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Mode of Action of Penicillin

Biochemical Basis for the Mechanism of Action of Penicillin and for Its Selective Toxicity

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In 1949, Park and Johnson (*1*) reported that uridine nucleotides accumulated in a *Staphylococcus aureus* that was inhibited by penicillin. Later, three previously unknown uridine nucleotides were separated and identified (*2*). The principal compound was uridine-5′-pyrophosphate linked to an unidentified N-acetyl-amino sugar and a peptide composed of D-glutamic acid, L-lysine, and DL-alanine in the ratio of 1/1/3 (Fig. 1). Several features of the accumulation of these nucleotides—the time sequence of accumulation after addition of penicillin, the relationship of accumulation to the threshold bacteriostatic concentration of penicillin, and the conditions under which accumulation could be demonstrated—have suggested that this phenomenon was a very early and specific effect of penicillin and might be closely related to the point of attack of penicillin within the bacterial cell (*2–4*). Uridine nucleotide accumulation was also found in a penicillin-sensitive *Lactobacillus helveticus* (*4, 5*) and similar compounds have been isolated from β-hemolytic streptococci (*6*).

The rate of accumulation of the peptide-containing uridine nucleotide in the presence of penicillin indicated that, if it were metabolized at this rate under normal conditions, it must be used in one of the principal synthetic reactions of the cell (*2–4*). At that time, the new amino sugar and D-amino acids were not known to be important in bacterial metabolism, and the product of the hypothetical reaction remained obscure. However, we now wish to present evidence that a structure analogous to part of the nucleotide is found in the cell wall of *Staphyloloccus aureus* and that the compound is presumably a biosynthetic precursor of the bacterial cell wall (*7*).

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Nature of the Cell Wall

In the past few years, a considerable amount of information concerning the nature of the bacterial cell wall has been obtained. Weibull (*8*) showed that, when a susceptible microorganism was treated with lysozyme in 0.2*M* sucrose, the lytic reaction was limited to removal of the external coat of the bacteria. The resulting spherical body, the protoplast, was composed of the bacterial cytoplasm with its limiting membrane (*9*). These observations clearly defined the cell wall as the rigid structure that gives shape to the bacterium and serves to protect the fragile protoplast. Cytological observations have also clearly differentiated the cell wall from the cell membrane (*10*), as well as from the extracellular capsule that is possessed by some microorganisms, and have indicated that growth of the cell wall during multiplication is always external to the cytoplasmic membrane (*11*).

Following the development of methods for preparing bacterial cell walls, a number of reports of their composition appeared (*12, 13*). Alanine, glutamic acid, and lysine (or, in some bacteria, diaminopimelic acid instead of lysine) are present in high concentration in the walls of all the gram-positive organisms that have been examined. Usually these are the only amino acids found in high concentration, although staphylococcal walls also contain glycine and the walls of some lactobacilli contain aspartic acid in high concentration (*13*). Recently, Snell and his coworkers (*14*) have reported that a large percentage of the alanine and glutamic acid in the cell walls of *Lactobacillus casei* and *Streptococcus faecalis* is in the D-form. These results suggest that DL-alanine, D-glutamic acid, and lysine (or diaminopimelic acid) are constituents of many bacterial cell walls.

Another line of investigation pertinent to understanding of the structure of the cell wall began with the observation of Strange and Powell (*15*) that a peptide derived from the spores of gram-positive bacilli was composed of alanine, glutamic acid, diaminopimelic acid, and an unidentified amino sugar. Cummins and Harris (*13*) have found this new amino sugar in the cell wall of a large variety of gram-positive bacteria, in more than 60 strains in all. The sugar was crystallized by Strange and Dark (*16*), and recently Strange (*17*) has proposed that its structure is 3-O-carboxyethyl hexosamine (see Fig. 1).

These analytic data strongly suggested a close similarity between the structures of a part of the cell wall and of a part of the uridine nucleotide that accumulates in penicillin-treated *Staphylococcus aureus*. Careful quantitative analyses of the cell walls of *S. aureus* were therefore carried out (*18*). The results are summarized in Table 1. The ratio of the new amino sugar, glutamic acid, lysine, and alanine in the cell wall approached 1/1/1/3, the ratio found in the nucleotide. Analysis of the optical rotation of the isolated alanine and glutamic acid by means of enzymes specific for D- or L-amino acids indicated that 45 percent of the alanine and 92 percent of the glutamic acid was in the D-form, compared with previously reported values of 52 percent and 100 percent for these amino acids in the nucleotide (*2*).

The unique amino sugar of the cell wall was identical with the amino sugar from the nucleotide, as is indicated by the following criteria which clearly distinguish it from glucosamine, galactosamine, or aminoglucuronic acid (*19*): (i) paper chromatography in three solvents; (ii) paper electrophoresis at several *p*H values from 2 to 6 (these data indicated that the compound contains a dissociable acid group with a *pK* around 2.5); (iii)

behavior of the N-acetylated amino sugar in a modified Morgan and Elson reaction (*20*) in different buffers; (iv) orange-pink color formed in a modified Elson and Morgan reaction (*21*) with maximum absorption at 506 millimicrons; (v) paper chromatography of the ninhydrin degradation product (*22*); and (vi) behavior in cation-exchange chromatography.

Furthermore, limited acid hydrolysis of the cell wall has yielded fragments that are very similar to fragments derived by similar treatment of the nucleotide (*23*). Exact comparison depends on further identification of these fragments. It may be emphasized that two of the compounds, D-alanine and 3-O-carboxyethyl hexosamine, have not been found elsewhere in nature and that D-glutamic acid is an unusual natural substance.

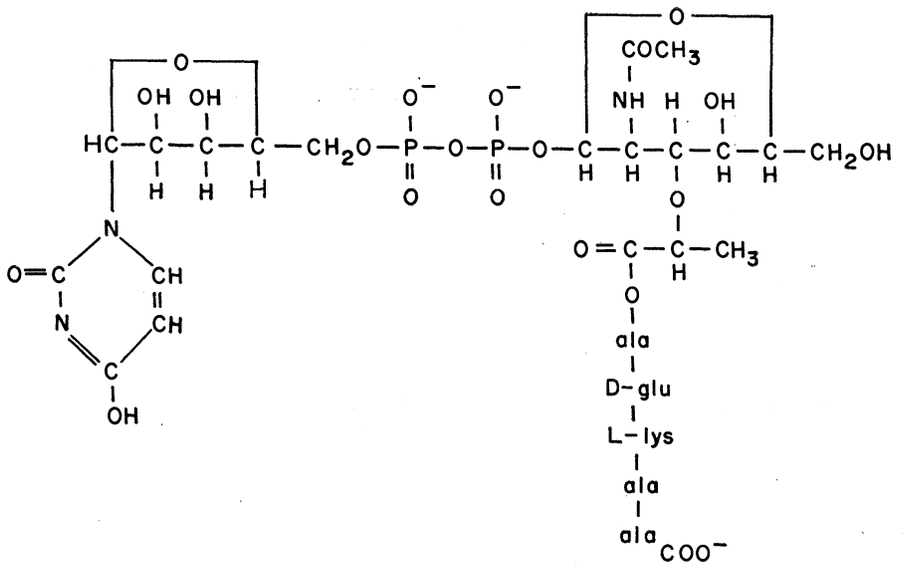

Fig. 1. Proposed structure of the principal nucleotide that accumulates in penicillin-treated *Staphylococcus aureus*. In addition to the original structural determination of Park (*2*), this structure incorporates a structure proposed for the amino sugar by Strange (*17*). The sequence of amino acids in the peptide is one of the possible sequences indicated by Strominger (*5, 32*).

Mechanism of Action

The uniqueness of the structures in the wall and in the nucleotide suggests that they may be metabolically related. Moreover, the striking similarity of structure, as well as the biological experiments, leads to the conclusion that the uridine pyrophosphate N-acetylamino sugar peptide is a biosynthetic precursor of the bacterial cell wall, and that the accumulation of this compound in penicillin-treated *Staphylococcus aureus* is the consequence of the interference by penicillin with the biosynthesis of the cell wall. Uridine pyrophosphate glycosyl compounds are activated intermediates in many biosynthetic transglycosidation reactions, and the N-acetylamino sugar peptide may be considered as a nucleotidyl fragment activated for such a synthetic reaction. The transfer of this fragment from the nucleotide to some cell wall acceptor would be a reaction for which many models now exist (*24*).

The exact nature of the interference by penicillin is a matter of speculation. It seems possible that penicillin is a specific inhibitor of the transglycosidation reaction involving this uridine nucleotide.

Data on the binding of penicillin by bacteria are consistent with this formulation. Maas and Johnson (*25*) and Cooper and Rowley (*26*) independently demonstrated that staphylococci specifically and firmly bound about 1000 molecules of penicillin per cell (possibly a titration of the number of molecules of a specific enzyme). Cooper (*27*) has shown that the penicillin is bound to a lipid-containing fraction close to the cell wall. The binding by these lipid particles was 7 to 12 times that of whole cells on a weight basis.

From the recent report of Mitchell and Moyle (*28*) it seems clear that the particles which bind penicillin were originally cell membrane. Thus the hypothetical transglycosidase, were it also the specific binding site of penicillin, is strategically located to transfer the N-acetylamino sugar peptide from uridine pyrophosphate, which is inside the membrane, to an acceptor (cell-wall site) outside the membrane. Indeed, the cell membrane may well be the location of many transglycosidases that appear to participate in synthesis of extracellular polysaccharides from intracellular uridine pyrophosphate glycosyl compounds.

It has been calculated (*3*) that if penicillin-binding inhibits the enzyme that normally utilizes the uridine pyrophosphate derivatives, this enzyme would have a turnover number of about 6000. It can now be calculated that a staphylococcus with a generation time of 1 hour and 1 percent of whose weight is 3-O-carboxyethyl hexosamine (the situation with strain 2) would require 1000 molecules of enzyme with a turnover number of about 6000 to incorporate the N-acetylamino sugar peptide into the wall at the observed rate. Therefore, on a kinetic basis, the rate of synthesis of uridine pyrophosphate derivatives is comparable to the rate at which they would normally be utilized if the N-acetylamino sugar peptide is incorporated into the cell wall. It must be pointed out that, until a membrane fragment is obtained which will catalyze the proposed transglycosidase reaction and until it is shown that this reaction is sensitive to penicillin, interference with cell-wall synthesis by blockage of a single reaction is not proved. More complicated mechanisms for transfer of the N-acetylamino sugar peptide through the membrane and into the cell wall can also be visualized. For example, accumulation of the nucleotide might occur if penicillin blocked the synthesis of the acceptor site in the cell wall.

Table 1. Comparison of analysis of the cell wall and of a uridine nucleotide that accumulates in penicillin-treated *Staphylococcus aureus*. Data are given as the ratio to the amount of glutamic acid. The cell walls were prepared by the method of Salton and Horne (*33*). After acid hydrolysis, the products were separated on Dowex-50. The only other ninhydrin-positive components present were glucosamine (ratio about 2 when corrected for loss due to acid hydrolysis), glycine (ratio 3.8), NH_3 and, in the case of strain 1, a small amount of an unidentified substance (*18*).

Compound	Cell walls		Uridine nucleotide
	Strain 1	Strain 2	
Glutamic acid	(1)	(1)	1
Lysine	0.95	1.17	1.0
Alanine	3.2	3.5	3.0
3-O-Carboxyethyl hexosamine	0.23*	0.93	1.0

* The low value for the new amino sugar in this case was owing to destruction as a result of prolonged acid hydrolysis (6*N* HCl at 100°C for 15 hours). When this value was corrected by the amount of ammonia present, a value close to 1 was obtained (*18*).

Selective Toxicity

The familiar effects of penicillin on morphology of bacteria—swelling, filamentous forms, large body formation, production of the penicillin-insensitive

L-forms of bacteria, and lysis—are all explained by the loss of the integrity of the cell wall that follows interruption of cell-wall synthesis. Simultaneously with our work, Lederberg has shown that *Escherichia coli* cells are quantitatively converted to protoplasts in the presence of penicillin and sucrose. The protoplasts reverted to bacilli after removal of penicillin, thus showing that the cells retained their full capacities. We believe that Lederberg has correctly interpreted these morphological observations as evidence that penicillin interferes with maintenance of the cell wall or with its synthesis (*29*). Hahn and Ciak have studied lysis of *E. coli* in the presence of penicillin and have also postulated that loss of cell-wall integrity induced by the drug is responsible (*30*).

In our view, therefore, the selective toxicity of penicillin is due to its interference in a metabolic sequence that is not found in animal cells, the biosynthesis of the cell wall. Crystal violet, another antibacterial substance also bound in the cell membrane or wall, seems to inhibit the same metabolic sequence, although at a different point (*31*). Possibly other antibacterial substances may owe their selective toxicity to interference at some point in this reaction sequence.

It seems attractive to speculate further that competitive inhibitors related to the several unique components of the wall and nucleotide, and which would be useful as chemotherapeutic agents, might now be devised. Purification and study of the reactions leading to synthesis of the wall might also contribute greatly to this goal, and the availability of uridine nucleotide intermediates suggests that this possibility lies in the not-too-distant future.

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Scientific Communications Should Be Improved

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Authentic, complete, prompt, and understandable reports of scientific developments have always been needed, and in the past they have always inestimably aided scientific achievement. The accelerated pace of science today requires more than ever the aid of full and accurate communications among science, business, and the public. However, during the past decade some questions of ethics and communications have grown until they now threaten to hamper critically the status of science in modern society. These questions demand review and action by the scientific community at large. The time is long overdue for all scientists to examine these questions in the contexts of their own professional fields and to share with other scientists their experiences in improving scientific communications.

Channels

Information about developments on the scientific frontier may reach the public through the channels of professional publications, newspapers, or advertising. All channels of communication are active when technologic development has economic implications. "Scientific facts" may then be proclaimed by several parties, and the public is thoroughly confused by sharply conflicting "scientific" conclusions.

Science is represented to society not only by qualified impartial scientists guided by objective logic but also by specialists and former scientists whose scientific conscience may have been more or less eroded by commercial interests, by specialists not broadly enough trained to speak with adequate perspective, by promoters operating on the fringe of science with little or no concern for the long-range growth of science, and by outright charlatans totally unqualified in science but yet accepted by many laymen as "scientists." Moreover, journalistic practices tend to aid the spectacular claims more than the cautious, qualified reports. Demands for brevity sometimes cause omission of essential features even in valid statements and leave erroneous impressions.

Several recent cases demonstrate a need for researchers to give special attention to examining their means of communication with one another and with the public on the results of scientific investigations. Conflicting reports have appeared, and in some cases controversy has raged over such subjects as battery

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