THE POSSIBLE IMPLICATION OF A BACTERIAL ENZYME IN THE BIOCHEMICAL MODE OF ACTION OF PENICILLINS ON GRAM NEGATIVE BACTERIA.

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On the basis of early cytological observations by Duguid (1946) and of experiments by Lederberg (1956) the idea was put forward that penicillins interfere with the synthesis of bacterial cell walls. Subsequently this view was strongly favoured when Park and Strominger (1957) demonstrated the accumulation of cell wall precursors by detecting the formation of small uridine mucopeptides in penicillin-treated cultures of staphylococci. Recently Rogers & Jeljaszewicz (1961) and Rogers & Mandelstam (1962) found a good correlation between growth inhibitory concentrations of different penicillins and the inhibition of mucopeptide synthesis in Staphylococcus aureus and Escherichia coli, provided that an effect of penicillinase was excluded.

Within the chain of biochemical and physico-chemical events induced by the antibiotic action of penicillins on bacteria the inhibition of mucopeptide synthesis must be viewed as a consequence of the inhibition of one or more defined enzymatic reactions. It is the purpose of this communication to point to the affinity of penicillins for an enzyme that is located in the cell wall-membrane complex of many gram negative and some gram positive bacteria. Originally this enzyme was shown to cleave the acyl

side chain of benzyl penicillin with the resulting formation of 6-aminopenicillanic acid (6-APA) and phenylacetic acid (Kaufmann and Bauer). A series of other penicillins are split more slowly or not at all. Further work on synthetic reactions with the enzyme disclosed its rather wide specificity as an acyl- and α-aminoacyltransferase (Kaufmann, Bauer and Offe). Thus it catalyses the transfer of acyl residues of monosubstituted and some disubstituted acetic acids from peptide-like-, amide-, ester-, and thioester linkages to 6-APA and probably other compounds bearing a primary NH_2-group. Moreover α-aminoacyl residues are transferable from amino acid amides and -esters.

The observation that the reaction (reaction I)

$$\text{DL-α-aminophenylacetyl-methyl ester} + \text{6-APA} \xrightarrow[\text{pH 6.0}]{\text{Enzyme}} \text{DL-α-aminobenzyl penicillin} + \text{methyl alcohol}$$

carried out with intact cells of E.coli ATCC 11105 as the enzyme, is under suitable conditions significantly inhibited by the presence of as little as 1 unit per ml. of phenoxymethyl penicillin, suggested a possible implication of this or of quite a similar type of enzyme in the biochemical mode of action of penicillins on gram negative bacteria. Therefore more systematic experiments were carried out to detect a correlation between the antibiotic action of different penicillins towards some gram negative bacteria and the inhibiting effect of these penicillins on the described enzymatic reaction I. Moreover the antibiotic activity of these penicillins was compared with their sensitivity toward enzymatic cleavage of the side chain. To avoid a distortion of the experimental results, the antibiotic sensitivity tests were carried out with strains of bacteria producing no substantial quantity of inductive or constitutive penicillinase.

Table I

Penicillins (as listed below)	Per cent inhibition of reaction I by 100 units per ml. of a penicillin.	Per cent enzymatic cleavage of penicillin to 6-APA + free acyl side chain acid.(Expressed relatively to the cleavage of benzyl penicillin).	Diameter (mm) of zones of inhibition in the agar plate assay with a.) E.coli ATTC 11105 b.) E.coli,strain "Pr" c.) Proteus OX 19 d.) Serratia marcescens as test organisms. Penicillin concentrations 2000 units per ml. (1), and 200 units per ml. (2).							
			a.)		b.)		c.)		d.)	
			(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1.)	0	83		30		26		31	26	
2.)		75		23						
3.)	80	100		20		18.5		31	16	
4.)	79	100	29	20	25	16.5	28	25	18	
5.)	82	20	25	12.5	21.5	10.5	20	0	12.5	
6.)	18	14	27		25		24.5		17	
7.)	1.5	10	19		17.5		16		11.5	
8.)	12	7	20		14		9.5		0	
9.)	67	0.1	24.5		23		23		14.5	
10.)	77	0.1	20.5		20		18.5		13	
11.)	28	0.5	20		17.5		18.5		0	
12.)	23	0	18.5		19		17		0	
13.)	0	0	0		0		0		0	
14.)	2	1.0	0		15		0		0	
15.)	5	0.1	14.5		0		0		0	
16.)	0	3.0	14		15		9.5		0	
17.)	0	trace	13		14		0		0	
18.)	4	0	14		14.5		9.5		0	
19.)	0	0	15		11.5		0		0	
20.)	0	0.1	0		0		0		0	
21.)	7	1.5	0		9.5		0		0	
22.)	-	-	19.5		22.5		18		20.5	

1.) D-α-aminobenzyl penicillin
2.) p-aminobenzyl penicillin
3.) p-hydroxybenzyl penicillin
4.) benzyl penicillin
5.) phenoxymethyl penicillin
6.) β-indolylmethyl penicillin
7.) hexahydrophenoxymethyl penicillin
8.) α-ethyl-n-propyloxymethyl penicillin
9.) β-phenylethyl penicillin
10.) heptyl penicillin
11.) benzyloxymethyl penicillin
12.) γ-phenylpropyl penicillin
13.) benzyloxy-α-propyl penicillin
14.) hexahydrobenzyloxymethyl penicillin
15.) α-n-propyl-n-butyloxymethyl penicillin
16.) α-phenoxyethyl penicillin
17.) α-phenoxypropyl penicillin
18.) 3-phenyl-5-methylisoxazolyl penicillin
19.) 2,6-dimethoxyphenyl penicillin
20.) 2,6-diethylphenoxymethyl penicillin
21.) (2,4-dimethylphenoxy)-methyl penicillin
22.) 6-aminopenicillanic acid (6-APA)

The results presented in Table I reveal that penicillins lacking or low in antibacterial potency neither affect reaction I, nor are substantially split to 6-APA + acyl side chain. Significantly, 6-APA is antibiotically much more active than penicillins of this type. An increasing antibiotic activity of the penicillins is associated with their increasing inhibitory action on reaction I or with their increasing sensitivity to enzymatic cleavage. Most of the compounds are endowed with both properties.

Thus it has been shown that the inhibitory action of penicillins on reaction I as well as their sensitivity to enzymatic cleavage is in fact correlated, at least qualitatively, with the antibiotic activity of these compounds towards gram negative bacteria. In other words, a strong affinity for the enzyme characterizes an antibiotically potent penicillin.

Regarding enzymatic reaction I merely as a model for a synthetic reaction in a particular step in the course of the

cell wall formation, penicillins would inhibit the normal function of the essential enzyme. However, it must be mentioned that reaction I is inhibited not only by penicillins, but also by the acyl side chain acids of some compounds listed in Table I. Most probably the mode of this inhibition is of the competitive type.

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