

AMMONIUM SALTS AS A SOLE SOURCE OF NITROGEN FOR THE GROWTH OF *STREPTOCOCCUS BOVIS*

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In preliminary experiments, a selective enrichment procedure was used to isolate organisms from bovine rumen fluid which could grow on a medium containing ammonium salts as a sole source of nitrogen (Manning, M.S. Thesis, University of Illinois, 1954). Ten ml of rumen fluid were inoculated into 100 ml of enrichment medium containing glucose, a vitamin solution, inorganic salts, thioglycolate, and $(\text{NH}_4)_2\text{SO}_4$ in test tubes which were flushed with CO_2 before capping with a rubber stopper. After 48 hr at 39 C, 10 ml of the incubated culture were transferred to fresh medium and incubated for 48 hr. Plating of serial dilutions of this culture in an agar medium of identical composition as the enrichment medium was then carried out. Agar plates were incubated under CO_2 . Single colony isolates were obtained. Some of these isolates were members of the species *Streptococcus bovis* (Breed, Murray, and Smith, *Bergey's manual of determinative bacteriology*, 7th ed., The Williams & Wilkins Co., Baltimore, 1957). These isolates were continuously subcultured in a medium identical to the enrichment medium in an atmosphere of CO_2 . It should be emphasized that the only source of nitrogen in the medium, aside from trace amounts in the vitamin supplement, was $(\text{NH}_4)_2\text{SO}_4$.

The ability of *S. bovis* to grow in a medium containing ammonium salts as the sole source of nitrogen has been corroborated in experiments with strains isolated by direct anaerobic plating of rumen fluid on media similar to that described by Bryant and Burkey (*J. Dairy Sci.*, **36**, 1, 1953). These strains were isolated from 10^{-7} or 10^{-8} ml of rumen fluid. The strains produce α -hemolysis on horse blood agar and do not ferment mannitol. The medium used in the studies is that of Prescott, Ragland, and Stutts (*J. Bacteriol.*, **73**, 133, 1957), except that purines and pyrimidines were omitted. Excellent growth of *S. bovis* is obtained in this medium with NH_4Cl or glutamine as sole sources of nitrogen if NaHCO_3 is included in the medium (table 1). Asparagine, or a combination of glutamic acid and arginine, used as the

nitrogen source also supports growth of *S. bovis* in the presence of NaHCO_3 . Glutamic acid, urea, biuret, and nitrate do not support growth. NH_4Cl also supports growth of a strain of *S. bovis* which is indifferent on horse blood agar and ferments mannitol.

Niven, Washburn, and White (*J. Bacteriol.*, **55**, 601, 1948) reported that glutamic acid and arginine would satisfy the nitrogen requirement

TABLE 1
*Growth response to NH_4^+ and L-glutamine**

Additions to 10 ml Basal Medium	Optical Density $\times 100^\dagger$		
	Hr		
	24	47	70
NH_4Cl , 7 mg	1	41	49
NH_4Cl , 7 mg + NaHCO_3 , 16.8 mg	95	120	130
L-Glutamine, 20 mg	1	15	51
L-Glutamine, 20 mg + NaHCO_3 , 16.8 mg	93	100	105
NaHCO_3 , 16.8 mg	2	3	4

* One drop of a 1:10 dilution of a washed culture was used as the inoculum. Incubation at 37 C; N_2 atmosphere.

† Bausch and Lomb Spectronic 20 at 660 $\text{m}\mu$.

of 14 of 15 strains of *S. bovis*, and a majority of strains could be cultivated in a medium containing arginine alone. Prescott and Stutts (*J. Bacteriol.*, **70**, 285, 1955) found that large amounts of arginine (as compared to the amounts used by Niven *et al.*) would support the growth of 2 strains of *S. bovis*, but the amount of arginine necessary for maximal growth could be spared with CO_2 . To the authors' knowledge, no member of the genus *Streptococcus* has been reported to grow on a medium containing ammonium salts as a sole source of nitrogen. Because of the occurrence of *S. bovis* in the rumen of animals in relatively large numbers, this organism can easily be conceived of as an important factor in the *de novo* synthesis of amino acids in the rumen.