangement of which leads to characteristic products. The primary intermediate formed (96, 327, 380, 391) is given as compound

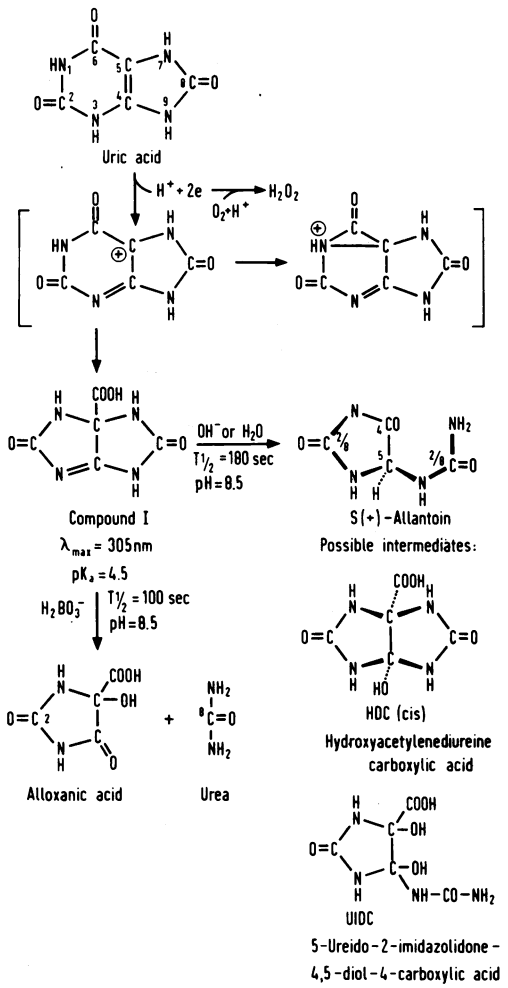


FIG. 4. *Mechanism of uricase action (45, 96, 327, 380, 391). The numbers in the molecules refer to the origin of the atoms with respect to the uric acid molecule (96, 124). $T_{1/2}$ refers to the half-life time of the molecules (327).*

I in Fig. 4. The rate of further degradation of this intermediate is independent of the amount of KCN-inhibited enzyme present in the incubation mixture (380). Pitts and Priest (380) assumed that the cyanide ion interacts with the oxygen site on the enzyme, thus preventing only the binding of oxygen, and concluded that the decay of compound I is independent of uricase. The final products of this decay can vary, depending upon the absence or presence of borate. Hydroxyl and borate ions act catalytically in these decays. Pitts and Priest assumed that an unstable and symmetrical intermediate is traversed prior to the formation of allantoin, but this view is hardly tenable since optically active allantoin is most probably the product. Therefore, the reactions involved in the decay of compound I to allantoin must proceed in an asymmetrical way, either due to the action of uricase or due to chemical reactions in which the asymmetry is conserved.

Hydroxyacetylenediureine carboxylic acid (HDC) was isolated as a silver salt from a uricase system and as a product of the chemical oxidation of uric acid by Schuler and Reindel (464). The participation of HDC in the degradation of uric acid needs further investigation, since the chemical conversion of HDC to allantoin appears to require relatively drastic conditions (464). If HDC is an intermediate in the uricase system, then the *cis* form is the likely candidate to conserve the asymmetry in the molecule (96).

Canellakis and Cohen (96) studied the degradation of uric acid-2-^{14}C and uric acid-8-^{14}C and proved that uric acid and a proposed intermediate UIDC (5-ureido-2-imidazolidone-4,5-diol-4-carboxylic acid), a hydrolytic product of HDC, decompose asymmetrically to urea and alloxanic acid at pH 7.2; urea is derived from the C8 atom of uric acid and alloxanic acid is derived from the C2 atom of uric acid. This asymmetry is not maintained in incubation mixtures at pH 9.0, indicating that one of the intermediates undergoes a secondary reaction.

Moreover, Canellakis and Cohen demonstrated the formation of an unidentified intermediate in allantoin production under these conditions (0.1 M borate, pH 9.0). According to these authors, the C2 atom of uric acid is equally distributed among the ureido group and the hydantoin moiety of allantoin, and Brown et al. (74) observed that the degradation of 1,3-^{15}N-labeled uric acid yielded in vivo allantoin which is equally labeled in both the ureido group and the hydantoin moiety. These results hardly tally with the production of S(+)-allantoin by uricase. However, the exchange of the label may be due to the conditions used in the hydroiodic acid degradation of allantoin.

Further studies are needed to reveal the exact mechanism of the uricase reaction, in which the asymmetry of the intermediates and the products, allantoin and alloxanic acid, is accounted for.

Uricase-like processes. Uric acid can be oxidized to allantoin by a large number of oxidizing reagents. In their study, Wöhler and Liebig (599) synthesized allantoin from uric acid by the use of lead dioxide, whereas Schieper (460), Von Gorup-Besanez (574), Wheeler (586), and Claus (112) used potassium ferricyanide, ozone, manganese dioxide, and potassium permanganate, respectively.

It is not unusual that besides uricase other biological systems are able to oxidize uric acid. Such a system was described by Canellakis et al. (97) to explain some degradation of uric acid in humans in the absence of uricase. Uric acid is degraded to allantoin by lactoperoxidase-H_2O_2, verdoperoxidase-H_2O_2, or horseradish peroxidase-H_2O_2 at a neutral pH in phosphate buffer. In borate alloxanic acid, allantoin and other products are formed.

Other uricase-like processes are catalyzed by the cytochrome-oxidase system (203) and by the pigments involved in the photosynthesis of cyanobacteria and algae. The photosynthetic pigment system is highly bleached by uric acid, and allantoin or other oxidized products are formed (11, 53, 180, 541, 542). These reactions will be discussed in the section dealing with cyanobacteria and algae.

Allantoin

In the 8th year of the French Revolution, the citizens Buniva and Vauquelin (83) reported on the isolation of a compound that they named "acide amniotique." It was obtained as needle-shaped crystals by evaporation of the amniotic fluid of cattle and extraction of the residue with boiling alcohol. Buniva and Vauquelin assumed it to be chemically related to uric acid, which was previously (in 1776), isolated from human urine by Scheele (457) and Bergman (47).

Gmelin (188) questioned in 1820 the acidic nature of this compound. In 1821 Lassaigne (304) distinguished between the allantoic and amniotic fluids and, since the compound could be isolated from the allantoic fluid, Lassaigne changed the name to allantoic acid. Wöhler and Liebig (599) changed the name to allantoin and described the synthesis of allantoin from uric acid. In 1876 Grimaux (205, 206) established the chemical structure of allantoin and synthesized it from simpler organic compounds, i.e., urea and glyoxylic acid. Fosse et al. (163-165) isolated the two optical isomers from allantoin. Dextrorotatory allantoin was isolated from leaves of *Platanus orientalis* and from the urine of cattle; levorotatory allantoin was obtained after incubation of the racemic mixture with ground beans of *Soja hispida*. The absolute configuration of the two optical antipodes of allantoin was established by 's-Gravenmade et al. (196). The dextrorotatory and levorotatory forms are S- and R-allantoin, according to the nomenclature of Cahn et al. (86, 87). The beneficial effect of allantoin in wound healing and the maintenance of normal skin properties is reported in a large number of papers. We refer to the reviews of Vitěz (561), Lubowe and Mecca (318), and Greenbaum (200). The therapeuthic effects of plants on wounds and the maggot therapy have been attributed to the presence of allantoin in these plants and the excretory products of maggots.

Allantoin racemase. The optical antipodes of allantoin are subject to a rapid racemization, especially at higher pH values. At pH 8.5 and 30 C, about 3% of an isomer will racemize per minute. The optical forms are rather stable at pH values between 1 and 4, where the racemization rate at 30 C amounts to about 0.1%/h (569).

In a number of *Pseudomonas* species (Table 3), the presence of allantoinase is accompanied by allantoin racemase (EC 5.1.99.3), thus enabling the organisms possessing an S(+)-allantoinase to use both forms of allantoin.

The enzyme from *P. testosteroni* was purified 133-fold and shows optimal activity at pH 8. The reaction follows pseudo first-order kinetics at different initial substrate concentrations since S- and R-allantoin are equally effective as the substrate. A number of other hydantoins are not racemized by the enzyme. In contrast to other racemases, allantoin racemase does not require metal ions, reducing compounds, flavins, or pyridoxal-5'-phosphate for full activity (553).

Allantoinase. Allantoinase (allantoin amidohydrolase [EC 3.5.2.5]) is a very common enzyme in nature. It occurs in many animals (168, 303), higher plants (514), and microorganisms.

The first studies on the enzyme were made in 1920 by Němec (365), but Fosse and Brunel (162) proved that allantoate is formed as the product of the reaction.

The enzymes from *Streptococcus allantoicus*, *P. rettgeri*, *E. coli* (570) and bakers' yeast (307) convert both R(−)- and S(+)-allantoin to allantoate. The aspecificity was not caused by the presence of allantoin racemase in the enzyme

TABLE 3. *Presence and absence of allantoin racemase in Pseudomonas (440, 453)*[a]

Enzyme present	Enzyme absent
P. fluorescens biotypes A through E	*P. fluorescens* biotype F
P. putida	*P. aeruginosa*
P. testosteroni	*P. stutzeri*
P. multivorans	*P. acidovorans*
P. mildenbergii	*P. alcaligenes*
P. tolaasii	*P. pseudoalcaligenes*
	P. maltophilia

[a] The allantoinases of *P. fluorescens*, *P. aeruginosa*, and *P. acidovorans* are proven to be specific for S(+)-allantoin.

preparations (553, 568). The enzymes of animal and plant sources and from *P. acidovorans*, *P. fluorescens* (517, 568, 570), *P. aeruginosa* (196), and *B. fastidiosus* (62) are specific for S-allantoin.

Allantoinases differ strongly as to the influence exerted on them by Mn^{2+} ions and reducing agents. The enzymes from plants, *S. allantoicus*, *P. rettgeri*, and *E. coli* are activated, but the enzymes from the *Pseudomonas* species are inhibited by Mn^{2+} ions. Cysteine and other reducing agents stimulate the activity of the enzymes from *S. allantoicus*, *P. rettgeri*, and *E. coli* but inhibit the plant and animal enzymes (571) as well as the enzyme from bakers' yeast (307). Besides allantoin, methylolallantoin and 5-aminohydantoin can serve as substrates. However, the latter compound is only degraded appreciably by the enzymes of *S. allantoicus* and *P. rettgeri* (565, 570).

Franke and Taha (171) reported on an oxygen-dependent degradation of allantoin by extracts of *Alternaria tenuis*. They postulated the presence of allantoin oxidase, which converts allantoin into oxonic acid. However, these results were not substantiated by further evidence.

Allantoate Amidohydrolase and Allantoicase

Allantoate was first synthesized by Schieper (460) in 1848. It is readily formed from allantoin under strongly alkaline conditions (569). Allantoate, in turn, is subject to a series of reactions which are given in Fig. 5. Equilibria are formed in each of the given reactions, which are examples of known processes in which nucleophilic reagents, i.e., urea and water, act on the carbon atom of a C=O group or a C=N bond. Water and urea are bound to glyoxylate with almost equal velocities, whereas urea is bound to allanturate about five times more rapidly than water (195). In aqueous solutions ureidoglycolate (195) and the hydrated form of glyoxylate (294) are formed from allanturate and glyoxylate, respectively. However, in this review we will follow the common practice of using the name glyoxylate also for the hydrated form.

Two enzymes are known to catalyze the degradation of allantoate, i.e., allantoicase (allantoate amidinohydrolase [EC 3.5.3.4]) and allantoate amidohydrolase (allantoate amidinohydrolase [decarboxylating] [EC 3.5.3.9]). Allantoicase was found independently by Krebs and Weil (290) in frog liver and by Brunel (77) in the mycelium of *A. niger (Sterigmatocystis nigra)*. Originally allantoicase was assumed to catalyze the conversion of allantoate into 2 mol of urea and 1 mol of glyoxylate, but Valentine et al. (530, 537) have shown that a separate enzyme is involved in the degradation of ureidoglycolate. Probably, a two-step reaction is involved in all biological systems which convert allantoate into 2 mol of urea and 1 mol of glyoxylate.

Allantoicases of *P. aeruginosa*, *Penicillum citreo-viride* (518), and frog liver (520) form S(−)-ureidoglycolate, which is probably the product of all allantoicase reactions. In contrast to the indication S, which refers to the absolute configuration, the notation (−) is almost meaningless in this instance. It refers to the sign of the optical rotation of a dilute, neutral, or alkaline solution of S-ureidoglycolate measured at wavelengths above 310 nm. If one of these conditions is not fulfilled, the solution is dextrorotatory (195).

Moreover, a second reaction appears to be catalyzed by allantoicase. The 190-fold purified enzyme of *P. aeruginosa* and the enzyme of frog liver (520) are able to degrade R-ureidoglycolate, the optical antipode of the product formed from allantoate, into glyoxylate and urea (519, 562). Apparently, R-ureidoglycolate is bound to the enzyme; its ureido group is cleaved off in a similar way as the ureido group that is split from allantoate. Both hydrolytic processes are reversible and may be represented by the reaction sequence (567):

$$E + S \leftrightarrow ES \qquad ES + H_2O \leftrightarrow EP + \text{urea}$$
$$EP \leftrightarrow E + P$$

in which E is allantoicase, S is allantoate or R-

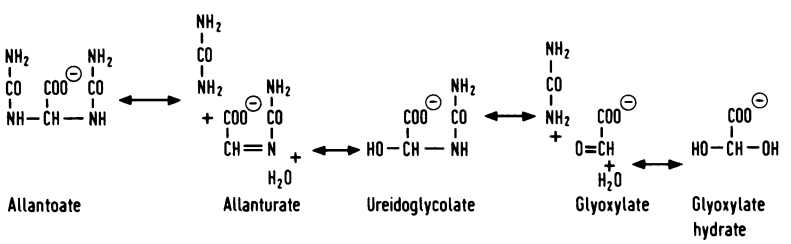


FIG. 5. *Equilibria formed in the hydrolytic degradation of allantoate (195, 294, 572, 573).*

ureidoglycolate, and *P* is S-ureidoglycolate or the hydrated form of glyoxylate. The dissociation constants of *ES* and *EP* are about equal (25 mM) for allantoate, ureidoglycolate, and the hydrated form of glyoxylate, but urea is not appreciably bound to the enzyme. The similarity between the chemical (Fig. 5) and enzymatic hydrolysis is obvious, and *ES* and *EP* must be regarded as activated forms of allanturate and glyoxylate. The equilibrium constant of the enzymatic hydrolysis of allantoate (519) amounts to:

$$K = \frac{[\text{allantoate}]}{[\text{urea}]\,[\text{S-ureidoglycolate}]} = 4.8\ \text{M}^{-1}\ (\text{pH } 7.5,\ 30\ \text{C})$$

Allantoicase of *P. aeruginosa* is a metalloenzyme containing strongly bound Mn^{2+} ions. The apoenzyme is inactive, but the activity can be restored by various cations, although with differing effectiveness (552). The binding subsites of the enzyme (567) and its subunit structure (194) were studied.

Allantoate amidohydrolase catalyzes the conversion of allantoate into 2 mol of ammonia, 1 mol of CO_2, and 1 mol of ureidoglycolate. A similar chemical hydrolysis of allantoate occurs slowly in alkaline media (572). Ureidoglycine is an intermediate in the enzymatic reaction:

$$\text{allantoate} \rightarrow NH_3 + CO_2 + \text{ureidoglycine}$$

$$\text{ureidoglycine} \rightarrow NH_3 + \text{ureidoglycolate}$$

The existence of a separate enzyme, ureidoglycine aminohydrolase, was proposed (565, 566), and Wu et al. (609) obtained evidence for it from studies with mutants of *P. acidovorans*. However, no firm proof has been obtained, partly due to the fact that ureidoglycine is not available as a substrate (545). Its accumulation during the enzymatic reaction is evident since it reacts with glyoxylate in a nonenzymatic transamination reaction in which glycine is formed (545, 566). Allantoate amidohydrolase from *S. allantoicus* and *B. fastidiosus* (62) produces S(−)-ureidoglycolate from allantoate (545, 549), whereas R(+)-ureidoglycolate is formed by the enzyme from *P. acidovorans* (517).

All allantoate amidohydrolases tested so far have one unique property in common: the enzyme is present in a partly inactive form in cell-free extracts. The activity can be increased considerably by pretreatment at pH values below 5 (517, 548, 550, 565, 566) or at pH values around 6 in the presence of complexing anions (548, 550). The enzyme exhibits optimal activity at pH 8.5 and requires Mn^{2+} ions (544). However, Mn^{2+} ions inactivate the enzyme of *S. allantoicus* at pH values between 5 and 8. The mechanism of the activation and inactivation processes can be explained by assuming an incorrect binding of Mn^{2+} ions to the enzyme at pH values below 8. The activation processes remove Mn^{2+} ions and, when the pH of the solution is brought to values above 8, the apoenzyme binds Mn^{2+} ions in the correct way (551). In our opinion the activation and inactivation must reflect some physiological function of the enzyme since it is conserved or has originated independently in the evolution of bacteria as different as *E. coli, P. rettgeri, B. fastidiosus, S. allantoicus,* and *P. acidovorans*.

In many instances it is not yet known whether either allantoate amidohydrolase or allantoicase is involved in the degradation of allantoate. In urease-negative organisms, a differentiation can be made, since ammonia is produced by the former enzyme and 1 mol of urea per mol of allantoate is produced by the latter enzyme. In urease-positive organisms, the enzyme must be separated from urease by purification procedures, or activation by acid or complexing agents may be used as an indication for the presence of allantoate amidohydrolase. The known data on the distribution of both enzymes in biological materials are presented in Table 4.

The presence of allantoicase is not yet proven unequivocally for *Desmidiales* (559), marine algae (560), basidiomycetes (76, 77, 79), *A. niger* (77), *Aspergillus fumigatus* (77), *Aspergillus oryzae* (77), *Penicillium chrysogenum* (7), *Geotrichum candidum* (26), *Fusarium moniloforme* (9), *Neurospora crassa* (419), *Saccharomyces cerevisiae* (115, 307), and *Histoplasma capsulatum* (316). In spite of the lack of definite evidence for the presence of allantoicase, all allantoate-degrading enzymes will be called allantoicases in this review, unless the presence of allantoate amidohydrolase is proven.

Ureidoglycolase

The intermediary role of ureidoglycolate in the catabolism of purines was first established by Valentine et al. (530, 537, 539). The compound can be synthesized by the incubation of a concentrated solution of urea and glyoxylate (517, 537) and hydrolyzes to these compounds in diluted solutions. These chemical reactions are catalyzed by bivalent cations and phosphate ions (183, 195, 573).

Besides allantoicase, which catalyzes the hydrolysis of R(+)-ureidoglycolate, two other enzymes are known to convert ureidoglycolate to glyoxylate and urea (Fig. 2). These ureidoglycolases (ureidoglycollate urea-lyase [EC 4.3.2.3]) differ as to the optical specifity. R-ureidoglyco-

TABLE 4. *Distribution of allantoicase and allantoate amidohydrolase in biological materials*

Allantoate amidohydrolase present	Reference	Allantoicase present	Reference
Bacillus fastidiosus[a]	62	Frog liver[a]	520
Streptococcus allantoicus[a]	549, 566	*Ochromonas malhamensis*	321
Alcaligenes eutrophus	251, 252	*Penicillium notatum*	518
Escherichia coli	566	*P. citreo-viride*[a]	518
E. coli var. *acidilactici*	566	*Aspergillus nidulans*	454
Citrobacter freundii	566	*Candida utilis*	106
Proteus rettgeri	566	*Brevibacterium linens*	16
Pseudomonas acidovorans[b]	517, 609	*Arthrobacter globiformis*	289
		Pseudomonas aeruginosa[a]	518
		P. fluorescens	518

[a] S-ureidoglycolate is formed.
[b] R-ureidoglycolate is formed.

lase is present in *P. acidovorans* (517) and S-ureidoglycolase is present in *P. aeruginosa, P. fluorescens, P. citreo-viride, S. allantoicus, B. fastidiosus*, and frog liver (62, 518–520, 530, 549, 568). The enzymes are slightly stimulated by Mn^{2+} ions (183, 517, 565), and the enzyme of *S. allantoicus* is strongly inhibited by *p*-chloromercuribenzoate, Hg^{2+} ions, and Zn^{2+} ions (183).

The enzymatic and nonenzymatic reactions led to the establishment of an equilibrium with a constant (183, 519):

$$K = \frac{[\text{ureidoglycolate}]}{[\text{urea}]\,[\text{glyoxylate}]} = 7.4\ M^{-1}\ (\text{pH } 7.5,\ 30\ \text{C})$$

The enzyme of *S. allantoicus* was purified 77-fold by Gaudy et al. (182).

Urea Degradation

Urea formed in the degradation of purines is degraded in various organisms by urease (urea amidohydrolase [EC 3.5.1.5]). Urease synthesis is strongly repressed by ammonia or NH_4^+ ions in various organisms, which in this way prevent an unlimited rise of the alkalinity in the medium (252, 287, 488, 556). Omura et al. reported on the presence of urea dehydrogenase in *Scenedesmus* species and higher plants (375, 376). The enzyme prefers NADP to NAD and acts also with α-ketoglutaric acid oxime and γ-glutamylhydroxamic acid as substrates.

In some reports an adenosine 5′-triphosphate (ATP)-yielding hydrolysis of urea was considered (241, 538), but the only known hydrolytic alternative of urease is an ATP-requiring reaction, which was first described by Roon and Levenberg (430) and is operative in yeast (429, 431, 592, 593), algae (224, 308, 472, 508), and some fungi (477). Two enzymes are involved in the reaction sequence (Fig. 6): urea carboxylase

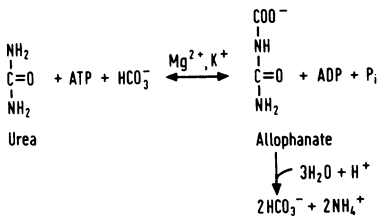


FIG. 6. *Allophanate pathway of urea degradation.*

(urea:CO_2 ligase [ADP] [EC 6.3.4.6]) and allophanate hydrolase (allophanate amidohydrolase [EC 3.5.1.13]). ATP is used by the first enzyme, to which biotin serves as a prosthetic group. Biotin is not necessary for allophanate hydrolase (593). In contrast to the enzymes from *Chlorella vulgaris* (508), the enzymes from *S. cerevisiae* are components of a multienzyme complex (592).

Glyoxylate Degradation

In most, if not all, instances glyoxylate is degraded in a reaction sequence involving tartronate-semialdehyde synthase (glyoxylate carboxy-lase [dimerizing] [EC 4.1.1.47]) and tartronate-semial dehyde reductase [D-glycerate:NAD (P)$^+$ oxidoreductase (EC 1.1.1.60)]. The former enzyme was first described by Krakow and Barkulis (284) for *E. coli* and is a flavoprotein (208), using thiamine pyrophosphate and Mg^{2+} ions as cofactors. It was shown to be present in various other microorganisms (236, 253, 281, 530, 565).

AEROBIC DEGRADATION OF PURINES BY VARIOUS MICROORGANISMS

Protozoa

The scant information on the purine degradation by protozoa is summarized in Table 5. *Tetrahymena pyriformis* requires preformed pu-

TABLE 5. *Enzymes involved in purine degradation by protozoa*

Organism	Enzyme[a] (reference)			
	Ade	Gua	XDH	Uricase[b]
Tetrahymena pyriformis	+(470) −(151, 306)	+(470) −(151, 221)	+(470, 557) −(221, 306)	+(470) −(221, 306, 355, 557)
Chaos chaos	±(356)		−(356)	+(356)
Amoeba proteus	±(356)		−(356)	+(356)
Paramecium aurelia			−(483)	−(483)
Acanthamoeba terricola				+(357)
Polytomella caeca				+(185)
Hartmanella culbertsoni				+(105)
Ochromonas malhamensis				+(321, 322)

[a] Ade, Gua, and XDH refer to adenine deaminase, guanine deaminase, and xanthine dehydrogenase or xanthine oxidase, respectively; + and − refer to the presence or absence of the enzyme, and ± refers to the presence of weak enzymatic activity.

[b] Presumably in all instances the enzyme is localized in peroxisomes, when present.

rines for growth since it is unable to synthesize the purine nucleus (219, 220). In *Ochromonas malhamensis* both allantoinase and allantoicase are present, but the intermediary position of ureidoglycolate has still to be established (321). *Epidinium ecaudatum caudatum* converts guanine and adenine to xanthine and hypoxanthine, respectively, and xanthine is degraded further along a pathway in which uric acid, allantoin, and allantoate may participate (113).

Algae

Reports on the degradation of purines by algae are restricted to studies on the use of these compounds as a nitrogen source for growth and on the occurrence of some of the enzymes involved in degradation (360). Most of the 38 chlamydomonad species tested by Cain (88) could use adenine, whereas about half of them used uric acid. *Chlamydomonas reinhardi* uses both xanthine and uric acid (53) and contains the urea carboxylase-allophanate hydrolase system, which is induced by urea and repressed by NH_4^+ ions (224, 472).

Xanthine and uric acid serve as a nitrogen source to *Chlorella pyrenoidosa*, *Chlorella vulgaris* (53), and *Monodus subterraneus* (337) but not to *Porphyridium cruentum* and *Euglena gracilis* (53). *C. pyrenoidosa* is able to use adenine as well as hypoxanthine and contains uricase (10). Uric acid is degraded in a photochemical process, when a solution of this substance is illuminated in the presence of chlorophyll *a* or *b* extracted from this organism. At least seven degradation products were observed, among which were allantoin, urea, cyanuric acid, and parabanic acid (11). Allantoin is not utilized as a nitrogen source for growth, presumably due to the inability to transport this compound, since allantoin formed from purines is degraded in the cell (10). Urea carboxylase of this organism is induced by urea and repressed by NH_4^+ ions, whereas allophanate hydrolase is a constitutive enzyme (224). The latter enzymes are also present in other *Chlorophyceae* (308).

Villeret (559) was the first author who demonstrated allantoinase and allantoicase activity in algae grown on mineral medium or peptone. All 21 freshwater algae investigated contained allantoinase, and only five species, all belonging to the *Desmidiales*, degraded allantoate. Allantoinase is also present in 90% of 51 species of marine algae (*Chlorophyta*, *Phaeophyta*, and *Rhodophyta*), but allantoicase activity was found in only about 20% of the strains (560).

Fungi

Basidiomycetes. Uricase, allantoinase, and allantoicase activities were demonstrated in a large number of *Basidiomycetes* by Brunel (76, 77) and Brunel and Capelle (79).

Phycomycetes The results for the *Phycomycetes* are summarized in Table 6.

Ascomycetes. Part of the data for the *Ascomycetes* is summarized in Table 7. *Penicillium chrysogenum* utilizes guanosine, adenosine (153), adenine, hypoxanthine, and xanthine (332) as sole sources of nitrogen for growth. The presence of adenine deaminase (8), a constitutive xanthine dehydrogenase (495), uricase (7, 172) and allantoinase (7), and an inducible al-

TABLE 6. *Degradation of purines by Phycomycetes*

Organism	Substrate used as N and C source		Enzyme[a]			Reference
	Uric acid	Allantoin	Ade	XDH	Uricase	
Phytophtora infestans	+		−			283, 377
Mucor boidin						283
Mucor spinosus (M. plumbeus)	+[b]				+	172
Mucor racemosus		−		+		495, 565
Rhizopus nigricans		−		+	+	172, 495, 565
Cunninghamella elegans					+	172

[a] Ade and XDH refer to adenine deaminase and xanthine dehydrogenase, respectively.
[b] Uric acid tested as sole nitrogen source.

TABLE 7. *Growth of Ascomycetes on uric acid or allantoin and the formation of xanthine dehydrogenase (XDH), uricase (Uri), allantoinase (An), allantoicase (Ac), and ureidoglycolase (Ug)*

Organism	Substrate[a,b]		Enzyme present[a]				
	Uric acid	Allantoin	XDH	Uri	An	Ac	Ug
Penicillium species							
P. brevicaule (Scopulariopsis brevicaulis)	CN (283)						
P. chrysogenum	N (7)		(495)	(7, 172)	(7)	(7)	
P. citreo-viride		CN (565)			(565)	(518)	(518)
P. frequentans (P. globrum)	N (172)			(172)			
P. glaucum	CN (172, 283)		(495)	(172)			
P. notatum		CN (565)		(172)	(565)	(518)	(518)
Aspergillus species							
A. flavus				(296)			
A. fumigatus	N (172)		(495)	(172)			
A. nidulans	N (126)	N (126)	(454)	(126)	(454)	(454)	(454)
A. niger	CN (77, 172, 283)	N (77)	(495)	(77, 172)	(77)	(77)	
A. niveo-glaucus (A. glaucus)	CN (283)						
A. oryzae		N (77)	(495)	(172)	(77)	(77)	
A. phoenicis	N (77)	N (77)		(77)	(77)	(77)	
Beavaria bassiana (Botrytis bassiana)	N (349)						
Geotrichum candidum	N (26)	N (26)		(26)	(26)	(26)	(26)
Gliocladium sp.				(172)			
Neurospora crassa	N (419)	N (367, 419)		(201, 419)	(367, 419)	(419)	(419)
N. sitophila				(172)			
Sporotrichum gougeroti	N (349)						
Trichophyta violaceum (Achorion violaceum)	N (349)						

[a] References are given within parentheses.
[b] N and CN refer to use of the substrates as sole nitrogen source or sole nitrogen and carbon source, respectively.

lantoicase (7) were demonstrated. *Penicillium roqueforti* can utilize methylpurines and xanthine as sole sources of either carbon or nitrogen for growth (292).

Hypoxanthine, xanthine, uric acid, allantoin, and allantoate serve as nitrogen sources for *Aspergillus nidulans*, which contains uricase (126), xanthine dehydrogenase, allantoinase, allantoicase, and ureidoglycolase (454). Two different xanthine dehydrogenases are reported to be present in this organism (125, 454, 455). One enzyme is constitutive; the other one

(as well as uricase) is induced by uric acid (454). Optimal induction of allantoinase requires both uric acid and allantoin. This phenomenon may be significant in view of the reported toxicity of allantoin to this organism (454). In the presence of histidine, *A. nidulans* can no longer use hypoxanthine, uric acid, allantoin, and urea as nitrogen sources. This compound represses the synthesis of urease and affects the activity of both xanthine dehydrogenase and uricase. The effects are not due to ammonia, which may be produced from histidine, but reflect a preferential use of the latter substance (385). *Aspergillus fumigatus* contains adenine deaminase (495).

Geotrichum candidum uses xanthine, uric acid, and allantoin as nitrogen sources (26). Uricase was present in spores of this organism germinated in the presence of glutamine or urea, but its level increased ninefold when uric acid was present during germination (26). Urea is degraded along the allophanate pathway, which is not induced by urea (477). Allantoinase and ureidoglycolase are constitutive enzymes in this organism, whereas allantoicase is induced by allantoin (26).

Neurospora crassa uses adenine, hypoxanthine (127), uric acid (419), allantoin (367, 419), and allantoate (420) as nitrogen sources. A common transport system is present for adenine, guanine, and hypoxanthine and also a specific one is present for adenine (326). Although McElroy and Mitchell (332) could not observe adenine deaminase in *Neurospora*, the presence of this enzyme could be deduced from the growth pattern of wild-type and mutant strains (127). Uricase of *N. crassa* is induced by uric acid and allantoinase is induced by either uric acid or allantoin. Both enzymes are repressed by ammonia. Ureidoglycolase and urease are constitutive enzymes (419).

Fungi imperfecti. Adenine deaminase, xanthine dehydrogenase, and uricase are demonstrated in *Alternaria tenuis* (167, 172). Growth of this organism on xanthine as the nitrogen source is almost negligible (495). Uricase is reported to be constitutive in *A. tenuis* and *Alternaria solani;* in *Alternaria tenuissime* and *Alternaria porri* the basal level could be enhanced by growth on uric acid (172). Uricase is present in *Alternaria gossypina* and *Stemphylium* (172). A species of *Stemphylium* is reported to utilize caffeine, theobromine, and xanthine as sources of either carbon or nitrogen for growth (292).

Fusarium moniliforme can grow on hypoxanthine, xanthine, uric acid, and allantoin as nitrogen sources. Xanthine dehydrogenase, uricase, allantoinase, and allantoicase were demonstrated in the cell-free extracts (9). Xanthine dehydrogenase and uricase of *Fusarium sambucinum* are constitutive enzymes (172). Uricase is also present in *Fusarium niveum, Fusarium semitietum* (172), and *Fusarium oxysporum* (330) but absent in *Fusarium equiseti* (172).

The tobacco-wilt organism *Fusarium oxysporum* var. *nicotianae* can use uric acid and allantoin as nitrogen sources (600). Uric acid serves as both a nitrogen and carbon source for *Cladosporium herbanum* and *Paecilomyces farinosus* (283) and as a nitrogen source for *Microsporum lanosum, Trichophyton ferrugineum* (490), *Mastigocladium blochi,* and *Epidermophyton inquinale* (349). Uricase is present in *Cladosporium, Trichoderma koningi,* and *Botrytis cinerea* (172). The fungal pathogen *Histoplasma capsulatum* is capable of utilizing uric acid as well as its degradation products as nitrogen sources for growth. Uricase, allantoinase, allantoicase, and urease are inducible (316).

Yeasts. The first experiment on the degradation of purines and its catabolites by microorganisms was performed by Wöhler (598) who in 1853 inoculated a solution of allantoin with an unidentified yeast. Among the end products formed after 4 days at 30 C were ammonia, urea, oxalate, and carbonate.

Growth of yeasts on purines is a widespread property. Adenine, hypoxanthine, xanthine, and guanine support the growth of 108, 82, 108, and 116 strains, respectively, out of a total of 123 yeast strains belonging to a wide variety of genera (302). *Candida utilis* readily uses the purine bases as a nitrogen source, whereas *Saccharomyces cerevisiae* utilizes adenine and guanine moderately well but is not able to grow on hypoxanthine and xanthine (137, 302).

S. cerevisiae, C. utilis, and *Schizosaccharomyces pombe* degrade the purines along the common pathways in which uric acid is an intermediate and adenine deaminase, guanine deaminase, xanthine dehydrogenase, and uricase are involved as inducible enzymes (299, 388, 434–437). In *S. pombe* another route may be involved in the conversion of adenine to hypoxanthine where AMP, adenosine, and inosine are intermediates (1).

Xanthine dehydrogenase could not be demonstrated in cell-free extracts of *C. utilis,* presumably due to the lability of the enzyme (436). The enzyme of *Candida guilliermondii* was reported to be unable to oxidize hypoxanthine, which is converted to xanthine via the intermediates inosinic acid and xanthylic acid (462, 463, 475).

Uric acid, allantoin, and allantoate sup-

ported the growth of 109, 113, and 118 species out of 123 tested yeasts from various genera. The ability of yeasts to use purines and their degradation products is so widely distributed that it is of no obvious value as a generic characteristic (302). Allantoin is degraded in a pathway involving allantoinase, allantoicase, ureidoglycolase, urea carboxylase, and allophanate hydrolase. Biotin is required for the function of urea carboxylase and, thus, its stimulating role in the degradation of allantoin, allantoate, and urea is obvious (136, 141, 348). The urea carboxylase-allophanate hydrolase system offers ureaseless yeasts a way of using nitrogen from urea and allantoin; its discovery resolved previous problems (114, 260). An inducible allantoinase is present in *Saccharomyces carlsbergensis* (543). Allantoinase, allantoicase, and ureidoglycolase of *S. cerevisiae* were reported to be constitutive (106, 108, 307) or inducible (141). Allophanate induces the allantoin degradative enzymes together with the multienzyme complex involved in the allophanate pathway (115) and the urea uptake system (116). The synthesis of the complex is furthermore repressed by NH_4^+ ions in *S. cerevisiae* (594), *C. utilis* (432), and *C. guilliermondii* (512).

Allantoinase, allantoicase, and ureidoglycolase of *C. utilis* are inducible enzymes (106–108, 307). *C. guilliermondii* contains allantoinase (462, 475), allantoicase (475), and the enzymes of the allophanate pathway. The latter pathway is induced by the presence of both urea and biotin (512).

Studies on the transport mechanism of purines and pyrimidines in yeast revealed that *S. cerevisiae* possesses a common active transport system for adenine, guanine, hypoxanthine, and cytosine (384, 416) and a specific one for uracil (202) and urea (116). Purine uptake is activated by protons and inhibited by K^+ ions, and it was concluded that the purine transport system in *S. cerevisiae* acts as a proton symporter and a K^+ antiporter (415). NH_4^+ ions exert an inhibitory effect on uric acid transport in *C. utilis* (436) and *Pichia guilliermondii* (476), but the mechanism of the inhibition is unknown.

Bacteria

Cyanobacteria. *Anacystis nidulans* and *Synechococcus cedrorum* can not use xanthine and uric acid as a sole source of nitrogen (53), but *Agmenellum quadruplicatum* is able to utilize adenine, hypoxanthine, xanthine, and uric acid as a nitrogen source for growth (256).

Several species of marine cyanobacteria show atypical growth when uric acid serves as a nitrogen source. The growth rate was much reduced and the photosynthetic pigment system was highly bleached (542). It was concluded that besides uricase, also a nonenzymatic oxidative attack on uric acid occurs in cyanobacteria. In *A. nidulans*, which could not grow on uric acid (53), this substance was degraded almost completely to allantoin in the light. This photooxidation was mediated by a pigment not identical to chlorophyll *a* or phycocyanin.

Pseudomonas. Various members of the genus *Pseudomonas* are able to grow on purines either as a nitrogen source or as a nitrogen and carbon source. Adenine, guanine, hypoxanthine, and xanthine serve as nitrogen sources for *P. aeruginosa* (170, 433), *P. acidovorans* (263), and an unidentified pseudomonad (139), and these purines are used also as sole carbon and nitrogen sources by *P. acidovorans* (478) and other pseudomonads (20, 90). Adenine inhibits the growth of some strains of *P. acidovorans* on hypoxanthine (263, 478), probably due to inhibitory effects of adenine which were discussed in a previous section. Adenine deaminase and guanine deaminase were demonstrated in *P. aeruginosa* (170), *P. acidovorans* (478), and *P. oleovorans* (448). Xanthine dehydrogenase is present in *P. aeruginosa* (49, 111, 139, 170, 344) and *P. acidovorans* (478); the enzyme can use oxygen as an electron acceptor.

Uric acid is used as carbon and nitrogen sources by *P. aeruginosa* (250, 314, 433), *P. putida (P. eisenbergii)* (132, 314), and *P. calcoacetica* (314). *P. fluorescens* utilizes this compound as a nitrogen source (170, 433) and probably also as carbon and nitrogen sources (132, 314). The presence of uricase was demonstrated for *P. aeruginosa* (49, 111, 139, 169, 250, 433), *P. fluorescens* (433), and *P. acidovorans* (478). The activity of this enzyme was lost after sonification of the cells (49), probably due to the fact that it is membrane bound (478).

The enzymes involved in the degradation of purines are inducible enzymes (20, 89, 169, 170, 250, 433, 440, 478).

Allantoin appears to be an attractive substrate to a large number of species. It is used as carbon and nitrogen sources, by *P. aeruginosa*, *P. fluorescens* (314, 570), *P. putida (P. eisenbergii)* (133, 314, 553), *P. calcoacetica* (314), *P. acidovorans* (570), *P. mildenbergii*, *P. tolaasii*, *P. testosteroni*, *P. multivorans* (440), and several unclassified strains (89, 132). It serves as a nitrogen source of *P. maltophilia*, *P. alcaligenes*, *P. pseudoalcaligenes*, *P. stutzeri* (553), *P. iodinum*, and *P. oleovorans* (440).

The various pathways involved in the degradation of purines are given in the enzymatic section on the aerobic degradation. Preliminary

studies on the genetics of allantoin metabolism in *P. aeruginosa* were made by Gaudy and Bruce (181).

Part of the pathway (Fig. 2) may be involved in alloxanate degradation by a soil pseudomonad and was studied by Gerhart et al. (186) and Gray et al. (198, 199). This microbe grows well aerobically in media containing alloxanic acid, allantoin, or uric acid as sole sources of carbon, nitrogen, and energy. Alloxanate is converted to 5-hydroxyhydantoin, and the latter yields glyoxylate and urea (Fig. 7). The authors mentioned allanturic acid as a possible intermediate. However, it seems more likely that 5-hydroxyhydantoin is hydrolyzed by hydantoinase into ureidoglycolate, which in turn is degraded by ureidoglycolase.

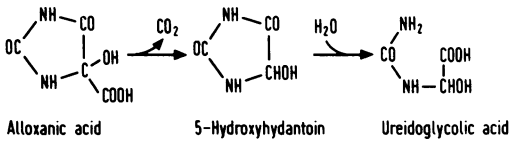


FIG. 7. *Degradation of alloxanic acid by a Pseudomonas species (186, 198, 199).*

Alcaligenes. Autotrophically grown *A. eutrophus* can use uric acid and allantoin either as a sole nitrogen or sole carbon source for growth when energy is available from the oxidation of H_2 gas, which is required for the incorporation of ammonia into cell material (12). Kaltwasser (248–252) demonstrated that this organism can use uric acid as a sole nitrogen and carbon source. A particle-bound and inducible uricase is involved. The presence of allantoate amidohydrolase is obvious since 1 mol of urea and 2 mol of ammonia are formed from 1 mol of uric acid (251, 252) in spite of the ability of the organism to produce urease. This enzyme is fully repressed by ammonia during growth with uric acid or allantoin (252). Glyoxylate is degraded along the tartronic semialdehyde pathway to glyceric acid and phosphoenolpyruvate (251). The enzymes of this pathway are induced when cells grow on uric acid, allantoin, or glyoxylate (251, 252).

Various heterotrophic strains of *Hydrogenomonas* utilize adenine, guanine, xanthine, hypoxanthine, uric acid, allantoin, and urea for growth (249). *Alcaligenes faecalis* can use uric acid as a source of nitrogen (169, 170, 516), but Rouf and Lomprey (433) reported the contrary. This organism contains uricase (612). Other species of *Alcaligenes* can use uric acid as a secondary carbon and energy source (458).

Arthrobacter* and *Brevibacterium. Krebs and Eggleston (289) demonstrated that *Arthrobacter globiformis* (*A. ureafaciens*) (110) can grow in media containing hypoxanthine, uric acid, or allantoin as the sole organic substrate. From uric acid 2 mol of urea is formed, and allantoin and allantoate are intermediates in the degradation. The results suggest the involvement of allantoinase and allantoicase, which are both induced by growth on urate.

The growth of *A. globiformis* on uric acid as the sole substrate was confirmed by Imshenetskii and Popova (232, 233) who isolated the organism (*A. ureafaciens* and *A. pascens*) from peat soil, but Rouf and Lomprey (433) found contrasting results. Uricase of *A. globiformis* (*A. pascens*) was studied by Arima and Nose (18).

Arthrobacter strains AC1 and AC207 are able to utilize uric acid and allantoin as sole sources of carbon, nitrogen, and energy, but in contrast to *A. globiformis* 4 mol of ammonia is formed instead of urea (16). *A. tumescens* and *A. simplex* are uricase negative (16).

Brevibacterium vitarumen var. *uricum* contains uricase, whose induction was stimulated by $FeSO_4$ (266), but Imshenetskii and Popova (232, 233) reported on a strain that appears to contain a constitutive uricase. *B. linens* degrades both xanthine and uric acid (481). The latter substance is used as a nitrogen source (481) but not as a nitrogen and carbon source (16). Washed cells convert 1 mol of uric acid or allantoin to 1 mol of urea (16).

Bacillus. Various reactions of the purine degradative pathway can be performed by a number of *Bacillus* species, but only a few species degrade these substances fully. Adenine is converted to hypoxanthine by *Bacillus anthracis* (345) and *B. subtilis* (131). The latter organism degrades also xanthine and uric acid (139), which is used as the sole source of nitrogen, carbon, and energy (433). This property is an adaptive one (433).

B. polymyxa utilizes xanthine and guanine as nitrogen sources (132). Moreover, uric acid and allantoin are used in such a way by this organism and by *B. megaterium, B. guano, B. hollandicus,* and *B. subtilis* var. *niger* (*B. vulgatus*) (132, 486). However, the results are not unequivocally confirmed (139, 169, 170, 516, 613, 614). Guanine, xanthine, uric acid, and allantoin are not used as nitrogen sources by *B. cereus* var. *mycoides* (132), and *B. stearothermophilus* cannot use allantoin in this way (433). An unidentified species of *Bacillus* grows at the expense of adenine, guanine, hypoxanthine, xanthine, uric acid, or allantoin as nitrogen sources. Growth on these compounds as sole organic substrates is slight. The growth on uric acid is an adaptive property (433).

B. fastidiosus was first isolated in 1929 by

Den Dooren de Jong (133). The organism is widely distributed in soil and was named for its fastidious demand for uric acid or allantoin as carbon and energy sources. No growth occurred when *B. fastidiosus* was inoculated in various rich media (133). Recently the bacterium was isolated and studied by Leadbetter and Holt (226, 305), Mahler (329), Kaltwasser (253), and Bongaerts and Vogels (62). The organism grows well in a synthetic medium containing uric acid, allantoin, or allantoate as the sole organic substrate but does not utilize common organic substrates other than these compounds (62, 133, 226, 305). Even adenine, guanine, xanthine, and hypoxanthine are not used for growth (253). A soluble uricase is induced by growth on urate and is almost absent in most strains when grown on allantoin. This enzyme is applied in clinical measurements of uric acid (213, 329). The degradative pathway of allantoin is catalyzed by S-allantoinase, allantoate amidohydrolase, and S-ureidoglycolase (62). Glyoxylate is degraded along the tartronic semialdehyde pathway (253).

Mycobacteria. A large number of mycobacteria were investigated for their ability to degrade purines or the products of purine degradation, either for taxonomic reasons or for testing tuberculostatic compounds (63, 132, 138, 169, 274, 428, 433, 494). In most instances the degradation was tested in suspensions of the bacteria in phosphate buffers containing the substrate (63, 138, 274, 428), and the production of ammonia, glyoxylate derivatives, or $^{14}CO_2$ was tested.

Most of the species investigated were unable to produce ammonia from adenine, hypoxanthine, xanthine, uric acid (428), or allantoin (63, 494). The degradative pathway is incompletely present in the investigated species, but the part which is present leads to formation of ammonia in most cases. It starts with adenine and guanine and is, therefore, most complete in the case of *Mycobacterium tuberculosis* BCG (138); the data of Bönicke (63) are inconsistent with these results, since he could not demonstrate the degradation of allantoin.

M. thamnopheos (63, 428) and *M. smegmatis* (138, 274, 428, 494) can degrade the compounds from hypoxanthine on. However, some of the data are contradictory for the latter organism as to hypoxanthine (138) and allantoin degradation (63, 494). *M. marinum* (428) starts with xanthine, but Bönicke (63) did not observe allantoinase activity in two strains tested. *M. butyricum* (138, 274) and *M. stercoris* start with uric acid, and the allantoin-degrading system is present in *M. fortuitum* (63). Allantoinase is reported to be present in *M. vaccae, M. peregrinum, M. fortuitum,* and *M. thamnopheos* (82). In *M. chelonei (M. borstelense)* ammonia is formed from xanthine but not from uric acid (428). The data concerning purine degradation by *M. phlei* are very inconsistent and contradictory (63, 132, 138, 169, 274, 433, 494) and do not allow any conclusion. As far as studied, the mycobacteria can use purines and their degradation products only as a source of nitrogen, but *M. phlei* is claimed to be capable of a limited growth on uric acid alone (433). Most, if not all, of the above-mentioned species contain urease as detected by a direct test (138, 274, 494) or by an obvious deduction: the production of 4 mol of ammonia from uric acid or allantoin (63, 274). Therefore, no conclusion can be made on the involvement of allantoicase or allantoate amidohydrolase in the pathways. *M. butyricum* was reported to be urease negative, but Klemperer et al. (274) reported the presence of urease.

Actinomycetales. The results obtained with *Nocardia, Saccharopolyspora,* and *Actinomadura* species are compiled in Table 8.

An unidentified streptomycete produces large amounts of uricase when cells grown in a peptone-glucose medium were incubated with uric acid or other purines (179, 582, 583). Uric acid degradation in these cells requires the presence of K^+ ions. Allantoin accumulates quantitatively because of the absence of allantoinase (583). Hypoxanthine is converted to xanthine and 6,8-dihydroxypurine in a ratio of 3 to 1 by various species of *Streptomyces*. Both products are converted to uric acid and are further degraded (584).

Various bacteria. *Aeromonas hydrophila* grows poorly in media containing uric acid as a sole nitrogen source (433). Adenine is utilized as a source of nitrogen by *Azotobacter chroococcum* and *A. vinelandii,* which contain adenine deaminase (227, 229). Guanine and allantoin are doubtful sources of nitrogen to these organisms and uric acid is not used (227, 433). *Corynebacterium xerosis, C. minutissimum, C. striatum, C. diphtheriae,* and *C. bovis* are able to degrade uric acid (481), but *C. pseudodiphtheriticum* is unable to grow in a medium containing uric acid as the sole nitrogen source (433). *Lactobacillus casei* does not degrade purines (24). A study was made on the xanthine dehydrogenase of this organism (558). *L. bulgaricus* cannot degrade allantoin when this compound is added to nutrient broth (613).

Micrococcus roseus (M. agilis) is unable to grow in a medium containing uric acid as a sole nitrogen source (433); however, this compound is used in this way by *M. varians,* in which the presence of uricase was demonstrated (266). *M.*

TABLE 8. *Hydrolysis of purines by Actinomycetales*

Organism	Substrate[a]						Reference
	Gua	Ade	Hypo	Xan	UA	All	
Nocardia otitidis-caviarum (N. caviae)		−	(+)	+	+	+[b]	82, 192, 298
N. brasiliensis	+	−[c]	+	−[c]	−[c]	+[b]	82, 192, 298
N. asteroides		−	−[c]	−	−[c]	+[b]	82, 192, 298
N. coeliaeca	+	+	+	+		+	82
N. polychromogenes		+	+	+	+		132
N. blackwellii						+	82
N. rubropertineta		+					82
N. rubra		+					428
N. opaca					+	+	132
N. erythropolis		+					82
N. pellegrino		+					428
N. corallina		−	+[d]	+[d]	−		428
Saccharopolyspora hirsuta		+	+	+			298
Actinomadura dassonvillei		+	+	+			298
A. madurae		−	(+)	−			298
A. pelletieri		−	+	−			298

[a] Abbreviations: Gua, guanine; Ade, adenine; Hypo, hypoxanthine; Xan, xanthine; UA, uric acid; All, allantoin.

[b] Allantoinase present (192).

[c] Reported to be positive for one strain (428).

[d] Reported to be negative for one strain (428).

luteus (Sarcina aurantiaca, S. lutea) utilizes guanine, xanthine, uric acid, and allantoin as nitrogen sources (139). Allantoin can replace urea as a requirement for T-strain mycoplasmas (331). *Myxococcus virescens, M. fulvus,* and *M. coralloides (Chondrococcus coralloides)* utilize adenosine and guanosine as sources of carbon, nitrogen, and energy (368). The nitrification process in *Nitrosomonas europaea* can occur at the expense of amino groups of guanine, uric acid, and allantoin, which suggests the presence of a degradation pathway for purines (439). *Paracoccus denitrificans (M. denitrificans)* can use uric acid as a sole source of nitrogen and carbon. Uricase is strongly induced by growth on uric acid (250). *Spirillum tenue* utilizes xanthine, guanine, uric acid, and allantoin as a source of nitrogen (132).

Both hypoxanthine and guanine can be converted to adenine by *Staphylococcus aureus*, but the reverse transition is not possible due to lack of the ability to deaminate adenine (601). Xanthine is not degraded by *S. aureus,* but uric acid and allantoin are used (481). *S. aureus (S. albus)* does not utilize xanthine, guanine, and uric acid as a nitrogen source, but allantoin is used (132). Purines are deaminated only slowly or not at all by *Vibrio cholerae* according to Agarwala et al. (4), but Dikstein et al. (139) demonstrated degradation of xanthine and uric acid by this organism.

DEGRADATION BY *ENTEROBACTERIACEAE* AND STREPTOCOCCI

Enterobacteriaceae

Various authors have studied the ability of *Enterobacteriaceae* to degrade uric acid and allantoin. The reported results are summarized in Table 9. Although the results are contradictory in some instances, one may conclude that the ability to degrade uric acid and allantoin is widely distributed among the *Enterobacteriaceae*.

Growth tests on uric acid and allantoin were proposed to differentiate the coli-aerogenes group (282, 343). *Escherichia coli* was assumed to be unable to use hypoxanthine (282), uric acid (27, 282, 343, 433, 481), or allantoin (343), whereas *Aerobacter aerogenes* and *A. cloacae* could use these compounds as a sole source of nitrogen (Table 9). However, a number of *E. coli* strains obtained from soil can use uric acid as a nitrogen source (104) and, when judged under anaerobic conditions, a majority of *E. coli* strains tested can use allantoin as a sole source of nitrogen, carbon, and energy (565). Moreover, the nitrogen of xanthine (132, 343), adenine (343), and guanine (132) was used by *E. coli*; cell suspensions of *E. coli* B convert isoguanine (6-amino-2-hydroxypurine) to xanthine (176). The capability of *E. coli* to use purines

TABLE 9. *Ability of Enterobacteriaceae to use uric acid or allantoin*[a]

Organism	Uric acid	Allantoin	Reference
Escherichia coli	−N		282, 433
	−N, +N[b]		104
	−N	−N	343
	(+)N, −CN	(+)N, −CN	132
	+O, −N		27, 517
		+O	613
	+N	+N	132
		+CN[c,d]	565
Citrobacter freundii	−N	−N	343
		+CN[d]	565
Aerobacter aerogenes		−CN	565
		+O	613
	+N		104, 282, 349, 517
	+N, −CN	+N, (−)CN	132
	+N, +CN	+N, +CN	343, 433
Klebsiella pneumoniae	+CN	+CN	433
Enterobacter cloacae (A. cloacae)		−CN	565
	+N, −CN	+N, −CN	343
Serratia marcescens (also *S. kiliensis)*		−CN	565
	+N, −CN	+N, (−)CN	132
	+CN		433
Proteus rettgeri		+CN[d]	565
P. vulgaris	+CN, (−)N	(−)CN, (−)N	132
	+O		517
		+O	613
	+N		516
P. mirabilis	(−)N		433
Erwinia herbicola (Bacterium herbicola)	(+)N	(+)N	132
Paracolobactrum aerogenoides	+CN	+CN	509

[a] Tests in which the compounds are tested as sole carbon, nitrogen, and energy source (CN), as sole nitrogen source (N), or as a secondary carbon and energy source (O) for growth are represented as: +, good growth; (+), weak growth; (−), uncertain growth; and −, no growth.

[b] Strains from feces −N; 50% of the strains from soils +N.

[c] Ten out of 16 strains were positive.

[d] Under anaerobic conditions.

has been underestimated in the past since various enzymes of the catabolic pathway are present in the cells, i.e., adenosine deaminase (68), adenine deaminase (68), uricase (496), and a set of enzymes similar to those found in *Streptococcus allantoicus*, as will be discussed below.

Guanine and xanthine are used as a nitrogen source by *Serratia marcescens* (132, 433), *A. aerogenes*, and *Erwinia herbicola (Bacterium herbicola)* (132). Adenine and hypoxanthine are used as a nitrogen source and to a less extent also as sole organic substrates by *A. aerogenes*, *Klebsiella pneumoniae*, and *S. marcescens (S. kiliensis)* (433). *Proteus vulgaris* can use xanthine but not guanine as a nitrogen source (132). In contrast to the above results, Dikstein et al. (139) found no degradation of xanthine by cell suspensions of *E. coli*, *A. aerogenes*, and *P. vulgaris*.

Salmonella typhimurium does not contain physiologically significant amounts of adenine deaminase. The conversion of adenine to hypoxanthine takes place via adenosine and inosine (225). The presence of uricase was demonstrated in *E. coli*, *Proteus morganii (Morganella)*, *P. inconstans (Providencia)*, *P. mirabi-*

lis, and *Serratia* species (496). Growth on uric acid is an adaptive property in *A. aerogenes*, *K. pneumoniae*, and *S. marcescens* (433).

The degradative route of allantoin used by *E. coli*, *Citrobacter freundii* (*E. freundii*), and *P. rettgeri* are similar to those described below for *S. allantoicus* and are perhaps common to all *Enterobacteriaceae*. The contradictory results represented in Table 9 are partly due to the conditions used during incubation. The catabolic routes involve uricase, which is operative only under aerobic conditions, whereas utilizable carbon compounds are produced under anaerobic conditions only.

STREPTOCOCCUS ALLANTOICUS

S. allantoicus was isolated in 1943 by Barker (28) from black mud from the shore of San Francisco Bay. Later, this organism was isolated from various sources according to the enrichment procedure of Barker, which involves anaerobic incubation in a medium containing allantoin and a small amount of yeast extract (536, 565), followed by isolation of the streptococci on agar plates containing glucose.

The same enrichment procedure resulted in the isolation of another bacterium, which was called *Arthrobacter allantoicus* (564, 565) but was identified later on as *P. rettgeri*.

S. allantoicus is a gram-positive, catalase-negative, nonmotile streptococcus, with spherical or ovoid cells that are 1.1 to 1.5 μm in diameter, mostly occurring in pairs or short chains; the chains are formed by pairs of lanceolate cells. No reaction was observed with group A-T sera according to Lancefield (565). Barker (28) reported the production of dextran as an extracellular polysaccharide for his isolate, but a negative cross-reaction with type II pneumococcus antiserum proves the absence of dextran in the isolate of Vogels (565). The action on blood is indifferent (gamma hemolysis) (28, 565). The colonies on agar media are partly transparent, circular (1 to 2 mm), and convex with undulate margin. The bacteria grow at 10 and 40 C but not at 45 C (565). Barker (28) did not observe growth above 36.5 C. Treatment for 15 min at 50 C kills the cells (565). They grow in yeast extract-glucose media containing 6.5% NaCl (28, 565). No growth was observed at pH 9.6 on blood agar containing 40% bile or in skim milk containing 0.005% methylene blue. Growth is strongly inhibited by bacitracin (5 U/ml). Litmus milk is weakly acidified with some reduction but not curdled. The final pH in yeast extract-glucose was 5.0 to 5.3. Acid, but no gas, was slowly formed from glucose, maltose, lactose, sucrose, trehalose, raffinose, mannitol, sorbitol, and salicin. Little or no acid was formed from arabinose, xylose, fructose, galactose, or rhamnose. No acid was formed from inulin, starch, or glycerol. Gelatin and sodium hippurate are not hydrolyzed, but esculin is split. No ammonia is formed from arginine and urea is not split. No diacetyl is formed in sugar media. It can be isolated from black shore mud (28), ditch mud (564, 565), and duck ponds (536). The following products are formed under anaerobic conditions per 100 mol of glucose: (+)-lactate, 106 mol; acetate, 36 mol; formate, 23.9 mol; CO_2, 9.7 mol; and ethanol, 27.6 mol (28). The isolate is able to grow very well in media containing no carbohydrate or polyhydroxyalcohol and is not identical to known *Streptococcus* species. Therefore, Barker (28) created the new species *Streptococcus allantoicus*.

The guanine plus cytosine content of deoxyribonucleic acid, determined by chemical analysis (73) of three different strains (V4012, V4031, and V4023), revealed values of 46.0, 47.5, and 49.7 ($\pm$ 0.3) mol% (unpublished data). These values are rather high for streptococci.

Catabolic Pathway of *S. allantoicus* and *Enterobacteriaceae*

Barker (28) observed that growth of *S. allantoicus* was much better under anaerobic than under aerobic conditions. The presence of a small amount of yeast extract in the growth medium is required, perhaps because biotin is a cofactor for growth. Under anaerobic conditions 100 mol of allantoin are converted to 226 mol of ammonia, 62.3 mol of urea, 14.8 mol of acetate, 1.5 mol of lactate, 168 mol of CO_2, 9.4 mol of formate, 44.8 mol of oxamate, and 13.8 mol of a compound which was thought to be glycolate (28). The identification and quantitative determination of oxamate as a new microbial product was a stimulus for further studies. Moreover, Barker pointed to the fact that the ratio of ammonia to urea is constant (3.64:1). He concluded that ammonia is formed directly from allantoin or some intermediate, rather than by hydrolysis of urea, since cell suspensions of *S. allantoicus* are unable to decompose urea (28, 565). Barker (29) proved also that allantoate is readily decomposed by whole cells of *S. allantoicus*.

Later studies (565) revealed that *P. rettgeri*, *E. coli*, and *C. freundii* produce oxamate from allantoin; the amounts formed per 100 mol of allantoin are 59, 51, and 53 mol of oxamate, respectively. Growth of the bacteria is strongly inhibited in the presence of oxygen, and under this condition *S. allantoicus* formed 80 mol of oxamate per 100 mol of allantoin degraded,

whereas the amount of urea dropped to 20 mol (565). These results suggest that urea and oxamate are formed along different pathways, i.e., an oxidative one resulting in the formation of oxamate and a reductive one in which urea is produced. In the presence of oxygen the interrelation between the pathways is disturbed, and the oxidative route prevails. The degradative routes were resolved by studies of Valentine et al. (59, 530, 531, 533, 535–539), Vogels (564, 565), and Van der Drift et al. (547).

Conversion of allantoin to ureidoglycolate. The catabolic pathway followed by *S. allantoicus* and *Enterobacteriaceae* (*E. coli*, *E. coli* var. *acidilactici*, *C. freundii*, and *P. rettgeri*) is described in Fig. 8. The enzymes involved in the degradation of allantoin to glyoxylate are discussed extensively in a previous section.

The allantoinases of this group of organisms exhibit a number of common properties which distinguish them from the allantoinases of other microorganisms, plants, and animals. They are activated by Mn^{2+} ions and reducing substances and are the only known aspecific allantoinases; i.e., R- and S-allantoin are degraded with about equal velocities (570, 571). This may reflect the scavenging character of the degradation of allantoin by these organisms, since the natural dextrorotatory S-allantoin (196) is subject to a rapid racemization in neutral and alkaline media (569). The product of the allantoinase reaction is allantoate, which was reported as an intermediate in the degradation by Barker (29).

Allantoate is not degraded by allantoicase, since less than 2 mol of urea and a substantial amount of ammonia are formed from allantoate by the urease-negative *S. allantoicus*, *E. coli*, and *C. freundii*. A new enzyme was found, allantoate amidohydrolase (allantoate amidinohydrolase [decarboxylating] [EC 3.5.3.9]), which catalyzes the conversion of allantoate into S(−)-ureidoglycolate, ammonia, and CO_2 (545, 550, 551, 565, 566). S(−)-ureidoglycolase is present in *S. allantoicus* (182, 183, 537, 565), in *P. rettgeri*, and probably also in *E. coli* and *C. freundii* (565). The intermediary function of glyoxylate in the degradation of allantoin by *S. allantoicus* was established by Valentine et al. (531).

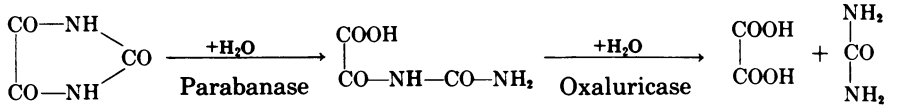
Reductive degradation of glyoxylate. Glyoxylate is converted to glycerate according to a reaction sequence similar to that described by Kornberg and Gotto (193, 281) and Dagley et al. (122) in the aerobic glycine metabolism by a pseudomonad. Tartronate-semialdehyde, which was identified in various ways (530, 533, 565), and CO_2 are formed in a reaction catalyzed by tartronate-semialdehyde synthase (Fig. 8), which is present in *S. allantoicus* (530, 533, 565), *P. rettgeri* (565), and *E. coli* (284). The reaction is stimulated by the presence of thiamine pyrophosphate (284, 530), Mg^{2+} ions (284, 533, 565), and anaerobic conditions (284, 565).

Tartronate-semialdehyde reductase. Tartronate-semialdehyde reductase of *S. allantoicus* (193, 530) catalyzes the reversible (533) conversion of tartronate-semialdehyde to glycerate in the presence of NAD. In tests in which both tartronate-semialdehyde synthetase and tartronate-semialdehyde reductase are allowed to act on glyoxylate, glycolate is formed (533). Glycerate is converted to 3-phosphoglycerate, which can yield pyruvate via the Embden-Meyerhof pathway and acetate and formate by pyruvate degradation (530). Acetate and formate were demonstrated as products of allantoin degradation by Barker (28).

Oxidative production of oxalurate. Ureidoglycolate is dehydrogenated to oxalurate by ureidoglycolate dehydrogenase in *S. allantoicus* (530, 538, 565), *P. rettgeri* (547, 565), and *E. coli* (565). The purified enzyme from *P. rettgeri* converts specifically S(−)-ureidoglycolate and uses both NAD and NADP equally well. A number of other hydroxy acids cannot replace ureidoglycolate as a substrate. The pH optimum is 8.0 to 8.4, and the enzymatic activity depends strongly on the ionic strength of the solution, being optimal at $I = 0.07$ to 0.12. The reversibility of the reaction could not be demonstrated, perhaps due to the fact that the equilibrium position of the reaction lies strongly in the direction of oxalurate formation (547).

The chemical relationship between uric acid and allantoin on one hand and oxalurate on the other hand has been known for a long time. Wöhler and Liebig (599) prepared oxalurate in 1838 by treatment of uric acid with moderately concentrated nitric acid. Biltz and Schauder (52) prepared oxalurate from allantoin by oxidation of the latter with H_2O_2 and from uric acid with $KMnO_4$ in alkaline solution. Oxalurate is formed among other products from ^{14}C-labeled urate by a catalase-ethyl hydrogen peroxide system (97).

The occurrence of oxalurate in human urine was claimed in 1867 by Schunck (467), in 1868 by Neubauer (366), and in 1938 by Flaschenträger and Müller (159). Oxalurate was supposed to be involved in the production of renal calculi composed wholly or partly of calcium oxalate (101). It was thought (101, 487) that uracil is converted to oxalurate in dogs; *N*-formyloxalurate was supposed to be an intermediate in this conversion. Parabanate was also reported as a source of oxalurate, which is formed by parabanase.



(Parabanic acid) (Oxaluric acid) (Oxalic acid) (Urea)

This enzyme was considered to be present in liver from frogs (290) and in microbes (78, 136, 198). Both reaction sequences leading to oxalurate are not well established and are questionable. The salts of parabanate are hydrolyzed readily to salts of oxalurate in aqueous solution (15), whereas the latter are relatively stable at pH values below 11 and are hydrolyzed to oxalate and urea in strongly alkaline solutions (143).

Oxaluricase was reported to be present in dogs (101, 487), in the livers of frogs (290), in *Aspergillus niger* (78), in *Saccharomyces cerevisiae* (136), and in an unidentified bacterium (198). New studies are required to prove the presence of this enzyme since at least a number of the results can be explained by assuming the presence of oxamate transcarbamoylase, which is described below.

Oxamate formation. The presence of oxamate among the end products of allantoin degradation by *S. allantoicus* was established by Barker (28).

Oxamate has been reported to occur in nature in only one other source. Kmínek (277) demonstrated its occurrence in beets. Since these plants also contain allantoin (276), a similar relationship between allantoin and oxamate may exist in plants as is described here for *S. allantoicus*. Oxamate is used as an inhibitor of lactate dehydrogenase (370).

The enzyme catalyzing oxamate formation in *S. allantoicus* was first studied by Valentine et al. (60, 531, 535, 539). A phosphorolytic or arsenolytic cleavage of oxalurate (carbamoyloxamate) is catalyzed by oxamate transcarbamoylase (carbamoylphosphate:oxamate carbamoyltransferase [EC 2.1.3.-]). Oxalurate accumulates during the degradation of allantoin or allantoate in the absence of phosphate and arsenate (530, 531, 539) and during the degradation of allantoin by cell suspensions in the presence of ethylenediaminetetraacetate (564).

The enzyme is also present in *P. rettgeri*, group D streptococci, and *E. coli* (511). However, Valentine et al. (530) reported its absence in *E. coli* K-12.

The enzyme catalyzes a reversible reaction (59, 511) (Fig. 8) with an apparent equilibrium constant (59):

$$K_{app} = \frac{[\text{oxalurate}]\,[\text{phosphate}]}{[\text{oxamate}]\,[\text{carbamoyl phosphate}]} = 0.623$$

The purified enzyme exhibits an absolute requirement for bivalent ions. Mg^{2+} and Mn^{2+} ions yield the highest activity, but Ca^{2+} and Co^{2+} ions can partly substitute (59, 511, 536, 565). The enzyme differs from ornithine carbamoyltransferase (carbamoylphosphate:L-ornithine carbamoyltransferase [EC 2.1.3.3]) (59) and is inactive with carbamoyl derivates of a number of amino acid tested and with *N*-formyloxalurate, acetylurea, formylurea, and biuret. Moreover, acetyl phosphate cannot replace carbamoyl phosphate (511, 565).

If tested in the presence of phosphate, equimolar amounts of oxalurate and phosphate are needed (536). Replacement of phosphate by arsenate increases the reaction rate, and ammonia and CO_2 are formed instead of carbamoyl phosphate (530, 535, 538). As a consequence, arsenate is not used in stoichiometric amounts (538).

The formation of carbamoyl phosphate was demonstrated by coupling the oxamate transcarbamoylase reaction to two transferases. (i) Other carbamoyltransferases, such as ornithine carbamoyltransferase (present in the cell-free extracts of *S. allantoicus* [530, 535, 538, 564] and *P. rettgeri* [564, 565]), can be used in this way, but the carbamoyl group can also be transferred to glycine or citrulline by enzymes present in both organisms (565). (ii) ATP:carbamate phosphotransferase (EC 2.7.2.2), present in *S. allantoicus* (531, 535, 539) and *P. rettgeri* (564, 565) can also be used.

The latter enzyme catalyzes the following reaction: $NH_2{-}CO{\sim}P + ADP \rightleftarrows NH_3 + CO_2 + ATP$. The enzyme is stimulated by Mg^{2+} and Mn^{2+} ions (242, 565) and furnishes energy for cell growth during allantoin degradation (536). Cell-free extracts of *P. rettgeri* contain also a hydrolytic enzyme which degrades carbamoyl phosphate in the absence of adenosine 5'-diphosphate (ADP) (565). A similar enzyme was found in *Clostridium uracilicum* by Campbell (92). The reaction sequence involved in ATP formation from oxalurate resembles the phosphorolytic cleavage of citrulline described by Jones et al. (243) for *Streptococcus faecalis*. Valentine and Wolfe (538) discussed a phosphorylytic cleavage of urea by *S. allantoicus*. Such cleavage could be brought about by the combined action of ureidoglycolase, ureidoglycolate dehydrogenase, oxamate transcarbamoylase, and enzymes hydrolyzing oxamate to oxa-

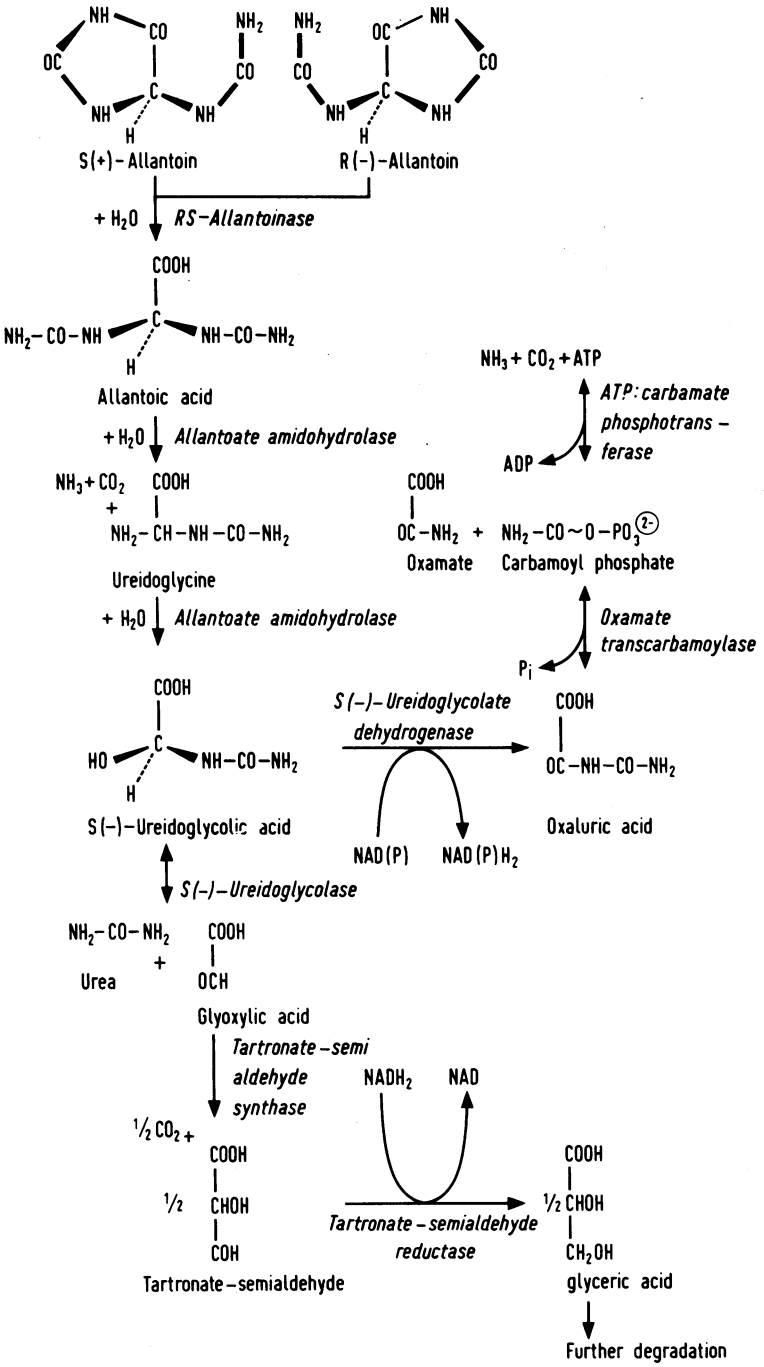


FIG. 8. *Degradation of allantoin by Streptococcus allantoicus and Enterobacteriaceae.*

late and converting the latter to glyoxylate. However, the two latter enzymes are not yet found in *S. allantoicus*. Moreover, urea appears to be a final product, and its apparent degradation in the urease-negative *S. allantoicus*, *P. rettgeri*, and *E. coli* can be explained by the presence of allantoate amidohydrolase instead of allantoicase.

Concluding remarks. The reactions involved in the anaerobic degradation of allantoin by *S.*

allantoicus and *Enterobacteriaceae* are given schematically in Fig. 9. The expected fermentation balance, accounting for the reduction equivalents but not for the products of further degradation of pyruvate, is represented: 100 allantoin + 33 P_i + 33 ADP + 266 H_2O → 33 oxamate (45) + 33 ATP + 66 urea (62) + 166 CO_2 (168) + 233 NH_3 (226) + 33 pyruvate (15 acetate, 15 lactate, 9 formate, 14 unknown). The values found by Barker (28) are given within the parentheses; they are very close to the expected values.

In the presence of oxygen, $NADH_2$ is oxidized by $NADH_2$ oxidase present in the cells of *S. allantoicus* and *P. rettgeri* (565). As a consequence, the amount of oxamate rises and the amount of urea drops, which results in a strong inhibition of the growth of these organisms since no carbon becomes available to the cells. Thus, the anaerobic character of the degradation of allantoin by these organisms can be explained on the basis of the reactions involved in the catabolism.

Substantial amounts of glycine are formed during the degradation of allantoate by proliferating cells, resting cell suspensions, and cell-free extracts of *S. allantoicus* but not of *P. rettgeri*. Glycine may result from an enzymatic or nonenzymatic transamination involving ureidoglycine and glyoxylate (545, 565).

The enzymatic system responsible for the catabolism of allantoin is inducible and only present in cells of *S. allantoicus* grown on allantoin (31). Cells grown on glucose contain no oxamate transcarbamoylase (59), no ureidoglycolase, and only trace amounts of tartronate-semialdehyde synthase (183). Tigier and Grisolia (511) demonstrated that oxamate transcarbamoylase is induced in group D streptococci by parabanate but only slightly by allantoin. Since these authors used autoclaved parabanate, it is highly probable that the enzyme was induced by oxalurate.

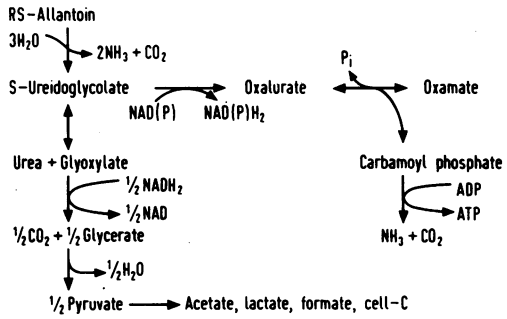


FIG. 9. *Schematic outline of allantoin degradation by Streptococcus allantoicus and Enterobacteriaceae.*

Purine Degradation by Other Streptococci

Among the streptococci only strains of *S. faecalis* exhibit activity against degradation products of purines. *S. agalactiae, S. lactis,* and a β-hemolytic streptococcus did not grow in a medium with uric acid as a sole nitrogen source (433). *S. hemolyticus* cannot decompose allantoin present in nutrient broth (613).

Mead (334) tested a number of strains of *S. faecalis, S. faecium,* and *S. durans* for their ability to degrade uric acid present in solid media. Positive results were only obtained with *S. faecalis*. When incubated in air, a few strains of *S. faecalis* were positive, although Young and Hawkins (613) found no degradation, but under anaerobic conditions a large majority of the strains decomposed uric acid. Highly active strains were found among the isolates from chicken cecal samples and samples of human feces. None of the strains was able to produce ammonia from adenine, guanine, or xanthine (334), but Dikstein et al. (139) presented evidence for xanthine degradation by *S. faecalis*.

These results taken in combination with the studies of Tigier and Grisolia (511) on the presence of oxamate transcarbamoylase in group D streptococci indicate that further studies are needed on uric acid and allantoin degradation by *S. faecalis*. The unique and similar routes followed by *S. allantoicus,* which may be ecologically related to *S. faecalis* and *Enterobacteriaceae,* should be considered against the background of possible gene transfer among these enteric organisms.

ANAEROBIC DEGRADATION OF PURINES

Clostridium acidiurici and *C. cylindrosporum*

Liebert, working in Beijerinck's institute at Delft, isolated in 1909 a sporeforming, obligatively anaerobic bacterium that decomposed uric acid only under anaerobic conditions. This organism, *C. acidiurici* (*Bacillus acidi-urici*), breaks down uric acid to CO_2, acetic acid, and ammonia. Glycine could not be detected as a product, but Liebert suggested that it is converted to acetate (314). Uricase is not involved in this degradation, since allantoin and allantoate are not converted to ammonia or CO_2 by cell suspensions (32) or cell-free extracts of *C. acidiurici* (409), nor are allantoin or urea attacked in the presence or absence of urate (32).

In 1941, Barker and Beck (32, 33) observed

that bacteria capable of decomposing uric acid under anaerobic conditions are widely distributed in soils, and they isolated two organisms from soil. Both organisms, *C. acidiurici* and *C. cylindrosporum*, grow in media containing certain purines (as the principal source of carbon and nitrogen) and small amounts of yeast autolysate. The organisms were initially differentiated on a morphological basis, but further studies also revealed differences in the fermentation products. *C. cylindrosporum* forms glycine as a product of purine fermentation, whereas this amino acid could not be detected in fermentation liquors of *C. acidiurici* (32). Considerable evidence was obtained, which implicated glycine as an intermediate in the fermentation of purines by *C. acidiurici* (32). Furthermore, *C. cylindrosporum* forms 1.0 mol of formic acid and 0.4 mol of acetic acid per mol of guanine fermented, whereas *C. acidiurici* forms 1.0 mol of acetic acid and only 0.2 mol of formic acid (397). A typical fermentation balance for *C. cylindrosporum* is given in Table 10. Growth of this organism is more rapid on uric acid than on guanine, but similar products are formed. However, the yields of formic acid and glycine from uric acid are only half those from guanine (397). *C. acidiurici* grows vigorously in media containing uric acid but develops poorly or not at all in media containing complex nitrogenous material such as tryptone or yeast extract in the absence of added purines. The ability of this organism to attack nitrogenous compounds is very restricted (32).

Degradation of purines. Cell-free extracts of *C. cylindrosporum* convert guanosine and inosine into guanine and hypoxanthine but only in the presence of phosphate (Fig. 10). Simultaneously, ribose-1-phosphate is formed, but no reaction was detected with adenosine, xanthosine, and uric acid riboside. These results suggest the presence of a rather specific purine-nucleoside phosphorylase (purine-nucleoside:orthophosphate ribosyl-transferase [EC 2.4.2.1]) in this organism (398). Washed cells of *C. cylindrosporum* grown on uric acid form CO_2 from guanosine but not from inosine and xanthosine (398). The nucleosides are poor growth substrates (if used at all) for both clostridia (32, 33, 398).

TABLE 10. *Typical fermentation balances of C. cylindrosporum and C. acidiurici*

Product	*C. cylindrosporum*		*C. acidiurici*
	Cells growing on guanine (mol/100 mol of substrate)	Cell-free extract and xanthine (mol/100 mol of substrate)	Cell suspensions and formiminoglycine (mol/100 mol of substrate)
NH_3	480	240	200
CO_2	280	160	92
Glycine	33	71	
Formic acid	99	97	
Acetic acid	38		112
Reference	(397)	(398)	(400, 404, 408)

Guanine is converted to xanthine by guanine deaminase (guanine aminohydrolase [EC 3.5.4.3]) present in *C. cylindrosporum* (398) and is decomposed by growing cultures of both clostridia (32, 33, 398). Adenine (32, 33, 398) and various guanine isomers (398) are not used.

Hypoxanthine is attacked by growing cultures and cell suspensions of *C. acidiurici* after adaptation (32) but less readily by *C. cylindrosporum* (398). This could be explained on the basis that hypoxanthine is less readily attacked by xanthine dehydrogenase (66, 398), but the lack of oxidizing equivalents formed in the fermentation may, as well, render hypoxanthine (398) and purine (32) as poor substrates for the organism. Hypoxanthine is converted to 6,8-dihydroxypurine by xanthine dehydrogenase of *C. cylindrosporum* (66). 6,8-Dihydroxypurine is oxidized by xanthine dehydrogenase to uric acid, which in turn is reduced to xanthine (66, 398). The coupling of these reactions does not result in a net requirement for reducing or oxidizing equivalents, and this may explain why 6,8-dihydroxypurine is decomposed by washed cell suspensions at a higher rate than either uric acid or hypoxanthine (398).

Uric acid and xanthine are readily decomposed by growing cultures of both clostridia (32, 33, 398). Uric acid is utilized much more rapidly by *C. acidiurici* (32), whereas cells of *C. cylindrosporum* use xanthine better than uric acid (398). These results indicate that the former organism is able to produce the reducing equivalents needed for uric acid conversion more readily and may even produce these equivalents superfluously.

The conversion of uric acid to xanthine is reversible (66, 398). In cell-free extracts urate decomposition is quickly and totally inhibited by oxygen (409), but cell-free extracts and dried cell preparations decompose xanthine both in the presence and absence of oxygen. Methylene blue is used as an electron acceptor and reduced benzyl viologen is used as an electron donor in the xanthine dehydrogenase reaction (398). The natural electron donor in uric acid degradation is reduced ferredoxin. Its role will be discussed below.

Conversion of xanthine to formiminogly-

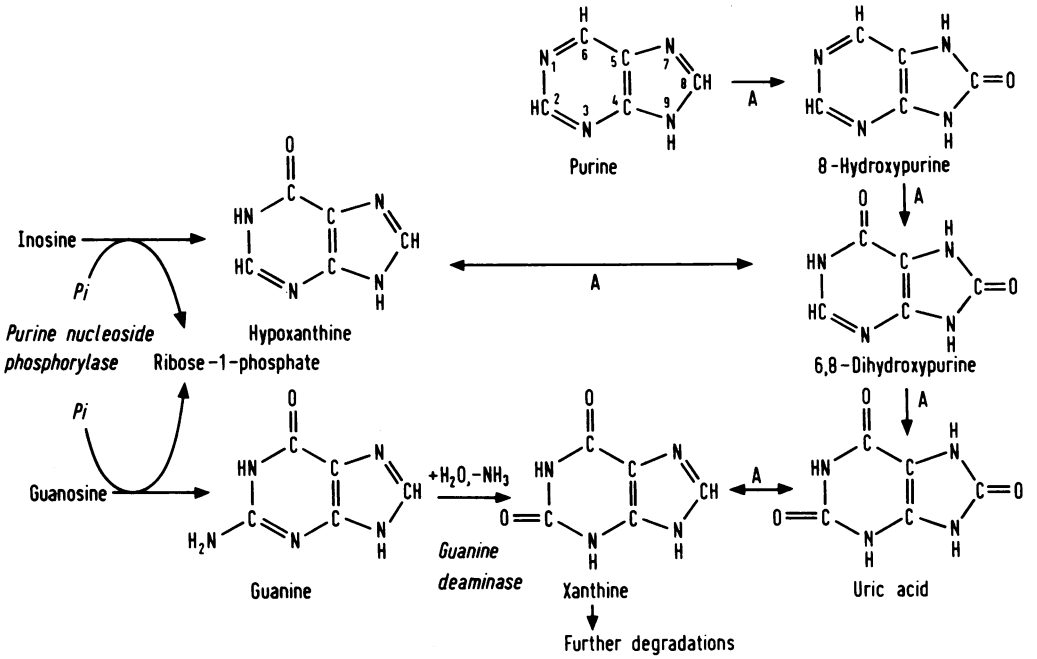


FIG. 10. *Conversion of purines to xanthine by Clostridium cylindrosporum. (A) refers to reactions catalyzed by xanthine dehydrogenase (66).*

cine. The degradative pathway of xanthine was resolved by two methods, i.e., studies with labeled xanthine, uric acid, formiminoglycine, glycine, and CO_2 and studies on the successive reactions.

The results of the experiments with labeled compounds were obtained with growing cells as well as with washed cell suspensions and are summarized in Fig. 11. Besides the main routes indicated in this figure, an extensive conversion of CO_2 to formic acid, acetic acid, and glycine was observed in studies with *C. cylindrosporum* (397). The methyl group of acetic acid contained 5.2 times the activity of the carboxyl group (397, 466). In contrast, Karlsson and Barker (259) and recently Schulman et al. (466) reported only 1.5 times more labeling in the methyl group in studies with *C. acidiurici*. These different results will be discussed later in this section.

Radin and Barker (409) observed that during the degradation of purines by *C. acidiurici* substances were formed that reacted positively in the Pauly test. Therefore, the initial split in the purines occurs in the pyrimidine ring to yield imidazole derivatives. Cell-free extracts of both clostridia degrade xanthine to the same products as growing cultures, but acetate is not formed (397, 409). Results of a typical experiment with cell-free extracts of *C. cylindrospo-*

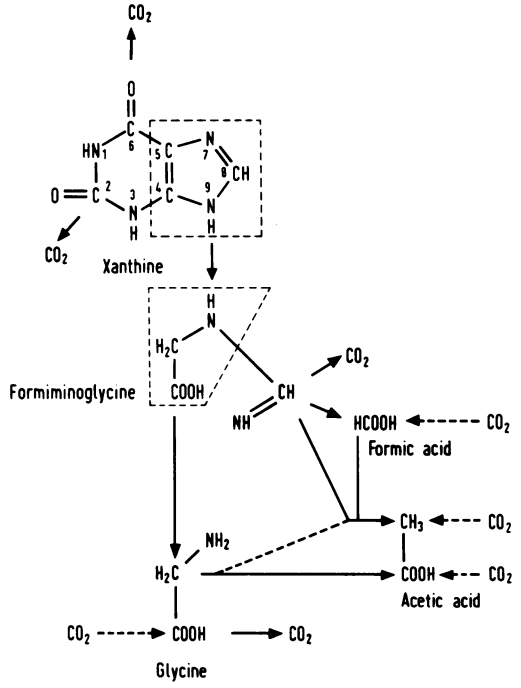


FIG. 11. *Degradation of purines by Clostridium cylindrosporum (34, 397) and C. acidiurici (259, 397, 408, 446). Main (solid lines) and secondary (dashed lines) routes of labeled carbon atoms.*

rum are presented in Table 10.

The successive reactions of the degradation of xanthine to formiminoglycine were studied with cell-free extracts of *C. cylindrosporum* (Fig. 12). However, the quantitative and qualitative similarity of the products formed and the studies with labeled compounds revealed strong evidence that the degradative routes are quite similar for both clostridia. Crude extracts convert xanthine quantitatively to 4-ureido-5-imidazolecarboxylic acid in the presence of sequestering agents, i.e., ethylenediaminetetraacetate, α,α'-dipyridyl, or potassium cyanide, since Mn^{2+}, Fe^{2+}, or, to a less extent, Mg^{2+} ions are required in the subsequent reaction (403). 4-Ureido-5-imidazolecarboxylic acid was isolated from the incubation mixture and was found to be identical to the authentic substance synthesized by the authors (396, 403). Rabinowitz and Pricer (403) pointed out the similarity of this reaction to those involved in the conversion of

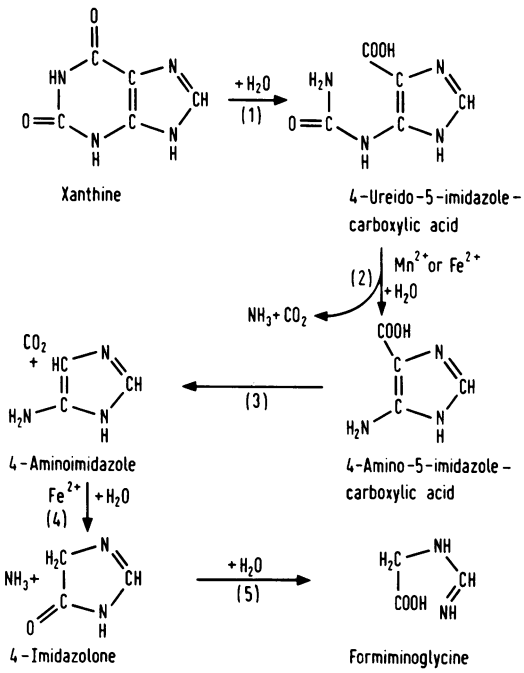


FIG. 12. *Conversion of xanthine to formiminoglycine by C. cylindrosporum and C. acidiurici. Enzymes involved: (1) xanthinase (xanthine amidohydrolase [EC 3.5.2.-]); (2) 4-ureido-5-imidazolecarboxylate amidohydrolase (EC 3.5.1.-); (3) 4-amino-imidazolecarboxylate decarboxylase (4-amino-5-imidazolecarboxylate carboxy-lyase [EC 4.1.1.-]; (4) 4-aminoimidazole deaminase (4-aminoimidazole aminohydrolase [EC 3.5.4.8]); (5) 4-imidazolonase (4-imidazolone amidohydrolase [EC 3.5.2.-]).*

allantoin, barbituric acid, and dihydroorotic acid to allantoic acid, ureidomalonic acid, and carbamoyl aspartic acid, respectively. These reactions will be discussed elsewhere in this review.

The subsequent intermediate, 4-amino-5-imidazolecarboxylic acid, accumulates when xanthine (393) or 4-ureido-5-imidazolecarboxylic acid (403) are degraded by extracts of *C. cylindrosporum* above pH 8.5. 4-Amino-5-imidazolecarboxamide is not degraded by the extracts (259, 403), nor does it accumulate under conditions favorable for xanthine decomposition (30).

The enzymatic conversion of 4-ureido-5-imidazolecarboxylic acid resembles the degradation of allantoate by allantoate amidohydrolase and the oxalurate degradation by oxamate transcarbamoylase. These enzymes are operative in *Streptococcus allantoicus* and *Enterobacteriaceae* and deliver ammonia and CO_2, and carbamoyl phosphate, respectively. No studies are available to establish whether carbamoyl phosphate is also produced from 4-ureido-5-imidazolecarboxylic acid, which would render the clostridia an extra source of energy as supposed by Barker (30).

The decarboxylation of 4-amino-5-imidazolecarboxylic acid to 4-aminoimidazole was demonstrated by Rabinowitz (393) who incubated the substrate with crude extracts in the presence of ethylenediaminetetraacetate. Both 4-amino-5-imidazolecarboxylic acid and 4-aminoimidazole formed in the latter two reactions were isolated and found identical to the compounds produced by catalytic reduction of the corresponding nitroderivatives (393).

Crude extracts convert 4-aminoimidazole to ammonia and formiminoglycine (404). The reaction is a two-step one, and 4-imidazolone was identified as an intermediate (174). The first enzyme involved in the degradation of 4-aminoimidazole, 4-aminoimidazole aminohydrolase (EC 3.5.4.8), was purified by Rabinowitz and Pricer (404) and requires Fe^{2+} ions for full activity. Co^{2+}, Mn^{2+}, or Ni^{2+} ions can replace Fe^{2+} ions to some extent, but the former two reduce the activity observed in the presence of Fe^{2+} ions. The enzyme was activated and stabilized by the presence of cysteine and various other reducing agents (404). 4-Imidazolone is easily transformed nonenzymatically into formiminoglycine (174) and into glycine under acid and alkaline conditions (404). An enzyme catalyzing the conversion of 4-imidazolone to formiminoglycine was found to be present in *C. cylindrosporum* (174).

Similar splits of imidazole rings to formimino derivatives were observed in the conversion of

imidazole via imidazolone to formiminoglycine by a pseudomonad, ATCC 23438, which is capable of growing on imidazole as a sole source of carbon and nitrogen (372), in the conversion of urocanic acid to L-4-imidazolone-5-propionate by urocanase (4-imidazolone-5-propionate hydrolyase [EC 4.2.1.49]), and in the conversion of the latter to *N*-formiminoglutamate by imidazolonepropionase (4-imidazolone-5-propionate amidohydrolase [EC 3.5.2.7]) (255, 413, 423). Formiminoglutamate may be converted in animal liver to glutamate by the transfer of the formimino group to tetrahydrofolic acid (493) or may be hydrolyzed to ammonia and *N*-formyl-L-glutamate, which yields glutamate and formate in a pseudomonad (595) and in *Pseudomonas fluorescens* (325, 492), or may be split to glutamate and formamide in *Aerobacter aerogenes* (323, 325, 423), *Salmonella typhimurium* (335), and *Bacillus subtilis* (255). Formamide cannot be metabolized further and accumulates in histidine-containing cultures of *A. aerogenes* (364); it also accumulates as a product of histidine fermentation by *Clostridium tetanomorphum* (576).

Utilization of formiminoglycine and glycine by whole cells. Formiminoglycine is not used for growth by either *C. cylindrosporum* or *C. acidiurici* (404, 408). It is converted to glycine, when incubated with washed cell suspensions of *C. cylindrosporum*, or to acetic acid, CO_2 and ammonia (Table 10) when incubated with washed cell suspensions of *C. acidiurici* (400, 404, 408). Cell suspensions of *C. acidiurici* decompose formiminoglycine, but glycine is not decomposed under comparable conditions, nor is the rate of formiminoglycine conversion affected by the addition of glycine. However, glycine is completely utilized if formiminoglycine is also present in the incubation mixture. Up to 3.7 mol of glycine could be utilized per mol of formiminoglycine degraded. A number of other formimino derivatives or one-carbon compounds, including formate and formaldehyde, were not able to replace formiminoglycine in its stimulating role in the utilization of glycine (400, 408). These results indicate that the formimino group derived from formiminoglycine plays an essential part in the degradation of glycine.

Glycine stimulates urate degradation by cell suspensions of *C. acidiurici*, probably because it serves as a reducing agent (409). Glycine is degraded by growing cultures in the presence of the fermentable substrates, uric acid, xanthine, and guanine. Approximately 1.5 mol of glycine was degraded per mol of uric acid utilized (32).

Conversion of formiminoglycine. Formiminoglycine is converted to glycine and (−)-5-formimino-tetrahydrofolate (THFA) (Fig. 13) by glycine formiminotransferase, which was first demonstrated in extracts of *C. cylindrosporum* and was partially purified from that source (402, 405, 523). 5-Formimino-THFA is labile at 37 C in aqueous solutions with a half-life time of 60 min and can be determined by

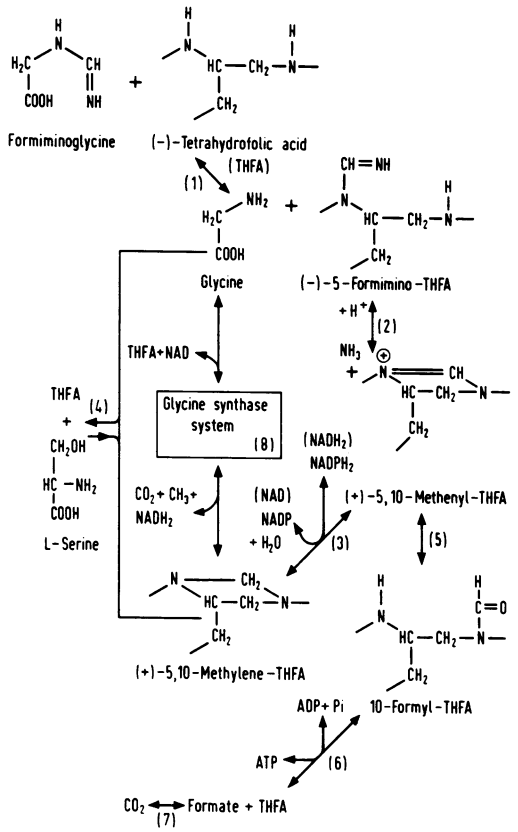


FIG. 13. *Role of tetrahydrofolic acid (THFA) derivatives in the degradation of formiminoglycine by C. acidiurici and C. cylindrosporum. Enzymes involved: (1) glycine formiminotransferase (5-formimino-THFA:glycine N-formiminotransferase [EC 2.1.2.4]); (2) Formimino-THFA cyclodeaminase (5-formimino-THFA ammonia-lyase [cyclizing] [EC 4.3.1.4]); (3) methylene-THFA dehydrogenase (5,10-methylene-THFA: NADP⁺ oxidoreductase [EC 1.5.1.5]); (4) serine hydroxymethyltransferase (5,10-methylene-THFA: glycine hydroxymethyltransferase [EC 2.1.2.1]); (5) methenyl-THFA cyclohydrolase (5,10-methenyl-THFA-5-hydrolase [decyclizing] [EC 3.5.4.9]); (6) formyl-THFA synthetase (formate:THFA ligase [ADP-forming] [EC 6.3.4.3]); (7) formate dehydrogenase; (8) glycine synthase (5,10-methylene-THFA:ammonia hydroxymethyltransferase [carboxylating, reducing] [EC 2.1.2.10]).*

heating the solution for a short period at 100 C in acidic medium. 5,10-Methenyl-THFA is formed and can be determined by measurement of the absorbancy at 350 nm (523).

The reaction catalyzed by the enzyme is readily reversible with an apparent equilibrium constant (523):

$$K = \frac{[\text{formimino-THFA}]\,[\text{glycine}]}{[\text{THFA}]\,[\text{formiminoglycine}]} = 0.32$$

Formiminoaspartate, formiminoglutamate, and *N*-formylglycine are inactive as substrates, but formiminoalanine and the methyl ester of formiminoglycine were active in one of the assays of the enzyme. Zn^{2+} and Fe^{2+} ions inhibit glycine formiminotransferase strongly (523).

Formimino-THFA cyclodeaminase catalyzes a reversible (524) reaction in which formimino-THFA is converted to (+)-5,10-methenyl-THFA and ammonia. The enzyme is present in both clostridia and was first demonstrated in these organisms by Rabinowitz and Pricer (405). Uyeda and Rabinowitz (524) purified the enzyme from *C. cylindrosporum* 40-fold, but it still contained 5,10-methenyl-THFA cyclohydrolase activity. The two enzyme activities could not be separated, which suggests that they are associated with one protein molecule. However, the formimino-THFA cyclodeaminase in cell-free extracts of *C. acidiurici* is accompanied by only small amounts of 5,10-methenyl-THFA cyclohydrolase activity (524).

(+)-5,10-Methenyl-THFA can be used in two ways. It can be reduced to 5,10-methylene-THFA and, thus, provides the condensing single-carbon moiety for the synthesis of serine from glycine, or it can be hydrolyzed to 10-formyl-THFA, subsequently yielding ATP for cell growth.

5,10-Methenyl-THFA cyclohydrolase catalyzes a reversible reaction that also proceeds rather rapidly in the absence of the enzyme under neutral and alkaline conditions (119, 394, 412, 525). The equilibrium constant

$$K = \frac{[\text{10-formyl-THFA}]\,[H^+]}{[\text{5,10-methenyl-THFA}]\,[H_2O]} = 2.4 \times 10^{-8}$$

indicates that the extents of hydrolysis at equilibrium are 40, 93, and 97% at pH 5.7, 7.0, and 7.4, respectively (493).

10-Formyl-THFA is degraded to formate, THFA, and ATP by formyl-THFA synthetase. This reaction constitutes one of the main sources of ATP in the clostridia (118). Rabinowitz and Pricer (407) calculated that this enzyme constitutes approximately 2 and 3% of the dry weight of *C. acidiurici* and *C. cylindrosporum*, respectively. The equilibrium of the reaction favors ATP utilization, but the activity of the enzyme in the direction of ATP synthesis can be demonstrated by trapping ATP in the presence of hexokinase (401). Under appropriate conditions, the rate of ATP formation by the enzyme of *C. cylindrosporum* is only eightfold lower than the rate of 10-formyl-THFA synthesis (118). The enzymes of both clostridia were purified, and the enzyme of *C. cylindrosporum* was obtained in crystalline form (407).

The enzymes of both bacteria are composed of four identical subunits (324, 469, 585) which reversibly dissociate at pH values below 7 (84, 585) or in the absence of monovalent cations (324, 585) and irreversibly at pH values above 11 (585). The monomeric units are catalytically inactive (324). The reassociation of the monomers is promoted by ATP, ADP, and to a less extent by AMP (324). One nucleotide binding site is present per monomer. This binding site is not altered by dissociation of the tetramer. The intact folate binding sites are created by the association of the monomers and render the enzyme catalytically active (120).

The catalytic mechanism of the enzyme was extensively studied by Buttlaire et al. (85), Joyce and Himes (244, 245), and Rabinowitz and Himes (394, 399). The enzyme requires Mg^{2+} ions or other bivalent cations for full activity, NH_4^+ ions or other monovalent ions for tetramer formation and possibly for catalytic activity, and a reducing agent, e.g., mercaptoethanol (84, 120, 223, 406, 585). It can be used for quantitative determination of formic acid (406).

(+)-5,10-Methylene-THFA dehydrogenase occurs in the extracts of both clostridia. The enzyme from *C. cylindrosporum* was purified 80-fold and is completely specific for $NADPH_2$ (525) as most microbial 5,10-methylene-THFA dehydrogenases, except that of *C. formicoaceticum*, which is completely specific for $NADH_2$. The enzyme of *C. acidiurici* is active with both $NADH_2$ and $NADPH_2$ (346). Uyeda and Rabinowitz (525) studied the reaction in the reverse direction and demonstrated that the product formed was 5,10-methenyl-THFA and not 10-formyl-THFA. The enzymatic dehydrogenation results in the formation of an equilibrium with an apparent equilibrium constant (525):

$$K = \frac{[\text{(+)-5,10-methenyl-THFA}]\,[NADPH_2]}{[\text{(+)-5,10-methylene-THFA}]\,[NADP]} = 0.14$$

ATP inhibits the enzyme of both clostridia (525) and may play an important role in the regula-

tory mechanism as suggested for the enzyme from a strain of *Salmonella* (123).

Serine hydroxymethyltransferase catalyzes the conversion of glycine and (+)-5,10-methylene-THFA to L-serine and (−)-THFA. The enzyme is present in both clostridia, and its activity depends on the presence of catalytic amounts of pyridoxal phosphate (526).

Specificity of the THFA derivatives. Three aspects of the specificity of the THFA derivatives (Fig. 14) will be discussed below: (i) the amount of glutamate residues present, (ii) the amino acid present, and (iii) the optical specificity of the coenzyme.

The purine-fermenting clostridia contain unusually high concentrations of folate coenzymes. The in vivo concentration of folate coenzymes in *C. acidiurici* was estimated to be 1 to 2 mM (119). Although Wright (606, 607) separated the folate derivatives from extracts of *C. cylindrosporum* into five groups containing different amounts of glutamate and phosphate, more recent studies indicate that the coenzyme in *C. cylindrosporum* and *C. acidiurici* occurs exclusively in the form of triglutamate derivatives of pteroic acid, presumably linked to each other by γ-linkages of L-glutamate (399, 525). Curthoys et al. (119) described a procedure to prepare (+)-5,10-methenyltetrahydropteroyltriglutamate (γ-linkage) from *C. acidiurici*. A total of 25 mg of this stable derivative was obtained per 100 g of wet cells. Out of this compound they prepared the natural isomer of tetrahydropteroyltriglutamate and its 10-formyl and 5,10-methylene derivatives. Besides the natural tetrahydropteroyltriglutamate derivatives, a number of other tetrahydropteroylderivatives can be used by the enzymes of clostridia (Table 11). However, the K_m values measured for the analogues differ substantially. The K_m values of the naturally occurring tetrahydropteroyltriglutamate coenzymes are considerably lower than those of tetrahydropteroate coenzymes for glycine formiminotransferase (523) and methylene-THFA dehydrogenase (525) and approximately 80-fold lower than that of the THFA coenzyme for formyl-THFA synthetase (32, 118, 223). The K_m value of the natural coenzyme is about half of that of THFA for serine hydroxymethyltransferase (526). In spite of these differences, the observed V_{max} values of these enzymes were equal or only twofold higher for the naturally occurring coenzyme as compared with the above-mentioned analogues (223, 523, 525). These results suggest that the additional glutamyl residues of the natural coenzyme are not involved in the enzymatic reaction mechanism. The folate binding sites of the enzymes appear to contain noncatalytic subsites that bind the additional glutamyl residues and increase the affinity for the folate coenzymes significantly (118).

All folate derivatives contain L-glutamate, but two optical antipodes can be distinguished on the basis of the configuration at the C6 atom

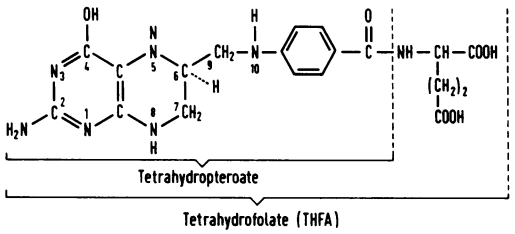


FIG. 14. *Structure of tetrahydropteroate and THFA.*

TABLE 11. *Coenzyme specificity of the enzymes involved in the single carbon conversions in C. cylindrosporum*

Enzyme	Tetrahydro(TH)-folate analogue tested						Reference
	TH-pteroate	TH-folate	TH-pteroyldiglutamate	TH-pteroyltri-L-glutamate[a]	TH-pteroyl-D-glutamate	TH-pteroyl-L-aspartate	
Glycine formiminotransferase	+	+	+	+	+	+	523
Formimino-THFA cyclodeaminase	+	+		+	+	+	524
Methylene-THFA dehydrogenase	+	+		+	ND[b]	ND	525
Serine hydroxymethyltransferase	+	+		+	ND	ND	526
Formyl-THFA synthetase	+	+		+[c]	+	+	118, 223

[a] γ-linkage; the naturally occurring coenzyme of *C. acidiurici* and *C. cylindrosporum*.
[b] ND, Not determined.
[c] TH-pteroyltri-α-glutamate is almost completely devoid of activity.

of the tetrahydrofolate derivatives. The optical rotation of the naturally occurring family of antipodes is given in Table 12. Only this family of isomers is active in the enzyme systems (119).

Degradation of serine. The studies described in this section were performed with *C. acidiurici*. As given in Table 10, acetate is also produced, albeit in lower amounts, by *C. cylindrosporum*, but studies on the mechanism of the acetate production in this organism are scarce.

The activities of the enzymes involved in the degradation of serine are considerably higher in crude extracts of *C. acidiurici* than the overall rate of uric acid fermentation by whole cells, which was estimated at 14 μmol/h per mg of cells (443). Pyruvate is formed from L-serine by L-serine dehydratase (L-serine hydro-lyase [deaminating][EC 4.2.1.13]) (42, 46, 409, 445) (Fig. 15). The enzyme from *C. acidiurici* was purified 370-fold by Sagers and Carter (445). Fe^{2+} ions and sulfhydryl reagents are essential for its activity (46, 445). The spectral properties of the enzyme strongly indicate the presence of pyridoxal phosphate in the enzyme (445). D-Serine is attacked only slowly, whereas L-threonine and L-cysteine are not degraded (46, 409, 445).

Pyruvate synthase (pyruvate: ferredoxin oxidoreductase [EC 1.2.7.1]) catalyzes the reversible production of acetyl-coenzyme A (CoA) and CO_2 from pyruvate. Part of this reaction is formed by an enzymatic exchange of the carboxylate group of pyruvate with CO_2, which can be measured separately by use of labeled compounds (395, 441).

The enzyme from *C. acidiurici* was purified 50-fold by Raeburn and Rabinowitz (410). It appears to contain 0.5 to 0.8 mol of thiamine pyrophosphate, 6 mol of nonheme iron, and 3 mol of acid-labile sulfur (527) and probably no lipoic acid (65) per 240,000 daltons, the molecular mass of the enzyme (527). It does not require coenzymes of the B_{12} group, as previously supposed (395). CoA, 2-mercaptoethanol, and certain transition state metal ions are required in the CO_2-exchange activity of the enzyme from *C. acidiurici* (410). Similar results were ob-

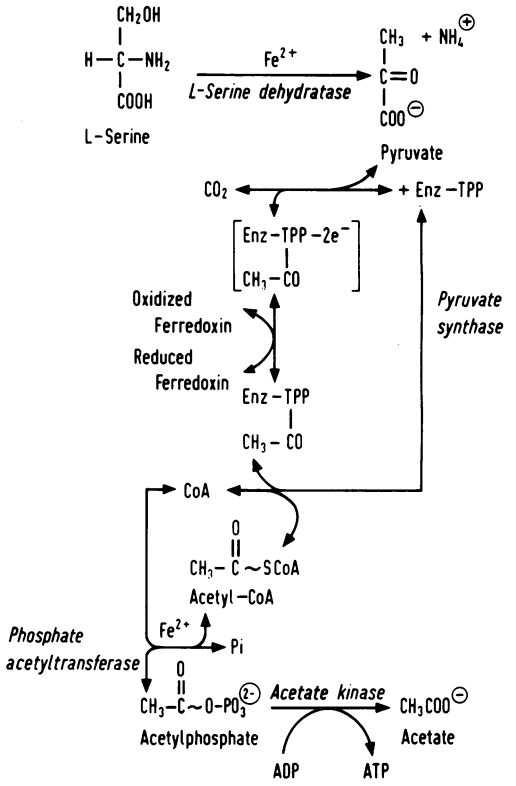


FIG. 15. *Degradation of L-serine by C. acidiurici.*

TABLE 12. *Specific optical rotations ($[\alpha]_D$) of tetrahydrofolic acid (THFA) derivatives*

Compound[a]	Natural isomer		Reference	Racemic mixture[b]		Reference
	$[\alpha]_D$	Temp (C)		$[\alpha]_D$	Temp (C)	
THFA[c]	−16.9	27	57	+14.9	27	57
5,10-Methenyl-THFA	+46 ± 3	23	57	−36	23	57
	+68	23	57			
5,10-Methylene-THFA	+163 ± 11	23.5	412			
	+142.5		611			
	+159 ± 9	27	56			
10-Formyl-THFA	−32 ± 3	23	57	−9	23	57
	−42	23	57			

[a] The natural isomer of formimino-THFA is levorotatory.
[b] With respect to the C6 atom.
[c] Measured in 0.1 N NaOH.

tained for the enzyme from *C. formicoaceticum* (13).

The reaction mechanism has been studied by Sagers et al. (443) and Uyeda and Rabinowitz (528). Thauer et al. (507) demonstrated that CO_2 rather than HCO_3^- (or H_2CO_3) is the active species of "CO_2" utilized by the enzyme from *Clostridium pasteurianum*. The natural electron acceptor is ferredoxin (529, 532), but FAD, 2,3,5-triphenyltetrazolium, and methyl viologen may also be used by the enzyme of *C. acidiurici* (410).

Phosphate acetyltransferase (acetyl-CoA: orthophosphate acetyltransferase [EC 2.3.1.8]) transfers reversibly acetyl units between CoA and phosphate. The enzyme from *C. acidiurici* requires a divalent metal, e.g., Fe^{2+} (444). Arsenate failed to uncouple the high-energy acyl compounds, as was observed for a number of other phosphotransacetylase systems. This may indicate a high degree of specificity giving preference to phosphate over arsenate. Relatively high concentrations of arsenate inhibit both the degradation of pyruvate and the formation of acetyl phosphate (444).

Acetate kinase (ATP:acetate phosphotransferase [EC 2.7.2.1]) of *C. acidiurici* was purified eightfold by Sagers et al. (443).

Glycine synthase system. Results of tracer studies with *C. acidiurici* indicate that a substantial amount of the methyl group of acetate is derived from the C5 urate atom and from the methylene group of glycine (Fig. 11) (259, 397). Sagers and Gunsalus (446) demonstrated that the mixing of carbons during acetate generation results from a cleavage reaction of glycine to one-carbon units in this organism. This cleavage was shown to be dependent on the presence of THFA and benzyl viologen. The methylene group of glycine is incorporated into 5,10-methylene-THFA. This cleavage allows the conversion of glycine to acetate, CO_2, and ammonia in the presence of an added electron acceptor (446).

The reaction is catalyzed by the glycine synthase system (5,10-methylene-THFA:ammonia hydroxymethyltransferase [carboxylating, reducing] [EC 2.1.2.10]). The glycine synthase system is also present in *Peptococcus glycinophilus*, which converts glycine to acetate (35, 98) according to the equation: $4\ NH_2CH_2COOH + 2H_2O \rightarrow 3\ CH_3COOH + 4\ NH_3 + 2\ CO_2$. The overall process involves, besides the glycine synthase system, also a conversion of CO_2 into acetate (35), but Schulman et al. (466) found only a small labeling of acetate by CO_2.

Extensive studies have been made on the glycine synthase system of *P. glycinophilus* (22, 270–273, 447). A similar enzyme system

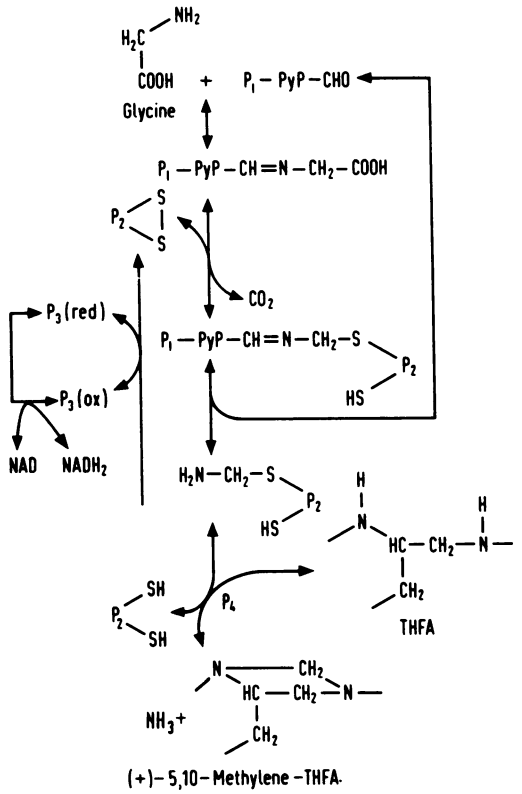


FIG. 16. *Reactions catalyzed by the glycine synthase system from Peptococcus glycinophilus (70), rat liver mitochondria (353), Arthrobacter globiformis (279), and probably also C. acidiurici (446). P_1, P_2, P_3, and P_4 are four proteins involved in the reaction (see text). P_2S_2 and $P_2(SH)_2$ represent the oxidized and reduced form of P_2. P_1-PyP-CHO represents protein P_1 with its pyridoxal phosphate prosthetic group.*

was later found in rat liver mitochondria (279, 351–353, 452, 453), in *Arthrobacter globiformis* (240, 279), and in *Escherichia coli* (383).

The reversible (272, 452) cleavage of glycine (Fig. 16) involves four proteins among which are a pyridoxal phosphate-containing enzyme, P_1 (270, 271, 351), and a colorless heat-stable protein, P_2 (270, 271), which is also called H (hydrogen carrier)-protein (279). Both proteins are involved in glycine decarboxylation and in the exchange of bicarbonate with the carboxyl group of glycine. P_2 is of low molecular weight and contains one functional disulfide group per molecule (279), which is part of a lipoic acid molecule (279, 425). As a result of these reactions, a complex is formed between P_2 and the product of glycine decarboxylation, which should be in the $-CH_2NH_2$ form. This complex was isolated by Kochi and Kikuchi using purified preparations of proteins P_1 and P_2 from *A. globiformis* (279).

Transfer of the glycine α-carbon to THFA to form 5,10-methylene-THFA and production of ammonia are catalyzed by protein P_4, which is also called T (catalyzing THFA-dependent step) protein (279, 353). Electron transfer to NAD (but not to NADP) or to a dye with a low oxidation-reduction potential is catalyzed by an FAD-containing enzyme P_3 (22, 272), which is also called L protein (279, 353) because it exhibits lipoamide dehydrogenase activity (22, 272, 279, 353). The above-mentioned complex between P_2 and the $—CH_2NH_2$ group is also formed on incubation of 5,10-methylene-THFA, NH_4Cl, P_3-protein, P_4-protein in the presence of some reducing system such as lipoamide dehydrogenase plus $NADH_2$, or dithiothreitol (279). Throughout the whole glycine cleavage process, the hydrogen atoms at the α-position are not labilized (279).

Formate dehydrogenase. Formate dehydrogenase from *C. acidiurici* was purified 30-fold by Kearny and Sagers (261). The purified enzyme catalyzes the following reaction:

$$HCOO^- + H_2O + X \rightleftarrows HCO_3^- + XH_2$$

(formate) (bicarbonate)

in which X represents a suitable electron acceptor. The purified enzyme may use benzyl viologen in the forward reaction but not other electron acceptors such as tetrazolium dyes, ferricyanide, dichloroindophenol, and methylene blue (261).

In crude extracts of *C. acidiurici*, ferredoxin is involved in the transfer of electrons from formate to NAD (69, 261, 529) and perhaps also to NADP (261). NAD is not reduced by the purified enzyme in the presence of ferredoxin and formate (261), probably due to the absence of $NADH_2$:ferredoxin oxidoreductase.

The enzymes of *C. acidiurici* (261) and *C. pasteurianum* (506) also catalyze an isotopic exchange between CO_2 and formate in the absence of ferredoxin. This exchange was also observed with whole cells of *C. acidiurici* (442).

The reverse formate dehydrogenation reaction, which expresses the CO_2-reductase activity of the enzyme, does not proceed when reduced benzyl viologen is used as the electron donor (261). $NADH_2$ and reduced ferredoxin are required for formate synthesis from CO_2 in *C. acidiurici* (503). In *Clostridium thermoaceticum* the reduction can be achieved by $NADPH_2$ (502) or reduced methyl viologen (14). The formate dehydrogenase of *C. acidiurici* (261) and *C. pasteurianum* (506) is rapidly inactivated by molecular oxygen, and light inhibits its activity (261). The production of the enzyme in growing cells of *C. formicoaceticum* is stimulated by Fe^{2+} ions, tungstate, and selenite (13). Formate dehydrogenase is probably a molybdoenzyme (504, 505).

Reduction of CO_2. Tracer studies performed with *C. acidiurici* and *C. cylindrosporum* revealed that CO_2 is reduced by these organisms and incorporated in the final products (Fig. 11). This is also evident from the fact that more than 1 mol of acetate is formed per mol of hypoxanthine by *C. acidiurici* (259). In fermentation experiments performed by Schulman and co-workers (466), *C. acidiurici* yielded 183 mol of acetate, 49 mol of butyrate, and 55 mol of formate from 100 mol of hypoxanthine and *C. cylindrosporum* yielded 168 mol of acetate, 29 mol of butyrate, and 21 mol of formate. Thirteen and 11% of acetate and 23 and 65% of formate produced by these organisms, respectively, were derived from CO_2 that was present in large excess during the fermentation. Studies with $^{13}CO_2$ and $^{14}CO_2$ performed with *C. acidiurici* revealed that 18.7% of the methyl groups and 9.3% of the carboxyl groups of acetate were derived from CO_2 and that 9.1% of the molecules were labeled in both the methyl and the carboxyl group and 9.5% only in one group.

Schulman et al. (466) assumed that CO_2 is reduced to formate, which in turn is reduced to 5,10-methylene-THFA by a reaction sequence depicted in Fig. 13. They proposed that this compound is further reduced to 5-methyl-THFA and methyl corrinoid in *C. thermoaceticum* (315). In this reaction sequence 5,10-methenyl-THFA and 5,10-methylene-THFA are common to the pathway of purine degradation and the pathway of CO_2 reduction. Thus, any methyl group of acetate that was derived from $^{13}CO_2$ and that passed through the common intermediates would have its ^{13}C concentration diluted by unlabeled carbon from the purine, and any acetate totally synthesized from CO_2 would involve dilution of the ^{13}C in the methyl group of acetate. However, the methyl group of acetate is labeled almost twofold more than the carboxyl group. Schulman et al. (466) assumed that this result may be explained by compartmentalization of the two pools of 5,10-methylene-THFA and/or 5,10-methenyl-THFA either by physical separation of the pathways or by use of different forms of THFA coenzymes. Later studies (465) demonstrated that in *C. thermoaceticum* the carboxyl of acetate was mainly derived from the carboxyl of pyruvate. If this reaction is also valid for *C. acidiurici*, this could explain the extra dilution of this group during the degradation of purines in the presence of labeled CO_2. However, such an explanation is hardly tenable if one takes into consideration the exchange of the carboxylate

group of pyruvate with CO_2, which is catalyzed by pyruvate synthase.

Schulman and co-workers overlooked another explanation of their results which does not involve the proposed (but not yet demonstrated) participation of methyl-THFA and methyl corrinoid intermediates. A total synthesis of acetate from CO_2 can be explained on the basis of a combination of the following processes: (i) synthesis of 5,10-methylene-THFA from CO_2, (ii) synthesis of glycine by a glycine synthase system, (iii) synthesis of serine from glycine and 5,10-methylene-THFA, (iv) conversion of serine to acetate. The occurrence of these reactions, which have been demonstrated in *C. acidiurici*, may also explain why the carboxyl group of acetate that originates via a longer bypass from CO_2 is less labeled than the methyl group. Glycine which is produced from the purines will introduce an additional dilution of the labeled C atom which finally delivers the carboxyl group of acetate.

Ferredoxin. The role of ferredoxin in the metabolism of *C. acidiurici*, *C. cylindrosporum*, and other organisms has been reviewed by Valentine (529). The ferredoxins of *C. acidiurici* and *C. cylindrosporum* were crystallized by Lovenberg et al. (317). They differ as to the crystal form, the absorption at 280 to 300 nm, and the amino acid composition (317). Moreover, the ferredoxin of *C. cylindrosporum* was unstable after diethylaminoethyl-cellulose purification (67, 317) and therefore is used less in metabolic studies. Both ferredoxins contain 5 mol of iron and 4 mol of inorganic sulfide per mol of protein (317).

Ferredoxin is generally found in hydrogen-evolving species, but *C. acidiurici* is an interesting exception to this rule. It possesses a high content of ferredoxin (81) but does not produce H_2 as a fermentation product (29) because of the low level or absence of hydrogenase in this organism (529, 532).

Ferredoxin is the primary electron acceptor and donor in the main redox reactions of the purine degradation (Fig. 17). The coupling of some of the reactions involving ferredoxin was shown by Valentine et al. (532), who demonstrated the ferredoxin-coupled pyruvate oxidation and urate reduction for *C. acidiurici*. The same could be demonstrated for *C. cylindrosporum* by Bradshaw and Reeder (67), who used the ferredoxin of *C. acidiurici* instead of the less stable ferredoxin from *C. cylindrosporum*. The primary electron acceptor of the glycine cleavage system has still to be determined.

Except for methylene-THFA, NAD and NADP are formed in secondary reactions. A key enzyme in these reactions is $NADH_2$:ferredoxin oxidoreductase, which was first found in *C. acidiurici*. In *C. pasteurianum* ferredoxin interacted specifically with NADP, no NAD being reduced under any of the experimental conditions (529).

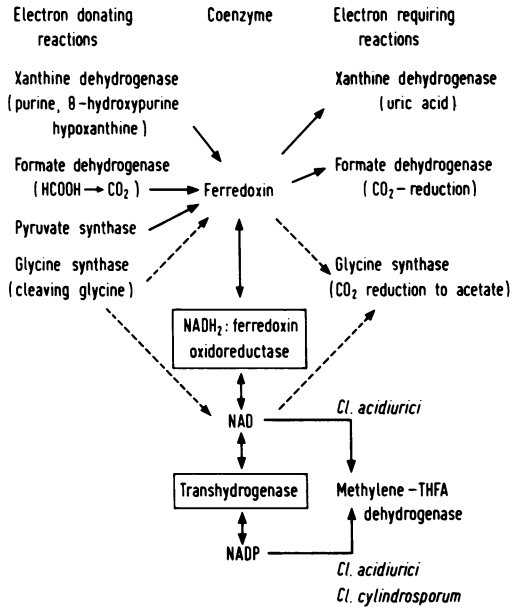


FIG. 17. *Role of ferredoxin, NAD, and NADP in the degradation of purines by C. acidiurici and C. cylindrosporum. The coenzyme involved in the glycine synthase reaction is not unequivocally established for the clostridia.*

Quantitative aspects. *C. cylindrosporum* forms relatively high amounts of formate; about 1 mol of this compound is formed per mol of xanthine or guanine degraded (Table 10). These results indicate that this organism converts the C8 urate atom preferentially to formate, which is not further degraded to CO_2. Under these conditions glycine degradation to acetate is hampered and the former compound accumulates (Fig. 18). Cell suspensions of this organism cannot convert formiminoglycine beyond the glycine level.

In contrast to *C. cylindrosporum*, *C. acidiurici* converts formiminoglycine readily to acetate (408). Probably due to the low activity of methenyl-THFA cyclohydrolase (524), methenyl-THFA can be used as an electron acceptor for the pyruvate synthase reaction, and about 1 mol of acetate is formed per mol of formiminoglycine degraded (Table 10). Accordingly, formate is only a minor product of uric acid fermentation in *C. acidiurici*, and tracer studies (259, 400) have shown that the C8 atom of the purine molecule is incorporated predominantly into the methyl carbon of acetate in this orga-

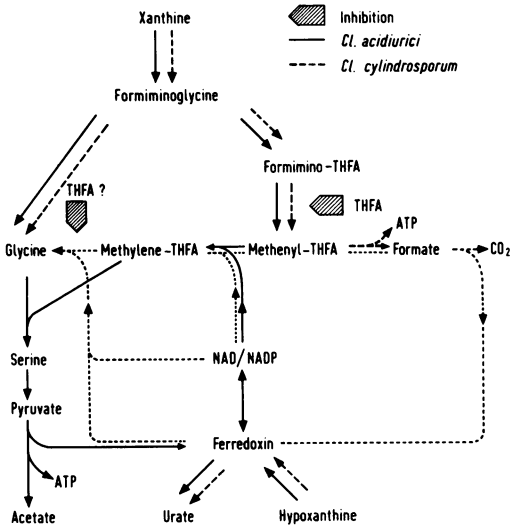


FIG. 18. *Main routes involved in purine degradation by C. acidiurici (solid lines) and C. cylindrosporum (dashed lines). The reduction equivalents needed in urate utilization or produced in the degradation of hypoxanthine are compensated by the interconversions indicated by dotted lines.*

nism. Therefore, it is unlikely that the 10-formyl-THFA synthetase reaction is a major energy-yielding step in this organism, even though the enzyme is present in very high amounts in the cells (407, 443). ATP is produced mainly by the acetate reaction in *C. acidiurici* and by the formyl-THFA synthetase pathway in *C. cylindrosporum* (Fig. 18).

If the cells grow on substrates that are more reduced (hypoxanthine, 8-hydroxypurine, and purine) or more oxidized (uric acid) than xanthine, the fermentation balance shifts by the involvement of other electron-requiring or -donating reactions, respectively (Fig. 17). *C. acidiurici* forms 125 mol of acetate and 250 mol of CO_2 per 100 mol of hypoxanthine and 75 mol of acetate and 350 mol of CO_2 per 100 mol of urate (259). The shift in the balance in the case of hypoxanthine fermentation as compared with xanthine fermentation may be explained by the following reactions:

$$\text{hypoxanthine} + X \rightarrow \text{6,8-dihydroxypurine} + XH_2 \quad (1)$$

$$\text{6,8-dihydroxypurine} \rightarrow \text{xanthine} \quad (2)$$

$$0{,}5\ CO_2 + 0{,}25\ \text{ATP} + XH_2 \rightarrow 0{,}25\ \text{acetate} + 0{,}25\ \text{ADP} + 0{,}25\ P_i \quad (3)$$

in which X is NAD, NADP, and/or ferredoxin. Reaction (3) represents the net result of the following processes: reduction of CO_2 to methylene-THFA (reactions 3, 5, 6, and 7, Fig. 13), reduction of half of the amount of methylene-THFA to glycine (Fig. 16), synthesis of serine and conversion of serine to acetate (Fig. 15). As a result of these processes the amount of ATP formed during the degradation is reduced by 25%. The shift in the balance of urate fermentation as compared with xanthine fermentation can be explained by a process in which one-quarter of the amount of glycine formed is converted to methylene-THFA. As a consequence, half of the amount of methenyl-THFA formed is superfluous to serine synthesis and can be converted to CO_2. Thus, the reduction equivalents needed to reduce uric acid are produced and 1.25 mol of ATP are formed instead of 1 mol in the case of xanthine. The higher yield of energy may explain why uric acid is a better substrate to *C. acidiurici* than xanthine and hypoxanthine (32).

Regulatory aspects of the coenzymes. Formimino-THFA cyclodeaminase is strongly inhibited by THFA and some other folate derivatives (524). The low K_i value (4.10^{-6} M) of THFA and the high in vivo concentration of folate coenzymes in *C. acidiurici*, which was estimated to be 1 to 2 mM (119), suggest that formimino-THFA cyclodeaminase is under stringent control in vivo (Fig. 18). Perhaps THFA also controls the glycine synthase system in the clostridia, since the exchange reaction catalyzed by purified preparations of the P_1 and P_2 proteins of *A. globiformis* are markedly inhibited by THFA (279). The regulatory aspects of the coenzymes involved in the reduction and oxidation processes, i.e., NAD, NADP, and ferredoxin, should be examined further. Ferredoxin plays a major role, but one crucial reaction catalyzed by methylene-THFA dehydrogenase uses NAD or NADP (Fig. 17).

Peptococcus aerogenes

Whiteley (587) demonstrated that cell suspensions of *Peptococcus aerogenes (Micrococcus aerogenes)* which had been grown in a rich medium without added purines or pyrimidines convert guanine, adenine, xanthine, hypoxanthine, thymine, uracil, and cytosine to ammonia, H_2, CO_2, acetic acid, and lactic acid. Thymine and uracil accumulate during the degradation of purines. Uric acid and allantoin are not degraded. Two pathways are perhaps involved, the greater portion being decomposed to yield an imidazole-containing compound and a small portion of the substrate undergoing cleavage to yield pyrimidines.

Later studies revealed the presence of xanthine dehydrogenase and 2-oxypurine dehydrogenase in this organism (605). In analogy to *C. cylindrosporum* and *Veillonella alcalescens*,

the specific activity of ^{10}N-formyl-THFA synthetase is high in *P. aerogenes* (590). The pathways of purine degradation remain to be solved.

Veillonella alcalescens

V. alcalescens (Micrococcus lactilyticus), an anaerobic bacterium indigenous to saliva, decomposes certain purines. Adenine and guanine are slowly deaminated. Xanthine and hypoxanthine are poor substrates for growth of certain strains of *V. alcalescens*, and other purines are not substrates (588). Growth of *V. parvula* is stimulated by hypoxanthine (427).

The products formed by cell suspensions of *V. alcalescens* from hypoxanthine, xanthine, and uric acid are given in Table 13. The possible intermediary state of glycine and uracil was considered by Whiteley and Douglas (588), but no ammonia is formed from uracil; other pyrimidines or amino acids are not attacked. Up to this moment the reaction pathway remains unknown. A point of interest is formed by the capability of one of the tested strains to hydrolyze allantoin completely to urea and an unidentified compound, which may be glyoxylic acid (588).

Xanthine dehydrogenase of *V. alcalescens* was extensively studied by Smith et al. (482) and is discussed in a previous section. A novel electron carrier was postulated to be involved in this reaction by Whiteley and Ordal (589) and was identified as ferredoxin by Valentine et al. (534). Ferredoxin is also involved in the pyruvate synthase reaction (529, 540, 591), the conversion of α-ketoglutarate to propionyl phosphate (540), and a large number of other reductions (540, 591). *V. alcalescens* contains hydrogenase which catalyzes also the production of hydrogen from hypoxanthine (Table 13). As a result of the reversibility of the xanthine dehydrogenase-hydrogenase system, the conversion of hypoxanthine to xanthine and the decomposition of hypoxanthine are inhibited in an atmosphere of H_2. Uric acid is reduced under such conditions (588, 589). The hydrogenase-ferredoxin system is involved in H_2 production from pyruvate and α-ketoglutarate (529, 540, 591) and the reduction of pyruvate, fumarate, and NADP, but not of NAD, by molecular H_2 (591).

TABLE 13. *Fermentation products of Veillonella alcalescens strain 416 (588)*

Product	Substrate (10 μmol/reaction vessel) incubated in N_2 atmosphere with cell suspensions:		
	Hypoxanthine (μmol/vessel)	Xanthine (μmol/vessel)	Uric acid (μmol/vessel)
CO_2	19.81	15.4	6.00
H_2	8.01	0.72	1.03
Acetic acid	7.20	3.67	0
Propionic acid	2.00	1.14	0
Ammonia	20.83	18.80	2.61
Urea	9.81	1.08	
Uric acid		4.75	9.0
Xanthine	0	0	0
Hypoxanthine	0	0	0

Extracts of *V. alcalescens* incubated in an atmosphere of H_2 reduce the following compounds (listed in order of decreasing activity): 2,4-dinitrophenol (2,4-diaminophenol), selenite (Se), metabisulfite ($S_2O_3^{2-}$), hydrosulfite, hydroxylamine (NH_3), tellurite (Te), arsenate (arsenite), nitrate (NH_3), and nitrite (NH_3). The reduced products are placed in parentheses. The presence of carrier amounts of benzyl viologen stimulates most of these reductions (604). The reduction of arsenate by H_2 is catalyzed by the enzymes hydrogenase and arsenate reductase. The overall reaction is markedly stimulated by K^+ or NH_4^+ but not by Na^+ and is inhibited by phosphate and arsenite. Arsenate reduction can be coupled to hypoxanthine oxidation (604).

DEGRADATION OF PYRIMIDINES

Until the early fifties, the only reported degradation route of pyrimidines was that proposed by Cerecedo for mammals (102). This pathway involves isobarbituric acid, formyloxaluric acid, and oxaluric acid as intermediates but has not been substantiated by later studies.

The main routes involved in pyrimidine degradation by microorganisms were established in 1951 to 1954 by studies of Lara (300, 301) with *Nocardia corallina*, by Lieberman and Kornberg (310–312) with *Clostridium oroticum*, by Hayaishi and Kornberg (214, 216) with strains of *Corynebacterium* and *Mycobacterium*, by Wang and Lampen (578–580) with a gram-positive, motile nonsporulating rod, and by Di Carlo et al. (135, 137) with yeasts. These studies provided the basis for elucidation of the degradative route of pyrimidines in animals and the synthetic route of pyrimidines by bacteria.

Fink et al. (156, 157) described a reductive degradation of pyrimidines in rat liver. In essence, this route resembles that used by certain bacteria (Fig. 19). Uracil and thymine are reduced to the dihydroderivatives, which in turn are hydrolyzed to carbamoyl-β-alanine and carbamoyl-β-aminoisobutyric acid, respectively. Dihydropyrimidinase (5,6-dihydropyrimidine amido-hydrolase [EC. 3.5.2.2]) of calf liver catalyzes not only the hydrolysis of dihydrouracil and dihydrothymine but also that of hydantoin

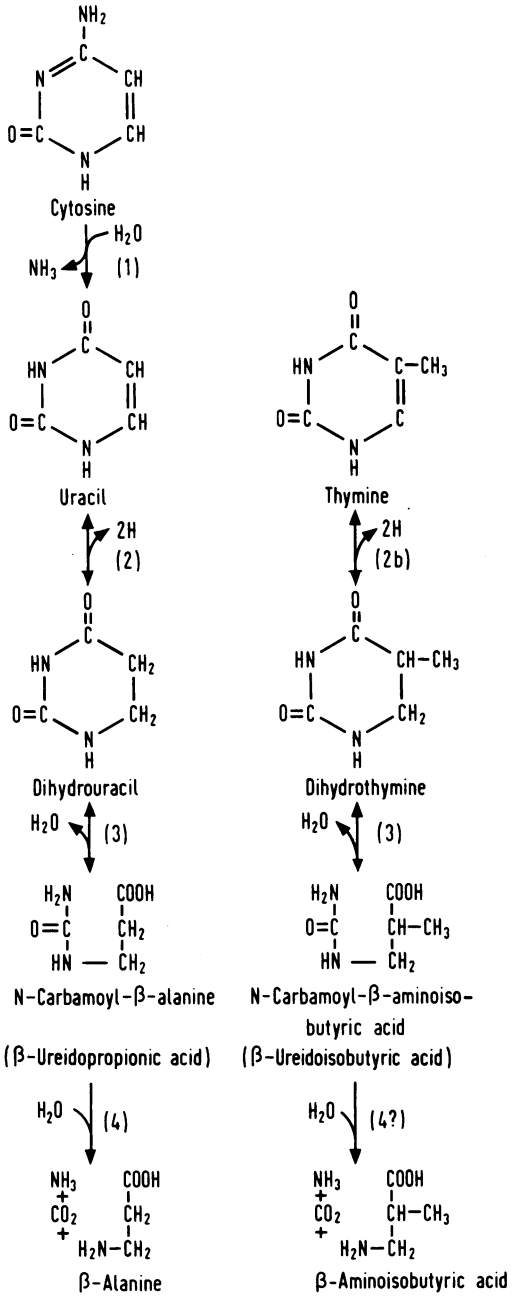


FIG. 19. *Reductive pathway of pyrimidine degradation. (1) Cytosine deaminase (cytosine aminohydrolase [EC 3.5.4.1]). Acts also on 5-methylcytosine. (2a) Dihydrouracil dehydrogenase (5,6-dihydrouracil:NAD$^+$ oxidoreductase [EC 1.3.1.1]). Active in C. uracilicum (93). (2b) Dihydrouracil dehydrogenase (NADP$^+$) (5,6-dihydrouracil: NADP$^+$ oxidoreductase [EC 1.3.1.2]). Acts also on dihydrothymine. (3) Dihydropyrimidinase (5,6-dihydropyrimidine amidohydrolase [EC 3.5.2.2]). Acts also on 5,6-dihydrouracil, 5,6-dihydrothymine, and hydantoin. (4) β-Ureidopropionase (N-carbamoyl-β-alanine amidohydrolase [EC 3.5.1.6]). The animal enzyme acts also on N-carbamoyl-β-aminoisobutyric acid.*

to *N*-carbamoyl glycine (577), of R(−)-5-phenylhydantoin to R(−)-2-phenylhydantoic acid (144), and of various R-amino acid hydantoins (100). The reductive degradation route of pyrimidines is also operative in plants (521).

The pyrimidine synthetic pathway was established by Yates and Pardee (610) and is presented in Fig. 20. A number of reactions resemble the route (Fig. 21) of orotate degradation by *C. oroticum* (310–313).

REDUCTIVE PATHWAY

A relatively small number of microorganisms (Table 14) is known to be able to degrade pyrimidines along a pathway involving the reduction of either uracil or thymine. This pathway is depicted in Fig. 19 and will be called the reductive pathway.

Neurospora crassa utilizes uracil, and to a less extent, dihydrouracil and *N*-carbamoyl-β-alanine as a source of nitrogen. Studies with mutant strains and identification of the degradation products revealed that the reductive pathway is operative in this organism (602). Thymine is oxidatively demethylated to uracil in *N. crassa* in a pathway involving 5-hydroxymethyluracil, 5-formyluracil, and uracil-5-carboxylic acid as intermediates (2, 473).

Di Carlo et al. (137) found that *Candida utilis* grows well on cytosine and uracil as a nitrogen source but not on thymine, whereas *Saccharomyces cerevisiae* grows moderately well on cytosine, but other pyrimidines are not used.

The presence of cytosine deaminase (cytosine aminohydrolase [EC 3.5.4.1]) in *S. cerevisiae* was shown 50 years ago (209), and the enzyme also degrades 5-methylcytosine to thymine (288). Di Carlo et al. (135) suggested, on the

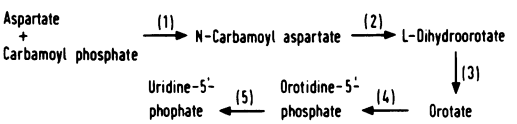


FIG. 20. *Pyrimidine biosynthetic pathway (610). (1) Aspartate carbamoyltransferase (carbamoylphosphate:L-aspartate carbamoyltransferase [EC 2.1.3.2]); (2) dihydro-orotase (L-5,6-dihydro-orotate amidohydrolase [EC 3.5.2.3]); (3) dihydro-orotate oxidase (L-5,6-dihydro-orotate:oxygen oxidoreductase [EC 1.3.3.1]); (4) orotate phosphoribosyltransferase (orotidine-5'-phosphate: pyrophosphate phosphoribosyltransferase [EC 2.4.2.10]); (5) orotidine-5'-phosphate decarboxylase (orotidine-5'-phosphate carboxy-lyase [EC 4.1.1.23]).*

TABLE 14. *Microorganisms known to degrade pyrimidines along a reductive pathway*

Pyrimidine tested	Microorganism	Reference
Uracil	*Chlorella fusca*	278
Uracil	*Neurospora crassa*	602
Uracil, cytosine	*Candida utilis*	135, 379
Uracil, cytosine, thymine	Various yeast species	302
Uracil	*Pseudomonas aeruginosa*	156
Uracil, cytosine, thymine	*P. facilis (Hydrogenomonas facilis)*	285, 286
Uracil, cytosine	*Mycobacterium* species[a]	428
Thymine	*Nocardia rubra*[a]	428
Uracil	*Clostridium uracilicum*	91-95
Uracil, cytosine, thymine	*C. sporogenes*[b]	222
Uracil	*C. botulinum* species[b]	222
Orotic acid	*C. oroticum*	310-313
Orotic acid	*Corynebacterium* species	424

[a] Evidence for the reductive pathway based only on the inability of the organisms to degrade barbiturate.
[b] The pyrimidines are reduced to the dihydroderivatives, but no evidence is available for further degradation of the latter.

basis of growth experiments, that dihydrouracil and dihydroorotic acid are intermediates in the degradation of uracil to urea by *C. utilis*, but later studies demonstrated that the reductive pathway is followed (379). Also the product of this route, β-alanine, is used as a nitrogen source by this organism (379).

The ability to use cytosine and uracil as nitrogen sources is widely distributed among yeast strains, but only a few yeasts degrade thymine (302). The degradations proceed according to the reactions given in Fig. 19 (302).

Pseudomonas aeruginosa accumulates dihydrouracil and *N*-carbamoyl-β-alanine when grown on uracil (156).

P. facilis (Hydrogenomonas facilis) grows at the expense of cytosine, uracil, thymine, 5-methylcytosine, orotic acid, and β-alanine as nitrogen sources, but barbituric acid is not used (286). *P. facilis* can use the carbon skeleton of pyrimidines since it is able to degrade β-alanine (286). Besides *C. utilis* and *P. facilis*, none of the microorganisms that degrade pyrimidines along the reductive pathway is known to use the carbon skeleton, probably due to the inability to degrade β-alanine. This compound can be degraded and used as sole organic substrate by *P. aeruginosa* (546). β-Alanine transaminase of *P. fluorescens* (217) and *Clostridium propionicum* (189) converts β-alanine into malonaldehydic acid, which may yield acetyl CoA. The enzymes involved in the pyrimidine degradation by *P. facilis* are induced by growth on uracil, but cytosine deaminase and the enzymatic system that degrades β-alanine are also present in cells grown in the presence of ammonium chloride (286).

Ammonia is formed from cytosine by *Mycobacterium smegmatis, M. vaccae, M. fortuitum*, and *M. diernhoferi* and from uracil by *M. smegmatis* and *M. chelonei (M. borstelense)* but in no case from barbituric acid (428). Probably, the reductive pathway is operative in these organisms. Ammonia is formed from thymine by *Nocardia rubra* and by one strain of *N. brasiliensis*. The latter strain attacks uracil, too. Other *Mycobacterium* and *Nocardia* species tested were inactive in the production of ammonia from pyrimidines (428).

Clostridium uracilicum was isolated by Campbell (91) from an enrichment medium containing uracil and yeast extract. Uracil is readily degraded, but it does not stimulate growth in a chemically defined medium, probably due to the inability of the organism to degrade β-alanine (91). The enzymes involved in the reductive pathway are induced by the respective substrates (92). Hilton et al. (222) tested a large number of *Clostridium* species for the ability to metabolize uracil. Only *C. sporogenes* and the proteolytic strains of *C. botulinum* types A and B convert uracil to dihydrouracil by an inducible dihydrouracil dehydrogenase, but growth of the organisms was not stimulated by uracil. Washed cells incubated in an H_2 atmosphere reduce uracil, 5-aminouracil, thymine, and isobarbituric acid to the corresponding dihydropyrimidines and cytosine to dihydrouracil (222). Thus, these cells contain several dihydropyrimidine dehydrogenases or a rather aspecific dihydrouracil dehydrogenase.

The dehydrogenases from *P. facilis* (285) and from animal liver reduce thymine, too. The enzymes of *C. sporogenes* (222), *P. facilis* (285), and plasma membranes of animal liver cells (480) are specific for NADP, but an NAD-dependent enzyme is present predominantly in the mitochondria of liver cells (480). In contrast to these results, dihydrouracil dehydrogenase of *C. uracilicum* is specific for NAD (92), and

the 27-fold purified enzyme does not react with other pyrimidines (93).

Dihydropyrimidinase of *P. facilis* hydrolyzes dihydrouracil and dihydrothymine (285). The enzyme from *C. uracilicum* needs Mg^{2+} or Mn^{2+} ions for activity and does not act on dihydrothymine (94).

β-Ureidopropionase of *C. uracilicum* was purified 100-fold and catalyzes a reaction that is essentially irreversible. Carbamoyl phosphate was not found as an intermediate (95).

Conclusively, it may be stated that the reductive pathway appears to be unattractive to most microorganisms. Some of them use the available nitrogen at the expense of one equivalent of $NADPH_2$ and leave the carbon skeleton as β-alanine for other microorganisms. *Clostridium* species are provided with an additional electron acceptor. Only *P. facilis* and perhaps *C. utilis*, too, also utilize the carbon atoms, but probably no energy is furnished by the degradation to the level of β-alanine.

Degradation of Orotic Acid

Orotic acid was isolated in 1905 by Biscaro and Belloni (54), and its structure was determined by Bachstez (21) in 1930. Two strains of the genus *Mycobacterium* isolated from soil grow on synthetic media containing orotic acid as the sole source of nitrogen (562). Orotic acid is converted to uracil, which in turn is degraded along the oxidative pathway that will be discussed below. In all other known instances orotic acid is degraded along a reductive pathway, either by conversion to uracil and dihydrouracil, as suggested for *P. facilis* (285), or by a separate route, as reported for *C. oroticum (Zymobacterium oroticum)* (310–313) and two unidentified corynebacteria (424).

Kornberg isolated from bay mud a bacterium capable of growing anaerobically in media containing orotic acid as the main organic substrate. It was characterized as *Z. oroticum* by Wachsman and Barker (575) and renamed *C. oroticum* (99) since it produces heat-resistant spores.

The first enzyme involved in the degradative pathway (Fig. 21), orotate reductase (L-5,6-dihydro-orotate:NAD^+ oxidoreductase [EC 1.3.1.14]), was recognized as a flavoprotein by Graves and Vennesland (197). It was purified and studied by Lieberman and Kornbery (311), Aleman et al. (5, 6, 210), and Miller and Massey (339, 340); Friedmann and Vennesland (177, 178) crystallized the enzyme. It contains equal amounts of FAD and riboflavine 5'-phosphate (FMN) (5, 178) and about 1 mol each of iron (5, 178, 339) and labile sulfide (5, 339) per mol of

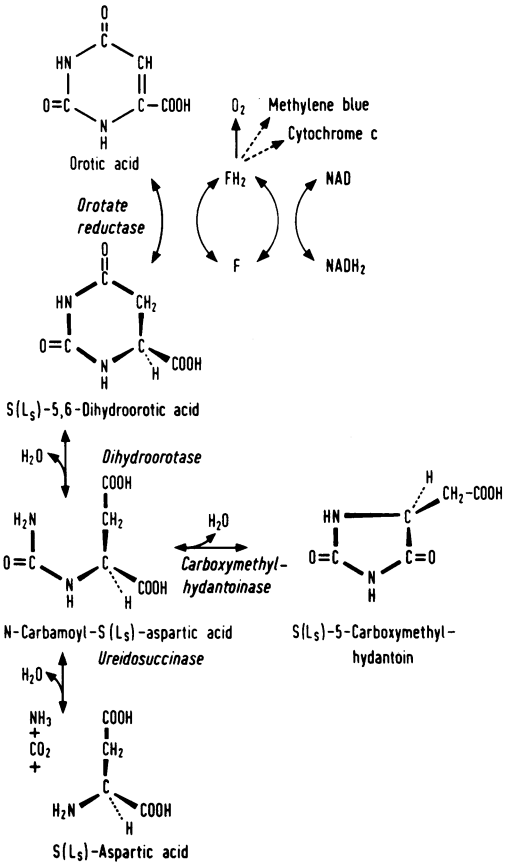


FIG. 21. *Degradation of orotic acid by Clostridium oroticum (177,310–313). F and FH_2 represent the oxidized and reduced flavin prosthetic group, respectively.*

flavin. The enzyme catalyzes: (i) the oxidation of $NADH_2$ by orotate or oxygen (177); (ii) the oxidation of dihydro-orotate by NAD or oxygen (58, 177); (iii) a diaphorase reaction catalyzing the anaerobic reduction of methylene blue (177), cytochrome *c* (411), or other electron acceptors (5) by $NADH_2$. The enzyme is rather specific for NAD, since the reaction rate with NADP was less than 2% of that observed with NAD (311); 5-fluoro-orotate (177) and 5-bromo-orotate (5) can substitute orotate in the reaction and are even more active substrates. The interaction of the enzyme with orotate or dihydro-orotate is dependent on added cysteine, whereas the interaction with $NADH_2$ is not (6, 177, 178). On addition of high amounts of $NADH_2$, not only flavins present in the enzyme are reduced but also other chromophoric groups, such as iron possibly in concert with the labile sulfide (339).

C. oroticum is reported to contain also a bio-

synthetic-type constitutive enzyme, dihydro-orotate oxidase (L-5,6-dihydro-orotate:oxygen oxidoreductase [EC 1.3.3.1]), which is induced by growth in media containing orotate (177, 499). Moreover, such conditions cause a threefold increase of both FMN and FAD in the cells, and Kondo et al. (280) presented evidence that this enhancement is related to the formation of this flavoprotein.

As a result of the orotate reductase action, S(L_s)-5,6-dihydro-orotate is formed. The free acid of this compound exhibits a specific optical rotation of [α](water) = +66.0° (312). The same optical isomer of dihydro-orotate is degraded by dihydroorotase (L-5,6-dihydro-orotate amidohydrolase [EC 3.5.2.3]) of *C. oroticum* (312). The enzyme requires a cation, probably Zn^{2+}, in its catalytic function (451) and is noncompetitively inhibited by substituted sulfonamide. A separate enzyme, carboxymethylhydantoinase (L-5-carboxymethylhydantoin amidohydrolase [EC 3.5.2.4]), enables the bacterium to convert *N*-carbamoyl-S(L_s)-aspartate reversibly into S(L_s)-5-carboxymethylhydantoin (312). The free acid of this compound exhibits a specific optical rotation of [α](water) = −98.9° (312). This reaction is a spur off the main pathway of metabolism (313).

The equilibria formed during both enzymatic conversions of *N*-carbamoyl-L-aspartate are described (312) by the equilibrium constants:

$$K = \frac{[N\text{-carbamoyl-S}(L_s)\text{-aspartate}]}{[\text{S}(L_s)\text{-5,6-dihydroorotate}]} = 1.9$$

$$K = \frac{[N\text{-carbamoyl-S}(L_s)\text{-aspartate}]}{[\text{S}(L_s)\text{-5-carboxymethylhydantoin}]} = 1.9$$

N-carbamoyl-L-aspartate is degraded to L-aspartate, ammonia, and CO_2 by ureidosuccinase (*N*-carbamoyl-L-aspartate amidohydrolase [EC 3.5.1.7]). The 10-fold purified enzyme from *C. oroticum* is not active with the R(D_s)-isomer, with S(L_s)-5-carboxymethylhydantoin, or with a number of other compounds (313). It showed an absolute requirement for metal ions; Mn^{2+} and Fe^{2+} ions were found to give the greatest effect. Cysteine stimulates the reaction several fold. Since phosphate is not required in the reaction, carbamoyl phosphate is most probably not an intermediate (313).

Reynolds et al. (424) described the metabolic pathway of orotic acid degradation by two unidentified corynebacteria isolated from soil. One of the organisms grows in a medium containing orotic acid as the sole organic substrate. The pathway is similar to that found in *C. oroticum*, except for the involvement of NADP instead of NAD in the orotate reductase (L-5,6-dihydro-orotate:$NADP^+$ oxidoreductase [EC 1.3.1.15]) and the absence of the enzyme involved in the reversible conversion of *N*-carbamoyl aspartate to 5-carboxymethylhydantoin (424).

Orotate reductase (NADP) is also present in an unidentified aerobic bacterium and possesses many characteristics similar to the enzyme from *C. oroticum* (522).

Pyrimidine Biosynthetic Pathway

The pyrimidine biosynthetic pathway (Fig. 20) has been reviewed by O'Donovan and Neuhard (371). The biosynthetic dihydro-orotate oxidase system differs markedly from orotate reductase described in the previous section. The biosynthetic system in *Escherichia coli* is constitutive, is composed of particle-bound enzymes, and is linked with the respiratory chain (257, 258, 264, 497, 498, 610). Similar results were obtained in studies on the enzyme of a pseudomonad (338, 499). However, when orotate was added to the growth medium, a new soluble NADP-linked orotate reductase was formed in this organism (499). Soluble and bound forms of enzymes catalyzing the conversion of dihydro-orotate to orotate are found in *Staphylococcus aureus*, which synthesizes pyrimidines according to the reaction given in Fig. 20 (333). Both forms are active in 2,6-dichlorophenol-indophenol reduction assays; the particulate enzyme normally links to oxygen via a cytochrome system. Orotate is also involved in the synthesis of pyrimidines in *Lactobacillus bulgaricus* (608). Dihydro-orotate oxidase of this organism is a soluble flavoprotein, containing only FMN as the prosthetic group. Redox dyes, oxygen, or cytochrome *c* are used as electron acceptors, but the purified enzyme is not active with pyridine nucleotides. The synthesis of the enzyme is repressed in cells growing in the presence of orotate and uracil (500).

The enzymic activity of pyrimidine synthesis in *S. aureus* is 6- to 20-fold higher in anaerobically or semianaerobically grown bacteria than in those grown in air, unless uracil is present. Aerobic cultures rapidly accumulate dihydro-orotate in the medium after transfer to anaerobic conditions. These results show that the requirement for uracil displayed by *S. aureus*, when growing anaerobically, is due to its inability to dehydrogenate dihydro-orotate in the absence of oxygen. The enzyme synthesis is derepressed in response to pyrimidine starvation (331). Dihydro-orotase is present in *E. coli* (450, 497, 610) and *P. fluorescens* (336). The

enzyme from *E. coli* was purified 145-fold and does not react with dihydrouracil and dihydrothymine (450).

Oxidative Pathway

In 1952 three groups of authors found an oxidative pathway of pyrimidine degradation (Fig. 22) in bacteria which are able to grow aerobically in media containing uracil or thymine as the sole source of nitrogen and carbon.

Hayaishi and Kornberg (216) isolated two bacteria from enrichment cultures which contained thymine and uracil as the sole organic substrates and which were inoculated with soil. One was tentatively assigned to the genus *Corynebacterium*, and the other was a *Mycobacterium*. The thymine-oxidizing enzymes of these cells are induced by growth on either thymine or uracil. These cells oxidize barbiturate as well, but a number of other pyrimidines, including cytosine and dihydrouracil, were not oxidized (216). Wang and Lampen (578–580) isolated from soil a gram-positive, motile, nonsporulating rod, which utilized uracil, cytosine, thymine, or barbituric acid as the sole source of C and N for growth, but orotic acid was not used. Lara (300, 301) observed that members of the genera *Corynebacterium* and *Nocardia* are able to grow aerobically in media containing either thymine or uracil as the only carbon, nitrogen, and energy source. Cells of *N. corallina* adapted to thymine were simultaneously adapted to uracil and barbituric acid (300).

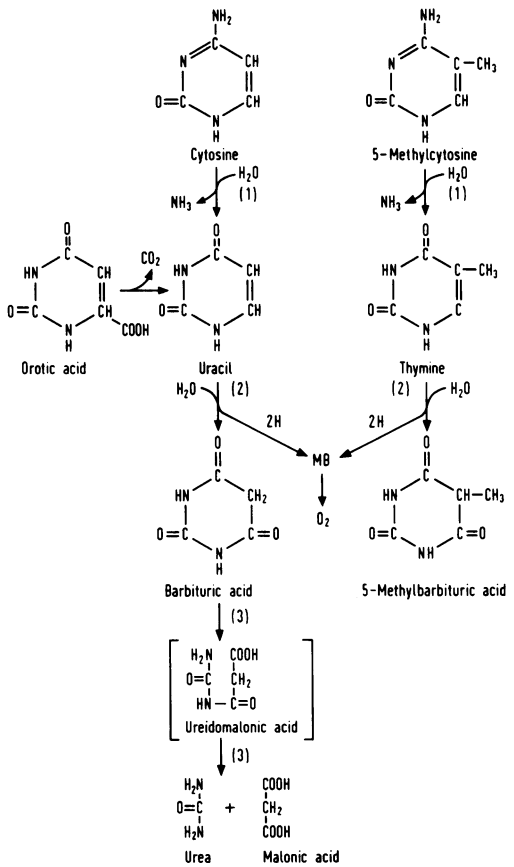


FIG. 22. *Oxidative pathway of pyrimidine degradation. (1) Cytosine deaminase (cytosine aminohydrolase [EC 3.5.4.1]). Acts also on 5-methylcytosine. (2) Uracil dehydrogenase (uracil:[acceptor] oxidoreductase [EC 1.2.99.1]). Also oxidizes thymine. (3) Barbiturase (barbiturate amidohydrolase [EC 3.5.2.1]). MB, Methylene blue.*

Later, Vitols et al. (562) reported on two strains of the genus *Mycobacterium* that grow on synthetic media containing orotic acid as the sole source of nitrogen. Cells of these bacteria convert orotic acid to uracil; barbituric acid and urea were identified in the culture liquid, whereas no dihydroorotic acid, carbamoyl aspartate, or aspartate was formed. Cytosine (216, 580) and 5-methylcytosine (216) are deaminated by an inducible (216) cytosine deaminase.

Uracil dehydrogenase is able to oxidize uracil, thymine (216, 580), and probably also 5-aminothymine (580). The electron acceptors of this enzyme are not NAD or NADP, but methylene blue may be used stoichiometrically under anaerobic conditions or in lesser amounts under aerobic conditions where it is autooxidizable (216, 580). Barbituric acid is formed, which is transformed to urea and malonic acid (214, 216, 301). Ureidomalonic acid may be an intermediate in this reaction that is catalyzed by barbiturase. Hayaishi and Kornberg (215, 216) purified barbiturase eightfold and showed that it does not react with a number of other compounds, including 5-methylbarbiturate. The fate of 5-methylbarbiturate is still unknown and, thus, the question is left open as to how the organisms gain carbon and nitrogen from thymine. *N. corallina* splits urea to CO_2 and ammonia (301), but the nonsporulating rod, studied by Wang and Lampen (580), does not contain urease. Nevertheless, 2 mol of ammonia is formed from uracil and thymine, which suggests that the ureido group is not released as a unit during the oxidative degradation.

The role of barbiturate in the metabolism of *Bacillus popilliae* is unclear. Barbiturate is required for constant growth in a synthetic medium, and this requirement was not replaced by the common pyrimidines and purines. It stimulates the synthesis of both nucleic acid and protein (117, 491).

Pyrimidine Degradation by Various Microorganisms

A number of microorganisms have been tested for their ability to use pyrimidines as a source of nitrogen or for the presence of cytosine deaminase (Table 15). *Tetrahymena pyriformis* requires preformed pyrimidines for growth since it is unable to synthesize the pyrimidine nucleus (220). Uracil is degraded by fruiting bodies of *Agaricus bisporus* and *Lycoperdon pyriforme* to urea. It was postulated that a reductive pattern implying ribotide derivatives might be operative in the degradation (417). The cyanobacterium *Agmenellum quadruplicatum* shows scant growth on thymine and uracil but no growth on orotic acid, dihydroorotic acid, and cytosine (256). *S. cerevisiae* possesses a common active transport system for adenine, guanine, hypoxanthine, and cytosine (384, 416) and a specific one for uracil (202). Pyrimidines are deaminated only slowly or not at all by *Vibrio cholerae* (4) and not at all by strains of *Streptococcus faecalis, S. faecium,* and *S. durans* (334). In *E. coli* the uptake of uracil is controlled by cyclic AMP, which may explain part of the inhibiting effect of cyclic AMP on the growth of *E. coli* on glucose (246). *E. coli* converts the C2 atom of thymine and uracil to CO_2. The induction of the enzymes involved in the degradation is prevented by the presence of NH_4^+ in the medium (25). The role of pyrimidines in the degradation of purines by *Veillonella alcalescens* was discussed in a previous section.

ECOLOGICAL ASPECTS

Large amounts of purines, pyrimidines, uric acid, and allantoin are produced in a number of ecosystems. Various bacteria appear to be particularly adapted to growth in such ecosystems. This adaptation and some examples of the ecosystems will be dealt with in this section.

Organisms

A special kind of adaptation to substrates like methylpurines, uric acid, and allantoin is observed in studies with *Clostridium* and *Bacillus* but may be more widely distributed in nature. Three examples are given here.

Clostridium acidiurici and *C. cylindrosporum* grow in media containing 0.2% uric acid and 0.5% (volume) yeast autolysate as sole organic substrates (33). No growth was observed in media containing peptone, tryptone, yeast autolysate, or other rich compounds instead of uric acid. Only very small amounts of ammonia were produced from these media when offered together with uric acid. Glucose was not degraded by cells growing on uric acid (33).

Kurtzman and Schwimmer (292) isolated a strain of *Bacillus coagulans* from soil on plates containing 0.01 M caffeine in Czapek medium.

TABLE 15. *Utilization of pyrimidines by various microorganisms and presence of cytosine deaminase*[a]

Organism	Substrate			Presence of cytosine deaminase	Reference
	Cytosine	Uracil	Thymine		
Tetrahymena pyriformis				−	152
38 Chlamydomonad algae	−N	−N	−N		88
Myxococcus virescens	[b]		+CN		368
M. fulvus	[b]		+CN		368
M. coralloides (Chondrococcus coralloides)	[b]		+CN		368
Pseudomonas acidovorans	+N	+N	+N, −C	+	263
P. oleovorans				+	448
Alcaligenes eutrophus H16 *(Hydrogenomonas* H16)	+N	−N	−N	+[c]	254
Azotobacter chroococcum	(−)N	−N			227
A. vinelandii	(−)N	−N			227
Aerobacter cloacae		+N			343
A. aerogenes		+N			343
Escherichia coli		+N[d]	+N	+	25, 103, 343
Salmonella typhimurium	+N	+N			41
Veillonella alcalescens (Micrococcus lactilyticus)	+N	+N	+N	+	587

[a] The substrates were tested as carbon (C), nitrogen (N), or carbon and nitrogen (CN) sources for growth. Symbols: +, positive results; (−), doubtful results; and −, negative results.

[b] Cytidine is utilized as the source of carbon, nitrogen, and energy.

[c] Inducible.

[d] A majority of *E. coli* strains use uracil as a sole source of nitrogen (343).

Subsequent cultures of the isolate degraded caffeine readily, but growth requirements on other media appeared to be rather fastidious. An authentic *B. coagulans* did not grow in the aforementioned medium. The authors suggest a degree of selective adaptation in this organism.

B. fastidiosus can be readily isolated from various sources, including soil. The fastidious requirement for uric acid and for its degradation products was described by Den Dooren de Jong (133) and confirmed by four independent groups (62, 253, 305, 329). No rich medium is known that supports growth of this organism to a level comparable to synthetic media containing uric acid, allantoin, or allantoate as the sole organic substrates (62). Besides these organisms, perhaps other ones are adaptively specialized in the degradation of purines and pyrimidines, but no studies have been made in this field. Moreover, it should be worthwhile to investigate the possible plasmid-born genetic information enabling some bacteria, especially those of the *Streptococcus allantoicus-Enterobacteriaceae* group, to degrade uric acid and allantoin. This might explain why *Escherichia coli* and *S. allantoicus* subcultured in media without uric acid or allantoin gradually lose the capacity to degrade these substances (Vogels and Van der Drift, unpublished data).

Ecosystems

Den Dooren de Jong noticed (133) that about 10% of the bacterial strains isolated from the Maas River and about half of the strains isolated from tap water were able to degrade uric acid. Antheunisse (16) tested the ability of soil microorganisms to degrade uric acid. About half of the microorganisms present in clay soil (2×10^7 to 4×10^7/g) were able to decompose uric acid in the presence of yeast extract and glucose. A large number of coryneform strains isolated from sandy soils, peaty soil, cheese, fish, seawater, and sewage decompose uric acid (16).

No quantitative data are known concerning the concentration of purines, pyrimidines, or their degradation products in marine environments, but the studies of Remsen et al. (421) demonstrated that urea is a major nitrogen source in the upper marine water layers as compared with inorganic nitrogen sources as ammonia, nitrate, and nitrite. The urea concentration in the open North Atlantic is about 0.28 μM. A relatively high concentration (1.7 μM) is found off the coast of Peru due to the huge quantities of bony fish and birds, both of which excrete large amounts of uric acid. By the same token, the water of Great South Bay, Lond Island, contains a high amount of organic nitrogen, particularly uric acid, due to the presence of duck farms located around the Bay. Estuarine waters contain about 10^6 urea-decomposing bacteria per liter, whereas the number in marine waters is around 3×10^3 to 4×10^3 cells/liter (421). Uric acid may be degraded also in the absence of microorganisms, since Antia and Landymore (17) reported uric acid degradation in a seawater medium mainly as a result of the presence of traces of metal ions.

Mammal intestines. The effect of feeding yeast or other single cells on the serum level of uric acid and the excretion of uric acid is discussed in the Introduction. Upon feeding higher amounts of yeast, the amount of uric acid excreted does not increase linearly with the amount of yeast ingested (149). Perhaps intestinal organisms are involved in the degradation of uric acid. Such degradation was found by many other authors (184, 319, 320, 501) for uric acid administered orally to humans and allantoin or uric acid fed to other mammals (231); intravenously administered uric acid was recovered almost completely as urinary uric acid (184), but perhaps part of a person's daily uric acid excretion is normally recycled to the intestine from the blood and degraded by the uricolytic bacteria. This was found to be true for urea (555). A number of the bacteria described above in the section on the anaerobic, degradative pathways of purines and allantoin are likely candidates for intestinal uricolysis, but in analogy to the avian caecum many other bacterial species may be involved (36).

Urea formed in these reactions can be converted to ammonia by *Selenomonas ruminantium* and *Peptostreptococcus productus,* which contain a urease that is strongly repressed by NH_4^+ ions (556). Ammonia is the major nitrogen source for growth of intestinal bacteria and is essential for growth of *Ruminococcus bromii* (80).

Avian caecum and insect intestines. Uric acid is the main excretory nitrogenous substance of birds (about 6% of dried poultry waste consists of uric acid [55]) and insects. Anaerobic uric acid-degrading bacteria were found by Barker and Beck (33) in fecal material of the yellow-shafted flicker and were demonstrated to occur in large numbers in intestines of termites and cockroaches (471) and poultry caeca (36, 449). In the latter case the number of uric acid-decomposing anaerobic bacteria amounts to between 5.4×10^8 and 1.8×10^{10}/g (wet weight) of caecal material (36). Among them were *Bacteroides, Sphaerophorus, Fusobacterium, Eu-*

bacterium, an anaerobic *Streptococcus*, *P. productus*, (as well as other *Peptostreptococcus* species), and *Clostridium malenominatum* (36). No studies were made on the degradation pathway of uric acid in these organisms.

The decomposition of uric acid in built-up poultry litter has been studied (458). The number of uric acid decomposers varied between 2.2×10^9 and 64×10^9/g (fresh weight) of the material. Their proportion of the total number of bacteria present was about 25% and included *Corynebacterium*, *Nocardia*, *Streptomyces*, *Pseudomonas*, *Alcaligenes*, *Achromobacter*, and *Cytophaga*, which were able to transform uric acid into ammonia or urea. Only small amounts of anaerobic uric acid decomposers were found, but the presence of organisms resembling *C. acidiurici* was suggested (458).

Rumen. Besides the purines and pyrimidines as constituents of nucleic acids, plants also contain free purines, uric acid, and allantoin. About half of the nonprotein nitrogen content of grassland herbage consists of purines and pyrimidines, either free or bound (154). Allantoin and uric acid are present in various *Gramineae* and *Leguminosae* (514) and are nitrogen sources for the rumen microflora (44). The concentration of uric acid in bovine rumen content is reported to be 5 mg/100 ml, but no data are available on the amount of allantoin, purines, or pyrimidines present (509). The bacteria may benefit the host by converting these compounds into a usable form. Cell suspensions of bovine rumen bacteria degrade xanthine, guanine, and uric acid to ammonia, fatty acids, and CO_2, but hypoxanthine and adenine are less readily attacked (247). Reports on the degradation of purines or pyrimidines by pure cultures of rumen bacteria are scant. Forty-two percent of the rumen coliform isolates are capable of growing on a medium that contained uric acid as the primary source of carbon and nitrogen. The predominant uricolytic organism in this group is *Paracolobactrum aerogenoides*. This bacterium degrades uric acid in a pathway involving allantoin and glyoxylate, and urea is an end product (509).

S. ruminantium can use adenine and uric acid as nitrogen source, but allantoin, xanthine, and uracil are not used (239).

Skin. Another ecosystem in which uric acid together with amino acids, ammonia, urea, and creatinine act as the most common nitrogen source is formed by the human integument and its autochthonous skin organisms. A considerable part of the staphylococci, the diphtheroids, and gram-negative bacteria present in the skin is able to degrade uric acid, but the first two groups appear to be unable to utilize uric acid as a sole nitrogen source (481).

Symbiotic uric acid-degrading bacteria. In the fat body and ovaries of cockroaches, special cells, mycetocytes, are found in which symbiotic bacteria are present. The bacteria have been isolated by Keller (262) and have been shown to grow in vitro under aerobic conditions on a medium containing uric acid as the sole source of carbon and nitrogen. The ability of the bacteria to utilize uric acid is of particular interest, since this compound is the main end product of nitrogen metabolism in the cockroach and is deposited within fat body cells as well as excreted. The symbiotic bacteria enable the host to utilize the waste material and perhaps offer a route for the remobilization of nitrogen stored as uric acid should this subsequently be required by the host.

Donnellan and Kilby (142) isolated a motile, gram-negative curved rod from the fat body of adult *Periplaneta americana*. The bacterium grows aerobically in a medium containing uric acid as the organic substrate in the presence of small amounts of Lab-lemco. Uric acid is degraded by uricase, which can be extracted from insoluble cell debris at pH 9. Allantoin is formed in this reaction and is subsequently degraded to allantoate, ureidoglycolate, and glyoxylate. Ammonia is formed from urea by urease, and glyoxylate is degraded along the tartronate semialdehyde pathway.

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