bound. New York: Interscience Publishers, Inc. 1944. $7.00.

THE first part of this book describes theory and practice of colorimetry and covers the subject of sampling, reagents, blanks, and the preparation of solutions. About a score of general colorimetric reagents, including dithizone, thionalide and hydrogen peroxide, are discussed.

The second part, from page 113 to the index, consists of procedures for the determination of traces of metals. The author has adopted the desirable practice of showing how to separate interfering elements so that the reader can see the limitations as well as the applicability of the methods. The discussions include quite a range of determinations, for example, chromium in silicate rocks, in iron ore, and in biological materials; lead in silicate rocks, in water, and in biological materials; and rhenium by various methods in molybdenite and pyrolusite. Usually more than one method is given so that the analyst has some choice depending upon the nature of the substance to be analyzed.

To an analyst it is always gratifying to read a book or use a method that conveys the feeling that the subject-matter is based on the experience of the author. This is such a book and should find a place on the desk of any analyst interested in the determination of traces of metals. It is to be regretted that more elements are not included, especially the more common non-metals that are so common in silicate rocks and metallurgical products. Another volume including these is desirable.

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SPECIAL ARTICLES

ON THE NATURE OF REFRACTORINESS OF CERTAIN GRAM-NEGATIVE BACILLI TO PENICILLIN[1,2,3]

GRAM-NEGATIVE bacilli are described as highly resistant to penicillin,[4] the fact being interpreted as an intrinsic difference between Gram-negative bacilli and the susceptible Gram-positive microorganisms. It was recently reported by the author of this article that *E. coli* is significantly more susceptible to the drug than hitherto assumed. The inhibitory effect may be obscured by tests against an excessively large number of organisms, the bacterial concentration at zero hours bearing a linear relationship to the concentration of penicillin producing complete inhibition. Furthermore, the susceptibility of *E. coli* could be significantly enhanced when the tests were made in mixtures of methionine with normal sera.[5] The observations suggested that the refractoriness of Gram-negative bacilli may at least in part result from the interference of some medium-ingredients or bacterial metabolites with the action of penicillin. In order to investigate this assumption autotrophic strains of Gram-negative bacilli, *i.e.*, capable of growing abundantly in synthetic media with ammonium as sole source of nitrogen, were selected for study. The measurements of penicillin activity were made in the manner previously described.[5] The organisms were cultured for a number of generations in the synthetic medium of Gladstone.[6] The same medium was used as diluent and for testing. As may be seen from Table 1, the susceptibility of the organisms to penicillin was markedly greater in the synthetic medium than in meat infusion broth. The following studies were made in order to determine the nature of the antagonism observed.

TABLE 1

COMPARISON OF INHIBITORY EFFECT OF PENICILLIN UPON E. COLI IN MEAT INFUSION BROTH AND SYNTHETIC MEDIUM (GLADSTONE)

Microorganism*	Medium	Penicillin in O.U./ml giving complete inhibition	Coefficient resistance†	Increase in susceptibility in Gladstone medium
E. coli No. 42	Broth	20	1000‡/50	20×
	Gladstone	1		
E. coli No. 742	Broth	>50	>2500/500	>5×
	Gladstone	10		
S. Newport	Broth	2	100/10	10×
	Gladstone	0.2		
S. Paratyphi B	Broth	3	150/75	2×
	Gladstone	1.5		
S. Enteriditis	Broth	8	400/50	8×
	Gladstone	1		

* The number of organisms at zero hours was 0.75×10^6 per ml.

† Taking as one unit of resistance the amount of penicillin required to produce complete inhibition of Staphylococcus H, 5×10^2 organisms per ml at zero hours.

‡ Lower concentrations of penicillin are required to inhibit smaller numbers of organisms at zero hours.[5]

[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] E. P. Abraham, *et al.*, *Lancet*, 2: 177, 1941; J. Florey, *Brit. Jour. Exp. Path.*, 23: 120, 1942; C. L. Hobby, R. Meyer and E. Chaffee, *Proc. Soc. Exp. Biol. and Med.*, 50: 281, 1942.

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

[6] G. P. Gladstone, *Brit. Jour. Exp. Path.*, 18: 322, 1937.

Anti-penicillin effect of casein hydrolysate: Casein hydrolysate[7] of a given concentration was added to Gladstone medium containing various amounts of penicillin; as well as various concentrations of the casein were tested against 1.5 O.U. of penicillin representing 1.5 times the amount necessary for complete inhibition of *E. coli,* strain No. 42, in Gladstone medium alone. The initial number of organisms was 0.75×10^6 cells per ml. In the presence of 2.5×10^{-3} and 1.25×10^{-3} ml of casein there were required 15 and 7.5 times greater amounts of penicillin, respectively, than in the absence of casein (*i.e.,* 15 and 7.5 O.U. as compared to 1 O.U.). Furthermore, 1.25×10^{-4} ml of the hydrolysate was capable of antagonizing 50 per cent. of the activity of 1.5 O.U. of penicillin per ml. It is obvious from the above that casein hydrolysate is an active antagonist of the effect of penicillin upon *E. coli.*

Effect of certain amino acids and asparagine upon inhibition of E. coli by penicillin: In this group of experiments there was studied the effect of various concentrations of some of the amino acids[8] entering into composition of casein hydrolysate and that of asparagine[8] upon the inhibition of *E. coli* by different concentrations of penicillin in synthetic medium. Glycine, 1.5×10^{-2}M and 0.75×10^{-2}M; histidine, 2×10^{-4}M; phenylalanine, 6×10^{-4}M; and serine, 1×10^{-4}M produced a somewhat variable antagonistic effect upon the minimal inhibitory dose of penicillin in synthetic medium (*i.e.,* 1 O.U. per ml), little effect having been observed with greater concentrations of penicillin. Glutamic acid, 6.2×10^{-3}M, possessed a greater but also variable anti-penicillin property. However, a distinct antagonism against penicillin was shown by asparagine. Thus 2 mg per ml of the substance completely antagonized the effect of 1 O.U., 50 per cent. of 1.5 O.U., 10 per cent. of 3 O.U. and 2 per cent. of 6 O.U. of penicillin. Valine, 1×10^{-2}M and 0.5×10^{-2}M, and methionine, 2.5×10^{-2}M, were totally devoid of anti-penicillin activity in Gladstone medium. It is evident that the anti-penicillin property of the substances described bears no relation to molar concentration and nitrogen contents.

Effect of dl-methionine upon anti-penicillin activity of casein hydrolysate and asparagine: In synthetic medium alone methionine failed to enhance the susceptibility of *E. coli* beyond one O.U. per ml. Methionine, 2.5×10^{-2}M, appeared capable of removing 50 to 60 per cent. of the anti-penicillin activity of casein hydrolysate, 1.25×10^{-4} ml and about 75 per cent. of the activity of asparagine, 1.5×10^{-2}M.

[7] Manufactured by SMACO as "Vitamin-free" Acid Hydrolysed Casein for use in culture media.

[8] l-Histidine, Hoffmann La Roche; dl-Serine, Eastman Kodak; Glycine, l(+)Glutamic acid, dl-Methionine, dl-Phenylalanine and dl-valine, SMACO; and Asparagine, Difco standardized.

Relation of rate of growth of E. coli to the penicillin effect of broth and casein digest: In these experiments there was studied at frequent intervals of time the optical density of cultures of *E. coli* in Gladstone medium alone, Gladstone medium containing various concentrations of casein hydrolysate and in meat infusion broth. The same inoculum was used in all media, all other conditions having been kept as uniform as possible (*i.e.,* size of tubes, amount of medium, H-ion concentration, etc.). In the absence of penicillin there was less than 10 per cent. difference in bacterial concentration of all the cultures studied at the expiration of 16 hours of incubation at 37° C. The initial lag period was 3½ to 4 hours. The generation time was 2 to 3 times longer during the initial 3 hours of the log phase in cultures made in the Gladstone medium than in the remaining media. Delay in growth is known to antagonize the effect of penicillin.[9] Inasmuch as the prolongation of generation time occurred in Gladstone cultures, obviously the fact could not be held responsible for the greater activity of penicillin in these cultures. In order to bring further support to this contention, a series of tubes with casein hydrolysate in Gladstone medium, and the same mixture containing 1.5 O.U. penicillin per ml were placed in the refrigerator for periods of time varying from zero to 5 hours prior to incubation at 37° C. for 16 hours. Variations in the amount of growths in the respective tubes did not exceed 5 per cent. It may be concluded that the differences in penicillin activity observed were not caused by changes in the rate of growth in the media used.

Summary: The susceptibility of certain strains of *E. coli* and Salmonella is significantly greater in synthetic medium than in meat infusion broth. Casein hydrolysate, asparagine, glutamic acid to a lesser degree, and possibly some other aminoacids partially antagonize the effect of penicillin upon *E. coli* in synthetic medium. The antagonism of casein hydrolysate and asparagine can be removed in greater part by methionine. The observations suggest that the refractoriness of Gram-negative bacilli to penicillin is at least to some extent extrinsic in nature.

GREGORY SHWARTZMAN

THE CHANGES IN RAT KIDNEY COCARBOXYLASE ASSOCIATED WITH THE INJURIOUS EFFECTS OF dl-SERINE[1, 2]

AN injurious action of *dl*-serine in rats has been observed following its administration by stomach tube[3]

[9] M. H. Dawson, *et al., Jour. Clin. Invest.,* 20: 434, 1941; G. L. Hobby and M. H. Dawson, *Proc. Soc. Exp. Biol. and Med.,* 56: 178 and 181, 1944; C. P. Miller and Foster A. Zimmerman, *Proc. Soc. Exp. Biol. and Med.,* 56: 205, 1944.