Communications to the editor

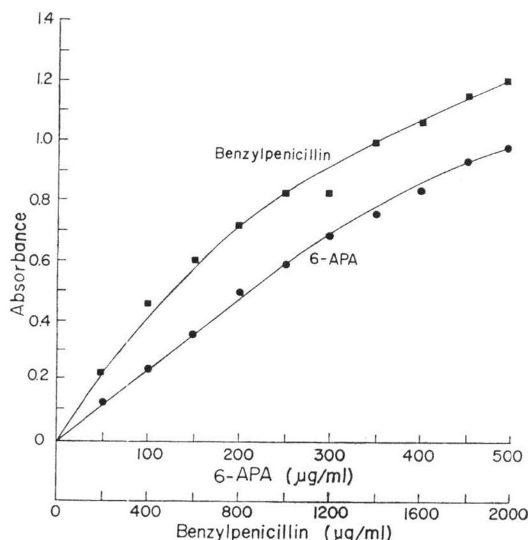
ESTIMATION OF PENICILLIN CONCENTRATIONS AND PENICILLIN METABOLISING ENZYME ACTIVITIES BY THE LOWRY REACTION

Sir:

As the present methods for determination of concentrations of penicillins, and activities of penicillin-metabolising enzymes, are not completely satisfactory we examined the possibility of using the LOWRY reaction with penicillins[1] for this purpose. All penicillins tested reacted with LOWRY reagent. Table 1 shows that ampicillin, 6-aminopenicillanic acid (6-APA), carbenicillin and several penicilloic acid derivatives reacted strongly. Other penicillins and some derivatives of 4-carboxythiazolidine gave a weaker reaction.

The plot of absorbance *versus* concentration of the reaction with benzylpenicillin and 6-APA is shown in Fig. 1. Under the conditions described by LOWRY *et al*[2], and reading at 500 nm, the curves were sufficiently regular for use as standards. They were sensitive up to 500 μg/ml for 6-APA and to 2,000 μg/ml for benzylpenicillin. Colour developed maximally after about 15 minutes at room temperature and was stable for at least one hour. In enzyme experiments protein was precipitated with 5% TCA prior to analysis. This slightly depressed colour development.

Fig. 1. Standard curves of 6-APA in the range up to 500 μg/ml and benzylpenicillin in the range up to 2,000 μg/ml.



Penicillin acylase was prepared in a manner similar to that already described[3] and assayed in a reaction mixture consisting of 550 μmoles of phosphate buffer pH 8, 77 μmoles of benzylpenicillin and 2.2 mg of enzyme protein in 11 ml. Incubation time was for 2 hours at 30°C and at the end of this time residual benzylpenicillin was extracted into cold butyl acetate. Samples from the aqueous phase were diluted, brought to a final concentration of 5% TCA without prior neutralisation, centrifuged and duplicate 1 ml samples used for analysis. The preparation used showed an activity of 23.4 μmoles 6-APA formed per mg protein over the 2 hour period. When checked by hydroxylamine analysis[4] after neutralisation

Table 1. Reaction of penicillins and related compounds with the LOWRY reagent.

Compound	Molar extinction litres moles^{-1} cm^{-1}	Coefficient relative to that of 6-APA
Penicillin VK	278	55.6
Cloxacillin	268	52.4
Carbenicillin	600	120
Methicillin	333	66.6
Ampicillin	795	159
Penicillin G	214	42.8
Phenethicillin	261	52.2
6-APA	500	100
Tyrosine	534	106.8
Penicilloic acids of		
Penicillin VK	432	86.4
Carbenicillin	835	167
Ampicillin	960	192
Penicillin G	294	58.8
Phenethicillin	484	96.8
4-Carboxythiazolidine = [4-CT]	282	56.4
2-Phenyl-[4-CT]	23.7	4.7
2-Isopropyl-[4-CT]	149	29.8
2-*n*-Propyl-[4-CT]	85	17
2-Dimethyl-[4-CT]	45	9

a result of 25.3 μmoles of 6-APA formed per mg protein was obtained. Thus the results agree to within 8% in spite of differences in sensitivity range for product analysis, dilution factors and neutralisation requirements for the hydroxylamine assay.

Narrow spectrum penicillinase activity was determined by substituting 10 μg of penicillinase protein in the above mixture and incubating for one hour only. LOWRY analysis showed that 1.98 μmoles of penicillin was hydrolysed per μg protein over that period whereas the result from iodometric analysis[5] was 2.12 μmoles of penicillin hydrolysed. Enzyme activity was lower when carbenicillin and penicillin VK were used as substrates and the results confirmed that methicillin and cloxacillin were resistant to the preparation used. Both methods of analysis gave close agreement when they were used to determine penicillinase activity of whole cells of *Klebsiella aerogenes* which produced a broad spectrum enzyme. In this case no attempt was made to purify the enzyme and soluble proteins and polypeptides caused considerable interference.

Although it lacks specificity the LOWRY reaction should have some application in analysis of penicillin concentrations where the hydroxylamine[4,6] or iodometric[5,7] methods are not appropriate. Stability of colour and low blank readings offer considerable advantages in this respect. There is also potential for automation. The sensitivity range for 6-APA allows the method to be used with convenience for determination of penicillin acylase activity but it may be used with less confidence for determination of penicillinase activity as it is less sensitive for benzylpenicillin. In enzyme studies problems may be encountered with soluble protein and adequate controls are required.

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References

1) SILVERMAN, D. J.: Interference with the FOLIN reaction by penicillin and some of its semisynthetic derivatives. Anal. Biochem. 27: 189~191, 1969
2) LOWRY, O. H.; N. J. ROSEBROUGH, A. L. FARR, & R. J. RANDALL: Protein measurement with the FOLIN phenol reagent. J. Biol. Chem. 193: 265~275, 1951
3) BAKER, W. L.: Estimation of 6-aminopenicillanic acid by the ninhydrin reaction. Aust. J. Pharm. Sci. 8: 86~90, 1979
4) BATCHELOR, F. R.; E. B. CHAIN, T. L. HARDY, K. R. L. MANSFORD & G. N. ROLINSON: 6-Aminopenicillanic acid. III. Isolation and purification. Proc. Roy. Soc. Lond. Ser. B. 154: 498~508, 1961
5) PERRET, C. J.: Iodometric assay of penicillinase. Nature 174: 1012~1013, 1954
6) FORD, J. H.: Hydroxylamine method of determining penicillins. Industr. Engng. Chem. (Anal. ed.) 19: 1004~1006, 1947
7) ALCINO, J. F.: Iodometric method for the assay of penicillin preparations. Industr. Engng. Chem. (Anal. ed.) 18: 619~620, 1946