NUTRITION OF *STREPTOCOCCUS BOVIS* IN RELATION TO DEXTRAN FORMATION

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ABSTRACT

BARNES, ISABEL J. (Cornell University, Ithaca, N. Y.), H. W. SEELEY, AND P. J. VANDEMARK. Nutrition of *Streptococcus bovis* in relation to dextran formation. J. Bacteriol. **82**:85–93. 1961.—Three nutritional types of *Streptococcus bovis* were encountered. Minimal media for their growth are described. Transferable growth in defined media was always accompanied by dextran production and the nutrition for dextran production could not be distinguished from the minimal nutritional requirements of the organism for growth. Measurable, free CO_2 was evolved from a complex medium during the growth of *S. bovis*. Several compounds were found to replace partially the CO_2 requirement of the species. When fractured cell preparations containing *S. bovis* dextran were employed as antigens, rabbits failed to yield sera which would precipitate *S. bovis* or leuconostoc dextrans.

Dain, Neal, and Seeley (1956) showed a relationship between dextran synthesis by *Streptococcus bovis* and availability of CO_2. In a study of this effect, the majority of cultures examined by these investigators formed large masses of slime on sucrose gelatin agar plates when they were incubated in CO_2-containing atmospheres, but no slime formation was apparent when the cultures were incubated in air. Bailey and Oxford (1958) showed that CO_2 was essential for the formation of dextran in a complex broth medium and reported that other prerequisites for polysaccharide formation were anaerobic growth conditions, the presence of sucrose, and adequate buffering action.

Several studies have dealt with the effect of CO_2 on the nutrition of *S. bovis* without specific relation to dextran synthesis. Prescott and his co-workers (1955, 1957) have shown that CO_2 will decrease the amino acid requirements of these organisms and that in the presence of CO_2, *S. bovis* will grow with arginine as the sole source of nitrogen in a glucose base medium. Wolin, Manning, and Nelson (1959) were able to obtain growth of rumen strains of *S. bovis* under CO_2 in a glucose-containing defined medium in which ammonium chloride was the sole nitrogen source. Wolin and Weinberg (1960) demonstrated that a substance produced by autoclaving glucose with phosphate was necessary for growth to take place above pH 7.0 when ammonium chloride was the sole source of nitrogen. They also showed that certain electron acceptors as acetaldehyde, acetone, and pyruvate would replace this product of autoclaving. Below pH 7.0, this product appeared to be unnecessary. Wright (1960) demonstrated that $C^{14}O_2$ was chiefly incorporated into aspartic acid with lesser amounts in threonine, glutamic acid, adenine, guanine, uridylic acid, and cytidylic acid. He also showed that the organism will preferentially fix CO_2 to form aspartic acid even though an adequate exogenous supply of aspartic acid is provided.

In the first specific study of the nutrition of *S. bovis*, Niven, Washburn, and White (1948) noted an absolute requirement for biotin and a strong growth stimulating effect of thiamine in a defined medium. More recent work by Ford, Perry, and Briggs (1958) indicated that no vitamins were required by the strains which they examined under anaerobic conditions in an atmosphere of CO_2. Their medium, however, included the oleic acid-containing compound, Tween 80 (polyoxyethylene sorbitan monooleate). Since oleic acid and Tween 80 have been shown to substitute for biotin in some of the lactic acid organisms, the evidence of Ford and his co-workers does not exclude a requirement for biotin in the absence of oleic acid.

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Oxford (1958) investigated the nutritional requirements of rumen strains of *S. bovis* in relation to dextran formation and found an absolute requirement for biotin for growth in a medium which contained acetate buffer and which was incubated in an atmosphere of CO_2. In addition, he found a requirement for calcium pantothenate for good dextran formation. On serial transfer, growth was maintained on this minimal medium but dextran was not readily formed unless eight vitamins of the B complex were present.

In the study being reported here, the general nutrition of *S. bovis* in relation to dextran formation was investigated as well as some aspects of the CO_2 metabolism of this organism.

MATERIALS AND METHODS

The strains of *S. bovis* utilized in this study were taken from the culture collection of Cornell University. The original source as well as some of the characteristics of these strains are shown in Table 1.

TABLE 1. *Sources and characteristics of the strains of Streptococcus bovis*

Strain no.*	Source	Action on mannitol	Type of hemolysis
141	Unknown	NC†	α
270	Infant feces	NC	γ
350	American deer feces	NC	α
444	Milk	Acid	γ
470	Bloat	NC	α
HO	Bovine rumen	NC	α
P10	Subacute bacterial endocarditis	Acid	γ

* All strains form dextran.
† NC = no change.

The basal medium contained sucrose, 200 mg; K_2HPO_4, 120 mg; sodium thioglycolate, 5 mg; NaCl, 20 mg; $MgSO_4 \cdot 7\ H_2O$, 0.8 mg; $FeSO_4 \cdot 7\ H_2O$, 40 μg; and $MnCl_2$, 12 μg; in a 10-ml volume. The vitamins used included biotin, niacin, calcium pantothenate, pyridoxin, riboflavin, and thiamine. These were added at levels of 0.01 mg per 10 ml of media with the exception of biotin which was added at a level of 0.01 μg per 10 ml. The nitrogen sources and purines were added to a 10-ml volume as follows: arginine, 10 mg; glutamic acid, 2 mg; ammonium chloride, 10 mg; adenine, 50 μg; and xanthine, 50 μg.

After the addition of amino acids, purines, and vitamins, the pH was adjusted to 6.8 and the medium was autoclaved for 7 min at 121 C. Sodium bicarbonate was autoclaved separately and added at a level of 16.8 mg per 10 ml. During the study of the vitamin requirements, arginine, glutamic acid, and either xanthine or adenine were included as nitrogen sources. The amino acid requirements were studied in the presence of biotin and thiamine, plus calcium pantothenate when needed.

In an attempt to find compounds which would replace CO_2 as an essential growth factor, the following substances were added to the complete defined medium in the amounts indicated: asparagine, 2 mg; aspartic acid, 10 mg; uracil, 50 μg; Tween 80, 100 mg; Na-acetate, 70 μg; and malic acid, 2 mg.

The initial inoculation into defined medium was made with a loop of a 24-hr culture which had been grown in a glucose yeast extract tryptone broth. Subsequent serial transfers in the defined media were made with a wire needle.

The amount of growth obtained is expressed in terms of optical density (OD) and was determined with a colorimeter at the end of 24 and 72 hr.

The presence of dextran was determined by the use of type II pneumococcus antiserum (commercial preparation of the Burroughs-Wellcome Co.; no. K1847). Sugg and Hehre (1942) reported that this extremely sensitive test would detect dextrans from leuconostoc and streptococcal sources, including *S. bovis*, at dilutions ranging from 1:500,000 to 1:2 million. Sugg, Hehre, and Neill (1942) showed that the polysaccharides from certain group H streptococci, *S. bovis*, and *Leuconostoc mesenteroides* had closely similar serological properties and that all were highly reactive with antisera obtained by immunization with cultures of any of these three bacteria which had been grown in sucrose broth. The capacity of these antisera to react with the polysaccharides was removed by adsorption with any of the bacteria providing that the adsorbing agent had been grown in a sucrose medium. Further evidence of the similarity of the three polysaccharides was that all reacted with type II and XX antipneumococcus sera, but not with other types.

The presence of dextran was examined in small vials by the standard technique in which sera were overlaid with Lancefield extracts of cells or by spent media which had been centrifuged clear of cells and neutralized.

The fact that *S. bovis* cultures do not produce dextran from glucose made it possible in the present work to use Lancefield extracts of glucose-grown cells as well as the spent medium as controls in the precipitin tests. These controls were designed to rule out reactions due to streptococcal antigens other then dextran. Cultures of *S. bovis* grown in glucose gave consistently negative reactions with type II antipneumococcus serum. Likewise, cultures of various strains of *S. bovis* used in the experiments, whether grown in glucose or sucrose media, gave no reaction with types I and III antipneumococcus serums, which are known not to react with dextran (Sugg and Hehre, 1942). These controls, which gave negative reactions, served to exclude precipitin reactions resulting from antigens common to the streptococci and pneumococci.

In work preliminary to the adoption of the precipitin test for detecting dextran, a number of dextran preparations from *S. bovis* were purified by repeated fractional alcohol precipitation. These purified dextrans (which upon hydrolysis gave ninhydrin-negative chromatograms) reacted with type II antipneumococcus serum at the 10^4 dilution, but not at all with sera of types I and III.

As a final precaution in insuring the specificity of the precipitin reaction, the defined media which contained sucrose were tested against the precipitating serum before they were inoculated, to rule out reactions which can result from dextran contamination of the sucrose. Dextran contamination of reagent grade sucrose preparations were reported by Neill et al. (1939).

Growth experiments, which involved the measurement of gas evolution, were conducted on a Warburg respirometer, employing sterile double side arm flasks. The main compartment contained 2.0 ml of a medium of the following percentage composition: sucrose, 8.5; tryptose, 1.4; yeast extract, 0.5; sodium acetate, 1.2; K_2HPO_4, 0.6; KH_2PO_4, 0.2; and 0.004 or 0.055 M K_2CO_3. One side arm contained 0.5 ml of cells that had been washed once and resuspended in physiological saline. The other side arm contained 0.5 ml of 3 N H_2SO_4. In some instances the center wall contained 0.15 ml of 40% KOH. The inoculum was tipped after an equilibration period. Readings were taken hourly for the first 12 hr and again at the end of 24 hr. At the termination of the experiment, the acid was tipped to release any CO_2 trapped in the medium and the final readings were taken. The atmosphere was nitrogen.

The amino acid decarboxylases were tested for using the method of Najjar (1955). The decarboxylation of malic and oxaloacetic acids were examined by a modification of the method of Ochoa (1955), which was originally developed for malic enzyme preparations from *Lactobacillus arabinosus*.

TABLE 2. *Composition of various basal media, expressed in weight per 10 ml*

Constituents	Medium		
	A	B	C
Sucrose	200.0 mg	200.0 mg	200.0 mg
K_2HPO_4	120.0 mg	120.0 mg	120.0 mg
Na-thioglycolate	5.0 mg	5.0 mg	5.0 mg
$NaHCO_3$	16.8 mg	16.8 mg	16.8 mg
Arginine	10.0 mg	10.0 mg	
Glutamic acid	2.0 mg	2.0 mg	
Ammonium chloride			10.0 mg
Xanthine	50.0 μg		
Adenine		50.0 μg	
Biotin	0.01 μg	0.01 μg	0.01 μg
Thiamine·HCl	0.01 mg	0.01 mg	0.01 mg
Calcium pantothenate		0.01 mg	
Salts*	+	+	+
Strains of *Streptococcus bovis*	350	270	444, 141, HO, P10, 470

* Salts present, *see* text.

For the production of antidextran sera in rabbits, a strain of *S. bovis* of rumen origin was grown for 48 hr at 37 C in 40 ml of yeast extract broth containing 0.5% sucrose. The cells were removed by centrifugation, washed once, and resuspended in 6 ml of sterile 0.85% saline. Cell suspensions were placed in a cup with 4.0 g of Ballotini beads and subjected to breakage for 1 hr on a Mickle disintegrator which had been adjusted to give a thrust of about ½ in. Cell breakage was determined microscopically. The supernatant liquid, after clarification by centrifugation, gave a strong precipitin test with type II pneumococcus serum, indicating the presence of dextran. A fresh, broken cell preparation was made for each injection.

Rabbits were given three intravenous injections of 0.5 ml (later increased to 1.0 ml) of broken cells at 2-day intervals and rested 1 week before the next series. Rest periods of 1 month followed by a repeated immunization schedule were employed in later experiments.

RESULTS

The exploratory study was done with *S. bovis* strain 350. This strain grew, although rather poorly, in a medium containing xanthine, arginine, glutamic acid, biotin, and thiamine. This is designated as medium "A" and is shown in Table 2. As additional strains were examined (Table 3), a marked difference in vitamin requirements became apparent. Strain 270 had an absolute requirement for calcium pantothenate in addition to biotin, whereas the other strains tested grew with biotin as their sole vitamin although growth was stimulated considerably by thiamine. The compositions of media that supported good growth of these strains are also shown in Table 2, and are listed as "B" and "C."

Variation of the strains with respect to the nitrogen requirements can be seen in Table 4. The strains other than 350 grew well in medium containing arginine, glutamic acid, and xanthine. Strain 270 required either xanthine or adenine for

TABLE 3. *Growth of Streptococcus bovis in defined media of varying vitamin composition*

Vitamins included	Strain no.				
	350	270	141	470	HO
All of the vitamins	0.158*				
Biotin	0.021	0.103	0.699	0.545	0.561
Biotin, thiamine	0.159	0.014	0.810	0.727	0.745
Biotin, Ca-panthothenate	0.035	0.429	0.710	0.538	0.585
Biotin, niacin	0.125		0.663	0.488	0.585
Biotin, niacin, thiamine	0.153	0.022			
Biotin, thiamine, Ca-pantothenate	0.101	0.611			
Thiamine		0.050	0.059	0.061	0.102
Biotin, Ca-pantothenate, niacin		0.432			

* Values in table are optical density after 72 hr growth.

Vitamins included in these media:	Basal medium per 10 ml:	
Biotin	Sucrose	200.0 mg
Niacin	K_2HPO_4	120.0 mg
Ca-pantothenate	Na-thioglycolate	5.0 mg
Pyridoxin	$NaHCO_3$ (for strain 350)	4.2 mg
Riboflavin	$NaHCO_3$ (other strains)	16.8 mg
Thiamine·HCl	Salts	
	Arginine	10.0 mg
	Glutamic acid	2.0 mg
	Xanthine (for strain 350)	50.0 μg
	Adenine (other strains)	50.0 μg

TABLE 4. *Effect of various nitrogen sources on the growth of Streptococcus bovis in defined media*

Strain no.	Miimal medium						
	Omissions						
	None	Xanthine	Xanthine, arginine	Xanthine, arginine	Xanthine, glutamic acid	Xanthine	Xanthine, arginine, glutamic acid
	Additions						
	None	Adenine	Adenine, 2 mg glutamic acid	Adenine, 10 mg glutamic acid	Adenine, 20 mg arginine	None	10 mg NH_4Cl
350	0.182*	0.113					
141	0.538	0.853	0.194	0.109	0.725	0.791	0.663
270†	0.299	0.423	0.272	0.213	0.502	0.0	0.0
444	0.796	0.824	0.194	0.221	0.710	0.820	0.745
470	0.561	0.733	0.168	0.150	0.241	0.660	0.530
P10	0.638	0.699	0.328	0.387	0.569		0.796
HO	0.648	0.704	0.128	0.187	0.611	0.726	0.613

* Optical density after 72 hr.

† Supplemented with Ca-pantothenate.

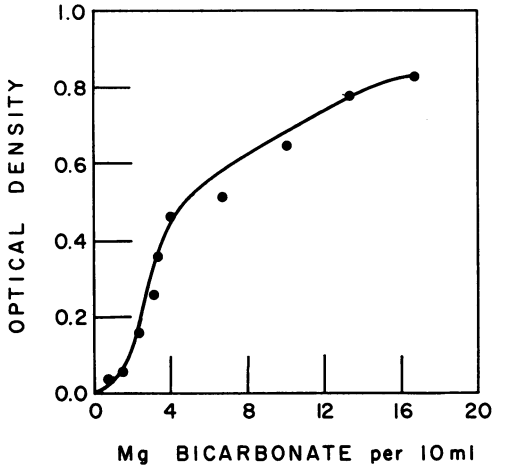

FIG. 1. *Growth response of Streptococcus bovis strain HO to graded levels of sodium bicarbonate.*

growth in the presence of arginine or glutamic acid. The additional five strains had no specific organic nitrogen requirement and could grow with ammonium chloride as the sole nitrogen source in the presence of CO_2. In all instances when transferable growth occurred, dextran could readily be detected in the supernatant fluid.

There was no growth in any of these media in the absence of a source of CO_2. Figure 1 shows the growth response of *S. bovis* strain HO to graded levels of sodium bicarbonate.

Since CO_2 appears to play such an important role in the growth of these organisms, various compounds were tested to determine their ability to substitute for CO_2. Of the 20 compounds tested, a few were able to replace CO_2 for growth and dextran formation, but, as can readily be seen from Table 5, none of the strains reached the same level of growth in the presence of these compounds as it did when CO_2 was supplied. In all instances where growth occurred with a CO_2 substitute, dextran was formed.

The observation that an occasional strain of *S. bovis* would grow well in a complex medium and would produce obvious slime without the deliberate addition of a known source of CO_2 led us to search for either a compound from which CO_2 could be secured by action of the organism or a compound which could fill the CO_2 requirement.

To demonstrate that CO_2 was evolved by growing cells at some period during the growth cycle, cultures were incubated in a complex medium in sterile Warburg cups for a period of 24 hr under an atmosphere of nitrogen. The results are shown in Table 6. In control flasks with KOH in the center well no measurable gas was evolved. It is clear from the results that CO_2 in excess of that required by the growing cells can be released by *S. bovis* from some constituent or a derivative of this constituent of the complex medium.

TABLE 5. *Growth response of Streptococcus bovis to compounds added as carbon dioxide substitutes*

Compound added	Strain no.	
	141	HO
$NaHCO_3$	0.810*	0.745
Asparagine	0.309	0.398
Aspartic acid	0.469	0.372
Uracil	0.469	0.166
Tween 80	0.398	0.344
Na-acetate	0.530	0.125
Malic acid	0.545	0.319

* Optical density at 72 hr.

TABLE 6. *Carbon dioxide evolved during the growth of Streptococcus bovis*

Conditions	Strain no.		
	350	470	444
	μl	μl	μl
Medium alone	24	101	101
Total from growing culture	90	196	297
Net from growing culture	66	95	196

Experiments with resting cells failed to clarify the origin of the gas. With sucrose, oxaloacetic or malic acids as substrates, no CO_2 was detected. The decarboxylation of arginine, aspartic acid, and glutamic acid could not be demonstrated. Employing these same strains Dunican (1960, *unpublished data*) could not demonstrate CO_2 formation from histidine, phenylalanine, lysine, ornithine, tyrosine, valine, isoleucine, serine, tryptophan, α-aminobutyric acid, uracil, adenine, or xanthine.

DISCUSSION

The correlation noted by Dain et al. (1956) between dextran-forming ability and the failure to ferment mannitol by a majority of strains of *S. bovis* was based upon visual observation of slime-forming colonies on the surface of sucrose gelatin agar plates. When a more critical method of detecting dextran was employed, i.e., the precipitin test, this relationship was found to be quantitative and dextran was produced by some cultures which also formed acid from mannitol. Although most of the dextran-forming cultures used in the present study did not ferment mannitol, strains P10 and 444, both of which formed dextran, produced acid from this substance.

It has been our experience that all organisms which could be identified culturally as *S. bovis* form readily detectable dextran when grown under the proper conditions. At very low CO_2 tensions, dextran production by the species occurs at a minimum; but as CO_2 becomes available in excess, polysaccharide formation increases out of proportion to the increased numbers of cells. This suggests that dextran-sucrase synthesis is contingent upon previous satisfaction by CO_2 of some more basic metabolic needs of the cell.

Among the seven strains of *S. bovis* that were investigated with respect to growth and dextran formation, three nutritional patterns emerged. Five of the strains grew with biotin as their only supplied vitamin and could use ammonium chloride as their sole nitrogen source. An additional type required calcium pantothenate in addition to adenine, biotin, thiamine, and arginine or glutamic acid. A third type appeared to require xanthine and additional amino acids for comparable growth. All of these strains, however, had the characteristic in common in that they all formed dextran in defined media.

A point to be emphasized here is that whenever transferable growth of *S. bovis* occurred, dextran was formed by the microorganism. It was impossible to distinguish between the nutritional requirements for growth and those for dextran production. The cultures were carried in phosphate-buffered media for more than 25 serial transfers and during this period, there was no decrease in the level of growth or loss of capacity for dextran production.

These findings differ in several respects from those of Oxford (1958). In his studies, he found that biotin and calcium pantothenate were essential for dextran production in acetate-buffered media. When his organisms were subcultured on media with biotin and calcium pantothenate alone, they readily lost their ability to form dextran although growth was still good on transfer. Thereby he was able to secure growth without concurrent dextran formation. If, however, serial transfers were made in acetate-buffered media containing all eight common vitamins of the B complex, the original level of dextran formation was maintained. When Oxford utilized media buffered with phosphate,

dextran production rarely accompanied growth. In his experience, the presence of 1.5% dialyzed or nondialyzed tryptone in addition to the eight B vitamins was necessary to insure the production of dextran simultaneously with growth in phosphate-buffered media.

The divergent results of these two investigations can probably be explained on a quantitative basis. The criteria used by Oxford to detect dextran in culture fluid included (i) a distinctly opalescent appearance; (ii) considerable increase in viscosity; (iii) difficulty in spinning down more than a small fraction of the organisms in an undiluted culture; (iv) the formation of a white precipitate upon addition of 4 volumes of ethanol to the undiluted culture supernatant. It is clear that small amounts or traces of dextran, which would be revealed by the precipitin test. would escape detection by these other methods. Thus the appearance of measurable dextran in Oxford's cultures might indicate a stimulation of existing dextran production to a measurable level rather than the initiation of dextran synthesis.

None of several attempts was successful in producing an antiserum which would precipitate either crude solutions of the dextran of *S. bovis* or purified preparations of the dextran of *L. mesenteroides*. Both of these dextran preparations reacted with type II pneumococcus serum. The haptene nature of purified leuconostoc dextran in rabbits is well known but an antidextran serum was obtained by Glynn, Holbrow, and Johnson, (1954) by employing as an antigen, a heat killed preparation of a type 4, group A streptococcus to which dextran had been adsorbed during a 1-hr contact period. The presence of cellular protein from *S. bovis* did not endow our broken-cell preparations containing dextran with antigenicity. Reactions, however, were obtained between the antisera so prepared, and HCl extracts (after Lancefield) of several strains of *S. bovis* which had been grown with glucose instead of sucrose to avoid dextran formation. The antisera, therefore, contained antibodies against other antigens common to the species, but not against dextran.

The strains of *S. bovis* used in this study were unable to grow in the defined medium containing the levels of arginine and glutamic acid described by Niven et al. (1948) unless bicarbonate was also included. The growth secured by Niven may possibly be attributed to the presence of factors found during the autoclaving of a complex medium with glucose. Field and Lichstein (1958) found that glucose autoclaved with amino acids supplied growth factors and would also satisfy the carbon dioxide requirement of *Propionibacterium* species. The failure of *S. bovis* to grow in the medium of Niven et al. had previously been observed by Prescott and his co-workers (1955, 1957). In addition, these investigators found that bicarbonate functioned in overcoming the inhibitory effects of various amino acid combinations.

With the strains used in the study being reported here growth in defined media varied directly, within limits, with the amount of bicarbonate present. In the presence of a sufficient concentration of bicarbonate, the nitrogen requirements of these organisms were very flexible. Wolin et al. (1959) reported the isolation of rumen strains of *S. bovis* that utilized ammonium salts as their sole nitrogen source in the presence of carbon dioxide. Five of the strains used in the present study also utilized ammonium chloride as a nitrogen source. The sources of these strains were varied and included the normal rumen, a case of bloat in sheep, and human subacute bacterial endocarditis.

Deibel and Niven (1955) showed that Tween 80 can replace CO_2 in the nutrition of the "minute" colony group G streptococci. Martin and Niven (1960) found that the "minute" streptococci fixed labeled CO_2 into oxaloacetic acid via the Wood-Werkman reaction, and that the oxaloacetic acid was then aminated to form aspartic acid. They found also that aspartic acid would satisfy the CO_2 requirement of these organisms in the presence of oleic acid. These investigators have proposed that oleic acid acts in some way to alter permeability, thus alleviating the need for CO_2.

The pathway of CO_2 fixation in *S. bovis* as noted earlier in the reference to the work of Wright (1960) is similar to that of the "minute" streptococci. Wright showed, in addition, that in the presence of Tween 80 increased amounts of labeled aspartic acid were taken up by the cell, although this event led to a negligible increase in cell protein carbon-14. Additional evidence indicated that, in the presence of carbon-14 aspartic acid, the marker appeared in an ethanol-soluble ether-soluble cell fraction which pre-

sumably was an aspartic acid-lipid complex. This observation suggested once again that Tween 80 functions in partially replacing CO_2 in the nutrition of *S. bovis* by altering the cell membrane to permit a greater uptake of aspartic acid.

The mechanisms by which the compounds other than aspartic acid take the place of the CO_2 are not known. Uracil, however, has been noted as a fixation product of CO_2 and may thus have a sparing effect on CO_2 in a manner similar to that of aspartic acid. Malic acid is readily converted to oxaloacetic acid by organisms having the enzymes of the Krebs cycle and although other related lactic acid bacteria can do so, there is no indication that *S. bovis* is able to carry out this reaction.

Although our attempts to demonstrate the substrate for gas production were unsuccessful with resting cells, the results of Jones (1958), which have recently come to our attention, show that CO_2 is formed by some growing cultures of *S. bovis* in a yeast extract medium containing 4% sodium malate plus 1% glucose. Seven of 12 strains tested showed visible gas production under agar seals. The ability of some strains to form gas from malate might explain the variability of different strains with respect to absolute need or critical levels of supplied CO_2 necessary for growth.

The widespread requirement for CO_2 by many heterotropic microorganisms points to the fact that there are several possible ways that this compound could function in the growth of the *S. bovis*, but nothing is known of the way in which it functions in the formation of dextran. The fact that cell-free dextransucrase forms dextran readily in the absence of any added CO_2 indicates that this substance is not functioning directly in polysaccharide formation. Since dextran synthesis and growth are inseparable, it would seem reasonable to postulate that CO_2 is functioning in a general role relating to protein synthesis.

Wright (1960) came to the same conclusion in his work, namely that the stimulation of growth by CO_2 is due to biosynthetic reactions involving CO_2 which lead to a sufficient supply of the intermediates required in nucleic acid and protein synthesis.

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