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Fermentation of Peptides by *Bacteroides ruminicola* B_14

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The maximum growth rate of *Bacteroides ruminicola* B_14 was significantly improved when either Trypticase or acetate and C_4-C_5 fatty acids were added to defined medium containing macrominerals, microminerals, vitamins, hemin, cysteine hydrochloride, and glucose. The organism was unable to grow with peptides as the sole energy source, but growth yields from glucose were significantly improved when Trypticase was added to batch cultures containing basal medium, acetate, and C_4-C_5 volatile fatty acids. During periods of rapid growth, very little peptide was deaminated to ammonia, but after growth ceased there was a linear increase in ammonia. Fifteen grams of Trypticase per liter resulted in maximum ammonia production. In glucose-limited chemostats, ammonia production from peptides was inversely proportional to the dilution rate, and 87% of the variation in ammonia production could be explained by retention time in the culture vessel. Chemostats receiving Trypticase had higher theoretical maximum growth yields and lower maintenance energy expenditures than similar cultures not receiving peptide. Cells from the Trypticase cultures contained more carbohydrate, and this difference was most evident at rapid dilution rates. When corrections were made for cell composition and the amount of peptides that were fermented, it appeared that peptide carbon skeletons could be used for maintenance energy. *B. ruminicola* B_14 was unable to grow on peptides alone because it was unable to utilize peptides at a fast enough rate to meet its maintenance requirement.

When feedstuffs are ingested by ruminants, proteins may be fermented to ammonia and volatile fatty acids by the rumen microflora (1, 3, 25, 29). If the rate of protein fermentation exceeds the rate of ammonia utilization for microbial growth, large quantities of ammonia can accumulate in the rumen (2, 21). Because ammonia can be absorbed into portal blood and converted to urinary urea, excessive ammonia production can decrease the nitrogen retention of the animal (19, 29, 39).

Since protein fermentation in the rumen is generally considered wasteful, ruminant nutritionists have sought ways of reducing its magnitude (16). Protein fermentation occurs in three steps: (i) proteolysis, (ii) transport of amino acids or peptides, and (iii) amino acid deamination and fermentation. Although knowledge about all three of these areas is lacking, proteolysis has received more attention because feedstuff proteins show different susceptibilities to this step (30, 47).

An understanding of amino acid deamination and fermentation has been complicated by several factors. The complexity or diversity of amino acids and rumen bacteria are both very great (21). Most rumen bacteria are unable to grow on amino acids or peptides alone and require carbohydrate (4, 21). Some of these organisms can transport peptides but are unable to take up many free amino acids (32, 33).

Batch culture studies by Bladen et al. indicated that a variety of rumen bacteria were able to produce ammonia from protein hydrolyzate. *Megasphaera elsdenii, Bacteroides ruminicola*, and *Selenomonas ruminantium* were most active (4). Scheifinger et al. also showed that different strains of rumen bacteria would utilize amino acids at different rates, but amino acid assimilation could not be differentiated from deamination because ammonia was not measured (41). More recently, Cotta and Russell reported that the growth yields of several rumen species could be increased if Trypticase was added to glucose-limited chemostats. This latter study did not elucidate whether the increased yields were due to a decrease in maintenance energy expenditure or an increase in theoretical maximum growth yield (13).

The following series of experiments was conducted with *B. ruminicola* because Bladen et al. concluded that "on a basis of number of strains and amount of ammonia produced, *B. ruminicola* is usually the most important ammonia-

producing bacterium in the rumen of mature cattle" (4). Most incubations were performed in continuous culture so that growth rate, maintenance energy, and growth yields could be measured under steady-state conditions. The primary objectives of the study were to see whether the rate of amino acid deamination was influenced by growth rate and to estimate how much energy could be derived from amino acid fermentation.

MATERIALS AND METHODS

Organisms, media, and cell growth. *B. ruminicola* B_14, obtained from the culture collection of M. P. Bryant, was used (8). The basal medium contained macrominerals, sodium carbonate (10), microminerals (40), vitamins (13), hemin (1 mg/liter), cysteine hydrochloride (0.5 g/liter), and glucose (amounts indicated in tables or figure legends). Trypticase (BBL Microbiology Systems, Cockeysville, Md.) or acetate, isobutyrate, valerate, isovalerate, and 2-methylbutyrate were sometimes added to the incubation medium, and the concentrations of these compounds are listed in the text, tables, or figure legends. Previous work has indicated that Trypticase is primarily composed of peptides and contains few free amino acids (J. B. Russell, C. J. Sniffen, and P. J. Van Soest, J. Dairy Sci., in press). The manufacturer indicated that Trypticase contains 10% ash, 11.7% total nitrogen, and 0% carbohydrate. The methods for batch and continuous culture media preparations have been previously described (35, 36). When the bacterium was grown in continuous culture, at least a 98% turnover of medium was allowed before a sample was taken at a new dilution rate. Previous work has indicated that a steady-state optical density is achieved by such a turnover if the change in culture conditions is not drastic (13, 36–38).

Sampling. After samples were withdrawn from the culture vessel, pH and optical density (Gilford spectrophotometer model 260 at 600 nm with cuvettes of 1-cm light path) were recorded. Cells were removed from the medium by centrifugation (0°C, 10,000 × *g*, 20 min). The cell pellet was washed, recentrifuged, and suspended in distilled water. The washed cell suspension and cell-free medium samples were stored at −15°C until analyzed.

Analyses. Bacterial weights were determined after the washed cell suspensions were dried on aluminum pans (105°C). A previous report indicated that this organism did not lyse during distilled water washing because 100% of the cellular DNA could be recovered in the pellet (20). We performed similar measurements and our results verified this earlier claim. Cells were analyzed according to methods described by Mink and Hespell (26). Cell nucleic acids were extracted with hot 0.5 N perchloric acid. DNA and RNA in the supernatants were determined by the diphenylamine and orcinol procedures of Burton (9) and Schneider (42), respectively. In each case, samples were compared against purified RNA and DNA (Sigma Chemical Co., St. Louis, Mo.). Protein from NaOH-hydrolyzed cells (0.2 N, 70°C, 30 min) was assayed by the method of Lowry (22) and compared to a bovine serum albumin standard curve. Cellular carbohydrate was measured by the phenol-sulfuric acid procedure (26) after the polysaccharides had been extracted with hot hydrochloric acid (9 N final concentration, 110°C, 4 h under a N_2 atmosphere). The resulting color was compared against a glucose standard curve. Fermentation acids and glucose were analyzed by high-pressure liquid chromatography (Beckman model 334 liquid chromatograph, model 156 refractive index detector, model 421 CRT data controller, CRIA integrator), using a Bio-Rad HPX-87 organic acid column (see reference 15). Ammonia was determined by the colorimetric assay of Chaney and Marbach (12). Cysteine interference to ammonia color formation was minimized by using six times as much reagent.

To ascertain the amounts of ammonia or peptides that were used for microbial protein synthesis, [^{15}N]ammonium sulfate was added to the incubations described in Table 2. Washed cell suspensions from this experiment were evaporated to dryness in a 100°C oven and digested in 3 ml of concentrated H_2SO_4 at 450°C with 1.1 g of catalyst (100:10:1 K_2SO_4/$CuSO_4$/selenium). Digested samples were diluted with water, made basic with 20 ml of 28% NaOH, and distilled into 2% boric acid. Total ammonia from cell-free supernatant was determined by distilling the sample with MgO and likewise collecting it into boric acid. Distillates were titrated with 0.100 N H_2SO_4 and dried in a 100°C oven. The ^{15}N content was determined with a Micromass 622 mass spectrometer, using an inlet system similar to that described by Porter and O'Deen (34).

Statistical methods. Batch culture incubations were performed in duplicate, and the data represent the average of these duplicates. Separate incubations were also performed during continuous culture studies, and the results of those separate inoculations are shown in Fig. 2 through 5. The correlation coefficients (*r*), intercepts to the ordinate (*a*), and slopes (*b*) are shown for the plots in Fig. 3 through 5 (43). Routine laboratory analyses (Tables 1 through 4) were performed in duplicate, and the variation between replicates was less than 10%.

RESULTS

When *B. ruminicola* B_14 was grown in basal medium without Trypticase or volatile fatty acids, the maximum growth rate was low (Table 1). The addition of acetate alone did not improve growth rate greatly, but C_4-C_5 volatile fatty acids or Trypticase were emendatory. Synergistic relationships among acetate, C_4-C_5 acids, and Trypticase were sometimes observed, but high maximum growth rates (0.68 h^{-1}) could be obtained only if Trypticase or acetate and C_4-C_5 acids were added. Similar results were reported by Bryant and Robinson (7), and subsequent incubations were conducted with Trypticase, 25 mM acetate plus 0.25 mM C_4-C_5 acids, or both.

To ascertain the level of Trypticase that would maximize peptide fermentation and ammonia production, *B. ruminicola* B_14 was grown in batch culture with various concentrations of Trypticase. If Trypticase concentration was increased from 0 to 15 g/liter, cell-free ammonia

TABLE 1. Influence of Trypticase or volatile fatty acids on the maximum growth rate of *B. ruminicola* B_14 in batch culture[a]

Addition[b]	Maximum growth rate (h^{-1})
Nothing	0.33
Low acetate	0.32
High acetate	0.39
C_4–C_5 acids	0.53
Trypticase	0.66
Low acetate + C_4–C_5 acids	0.46
Low acetate + Trypticase	0.69
Low acetate + C_4–C_5 acids + Trypticase	0.71
High acetate + C_4–C_5 acids	0.68
High acetate + Trypticase	0.72
High acetate + C_4–C_5 acids + Trypticase	0.84

[a] Basal medium with 6.0 g of glucose per liter; growth rates were measured from 0.05 to 0.60 optical density.

[b] Concentrations used: low acetate, 7.6 mM acetate; high acetate, 25.0 mM acetate; C_4–C_5 acids, 0.25 mM isobutyrate, valerate, isovalerate, and 2-methylbutyrate; Trypticase, 15 g/liter.

rose, but further increments of Trypticase (20 and 25 g/liter) did not yield additional ammonia after 48 h of incubation (data not shown). At a Trypticase concentration of 15 g/liter, cell protein synthesis proceeded at a logarithmic rate until glucose was depleted (Fig. 1). Further growth was not observed even though there was a large amount of undegraded Trypticase left in the medium. During the growth phase, there was little change in the concentration of extracellular ammonia, but thereafter ammonia increased at a linear rate of 1.52 mg/liter per h. When Trypticase was not available, growth lagged for 4 h. At 4 h, rapid growth ensued, but the conversion of glucose to cell protein was less than when Trypticase was provided. Cultures not receiving Trypticase used a considerable amount of ammonia during the growth phase (0 to 7 h), but after this time, some accumulation (approximately 0.7 mg/liter per h) was observed.

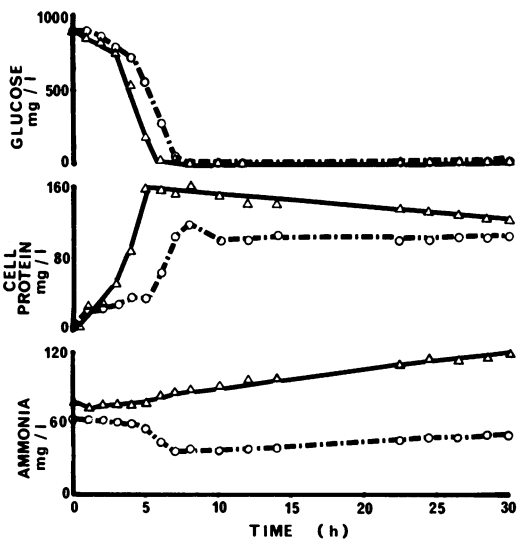

FIG. 1. Effect of 15 g of Trypticase per liter (△) or no added Trypticase (○) on glucose utilization, cell protein synthesis, and ammonia production or utilization by *B. ruminicola* B_14. Both cultures contained basal medium plus 1 g of glucose per liter, 25 mM acetate, and 0.25 mM isobutyrate, valerate, isovalerate, and 2-methylbutyrate.

Whenever Trypticase was added to the medium at 15 g/liter, the net utilization of extracellular ammonia was small (Fig. 1). This net change, however, did not necessarily represent total flux. If Trypticase was fermented to extracellular ammonia, and if this extracellular ammonia was then used for microbial growth, the gross production of ammonia could have been greater. When *B. ruminicola* B_14 was incubated with ^{15}N-labeled ammonium sulfate and 15 g of Trypticase and 1.0 g of glucose per liter, little ^{15}N accumulated in the cells, and the specific activity of the ammonia pool was relatively constant (Table 2). After growth ceased, there was some decline in ammonia specific activity, but this decline could be explained by the net accumulation of ammonia. Such results would indicate that a minor amount of Trypticase nitrogen was cycled through the extracellular ammonia pool and back into cell nitrogen. It was then assumed that net changes in extracellular ammonia could be used to estimate Trypticase fermentation.

When *B. ruminicola* B_14 was grown in continuous culture with acetate and C_4-C_5 acids, a considerable amount of ammonia was used (see Fig. 2). Substitution of Trypticase for acetate and C_4-C_5 acids in the medium reservoir resulted in higher steady-state levels of ammonia, but net production was dependent on the dilution rate. At high dilution rates, very little increase in ammonia level was noted, but as the dilution rate was decreased, ammonia concentrations rose. A plot of the increase in ammonia per milligram of bacterial protein versus time (inverse of dilution rate) was linear, with a correlation coefficient of 0.93 (Fig. 3). By squaring the correlation coefficient, one could estimate that 87% of the variation in ammonia concentration was explained by retention time in the culture vessel.

When 1/yield was plotted against 1/dilution rate, according to the method of Pirt (31), the theoretical maximum growth yield from glucose was greater when Trypticase was available (Fig. 4). Because *B. ruminicola* B_14 produced little

TABLE 2. The incubation of *B. ruminicola* B_14 with ^{15}N-labeled ammonium sulfate, Trypticase, and glucose[a]

Time (h)	Optical density	Cell nitrogen (mg/liter)	Sp act of cell nitrogen (% ^{15}N)	Ammonia nitrogen (mg/liter)	Change in ammonia nitrogen (mg/liter)	Sp act of ammonia nitrogen (% ^{15}N)
0	0.093	3	−0.04	79.5	0	22.00
2	0.250	11	0.42	80.1	0.6	20.80
4	0.700	29	2.03	80.2	0.7	20.46
6	1.255	45	1.99	81.9	2.4	18.60
8	1.048	35	1.94	99.6	20.1	15.28

[a] Trypticase, glucose, and ammonia were provided at 15, 1.0, and .065 g/liter, respectively.

ammonia at high dilution rates, this difference in yield must have resulted from a direct incorporation of Trypticase into cell material. Maintenance energy, the slope of the double-reciprocal plot, was less when Trypticase was present (0.051 versus 0.061 mg of glucose per mg of bacteria per h), and this contrast suggested that *B. ruminicola* B_14 was able to derive some energy from Trypticase fermentation. The maintenance plots showed some divergence from linearity at high dilution rates (0.55 h^{-1}), and these points were not included in the linear regressions (Fig. 4). Similar results were obtained by Neijssel and Tempest, and they argued that fast-growing cells have greater concentrations of osmotically active or labile molecules and should exhibit somewhat higher maintenance energy expenditures (28).

Analyses of the cells for protein, carbohydrate, RNA, and DNA indicated that cell composition was influenced by Trypticase and dilution rate (Table 3). Many bacteria regulate macromolecular synthesis to accommodate changes in growth rate (24), but the nucleic acid content of these two cultures did not vary greatly. However, the Trypticase chemostats contained cells that were richer in carbohydrate, and this difference was more pronounced at high dilution rates. From 68 to 86% of bacterial dry matter could be accounted for in the carbohydrate, protein, RNA, and DNA pools. Lipid, peptidoglycan, and ash were not measured, but Hespell and Bryant indicated that rumen bacteria contain 7 to 25% lipid, 2% peptidoglycan, and 4.4% ash (18). Given this, nearly all of the bacterial dry matter could be accounted for as

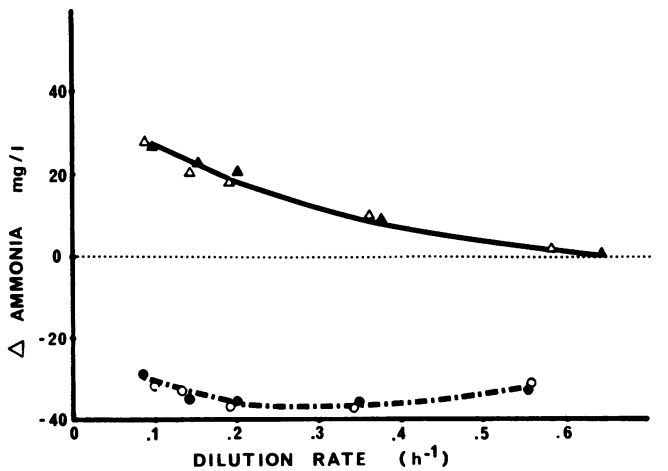

FIG. 2. Effect of 15 g of Trypticase per liter (△, ▲) or no added Trypticase (○, ●) on the production or utilization of ammonia by *B. ruminicola* B_14 in continuous culture. Ammonia production or utilization was calculated by taking the difference in ammonia concentrations between the medium reservoirs and culture vessels. The 15 g of Trypticase per liter medium reservoirs (△, ▲) and without Trypticase medium reservoirs (○, ●) contained 77 and 64 mg of ammonia per liter, respectively. Glucose was provided at 970 mg/liter. Open and closed symbols refer to separate chemostat incubations.

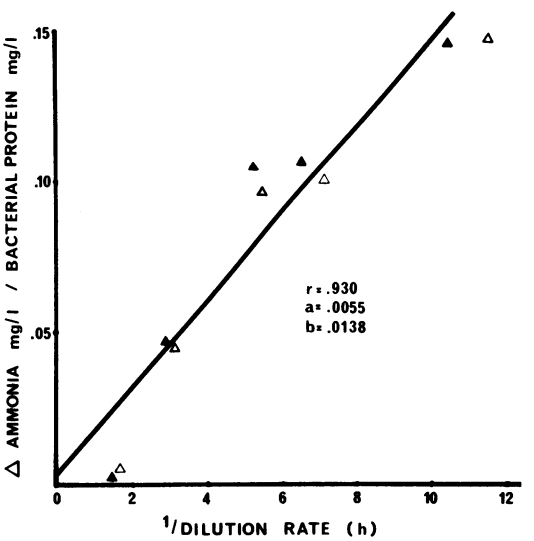

FIG. 3. Change in ammonia per milligram of bacteria per hour during the continuous culture of *B. ruminicola* B_14 with 15 g of Trypticase per liter. The medium reservoir concentrations of glucose and ammonia were 97 and 77 mg/liter, respectively. Open and closed symbols refer to separate chemostat incubations.

cell components. Since cellular carbohydrate requires very little energy for its formation, very high cell yields can be obtained if storage polysaccharide levels are high (44, 45). In Fig. 5, the yields were corrected for cellular carbohydrate by a method similar to that described by Howlett et al. (20). In this case, total cell carbohydrate was subtracted from the bacterial dry weights and the glucose that was fermented.

Analysis of the fermentation products (Table 4 and Fig. 2) indicated that a significant amount of Trypticase was incorporated into cell material or fermented to ammonia and organic acids. To obtain more realistic comparisons of energy availability between the cultures receiving and lacking Trypticase, the double-reciprocal plot in Fig. 5 was also corrected for peptide fermentation. The amount of fermented peptide was calculated by multiplying the increase in ammonia nitrogen by 7.50 (Trypticase on an ash-free basis is approximately 13.3% nitrogen). A carbohydrate equivalent was calculated by multiplying the peptide equivalent by 86%. This 86% factor was used because approximately 14% of the weight of an average amino acid is derived from the amino group and cannot be used for ATP generation. Glucose equivalents are the sum of glucose that was used for carbohydrate-free cell synthesis and the carbohydrate equivalent that resulted from ammonia formation (Fig. 5).

Correction for cell carbohydrate and Trypticase fermentation caused the cell yields to decrease, but the Trypticase cultures once again had higher yields than the cultures not receiving Trypticase (Fig. 5). The slopes of these double-reciprocal plots were nearly identical. Because 0 to 15% of the glucose equivalent in the Trypticase plot was derived from peptide fermentation, it was apparent that *B. ruminicola* B_14 derived a significant amount of energy from

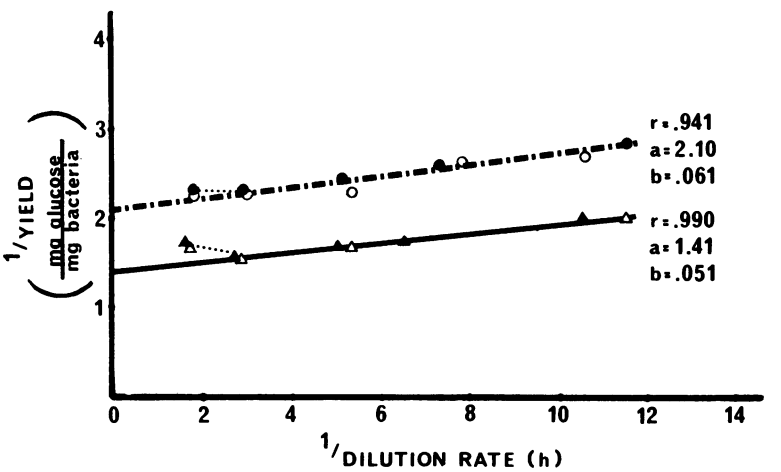

FIG 4. Double-reciprocal plot of 1/yield versus 1/dilution rate for the continuous culture of *B. ruminicola* B_14 with 15 g of Trypticase per liter (△, ▲) and without Trypticase (○, ●). Open and closed symbols refer to separate chemostat incubations. Theoretical maximum growth yields were 71 mg of bacteria per 100 mg of glucose (128 mg of bacteria per mmol of glucose) and 48 mg of bacteria per 100 mg of glucose (85.7 mg bacteria per mmol of glucose) for the 15 g of Trypticase per liter and without Trypticase cultures, respectively.

TABLE 3. Cell compositions for the growth of *B. ruminicola* B_14 in continuous culture with and without Trypticase

Culture and dilution rate (h^{-1})	Cell composition (%)				
	Cell protein	Cell carbohydrate	RNA	DNA	Total
No Trypticase					
0.550–0.640	43	9	19	1	72
0.337–0.373	41	6	21	1	69
0.187–0.200	44	5	18	1	68
0.128–0.153	46	6	17	1	70
0.087–0.095	48	7	18	1	74
Trypticase (15 g/liter)					
0.550–0.640	38	21	18	1	78
0.337–0.373	43	16	16	1	76
0.187–0.200	51	14	19	2	86
0.128–0.153	45	13	22	2	82
0.087–0.095	52	6	18	2	78

peptide fermentation even though it is unable to grow on peptides alone.

DISCUSSION

B. ruminicola B_14 was unable to grow on Trypticase alone, but the growth yields of glucose-limited cultures were significantly enhanced by Trypticase addition (Fig. 1 and 4). These increases in bacterial dry matter suggested that Trypticase metabolism was improving the energetics of bacterial growth. However, the synthesis of cell material is not always proportional to the availability of an energy source. Changes in cell composition (44), fermentation pathways (17), maintenance energy (31, 45, 46), and "uncoupling of growth" (45) can all affect the efficiency of energy source utilization for growth. In our experiments *B. ruminicola* B_14 was grown in glucose-limited chemostats and uncoupled growth was unlikely. Because growth rate could be ascertained under steady-state conditions, the influence of maintenance energy could likewise be determined. Glucose was primarily metabolized to acetate and succinate, but the ratio of these products was not always constant (Table 4). Nonetheless, when 1/yield was plotted against 1/dilution rate, the relationship was linear over a wide range of dilution

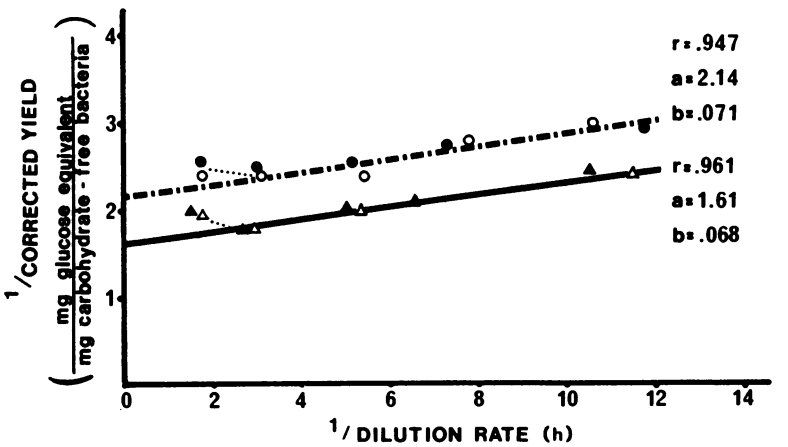

FIG. 5. Corrected double-reciprocal plot of 1/yield versus 1/dilution rate for the continuous culture of *B. ruminicola* B_14 with 15 g of Trypticase per liter (△, ▲) and without Trypticase (○, ●). Open and closed symbols refer to separate chemostat incubations. Theoretical maximum growth yields were 62 mg of carbohydrate-free bacteria per 100 mg of glucose equivalent (117 mg of carbohydrate-free bacteria per mmol of glucose) and 47 mg of carbohydrate-free bacteria per 100 mg of glucose equivalent (84 mg of carbohydrate-free bacteria per mmol of glucose) for the 15 g of Trypticase per liter and without Trypticase cultures, respectively.

TABLE 4. Carbon balance for the growth of *B. ruminicola* B_14 in continuous culture with and without Trypticase

Culture and dilution rate (h^{-1})	Carbon balance (mg of C atoms/liter)								
	Succinate[a]	Acetate	Formate	Propionate	Cells[b]	Total product	Glucose used	Glucose equivalents used[c]	% Recovery
No Trypticase									
0.550–0.640	105	15	ND[d]	ND	178	298	326		92
0.337–0.373	143	12	ND	ND	208	363	383		95
0.187–0.200	131	14	ND	ND	205	350	383		91
0.128–0.153	149	28	ND	ND	184	361	383		94
0.087–0.095	150	32	ND	4	176	362	383		95
Trypticase									
0.550–0.640	142	67	9	ND	234	452		324	140
0.337–0.373	186	63	ND	4	296	549		389	141
0.187–0.200	229	73	ND	ND	287	589		426	138
0.128–0.153	224	71	ND	ND	275	588		432	136
0.087–0.095	250	78	ND	ND	246	574		448	129

[a] The value for succinate was multiplied by 0.63 to correct for carbon dioxide incorporation.
[b] Cells were assumed to be 50% carbon.
[c] Also includes amino acid carbon skeletons that were fermented. See the text for explanation.
[d] ND, Not detectable.

rates (Fig. 4 and 5), and it did not seem that the ATP yield per mole of glucose was changing. If *B. ruminicola* has a cytochrome-linked fumarate reductase like *B. fragilis*, one would expect the ATP yields from acetate and succinate production to be similar (17, 23, 49). The cell composition and the amount of energy from Trypticase fermentation were not constant, and corrections were employed so that the energetic effects of Trypticase utilization could be evaluated.

In continuous culture, Trypticase fermentation to ammonia was inversely proportional to dilution rate (Fig. 2), but even at a dilution rate of 0.087 h^{-1}, less than 5% of the available Trypticase was degraded. The high background of undegraded material made direct assessment of Trypticase disappearance unfeasible. Increases in ammonia, however, could be made with confidence, and the use of ammonia to estimate total Trypticase fermentation seemed appropriate because little nitrogen was cycled from Trypticase through the ammonia pool and back into cell nitrogen (Table 2).

When plots of 1/yield versus 1/dilution rate were compared (Fig. 4 and 5), it was apparent that Trypticase could be used for maintenance energy. The question then arises, if energy from amino acid fermentation could be used for maintenance, why was *B. ruminicola* B_14 unable to grow on peptide alone? In continuous culture, ammonia was produced at a rate of 0.0138 mg of ammonia per mg of bacterial protein per h (Fig. 3) or 0.006 mg of ammonia per mg of bacteria per h (the bacteria were approximately 45% protein, see Table 3). This rate then corresponded to 0.039 mg of carbon skeleton per mg of bacteria per h when peptide fermentation was calculated from ammonia production (see above for explanation). Assuming that the carbon skeletons from peptide fermentation were used with equal efficiency to glucose, a rate of 0.039 mg of glucose equivalent per mg of bacteria per h was still less than the maintenance energy requirement of 0.068 (Fig. 5). It appeared that *B. ruminicola* B_14 was unable to grow on peptides alone because it is unable to transport or ferment (or both) peptides at a fast enough rate to meet its maintenance energy requirements.

When Trypticase was present at 15 g/liter, cell yields from glucose were high (Fig. 1, 4, and 5), ammonia utilization was minimal (Table 2, Fig. 2), and the recovery of cells and fermentation acids was more than could be explained by glucose or peptide fermentation (Table 4). Such results indicate that much of the cell nitrogen and carbon was derived from Trypticase. Previous experiments showed that many rumen bacteria prefer ammonia to amino nitrogen and grow poorly if amino acids or peptides are the sole nitrogen source (1, 5). When strains of *B. ruminicola* were incubated with ammonia and ^{14}C algal protein hydrolyzate, the B_14 strain incorporated approximately twice as much ^{14}C as strains 23, 85, and GA33 (6). Even though the B_14 strain was more efficient at utilizing amino nitrogen, the specific incorporation by other rumen bacteria was often greater (6). The ability of *B. ruminicola* to incorporate more amino nitrogen in these experiments may be related to the source (Trypticase versus algal protein hy-

drolyzate) or concentration (15 versus 4 g/liter) of the amino acid source.

Fermentation analyses revealed that succinate and acetate were the major products of *B. ruminicola* B_14 (Table 4). Other investigators have shown that *B. ruminicola* can produce formate (8, 20, 27) and propionate (14, 48), but we were only able to detect trace amounts of these compounds. The lack of propionate may be related to the absence of rumen fluid in the medium. Because acetate production was low and bacterial cells were somewhat reduced, one would not have expected high levels of formate (50). The failure of *B. ruminicola* B_14 to produce significant levels of propionate may reflect differences between strain B_14 and strain GA33 or an interaction between propionate production and rumen fluid in the medium (48). Fermentation of branched-chain amino acids would theoretically give rise to branched-chain volatile fatty acids, but Trypticase interference during the high-pressure liquid chromatography analyses made quantitation of these acids impossible. Based on the ammonia production data, total branched-chain volatile fatty acid production would have been less than 22 mg/liter.

B. ruminicola strains differ significantly in their nutritional requirements (11), abilities to use amino versus ammonia nitrogen (6), and resistance to low pH (38). Previously, Russell and Baldwin reported that the GA33 strain had a maintenance energy requirement of 0.135 mg of glucose per mg of bacteria per h (37). In these experiments, the B_14 strain exhibited maintenance energy requirements that were less than 0.071 mg of glucose equivalent per mg of bacteria per h. The B_14 strain was chosen for this investigation because Bladen et al. found that it was one of the most active ammonia producers isolated from the rumen (4).

In the batch culture studies of Bladen et al., ammonia accumulation was not measured until 96 h, and the magnitude of this increase was not great (4). In our studies, the rates of ammonia generation were also slow and within the range reported by Bladen et al. (4). Ammonia production rates in the rumen can be as great as 375 mg/liter per h (21), and this rate is approximately 250 times the rate we determined in batch culture. Some of this difference is obviously due to cell density. Laboratory cultures usually contain 10^8 bacteria per ml and total cell densities in vivo can be greater than 10^{10} per ml. One must also consider the matter of carbohydrate availability. When the rate of carbohydrate metabolism was rapid in batch or continuous culture (Fig. 1 and 2), little ammonia accumulation was detected. One must interpret these data to mean that carbohydrate availability is at times very restricted in vivo or that there are major differences between the in vivo and in vitro growth of rumen bacteria.

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