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Influence of Nitrogen Source on Growth of *Streptococcus Bovis.** (25292)

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Niven *et al.*(1) showed that a number of strains of *Streptococcus bovis* have no absolute requirement for any single amino acid, and that most strains studied could grow in a medium containing arginine as the only source of amino nitrogen. Our previous work has shown growth dependence of 2 strains of *S. bovis* on CO_2 when arginine served as the nitrogen source(2), and demonstrated certain interactions between CO_2 and various individual amino acids during growth in this medium(3). Ability of the organism to grow well in media containing arginine as sole source of amino nitrogen suggested that other compounds might similarly serve in this capacity. This report describes ability of various individual amino acids and other nitrogenous compounds to support growth of 3 strains of *S. bovis* in the presence of added CO_2.

Materials and methods. The cultures were *Streptococcus bovis* ATCC 9809 and 2 cultures from Cornell University collection designated as P-10 and P-2. Maintenance of cultures and preparation of inocula have been described(2). *Basal medium* consisted of glucose, K_2HPO_4, sodium thioglycollate, adenine, guanine, uracil, a vitamin mixture, and salts mixture in the same proportions described by Prescott and Stutts(2). Various nitrogen sources were added for each experiment, usually at levels of 0.05 or 0.1 m mole/6 ml culture, although graded levels of these compounds were frequently tested. Also, some experiments compared responses to different amino acids supplied at the same level of total nitrogen. Filter sterilized $NaHCO_3$ was added to autoclaved medium at 0.05 or 0.2 m mole/6 ml culture to supply the CO_2 requirement (2,3). *Procedures for growth tests* were identical to those used in earlier experiments(2). Growth was determined turbidimetrically, either at a fixed time, or more commonly, at different times during the growth phase. Incubation was usually terminated at 72 hours or less, and was never carried beyond 96 hours.

Results. Ability of compounds to serve as sole nitrogen source.† Thirty-seven amino acids and related organic nitrogenous compounds were tested for ability to serve as sole source of amino nitrogen for 3 strains of *S. bovis*. A list of compounds tested and their effects on 2 strains are shown in Table I. These data represent average responses, and evaluations shown are based on the following factors: (a) total growth, (b) rate of growth, and (c) consistency of growth response from one experiment to another. Results obtained with strain P-2 are not shown, since this culture did not show consistent heavy growth with any of the individual compounds listed in Table I, although it grew well with a complete amino acid mixture, as did the other 2 strains.

Relatively few amino acids tested supported

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† Exclusive of vitamins, purines, and pyrimidines. Purines and pyrimidines are not essential for growth under conditions employed(1), but were routinely included in the basal medium to insure more rapid and regular growth.

TABLE I. Ability of Individual Compounds to Serve as Primary Nitrogen Source for Growth of 2 Strains of *S. bovis*.*†

Addition to basal medium	Strain P-10	Strain 9809
DL-α-Amino-*n*-butyric acid	+	—
L-Arginine • HCl	++++	++++
DL-Aspartic acid	+	+
DL-Citrulline	+++	+++
L-Cysteine • HCl	+	+
α, ε Diaminopimelic acid	++	±
D-Glucosamine • HCl‡	+++	++
L-Glutamic acid	±	±
L-Glutamine§	++++	+++
Glutathione (reduced)	±	±
Glycocyamine	±	±
L-Histidine • HCl	+++	++
L-Lysine • HCl	±	±
DL-Methionine	±	±
L-Ornithine • HCl	++	+
DL-Phenylalanine	+	+
L-Proline	±	±
DL-Serine	+++	+++

* Other compounds tested which failed to support growth of either strain were *DL*-Alanine, *DL*-α-Aminoadipic acid, γ-Amino-*n*-butyric acid, α-Aminoisobutyric acid, *DL*-α-Amino-*n*-caproic acid, *DL*-α-Aminophenylacetic acid, *DL*-α-Amino-*n*-valeric acid, *L*-Canavanine • HCl, Glycine, Guanidine, *L*-Homoarginine‖, Hydroxy-*L*-proline, *DL*-Isoleucine, *L*-Leucine, *DL*-Norleucine, *DL*-Norvaline, *DL*-Threonine, *L*-Tyrosine, and *DL*-Valine.

† Some compounds showed variation from one experiment to another in ability to support growth. Serine, for example, produced moderate to good growth in one experiment, yet little or none in the next, thus suggesting existence of some variation in cultural conditions. Careful attention to level of $NaHCO_3$ supplied was frequently beneficial, but did not completely eliminate the inconsistencies. These erratic growth responses were not eliminated by changing the density of inoculum, varying autoclaving time, or by filter sterilizing the nitrogen source.

‡ Effective when filter sterilized and added to autoclaved medium.

§ Effective either filter sterilized or autoclaved with medium.

‖ Kindly supplied by Dr. James B. Walker.

vigorous growth of *S. bovis,* indicating a definite selectivity with respect to nitrogen sources. Of amino acids tested, arginine was, by above criteria, the best nitrogen source for both strains P-10 and 9809. Superiority of arginine was not simply a result of its high nitrogen content, since it supported better growth than other compounds when compared at equal levels of nitrogen. Nutritional selectivity of these organisms is emphasized by the observation that 2 closely related structural analogues of arginine, canavanine and homoarginine, did not support rapid or heavy growth. On the other hand, neither of these compounds inhibited growth in medium containing arginine(3). Several amino acids failed to produce even traces of growth in repeated experiments (Table I); some of these compounds were identical to those which retard growth of this organism in the arginine medium(3).

The possibility existed that the organism might utilize any amino acid as a primary nitrogen source once growth had been initiated. A complete amino acid mixture(4) was therefore added to the basal medium in amounts just sufficient to initiate growth, and some 20 amino acids were tested individually for ability to support growth under these conditions. These low levels of complete amino acid mixture did not markedly increase ability of individual amino acids to serve as nitrogen source.

Unlike most organic nitrogenous compounds, simple ammonium salts produced excellent growth which frequently equalled or exceeded that obtained with arginine. Furthermore, strain P-2, which did not grow consistently well on any single amino acid tested, showed heavy growth in basal medium containing ammonium ions. These results provide an additional example of simple nutritional requirements of *S. bovis,* and also emphasize a rather high degree of nitrogen source selectivity, since most preformed amino acids did not support heavy growth. Fig. 1 compares growth obtained when arginine, ammonium acetate, and a complete amino acid mixture (4) served as nitrogen sources. Although the lag phase was sometimes longer with the ammonium medium, total growth was frequently greater than in the arginine medium. Removal of CO_2 from the ammonium medium by the method of Lyman *et al.*(5) resulted in growth failure as it did in the arginine medium(2).

That ammonium ions were capable of serving as a nitrogen source suggested that the effectiveness of arginine might be due in part to its degradation to ammonia, perhaps by action of arginine desimidase, which converts arginine to citrulline and ammonia in *Streptococcus faecalis*(6,7). This possibility appeared especially likely, since citrulline plus ammonium acetate produced rapid, heavy

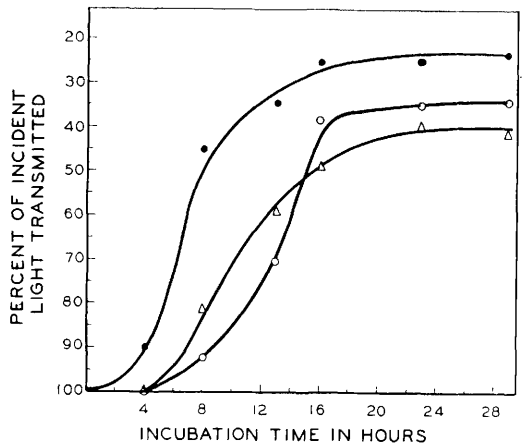

FIG. 1. Growth curves for *Streptococcus bovis* strain P-10, with different nitrogen sources. ●—●, Complete amino acid mixture (30 mg/tube). △—△, *L*-Arginine · HCl (10.5 mg/tube). ○—○, Ammonium acetate (3.85 mg/tube).

growth of all 3 strains. Ability of resting *S. bovis* cells to degrade arginine was therefore tested by incubating suspensions of washed cells with arginine. Neither appearance of ammonia nor destruction of arginine (measured colorimetrically(6)) was observed under conditions employed, nor did addition of glucose or of ATP plus a mineral mixture to resting cells produce detectable destruction of arginine. Experiments with cell free extracts also failed to show a perceptible reduction of arginine. Failure to detect arginine destruction by resting cells does not mean, however, that such reactions do not occur in actively growing cultures.

Ammonium acetate frequently produced more rapid growth than equivalent amounts of either ammonium sulfate or ammonium chloride, and growth failures sometimes occurred with the latter 2 compounds. This suggested either that sulfate and chloride were toxic, or that acetate was stimulatory. The latter was shown to be the case, since addition of 20 mg of sodium sulfate/6 ml culture failed to reduce growth in the medium containing ammonium acetate, and a marked increase in growth response occurred when medium containing ammonium sulfate was supplemented with 10 mg of sodium acetate/tube. Also, cells of strain P-10 growing in ammonium sulfate medium rapidly incorporated C^{14} labeled acetate. *Effects of amino acids on growth in ammonium medium.* Individual addition of certain amino acids to media containing arginine results in either retardation or stimulation of growth of *S. bovis*(3,8). A similar effect was observed when such additions were made to a medium containing ammonium acetate as the nitrogen source. Amino acids which retarded growth in the arginine medium also retarded growth in the ammonium medium. Citrulline and glutamic acid were extremely effective in stimulating growth in the ammonium medium by reduction of lag phase.

It is somewhat curious that these strains of *S. bovis* exert a decided selectivity toward amino acids which they utilize for growth, yet grow luxuriantly in media containing ammonium salts as the primary nitrogen source. This suggests the possibility that while the enzymatic make-up exists for synthesizing amino acids *de novo* from simple precursors, pathways are not available for certain interconversions among amino acids or for degradation of some amino acids to simpler compounds from which cellular amino acids may be synthesized.

Summary. Of 37 organic nitrogeneous compounds tested, arginine was the most effective as a primary nitrogen source for 2 strains of *Streptococcus bovis*. Other compounds supporting growth were glutamine, citrulline, serine, histidine, and glucosamine. The majority of compounds tested, including most amino acids, failed to support growth. Another strain of *S. bovis* did not grow well on any one organic compound tested as a primary nitrogen source. Simple ammonium compounds, however, were extremely effective in serving as nitrogen source for all 3 strains.‡ Growth in media containing ammonium salts was stimulated by acetate, and C^{14} labeled acetate was rapidly incorporated. No evidence was found for a mechanism involving degradation of arginine by resting cells or cell free extracts.

Technical assistance of Ann Stipp and Bettye Jones is gratefully acknowledged.

‡ *Added in proof.* After this paper was submitted for publication, Welin, *et al.* reported utilization of ammonium salts as sole nitrogen source by other strains of *S. bovis*(9).

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Effect of Adrenergic Blocking Agents on Fatty Acid Mobilization during Fasting.* (25293)

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Recent studies by Dole and by Gordon and their collaborators(1,2) indicate that a major portion of lipide mobilized from peripheral depots is transported in the form of non-esterified fatty acids (NEFA). It has been demonstrated that concentration of plasma NEFA increases during fasting and that venous blood draining areas rich in adipose tissue contains higher concentrations of NEFA than the blood supplying these areas(2). The additional observation that adipose tissue from fasting rats releases more NEFA *in vitro* than identically treated tissue from fed rats (3) leaves little doubt that the increase in plasma NEFA seen during fasting reflects lipide mobilization. A large body of evidence has been adduced to support the view that fat mobilization from adipose tissue during fasting is a consequence of increased sympathetic discharge(4). Although epinephrine and norepinephrine have been shown to be potent mobilizers of NEFA(2,5), we have shown (to be published) that the adrenal medulla does not play a major role in NEFA mobilization of fasting since adrenalectomized rats maintained with adrenal cortical replacement therapy respond to fasting in the normal fashion with an increase in plasma NEFA. The present paper reports experiments designed to assess the effects of sympathetic blockade at the ganglionic and postganglionic levels on NEFA mobilization during fasting.

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† U.S.P.H.S. Predoctoral Fellow, Nat. Cancer Inst.

Materials and methods. Female rats (Holtzman) weighing approximately 200 g were used in this study. *Blockade of epinephrine induced NEFA mobilization*: Epinephrine was administered subcutaneously as an oil suspension (1 mg in 0.5 ml peanut oil) to 2 groups of rats. One group received in addition 0.5 mg of ergotamine tartrate in a separate subcutaneous injection. A third group received 0.5 ml of the peanut oil vehicle subcutaneously. The animals were deprived of food at time of injection and were killed 3 hours later. *Blockade of NEFA mobilization during fasting*: Ergotamine tartrate (1.5 mg/rat, total dose) and hexamethonium bromide (15 mg of ion per rat, total dose) were administered in 3 equal subcutaneous injections 0, 4, and 8 hours following removal of food. Dibenzyline was injected under light ether anesthesia as a single dose of 0.5 mg into the tail vein of rats at the beginning of the fasting period. In all cases, control animals were injected with an equal volume of saline by the same route and at the same times. The animals were sacrificed 12 hours after initiation of the fast. At the end of the fasting interval, the animals were anesthetized with ether and blood for NEFA analysis was drawn into a heparinized syringe from the abdominal aorta. NEFA analyses were performed in duplicate by the method of Dole(6) on one ml aliquots of plasma.

Results. The NEFA mobilizing action of epinephrine is completely blocked by 0.5 mg of ergotamine (Table I). None of the au-