JOURNAL OF BACTERIOLOGY, Dec. 1982, p. 1105–1110
0021-9193/82/121105-06$02.00/0

Vol. 152, No. 3

Enzymatic Activities for Interconversion of Purines in Spirochetes

ERCOLE CANALE-PAROLA[1]* AND GEORGE W. KIDDER[2]

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003,[1] and Biological Laboratories, Amherst College, Amherst, Massachusetts 01002[2]

Received 28 June 1982/Accepted 8 September 1982

Enzymatic activities that catalyze the interconversion of purines and purine derivatives were detected in cell extracts of *Spirochaeta aurantia*, *Spirochaeta stenostrepta*, *Treponema succinifaciens*, and *Treponema denticola*. Phosphoribosyltransferase activities present in cell extracts of each of the four spirochete species functioned in the conversion of adenine, hypoxanthine, and guanine to AMP, IMP, and GMP, respectively. Nucleotidase activities in the extracts mediated the formation of nucleosides from nucleotides. The conversion of adenosine, inosine, and guanosine to the respective purine bases was catalyzed by nucleoside phosphorylase and, in some instances, by nucleoside hydrolase activities. Guanine deaminase activity was found in both *S. aurantia* and *S. stenostrepta*, whereas adenosine deaminase activity was detected only in *S. aurantia*. Adenine deaminase activity in *T. succinifaciens* extracts was sensitive to O_2 and was relatively resistant to heating. Our results indicate that the four species of spirochetes studied possess a broad spectrum of purine interconversion enzymes. It is suggested that these enzymes may function in metabolic processes important for the survival of spirochetes in nutrient-poor natural environments.

Free purine bases and purine derivatives, either present in the external environment or generated intracellularly through degradative processes, may be "salvaged" by microorganisms that possess appropriate enzymes (8). The utilization of these purine compounds by microorganisms involves the participation of a complex array of enzymes which serve to interconvert purine bases, nucleosides, and nucleotides (Fig. 1) (22 and references therein). Such interconversion activities provide cells with a variety of purine compounds which are required for the synthesis of nucleic acids and coenzymes and for other metabolic processes. A single purine base available to a bacterium that possesses the necessary interconversion enzymes (Fig. 1) can serve as the sole source of a wide spectrum of purine derivatives. For example, adenine can be converted to AMP, IMP, XMP, and GMP (15, 22), as well as to nucleosides such as adenosine, inosine, and guanosine, and to free bases (e.g., hypoxanthine, guanine, and xanthine). These interconversions frequently involve numerous enzymatic steps and may proceed through rather tortuous metabolic routes (22).

The objective of the research described in this article was to identify purine interconversion enzymatic activities in four species of spirochetes. When this work was initiated, an extremely limited amount of information was available on the occurrence of these enzymes in spirochetes. In fact, purine interconversion enzymes of few bacterial species have been studied (14), and frequently these studies have been concerned with only one or two enzymes in any one species. We were especially interested in investigating purine interconversion enzymes of spirochetes because in recent years we have been studying physiological mechanisms that these bacteria utilize to survive in environments in which nutrients necessary for growth are present at very low concentrations or are absent (10, 11). The research we report here was prompted by the possibility that purine interconversion enzymes play an important role(s) in the survival of spirochetes in the natural environments which they inhabit.

MATERIALS AND METHODS

Spirochetes and cultural conditions. *Spirochaeta aurantia* M1, a facultative anaerobe isolated from a freshwater pond (2), was grown in GTY broth (3) at 30°C. *Spirochaeta stenostrepta* Z1, an obligate anaerobe isolated from freshwater mud, was grown in liquid medium GYPT (3) at 30°C. *Treponema denticola* 10, an obligate anaerobe isolated from the human mouth, was grown in liquid medium GM-1 (1) at 37°C in an N_2 atmosphere. *Treponema succinifaciens* ATCC 33096, an obligate anaerobe isolated from a swine colon, was grown in RF broth (6) at 39°C. in an N_2 atmosphere.

Preparation of cell extracts. Cultures of spirochetes were incubated until they reached the late exponential phase of growth. The cells were then harvested by

centrifugation at 5°C and washed in a buffer solution, except as noted below. Whole cells of obligately anaerobic species were kept in an N_2 atmosphere during the centrifugation and washing steps. *S. aurantia* cells harvested from 1,800 ml of GTY broth were washed by suspending them in 30 ml of cold (5 to 10°C) 0.1 M potassium phosphate buffer (pH 7). *T. denticola* or *T. succinifaciens* cells harvested from 6,000 ml of GM-1 broth or 2,000 ml of RF broth, respectively, were suspended in 30 ml of cold 0.1 M potassium phosphate buffer solution (pH 7.4) containing 5×10^{-3} M (final concentration) dithiothreitol. The cells were harvested from the buffer solution by centrifugation at 5°C and suspended in 10 ml of the same buffer solution used in the washing step. The washed spirochetes were then disrupted by passing the cell suspensions through a French pressure cell at 10,000 lb/in^2 (7×10^6 kg/m^2). Cell debris was removed by centrifugation at 5°C for 20 min at 27,000 × *g*. Disrupted cells of obligately anaerobic spirochetes were kept in an N_2 atmosphere during centrifugation. The supernatant fluid (cell extract) was stored either in N_2 (for the obligate anaerobes) or in air (for *S. aurantia*) at 5°C and was used shortly after preparation.

Cells of *S. stenostrepta* were not washed. *S. stenostrepta* cells harvested from 4,000 ml of GYPT medium were suspended in 10 ml of 0.03 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer solution (pH 7) containing 0.001 M (final concentration) dithiothreitol. This suspension was passed through a French pressure cell and used to prepare cell extracts as described above for *T. denticola* and *T. succinifaciens*.

The protein content of cell extracts was determined by means of the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.), Cell extracts used in some of the reaction mixtures were dialyzed against 0.05 M Tris-hydrochloride buffer (pH 7.5) solution or against distilled water (at 5 or 15°C) to remove phosphates, endogenous phosphoribosylpyrophosphate, and other dialyzable substances.

Enzyme assays. Each reaction mixture contained the following in a total volume of 0.14 ml: 8-^{14}C-purine base, -nucleoside, or -nucleotide, 20 nmol; $MgCl_2$, 50 nmol; potassium phosphate buffer (pH 7.5) or Tris-hydrochloride buffer (pH 7.5), 5 μmol; and cell extract, 0.03 ml. In some experiments 20 nmol of 5-phosphoribosyl-1-pyrophosphate (PRPP) was added to the reaction mixture. The reaction mixtures were incubated for 10 min at 35°C in air. The reaction was stopped by the addition of a drop of glacial acetic acid, and samples of the reaction mixture were streaked on Whatman no. 1 paper for descending chromatography or for electrophoretic separation (17). Chromatograms

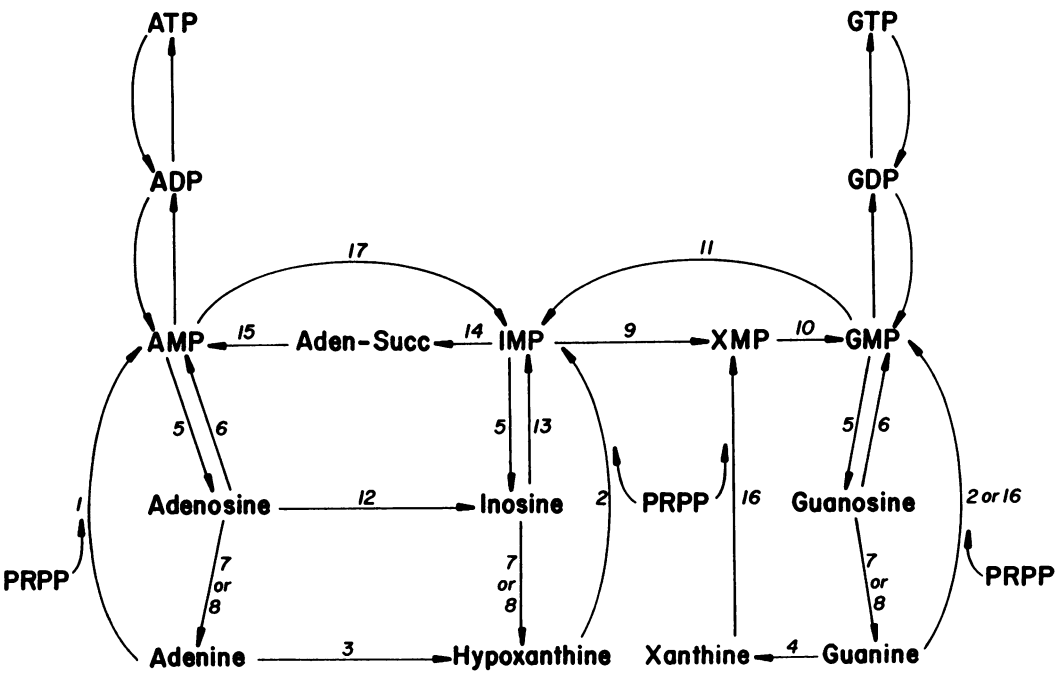

FIG. 1. Some of the reactions involved in interconversions of purines and purine derivatives. This is a compendium of reactions present in various organisms. Aden-Succ, Adenylosuccinate. Enzymes: 1, adenine phosphoribosyltransferase (EC 2.4.2.7); 2, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); 3, adenine deaminase (EC 3.5.4.2); 4, guanine deaminase (EC 3.5.4.3); 5, 5'-nucleotidase (EC 3.1.3.5); 6, adenosine kinase (EC 2.7.1.20); 7, purine-nucleoside hydrolase (EC 3.2.2.1); 8, purine-nucleoside phosphorylase (EC 2.4.2.1); 9, IMP dehydrogenase (EC 1.2.1.14); 10, GMP synthetase (EC 6.3.4.1); 11, GMP reductase (EC 1.6.6.8); 12, adenosine deaminase (EC 3.5.4.4); 13, inosine kinase (EC 2.7.1.73); 14, adenylosuccinate synthetase (EC 6.3.4.4); 15, adenylosuccinate lyase (EC 4.3.2.2); 16, guanine phosphoribosyltransferase (bacterial); 17, AMP deaminase (EC 3.5.4.6).

were developed in isobutyric acid-ammonia-water (66:1:33, vol/vol/vol) or in *n*-butanol-acetic acid-water (20:3:7, vol/vol/vol). The electrolyte for electrophoresis (at 400 V) was formic acid-water (8:300, vol/vol). After development, the radioactive compounds were located by using a Tracerlab 4 π strip scanner and were identified by comparison with cochromatographed known compounds. Quantitation was by planimetry (19).

Adenine deaminase (adenine + $H_2O \rightarrow$ hypoxanthine + NH_3) activity was determined by measuring the formation of hypoxanthine from adenine in the absence of PRPP. Guanine deaminase (guanine + $H_2O \rightarrow$ xanthine + NH_3) activity was determined by measuring the formation of xanthine from guanine in the absence of PRPP. Purine phosphoribosyltransferase (purine + PRPP $\rightarrow$ nucleotide + pyrophosphate) activity was assayed by measuring the PRPP-dependent conversion of adenine, hypoxanthine, or guanine to AMP, IMP, or GMP, respectively. The term "nucleotidase activity" is used to indicate any enzymatic activity (or activities) that mediated the conversion of nucleotides to nucleosides or purine bases or both. Adenosine deaminase (adenosine + $H_2O \rightarrow$ inosine + NH_3) activity was determined by measuring the formation of inosine from adenosine and by assessing the inhibitory effect of coformycin (3-β-D-ribofuranosyl-6,7,8-trihydroimidazo [3,4-*d*]-[1,3]-diazapin-8-(*R*)-ol) and of deoxycoformycin (12) on the reaction. Another possible route for the conversion of adenosine to inosine would involve adenine deaminase (18) (Fig. 1). However, adenine deaminase activity was detected only in *T. succinifaciens* cell extracts, which lacked adenosine deaminase activity. Nucleoside phosphorylase (nucleoside + $P_i \rightleftarrows$ purine + ribose 1-phosphate) activity was assayed by measuring the phosphate-dependent conversion of nucleosides to purine bases. Nucleoside hydrolase (nucleoside + $H_2O \rightarrow$ purine + ribose) activity was determined by measuring the conversion of nucleosides to purine bases in the absence of phosphate. Dialyzed (see above) cell extracts were used in the assays for nucleoside phosphorylase and nucleoside hydrolase activities. Xanthine oxidase (xanthine + $H_2O + O_2 \rightarrow$ uric acid + superoxide) was assayed by measuring the disappearance of xanthine and the formation of uric acid from xanthine.

8-^{14}C-purines, 8-^{14}C-nucleosides, and 8-^{14}C-nucleotides (all at 50 mCi/mmol) were purchased from Amersham Corp. (Arlington Heights, Ill.). Bovine liver catalase, bovine blood superoxide dismutase, and other chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.). Coformycin and 2'-deoxycoformycin were gifts from H. W. Dion (Warner-Lambert/Parke-Davis).

RESULTS

Enzyme activities that function in interconversions of purine bases, nucleosides, and nucleotides were detected in cell extracts of the four spirochete species tested.

The results outlined in Table 1 indicate that adenosine deaminase activity is present in *S. aurantia*, inasmuch as cell extracts of this bacterium catalyzed the conversion of adenosine to inosine. Some of the inosine formed from adenosine was converted to hypoxanthine by phosphorylase and hydrolase activities which were detected in the cell extracts (see below). When phosphate was present, adenosine phosphorylase activity (see below) catalyzed the formation of adenine from adenosine (Table 1). The occurrence of adenosine deaminase activity in *S. aurantia* was confirmed by the inhibitory effect of coformycin (Table 1) and deoxycoformycin (data not shown) on the conversion of adenosine to inosine. Coformycin and deoxycoformycin also inhibit adenine deaminase activity (18), but this enzyme was not detected in cell extracts of *S. aurantia*. The lack of adenine deaminase activity was indicated by the inability of *S. aurantia* cell extracts to catalyze the formation of hypoxanthine from adenine in the absence of PRPP (Table 1). In the absence of the latter compound, adenine was not metabolized to any product. On the other hand, in the presence of PRPP, adenine was converted to adenosine, inosine, and hypoxanthine (Table 1). These observations indicated that adenine phosphoribo-

TABLE 1. Metabolism of adenine, adenosine, and AMP by *S. aurantia* cell extracts

^{14}C-labeled substrate	Conditions	Amt of product formed[a]				
		Adenine	AMP	Adenosine	Inosine	Hypoxanthine
Adenine	PRPP present	Substrate	ND	5	22	25
Adenine	PRPP absent	Substrate[b]	ND	ND	ND	ND
Adenosine	Phosphate present	12	ND	Substrate	11	44
Adenosine	Phosphate absent[c]	ND	ND	Substrate	62	38
Adenosine	Coformycin added[d], phosphate present	31	ND	Substrate	ND	ND
AMP	PRPP absent	ND	Substrate	ND	38	28

[a] Expressed as nanomoles of product per milligram of protein per 10-min incubation period. All radioactivity in each sample tested was recovered in the products detected and in the remaining substrate. Thus, no other purine compounds were present in addition to those listed. Substrates were radioactively labeled at C-8. ND, Not detected.

[b] All radioactivity was recovered in the substrate after the 10-min incubation period.

[c] Dialysis was used to remove phosphate from the cell extract (see text).

[d] The final concentration of coformycin in the reaction mixture was 1.4×10^{-6} M.

syltransferase activity was present and that AMP formed from adenine did not accumulate in the reaction mixture but was metabolized via a reaction(s) catalyzed by nucleotidase activity (see below).

The occurrence of AMP nucleotidase activity in cell extracts of *S. aurantia* was inferred because AMP was metabolized to inosine and hypoxanthine (Table 1). Probably, metabolism of AMP was mediated by 5'-nucleotidase (Fig. 1), with adenosine not accumulating in the reaction mixture but being converted to inosine by adenosine deaminase. Adenosine deaminase activity was demonstrated in the extracts, as mentioned above. Alternatively (or in addition), AMP may be converted to IMP by AMP deaminase (Fig. 1) and the IMP formed converted to inosine. Another enzyme that could act on AMP is AMP nucleosidase (AMP + $H_2O \rightarrow$ adenine + ribose 5-phosphate). However, this possibility seems unlikely since no adenine was formed from AMP (Table 1).

The conversion of adenosine to adenine by cell extracts of *S. aurantia* required phosphate (Table 1). This observation indicated the presence of purine-nucleoside (adenosine) phosphorylase activity and the absence of adenosine hydrolase activity. However, hydrolase activity specific for inosine was present because, in the absence of phosphate, inosine derived from adenosine was converted to hypoxanthine (Table 1). The occurrence of inosine hydrolase activity in *S. aurantia* cell extracts was confirmed by other experiments in which [8-^{14}C]inosine was added to the reaction mixture as the substrate in the absence of phosphate (data not shown).

Experiments similar to those summarized in Table 1 were carried out to study the metabolism of guanine, guanosine, and GMP by *S. stenostrepta* cell extracts. These experiments (Table 2) showed that in the cell extracts (i) guanine phosphoribosyltransferase activity was present, as indicated by the PRPP-dependent formation of GMP from guanine, (ii) nucleotidase activity was present, inasmuch as GMP was metabolized to guanosine, and (iii) purine-nucleoside (guanosine) phosphorylase activity was present and guanosine hydrolase activity absent, because phosphate was required for the conversion of guanosine to guanine (Table 2). Furthermore, guanine deaminase activity was demonstrated by showing that the extracts catalyzed the formation of xanthine from guanine (Table 2). In the absence of PRPP, xanthine was the only product formed from guanine. Xanthine was not metabolized by *S. stenostrepta* cell extracts, an indication that xanthine oxidase was absent (Table 2).

Experiments analogous to those described above were carried out to study enzymatic activities involved in the metabolism of purine bases, nucleosides, and nucleotides in *T. succinifaciens* and *T. denticola*, as well as to investigate additional enzymatic activities of *S. aurantia* and *S. stenostrepta*. The results of these studies and of those presented in Tables 1 and 2 may be summarized as follows. All four species of spirochetes have phosphoribosyltransferase activities (for adenine, hypoxanthine, and guanine), nucleotidase activities (for AMP, IMP, and GMP), and purine-nucleoside phosphorylase activities (for adenosine, inosine, and guanosine) (Table 3). Purine-nucleoside hydrolase activities specific for some of the nucleosides tested were detected in three of the four spirochete species (Table 3).

Adenine deaminase activity, detected only in *T. succinifaciens* (Table 3), was especially sensitive to molecular oxygen. In *T. succinifaciens* cell extracts (1-ml volumes, 20 to 25 mg of protein per ml, in test tubes 10 by 44 mm) the adenine deaminase activity was totally lost after 90 min of exposure to air, whereas the activity

TABLE 2. Metabolism of guanine, guanosine, and GMP by *S. stenostrepta* cell extracts

^{14}C-labeled substrate	Conditions	Amt of product formed[a]			
		Guanine	GMP	Guanosine	Xanthine
Guanine	PRPP present	Substrate	34	12	15
Guanine	PRPP absent	Substrate	ND	ND	30
Guanosine	Phosphate present	11	15	Substrate	9
Guanosine	Phosphate absent[b]	ND	ND	Substrate[c]	ND
GMP	Phosphate present	13	Substrate	53	ND
Xanthine	PRPP absent	ND	ND	ND	Substrate[c]

[a] Expressed as nanomoles of product per milligram of protein per 10-min incubation period. All radioactivity in each sample tested was recovered in the products detected and in the remaining substrate. Thus, no other purine compounds were present in addition to those listed. Substrates were radioactively labeled at C-8. ND, Not detected.

[b] Dialysis was used to remove phosphate from the cell extract (see text).

[c] All radioactivity was recovered in the substrate after the 10-min incubation period.

was fully retained when the extracts were kept in an argon atmosphere under identical conditions for the same period of time. When either catalase or superoxide dismutase was added to the cell extracts (70 U or either enzyme per ml of extract), 37 to 45% of the original deaminase activity was retained after 90 min in air.

The adenine deaminase activity of *T. succinifaciens* was relatively (17) resistant to heating. Cell extracts of *T. succinifaciens* (20 mg of protein per ml) that were heated in an argon atmosphere for 5 min at 55°C retained 100% of their adenine deaminase activity. Approximately one-third of the original activity was still present after the extracts were heated for 5 min at 65°C.

Both *S. aurantia* and *S. stenostrepta* had guanine deaminase activity (Table 3).

DISCUSSION

The results of this study show that the four *Spirochaeta* and *Treponema* strains that were investigated possess a broad range of purine interconversion enzymes.

Most likely, the phosphoribosyltransferase activities we detected (Table 3) are important with regard to the energy economy of the four spirochete strains, inasmuch as these enzymes enable the cells to salvage preformed purine bases and to channel them, as nucleotide components, into metabolic processes. Spirochetes that require exogenous purines depend on these salvage activities for growth and survival. Two spirochetes of the genus *Leptospira* have been reported to have phosphoribosyltransferases (16).

Our experiments show that the spirochetes we studied possess nucleoside phosphorylase and nucleoside hydrolase activities that catalyze reactions in which ribose 1-phosphate, ribose, adenine, hypoxanthine, and guanine are formed from purine nucleosides (Fig. 1 and Table 3). These observations are of interest in view of the finding that bacterial cells break down intracellular rRNA when exogenous nutrients necessary for growth are not available to them (7, 9, 20, 21, 23, and references therein). It is possible that in starving spirochete cells, nucleosides derived from the breakdown of rRNA serve as substrates in reactions catalyzed by phosphorylases and hydrolases. Ribose and ribose 1-phosphate formed in these reactions may be used as endogenous energy and carbon sources by the starving spirochetes. Free purine bases, generated in the phosphorylase and hydrolase reactions, may be converted to derivatives for utilization in cellular processes, or, if the spirochetes are able to cleave the purine ring, the purine bases could be

TABLE 3. Enzymatic activities involved in purine salvage and interconversion

Enzymatic activity and substrates	Presence[a] in:			
	S. aurantia	*S. stenostrepta*	*T. succinifaciens*	*T. denticola*
Adenine deaminase	−	−	+	−
Adenosine deaminase	+	−	−	−
Guanine deaminase	+	+	−	−
Phosphoribosyltransferase				
Adenine	+	+	+	+
Hypoxanthine	+	+	+	+
Guanine	+	+	+	+
Nucleotidase				
AMP	+	+	+	+
IMP	+	+	+[b]	+
GMP	+	+	+[b]	+
Nucleoside phosphorylase				
Adenosine	+	+	+	+
Inosine	+	+	+	+
Guanosine	+	+	+	+
Nucleoside hydrolase				
Adenosine	−	−	−	+
Inosine	+	−	+	+
Guanosine	−[b]	−	+	−

[a] +, Activity present; −, activity not detected.
[b] As indicated or weak activity.

used as energy, carbon, and nitrogen sources. Thus, it is possible that purine interconversion enzymes, such as nucleoside phosphorylases and hydrolases, function in survival processes used by spirochete cells to obtain energy, carbon, and nitrogen when exogenous growth substrates are not available.

Other purine interconversion enzymatic activities present in spirochetes yield NH_3 that may be utilized for cell survival under conditions of nitrogen starvation. For example, it is possible that NH_3 formed in reactions catalyzed by adenine deaminase, adenosine deaminase, and guanine deaminase (Fig. 1 and Table 3) is used as a nitrogen source. It would not be necessary for the spirochetes to cleave the purine ring to generate NH_3 via these reactions. None of the three deaminase activities mentioned above was detected in *T. denticola*. However, it may be possible to demonstrate the presence of these and other undetected enzymatic activities in cell extracts by using different experimental conditions (e.g., anaerobic incubation of reaction mixtures).

Adenine deaminase activity in *T. succinifaciens* extracts was rapidly inactivated by exposure to air, but was quite stable in an argon atmosphere. Adenine deaminases from other microorganisms (13, 17) are much more stable in an air atmosphere.

Spirochetes are widespread in nature and occur in a large variety of environments (4, 5). It may be inferred that to compete with other microorganisms and to survive in so many different habitats, spirochetes have adapted by evolving a multiplicity of physiological mechanisms that allow them to cope with frequent changes in levels of available nutrients and with other environmental stresses. It would be of great interest to (i) determine whether the purine interconversion enzymatic activities that we detected in spirochetes contribute significantly to the ability of these bacteria to deal with nutrient deprivation and with other selective pressures in natural environments and (ii) elucidate the biochemical mechanisms involved. We are directing our research efforts toward these goals.

ACKNOWLEDGMENTS

We are grateful to L. L. Nolan for helpful discussions and criticism and to W. M. Cwyk for excellent technical assistance.

This research was supported by Public Health Service research grants AI-17737 and AI-12482 from the National Institute of Allergy and Infectious Diseases (to E.C.-P.) and by National Science Foundation research grant PCM 8103669 (to G.W.K.).

LITERATURE CITED

1. **Blakemore, R. P. and E. Canale-Parola.** 1976. Arginine catabolism by *Treponema denticola*. J. Bacteriol. **128**:616–622.
2. **Breznak, J. A., and E. Canale-Parola.** 1975. Morphology and physiology of *Spirochaeta aurantia* strains isolated from aquatic habitats. Arch. Microbiol. **105**:1–12.
3. **Canale-Parola, E.** 1973. Isolation, growth and maintenance of anaerobic free-living spirochetes, p. 61–73. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 8. Academic Press, Inc., New York.
4. **Canale-Parola, E.** 1977. Physiology and evolution of spirochetes. Bacteriol. Rev. **41**:181–204.
5. **Canale-Parola, E.** 1978. Motility and chemotaxis of spirochetes. Annu. Rev. Microbiol. **32**:69–99.
6. **Cwyk, W. M., and E. Canale-Parola.** 1979. *Treponema succinifaciens* sp. nov., an anaerobic spirochete from the swine intestine. Arch. Microbiol. **122**:231–239.
7. **Dawes, E. A.** 1976. Endogenous metabolism and the survival of starved prokaryotes. Symp. Soc. Gen. Microbiol. **26**:19–53.
8. **Gots, J. S.** 1971. Regulation of purine and pyrimidine metabolism, p. 225–255. *In* H. J. Vogel (ed.), Metabolic pathways, 3rd ed., vol. 5. Academic Press, Inc., New York.
9. **Gronlund, A. F., and J. J. R. Campbell.** 1965. Enzymatic degradation of ribosomes during endogenous respiration of *Pseudomonas aeruginosa*. J. Bacteriol. **90**:1–7.
10. **Harwood, C. S., and E. Canale-Parola.** 1981. Branched-chain amino acid fermentation by a marine spirochete: strategy for starvation survival. J. Bacteriol. **148**:109–116.
11. **Harwood, C. S., and E. Canale-Parola.** 1981. Adenosine 5′-triphosphate-yielding pathways of branched-chain amino acid fermentation by a marine spirochete. J. Bacteriol. **148**:117–123.
12. **Henderson, J. F., L. Brox, G. Zombar, D. Hunting, and C. A. Lomax.** 1977. Specificity of adenosine deaminase inhibitors. Biochem. Pharmacol. **26**:1967–1972.
13. **Heppel, L. A., J. Hurwitz, and B. L. Horecker.** 1957. Adenine deaminase of *Azotobacter vinelandii*. J. Am. Chem. Soc. **79**:630–633.
14. **Hoffee, P. A., and M. E. Jones (ed.).** 1978. Purine and pyrimidine nucleotide metabolism. Methods Enzymol. **51**:1–654.
15. **Hoffmeyer, J., and J. Neuhard.** 1971. Metabolism of exogenous purine bases and nucleosides by *Salmonella typhimurium*. J. Bacteriol. **106**:14–24.
16. **Johnson, R. C., and P. Rogers.** 1967. Metabolism of leptospires. II. The action of 8-azaguanine. Can. J. Microbiol. **13**:1621–1629.
17. **Kidder, G. W., V. C. Dewey, and L. L. Nolan.** 1977. Adenine deaminase of a eukaryotic animal cell, *Crithidia fasciculata*. Arch. Biochem. Biophys. **183**:7–12.
18. **Kidder, G. W., and L. L. Nolan.** 1979. Adenine aminohydrolase: occurrence and possible significance in trypanosomid flagellates. Proc. Natl. Acad. Sci. U.S.A. **76**:3670–3672.
19. **Kidder, G. W., L. L. Nolan, and V. C. Dewey.** 1979. The purine phosphoribosyltransferases of *Crithidia fasciculata*. J. Parasitol. **65**:520–525.
20. **Mink, R. W., and R. B. Hespell.** 1981. Survival of *Megasphaera elsdenii* during starvation. Curr. Microbiol. **5**:51–56.
21. **Mink, R. W., and R. B. Hespell.** 1981. Long-term nutrient starvation of continuously cultured (glucose-limited) *Selenomonas ruminantium*. J. Bacteriol. **148**:541–550.
22. **Moat, A. G.** 1979. Microbial physiology, p. 301–323. John Wiley & Sons, Inc., New York.
23. **Postgate, J. R., and J. R. Hunter.** 1962. The survival of starved bacteria. J. Gen. Microbiol. **29**:233–263.