Requirements for Growth of *Streptococcus agalactiae* in a Chemically Defined Medium¹

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Despite extensive work on the group A streptococci (A. W. Bernheimer and A. M. Pappenheimer, Jr., *J. Bacteriol.* **43**:481, 1942; H. D. Slade and W. C. Slamp, *J. Exptl. Med.* **102**:291, 1955), very few investigators have been interested in the group B organisms which may or may not have similar physiological requirements for growth. The vitamin requirements of 19 human and bovine strains and the amino acid requirements of 2 of these strains were determined by C. F. Niven, Jr. (*J. Bacteriol.* **46**:573, 1943). G. Redaelli and L. Bertoni (*Boll. Ist. Sieroterap. Milan.* **41**:204, 1962) carried out similar studies on 17 serotypes of *Streptococcus agalactiae*. Previous studies from this laboratory (N. P. Willett and G. E. Morse, *J. Bacteriol.* **91**:2245, 1966) demonstrated that *S. agalactiae* could be grown in a chemically defined medium which supported a rate and an amount of growth comparable to that obtained in complex medium.

The purpose of this investigation was to determine the minimal nutritional and physiological requirements of a specific strain of *S. agalactiae* which has been used widely in experimental studies of bovine mastitis (J. M. Murphy, *Cornell Vet.* **49**:411, 1949). *S. agalactiae* is becoming increasingly important to the medical profession as well as to the veterinarian owing to its implication as a cause of neonatal sepsis (T. C. Eickhoff et al., *New Engl. J. Med.* **89**:421, 1964).

The strain of *S. agalactiae*, preparation of the inoculum, and description of the complete chemically defined medium have been reported previously (N. P. Willett and G. E. Morse, *J. Bacteriol.* **91**:2245, 1966). Minimal nutritional requirements of the organism were determined by eliminating individual nutrients from the defined medium and determining whether the resultant medium would support growth. Amounts of 1% were serially transferred into each deficient me-

dium to determine whether the particular nutrient was essential for continued transfer. Tubes were incubated at 37 C for 24 hr, and optical density (OD) was read at 550 m μ on the Spectronic-20 colorimeter. Growth was expressed either as per cent growth of control, as described previously (N. P. Willett and G. E. Morse, *J. Bacteriol.* **91**:2245, 1966), or read directly as OD.

The amino acid requirements of this strain of *S. agalactiae* are shown in Table 1. They have been arbitrarily divided into three categories: those amino acids which omitted from the medium result in reduction of growth from the initial transfer to 80% or less than that of the control; those which result in reduced growth of a smaller magnitude as a result of continued transfer into deficient medium; and those which have little or no effect in either two categories. With the exception of glycine, histidine, isoleucine, and methionine, absence of any other amino acid in the first category resulted in complete absence of growth. These results compare favorably with those of Niven (*J. Bacteriol.* **46**:573, 1943); however, he did not report requirements for threonine, tyrosine, methionine, or glycine. G. Redaelli and L. Bertoni (*Boll. Ist. Sieroterap. Milan.* **41**:204, 1962) reported requirements for threonine and tyrosine, but the requirement of a reducing agent such as cysteine or sodium thioglycolate was not observed. Growth in succeeding transfers in the absence of histidine, methionine, and isoleucine was similar to that of the initial transfer. After the second transfer, in the absence of glycine, growth was comparable to that of the control. Glutamine could be substituted for glutamic acid.

In general, the requirements for vitamins were not as critical qualitatively and quantitatively as those for the amino acids and were very close to those previously reported (C. F. Niven, Jr., *J. Bacteriol.* **46**:573, 1943). Elimination of calcium pantothenate resulted in complete lack of growth, whereas omission of thiamine, nicotinic acid, or biotin resulted in lesser degrees of inhibition.

Since elimination of individual "nonessential"

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TABLE 1. *Effect of omission of individual amino acids on growth of Streptococcus agalactiae in a chemically defined medium*

Amino acids ^a	Per cent of growth in complete medium ^b
Essential for continued transfer	
Alanine	84-44
Proline	85-80
Aspartic acid or asparagine	93-79
Nonessential	
Cystine	99-100
Serine	99-100

^a The following amino acids are essential for growth resulting from initial transfer. Figures in parentheses represent per cent of growth in complete medium: arginine (0); cysteine (0); glycine (37), growth equal to control after second transfer; glutamic acid or glutamine (0); histidine (21); isoleucine (15); leucine (0); lysine (0); methionine (18); phenylalanine (0); threonine (0); tyrosine (0); tryptophan (0); and valine (0).

^b The higher values represent per cent of growth in complete medium resulting from initial transfer. Lower values represent per cent of growth in complete medium after four serial transfers.

nutrients does not preclude a cumulative action by these compounds, it was decided to omit simultaneously all those compounds which did not appear to be required, either for initial or continuous transfer, i.e., cystine, serine, asparagine, riboflavine, folic acid, and xanthine. The results (Fig. 1) demonstrate that both total growth and rate of growth were reduced markedly in the minimal medium. Differences at 24 hr were essentially the same as at 7 hr.

When individual "nonessential" compounds were added back to the minimal medium, serine was the most effective, restoring growth almost to the level of the complete medium. Asparagine, cystine, and riboflavine were less effective, in the order named. Folic acid and xanthine had little or no effect when added to the minimal medium. The reason for stimulation of growth by individual "nonessential" compounds is unclear. In the case of the amino acids, it may be due to a general requirement for amino nitrogen.

Substitution of other reducing agents at various inoculum levels as shown in Table 2 demonstrated that thioglycolic and thiomalic acids were as effective as cysteine for initiation of growth at all inoculum levels, whereas ascorbic acid was less efficient. All compounds were capable of initiating growth from an inoculum as low as 3,100 colony-forming units per ml. Absence of any reducing agent resulted in almost complete elimination of

FIG. 1. *Effect of removal of "nonessential" nutrients on growth.*TABLE 2. *Effect of reducing agents^a on initiating growth of Streptococcus agalactiae from various inoculum levels*

Inoculum (colony-forming units/ml)	OD ^b		
	Cysteine HCl	Ascorbic acid	Thioglycolic acid
31×10^6	0.77	0.63	0.78
31×10^5	0.73	0.53	0.69
31×10^4	0.67	0.47	0.60
31×10^3	0.60	0.38	0.57
31×10^2	0.60	0.25	0.53

^a Cysteine HCl at 3.7×10^{-3} M; all other reducing agents at 5×10^{-3} M. Results with thiomalic acid identical to cysteine and thioglycolic acid.

^b OD readings at 550 m μ on Spectronic-20 colorimeter.

growth. Initiation of growth from small inocula by cysteine or other reducing compounds confirmed observations with *S. pyogenes* (H. D. Slade and G. A. Knox, J. Bacteriol. 60:301, 1950).

Determination of the physiological requirements of growth provides a basis from which to investigate parameters which govern the pathogenicity of a group of streptococci in which basic knowledge is lacking. The increasing incidence of human infection with a *Streptococcus* strain more commonly classified as an "animal pathogen" poses an interesting problem in host-parasite relationships which deserves to be explored further on a biochemical level.

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