Synthesis and Microbiological Testing of 1,1-Aminopropanesulfonic Acid.* (17737)

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McIlwain(1) has shown that the 1,1-aminosulfonic acid analogues of glycine, alanine, valine, and leucine inhibit the growth of several bacteria. In some cases the inhibition was reduced only by the corresponding aminocarboxylic acid; in others, however, the inhibition was reduced by other amino acids as well. It was the purpose of these investigations to synthesize 1,1-aminopropane sulfonic acid, analogous to the aminocarboxylic acid, α-aminobutyric acid, which does not occur in nature, and to test the sulfonic acid for its inhibitory or growth-promoting effects in a bacterial system.

Synthesis. 1,1-aminopropanesulfonic acid was synthesized by the addition of 50% aqueous ammonium bisulfite solution to a five per cent excess of propanal. The volume of the mixture was reduced under vacuum to one quarter the original volume. Ethyl alcohol was added till precipitation began and the mixture was placed in the refrigerator for crystallization. The crystals collected were purified by redissolving them in a minimum of water, adding ethyl alcohol, and again crystallizing in the cold.

$C_3H_9O_3NS$. Calculated, C 25.90, N 10.06, S 23.04: Found, C 26.65, N 9.97, S 23.33.

Microbiological method. The organism used for testing was *Lactobacillus casei* carried in tomato agar stabs. For the experiments, a 2% dextrose, 2% yeast extract broth was used as the basal medium. For a given test, 5 ml of basal medium were added to each test tube in a series, the aminocarboxylic acids and water were added. The tubes were then sterilized by autoclaving at 15 lb pressure for 15 minutes. A solution of the sulfonic acid, sterilized by filtration through a Seitz filter, was added to the autoclaved tubes after they were cooled. The final volume of each tube was 10 ml. The tubes were inoculated with *Lactobacillus casei* and incubated at 37°C for 24 hours; the growth was determined by turbidimetric measurements.

TABLE I.
Effect of 1,1-Aminopropanesulfonic Acid on Growth of *Lactobacillus casei*.

1,1-aminopropane-sulfonic acid, M	Growth (turbidity)
0	103
1×10^{-5}	103
5×10^{-5}	102
1×10^{-4}	94
$2 \times$ "	92
$4 \times$ "	90
$6 \times$ "	82
$8 \times$ "	67
1×10^{-3}	57
$2 \times$ "	42
$4 \times$ "	28
$6 \times$ "	20
$8 \times$ "	10
1×10^{-2}	0

Results. The 1,1-aminopropanesulfonic acid began to inhibit growth at a concentration of 1×10^{-4} M; complete inhibition was obtained at 1×10^{-2} (Table I). Alpha-aminobutyric acid did not reverse the inhibition produced by the sulfonic acid analogue, but instead inhibited the growth still more. This result is not surprising since Gladstone(2) has shown that α-aminobutyric acid is toxic to microorganisms. In view of the investigations showing the overlapping growth-reversal effect produced by aminocarboxylic acids of aminosulfonic acid inhibition(1), it might be expected that the inhibition produced by 1,1-

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1. McIlwain, H., *Brit. J. Exp. Path.*, 1941, v22, 148.

2. Gladstone, G. P., *Brit. J. Exp. Path.*, 1939, v20, 189.

aminopropanesulfonic acid could be reversed by such natural amino acids as glycine, alanine, valine, or the leucines. None of these amino acids, however, produced an increase in growth at the concentrations used. In addition, simple mixtures of natural amino acids were ineffective in reversing the inhibition. The addition of from one to three milliliters of a solution of sixteen amino acids† to the basal medium in the presence of the aminosulfonic acid produced about a 2% increase in growth. However, addition of the same amino acid mixture to the basal medium in the absence of the aminosulfonic acid resulted in a similar increase in growth. This would indicate that the increased growth was due to a general improvement in the nutritive value of the medium rather than a reversal of inhibition by a specific amino acid.

Further investigations will be necessary to determine whether or not inhibition produced by 1,1-aminopropanesulfonic acid is reversed by substrates other than amino acids.

Summary. 1,1-aminopropanesulfonic acid was synthesized by the addition of ammonium bisulfite to propanal. The aminosulfonic acid inhibited the growth of *Lactobacillus casei* at levels from 10^{-4} to 10^{-2} molar. Single amino acids as well as combinations of amino acids were ineffective in relieving the inhibition.

† The amino acid mixture contained in each ml, 0.8 mg each of DL-leucine, DL-serine, DL-phenylalanine, DL-glutamic acid, DL-valine, DL-aspartic acid, L-cystine, DL-arginine hydrochloride, L-tryptophane, L-tyrosine, DL-threonine, DL-methionine, DL-alanine, DL-isoleucine, DL-lysine and L-histidine hydrochloride.

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Growth of *Mycobacterium tuberculosis* in Egg Yolk Mediums. (17738)

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Egg yolk is widely used as the chief ingredient in a variety of solid mediums for the cultivation of *Mycobacterium tuberculosis;* in fact, no other nutrients need to be added(1). That it can be equally useful in liquid and semi-liquid mediums is not so well known. Besredka(2) described a liquid medium made by autoclaving egg yolk diluted with alkaline water; this preparation, however, does not support the growth of small inocula. Tubercle bacilli will grow well within the yolks of market eggs(3) even from inocula as small as 10 tubercle bacilli(4), as well as in the yolks of embryonated eggs(5). The widespread use of the Dubos Tween-albumen mediums has demonstrated the value of liquid mediums in metabolic and chemotherapeutic studies of the tubercle bacillus. The measure of the adequacy of such mediums is their ability to promote rapid growth from small inocula (10 organisms or less). Several easily prepared mediums that will do this can be made from egg yolk.

Methods. Strictly fresh chicken eggs were used. The yolks were removed aseptically and diluted with sterile water, salt solutions, or a basal medium made up according to a formula given by Dubos *et al.*(5):

	g
Casein digest (N-Z Amine)* or asparagine	2.0
$Na_2HPO_4 \cdot 12H_2O$	6.3
KH_2PO_4	1.0
Na citrate $\cdot 2H_2O$	1.5
Dissolve, then add	
Ferric ammonium citrate	0.1
$MgSO_4 \cdot 7H_2O$	0.6
Distilled water to 1000 ml. Adjust to pH 6.8.	

*N-Z Amine (Type B), Sheffield Farms Co., Inc.

1. Corper, H. J., and Cohn, M. L., *Am. Rev. Tuberc.*, 1942, v46, 560.

2. Besredka, A., *Ann. de l'Inst. Pasteur,* 1921, v35, 291.

3. Gelarie, A. J., *J. Lab. Clin. Med.*, 1944, v29, 532.

4. Eggerth, A. H., Dresher, E., and McOsker, V. C., *Am. Rev. Tuberc.*, 1948, v57, 632.