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Carbon Dioxide Requirement of Various Species of Rumen Bacteria[1]

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The carbon dioxide requirement of 32 strains of rumen bacteria, representing 11 different species, was studied in detail. Increasing concentrations of CO_2 were added as $NaHCO_3$ to a specially prepared CO_2-free medium which was tubed and inoculated under nitrogen. Prior depletion of CO_2 in the inoculum was found to affect the level of requirement; however, the complexity and buffering capacity of the medium did not appear to be involved. An absolute requirement for CO_2 was observed for eight strains of *Bacteroides ruminicola*, three strains of *Bacteroides succinogenes*, four strains of *Ruminococcus flavefaciens*, two strains of *Lachnospira multiparus*, one strain of *Succinimonas amylolytica*, and two strains of *Butyrivibrio fibrisolvens*. Inconsistent growth responses were obtained in CO_2-free media with one strain each of *B. fibrisolvens*, *Ruminococcus albus*, and *Selenomonas ruminantium*. Growth of six additional strains of *B. fibrisolvens*, and single strains of *Eubacterium ruminantium* and *Succinivibrio dextrinosolvens* was markedly increased or stimulated by increasing concentrations of CO_2. *Peptostreptococcus elsdenii* B159 was the only organism tested which appeared to have no requirement, either absolute or partial, for CO_2. Higher concentrations of CO_2 were required for the initiation of growth, as well as for optimal growth, by those species which produce succinic acid as one of their primary end products.

One of the criteria commonly used in characterizing certain species of rumen bacteria is their requirement for carbon dioxide. While attempting to measure this parameter in some recent studies, difficulty was encountered in that some species which should require CO_2, presumptively identified by other criteria, did not. It was assumed that the medium was not depleted of CO_2, and extra care was taken in this regard when new medium was prepared. Those species then required CO_2 for growth; however, growth of other strains which supposedly should not require CO_2 was very inconsistent in the CO_2-free medium. When previously characterized strains not requiring CO_2 were included as controls, the same variability was observed.

The only obvious answer to this variation appeared to be that the particular strains might have a CO_2 requirement at or near the residual CO_2 level in this specially treated medium. Several of the strains involved belonged to the species *Butyrivibrio fibrisolvens*, and, although 48 strains studied by Bryant and Small (7) did not require CO_2, Gill and King (15) have described a CO_2-requiring strain of this species. Recently, since the present study was completed, Shane, Gouws, and Kistner (21) reported the isolation of 19 strains of gram-negative curved rods belonging to the genus *Butyrivibrio*. Only one isolate completely fit the description for *B. fibrisolvens*, whereas 10 additional strains were quite similar to this species. It is of interest that 7 of these 10 similar strains required CO_2.

The other strains in the present study which also exhibited variation in CO_2 requirement were subsequently identified as belonging to the species *Lachnospira multiparus*. The type species and four other strains were described by Bryant and Small (8) as not requiring CO_2 for growth.

This study was undertaken to investigate the requirement of various species of rumen bacteria for CO_2 under as rigorously controlled conditions as possible. Preliminary results prompted an expansion of the study to quantitatively estimate the CO_2 requirement of different species and strains within species. In addition, determination of CO_2 requirement in an enriched or complex medium versus a nonenriched medium was investigated, as well as possible changes in fermenta-

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tion patterns associated with CO_2 level in the medium.

MATERIALS AND METHODS

A total of 32 strains of rumen bacteria were used in this study. The majority of strains were previously isolated and characterized in this laboratory (12–14); the additional strains, the designations of which are italicized, were obtained from M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, and have been described in the literature (2–4, 6, 8–10). The number of strains, species, and individual strain designations were as follows: nine strains of *B. fibrisolvens* (H4a, H10b, H13b, H16a, H17c, D16f, D23g, D29d, and D30g); eight strains of *Bacteroides ruminicola* (H2b, H8a, H15a, *23*, *GA33*, D28f, D31d, and D42f); two strains of *Lachnospira multiparus* (D15d and D25e); three strains of *Bacteroides succinogenes* (A3c, B21a, and *S-85*); four strains of *Ruminococcus flavefaciens* (B1a, B34b, C1a, and *C-94*); one strain each of *Ruminococcus albus* (*7*), *Eubacterium ruminantium* (*B_1C23*), *Succinivibrio dextrinsolvens* (*24*), *Selenomonas ruminantium* (*GA 192*), *Succinimonas amylolytica* (*B24*), and *Peptostreptococcus elsdenii* (*B159*). The anaerobic cultural techniques were similar to those reported in earlier publications (12–14). Unless otherwise specified, all inoculations were carried out under nitrogen.

Reagent grade $NaHCO_3$ was used as a source of CO_2. Stock solutions were prepared with CO_2-free water, tubed, autoclaved, and stored under nitrogen.

Preparation of the different CO_2-free media was accomplished by mixing all of the ingredients except bicarbonate and cysteine in a round-bottomed flask, adjusting the *p*H to approximately 5.5 with 3 N HCl, and bringing the contents to a boil while gassing with nitrogen. Gassing was continued for at least 1 hr, after which the *p*H was adjusted to 6.7 with CO_2-free 5% NaOH. Cysteine and $NaHCO_3$, as required, were then added. Bubbling with the nitrogen gas was discontinued at this point to minimize CO_2 loss, and anaerobiosis was maintained in the medium by flushing the surface with a stream of nitrogen. The *p*H was measured and adjusted back to approximately 6.7 if required. The medium was then tubed under nitrogen and autoclaved in the tube (14). Glucose was used as a substrate in all media except for the final study involving the cellulolytic ruminococci, in which cellobiose was employed.

Optical density, as a parameter of growth, was measured at 600 nm with a Spectronic-20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.).

Organic acid fermentation products were determined by silica gel chromatography (E. G. Linke, M. S. Thesis, The Ohio State University, Columbus, 1952).

Most of the reported mean values are based on replicate fermentations run in duplicate, or four fermentation tubes. In a few instances, however, data from only two individual fermentation tubes were available.

RESULTS

In the first series of experiments, graded levels of $NaHCO_3$ were added to a CO_2-free basal medium containing 0.5% glucose, 30% clarified rumen fluid (CRF), 0.25% Trypticase, and 0.1% yeast extract. A 2-mm loopful of cells from an 18- to 24-hr rumen fluid-glucose-cellobiose-agar (RGCA) slant was used as an inoculum. The individual culture tube data for three strains of *B. fibrisolvens* included in this study are shown in Table 1. In general, these strains illustrate three relatively different types of response which were obtained. Inconsistent growth was observed with strains H10b and D30g at the two lowest levels of CO_2. In those tubes of H10b which grew, only partial growth occurred after an extended lag phase. This would contrast to strain D30g, which showed an almost optimal growth response in which only lag phase was affected. At the two lower levels with strain D30g, 0 and 0.005% $NaHCO_3$, absolutely no growth was observed for the first 72 hr, after which, for those tubes showing growth, the rate appeared similar to that observed at an earlier time with the higher $NaHCO_3$ concentrations. On the other hand, the extent of growth appeared to be the primary criterion affected by increasing $NaHCO_3$ levels with strain D16f.

The mean values for all strains tested are presented in Table 2. Examination of these data suggest that two factors should be considered in estimating CO_2 requirement, i.e., extent of growth and lag period. Complete lack of growth in the absence of CO_2 would indicate an absolute requirement for this nutrient; however, this criterion is confounded by the variation observed between duplicate and replicate tubes as indicated in Table 1. Limited growth, optimal growth after an extended lag phase, or a graded response to increasing concentrations would suggest that CO_2 is highly stimulatory, and the organism can probably best be described as having a partial requirement. On this basis, all of the strains listed in Table 2 would have some requirement for CO_2. The highest level of $NaHCO_3$ (0.026% CO_2) will not support optimal growth of *B. ruminicola* or *L. multiparus* strains, but it appears to be approaching that level for the various strains of *B. fibrisolvens*.

The possibility that a carry-over of CO_2 with the inoculum might be responsible for the variation observed in growth response was investigated. As mentioned previously, the regular inoculum was a 2-mm loopful of cells from an RGCA slant into 7 ml of medium. A CO_2-depleted inoculum was obtained by growing the organisms for 18 to 20 hr in a 0.10% CO_2 broth culture under N_2. With several of the high CO_2-requiring strains, however, a 10% CO_2-90% N_2 gaseous phase was required for adequate growth. A 3-mm loopful of the broth culture per tube was used for the CO_2-depleted inoculum. Some typical results (Table 3) indicate that a small amount

TABLE 1. *Growth response of three strains of Butyrivibrio fibrisolvens to increasing concentrations of CO_2*

Strain	Replicate	Maximal increase in optical density (600 nm)			
	Gas:	N_2	N_2	N_2	10% CO_2
	%$NaHCO_3$ (% CO_2)	0 (0)	0.005 (0.0026)	0.05 (0.026)	0
H10b	I	0.84 (168)[a]	0.40 (168)	1.34 (42)	1.30 (24)
	I	0 (168)	0 (168)	0.96 (48)	
	II	0.45 (72)	0.65 (40)	0.76 (40)	0.74 (40)
	II	0 (168)	0.56 (72)	0.60 (40)	
D16f	I	0.36 (24)	0.36 (30)	0.74 (30)	0.85 (18)
	I	0.31 (30)	0.40 (24)	0.74 (30)	
	II	0.34 (24)	0.36 (16)	0.68 (24)	0.70 (16)
	II	0.28 (24)	0.38 (16)	0.58 (24)	
D30g	I	0.04 (120)	0 (168)	1.40 (30)	1.35 (18)
	I	1.38 (120)	1.35 (120)	1.39 (18)	
	II	0 (168)	1.29 (168)	1.34 (16)	1.25 (16)
	II	0 (168)		1.35 (24)	

[a] Figures in parentheses indicate the hours of incubation required to reach maximum optical density.

TABLE 2. *Growth response of different strains and species of rumen bacteria to increasing concentrations of CO_2 in the medium*

Species	Strain	Maximal increase in optical density (600 nm)			
	Gas:	N_2	N_2	N_2	10% CO_2
	%$NaHCO_3$ (% CO_2)	0 (0)	0.005 (0.0026)	0.05 (0.026)	0
Butyrivibrio fibrisolvens	H10b	0.32 (144)[a]	0.54 (112)	0.92 (42)	1.02 (32)
	D16f	0.32 (26)	0.38 (22)	0.68 (27)	0.78 (17)
	D23g	0.36 (98)	0.38 (90)	0.42 (44)	0.49 (20)
	D29d	0.42 (136)	0.39 (70)	0.60 (46)	0.52 (44)
	D30g	0.36 (144)	0.88 (152)	1.37 (22)	1.30 (17)
Bacteroides ruminicola	H8a	0 (168)	0 (168)	0.84 (84)	1.17 (20)
	D28f	0 (168)	0.09 (168)	0.48 (27)	1.07 (16)
	D31d	0.16 (156)	0.68 (120)	0.53 (32)	1.26 (17)
	D42f	0 (168)	0 (168)	0.25 (120)	0.94 (27)
Lachnospira multiparus	D15d	0.35 (156)	0.24 (156)	0.56 (132)	1.27 (24)
	D25e	0 (168)	0.34 (168)	0.53 (144)	1.38 (20)

[a] Figures in parentheses indicate the hours of incubation required to reach maximum optical density.

of CO_2 was apparently being carried over with the inoculum, and that this did affect the level of CO_2 required for the initiation of growth. On the other hand, variation between duplicates still occurred at the lowest level allowing growth, i.e., the 0.005% CO_2 level for H10b. The individual tubes comprising the mean (next to last column, Table 3) varied in optical density between 0.03 and 0.67.

The possible effects of a nonenriched versus an enriched rumen fluid medium were investigated, and some typical results are presented in Tables 4 and 5. It can readily be seen that neither the minimum level of CO_2 required for growth nor the extent of growth at the various levels appears to be markedly influenced by the addition of Trypticase and yeast extract to the rumen fluid medium.

One additional factor which had to be considered was whether a lowered *p*H, caused by a lack of buffering capacity in the zero and low CO_2 media, might be limiting the extent of growth. A medium buffered to *p*H 6.75 with KH_2PO_4-K_2HPO_4 (1%) was compared to the normal medium at several lvels of added CO_2 (0 to 0.10%). Almost identical results were obtained on both

TABLE 3. *Effect of inoculum depletion on the CO_2 requirement of several rumen bacteria*

Organism	CO_2 (%)[a]	Maximal increase in optical density (600 nm)			
		Regular inoculum		Depleted inoculum	
Butyrivibrio fibrisolvens H10b	0	0.32 (144)[b]	0.52 (120)	0 (488)	0 (356)
	0.0026	0.54 (112)			0 (356)
	0.0039		0.44 (168)		
	0.005			0.50 (96)	0.37 (64)
	0.010			0.80 (52)	0.45 (40)
Lachnospira multiparus D15d	0	0.35 (156)	0.14 (168)		0 (168)
	0.0026	0.24 (156)			
	0.0039		0.13 (144)		
	0.0050				0 (168)
	0.0131		0.35 (144)		
	0.020				0.51 (116)

[a] Under N_2 gas.
[b] Figures in parentheses indicate the hours of incubation required to reach maximum optical density.

TABLE 4. *CO_2 requirement of Bacteroides ruminicola in nonenriched and enriched rumen fluid media*

Strain	CO_2 (%)	Gas	Maximal increase in optical density (600 nm)	
			Nonenriched medium[a]	Enriched medium[b]
H8a	0	N_2	0 (448)[c]	0 (356)
	0.005	N_2	0 (448)	0 (356)
	0.01	N_2	0 (448)	0 (356)
	0.02	N_2	0.24 (372)	0.64 (138)
	0.05	N_2	0.87 (50)	0.65 (31)
	0.10	N_2	1.48 (46)	1.34 (64)
	0.10	10% CO_2		1.38 (22)
	0.20	10% CO_2	1.78 (46)	
GA33	0	N_2	0 (485)	0 (356)
	0.005	N_2	0.12 (456)	0 (356)
	0.01	N_2	0.12 (485)	0 (356)
	0.02	N_2	0.15 (358)	0 (356)
	0.05	N_2	0.52 (181)	0.60 (223)
	0.10	N_2	1.04 (87)	1.32 (138)
	0.10	10% CO_2	1.41 (31)	1.34 (40)
	0.20	10% CO_2	1:30 (64)	

[a] Clarified rumen fluid (40%).
[b] Clarified rumen fluid (40%) plus 0.5% Trypticase plus 0.25% yeast extract.
[c] Figures in parentheses indicate the hours of incubation required to reach maximum optical density.

media for all 10 strains tested, and thus it was concluded that a *p*H drop was not responsible for the limited amount of growth obtained with the lower concentrations of CO_2.

The CO_2 requirement of a number of different strains and species of rumen bacteria was then determined; Table 6 illustrates the various types of growth curves obtained. From these data, the levels of CO_2 required for initiation of growth and for near optimal growth were estimated. This was relatively easy in the case of strain D25e, 0.02 and 0.05%, respectively; however, this was somewhat more complicated in the case of strain D30g.

After summarizing all of the data, estimates were made of the CO_2 requirement for all 32 strains of rumen bacteria used in the present study. Both the levels of CO_2 required for the initiation of growth and near optimal growth were estimated (Table 7). From a total of nine strains of *B. fibrisolvens*, two had an absolute requirement for CO_2 (H10b and H16a), and a third strain (D30g) gave an inconsistent growth response in the absence of CO_2. Growth of all strains of this species was stimulated or increased by increasing concentrations of CO_2, most strains reaching optimal growth in the vicinity of 0.05% CO_2 in the medium.

TABLE 5. *CO_2 requirement of Butyrivibrio fibrisolvens in nonenriched and enriched rumen fluid media*

Strain	CO_2 (%)	Gas	Maximal increase in optical density (600 nm)	
			Nonenriched medium[a]	Enriched medium[b]
H16a	0	N_2	0 (448)[c]	0 (356)
	0.005	N_2	0.38 (162)	0 (356)
	0.01	N_2	0.77 (78)	0.92 (100)
	0.02	N_2	0.55 (49)	0.62 (77)
	0.05	N_2	0.87 (46)	0.92 (52)
	0.10	N_2	1.29 (26)	0.98 (40)
	0.10	10% CO_2		0.84 (40)
	0.20	10% CO_2	0.94 (22)	
H17c	0	N_2	0.20 (36)	0.37 (136)
	0.005	N_2	0.30 (26)	0.34 (90)
	0.01	N_2	0.30 (26)	0.46 (64)
	0.02	N_2	0.41 (20)	0.34 (52)
	0.05	N_2	0.66 (18)	0.43 (22)
	0.10	N_2	0.98 (20)	0.68 (22)
	0.10	10% CO_2		0.72 (15)
	0.20	10% CO_2	1.06 (15)	

[a] Clarified rumen fluid (40%).
[b] Clarified rumen fluid (40%) plus 0.5% Trypticase plus 0.25% yeast extract.
[c] The figures in parentheses indicate the hours of incubation required to reach maximum optical density.

TABLE 6. *Growth response of several species of rumen bacteria to increasing concentrations of CO_2*

CO_2 (%)	Maximal increase in optical density (600 nm)[a]				
	24	D25e	D16f	D30g	A3c
0	0.71 (186)[b]	0 (168)	0.54 (100)	0.70 (152)[c]	0 (168)
0.0025	0.74 (161)				
0.005	0.69 (186)	0 (168)	0.57 (64)	1.38 (116)	0 (168)
0.01	0.72 (186)				
0.02	0.68 (69)	0.81 (94)	0.84 (40)	1.45 (52)	0 (168)
0.05	0.88 (161)	1.34 (20)	1.06 (40)	1.54 (30)	0.30 (116)
0.10	1.09 (18)	1.34 (20)	1.19 (40)	1.70 (24)	1.24 (93)
Control[d]	1.53 (22)	1.40 (20)	1.33 (24)	1.70 (22)	1.36 (24)

[a] Species of rumen bacteria: 24, *Succinivibrio dextrinosolvens;* D25e, *Lachnospira multiparus;* D16f, *Butyrivibrio fibrisolvens;* D30g, *B. fibrisolvens;* A3c, *Bacterioides succinogenes.*

[b] Figures in parentheses indicate the hours of incubation required to reach maximum optical density.

[c] The tubes showing growth reached an optical density of 1.40 after a long lag phase; however, the cultures in some tubes did not grow.

[d] Control medium contained 0.10% CO_2 and was prepared, tubed, and inoculated under 10% CO_2 gas.

TABLE 7. *CO_2 requirement of several species of rumen bacteria*

Species	Strain	CO_2 (%) required in medium for	
		Initiation of growth	Near-optimal growth
Butyrivibrio fibrisolvens	H4a	0	0.05
	H10b	0.005	0.02-0.05
	H13b	0	0.02-0.05
	H16a	0.005-0.01	0.05
	H17c	0	0.10
	D16f	0	0.05
	D23g	0	0.05
	D29d	0	0.005
	D30g	0-0.005	0.02
Bacterioides ruminicola	H2b	0.05-0.10	≥0.10
	H8a	0.02-0.05	≥0.10
	H15a	0.02-0.05	>0.10
	23	0.005	>0.10
	GA33	0.05	>0.10
	D28f	0.05	>0.10
	D31d	0.02	>0.10
	D42f	0.05	>0.10
Lachnospira multiparus	D15d	0.02	0.05
	D25e	0.02	0.05
Bacteroides succinogenes	A3c	0.05	0.10
	B21a	0.05	≥0.10
	S-85	0.02-0.05	≥0.10
Ruminococcus flavefaciens	B1a	0.10	≥0.10
	B34b	0.05	0.10
	C1a	0.05-0.10	≥0.10
	C-94	0.05-0.10	>0.10
Ruminococcus albus	7	0-0.005	0.05-0.10
Eubacterium ruminantium	B_1C23	0	>0.10
Succinivibrio dextrinosolvens ..	24	0	0.10
Selenomonas ruminantium	GA192	0-0.02	0.05
Succinimonas amylolytica	B_24	>0.10	>0.10
Peptostreptococcus elsdenii ...	B159	0	0

All eight strains of *B. ruminicola*, two strains of *L. multiparus*, three strains of *B. succinogenes*, four strains of *R. flavefaciens*, and one strain of *S. amylolytica* have an absolute requirement for CO_2. The concentration of CO_2 required for growth initiation varied considerably between strains as well as species. With the exception of the two strains of *L. multiparus*, most of the organisms in this group required a level of 0.10% CO_2 or higher in the medium for optimal growth.

One strain each of *R. albus* and *S. ruminatium* were variable with respect to growth initiation in a CO_2-free medium, whereas *E. ruminantium*, *S. dextrinsolvens*, and *P. elsdenii* all exhibited growth. With the exception of *P. elsdenii*, growth of all these strains was increased by increasing the concentration of CO_2. *P. elsdenii* B159 was the only organism tested which appears to have no requirement, either absolute or partial, for CO_2.

End products were measured for various strains of the species *B. fibrisolvens*, *B. ruminicola*, *B. succinogenes*, *R. flavefaciens*, and *R. albus*. In general, total acid production was proportional to growth and CO_2 concentration in the medium. The relative proportions of the individual acids were not markedly changed when growth was limited or an extended lag phase occurred before growth initiation. The only exception to this was observed with *R. albus*. Almost all the acid produced at the low CO_2 levels was acetic, and as CO_2 concentration increased the proportion of acetic acid decreased to about 50%, whereas formic acid rose from essentially zero to 50%.

DISCUSSION

Although Bryant and Small (7) found that all 48 of their *B. fibrisolvens* strains would grow well in a medium in which N_2 replaced CO_2, the reports by Gill and King (15) and Shane et al. (21), along with the present study, suggest that this property can vary among strains. The data obtained in this study indicate that the conditions of growth and medium preparation would also be of extreme importance in determining the CO_2 requirement for a particular strain. Thus, CO_2 requirement is probably not a good characteristic to be used in the identification of this species. An additional point of interest in the study reported by Gill and King (15) was the observation that growth of their *Butyrivibrio* strain was restricted to media which contained at least 0.02 M $NaHCO_3$. This would be equivalent to approximately 0.088% CO_2, which is considerably higher than the requirements found in the present study for this species.

Bryant and Small (8) studied the characteris-

tics of five strains of *L. multiparus*, all of which grew well in media without added CO_2 or bicarbonate. The two strains of this species used in the present study both had a low but absolute requirement for CO_2. However, the extra precautions taken in medium preparation and growth of the inoculum culture may well account for this discrepancy.

In general, absolute requirements for the remaining species are in agreement with the original species descriptions (2-4, 6-10).

The results obtained in this study suggest that two types of CO_2 requirement are being observed. The first is a biosynthetic type in which CO_2 is required for cell growth and multiplication; the second type includes a requirement for CO_2 in the formation of metabolic end products (succinate) as well as biosynthesis.

The biosynthetic requirement can occur even in those species which produce CO_2 as a fermentation product and is probably observed as an extended lag phase with eventual near optimal growth. The partial requirement for CO_2 mentioned previously, where lag phases were markedly reduced and growth of certain strains was stimulated by increasing concentrations of CO_2, is probably of the biosynthetic type. The CO_2 requirement of *Streptococcus bovis* has been studied in detail (19, 20, 22), and it has been found that this requirement can be replaced or spared by adding a mixture of amino acids or hydrolyzed protein to the medium. This would suggest that CO_2 is required by *S. bovis* primarily for amino acid biosynthesis. Huhtanen et al. (17) reported that radioactive CO_2 was primarily incorporated into fatty acids and amino acids by several strains of unidentified rumen bacteria. No measureable change in CO_2 requirement or sparing effect was noted in the present study when 19 strains were grown on a basal rumen fluid medium enriched with Trypticase (a pancreatic digest of casein) and yeast extract. Presumably these strains require CO_2 in some other metabolic function, or the preformed essential nutrients were unable to enter the cell.

Those organisms which produce succinic acid as one of their primary end products, *B. ruminicola*, *B. succinogenes*, *R. flavefaciens*, and *S. amylolytica*, all exhibited an absolute CO_2 requirement and required considerably higher concentrations of CO_2 for the initiation of growth as well as optimal growth. The only exception observed was with the single strain of *S. dextrinosolvens* studied, in which limited growth occurred in the absence of added CO_2 after an extended lag phase. However, a level of 0.10% CO_2 was required for near optimal growth. Recent studies by Caldwell et al. (11) indicate that three succinogenic rumen *Bacteroides* species, *B. amylophilus*, *B. ruminicola*, and *B. succinogenes*, all require CO_2 for the production of succinate, either through a direct fixation pathway involving phosphoenolpyruvate or pyruvate, or an as yet unknown route of direct CO_2 fixation. Similar results were obtained by Hopgood and Walker (16) with a single strain of *R. flavefaciens*.

The complete lack of CO_2 requirement observed with *P. elsdenii* indicates that this organism does not require an exogenous source of CO_2 in a complex medium. Previous studies with this strain have shown that, not only will it take up exogenous amino acids in preference to synthesizing them from NH_3, but it has an absolute requirement for certain components of casein hydrolysate, presumably amino acids (4, 5). Either its CO_2 requirement is thus spared in the complex medium, or it can produce an adequate amount of CO_2 for any other critical metabolic functions by the anaerobic oxidation of pyruvate (18).

The shift in end products observed for *R. albus* 7 with increasing concentrations of CO_2 was of considerable interest. The only acid produced in the basal or low CO_2 medium was acetate; however, as CO_2 concentration in the medium was increased, acetate decreased and formate increased until they were approximately equal in the positive controls. This would agree with the ratio originally reported by Bryant et al. (10) for this strain. Since this strain does not produce succinate, normally produces H_2 and CO_2 as end products, and has a very low, if any, requirement for exogenous CO_2, it appears that it can possibly satisfy its own CO_2 requirements by the cleavage of formate to CO_2 and H_2 (1).

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