LETTERS TO THE EDITORS

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Cephalosporin *N*: a New Type of Penicillin

A SPECIES of *Cephalosporium* has been found to produce two different kinds of antibacterial substance[1,2]. The first consists of a group of acidic antibiotics which are soluble in common organic solvents, are active mainly against Gram-positive organisms[2], and show similarities to helvolic acid[2–4]. The second consists of a substance (or group of substances) which is insoluble in most organic solvents and is active against a number of Gram-positive and Gram-negative organisms. This has been called 'cephalosporin *N*'. Evidence has now been obtained that cephalosporin *N* is a new type of penicillin.

The reasons for believing that the antibiotic is a penicillin are:

(1) It was inactivated by preparations of the enzyme penicillinase in high dilution, and, like benzylpenicillin[5], it stimulated the adaptive production of penicillinase by suspensions of *Bacillus cereus*.

(2) It was rapidly inactivated at room temperature in aqueous solution below *p*H 4 or above *p*H 9, and also at *p*H 7 in the presence of heavy metal ions such as those of copper, lead and tin.

(3) Acid hydrolysis of purified material resulted in the liberation of carbon dioxide. On addition of mercuric chloride to the hydrolysate a mercaptide was precipitated. Decomposition of the latter with hydrogen sulphide and reaction of the product with hot acetone yielded the hydrochloride of *iso*propylidenepenicillamine[6] (2,2-5,5-tetramethylthiazolidine-4-carboxylic acid), melting point 198°, $[\alpha]_D^{20} = +94°$ (found: C, 40·6; H, 7·7; N, 6·0; S, 12·4; Cl, 14·8 per cent; $C_8H_{15}O_2NS,HCl,1/2H_2O$ requires: C, 40·9; H, 7·3; N, 6·0; S, 13·6; Cl, 15·1 per cent). After boiling with dilute hydrochloric acid a solution of the *iso*propylidenepenicillamine hydrochloride showed a strong nitroprusside reaction, and evaporation *in vacuo* yielded the crystalline hydrochloride of penicillamine (β-thiolvaline)[6]. Oxidation of the latter with bromine resulted in the formation of penicillaminic acid (ββ-dimethylcysteic acid).

On addition of 2 : 4-dinitrophenylhydrazine in 2*N* hydrochloric acid to the supernatant from the mercaptide, only a very low yield of a precipitate consisting of a mixture of 2 : 4-dinitrophenylhydrazones was obtained. A small proportion of this precipitate was soluble in carbon tetrachloride and crystallized in orange-coloured needles from aqueous ethanol, melting point 183–185° (found: C, 48·2; H, 4·8 per cent). The 2 : 4-dinitrophenylhydrazone of *iso*butyraldehyde, melting point 182°, requires C, 47·6, H, 4·8 per cent. A further small proportion of the precipitate, which separated in crystalline form from cold pyridine, was glyoxal *bis*-2 : 4-dinitrophenylhydrazone[6].

The thiazolidine hydrochloride, penicillamine hydrochloride, penicillaminic acid and glyoxal *bis*-2 : 4-dinitrophenylhydrazone were kindly identified by X-ray powder photographs by Dr. D. Hodgkin, Miss P. Cowan and Dr. J. Robertson.

Cephalosporin *N* differs strikingly from the common penicillins in its hydrophilic character and its antibacterial activity. It behaves like an acidic substance on ion-exchange resins, and ionophoresis on paper shows that it carries a negative charge at *p*H 6–7. However, the purest preparations so far available contain a basic group titrating in the *p*H range 8–11, and the way in which the partition coefficient of the activity varies with *p*H in the phenol–water system indicates that a basic group, as well as acidic groups, may be present in the active substance itself. To explain the fact that the antibiotic is only soluble in highly polar solvents, it would seem necessary to assume that the essential core of the penicillin structure is linked to a residue which is rich in hydroxyl groups and/or contains a basic group which confers a zwitterionic character on the molecule. The nature of this residue is under investigation.

Cephalosporin *N* shows activity of the same order of magnitude against a number of Gram-positive and Gram-negative bacteria. For example, its activity against a strain of *Staph. aureus* is similar to that against a strain of *Salm. typhi*, whereas benzylpenicillin is more than a hundred times as active against the former as against the latter. An attempt has been made to obtain some idea of the purity of the present preparations of cephalosporin *N* by the application of two chemical methods for assaying penicillin[7,8] and by measuring the carbon dioxide evolved on hydrolysis. The results of these procedures agree in indicating that the preparations contain about 35 per cent of active material if the latter has the same molecular weight as sodium benzylpenicillin. Alternatively, they contain nearly 100 per cent of active material if the molecular weight is about 1,000. On the basis of these results, it appears likely that the activity of pure cephalosporin *N* against many Gram-negative bacteria will be of the same order as that of benzylpenicillin. The relatively low activity of the antibiotic against Gram-positive bacteria suggests that it reaches the sensitive part of these organisms much less readily than the other penicillins.

It is of interest that *Cephalosporium salmosynnematin* and certain other members of the genus *Cephalosporium* have been found to produce an antibiotic called 'synnematin', which is active against a number of Gram-positive and Gram-negative bacteria[9,10]. Although no evidence has been published to indicate that synnematin is a penicillin, the data which are available about its physical and chemical properties do not distinguish it from cephalosporin *N*.

We are indebted to the director and staff of the Antibiotics Research Station, Clevedon, Somerset, for the production of cephalosporin *N*.

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