THE EFFECT OF METHIONINE AND METHIONINE PRECURSORS ON THE GROWTH OF *STREPTOCOCCUS LACTIS*¹

D. E. KIZER, M. L. SPECK, AND L. W. AURAND

Department of Animal Industry, North Carolina State College, Raleigh, North Carolina

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During the course of a study on the amino acid requirements of a strain of *Streptococcus lactis*, it was observed that the organism manifested adequate growth in the absence of added methionine. Further investigations with a number of strains of *S. lactis* revealed that all were able to manifest appreciable growth in the absence of added methionine. A detailed study using one of these strains showed that it responded favorably to the addition of amino acids previously shown to be precursors of methionine in the rat. It appeared that the organism readily synthesized methionine and that the pathway of the synthesis was strikingly similar to that established for the rat.

EXPERIMENTAL METHODS

The strain of *S. lactis* used in this study was from the departmental culture collection and was selected on the basis of its excellent growth in a synthetic medium. Throughout the experimental work the culture was carried in litmus milk and routinely transferred every seven days. The synthetic medium used was that of Henderson and Snell (1948). The modifications of this medium by Anderson and Elliker (1953) were not adopted *in toto*, the exception being the ascorbic level which was lowered to 20 mg per 100 ml of medium. The original medium was further modified to the extent that the citrate level of the medium was reduced to 10 mg per ml and acetate was deleted (Kizer and Speck, 1954).

Each medium tested was inoculated initially from a 24 hr litmus milk culture using 0.05 per cent inoculum and incubation at 32 C for 24 hr. This was followed by two serial 24 hr subcultures in the same medium using 0.05 per cent of the previous 24 hr culture for inoculum. Cultures were tested in 2 ml of medium, and inoculation

was with a calibrated 0.001 ml platinum loop. Incubation was at 32 C for 24 hours for each transfer. The data reported throughout this paper were recorded from the growth response of the organism in the third serial culture grown in synthetic medium. This was necessary to insure that metabolite carryover from the original litmus milk inoculum exerted no measurable effect. The inoculation procedure provided a 2,000-fold dilution per transfer, thus adequately eliminating methionine contamination from the original milk inoculum.

Growth was determined turbidimetrically and expressed as per cent light transmission using a model no. 401 Lumetron colorimeter equipped with a 550 m μ light filter.

RESULTS

The removal of methionine from an otherwise complete medium resulted in a significant reduction in growth as shown in table 1; however, the residual growth was still appreciable and manifested a high degree of stability as evidenced by the low standard deviation. Growth in the absence of serine was reduced to an amount nearly equivalent to that observed when methionine was removed. Upon comparing the growth in the presence of glycine and in its absence, it would appear that this amino acid had no significant effect. Further, when methionine or methionine plus glycine were omitted, the growth responses were essentially the same. Consideration of the responses, when serine and serine plus glycine were deleted, lead to a similar conclusion concerning the effect of glycine. Upon the deletion of methionine, serine, and glycine simultaneously, less growth was observed than in any of the previous media tested although the culture was still able to manifest adequate growth, and the response appeared to be quite stable.

The ability to grow in the absence of added methionine was tested using seven additional strains of *S. lactis* (table 2). All of the strains

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tested were able to manifest appreciable growth on deletion of methionine from the medium although less growth occurred than when methionine was supplied.

A comparison of the growth in the complete medium against one in which methionine was replaced with homocysteine (table 3) indicated that homocysteine exerted a definite replacement effect for the methionine requirement. In the absence of serine, this effect of homocysteine was definitely reduced, and the growth was the same as when methionine, serine, and glycine were omitted simultaneously (table 1).

TABLE 1

The effect of methionine, serine, and glycine, deleted singly or in combinations, on the growth of Streptococcus lactis, strain RS

Amino Acids Deleted from Medium	Per cent Transmission	Standard Deviation	No. of Observations
	avg		
None.....	33.3	0.10	15
Glycine.....	33.9	0.01	8
Methionine.....	47.9	0.01	10
Serine.....	50.7	0.33	17
Glycine and methionine.....	46.1	0.10	16
Glycine and serine.....	54.5	0.87	6
Methionine, serine, and glycine.....	64.6	0.21	6

TABLE 2

The response of strains of Streptococcus lactis to growth in the presence and absence of added methionine

Strain	Added Methionine	Per cent Transmission	
	mg/100 ml		
24	10	37.0	35.5
	—	52.0	52.5
MS	10	41.0	40.5
	—	53.5	53.5
UM	10	45.5	47.5
	—	55.5	60.0
51	10	35.5	36.0
	—	51.5	53.0
27	10	33.5	35.5
	—	54.5	56.5
WS	10	40.5	37.5
	—	56.5	57.5
E	10	45.5	43.5
	—	55.5	56.5

TABLE 3

The effect of homocysteine upon the growth of Streptococcus lactis, strain RS

Amino Acids Deleted from Medium	Homocysteine	Per cent Transmission	Standard Deviation	No. of Observations
	mg/100 ml	avg		
None.....	—	33.3	0.10	15
Methionine.....	—	47.9	0.01	10
Glycine.....	—	33.9	0.01	8
Serine.....	—	50.7	0.33	17
Glycine and methionine.....	10	40.2	0.11	13
Glycine, serine, and methionine.....	10	65.0	0.39	6
Glycine, serine, and methionine.....	—	64.6	0.21	6

TABLE 4

The effect of vitamin B₁₂ or folic acid on the growth of Streptococcus lactis, strain RS, in the absence of methionine, glycine, and glycine plus methionine

Amino Acids Deleted from Medium	Vitamin Added (1 µg/100 ml)		
	None	B ₁₂	Folic acid
	% T	% T	% T
None.....	35.0	35.75	35.0
Methionine.....	52.25	51.75	53.0
Glycine.....	38.0	38.0	36.5
Methionine and glycine...	50.25	49.5	51.75

Since Arnstein and Neuberger (1953) had shown the involvement of vitamin B₁₂ in this cycle, and since folic acid had also been shown to operate (Binkley and Christensen, 1951), these two compounds were tested in this system. Vitamin B₁₂ was added to the complete medium and to this medium from which methionine and glycine were omitted singly and together. Serine was not removed from any of these media on the assumption that it was a primary carbon donor to the methyl group of methionine. Folic acid was tested under identical conditions. Under the conditions employed no effect could be attributed to either vitamin B₁₂ or to folic acid (table 4).

DISCUSSION

The present observations that *S. lactis* was able to grow in the absence of methionine appear to have no precedence in published literature.

Niven (1944) and Anderson and Elliker (1953) indicated that under the conditions they employed none of the lactic group of streptococci was able to grow in the absence of methionine. It is possible that the difference in results may lie in the culturing methods employed. These investigators cultured the test organisms in another medium prior to inoculation of the test medium. In the present study, it appears unlikely that growth of *S. lactis* in the absence of methionine was due to methionine carry-over from the litmus milk inoculum. According to McMeekin and Polis (1949) the methionine percentage in milk protein is 6.3. If all of the methionine were free in the milk cultures used for inocula, its maximum concentration (based on average composition figures for milk) would be 0.01 g per ml in the first transfer tubes; this level would be diluted 2,000-fold in the second transfer and again in the third transfer which was used for analysis. Our studies have indicated no response below 0.01 g per ml of methionine.

The growth of seven other strains of *S. lactis* in the absence of added methionine indicates that this property appears to be shared generally by *S. lactis* (table 2).

Umbreit (1952) integrated the work of a number of investigators concerning the precursors of methionine and cystine. This integration has allowed the formulation of a pathway for the synthesis of methionine. The reactions of this pathway are as follows: homoserine, which arises from threonine, condenses with cysteine to form cystathionine; cleavage of cystathionine gives rise to homocysteine; homocysteine is methylated by the β -carbon of serine, forming methionine. The results of this study suggest that this pathway of methionine synthesis may be in operation in this organism. This is supported by the fact that the organisms grew adequately in the presence of threonine, cystine, and serine and in the absence of added methionine (tables 1 and 2). It is further supported by the apparent replacement effect exerted by homocysteine for methionine in the growth of *S. lactis*, strain RS (table 3). A similar observation was made by Teas *et al.* (1948) while working with a strain of *Neurospora* that required methionine and threonine. They found that both of these requirements could be satisfied by homoserine. Binkley (1951) studied the enzymatic formation of cystathionine and found that homocysteine

and serine were the precursors. Rachele *et al.* (1950) reported that upon feeding rats cystathionine labeled with S^{35} radioactive cystine was isolated from the hair.

The response of the organism to the deletion of serine in the presence of homocysteine is in accord with studies on the rat. Arnstein and Neuberger (1953) found that serine was a major carbon donor to the methyl groups of methionine and choline. Furthermore, serine was a more efficient carbon donor than was glycine. Gibson and Woods (1952) noted that cell suspensions of a mutant strain of *Escherichia coli* requiring either serine or glycine for growth failed to synthesize methionine when both amino acids were deleted. Since synthesis proceeded more rapidly in the presence of serine, they concluded that serine was the intermediate more directly concerned. In the present observations, the removal of serine from the complete medium resulted in a growth retardation closely paralleling that experienced upon deletion of methionine from the complete medium (table 1). This indicates that the absence of serine as a methyl donor exerts a rate limiting reaction nearly equivalent to that experienced when methionine is withdrawn. This may be a reflection of a continual methionine synthesis reaction even in the presence of optimal levels of methionine; a portion of the growth retardation upon the omission of serine may be due to the loss of potential methyl groups for methionine synthesis in addition to the anabolic requirement for serine.

The apparent failure of the organism to respond to either vitamin B_{12} or folic acid does not necessarily indicate a lack of involvement of these two compounds in the system under study. In a previous study, Anderson and Elliker (1953) found folic acid to be stimulatory but not essential. Folic acid, according to Binkley and Christensen (1951), markedly increased the formation of pyridoxal phosphate when pyridoxine was incubated with adenosine triphosphate and liver homogenate. Prior to this, it has been reported (Speck and Pitt, 1947) that certain lactic acid bacteria required pyridoxine for growth in the absence of cystine. Later, it was found that pyridoxine was involved in the formation of cystine from homocysteine and serine (Braunstein and Goryachonkova, 1950). The failure to respond may indicate adequate syn-

thesis of folic acid and vitamin B₁₂ or, in the case of the latter, an expression of failure to deplete the cells sufficiently of existing stores of vitamin B₁₂. A necessity for such depletion was noted by Kratzer (1953) while studying the effect of vitamin B₁₂ on transmethylation reactions in poult.

SUMMARY

A synthetic medium containing no added methionine was able to support adequate growth of a strain of *Streptococcus lactis* although growth was less than when methionine was present. Growth was also obtained in a medium from which glycine, serine, and methionine had been deleted. Tests with seven additional strains of *S. lactis* showed that all were able to grow in the absence of added methionine.

The addition of homocysteine in the presence of serine resulted in a reversal of the growth retardation noted when methionine was omitted; however, it exerted little replacement effect in the absence of serine. The data suggested that methionine was actively synthesized and that the synthesis proceeded via cystathionine, homocysteine, and subsequent methylation to methionine.

Folic acid and vitamin B₁₂ additions to the medium failed to show any effect upon the growth of the organisms in the presence or absence of added methionine.

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