Antibiotic Activity of Subtilin and Streptomycin in the Presence of BAL[1]

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The sulfhydryl groups in enzymes have been stressed by some authors as responsible for the attack of antibiotic (*4*) and metallic antiseptics (*9*) on microorganisms as well as for the arsenic poisoning of mammalian tissues (*1*). The inactivation of antibiotic substances by sulfhydryl compounds in certain cases (*6, 7*) and the effectiveness of using BAL as an antidote to arsenic and other heavy metal poisoning (*3, 11, 14*) gave support to this postulation.

BAL (2,3-dimercaptopropanol), a simple compound with two sulfhydryl groups, has been reported by Johnson, *et al.* (*12*) to be able to inactivate bacitracin, an antibiotic produced by a strain of *B. subtilis*. Subtilin, which is also produced by a strain of *B. subtilis*, has an antitubercular activity *in vitro* comparable to that of streptomycin. This property of subtilin is being carefully studied in this laboratory. Chin (*5*) has reported that sodium chloride does not alter the antibiotic action of subtilin. Since Loo, *et al.* (*13*) and Berkman, *et al.* (*2*) have reported that sodium chloride antagonized the antibiotic action of streptomycin, which was dissimilar to the case of subtilin, it is of interest to know whether sulfhydryl compounds which have been known to inactivate streptomycin (*7, 16*) affect subtilin similarly or dissimilarly. While experiments on other sulfhydryl compounds are still in progress, the study on BAL has brought about interesting results.

This study has been made upon four strains of *Mycobacterium*, two human strains (H37Rv and R1), one bovine, and one nonpathogenic fast-growing # 599, and a strain of *Micrococcus lysodeikticus*.[3] *M. lysodeikticus* was cultured in beef-heart infusion medium, and tests were also made in the same medium. Mycobacteria were cultured and tested in Dubos medium (*8*). Since BAL at a dilution of 1:10,000 has been found to inhibit the growth of mycobacteria, a dilution of 1:100,000 was used. The results, as shown in Table 1, indicate that BAL was synergistic with subtilin in bacteriostatic action on all strains of mycobacteria studied but not on *M. lysodeikticus*. The antibiotic action of streptomycin was affected in various ways. The bacteriostatic activity against pathogenic mycobacteria was potentiated, though not as remarkably as in the case of subtilin, whereas against the nonpathogenic strain # 599 its action was not affected. Streptomycin was inactivated in the presence of BAL against *M. lysodeikticus*.

The mechanism of synergism between subtilin and BAL is entirely unknown. A similar potentiation, though to a lesser degree, of the action of streptomycin against a pathogenic mycobacterium is remarkable and may reflect that BAL itself acted somehow on the organisms so that they were rendered more susceptible to antibiotics.

Shwartzman (*15*) has reported synergism between methionine, methionine sulfoxide, and threonine and penicillin and suggested that these amino acids reverse the action of other antagonistic substances. The same explanation is unlikely and not applicable to the case of BAL, which potentiates both subtilin and streptomycin similarly.

BAL has been known to attack enzyme systems (*17*). Is it possible that some enzyme systems of mycobacteria are susceptible to BAL? Since BAL has a structure very similar to that of glycerol, which is utilized especially by pathogenic mycobacteria as a source of carbon (*18*), and it has been found to inhibit the growth of these organisms at 1:10,000, does it mean that Fildes' theory (*10*) of biochemical antagonism may be extended to the field of nonessential metabolites? Even if the action of BAL on a mycobacterium can thus be explained, the question of synergism remains to be elucidated. An elucidation of this mechanism is practically impossible at present when so much about the metabolic processes of mycobacteria remains unknown and is awaiting further study.

TABLE 1

EFFECT OF BAL ON THE BACTERIOSTATIC LEVEL OF SUBTILIN AND STREPTOMYCIN

Organism	Effective level of subtilin		Effective level of streptomycin		Conc. of BAL
	with BAL	without BAL	with BAL	without BAL	
Pathogenic *Mycobacterium*					
Human H37Rv	1:32 × 10^5	1:2 × 10^5	1:32 × 10^5	1:8 × 10^5	1:10^5
Human R1	1:64 × 10^5	1:4 × 10^5	1:64 × 10^5	1:16 × 10^5	1:10^5
Bovine	1:128 × 10^5	1:32 × 10^5	1:64 × 10^5	1:32 × 10^5	1:10^5
Nonpathogenic *Mycobacterium* # 599	1:16 × 10^5	1:8 × 10^5	1:32 × 10^5	1:32 × 10^5	1:10^5
Micrococcus lysodeikticus	1:16 × 10^7	1:16 × 10^7	1:2 × 10^5	1:8 × 10^5	1:10^4

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[3] H37Rv was obtained from the standard culture depot, National Tuberculosis Association; R1, bovine, and # 599 strains, from the Lilly Research Laboratories; *M. lysodeikticus*, from H. B. Woodruff.

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Inadequate Maternal Nutrition and Hydrocephalus in Infant Rats[1]

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Richardson and Hogan (*1*) observed hydrocephalus in infant rats as the result of feeding an inadequate diet to the mother. In this abnormality the head is dome shaped and greatly enlarged. The brain cavity is filled with serum and transmits light readily. In some cases the eyes are abnormally small and muscular incoordination usually develops if the affected rat survives long enough.

In order to demonstrate more conclusively that hydrocephalus is caused by a nutritional deficiency it seemed desirable to produce the abnormality in an unrelated colony of rats. The colony at the Texas Station, which has been maintained for more than 10 years without the introduction of any new strains, was suitable for such a study. A total of 38 females have received Diet A, which is essentially the same in composition as that used by Richardson and Hogan. This diet is composed of casein (acid washed), 25 grams; Cerelose, 57 grams; wood pulp, 3 grams; salts, 5 grams; lard, 10 grams; choline chloride,[2] 0.1 gram; inositol,[2] 0.01 gram; p-aminobenzoic acid,[2] 0.05 gram; vitamin A, 3,000 I.U.; vitamin D, 425 I.U.; α-tocopherol,[2] 2.5 mg.; Menadione,[2] 2.5 mg.; thiamine chloride,[2] 1.0 mg.; riboflavin,[2] 1.0 mg.; pyridoxine hydrochloride,[2] 1.0 mg.; calcium pantothenate,[2] 4.0 mg.; niacin,[2] 5.0 mg.; and biotin,[2] 0.02 mg.

Some of the experimental females were from mothers which received a stock diet and others were from mothers which received a synthetic diet, but in every case they received Diet A from 28 days of age until the observations were discontinued. A female was observed until it was evident that she would not produce any additional young.

A total of 10 young have developed typical hydrocephalus. The incidence was 1.5 per cent, or approximately the same as that given in the earlier report. None has occurred in the offspring of females which received a stock diet composed of natural feedstuffs. The hydrocephalus in 5 of the 10 young was identified at birth. It was identified again in these same young when they were 10 days old by observing the transmission of light through the brain cavity, and finally by autopsy. It was not identified in the other 5 until they were about 10 days old. Twelve additional young appeared to be hydrocephalic at birth, but none of these survived longer than two days, and these early identifications have not been entirely reliable.

Richardson and Hogan observed one case of hydrocephalus in the offspring of a mother which received 5 per cent of dried yeast in the diet. This observation suggested that a small amount of yeast in the diet would furnish very little, if any, of the factor which prevents hydrocephalus, and at the same time it would supply sufficient pteroylglutamic acid for normal reproduction.

Intestinal synthesis of some unrecognized factor might decrease the incidence of hydrocephalus, even though the diet itself was low in this factor. The addition of a sulfonamide to the diet would decrease this intestinal synthesis and thus increase the incidence of the abnormality. In order to test this possibility 12 females were given Diet B, which is the same as Diet A with 2 per cent of dried yeast and 1 per cent of sulfasuxidine substituted for equal amounts of Cerelose. So far, these 12 females have produced 92 young and none has been hydrocephalic. These data are too insufficient to be conclusive, but they indicate that the addition of sulfasuxidine to the diet does not increase the incidence of the abnormality under these conditions.

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[2] Kindly supplied by Merck and Company, Rahway, New Jersey.

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Effect of Flavonols on *Clostridium botulinum*

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While conducting experiments on the microbial spoilage of vegetables, the authors found that, although asparagus is readily attacked by many organisms, it is a poor medium for growth of *Clostridium botulinum*. The thought was entertained that the flavonol compound described by Campbell (*1*) as occurring plentifully in asparagus, and subsequently shown by DeEds and Couch (*2*) to be rutin, might be responsible. Following the report by Naghski, Copley, and Couch (*3*) on the suppression of *Staphylococcus aureus* by quercetin, an aglucone derivative of rutin, tests were made to determine the action of rutin, quercetin, and quercitrin[2] (a rhamnoside of quercetin) on *Cl. botulinum*. Three sets of flasks, each flask containing 15 grams of green peas and 15 ml. of corn steep-casein medium, were inoculated with a mixture of approximately 1,000 detoxified spores of *Cl. botulinum*, Types A and B, per flask. The reaction of the medium was pH 6.60. One flask in each set was left untreated as control. Weighed amounts

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

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