JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1982, p. 334–340
0095-1137/82/080334-07$02.00/0

Vol. 16, No. 2

Nutritional Requirements of *Corynebacterium pyogenes*†

ANNEMARIE M. FRAGA AND C. ADINARAYANA REDDY*

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

Received 26 January 1982/Accepted 17 May 1982

The nutritional requirements of *Corynebacterium pyogenes* (strains C100, 5, and 1909), a commonly encountered animal pathogen, were determined in this study. A semidefined medium (SDM) containing glucose, HCO_3^-, hemin, charcoal-treated Trypticase, and a defined mixture of purines and pyrimidines, amino acids, and minerals which supported optimal growth of *C. pyogenes* was employed in all nutritional studies. Adenine and uracil were required for optimal growth of strains 5 and C100 but were not required for strain 1909. Riboflavin and nicotinic acid were required for good growth of all three strains; biotin and thiamin were stimulatory but did not appear to be required for growth. Hemin and $NaHCO_3$ were stimulatory for growth, whereas lipoic acid and Tween 80 were neither stimulatory nor required for growth. The replacement of Trypticase with a specific peptide fraction (obtained by fractionation of Trypticase on Sephadex G-25) rich in dipeptides gave growth comparable to that in SDM, indicating a peptide requirement for the growth of *C. pyogenes*. It was of considerable interest that growth comparable to that in SDM was obtained when Trypticase was replaced by inositol (1 μg/ml of SDM).

Corynebacterium pyogenes (23) is one of the most frequently isolated pathogens from a variety of pyogenic disease conditions in domestic animals. It apparently exists as a commensal organism on the mucous surfaces of warm-blooded animals (1, 14, 28). Although *C. pyogenes* was first isolated almost one hundred years ago, physiological and metabolic studies on this organism have been hampered because it grows poorly on laboratory media unless supplemented with blood or serum (1, 23). Reddy et al. (20) reported that hemin is stimulatory for the growth of this organism and recently described a semidefined medium and a chemically defined medium which supported excellent growth of *C. pyogenes* (19). The development of these media facilitated the present study, in which we determined the vitamin, nucleotide, nitrogen, and other nutritional requirements of *C. pyogenes*.

MATERIALS AND METHODS

Bacterial strains. The sources of *C. pyogenes* strains C100 (NCTC 5224; ATCC 19411), NCDO 1909 (NIRD 212), and 5 used in this study were described previously (19).

Culture maintenance and media. All cultures were maintained on plates of tryptose agar (Difco Laboratories) plus 5% sheep blood as previously described (19).

The semidefined broth medium (SDM) used for the nutritional studies contained (per 100 ml): glucose, 0.5 g; charcoal-treated Trypticase (BBL Microbiology Systems), 0.4 g; cysteine hydrochloride, 0.05 g; hemin, 0.0002 g; $NaHCO_3$, 0.4 g; salts solution, 25 ml; trace minerals solution, 1 ml; purines and pyrimidines, 1 ml; vitamin mixture, 1 ml; and amino acid mixture, 10 ml. The salts solution contained (per liter): KH_2PO_4, 60 g; $(NH_4)_2SO_4$, 4 g; $MgSO_4 \cdot 7H_2O$, 0.8 g; and $CaCl_2$, 0.08 g. The composition of the trace minerals stock solution, the purine and pyrimidine solution, the vitamin solution, and the amino acid stock solution have been previously described (19). Unless otherwise mentioned, Trypticase was treated with charcoal (4) to remove nucleotides and vitamins.

In one experiment (see Table 1), SDM also contained (per 100 ml): Tween 80, 50 mg; lipoic acid, 0.1 mg; and myoinositol, 0.1 mg.

Acid-washed glassware was used in all cases. All ingredients of the broth medium except $NaHCO_3$ were mixed in double-distilled water. The pH was then adjusted to 6.5 with 10 M NaOH, and the medium was dispensed into foam-plugged tubes (18 by 150 mm; 9.5 ml per tube) and sterilized by autoclaving (15 min at 121°C). Before inoculation, 0.5 ml of a sterile 8% $NaHCO_3$ solution was added to each tube unless indicated otherwise.

Vitamin requirements were determined by adding one or more individual, filter-sterilized vitamin solutions as needed to a sterile basal medium consisting of SDM without vitamins.

Purine and pyrimidine requirements were determined by adding one or more sterile, individual purine or pyrimidine solutions or both to sterile SDM without nucleotides.

Preparation of inoculum and growth conditions. An isolated colony of *C. pyogenes* grown on tryptose blood agar plates was transferred to SDM broth without amino acids and incubated for approximately 24 h.

† Journal article no. 9149 from the Michigan Agriculture Experiment Station, East Lansing.

Cells from the third serial transfer in this medium were used as the inoculum, as previously described (19).

Growth was estimated by measuring the absorbance at 600 nm (A_{600}) with a Bausch & Lomb Spectronic 20 spectrophotometer. In all cases, the mean absorbance of three replicate tubes is reported, and less than 5% variation in absorbance between replicate cultures was noted. Other studies not reported here (C. A. Reddy and C. P. Cornell, unpublished data) showed good correlation between A_{600} values, cell numbers, and the dry weight of the cells (A_{600} of 1.0 = 1.83 (± 0.18) × 10^9 cells per ml = 0.45 mg dry weight).

Fractionation of Trypticase. To determine which Trypticase fraction was most stimulatory to growth, we fractionated charcoal-treated Trypticase on a 1.5- by 30-cm Sephadex G-25 column (medium grain; Pharmacia Fine Chemicals) with 50 mM KPO_4 buffer (pH 7.0) used as the eluant (flow rate, ~60 ml/h). A 20% (wt/vol) Trypticase solution (5 ml) was applied to the column and, after the void volume had passed, 5-ml fractions of effluent were collected. The fractions were autoclaved for 10 min and then stored at −20°C until used.

The α-amino nitrogen content of the fractions was determined by the method of Rosen (24). A sample of each fraction was hydrolyzed with an equal volume of 12 N HCl for 11 h at 121°C. The average peptide size for each fraction was calculated by dividing the value for α-amino nitrogen from the hydrolyzed fraction by the corresponding value for the unhydrolyzed sample.

Analysis of myoinositol. The myoinositol (chromatographic grade; Applied Science Laboratories, Inc.), hereafter called inositol, used in this study appeared to be pure as determined by thin-layer chromatography on silica gel plates (29). Acetone-water (85:15) was used as the solvent, and acetone–$AgNO_3$–ethanolic NaOH was used for detection of the spots.

RESULTS

Deletions from SDM. The effect of deleting a single component at a time from SDM on the growth of all three strains of *C. pyogenes* was investigated to determine which components were essential, stimulatory, or without effect on growth (Table 1). All three strains grew well in SDM. The salts solution appeared to be stimulatory for the growth of all of the strains, especially strain 5. Trace minerals were stimulatory for all of the strains. Purines and pyrimidines were stimulatory for the growth of strains 5 and C100 but not for strain 1909. Vitamins were stimulatory for the growth of all of the strains. The Trypticase used in this experiment was not pretreated with charcoal, which is known to remove trace amounts of purines, pyrimidines, and vitamins (2); however, when charcoal-treated Trypticase was used, vitamins, purines, and pyrimidines were either required or highly stimulatory for growth (see below). The deletion of hemin resulted in a long lag period (40 to 50 h), but growth equivalent to 50 to 75% of that observed in SDM was finally obtained. Deletion of the amino acid mixture had no noticeable effect on growth. Deletion of Trypticase, even in the presence of a full complement of amino acids and $(NH_4)_2SO_4$ (present in the salts solution), resulted in a 40 to 50% decrease in growth, suggesting a peptide requirement for the growth of *C. pyogenes*. Deletion of glucose resulted in little or no growth, indicating that amino acids, peptides, and other components present in the medium do not serve as energy sources for growth under these conditions. Cysteine appeared to be stimulatory but was not a requirement for growth. Growth decreased by ~50% after the deletion of bicarbonate.

TABLE 1. Effect of single deletions of various components from SDM on the growth of three strains of *C. pyogenes*[a]

Deletion from SDM	Growth[b] of strain:		
	5	1909	C100
None	1.50 (36)	1.50 (31)	1.50 (35)
Salts	0.92 (65)	1.26 (38)	1.30 (35)
Trace minerals	1.43 (65)	1.20 (38)	1.00 (66)
Purines and pyrimidines	0.44 (56)	1.50 (31)	0.64 (66)
Vitamins	1.16 (42)	1.15 (42)	1.20 (42)
Hemin	1.11 (65)	0.62 (56)	1.00 (70)
Amino acids	1.50 (37)	1.50 (31)	1.50 (35)
Trypticase	0.79 (65)	0.69 (56)	0.93 (60)
Glucose	0.00 (70)	0.00 (70)	0.07 (52)
Cysteine	1.06 (56)	1.14 (34)	1.36 (35)
$NaHCO_3$	0.75 (56)	0.80 (38)	0.79 (42)
Inositol	1.50 (35)	1.40 (31)	1.50 (32)
Lipoic acid	1.50 (35)	1.50 (31)	1.50 (32)
Tween 80	1.50 (35)	1.50 (31)	1.50 (42)

[a] In this experiment the SDM additionally contained inositol, lipoic acid, and Tween 80, and the Trypticase used was not charcoal treated.

[b] Growth is reported as the mean maximal A_{600} of three tubes (18 by 150 mm; 10 ml per tube) after three serial transfers. Numbers in parentheses refer to hours of incubation at 37°C for maximal growth. Washed inoculum, grown in SDM to an A_{600} of 0.8, was used (0.05 ml per tube).

Tween 80 was suggested to be a requirement for the growth of *C. pyogenes* by Jayne-Williams and Skerman (12). However, our results showed that the deletion of Tween 80, lipoic acid, or inositol singly from SDM had no measurable effect on growth.

Vitamin requirements. Strains 1909 and C100 showed negligible growth in SDM without vitamins (Table 2), whereas the growth of strain 5 was ~12% of that observed in SDM (Fig. 1). The addition of nicotinic acid to SDM without vitamins did not stimulate growth. The growth of strains 1909 and C100 was only slightly stimulated by the addition of riboflavin, but this vitamin supported growth of strain 5 equivalent to ~50% of that observed in SDM. The growth of strain 5 was ~80% and that of 1909 and C100 was 64% of

TABLE 2. Effect of various vitamins on growth of *C. pyogenes*

Addition[a] to SDM	Growth[b] of strain:		
	5	1909	C100
None	0.17	0.03	0.02
R	0.65	0.23	0.14
N	0.10	0.08	0.03
R + N	1.20	0.90	0.90
R + N + biotin + thiamine		1.00	1.40
Complete	1.50	1.40	1.40

[a] None, SDM without vitamins; R, riboflavin; N, nicotinic acid; complete, complete vitamin mixture as used in SDM. Individual vitamins were added to the medium at the same concentration as used for SDM. SDM contained charcoal-treated (4) Trypticase.

[b] See Table 1, footnote *b*, for inoculum preparation and growth measurements. Incubation was for 50 h at 37°C.

the growth observed in SDM after the addition of both riboflavin and nicotinic acid. Typically, both the rate of growth and the maximal growth were affected by the addition of vitamins (Fig. 1). C100 appeared to require only riboflavin, nicotinic acid, biotin, and thiamine for optimal growth, whereas the complete vitamin mixture was required by 1909. Further results showed that a combination of riboflavin, nicotinic acid, thiamine, pyridoxal, *p*-aminobenzoic acid, and biotin were required for the maximal growth (A_{600}, 1.5) of strain 5 (data not shown). Deletion of riboflavin from the above combination resulted in growth of strain 5 comparable to that observed in SDM without vitamins, whereas the deletion of nicotinic acid from the above combination resulted in growth equal to that obtained with riboflavin alone. Additions of thiamine, pyridoxal, *p*-aminobenzoic acid, or biotin along with riboflavin and nicotinic acid provided no additional stimulation of growth over that obtained with riboflavin and nicotinic acid only. Apparently, thiamine, pyridoxal, *p*-aminobenzoic acid, biotin, riboflavin, and nicotinic acid interact to support growth of strain 5 equivalent to that observed in SDM.

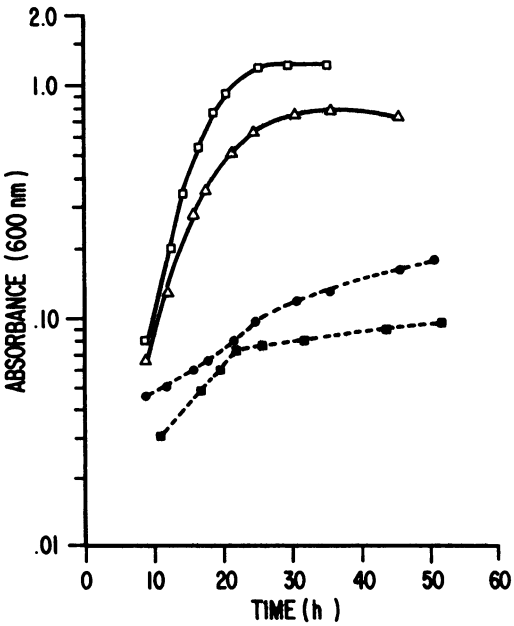

FIG. 1. Vitamin requirements for growth of *C. pyogenes* strain 5. Various vitamins were added to SDM without vitamins. Growth measurements and preparation of inoculum are described in footnote *b*, Table 1. Addition: ■, none; ●, nicotinic acid; △, riboflavin; □, riboflavin plus nicotinic acid.

Requirements for purines and pyrimidines. Strain 1909 did not require purines or pyrimidines for growth and was actually inhibited by the addition of adenine to SDM without the purine-pyrimidine mixture (Table 3). In contrast, strains 5 and C100 showed scant growth in the latter medium (Table 3). There was no increase in growth after the addition of adenine or uracil, but adenine plus uracil supported growth comparable to that in SDM. Typically, total growth but not the growth rates were affected in each of the media tested (data not shown). The addition of adenine plus thymine or of xanthine plus guanine plus uracil did not stimulate growth greater than that observed in SDM without the purine-pyrimidine mixture. Furthermore, thymine could not replace uracil in the presence of adenine, and xanthine and guanine could not replace adenine in the presence of uracil (data not shown).

Nitrogen requirements. Trypticase appeared to be essential for growth, since its deletion from SDM gave negligible growth (Table 4). This indicated that the amino acid mixture and cysteine did not satisfy the organic nitrogen requirements of the strains tested and that peptides are required for optimal growth. Only a slight inhibition of growth of strain 5 was seen after deletion of the amino acid mixture, cysteine, or both, indicating that Trypticase can satisfy the major organic nitrogen requirements of this organism. Cysteine, but not the amino acid mixture, appeared to be stimulatory for the growth of both C100 and 1909 (Table 4). As expected, there was negligible growth when both Trypticase and the amino acid mixture or both Trypticase and cysteine were omitted from SDM.

Peptide requirements for growth. A requirement for Trypticase even in the presence of the amino acids and cysteine suggested that a peptide(s) in Trypticase is required for growth. Therefore, Trypticase was fractionated on a Sephadex G-25 column, and the ability of each

TABLE 3. Purine and pyrimidine requirements for growth of *C. pyogenes*

Additions[a]	Growth[b] of strain:		
	5	1909	C100
None	0.09	1.5	0.08
A	0.22	1.0	0.11
U	0.25	1.5	0.06
A + U	1.50	1.2	1.50
A + thymine	0.15	1.1	0.15
Xanthine + guanine + U	0.04	1.3	0.04
Complete	1.50	1.4	1.50

[a] None, SDM without the purine-pyrimidine mixture; A, adenine; U, uracil; complete, the purine-pyrimidine mixture as used in SDM. Concentration of individual purines or pyrimidines in the medium was the same as that used for SDM. Trypticase was charcoal treated (4).

[b] See Table 1, footnote *b*, for growth measurements and inoculum preparation. The length of incubation was 62 h.

fraction to stimulate the growth of strain 5 when added to SDM without Trypticase was determined. The addition of fraction 4, with an average peptide size of 1.5, resulted in growth comparable to that observed in SDM (Table 5). Peptides containing 5 to 11 amino acid residues (fractions 1 and 2) gave little or no growth response. Maximum growth stimulation was obtained when an amount of fraction 4 containing 50 μmol of α-amino nitrogen was added per tube of SDM without Trypticase (Fig. 2). The addition of fraction 4 to SDM without Trypticase and amino acids gave no growth, indicating that the organism required amino acids for growth when Trypticase fractions were used, but not when unfractionated Trypticase was used (Table 5).

Inositol requirement and description of a chemically defined growth medium. Deletion of Trypticase from SDM in the presence of inositol, Tween 80, and lipoic acid resulted in appreciable growth (A_{600}, 0.69 to 0.93; Table 1). However, growth was negligible when Trypticase was deleted from SDM in the absence of the above three growth factors, suggesting that one or more of these three factors is required for growth in the absence of Trypticase (see Tables 4 and 5). There was negligible growth of *C. pyogenes* when no growth factors were added or when Tween 80 or lipoic acid was added to SDM without Trypticase (Table 6). The addition of

TABLE 4. Nitrogen requirements for optimal growth of *C. pyogenes*

Deletion[a] from SDM	Growth[b] of strain:		
	5	1909	C100
None	1.5	1.40	1.40
Try	0.07	0.05	0.06
Try + AA	0.04	0.02	0.02
Try + Cys	0.07	0.08	0.05
AA + Cys	1.20	1.20	1.10
AA	1.23	1.50	1.50
Cys	1.30	1.14	1.06

[a] None, SDM as described in the text; Try, Trypticase (charcoal treated); AA, amino acids; Cys, cysteine.

[b] See Table 1, footnote *b*. Incubation was for 74 h at 37°C.

TABLE 5. Effect of addition of various Trypticase fractions on growth of *C. pyogenes* strain 5.

Fraction no. added to SDM[a]	Amino nitrogen concn (μmol/10 ml)	Average no. of amino acids per peptide	Growth[b]
1	34	11.3	NG
2	65	5.0	0.04
3	72	2.2	0.95
4	66	1.5	1.20
5	65	1.4	1.10
6	52	1.5	0.95
7	32	1.5	0.75
None			NG
SDM			1.3
4 (without AA)	66	1.5	NG

[a] Trypticase was fractionated on a Sephadex-G25 column as described in the text. Each fraction was added to SDM without Trypticase. None, No fraction added; SDM, medium as described in the text with charcoal-treated Trypticase served as a positive control; 4 (without AA), Trypticase fraction 4 added to SDM without Trypticase or amino acids.

[b] See Table 1, footnote *b*. Incubation was for 48 h at 37°C. NG, No measurable growth.

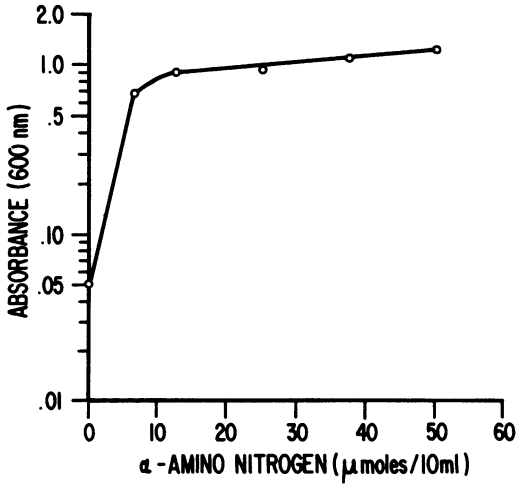

FIG. 2. Growth response of *C. pyogenes* strain 5 to various concentrations of Trypticase fraction 4 added to SDM without Trypticase. Absorbance values represent maximum absorbances at the end of 48 h of incubation at 37°C.

TABLE 6. Effect of addition of various growth factors to SDM without Trypticase on growth of *C. pyogenes*

Addition[a] to SDM	Growth[b] of strain:		
	5	1909	C100
None	0.07 (72)	0.06 (72)	0.06 (72)
I	1.20 (47)	1.20 (48)	1.30 (48)
T	0.03 (47)	0.02 (72)	0.03 (70)
L	0.09 (47)	0.05 (56)	0.07 (47)
I + T + L	1.2 (47)	0.90 (48)	1.0 (47)
Trypticase	1.2 (47)	1.2 (43)	1.4 (47)

[a] None, SDM without Trypticase. Inositol (I) and lipoic acid (L) were added to the medium at 1 μg/ml, and Tween 80 (T) was added at 500 mg/ml. Where indicated, Trypticase was present at the same concentration as in SDM.

[b] Growth measurements and preparation of inoculum were done as described in footnote *b*, Table 1, except that washed cells were suspended to give an absorbance of 0.2. The incubation in hours required to reach maximum absorbance are given in parentheses.

inositol at 1 μg/ml to SDM without Trypticase gave growth comparable to that in SDM with Trypticase. The combined addition of inositol, Tween 80, and lipoic acid to SDM without Trypticase gave growth comparable to or lower than that obtained with SDM plus inositol (Table 6). The effect of inositol concentration versus growth of strain 5 indicated that good growth was obtained with as little as 10 ng of inositol per ml (Fig. 3 and Table 7).

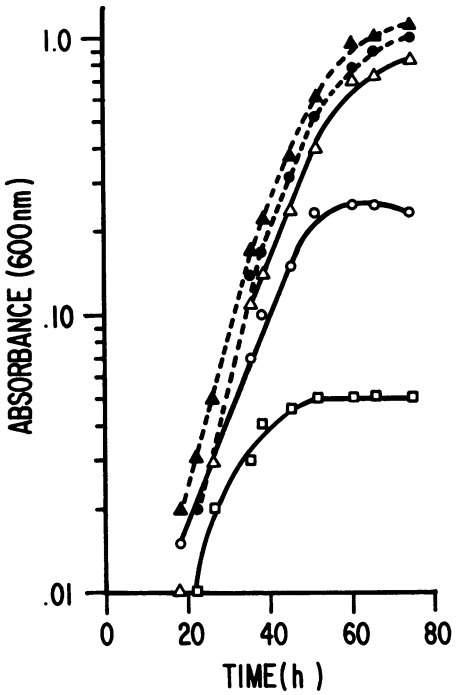

FIG. 3. Effect of inositol concentration on growth. All experimental details are described in footnotes *a* and *b*, Table 6. Addition: □, none; ○, 0.01 μg; △, 0.1 μg; ●, 1 mg; or ▲, 10 mg of inositol added to 100 ml of SDM without Trypticase.

DISCUSSION

The results show that deletion of HCO_3^- from SDM led to about a 50% decrease in growth for all three strains tested. Similar results were obtained by T. Skerman (Ph.D thesis, University of Reading, England, 1966). These results are further supported by the findings of Cornell and Reddy (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, D9, p. 41), who showed that *C. pyogenes* produces formate, acetate, and succinate as major products in a medium containing HCO_3^- and that a Y_{glc} (gram of cells [dry weight] per mole of glucose metabolized) value of 31 is obtained. In contrast, in the same medium without HCO_3^-, Y_{glc} is only 23 and the major product is lactate; acetate and succinate are minor products. These results suggest that *C. pyogenes* apparently fixes CO_2 (or HCO_3^-) and produces succinate as a major electron sink product in SDM, whereas in SDM without CO_2 lactate appears to be the major electron sink product.

The results showed that strain 1909 grew poorly and strains 5 and C100 grew reasonably well in SDM without hemin (Table 1); however, growth occurred only after a lag period of 40 to 50 h. This pattern was consistently shown by these strains even after three serial transfers. Heme-requiring strains of *Bacteroides melaninogenicus* (22), *Bacteroides ruminicola* (32), and *Bacteroides fragilis* (16) utilize hemin for the synthesis of a *b*- or *c*-type cytochrome. The stimulatory nature of heme for *C. pyogenes* strains 1909 and C100 may be due to its involvement in *b*-type cytochrome synthesis, as was previously demonstrated for strain 5 (20). The *b*-type cytochrome in *C. pyogenes* may be part of a primitive electron transport system which me-

TABLE 7. Effect of inositol concentration on growth of strains 1909 and C100 of *C. pyogenes*

Inositol concn (μg/100 ml)[a]	Growth[b]	
	1909	C100
1.0	1.5 (60)	0.87 (74)
0.1	0.94 (74)	0.71 (74)
0.01	0.27 (51)	0.30 (60)
None	0.03 (60)	0.07 (60)

[a] Basal medium consisted of SDM without Trypticase.

[b] Growth measurements and inoculum preparation are described in footnote *b*, Table 1. Incubation time to maximal growth (in hours) is shown in parentheses.

diates the reduction of fumarate to succinate with concomitant production of ATP (8, 30).

Riboflavin and nicotinic acid were required for the optimal growth of all three strains of *C. pyogenes*. Biotin and thiamine were stimulatory for growth in the presence of riboflavin and nicotinic acid. These results are at variance with the results of Skerman (Ph.D. thesis, University of Reading, England, 1966), who reported that riboflavin, nicotinic acid, biotin, and pantothenic acid were all essential for growth. The reasons for this discrepancy are not clear at this time. The vitamins are probably required to support the mixed acid fermentation of *C. pyogenes*.

The results showed that *C. pyogenes* requires both free amino acids and peptides for growth. Peptides are required because the organism probably has a lesion for the transport of one or more free amino acids. The amino acid mixture could be deleted from SDM in the presence of Trypticase because the latter contains both free amino acids and peptides. A specific peptide, fraction 4 (Table 5), presumably contains a peptide(s) which is transported into the cell and, after hydrolysis, releases the free amino acid(s) for which a transport function is missing in the organism. A strong growth requirement for peptides even in the presence of a full complement of free amino acids has been previously demonstrated for other organisms (17, 18).

Peptides were shown to be either required or stimulatory for growth when the growth medium contained amino acids at such a concentration that the transport of a limiting amino acid into the cell was blocked or severely inhibited by an excess of a competing amino acid (13, 18). This is a possible, albeit unlikely, alternate explanation for the peptide requirement of *C. pyogenes*. If this hypothesis were correct, optimal growth would not have been expected when the Trypticase was replaced by inositol in SDM. The results show that there was optimal growth in the latter medium, indicating that free amino acids are not present at a concentration which is inhibitory for the utilization of one or more of the required amino acids.

The ability of inositol to replace the peptide requirement for the growth of *C. pyogenes* is an interesting phenomenon, but it is not clear at this time how two substances as different as these are able to substitute for each other in supporting maximal growth of the organism. A likely explanation is that, as hypothesized above, the cells have a membrane lesion rendering them unable to transport one or more of the free amino acids required for growth. This transport problem can be alleviated by presenting the cell with a peptide(s) that contains the required amino acid(s) and can be transported into the cell by peptide permeases. Alternately, by growing the cell in the presence of inositol the membrane lesion is cured and transport activity for the amino acid(s) in question is restored. Several reports on the inositol requirement of yeasts and KB cells and the demonstration that inositol deficiency impairs the transport of amino acids in KB cells support the latter explanation (3, 11, 15).

Although inositol has an almost ubiquitous distribution in plant and animal tissues and has long been recognized as a growth factor for yeasts and fungi (9, 11, 25, 33), there have been no previous publications demonstrating an inositol requirement in bacteria, except for two reports concerning an apparent inositol requirement by a strain of *Actinomyces israelii* (5) and several strains of *C. pyogenes* (T. Skerman, Ph.D. thesis, University of Reading, England, 1966). Both *C. pyogenes* and *A. israelii* have ready access to inositol in the animal and human tissues which they parasitize, and hence their inability to synthesize inositol reflects this adaptation to their natural niche.

Reddy and Kao (21) recently reviewed evidence supporting the proposal that *C. pyogenes* should be excluded from the genus *Corynebacterium* because it is different from the type species, *C. diphtheriae*, and related corynebacterial species of animal origin in cell wall composition (7), biochemical characteristics, acid metabolic products, and the absence of mycolic acids. The data presented here show that *C. pyogenes* is also different from *C. diphtheriae* (23), *C. renale* (10, 31), and *C. bovis* (26) in its inability to use ammonia as a main nitrogen source, its stimulation by hemin, its requirement for riboflavin, nicotinic acid, adenine, and uracil, and its requirement for inositol when grown in the presence of amino acids with no peptides present. Reddy and Kao (21) and Slack and Gerencser (27) have noted the close relationship between *C. pyogenes* and *Actinomyces* species. In this regard it is of interest that the vitamin requirements of *C. pyogenes* are very similar to those of *A. israelii* in that both require riboflavin, nicotinic acid, biotin, and inositol in a medium containing amino acids as the major nitrogen source (6).

LITERATURE CITED

1. **Bruner, D. W., and J. H. Gillespie (ed.).** 1973. Hagan's infectious diseases of domestic animals, 6th ed., p. 308–323. Cornell University Press, Ithaca, N.Y.
2. **Bryant, M. P., and I. M. Robinson.** 1961. Some nutritional requirements of the genus *Ruminococcus*. Appl. Microbiol. 9:91–95.
3. **Charalampous, F. C.** 1969. Metabolic functions of myo-inositol. VII. Role of inositol in the transport of α-aminoisobutyric acid in KB cells. J. Biol. Chem. 244:1705–1710.
4. **Chen, R. F.** 1967. Removal of fatty acids from serum albumin by charcoal treatment. J. Biol. Chem. 25:173–181.

5. **Christie, A. O., and J. W. Porteous.** 1962. The growth factor requirements of the Wills strain of *Actinomyces israelii* growing in a chemically defined medium. J. Gen. Microbiol. **28**:455–460.
6. **Christie, A. O., and J. W. Porteous.** 1962. The cultivation of a single strain of *Actinomyces israelii* in a simplified and chemically defined medium. J. Gen. Microbiol. **28**:443–454.
7. **Cummins, C. S., and H. Harris.** 1956. The chemical composition of the cell wall in some Gram-positive bacteria and its possible value as a taxonomic character. J. Gen. Microbiol. **14**:583–600.
8. **DeVries, W., W. M. C. van Wijck-Kapteijn, and A. H. Stouthamer.** 1973. Generation of ATP during cytochrome-linked anaerobic electron transport in propionic acid bacteria. J. Gen. Microbiol. **76**:31–41.
9. **Halliday, J. Q., and L. Anderson.** 1955. The synthesis of myo-inositol in the rat. J. Biol. Chem. **217**:797–802.
10. **Harai, K., and R. Yanagawa.** 1967. Nutritional requirements of *Corynebacterium renale*. Jpn. J. Vet. Res. **15**:121–134.
11. **Henry, S. A., K. D. Atkinson, A. I. Kolat, and M. R. Culbertson.** 1977. Growth and metabolism of inositol-starved *Saccharomyces cerevisiae*. J. Bacteriol. **130**:472–484.
12. **Jayne-Williams, D. J., and T. M. Skerman.** 1966. Comparative studies on coryneform bacteria from milk and dairy sources. J. Appl. Bacteriol. **29**:72–92.
13. **Kihara, H., W. G. McCullough, and E. E. Snell.** 1952. Peptides and bacterial growth. I. L-Alanine peptides and growth of *Lactobacillus casei*. J. Biol. Chem. **197**:791–800.
14. **Knight, H. D.** 1979. Corynebacterial diseases, p. 311–326. *In* A. Laskin and H. Lechevalier (ed.), CRC handbook of microbiology, vol. 1, section A: bacterial, rickettsial and mycotic diseases. CRC Press Inc., Boca Raton, Fla.
15. **Lembach, K., and F. C. Charlampous.** 1967. Metabolic functions of myoinositol. VI. Impairment of amino acid transport in KB cells caused by inositol deficiency. J. Biol. Chem. **242**:2606–2614.
16. **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.
17. **Phillips, A. W., and P. A. Gibbs.** 1961. Techniques for the fractionation of microbiologically active peptides derived from casein. Biochem. J. **81**:551–556.
18. **Pittman, K. A., and M. P. Bryant.** 1964. Peptides and other nitrogen sources for the growth of *Bacteroides ruminocola*. J. Bacteriol. **88**:401–410.
19. **Reddy, C. A., C. P. Cornell, and A. M. Fraga.** 1980. Chemically defined growth medium for Corynebacterium pyogenes. Am. J. Vet. Res. **40**:843–845.
20. **Reddy, C. A., C. P. Cornell, and M. Kao.** 1977. Hemin-dependent growth stimulation and cytochrome synthesis in *Corynebacterium pyogenes*. J. Bacteriol. **130**:965–967.
21. **Reddy, C. A., and M. Kao.** 1978. Value of acid metabolic products in identification of certain corynebacteria. J. Clin. Microbiol. **7**:428–433.
22. **Rizza, V., P. R. Sinclair, D. C. White, and P. R. Cuorant.** 1968. Electron transport system of the protoheme-requiring anaerobe *Bacteroides melaninogenicus*. J. Bacteriol. **96**:665–671.
23. **Rogosa, M., C. S. Cummins, R. A. Lelliott, and R. M. Keddie.** 1974. Coryneform group of bacteria, p. 599–632. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
24. **Rosen, H.** 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. **67**:10–15.
25. **Sebrell, W. H., and R. S. Harris (ed.).** 1954. The vitamins, p. 366–371. Academic Press, Inc., New York.
26. **Skerman, T. M., and D. J. Jayne-Williams.** 1966. Nutrition of coryneform bacteria from milk and dairy sources. J. Appl. Bacteriol. **29**:119–130.
27. **Slack, J. M., and M. A. Gerencser.** 1975. *Actinomyces*, filamentous bacteria biology and pathogenicity. Burgess Publishing Co., Minneapolis.
28. **Smith, J. E.** 1966. *Corynebacterium* species as animal pathogens. J. Appl. Bacteriol. **29**:119–130.
29. **Stahl, E. (ed.).** 1969. Thin layer chromatography, 2nd ed. Springer, New York.
30. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
31. **Van Eseltine, W. P., W. M. Cox, and S. Kadis.** 1978. Minimal nitrogen requirements of *Corynebacterium renale* strains. Am. J. Vet. Res. **39**:123–127.
32. **White, D. C., M. P. Bryant, and D. R. Caldwell.** 1962. Cytochrome-linked fermentation in *Bacteroides ruminicola*. J. Bacteriol. **84**:822–828.
33. **Yarbrough, H. F., Jr., and F. M. Clark.** 1957. Utilization of inositol, an essential metabolite for *Schizosaccharomyces pombe*. J. Bacteriol. **73**:318–323.