

hydrolyse benzylpenicillin because of its specificity for the phenylacetyl group. Liberation of 6-APA by deacylation of phenoxymethylpenicillin using preparations of animal tissue has already been reported<sup>14</sup>. Since then we have found that pig kidney acylase I (British Drug Houses, Ltd.), which will hydrolyse phenoxyacetyl-glycine, octoyleglycine and hexoyleglycine but not phenylacetyl-glycine, will hydrolyse the corresponding penicillins, phenoxymethylpenicillin, heptylpenicillin and pentylpenicillin, but not benzylpenicillin. It seems probable that the liberation of 6-APA by enzymatic splitting of penicillins by preparations from various micro-organisms<sup>1-11</sup>, animals<sup>14</sup> and plants<sup>15</sup> is due to acylase enzymes which are specific for the acyl group present in the penicillin which is cleaved.

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M. COLE

Beecham Research Laboratories,  
Research Division,  
Brockham Park,  
Betchworth, Surrey.

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### Variety of Substrates for a Bacterial Benzyl Penicillin-splitting Enzyme

PREVIOUS work on a cell-bound bacterial enzyme which catalyses a reversible enzymatic cleavage of benzyl penicillin to 6-aminopenicillanic acid (6-APA) and phenylacetic acid had shown that this enzyme represents an acyl transferase which, under suitable conditions, is able to synthesize penicillins by transferring acyl- and  $\alpha$ -aminoacyl residues from acylamino acids, acyl amides, acylated hydroxycarboxylic acids, mercaptocarboxylic acids, alcohols, phenols and thiophenols to the amino group of 6-APA (refs. 1-3). Free amino-acids, ammonia, hydroxycarboxylic acids, mercaptocarboxylic acids, alcohols phenol and thiophenol, liberated in the course of these reactions, may be considered to be hydrolysis products of the acyl compounds used.

Table 1. HYDROLYSIS OF VARIOUS ACYL-COMPOUNDS BY THE PENICILLIN-SPLITTING ENZYME AT NEUTRAL REACTION

- (1) Phenylacetyl L-glutamic acid  $\xrightarrow{+H_2O}$  glutamic acid + phenylacetic acid  
 (2) DL- $\alpha$ -Aminophenylacetyl glycine  $\xrightarrow{+H_2O}$   $\alpha$ -aminophenylacetic acid + glycine  
 (3) DL- $\alpha$ -Phenoxypropionyl amide  $\xrightarrow{+H_2O}$   $\alpha$ -phenoxypropionic acid + ammonia  
 (4) D- $\alpha$ -Aminophenylacetyl methyl ester  $\xrightarrow{+H_2O}$   $\alpha$ -aminophenylacetic acid + methyl alcohol  
 (5) Phenoxyacetyl glycollic acid  $\xrightarrow{+H_2O}$  phenoxyacetic acid + glycollic acid  
 (6) p-Methoxy-phenoxyacetyl thioglycollic acid  $\xrightarrow{+H_2O}$  p-methoxy-phenoxyacetic acid + thioglycollic acid

By means of some representative reactions listed in Table 1 we wish to demonstrate that a hydrolytic cleavage of acyl- and  $\alpha$ -aminoacyl compounds as mentioned above is not dependent on the presence of 6-APA.

On the basis of these findings it is not the penicillin nucleus which seems to be important for the substrate specificity of the enzyme, but rather the acyl residue, which may be linked to different compounds. Considering the impressive variety of enzymatic reactions including those not involving penicillins, the synonymous names 'penicillin amidase' and 'penicillin acylase' proposed for this enzyme by several authors and discussed separately by Claridge *et al.*<sup>4</sup> and Huang *et al.*<sup>5</sup> are not satisfactory.

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WILFRIED KAUFMANN  
KLAUS BAUER

Biochemical Laboratory,  
Farbenfabriken Bayer AG.,  
Wuppertal-Elberfeld, Germany.

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### Influence of Extraneous Ribonucleic Acid on the Differentiation of Haematopoietic Tissue in Chick Embryos

EXPERIMENTAL results are given here which seem to confirm the work of several investigators<sup>1-4</sup> concerning the ability of phenol-extracted RNA to stimulate protein synthesis in embryonic cells.

It has, indeed, been found possible to increase somewhat the amount of haemoglobin synthesized by the isolated vascular area of the chicken embryo by the addition of phenol-extracted chicken reticulocyte RNA, rabbit reticulocyte RNA or total 3-5-day chick embryo RNA. Moreover, isolated fragments of the extra-embryonic region of the blastodisk have also been found to increase their potential for haemoglobin synthesis in the presence of chick embryo RNA added to their culture medium.

RNA extracted by a modification of the method described by Fraenkel-Conrat<sup>5</sup> was suspended in chick Ringer and added to a yolk agar medium of the type recommended by Britt and Hormann<sup>6</sup>. Chick embryo blastodisks were explanted, washed in Ringer and transferred to the experimental medium after removal of the embryo at stages between 6 and 11 (Hamilton-Hamburger classification) such that the explanted tissue cultivated consisted mainly of presumptive haematopoietic tissue, particularly favourable for examining variations in net haemoglobin synthesis over a limited experimental period.

After culture for 24 h, the explants were homogenized separately and determinations of haemoglobin, total RNA and residual proteins were carried out by the methods of O'Brien<sup>7</sup>, Ogur and Rosen<sup>8</sup> and Lowry<sup>9</sup>, respectively.

It has thus been possible to record, in certain experimental series, an increase in the net quantity of haemoglobin synthesized over a 24-h period by the treated explants, cultivated on an agar yolk medium containing additional RNA. Similar effects were recorded with chicken reticulocyte RNA, rabbit reticulocyte RNA or chick 3-5-day embryo RNA. A difference, varying from 4 to 39 per cent was recorded between treated and control embryos (Table 1). It has not been found possible to detect a linear relationship between increase in concentration of added RNA and the increase in haemoglobin synthesis. In fact, a concentration of 2 mg/ml., which