tion of a role of the spleen in resistance has thus far been demonstrated only for infections in which intra-erythrocytic parasitism is prominent(6). Conceivably this situation is related to the function of the spleen in removing abnormal red cells and their inclusion bodies(8).

Summary. No difference in susceptibility to infection by a highly virulent strain of pneumococci could be demonstrated between normal and splenectomized suckling rats. The implications of this finding are briefly discussed.

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Antimetabolite Activity of Lysine Analogues on Lysine Incorporation into Rat Bone Marrow Protein *in vitro*. (24579)

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Lysine deficiency in the young rat is attended by anemia(1) brought on by retarded development of the hematopoietic system(2). Recently, Rohdenberg(3) reported that rats fed a lysine-deficient ration accumulated abnormal levels of fat in their bone marrow. It is apparent that integrity and activity of this tissue is markedly sensitive to lysine deprivation. As part of a study on action of amino acid analogues on protein synthesis by isolated mammalian tissues, we investigated the effect of 4 lysine analogues on *in vitro* incorporation of this amino acid into proteins of bone marrow.

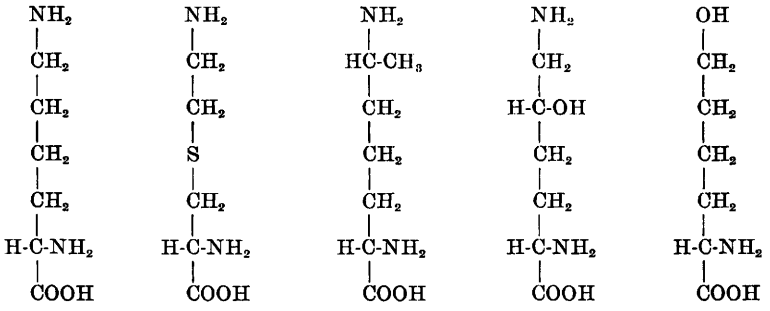
Materials and methods. Rats of both sexes maintained on Purina Lab. Chow and weighing 90 to 125 g were decapitated and their femurs rapidly removed and placed in chipped ice. Twenty-five rats furnished sufficient bone marrow for 12 flasks in each experiment. Both ends of bone were cut with scissors and the bone marrow removed by forcing ice cold Krebs Ringer Phosphate (KRP) buffer through the bone with #23 hypodermic needle. The buffer, which was also used as incubation medium, contained .12 M sodium chloride, 1.3 x 10^{-2} M potassium chloride, 1.8 x 10^{-3} M calcium chloride, 6.5 x 10^{-4} M magnesium sulfate, and 0.01 M sodium phosphate, pH 7.2. This procedure dissociated most of the tissue into free cells. The fraction which remained intact was passed through 60 mesh, monel metal sieve and then combined with the free cells. The bone marrow cells were washed 3 times with cold KRP by alternate suspension and sedimentation in refrigerated centrifuge at 2°C and were then resuspended in 2 volumes of buffer. S-(β-Aminoethyl)-L-cysteine hydrochloride had been synthesized by the method of Cavallini *et al.*(4) and was a gift of Dr. F. Tietze of Nat. Inst. for Arthritis and Metabolic Diseases. The ϵ-C-methyl lysine (2,6-diamino heptanoic acid) was prepared as the dihydrochloride according to the method of McLaren and Knight(5). The δ-hydroxylysine hydrochloride was purchased from Calif. Corp. for Biochemical Research, Los Angeles. The latter 2 analogues were present in their 4 diastereoisomeric forms. Hexahomoserine was prepared as described by Gaudry(6). DL-Lysine-1-C^{14} was a gift of Dr. M. Rothstein, Univ. of California, Berkeley, which had been prepared by a new method(7). Solutions of all compounds were pre-

pared in KRP at pH 7.2. The main compartment of Warburg flasks contained the analogue at a single concentration and DL-Lysine-1-C^{14} at several concentrations. Buffer was added to adjust volume of main compartment to 1.5 ml. One-half ml of bone marrow cells in 2 volumes of buffer was added to one side arm and 0.05 ml of 40% trichloroacetic acid (TCA) was added to second sidearm. After temperature equilibration at 37.5°C for 5 minutes, bone marrow was tipped into the main compartment and incubated 15 minutes. The contents were mixed with TCA to terminate the incorporation. Precipitated material was washed 3 times with 5% TCA, once with 5% TCA at 90°C for 15 minutes, once with 5% TCA at room temperature, once with absolute ethanol, 3 times with 3:1 ethanol-ether mixture at 62°C for 5 minutes, and twice with absolute ether. The protein was plated on aluminum discs and counted in flow-gas counter.

Results. Of the 4 analogues of lysine tested only 2, S-(β-aminoethyl)-cysteine and ϵ-C-methyl lysine, were effective competitive inhibitors of lysine incorporation into bone marrow protein. These 2 analogues have been shown to be effective lysine antagonists in bacterial growth studies(8,5). Double reciprocal plots showing characteristics of their inhibition are shown in Figs. 1 and 2. The kinetics resemble those found for the action of phenylalanine analogs(9) and ethionine(10) on incorporation of their corresponding metabolites into Ehrlich ascites tumor protein. Inhibition indices (mol analogue/mol lysine for 50% inhibition) estimated on basis of L forms of both analogs and lysine present, were 3 for S-(β-aminoethyl)-cysteine and 20 for ϵ-C-methyl lysine.

Hexahomoserine was found by Gaudry and co-workers(11) to induce anemia when fed to rats and thus served as basis for explanation of a similar syndrome(12) in rats fed diazotized casein. Prevention of anemia by dietary lysine suggested that hexahomoserine was a potent lysine antagonist(11). It is of interest that this compound was not a lysine antagonist in the *in vitro* system (Fig. 2). We found similar completely negative results when lysine incorporation into hemoglobin of rabbit reticulocytes was used as test system. It is probable that toxic manifestations observed when hexahomoserine is included in rations of rats is brought about by a systemic effect, such as an amino acid imbalance and that the results should not be interpreted to mean that the compound is a lysine antagonist.

Not only does substitution of a hydroxy for an amino group result in a non-inhibitory analog, but the presence of a hydroxy group on the penultimate carbon of lysine also produces an ineffective analog. Thus, δ-hydroxylysine did not competitively inhibit lysine incorporation. This analog produced a non-competitive inhibton of about 30% for all levels of lysine tested (Fig. 3). A similar inhibition by δ-hydroxylysine was earlier observed under identical conditions with leucine incorporation into Ehrlich ascites tumor protein(13). The latter inhibition was an indirect result of the action of δ-hydroxylysine as an inhibitor of



Compound	lysine	S-(β-aminoethyl) cysteine	ϵ-C-methyl lysine	δ-hydroxylysine	hexahomoserine
Antimetabolic action		very active, competitive	moderately active, competitive	low activity, not competitive	inactive
Inhibition index		3	20		

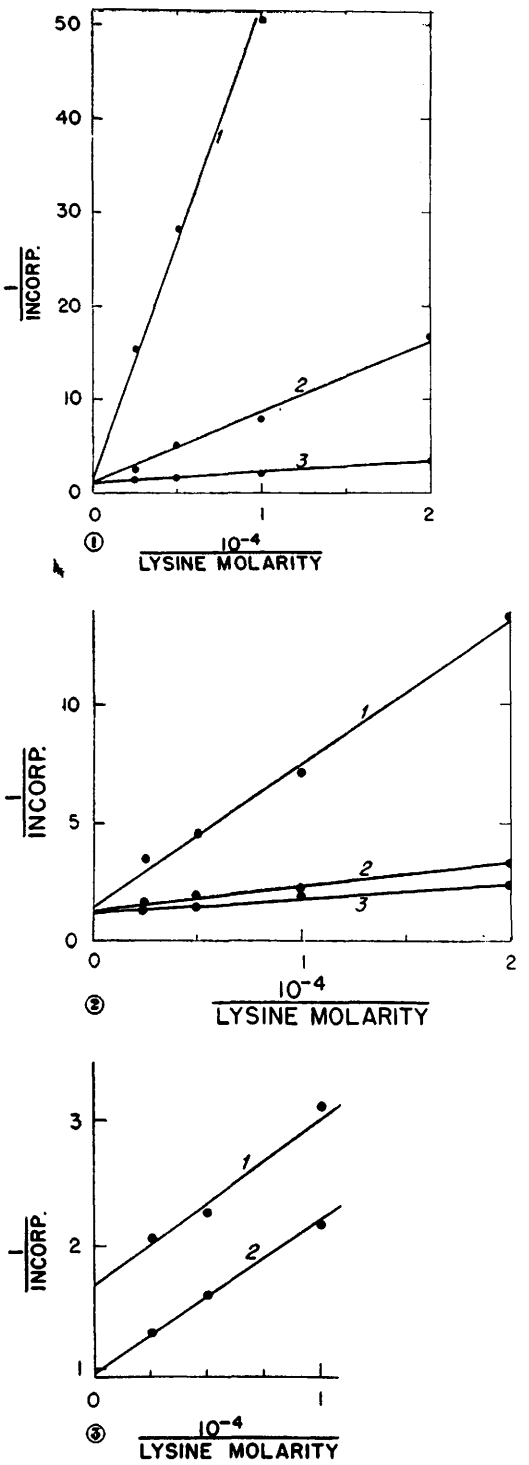


FIG. 1. Double reciprocal plot showing competitive inhibition by S-(β-aminoethyl)-cysteine of lysine incorporation into bone marrow protein. Curve 1, inhibition by S-(β-aminoethyl)-cysteine, 5×10^{-3} M; Curve 2, inhibition by S-(β-aminoethyl)-cysteine, 1×10^{-3} M; Curve 3, uninhibited incorporation. Incorporation expressed as lysine incorporated/g of protein during 15-min. incubation period.

FIG. 2. Double reciprocal plot showing competitive inhibition by ϵ-C-methyl lysine and hexahomoserine of lysine incorporation into bone marrow protein. Curve 1, inhibition by ϵ-C-methyl lysine, 1×10^{-2} M; Curve 2, inhibition by hexahomoserine, 1×10^{-2} M; Curve 3, uninhibited incorporation. Incorporation expressed as in Fig. 1.

FIG. 3. Double reciprocal plot showing noncompetitive inhibition by δ-hydroxylysine of lysine incorporation into bone marrow protein. Curve 1, inhibition by δ-hydroxylysine, 1×10^{-2} M; Curve 2, uninhibited incorporation. Incorporation expressed as in Fig. 1.

glutamine synthesis; and it is possible that it may also act in this manner with bone marrow.

The results are summarized in the accompanying tabulation.

Summary. Four lysine analogs were studied for antimetabolic action against radioactive lysine incorporation into rat bone marrow protein *in vitro*. S-(β-Aminoethyl)-cysteine was a potent competitive inhibitor and ϵ-C-methyl lysine was moderately active. δ-Hydroxylysine inhibited slightly and in a non-competitive manner. Hexahomoserine was inactive.

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