A CONTRACT has been made between Union College and the American Locomotive Company, in which it is agreed that the company will enlarge the physics laboratories of the college in exchange for the use of a laboratory and other facilities and help on specific technical problems by members of the college faculty. The company has agreed to provide and supervise the building of ten or twelve new laboratories, and the facilities and faculty at the college are to be available to the company for five years. Faculty and research personnel will be available for at least four meetings a year. It has been arranged that representatives of the college and company will make shop inspection trips, followed by discussion of any company problems submitted.

A PROPOSAL is now before Governor Frank J. Lausche of the State of Ohio and members of the legislature for the erection of a State Health Center at the Ohio State University. An appropriation of five million dollars is requested to cover the cost of the center as the result of two years of planning and study by members of the medical and dental faculties and by representatives of these professions. The proposed center has been approved by official representatives of the medical colleges of Western Reserve University and the University of Cincinnati. It has also been approved by the Inter-University Council, which includes representatives of the six state universities—Bowling Green, Kent, Miami, Ohio, Ohio State and Wilberforce. Joining also in active support of the project are medical, dental and nursing alumni of the Ohio State University, under the chairmanship of Dr. Russel G. Means, Columbus.

IT is reported in the daily press that the will of Vincent Bendix, inventor and founder of the Bendix Aviation Corporation, who died on March 29, provides that 78 per cent. of the residue of his estate be left in trust for the establishment of the Bendix Foundation to assist in the teaching of science.

DR. JAMES GREENWOOD, JR., has given a fund to the Medical Branch at Galveston of the University of Texas for the establishment of the James Greenwood lectureship in neurology and neuro-surgery, in honor of his father. The lectureship will provide for meetings both at the Baylor Medical College in Houston and at the Medical Branch at Galveston of the university.

THROUGH an error, it was stated in SCIENCE that the *Biometrics Bulletin* was issued monthly. It appears bi-monthly. The first issue appeared in February of this year. It is the official organ of the Biometrics Section. The American Statistical Association has long published a journal and a bulletin which are concerned with the more general aspects of statistics. The new *Biometrics Bulletin* attempts to meet the specific needs of biologists who use statistical method in their work and of statisticians who work with biological data. Inquiries concerning the Bulletin should be addressed to the American Statistical Association, 1603 K Street, N.W., Washington 6, D. C.

SPECIAL ARTICLES

CONCERTED ANTIBIOTIC EFFECT OF PENICILLIN, METHIONINE, THREONINE AND METHIONINE SULFOXIDE UPON BRUCELLA, EBERTHELLA, SALMONELLA, AND SHIGELLA[1,2,3]

PREVIOUSLY published[4,5] and more recent unpublished observations demonstrated that broth, casein digest and blood serum antagonized the effect of penicillin upon certain Gram-negative microorganisms. The antagonism was at least in part due to the amino-acid contents, inasmuch as it could be also obtained with monoaminodicarboxylic acids, and possibly, histidine and arginine. No antagonistic effect was shown by hydroxyamino and diaminomonocarboxylic acids and methionine sulfoxide. Methionine was capable of reversing fully the antipenicillin effect of the monoaminodicarboxylic acids and only incompletely that of casein hydrolysate, broth and blood serum. However, upon addition of methionine in combination with threonine and methionine sulfoxide[6] there appeared a marked enhancement of penicillin activity against highly refractory Gram-negative microorganisms in the presence of the antipenicillin factors as illustrated by the following experiments which were all carried out in meat inferior broth:

The measurements of the effect of penicillin were carried out in the manner previously described. The amount of bacterial growth was expressed in optical

[1] From the Division of Bacteriology, Laboratories of the Mount Sinai Hospital, New York, N. Y.

[2] The author wishes to acknowledge thankfully the accurate and capable assistance of Miss Alice Fisher.

[3] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

[4] Gregory Shwartzman, SCIENCE, 100: 477, 1944.

[5] *Idem*, SCIENCE, 101: 276, 1945.

[6] The methionine sulfoxide was generously supplied by Dr. Alfred Barol, director of the Department of Pharmacology of Wyeth Institute of Applied Biochemistry, at the recommendation of Dr. Paul György. SMACO dl-methionine and dl-threonine were used in these studies.

density.[4] Solutions of amino acids in water were adjusted to pH 7.0–7.2 with sodium bicarbonate and sterilized by filtration through Berkefeld V candles. Forty-eight-hour-old cultures of Brucella and five-hour-old cultures of the remaining organisms served as inoculum. In all tests the initial number of organisms was 0.75×10^6 cells per ml. In repeated experiments methionine, 3.75 mg per ml, used alone and in mixture with threonine, 1.25 mg per ml, gave variable inhibition of the organisms studied (*i.e.*, 10 to 55 per cent.). The inhibitory effect of mixtures of methionine, threonine and methionine sulfoxide with and without penicillin is shown in Table 1.

TABLE 1

ENHANCEMENT OF PENICILLIN SUSCEPTIBILITY OF REFRACTORY GRAM-NEGATIVE ORGANISMS WITH THE AID OF METHIONINE, THREONINE AND METHIONINE SULFOXIDE

Microorganism	Per cent. inhibition in broth and M.T.S.*	Penicillin tests: Coefficient of resistance†	
		Broth	Broth and M.T.S.
Eb. ty. Rawlings ...	gr. p.‡	250	125
" " H (Oxford) .	0§	375	100
" " TLM	16	500	75
" " O (Oxford) .	gr. p.	1500	75
" " Mt. S.	13	75	30
S. Newport, 563	0	37.5	37.5
" typhi murium, var. Binns	50	37.5	18.75
" typhi murium, var. Aertrycke ...	gr. p.	5000	1500
" paratyphi A, var. Durozzo	0	2500	500
" paratyphi B	0	625	50
Sh. Flexner I	50	4500	1500
" Sonnei	0	8000	2500
" dysenteriae (Shiga)	20	375	75
Br. Melitensis	40	100	25
" " '73 ...	46	1250	100
" Caprini (Oxford)	50	3750	100

* M.T.S. = Methionine, 3.75 mg + Threonine, 1.25 mg + Methionine sulfoxide, 3.125 mg per ml.
† Ratio of the minimal amount of penicillin giving complete inhibition of the organism studied (initial concentration 0.75×10^6 cells per ml) to the minimal amount of penicillin required for complete inhibition of staphylococcus, strain H, initial concentration 5×10^3 cells per ml.
‡ gr. p. = Better growth than in broth alone.
§ 0 = The same amount of growth as in broth alone.

As may be seen from Table 1, penicillin susceptibility of broth cultures of highly refractory Gram-negative organisms may be significantly enhanced in the presence of methionine, threonine and methionine sulfoxide. The amino acids alone produced variable degrees of inhibition of some organisms, and no inhibition or even some growth promotion of others. Apparently, these effects bore no relation to the enhancement of susceptibility to penicillin obtained in the presence of the acids.

In the following experiments the enhancing properties of the amino acids were studied quantitatively. *Eb. typhosa,* strain T_LM selected for the studies, was completely inhibited in broth alone by 10 O.U. of penicillin per ml. Methionine, threonine and methionine sulfoxide were tested separately and in various combinations against 0.1–2.5 O.U. of penicillin per ml, *i.e.,* the highest concentration of penicillin being 4 times smaller than the minimal amount necessary for complete inhibition in the absence of the acids. As may be seen from Table 2, enhancement of susceptibility to penicillin results from synergistic rather than additive action of the amino acids. No enhancement was found upon addition of each of the amino acids separately and of mixture of methionine sulfoxide and threonine. The presence of methionine in sufficient concentration was essential for the enhancement. Methionine sulfoxide and threonine facilitated reciprocally the effect of methionine, the same degree of enhancement having been obtained with decreasing amounts of methionine, provided the concentration of threonine and methionine sulfoxide was correspondingly increased. It is of interest to note that using optimum concentrations of the acids the increase in susceptibility of the organism tested to penicillin was one hundred fold as compared to its sensitivity in broth alone, *i.e.,* 0.1 and 10 O.U. per ml, respectively.

TABLE 2

SYNERGISTIC ANTIBIOTIC EFFECT OF PENICILLIN, METHIONINE, THREONINE AND METHIONINE SULFOXIDE

Amino acids in mg/ml of broth			O.U./ml of penicillin required for complete inhibition
Methionine	Threonine	Methionine sulfoxide	
0.3–3.75	....	....	> 2.5
....	1.25–2.5	....	> 2.5
....	....	0.3–6.25	> 2.5
0.3–0.94	1.25–2.5	3.125–6.25	> 2.5
1.875	1.25	3.125–6.25	> 2.5
1.875	2.5	3.125	> 2.5
1.875	2.5	6.25	2.5
3.0	0.125–2.5	....	> 2.5
3.0	....	0.3–3.125	> 2.5
3.0	....	6.25	2.5
3.0	0.125–0.625	3.125	> 2.5
3.0	1.25	3.125	2.5
3.0	2.5	0.3	> 2.5
3.0	2.5	0.625	2.5
3.0	2.5	3.125	1.5
3.0	2.5	6.25	0.5
3.75	1.25	3.125	1.5
3.75	2.5	6.25	0.1

0 = 0 = 40 per cent inhibition.
The organism used was Eb. ty. TLM in initial concentration of 0.75×10^6 cells per ml. When tested in broth alone 10 O.U. per ml of penicillin were required for complete inhibition.

Observations were also made at short-time intervals on the rate of growth and H-ion concentration of cultures of *Eb. typhosa,* strain T_LM, in the presence and absence of the amino acids. The mixture of the amino acids in highest concentrations employed in these studies brought about some prolongation of generation time during several initial hours of the log phase. The H-ion concentration of the cultures varied from pH 6.8 to 7.1.

SUMMARY

Penicillin susceptibility of highly refractory Gram-

negative organisms may be greatly enhanced upon addition of methionine, threonine and methionine sulfoxide. The action of amino acids appears to be synergistic rather than additive. Methionine is essential for the enhancement. Threonine and methionine sulfoxide facilitate the effect of methionine following a reciprocal quantitative relationship.

GREGORY SHWARTZMAN

THE BENZYL ESTER OF PENICILLIN

DURING the past several years, various types of esters of penicillin have been made in this laboratory in a search for derivatives possessing greater stability, a depot action or effectiveness by mouth. Crude preparations of the methyl, ethyl, n-butyl and benzhydryl esters have been described by others,[1] but were reported to hold "no great promise." However, we have found that the benzyl ester promises to be extremely valuable because of its ease of preparation, enhanced stability, ready oral absorption and powerful chemotherapeutic action.

Our benzyl penicillin G has been obtained as a colorless, hard glassy solid which shows a sharp increase in fluidity at 50° C. The ester was prepared by treating free penicillin in an inert organic solvent with an excess of phenyl diazomethane, any unreacted penicillin being extracted with sodium bicarbonate solution. Evaporation of the solvent yielded a resinous product which was readily purified.

Benzyl penicillin is stable at temperatures above 100° C., in contrast to penicillin salts. The ester is also much more stable in alcoholic solvents than any known penicillin salt. It has a very low solubility in water, but is soluble in alcohol, ether, chloroform, ethyl acetate and in the polyethylene glycol type of polymers. It is approximately 2.5 per cent. soluble in sesame oil or propylene glycol.

In vitro, pure benzyl penicillin exhibits about one thirtieth as much bacteriostatic activity as pure sodium penicillin against broth cultures of *Staphylococcus aureus.* This was shown by serial dilution tests in which the bacteriostatic concentration was found to be 1 microgram per cc; under similar conditions, 0.03 microgram or 0.05 international unit of pure sodium penicillin sufficed. By the cup-plate method, benzyl penicillin is relatively much less potent and shows approximately one four-hundredth of the activity of sodium penicillin.

In vitro, a substantial proportion of the theoretical activity can be demonstrated after splitting the ester by incubating with an aqueous extract of rat kidney. Presumably this regeneration is caused by enzymatic cleavage since boiled kidney extract fails to liberate any activity. Rat serum is almost as effective as rat kidney extract, guinea pig serum shows some activity, while human, horse, rabbit and dog sera are inactive. The cup-plate, serial dilution and the Warburg respirometer procedures have been used to measure the activity so liberated. The observed activity is approximately one half that to be expected theoretically from the penicillin content of the ester. The highest value observed was 54 per cent. (using the cup-plate method). Hickey[2] recently reports obtaining a maximum of 26 and 16 per cent. regeneration from methyl and ethyl penicillin, respectively, using alkaline hydrolysis.

Benzyl penicillin dissolved in a vegetable oil and injected subcutaneously or given by mouth protects mice against lethal doses of streptococci and pneumococci. Mice were inoculated intraperitoneally with 0.3 cc of a 1:100,000 dilution of an 18-hour broth culture of *Streptococcus hemolyticus* (Strain C 203). This inoculation kills 92 per cent. of the unmedicated control mice within 18 to 46 hours (average of 15 experiments). In the subcutaneous injection experiments 0.1 cc of a solution containing the desired dose of benzyl penicillin was injected one hour after the inoculation. For the oral experiments 0.1 cc was given by stomach tube one hour before and 4 hours after the inoculation. Observations were made at convenient intervals for the succeeding 72 hours; rarely were there any deaths after this time. The relative efficacies of 2 or more preparations were

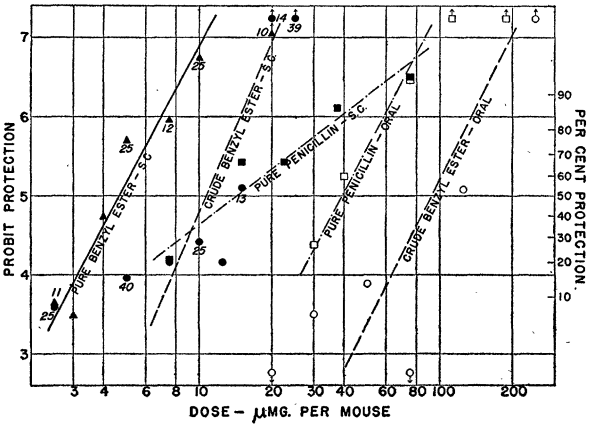

FIG. 1. Graph showing protection afforded by benzyl penicillin and pure penicillin against experimental streptococcal infection in mice.

Legend: ▲—Pure benzyl penicillin given subcutaneously, ●—Crude benzyl penicillin given subcutaneously ■—Pure penicillin given subcutaneously, □—Pure penicillin given orally, ⊙—Crude benzyl penicillin given orally. Each point represents 15 mice unless otherwise indicated. The points to which arrows are attached represent survivals of either 0 per cent. (downward arrow) or 100 per cent. (upward arrow). In these experiments, 5 of the 63 inoculated control mice survived without any penicillin treatment.

[1] K. Mayer, G. L. Hobby and E. Chaffee, SCIENCE, 97: 205–206, 1943.

[2] R. J. Hickey, SCIENCE, 101: 462–463, 1945.