JOURNAL OF BACTERIOLOGY, Jan. 1981, p. 466-471
0021-9193/81/010466-06$02.00/0

Vol. 145, No. 1

Influence of Heme and Vitamin B_{12} on Growth and Fermentations of *Bacteroides* Species

MIN CHEN* AND M. J. WOLIN

Division of Laboratories and Research, New York State Health Department, Albany, New York 12201

We examined the effects of heme on the growth and fermentations of *Bacteroides* species. *Bacteroides fragilis* ATCC 25285 required heme for growth and produced malate and lactate as major products of glucose fermentation when the concentration of heme was 1 ng/ml. With 1 μg of heme per ml, malate was not formed, lactate production decreased, and succinate and acetate were the major fermentation products. *B. eggerthii* ATCC 27754 grew without heme, with the production of mainly malate and lactate from glucose. Its fermentation with 1 μg of heme per ml was similar to that of *B. fragilis* grown with the same concentration of heme. *B. splanchnicus* VPI 6842 grew without heme, with the production of mainly malate, acetate, and H_2 from glucose. With 1 μg of heme per ml, malate disappeared, H_2 decreased significantly, and succinate, acetate, and butyrate were the major products. The addition of vitamin B_{12} to media containing 1 μg of heme per ml caused all species to produce propionate at the expense of succinate and, with *B. splanchnicus*, also at the expense of butyrate. Thus, the concentration of heme and the presence of vitamin B_{12} significantly influenced the course of glucose fermentation by these bacteria.

The growth of several important species of *Bacteroides* of the human large intestine, from human clinical isolates, and of the rumen ecosystem is dependent on or is stimulated by heme (4, 9, 15, 16). Varel and Bryant (16) demonstrated that *Bacteroides fragilis* and closely related species require either vitamin B_{12} or methionine for growth. The kinds of fermentation products formed by these organisms are also affected by the presence or absence of heme and the concentration of heme and the presence or absence of vitamin B_{12}. Macy et al. (9) showed that succinate and propionate are produced from glucose by *B. fragilis* ATCC 23745 in the presence of heme, but fumarate is produced instead when heme is absent. This report extends these findings on the effects of heme to another strain of *B. fragilis* ATCC 25285 and to two other species, *B. splanchnicus* and *B. eggerthii*. Malate rather than fumarate is their major product of glucose fermentation in the absence of or with low concentrations of heme. Heme is also required for the formation of butyrate by *B. splanchnicus*. Vitamin B_{12} is required for the production of propionate by all three species.

MATERIALS AND METHODS

Growth of bacteria. *B. splanchnicus* VPI 6842 and *B. eggerthii* ATCC 27754 were obtained from the culture collection of the Anaerobe Laboratory, Virginia Polytechnic Institute (VPI), and *B. fragilis* ATCC 25285 was from M. P. Bryant. The Hungate technique as modified by Miller and Wolin (10) was used for preparation of media and cultivation. Bacteria were grown in a 10- or 100-ml serum bottle containing 5 or 25 ml of medium, respectively. A complex medium was used that contained, per liter: glucose, 5.0 g; Trypticase (BBL Microbiology Systems), 5.0 g; yeast extract (Difco Laboratories), 2.0 g; K_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$, 0.24 g each; Na_2CO_3, 4.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2 \cdot H_2O$, 0.06 g; resazurin, 1.0 mg; cysteine $HCl \cdot H_2O$, 0.375 g; $Na_2S \cdot 10H_2O$, 0.375 g; dithioerythritol, 15.4 mg; isobutyric, 2-methylbutyric, *n*-valeric, and isovaleric acids, 0.1 ml each. The pH was adjusted to 7.0 with NaOH before gassing with 100% CO_2 and the addition of Na_2CO_3. Unless otherwise stated, 1 μg of hemin per ml was added to the medium. Cysteine and sulfide were added as described previously (5). A defined medium for the growth of *B. fragilis* contained, per liter: glucose, 5.0 g; K_2HPO_4, 5.0 g; L-methionine, 7.5 mg; NaCl and $(NH_4)_2SO_4$, 1.0 g each; Na_2CO_3, 4.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 4.0 mg; resazurin, 1.0 mg; dithioerythritol, 15.4 mg; isobutyric, 2-methylbutyric, *n*-valeric, and isovaleric acids, 0.1 ml each; folinic acid, 10 μg; thiamin-HCl, calcium D-pantothenate, nicotinamide, riboflavin, and pyridoxine-HCl, 2 mg each; biotin, 1 mg; *p*-aminobenzoic acid, 100 μg; folic acid, 50 μg. Heme, cysteine, and sulfide addition, pH adjustment, and gassing were as for the complex medium.

All incubations were at 37°C. Cell turbidity was measured in 10-ml serum bottles at 660 nm with a Bausch & Lomb Spectronic 70 spectrophotometer; the light path was 2 cm. Washed cells of *B. fragilis* were prepared by centrifuging a 5-ml culture three times and suspending each pellet in 5 ml of 10 mM KH_2PO_4–0.85% NaCl–1 mM dithioerythritol, pH 7.0, prepared anaerobically under N_2.

Fermentation analyses. Total gas volume was

determined by adding the calibrated gas space of the serum bottles to the amount of gas displaced into a disposable syringe. Gas composition was determined by gas chromatography as described previously (5). Cultures were then centrifuged at 12,000 × *g* for 15 min at 4°C. Supernatant solutions were stored at −15°C.

For analysis of volatile fatty acids, 0.2 ml of 2 M formic acid was added to 1 ml of culture supernatant solution, which was then injected directly into the gas chromatograph. Dimethyl esters of dicarboxylic acids were prepared by freeze-drying 1.0 ml of supernatant solution (acidified with 0.05 ml of 2 N HCl) and then adding 1.0 ml of 2% H_2SO_4 in dry methanol (vol/vol) in sealed tubes and heating for 2 h at 90°C. The tubes were cooled at room temperature, briefly opened for the addition of approximately 0.05 g of Na_2CO_3, and resealed. Gas chromatography was performed with stainless-steel columns (183 by 0.32 cm) containing 80- to 100-mesh Chromosorb 101 (Supelco, Inc.) for volatile fatty acids and SP-1200–1% H_3PO_4 on 80- to 100-mesh Chromosorb WAW (Supelco, Inc.) for dimethyl esters of dicarboxylic acids. A helium carrier gas flow rate of 120 ml/min was used, and peaks were detected with a flame ionization detector. The column temperature was 190°C for volatile fatty acids and 120°C for dimethyl esters of dicarboxylic acids. Acids were identified and quantitated by comparison of retention times and peak heights with those of standards.

For other analyses, the culture supernatant solutions were clarified by the Somogyi procedure (14). Fumaric and malic acids were assayed with fumarase, malic dehydrogenase, phosphotransacetylase, and citrate synthase as described by Kröger (7). Carbohydrate in the medium was analyzed either by the anthrone procedure with a glucose standard (12) or by the glucose oxidase procedure as described previously (5). Formate was determined by the formyltetrahydrofolate synthetase method of Rabinowitz and Pricer (13). The synthetase was a gift from J. C. Rabinowitz. Ethanol was measured by the alcohol dehydrogenase method of Bonnichsen (3), and lactic acid was measured by the method of Barker and Summerson (2). Glycerol was measured with glycerokinase and L-glycerol-3-phosphate dehydrogenase (18).

RESULTS

Heme requirement for growth. Figure 1 shows that the growth rate of *B. splanchnicus* was increased in the complex medium by the addition of heme up to a concentration of 10 ng/ml.

B. eggerthii did not require heme and grew slowly with a generation time of 7.8 h in its absence, which decreased to 2.0 h in the presence of 100 ng of heme per ml. *B. fragilis* did not grow without heme and required at least 1 ng/ml for growth in the complex medium (Fig. 2). Growth rate and total growth increased with increasing concentrations of heme.

Effect of heme on fermentation products. Table 1 shows the fermentation balances obtained with *B. fragilis* grown with low and high concentrations of heme. The balances were calculated by equating each C_4 product with the corresponding decarboxylation product, i.e., succinate with propionate, malate with lactate, and fumarate with acrylate. This eliminated the necessity of introducing CO_2 as a substrate and permitted the calculation of all fermentation parameters solely in terms of the fermented glucose. With 1 ng of heme per ml, the malate formed by *B. fragilis* comprised about 50% and lactate comprised about 25% of the fermented glucose carbon. Acetate, succinate, propionate, formate, and CO_2 were the minor products (each represented less than 10% of the glucose consumed). With 1 μg of heme per ml, succinate, propionate, acetate, and CO_2 were the major products, and malate, lactate, and formate were the minor products. Butyrate and fumarate were not produced in either medium, and ethanol and H_2 were formed in trace amounts.

Fermentation products of *B. eggerthii* grown without or with 1 μg of heme per ml were very similar to those produced by *B. fragilis* with low and high heme, respectively (Table 2). The major difference was that propionate was not formed by *B. eggerthii* without or with heme.

Analyses of analogous fermentations by *B. splanchnicus* showed that, in contrast to the

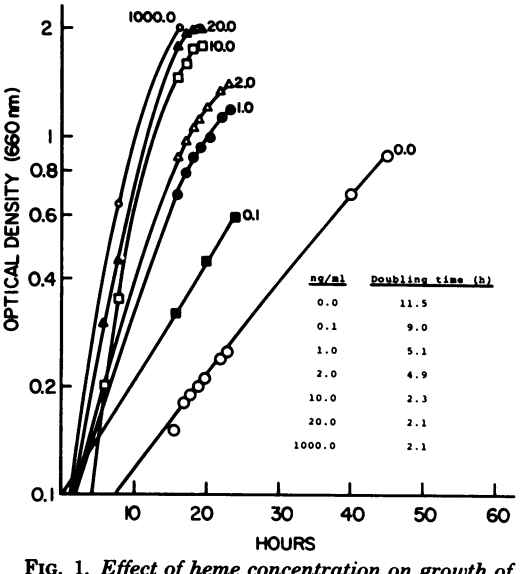

FIG. 1. *Effect of heme concentration on growth of B. splanchnicus. The numbers at the end of each curve are the heme concentrations in nanograms per milliliter. The insert shows the doubling times estimated from the data in the figure for the increase from 0.2 to 0.4 optical density unit. The inoculum was from a culture that had been transferred sequentially by inoculating 0.1 ml in 5 ml of the heme-free medium five times to deplete heme.*

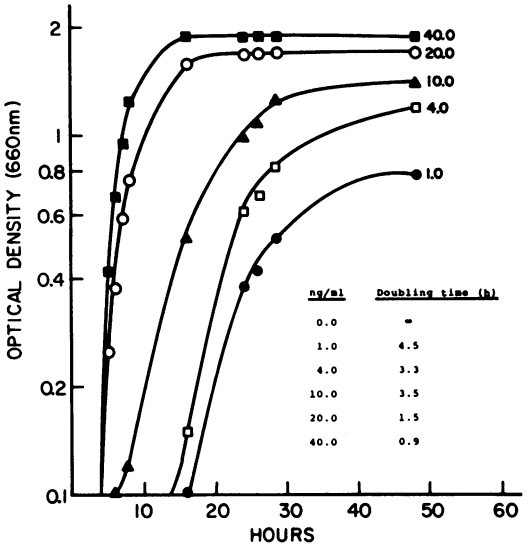

FIG. 2. *Effect of heme concentration on growth of B. fragilis. The numbers at the end of each curve are the heme concentrations in nanograms per milliliter. The insert shows the doubling times estimated from the data in the figure for the increase from 0.2 to 0.4 optical density unit. The inoculum was from a culture that had been transferred in the heme-free medium until a 0.1-ml inoculum contained insufficient heme to support growth in 5 ml of the heme-free medium.*

other two species, it produced significant amounts of H_2 in the absence of heme, and only small amounts of lactate were formed (Table 3). The other major organic products were similar to those formed by the other two species. When heme was present, there was an increase in succinate and a significant depression in the production of H_2 and malate. In addition, butyrate was formed in the presence of heme but not in its absence.

Effect of heme concentration on growth rate and fermentation products of *B. fragilis*. Concentrations of heme between 1 and 40 ng/ml increased the growth rate of *B. fragilis* to its maximum in the complex medium with little or no change in the pattern of fermentation products; significant production of malate occurred with up to 40 ng of heme per ml (Table 4). Little malate was formed with 200 ng of heme per ml, but the growth rate did not increase with heme at between 40 and 200 ng/ml. Lactate production fell to insignificant levels in the presence of 200 ng of heme per ml.

Vitamin B_{12} and fermentation. Addition of vitamin B_{12} to heme-containing media decreased the production of succinate and increased the production of propionate by all three species (Table 5). Succinate, but not propionate, was the major product formed by *B. fragilis* and *B.*

TABLE 1. *Glucose fermentation by B. fragilis*[a]

Product	With heme (1 ng/ml)		With heme (1 μg/ml)	
	mol/100 mol of C_6[b]	% Carbon	mol/100 mol of C_6	% Carbon
Ethanol	Tr	Tr	Tr	Tr
Lactate	53	26.5	1	1.5
Acetate	20	6.7	76	25.3
Butyrate	0	0.0	0	0.0
Malate	107	53.5	11	5.5
Fumarate	0	0.0	0	0.0
Succinate	18	9.0	85	42.5
Propionate	9	4.5	34	17.0
Formate	16	2.7	13	2.2
CO_2[c]	4	0.6	63	10.5
H_2	Tr	Tr	Tr	Tr
Carbon recovery (%)	99		91	
O/R	0.89		1.17	

[a] Fermentation of 5 ml of 0.5% glucose in the complex medium. Incubations were for 48 and 96 h with 1 μg and 1 ng of heme per ml, respectively. Optical densities were ≥2.0 and 1.6, respectively, and glucose disappearance was 26.5 and 25.5 mM, respectively.

[b] The anthrone procedure was used. Blank values for products in uninoculated media were subtracted.

[c] Calculated as equal to acetate minus formate.

TABLE 2. *Glucose fermentation by B. eggerthii*[a]

Product	Without heme		With heme (1 μg/ml)	
	mol/100 mol of C_6[b]	% Carbon	mol/100 mol of C_6	% Carbon
Ethanol	1	0.3	0	0.0
Lactate	73	36.5	22	11.0
Acetate	24	8.0	48	16.0
Butyrate	0	0.0	0	0.0
Malate	73	36.5	0	0.0
Fumarate	0	0.0	0	0.0
Succinate	31	15.5	90	45.0
Propionate	0	0.0	0	0.0
Formate	0	0.0	3	0.5
CO_2[c]	24	4.0	45	7.5
H_2	Tr	Tr	Tr	Tr
Carbon recovery (%)	101		80	
O/R	1.45		1.03	

[a] Fermentation of 5 ml of 0.5% glucose in the complex medium. Incubations were for 48 and 96 h in the presence and absence of heme, respectively; optical densities were ≥2.0 and 1.6, respectively, and glucose disappearance was 26.8 and 18.4 mM, respectively.

[b] The anthrone procedure was used. Blank values for products in uninoculated media were subtracted.

[c] Calculated as equal to acetate minus formate.

TABLE 3. *Glucose fermentation by B. splanchnicus*[a]

Product	Without heme		With heme (1 μg/ml)	
	mol/100 mol C_6[b]	% Carbon	mol/100 mol of C_6	% Carbon
Ethanol	5	1.7	0	0.0
Lactate	5	2.5	1	0.5
Acetate	60	20.0	63	21.0
Butyrate	0	0.0	28	18.6
Malate	105	52.5	0	0.0
Fumarate	10	5.0	0	0.0
Succinate	20	10.0	51	25.5
Propionate	0	0.0	0	0.0
Formate	0	0.0	0	0.0
CO_2[c]	65	10.8	119	20.0
H_2	91		14	
Carbon recovery (%)	103		86	
O/R	1.07		2.00	

[a] Fermentation of 5 ml of 0.5% glucose in the complex medium. Incubations were for 48 and 96 h in the presence and absence of heme, respectively; optical densities were ≥2.0 and 1.8, respectively, and glucose disappearance was 29.6 and 13.0 mM, respectively.

[b] The anthrone procedure was used. Blank values for substrate and products from inoculated media without added glucose were subtracted.

[c] Calculated as equal to acetate plus 2 × butyrate.

TABLE 4. *Heme concentration and B. fragilis fermentation*[a]

Heme (ng/ml)	Doubling time (h)[b]	Products (mol/100 mol of C_6)				
		Acetate	Lactate	Malate	Succinate	Propionate
1	4.5	24	24	99	14	8
4	3.3	38	42	107	18	9
10	3.5	25	39	109	16	7
20	1.5	52	36	68	28	7
40	0.9	52	40	55	54	8
200	0.9	73	3	17	81	18
1,000	0.9	56	6	11	58	35

[a] Complex medium with 26.7 mM glucose without vitamin B_{12}. Incubation was for 48 h, and glucose concentrations used were 5.0, 9.9, 20.1, and 26.7 mM for 1, 4, 10, and 20 to 1,000 μg of heme, respectively, per ml.

[b] The time for increase from 0.2 to 0.4 optical density unit.

splanchnicus when cobalt chloride (0.1 mM) instead of vitamin B_{12} was present in the heme-containing medium (data not shown). Fermentations of glucose by *B. fragilis* were also carried out in a defined medium containing heme which did not support growth unless either 7.5 μg of methionine or 20 ng of vitamin B_{12} per ml was present. Succinate and propionate formation (per 100 mol of glucose) were 65 and 7 mol in the absence and 36 and 46 mol in the presence of vitamin B_{12}. The results were similar to those obtained with the complex medium (Table 5).

With *B. splanchnicus*, the addition of vitamin B_{12} caused not only a change in the relative amounts of succinate and propionate formed, but also a decrease in the production of butyrate (Table 5). The ratio of oxidized to reduced products (O/R) was close to 1 when both vitamin B_{12} and heme were present, whereas with heme alone it was significantly greater than 1 (Table 5). When vitamin B_{12} and heme were both absent, the O/R was also close to 1 (Table 3).

DISCUSSION

Sperry et al. (15) showed that heme was required for growth of several species of saccharolytic bacteroides commonly found in human feces or as human clinical isolates. Other investigators have reported either a requirement for heme or a significant stimulation of growth by heme with strains of *B. fragilis* (9, 15, 16).

Our results with *B. fragilis* ATCC 25285 agree with those of Sperry et al. (15). We were unable to demonstrate an absolute requirement for heme by *B. splanchnicus* and *B. eggerthii*, but heme was highly stimulatory. Sperry et al. reported an absolute heme requirement for *B. eggerthii* VPI B8-51 (15). Strain and medium differences as well as differences in methods used to deplete cells of heme may have contributed to the differences in results reported by

TABLE 5. *Effect of vitamin B_{12} on fermentation*[a]

Product	mol of product per 100 mol of C_6[b]					
	B. splanchnicus		*B. fragilis*		*B. eggerthii*	
	Without B_{12}	With B_{12}[c]	Without B_{12}	With B_{12}	Without B_{12}	With B_{12}
Acetate	76	48	58	61	65	49
Butyrate	33	13	0	0	0	0
Succinate	49	30	92	33	115	70
Propionate	0	69	9	49	0	56
Formate	0	NA[d]	36	36	0	0
H_2	10	14	9	1	0	0
% Carbon[e]	96	89	83	72	90	88
O/R[e]	2.27	1.06	0.72	1.04	1.13	0.78

[a] Fermentation of 25 ml of 28 mM glucose in the complex medium with 2 μg of heme per ml. Incubations were for 72 h, the optical density was ≥2.0, and glucose was completely used.

[b] The glucose oxidase procedure was used. Blank values for products in uninoculated media were subtracted.

[c] Vitamin B_{12}, 20 ng/ml.

[d] NA, Not analyzed.

[e] Calculated CO_2 values (not shown) were included in the calculations.

various investigators. It is also necessary to follow growth for extended periods of time in the absence of heme to be certain of an absolute requirement because of the slow growth rate of stimulated strains grown without heme. With *B. splanchnicus*, the doubling time was approximately 11.5 h in the absence and 2.1 h in the presence 1 μg of heme per ml.

All species produced large amounts of malate when grown with no or low concentrations of heme. Macy et al. (9) reported that fumarate was produced in large amounts by *B. fragilis* grown in the absence of heme, and they did not report that malate was produced. This discrepancy may be due to either the failure of the enzymatic assay used by Macy et al. to distinguish between fumarate and malate or a difference between strains. Using the same assay, which depends on malic dehydrogenase to measure malate and malic dehydrogenase and fumarase to measure fumarate, we found that there was no increase in the rate and amount of reduction of 3-acetylpyridine adenine dinucleotide when fumarase was added to the assay system. This was also the case with authentic L-malate, but not with authentic fumarate, when fumarase was required for rapid reduction of the pyridine nucleotide. Although gas chromatography did not distinguish between succinate and fumarate, there was no correspondence between the amount of dicarboxylic acid detected in the "succinate-fumarate" peak and the amount of malate detected enzymatically. There was a separate peak that corresponded in retention time to the peak of the dimethyl ester of malic acid and in amount to that detected in the enzymatic assay. Sperry et al. (15) used the gas chromatography method of Holdeman and Moore (8), which is not reported to detect malate but detects fumarate, to measure dicarboxylic acid formed by *B. fragilis* and did not find any malate or fumarate in low-heme media. It is likely that malate was formed and was not detected by the analytical method.

Other than malate, there are differences in the products formed by the strains of the three species we examined when they were grown with no or low heme. *B. eggerthii* and *B. fragilis* formed significant amounts of lactate, but *B. splanchnicus* did not. Macy et al. reported the production of large amounts of lactate (measured enzymatically) for *B. fragilis* ATCC 23745 grown without heme (9), and Sperry et al. reported relatively small amounts for the same strain grown with low heme and none by *B. fragilis* ATCC 25285 grown under the same conditions (15). *B. splanchnicus* is the only one of these species that produced significant amounts of H_2 and only when no or low heme was present. None of the species produced significant amounts of glycerol. Although malate appears to be a major electron sink product for these bacteria grown with no or low heme, there are alternatives which lead to differences in other products. Additional investigation is necessary to see if there are any relationships between these differences and other taxonomic or physiological features of different strains of these species.

With all strains, there is a common effect on the course of glucose fermentation produced by the presence of relatively high concentrations of heme. There is a shift to the formation of succinate and acetate at the expense of those products that accumulate in low or no heme-containing media. This is presumably a consequence of the ability of the cells to produce a cytochrome from added heme that is necessary for the reduction of fumarate to succinate (9, 17). In the case of *B. splanchnicus* formation of an additional product, butyrate, is dependent on high concentrations of heme. Whether succinate or propionate accumulates depends on the absence or presence of vitamin B_{12}. The organisms appear to have the capability of producing the apoenzyme necessary for isomerizing succinyl-coenzyme A to methylmalonyl-coenzyme A but require vitamin B_{12} to synthesize the cobalamin coenzyme necessary for the reaction (1). *B. fragilis* was the only one of the three species that produced some propionate in the absence of vitamin B_{12} (Table 5). This might indicate a limited ability to synthesize a small amount of vitamin B_{12} or a B_{12}-independent pathway for production of propionate. In a few experiments, *B. fragilis* produced significant amounts of propionate when large amounts of heme were added in the absence of vitamin B_{12} (Tables 1 and 4). This might have been caused by a small contamination of heme with vitamin B_{12}.

The fermentation by *B. splanchnicus* is complicated and not well understood. When high heme is present, the O/R ratio is closer to 2.0 than to the expected 1.0 despite the fact that carbon recoveries based on glucose disappearance approach 100%. Without heme or with heme and vitamin B_{12}, the O/R ratio is close to 1.0 and carbon recoveries are about 90 to 100%. The results in this study indicate that butyrate formation is strongly dependent on nutritional conditions. The acid is not produced in the absence of heme nor is it produced when both heme and vitamin B_{12} are present.

When heme was added to the medium in increasing concentrations, all of the species examined showed increasing rates of growth. Macy et al. attributed the increased growth rate and yield obtained with *B. fragilis* when heme was

added to a medium containing growth-limiting glucose to a shift to succinate formation which could provide more energy for growth (9). The amount of ATP formed per mole of glucose used and its rate of formation are increased when heme is added, and there is a switch from a predominantly malate to a predominantly succinate or propionate fermentation. Although this explanation probably accounts for the major portion of the increase in growth rate we observed, increases in the growth rate of *B. fragilis* occurred with amounts of heme that did not produce a significant shift in the fermentation (Table 4). In addition to causing a fermentation shift, it is possible that heme has an additional role that influences growth rate, perhaps by participating in a rate-limiting biosynthetic reaction.

It is not clear why these saccharolytic bacteroides have a multiplicity of potential fermentation pathways. In natural habitats, i.e., the intestinal tract and tissues, they probably would carry out a propionate fermentation because of the availability of heme produced by either other bacteria or the host. Bacteroides species should have access to vitamin B_{12} or other cobalamins. Cullen and Oace showed a significant increase in urinary methylmalonate excretion and fecal vitamin B_{12} excretion in B_{12}-depleted, pectin-fed rats (6). This may have been due to increased growth of organisms similar to the saccharolytic bacteroides we have studied when increased energy source (pectin) was available in the large intestine. The bacteria could have taken up the endogenous vitamin B_{12} excreted into the large intestine in bile and produced large amounts of propionate that was absorbed by the rat and converted in its liver to methylmalonate. Excretion rather than utilization occurred because of the vitamin B_{12} deficiency. Rumen saccharolytic bacteroides never produce propionate by the succinate pathway even when supplied with vitamin B_{12} (11). They accumulate succinate. Although some strains of *B. ruminicola* require heme (4), there has been no report that they produce malate when grown with limiting heme, although this possibility has not been investigated. It is possible that there are some special environmental scenarios in human habitats in which heme or vitamin B_{12} is deficient and the malate or succinate fermentations are significant for the growth of the saccharolytic bacteroides.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-12461 from the National Institute of Allergy and Infectious Diseases.

We thank E. Currenti, M. Garfinkel, P. Maleck, and R. Toombs for technical assistance.

LITERATURE CITED

1. **Barker, H. A.** 1967. Biochemical functions of corrinoid compounds. Biochem. J. **105**:1-15.
2. **Barker, S. B., and W. H. Summerson.** 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. **138**:535-554.
3. **Bonnichsen, R.** 1965. Ethanol, p. 285-287. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
4. **Caldwell, D. R., D. C. White, M. P. Bryant, and R. N. Doetsch.** 1965. Specificity of the heme requirement for growth of *Bacteroides ruminicola*. J. Bacteriol. **90**: 1645-1654.
5. **Chen, M., and M. J. Wolin.** 1977. Influence of CH_4 production by *Methanobacterium ruminantium* on the fermentation of glucose and lactate by *Selenomonas ruminantium*. Appl. Environ. Microbiol. **34**:756-759.
6. **Cullen, R. W., and S. M. Oace.** 1978. Methylmalonic acid and vitamin B_{12} excretion of rats consuming diets varying in cellulose and pectin. J. Nutr. **108**:640-647.
7. **Kröger, A.** 1974. Electron transport phosphorylation coupled to fumarate reduction in anaerobically grown *Proteus rettgeri*. Biochim. Biophys. Acta **347**:273-289.
8. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1975. Anaerobe laboratory manual, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg.
9. **Macy, J., I. Probst, and G. Gottschalk.** 1975. Evidence for cytochrome involvement in fumarate reduction and adenosine 5'-triphosphate synthesis by *Bacteroides fragilis* grown in the presence of hemin. J. Bacteriol. **123**: 436-442.
10. **Miller, T. L., and M. J. Wolin.** 1974. A serum bottle modification of the Hungate technique for cultivating anaerobes. Appl. Microbiol. **27**:985-987.
11. **Miller, T. L., and M. J. Wolin.** 1979. Fermentations by saccharolytic intestinal bacteria. Am. J. Clin. Nutr. **32**: 164-172.
12. **Morris, D. L.** 1948. Colorimetric determination of total carbohydrates. Science **107**:254-255.
13. **Rabinowitz, J. C., and W. E. Pricer, Jr.** 1965. Formate, p. 308-312. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
14. **Somogyi, M.** 1930. A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem. **86**:655-663.
15. **Sperry, J. F., M. D. Appleman, and T. D. Wilkins.** 1977. Requirement of heme for growth of *Bacteroides fragilis*. Appl. Environ. Microbiol. **34**:386-390.
16. **Varel, V. H., and M. P. Bryant.** 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl. Microbiol. **28**:251-257.
17. **White, D. C., M. P. Bryant, and D. R. Caldwell.** 1962. Cytochrome-linked fermentation in *Bacteroides ruminicola*. J. Bacteriol. **84**:822-828.
18. **Wieland, O.** 1965. Glycerol, p. 211-214. *In* H. U. Bergmeyer (ed.), Methods in enzymatic analysis. Academic Press, Inc., New York.