Expressed in absolute units (35·2 for normals; 79·1 for schizophrenics) or in units relative to the total ATPase (16 per cent for normals; 26 per cent for schizophrenics) there is significance ($P = 0{\cdot}001$ and $P = 0{\cdot}06$, respectively). We used EDTA 0·1 mM in the incubation medium and we found that our inhibition (16 and 26 per cent) with glycoside was of the same order as that of Post *et al.*[3].

It is possible that these small but definite differences in sodium-potassium-ATPase might be 'magnified' if cerebrocortical biopsy or autopsy material were examined, since it is known[4] that grey matter contains roughly about 700 times more sodium-potassium-ATPase than red cells.

Philip M. Seeman

Rockefeller Institute,
New York, 21.

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Penicillin Amidase from Coliforms: its Extraction and some Characteristics

It is known that many fungi and some bacteria produce penicillin-splitting amidases or acylases[1,2]. With the exception of the amidase from *Streptomyces lavendulae* described by Batchelor, Chain, Richards and Rolinson[3], few details have been published about these enzymes.

A survey of 310 clinical isolates of *E. coli* types I and II showed that 40 per cent possessed to varying degree a specific amidase acting at the CO : NH linkage and thereby removing the side chain of benzyl- and phenoxymethyl-penicillin[4]. The root molecule of 6-aminopenicillanic acid (6-APA) was left intact, showing that these amidase-producing coliforms possessed no β-lactamase; the presence of residual 6-APA was readily demonstrated by reconverting it to benzylpenicillin with phenyl-acetyl chloride. Some of the coliform strains appeared to produce penicillin-amidase in considerable quantity, and one such strain (*C.*15) was used in an attempt to extract and purify the enzyme. (The strain of *E. coli* used in preparing this enzyme (penamidase) has been accepted by the Department of Scientific and Industrial Research, Torry, Aberdeenshire, with the number N.C.I.B. 9465.)

Strain *C.*15 was grown in nutrient broth at 37° C for 48 h under inducement from 100 μg/ml. benzylpenicillin. Initial efforts at enzyme precipitation by chemical means had yielded inactive products, so 100-ml. lots of *C.*15 broth cultures were filtered through cellulose-acetate membranes, and the filtrate vacuum freeze-dried. Approximately 0·5 g of pale brown flakes, readily soluble in water, was recovered from each 100 ml. of culture. 0·2 g of flake, dissolved in 5 ml. water, was dialysed overnight through narrow 'Viskene' sleeves against 1,000 ml. water at 4° C. The resultant pale yellow fluid, about 5 ml. volume, was again rapidly vacuum freeze-dried, to yield 4·9 mg of dry yellow powder. At each stage throughout the experiment, small aliquots were checked for full amidase activity.

The dialysed powder was found by protein–nitrogen determination to contain 53 per cent w/w protein; extractable lipids represented 3·7 per cent by weight. Ashing to constant weight left inorganic residues of 9·4 per cent w/w; this inorganic residue contained 5·1 per cent potassium, 11 per cent sodium, 6 per cent calcium and no detectable magnesium.

The amidase activity of both the crude and dialysed powders was estimated by the addition of unit volumes of enzyme solutions to various substrate concentrations (benzylpenicillin solutions ranging from 1 to 100 μg/ml.). The system was buffered at *p*H 5·5 determined by prior experiment to be optimum, and optimum temperature at 40° C. At close intervals, so that the initial velocity of reaction might be precisely established, aliquots were removed and the enzyme immediately inactivated by raising mixture temperature to 75° C for 10 min.

Residual benzylpenicillin was now assayed by conventional microbiological techniques, using *Sarcina lutea* as assay organism. It was found that whereas 500 μg of the crude product was required to destroy 1 μg of benzylpenicillin per h, this amount was split by 6 μg of the dialysed enzyme. Graphical determination of the Michaelis constant was then made (Fig. 1) giving $K_s = 4{\cdot}0 \times 10^{-4}$ mole/l. at *p*H 5·5 and 40° C.

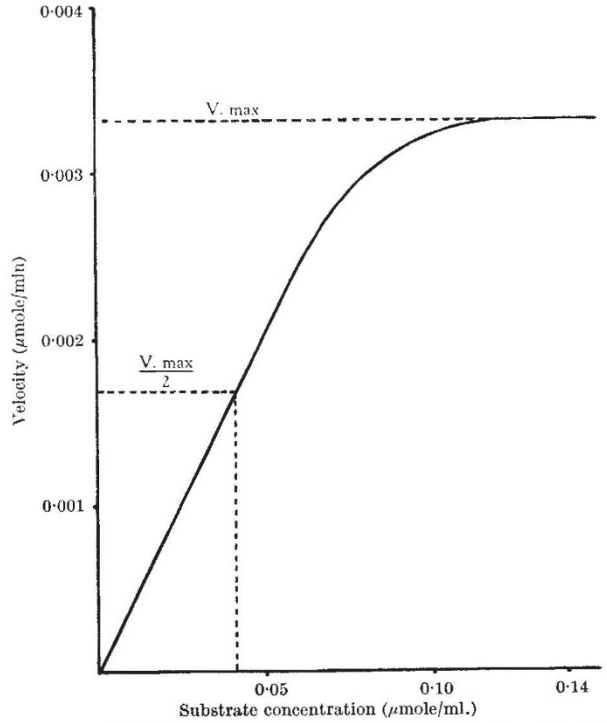

Fig. 1. Hydrolysis of benzylpenicillin by amidase from *E. coli*

The effect of enzyme inhibitors was tested on the dialysed extract; copper, iron, stearic acid and *p*-chloromercuriphenyl sulphonic acid had no inhibitory effect up to 10^{-3} M concentration. 'Versene' showed some inhibition at that concentration.

A proportion of coliforms produce β-lactamase as well as amidase; in our series of 310 strains of *E. coli* types I and II, we identified 12 per cent in this category. The optimal conditions for this enzyme are different from those for amidase, though benzylpenicillin is an ideal substrate for both. The semi-purified amidase described here had no action on 6-APA in a *p*H range 5 8, and contained therefore no β-lactamase. Under the conditions described, amidases from *E. coli* showed maximal activity against benzylpenicillin and phenoxymethyl-penicillin, but possessed also activity of a lower order against other derivatives of 6-APA such as α-aminopenicillanic acid (ampicillin) and, in some instances, against derivatives of 7-aminocephalosporanic acid.

R. J. Holt
G. T. Stewart

Queen Mary's Hospital for Children and the
Medical Research Council Laboratories,
Carshalton.

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