ANTIBACTERIAL CHEMOTHE

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The field of antibacterial chemotherapy is now so vast and the results of research so profitable that to do justice to the subject while retaining a measure of readability would require many more pages than can be spared for inclusion in this volume. I have presented, therefore, no more than a few facets of a many-sided picture. These were chosen to permit an appreciation of the developments over the past year or so of new antibacterial substances which have already shown some application to therapeutics. Little or no mention has been made of substances still in the stage of development. A brief discussion of penicillin resistance and penicillin hypersensitivity has also been included because of the interest and importance of these topics at this time.

PENICILLINS AND CEPHALOSPORINS

Semisynthetic Penicillins

Nafcillin.—Nafcillin (Fig. 1) is another semisynthetic β-lactamase-resistant penicillin introduced recently into clinical practice (1).

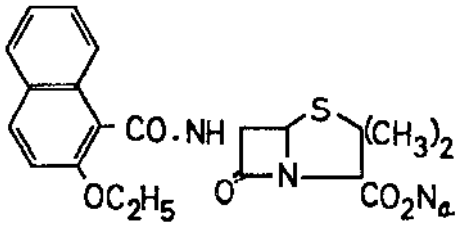

Fig. 1. Nafcillin.

Its antibacterial activity *in vitro* against *Diplococcus pneumoniae* and *Streptococcus pyogenes* has been found to be slightly higher than that of other penicillins of this type (1–4). Indeed, in a controlled trial, it was concluded that nafcillin was as effective in treating "penicillin treatable infections" as benzylpenicillin, and as effective as methicillin in treating "staphylococcal infections" (5). However, potency differences between the drugs were obscured by the use of dosages much greater than those minimally curative (6). In the laboratory, nafcillin appeared to be more active against animal infection with staphylococci, streptococci, and pneumococci than methicillin, oxacillin, and cloxacillin [see (7)].

The greater potency of nafcillin *in vivo,* if confirmed, may have sev-

[1] The survey of the literature pertaining to this review was concluded on June 1, 1965.

eral explanations quite apart from its slight advantage in terms of minimum inhibitory concentration *in vitro*. Its distribution in the body may be one of the reasons. Glassman et al. (8) have shown that biliary excretion constitutes a much more important pathway in the disposition of nafcillin than elimination by the kidney. The reverse is true for methicillin and benzylpenicillin. Hence, the process of reabsorption and re-excretion continuing for a considerable period of time may be responsible for the greater therapeutic efficacy of nafcillin in laboratory animals.

However, quite apart from the demonstrable antibacterial activity of any antibiotic lies the effect of host response to infection and the interaction of unknown factors with bacteria which have been damaged but not killed by the agent. These factors cannot be determined directly as yet. Nevertheless, the action of the enzymes lysozyme and trypsin on bacteria may provide an insight into such processes occurring in the tissues of an infected host.

Warren & Gray (9) have demonstrated that subinhibitory concentrations of nafcillin, oxacillin, and cloxacillin decreased the resistance of *Staphylococcus aureus* to lysis by these enzymes. The results obtained with the three penicillins differed quantitatively one from another, although growth of the particular strain used was inhibited by approximately equal concentrations of each penicillin. Nafcillin was found to be considerably more potent that the other two penicillins in reducing resistance to lysis. The results of this study suggest the subinhibitory concentrations of nafcillin readily produce alteration in cell wall structure, which allows access of the lytic enzymes and consequent death of the cell. Differences in potency in this respect between nafcillin and other penicillins might, at least in part, explain the differences in potency observed in experimental infections. Similar changes in lysozyme sensitivity have also been produced by subinhibitory concentrations of methicillin (10).

Quinacillin.—The very recent introduction of quinacillin into clinical practice resulted from studies on a series of heterocyclic side-chain penicillins. Early studies were based on the observation that 2-pyridylpenicillin was inactivated more slowly than was benzylpenicillin by staphylococcal β-lactamase (11). Subsequent work was directed towards the preparation of bis-penicillins, particularly from vicinal dicarboxylic acids, on the supposition that the two penicillanic acid moieties might provide mutual steric protection against attack by the enzyme. Expectation was fully realized when it was shown that the penicillin prepared from quinoxaline-2,3-dicarboxylic anhydride was as active against highly penicillin-resistant strains of *S. aureus* as against normally sensitive strains. Further investigation showed, however, that this anhydride reacted with 6-aminopenicillanic acid to give a single product, a monopenicillin, with the formula shown in Figure 2.

Quinacillin has unusual properties and in many respects comes close to meeting the demands of Pollock (12) for improvements in β-lactamase-resistant penicillins. He required that the ideal penicillin should have the

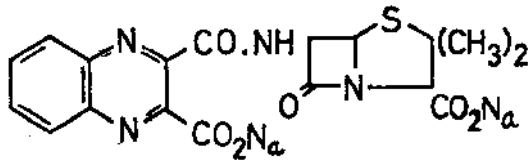

FIG. 2. Quinacillin.

antibiotic potency of benzylpenicillin, the insusceptibility to hydrolysis of cephalosporin C, an affinity for the enzyme as low as that of methicillin, and the property of not inducing β-lactamase formation or a substantially decreased inducing power. To the biochemist, the properties of quinacillin are certainly of interest. To the clinician, data are as yet inadequate to foretell what part it will play in the treatment of staphylococcal infection.

The antibacterial activity of quinacillin is low against all species tested so far with the exception of *S. aureus* and *Streptococcus faecalis*. Against the former, its activity was found to be of the same order as that of methicillin; against some strains of the latter, it was the most active of the resistant penicillins (2, 11, 13). As a substrate for staphylococcal β-lactamase, quinacillin was found to have a V_{max} 8 per cent that of benzylpenicillin at pH 7.4 (14)—5 per cent at pH 6.5 (11)—compared to a value of 0.18 for cephalosporin C and 1.6–3.3 for methicillin [see (12)]. However, its affinity for the enzyme is so low, K_m—61 m*M* at pH 7.4, 11 m*M* at pH 6.5 [K_m methicillin—28 m*M* (15)], that at 4.3 m*M* no appreciable hydrolysis was observed. The induction constant of quinacillin was found to be considerably higher than that for methicillin. Richards, Housley & Spooner (11) reported a 25-fold difference while Smith, Hamilton-Miller & Knox (14), using a different strain of *S. aureus*, observed an eightfold difference.

These data indicate, therefore, that the side-chain of quinacillin is responsible for a loss of affinity for β-lactamase, for the induction centre in staphylococci, and for the site responsible for antibacterial activity in most bacteria but not in staphylococci. Under the conditions of these experiments, however, the possibility existed that a change occurred in the structure of the molecule to produce a less reactive substance and that either this or the intact molecule was capable of inhibiting growth of staphylococci without producing the now well-known penicillin-induced biochemical lesion. On investigation, however, there was no evidence of any degradation (14), and at minimum inhibitory concentrations, 46.6 per cent of maximum intracellular accumulation of N-acetylamino sugars was observed compared to a figure of 59 per cent for benzylpenicillin (16). Hence, it appears that the substance itself is the active moiety and that it has a typical penicillin-like mode of action. The reasons for its lesser antibacterial activity against other species is as yet unknown.

Pollock's demands might be extended to include lack of cross-resistance

to clinically isolated methicillin-resistant strains of *S. aureus* and inhibition of the action of β-lactamase against penicillins sensitive to the action of the enzyme. Evidence on the first point is contradictory. Barber & Waterworth (2) have shown that against such strains, quinacillin was much less active than methicillin itself, while Smith, Hamilton-Miller & Knox (14) maintain that the organisms were slightly more susceptible to quinacillin than to methicillin. Obviously, experimental protocols differed in these experiments *in vitro*. Final proof would depend on the successful use of quinacillin in clinical situations where other penicillins have proved to be ineffective. Here, there is little to go on except for the statement by Hale et al. (17) that one patient with staphylococcal meningitis recovered dramatically following treatment with quinacillin, having already been treated unsuccessfully with cloxacillin, methicillin, and chloramphenicol. Evidence on inhibition of the action of β-lactamase is not encouraging. Smith, Hamilton-Miller & Knox (14) have shown that quinacillin does not inhibit the hydrolysis of benzylpenicillin by β-lactamase prepared from several gram-positive bacteria including *S. aureus*. β-Lactamases from gram-negative bacteria, with the exception of *Klebsiella aerogenes* also are not inhibited by quinacillin.

Little is known of the pharmacology of the substance except that, although highly acid-stable, it is poorly absorbed after oral administration. It is more highly bound to serum proteins than is methicillin but less so than other β-lactamase-resistant penicillins (11).

Dicloxacillin.—Dicloxacillin is the latest of the acid-stable, β-lactamase-resistant semisynthetic penicillins. It has a structure identical to that of cloxacillin except that a second chlorine atom has been added in the meta position relative to the first (Fig. 3).

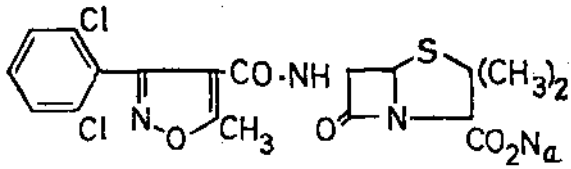

FIG. 3. Dicloxacillin.

Its stability at pH 2 is almost identical with that of phenoxymethylpenicillin and appreciably greater than the stability of either oxacillin or cloxacillin (18). Against staphylococci and other gram-positive bacteria, the activity of dicloxacillin is little different from that of the other isoxazolyl penicillins (18–21). There is, however, an important difference in its physiological disposition. After a single oral dose, dicloxacillin appears and persists in greater concentrations in the blood than after single doses of the other acid-stable β-lactamase-resistant penicillins (18–22).

A large proportion is, however, bound to serum proteins. At a concentration of 10 μg/ml, only 5 per cent is unbound in human serum, less at

lower concentrations. The degree of binding is, therefore, greater than that of oxacillin and cloxacillin (18). Hence, the benefit of high blood concentration which is probably caused at least in part by this factor, may be offset by the relatively small proportion of free material available for antibacterial activity and tissue penetration.

Penicillin Resistance

Fortunately for clinical practice, the all-pervading and often unnecessary use of penicillin has resulted in the development of resistance to a serious degree in only two organisms—the staphylococcus and the gonococcus. The development of the semisynthetic penicillins resistant to hydrolysis by β-lactamase produced by resistant *S. aureus* answered, for a time at least, problems posed by this versatile organism. Penicillin resistance in the gonococcus, however, raises different problems in that resistance involves an intrinsic relative insusceptibility to penicillin and not the development of an antibiotic-destroying enzyme. Hence, resistance to benzylpenicillin involves resistance to other penicillins and is similar to the resistance to methicillin observed in some strains of staphylococci (*vide infra*). Added to this are public health problems concerned with a rising incidence of gonorrhoea and the necessity for continuing success of "one-shot" curative chemotherapy.

Resistance in gonococci.—Various explanations have been forwarded for failure of "routine" doses to cure gonorrheal infections. Erroneous diagnosis, the presence of the morphologically similar *Mimea* spp. (23, 24), and the production of β-lactamase by concomitant staphylococcal infections (25, 26) have been invoked. However, evidence from *in vitro* sensitivity tests carried out on isolates from patients with inadequate response to treatment have shown that strains of *Neisseria gonorrhoeae* with sensitivity much reduced when compared with earlier isolates are now prevalent. In a recent survey (27), the *in vitro* sensitivity of gonococci isolated from treatment failures differed appreciably from that of gonococci isolated from those treated successfully. Treatment consisted of intramuscular injection of either 600,000 units procaine penicillin plus 600,000 units benzathine penicillin or one million units of procaine penicillin. The minimum inhibitory concentrations of benzylpenicillin against "sensitive" gonococci ranged from 0.01 to 0.3 units/ml with an average of 0.103 units/ml, while the "resistant" organisms were sensitive to 0.1 to 0.5 units/ml with an average of 0.26 units/ml. Similar changes in *in vitro* sensitivity have been noted elsewhere (28). Fortunately, these changes represent, as yet, fairly minor changes in susceptibility which can be catered for by increasing the dosage of penicillin. Nevertheless, the situation is uncomfortable and calls for frequent review of established procedures.

Methicillin resistance.—Staphylococcal resistance to the newer semisynthetic β-lactamase-resistant penicillins, usually termed methicillin resistance, is similar in character to the resistance of gonococci to benzylpeni-

cillin. It represents an intrinsic insusceptibility, albeit of a greater degree, to the group of penicillins which are more or less unaffected by the β-lactamase produced by the organisms. Such strains usually are resistant to streptomycin and tetracycline and sometimes to chloramphenicol and erythromycin, and belong to phage group III (29).

While the authors of several studies have concluded that inactivation by β-lactamase plays little or no part in resistance to methicillin, the "type" compound (30–33), others maintain it appears highly probable that β-lactamase activity contributes significantly to the resistance of naturally occurring methicillin-resistant strains (34, 35). The truth of the matter is obscure and subject to dispute. A possible explanation is that the growth of methicillin-resistant strains is not inhibited by "therapeutically available" concentrations by virtue of the intrinsic insensitivity of the organisms and that increased and, in some strains, very large concentrations of β-lactamase induced by the presence of the penicillin cause a subsequent decrease in concentration of the antibiotic. Nevertheless, some strains of methicillin-resistant staphylococci are unable to inactivate methicillin even after induction (35, 36, 37).

Since the first report of the occurrence of methicillin-resistant strains of *S. aureus,* three strains of a total of 5440 isolates (38), many such strains have been isolated [see (39, 40)]. Generally, their incidence is quite low. For example, 0.48 per cent of strains received at the British staphylococcal reference laboratory were found to be resistant to methicillin (41). However, within individual hospitals the incidence may be very much higher (40, 42). Most investigators have been unable to find any correlation between the use of β-lactamase-resistant penicillins and acquisition of resistance to them, hence such strains have been dubbed "naturally resistant." In one case, however, a fourfold decrease in susceptibility was observed after treatment of a patient with methicillin. In this case, a concomitant infection with a gram-negative bacillus possessing methicillin-inactivating properties may have contributed to a local reduction in the concentration of methicillin which permitted conditions suitable for development of resistance (43).

Examination of several strains of methicillin-resistant *S. aureus* has shown that even at subinhibitory concentrations of methicillin, growth on nutrient agar is much less luxuriant than on agar without antibiotic, and tends to be confined to the site of heavy inoculum (29). This is accounted for by the fact that all such strains consist of mixed populations in which the majority of cells are of normal sensitivity to methicillin with a minority showing methicillin resistance. In one such strain, it was found that 250 per million of the population grew at a concentration of 12.5 μg/ml, and that only 2.5 cells per million were capable of growing at 500 μg/ml of methicillin. However, despite this marked variation in sensitivity across the population, a much more uniform response to other antibiotics was found whether the strain was sensitive to them or not (39).

Because highly resistant organisms form such a small proportion of

the total population, and because these organisms have been found to grow much more slowly than normally sensitive cells, the usual disc method of sensitivity testing is unsuitable for determining whether or not a strain is resistant to methicillin. A serial dilution test using a heavy inoculum of the suspect organism and incubation for 48 hours has been recommended as being more reliable. Under these conditions, the growth of methicillin-sensitive staphylococci is inhibited by concentrations of 5 μg/ml or less while methicillin-resistant staphylococci are prevented from growing only by concentrations of 125 μg/ml or more (39).

Penicillin Hypersensitivity

Hypersensitivity to penicillin is considered to be the most common cause of allergic reactions produced by therapeutic agents today (44). Its incidence has been variously estimated, published figures ranging from 1 per cent to over 10 per cent. The differences probably reflect the composition of the populations studied. Little or nothing is known about factors predisposing individuals to sensitivity, but the risk seems to be less in patients receiving continuous prophylactic treatment than in those undergoing interrupted therapy (45). There is little danger of death resulting from the most acute manifestation of hypersensitivity, anaphylaxis, providing proper precautions are observed; nevertheless, irreversible residual brain damage caused by secondary anoxia is a hazard that makes prompt treatment mandatory (46). A previous history of reaction after the administration of a penicillin is good justification for the use of an alternative agent, but the lack of such a history is no basis for assuming that a subsequent course of penicillin treatment will prove uneventful. For this reason, and the fact that the penicillins are perhaps the most widely used of all antibacterial agents, it is of considerable importance to be able to determine, in advance of treatment, whether or not a patient harbours antibodies to these substances. It is unfortunate that hypersensitivity to any one of the penicillins manifests as hypersensitivity to any other homologue containing the 6-aminopenicillanic acid nucleus (45), although there are isolated reports of semisynthetic penicillins being used uneventfully in patients presenting histories of sensitivity to benzylpenicillin and showing positive results to intradermal and scratch test (47).

Much work has been carried out in recent years to devise tests which will tell the clinician whether or not a patient can be treated safely with a penicillin. Arising out of this work has been the finding that hypersensitivity to penicillin provides a "comparatively well-defined haptenic system for the study of fundamental immune mechanisms involved in human allergic diseases" (48). It is generally agreed that in penicillin hypersensitivity the antigenic determinant is not the intact molecule, but rather the penicilloyl group. The penicillin molecule as such is capable of reversible binding with proteins, but these complexes are thought to be incapable of inducing the hypersensitive state. Rather, it was thought that benzylpenicillin rearranges to form an isomer, D-benzylpenicillenic acid, which reacts

irreversibly with lysine ε-amino groups of proteins to form benzylpenicilloyl-amine haptenic groups. The intermediate substance, D-benzylpenicillenic acid, is also found as an impurity in dry crystalline benzylpenicillin. However, most recent evidence has shown that some semisynthetic penicillins form their corresponding penicillenic acids at very much reduced rates and yet give rise readily to penicilloyl-specific antibody in experimental animals. In addition, many penicillin hypersensitivities result from the presence of antibodies specific for D-α-penicilloyl rather than the mixture of diastereoisomers which would result from the intermediate formation of penicillenic acid. Therefore, it is thought currently that direct acylation of lysine ε-amino groups of protein by the intact penicillin can occur, and that both processes are accountable for the formation of antibody. The steps involved are shown diagrammatically in Figure 4. For a

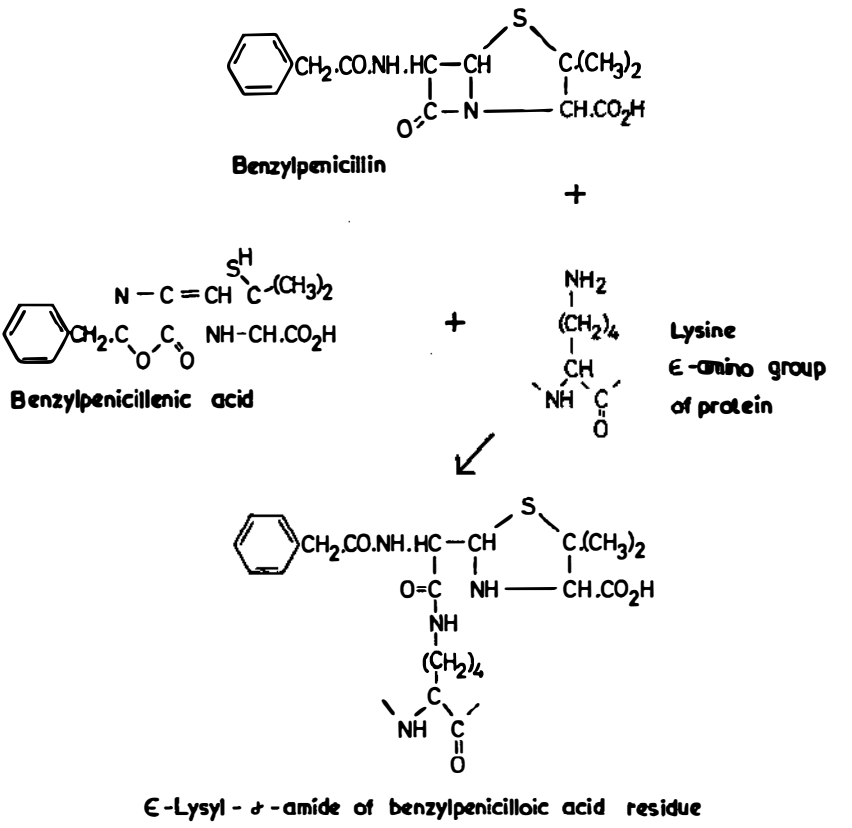

FIG. 4. Possible steps involved in the formation of benzylpenicilloyl-amide haptenic groups. [After Levine (48)].

more complete discussion of this subject see references (48, 49, 50). Several tests have been devised to assess a patient's sensitivity to penicillin and these may be classified as follows: skin-tests, serological tests, and tests of histamine release.

Skin testing.—Skin testing with solutions of crystalline benzylpenicillin is the oldest and the simplest of the techniques used. Unfortunately it has, by itself, little useful value. False positives and false negatives occur with sufficient frequency that little reliance can be placed upon the results. In addition to which, anaphylactic reactions, including at least one fatal reaction, have been reported after the intradermal injection of minute amounts of penicillin (51, 52). The same comments apply to conjunctival testing.

To be useful, a suitable preparation should enable a correct assessment to be made of an individual's state of sensitivity without itself sensitizing or producing a hypersensitivity reaction. Recent work based on knowledge of the scheme outlined above has resulted in the synthesis of penicilloyl-polylysine. It was hoped that this substance would overcome all the disadvantages inherent in the use of penicillin itself (53). A preparation containing, on average, 20 lysine groups per molecule, bearing 12 to 15 penicilloyl groups, has been found to be the most suitable (54).

Penicilloyl-polylysine is not sensitizing. Repeated skin testing has failed to produce antibody in experimental animals (55). In addition, there is no record of a reaction having occurred in a human being as a result of testing with this material. On the other hand, its effectiveness in screening patients for hypersensitivity is, at the moment, in question. Some patients, a very small proportion, may develop manifestations of hypersensitivity without reacting to penicilloyl-polylysine. Others, a larger proportion, may exhibit a strongly positive skin test and yet experience no clinical reaction following penicillin treatment. There is, indeed, some evidence to indicate that crystalline benzylpenicillin may be a more effective detector of the potential "immediate reactor" than penicilloyl-polylysine (56, 57). To detect such a reactor, it is recommended that the test be carried out with both reagents with the knowledge that a strongly positive reaction only increases the probability that an immediate reaction following penicillin treatment will occur. Based on what is known to date, a negative reaction to both materials would indicate that an immediate reaction following the administration of penicillin will not occur, but this view is based on only a relatively small number of cases. In addition, a negative reaction does not rule out the occurrence of a late urticarial or serum-sickness type of reaction (45, 48).

Serological testing.—It has been known for some time that red blood cells preincubated with benzylpenicillin could be agglutinated by some human sera (58). The antibodies detected by this technique are specific, mainly for the penicilloyl-amide structure described above, but only low titres have been obtained. The sensitivity of this reaction can be increased

considerably if the red cells are preincubated with benzylpenicillin at alkaline pH. These conditions accelerate the direct acylation of protein amino groups by the penicillin molecule.

Using this modified technique, titres of human antipenicilloyl antibodies as high as 1/20,000 have been observed. However, although hemagglutinating antibodies accompany allergic manifestations, no correlation has been found between the titre obtained and the level of hypersensitivity as estimated by skin reaction to penicilloyl-polylysine. The fact that high titres persist for only a few weeks after an allergic reaction also limits its usefulness. The test, therefore, may be useful to confirm a diagnosis of penicillin hypersensitivity but is not suitable for determining routinely whether or not a patient, lacking a history of previous reactions, will react to the administration of penicillin. For references to this and earlier work, see DeWeck (59).

Tests for histamine release.—Skin testing with either benzylpenicillin or penicilloyl-polylysine depends on observation of the effects resulting from release of histamine from the mast-cell. The release of histamine from other cells, basophils, has been studied by microscopic examination of the cells themselves. The assumption made is that during the reaction between antigen and antibody, the granules of living basophils release histamine and visible changes occur in the cells (60). Variations in this test are possible using either the patients own blood as a source of basophils or, if basophils are scarce in the sample, rabbit leukocytes. Benzylpenicillin has been used as the antigen. In general, a high proportion of positive results have been observed in patients with a history of reaction less than two years previously. If a larger period had ensued, results were more variable, and in the temporary anergic state following a severe reaction, results of the test were negative. The test may be criticized on the grounds that the results depend largely on the personal judgement of the technician observing subtle alterations in the cytology of the basophil. In addition to which, serum controls may contain several abnormal cells making interpretation of the test difficult. In a modified test (61), both serum control and serum penicillin sample are applied to the same slide, making it easier to compare the cytology of the control basophils with that of the test basophils.

A refinement of this technique, which removes sources of error caused by personal bias in the observer, has recently been described (62). In this, the amount of histamine released from a system containing benzylpenicillin and rabbit blood passively sensitized with fresh human serum is measured fluorometrically. The results obtained have been similar to those described above.

Cephalosporins

Research towards new derivatives of cephalosporin C is reminiscent of the early days of semisynthetic penicillins. To the first clinically useful derivative, cephalothin (63, 64), have been added cephaloridine (65) and

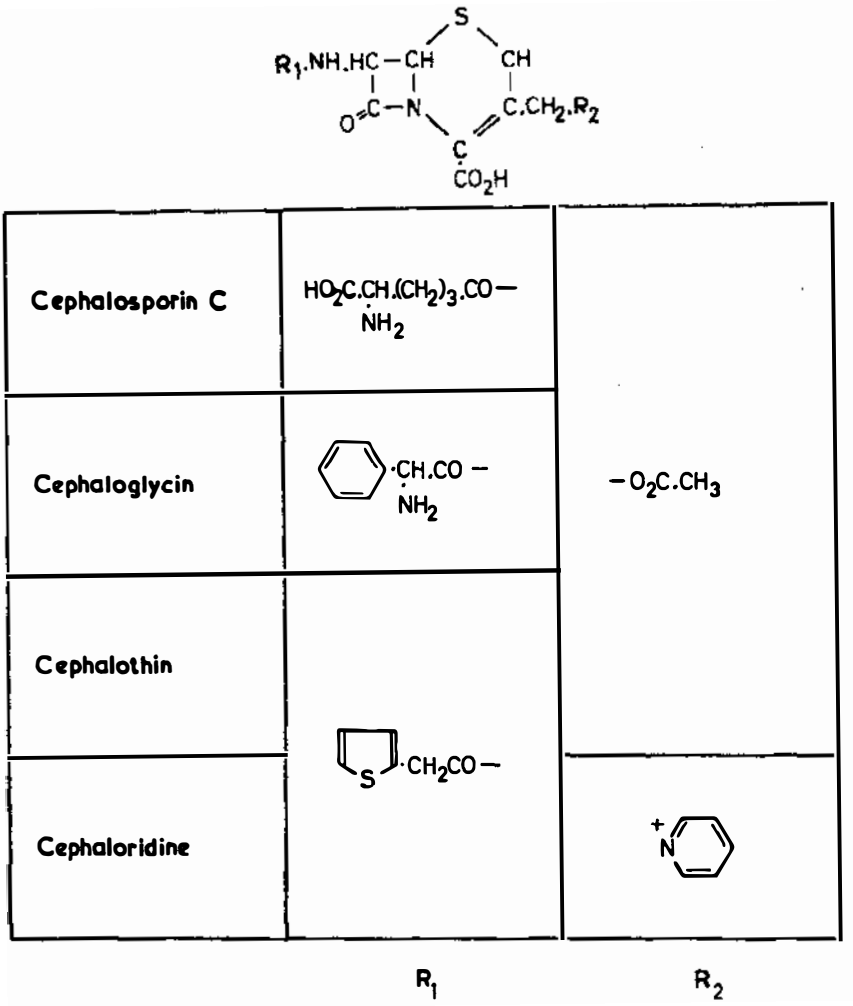

FIG. 5. Cephalosporins.

cephaloglycin (66). The structural formulae of these substances are shown in Figure 5. Other closely related substances described recently are cephaloram, the phenylacetamido derivative of cephalosporin C (67), and CAPH (3-pyridinomethyl-7-[4-(3-phenylhydantoin-5-yl)-*n*-butyramido]-Δ^3-cephen-4-carboxylic acid) (68). The literature on cephalothin has been reviewed recently (69, 70); further reports on its behaviour in the laboratory and its clinical usefulness confirm the earlier findings (2, 71, 72, 73).

A feature of the clinical reports is that cephalothin has been given with no untoward reactions to patients with either known or suspected hyper-

sensitivity to penicillin. In one patient, however, with known hypersensitivity, the first injection of cephalothin was followed immediately by an anaphylactoid reaction (74). It seems unlikely that this was a case of cross-sensitization because of dissimilarities between the structures of the two substances. The patient suffered from a chronic dermatophyte infection and it is probable that cephalosporins produced by the organisms were responsible for the hypersensitive state. Batchelor, Dewdney & Gazzard (50) have shown that cephalosporin C and its derivatives can readily acylate amino groups of proteins, and in several clinical studies, the occurrence of sensitivity reactions in patients treated with cephalothin has been noted. One may assume, therefore, that indiscriminate use of such derivatives will result in an increasing incidence of hypersensitivity reactions.

Quite apart from their value in treating patients hypersensitive to penicillins, definite clinical advantages would accrue the cephalosporins if they combined high activity against all strains of staphylococci with high activity against other gram-positive and gram-negative organisms.

Cephaloridine has been shown to possess high activity against a range of gram-positive organisms and to retain activity against β-lactamase-producing staphylococci. In a medium containing 95 per cent human serum, cephaloridine inhibited the growth of a large inoculum of β-lactamase-producing *S. aureus* at a concentration lower than was required for any of the other "resistant" penicillins and cephalosporins (2). It was also moderately active against naturally occurring methicillin-resistant strains of *S. aureus*.

In contrast to the "resistant" penicillins, none of which had significant activity against any of the coliform bacilli, both cephaloridine and cephalothin were shown to have activity similar to that of ampicillin against *Escherichia coli,* salmonellae and shigellae, and to be more active against some strains of *Proteus* and *Klebsiella* spp. The effectiveness of cephaloridine in treating some gram-negative infections has been demonstrated in preliminary clinical trials (75, 76). The suggestion has been made that because cephaloridine is nontoxic and is bactericidal against a wide range of organisms, its use should be seriously considered when a patient is obviously septicaemic, especially in the presence of renal failure, but before the organism can be identified.

Cephaloridine is not readily absorbed after oral administration. After intramuscular injection, high concentrations appear in the serum. Urinary recovery is good and high concentrations appear in the urine. The instability *in vivo* of the acetoxy group, induced by replacement of the 7-α-aminoadipic acid side-chain of cephalosporin C with various substituted acetic acids, is circumvented in cephaloridine by replacement of this group with pyridine (77).

Unlike the earlier derivatives of cephalosporin C, cephaloglycin is well absorbed after oral administration and, in mice at least, higher concentrations are found in the blood than after the oral administration of the same

dose of phenoxymethylpenicillin (66). Its activity against gram-positive bacteria is considerably less than that of cephaloridine, however, against sensitive gram-negative organisms their activities are comparable. Cephaloglycin is rather unstable, and after 12 hours incubation in broth solutions little biologically active material remains.

The spectrum of activity of CAPH (see above) is interesting when compared with the activity of other cephalosporins. Although it retains a fairly high degree of activity against gram-positive bacteria, it has lost all activity against gram-negative organisms. In this respect it resembles the β-lactamase-resistant penicillins.

NEW ANTIBACTERIAL AGENTS

Antibiotics

Lincomycin.—Lincomycin, first described in 1962 (78), is unrelated chemically to any other commercially available antibiotic (79). Its structure has not yet been determined, but its hydrochloride has an empirical formula $C_{18}H_{34}N_2O_6S.HCl.1/2\ H_2O$. Group analysis indicates 2 C-CH_3, 1 N-CH_3, and no acetyl or methoxy groups. It was isolated from a fermentation broth of a new actinomycete designated *Streptomyces lincolnensis* var. *lincolnensis* sp. n. Its antibacterial activity is directed against a very narrow range of organisms. Gram-positive cocci, with the exception of enterococci, are sensitive to low concentrations, whereas gram-negative bacteria are unaffected by concentrations up to several orders of magnitude higher (78, 80-86). Lincomycin's lack of activity towards the gram-negative cocci, which are usually susceptible to other "narrow-spectrum" antibiotics, such as the penicillins and erythromycin, is notable. Data on the *in vitro* activity of lincomycin and, for comparison, the activity of erythromycin are summarized in Table I. Lincomycin, like erythromycin, is bacteristatic at low concentrations. Bactericidal activity is observed only at very much higher concentrations.

Staphylococci, as might be expected, can develop resistance to lincomycin. In one study (81), multiple passage *in vitro* in the presence of lincomycin permitted a 14-fold increase in resistance after 15 transfers. In a parallel series, resistance of erythromycin increased 170-fold. There was no evidence that either antibiotic allowed resistance development of the "one-step" type. In another study (85), a several hundredfold increase in resistance to lincomycin after 32 transfers, with concomitant cross-resistance to erythromycin, occurred in one strain. Four others similarly treated showed little or no change in sensitivity to both substances. After exposure to erythromycin, some strains became cross-resistant to lincomycin, others showed little or no change in sensitivity although all became resistant to erythromycin. Using one of the strains showing this dissociated type of resistance (88), the activity of lincomycin was found to be markedly reduced in the presence of small concentrations of erythromycin.

In a clinical study (89), MacLeod et al. found a very low incidence of

TABLE I

ANTIBACTERIAL ACTIVITY *In Vitro* OF LINCOMYCIN (85) COMPARED WITH THAT OF ERYTHROMYCIN (87)

	Lincomycin	Erythromycin
Gram-positive:		
Staphylococcus aureus	0.5–2	0.01–1.6
Streptococcus pyogenes	0.06–0.12	0.02–0.2
Diplococcus pneumoniae	0.06–0.5	0.01–0.2
Streptococcus faecalis	4–16	0.6–3.1
Gram-negative:		
Neisseria gonorrhoeae	32	0.04–0.4
Neisseria meningitidis	>32	0.2–1.5
Haemophilus influenzae	4–16	0.4–3.1
Escherichia coli	>128	50–300
Shigella spp.	128–>128	100–200
Salmonella spp.	>128	100–200

Figures represent minimum inhibitory concentrations (μg/ml), obtained using an agar dilution technique.

lincomycin resistance in over a thousand isolates of *S. aureus*. However, Daikos et al. (82) observed 19 per cent of their strains to be resistant to more that 2 μg/ml. Their isolates showed a 30 per cent incidence of erythromycin-resistant strains. Daikos et al. associated several failures in lincomycin-treated staphylococcal urinary tract infection with development of resistance by the organisms. This appeared a short time after starting treatment, with no phase of initial improvement in the patients' condition. Others have noted the development of resistance by staphylococci in urinary tract infection (83).

Lincomycin is effective in preventing growth of L-forms of staphylococci at much lower concentrations than those necessary to inhibit the intact coccal forms (90). This suggests that its primary site of action is not in the cell wall. Indeed, this high degree of activity suggests its use in controlling the growth of such forms in human tissue if the hypothesis is correct that L-forms are of account in relapsing states of staphylococcal disease (91). This effect is consistant with earlier findings that high concentrations of lincomycin did not affect the incorporation of L-lysine into the cell wall fraction of logarithmically growing cultures of *S. aureus* (92). Subsequent experiments showed that protein synthesis was inhibited by lincomycin and that this occurred immediately on adding the antibiotic to the growing culture. Nucleic acid synthesis was unaffected for 15 to 30 minutes in the case of DNA and at least 60 minutes in the case of RNA, suggesting that lincomycin acts by inhibiting the activation, transfer, or polymerization of amino acids.

Lincomycin is rapidly but incompletely absorbed from the gastroin-

testinal tract. Absorption is primarily from the duodenum. Little or none is absorbed from the stomach (93). In human beings, an oral dose of 500 mg every six hours maintains serum concentrations between 2.4 and 3.9 μg/ml. Only 3 to 5 per cent of the administered dose is excreted in the urine within 24 hours. After intramuscular or intravenous administration, about one quarter to one half of the dose is recoverable in the urine in 24 hours (94). Other investigators, however, have obtained lower figures. Lincomycin is distributed throughout a space larger than that occupied by extracellular water and almost equivalent to that of total body water (95). Higher concentrations than those present in serum are found in bile, but little appears in the cerebrospinal fluid in the absence of meningeal inflammation. Lincomycin passes readily the placental barrier. In nursing mothers, the milk contains concentrations similar to those of the maternal peripheral blood (96). Lincomycin has been found in bone at concentrations of 1 to 2 μg/g after either oral or intramuscular administration (83).

Lincomycin has been used successfully in the treatment of a variety of infections caused by gram-positive bacteria (82, 83, 84, 97). Encouraging results have been obtained in the treatment of severe staphylococcal infections caused by organisms demonstrating patterns of multiple resistance, including resistance to erythromycin (89). Lincomycin has been used successfully in the treatment of staphylococcal osteomyelitis (98).

In laboratory animals, lincomycin has proved to be remarkably nontoxic even at high dosage (99). The clinical use of this antibiotic has been attended by pain at the site of intramuscular injection and, not uncommonly, diarrhoea during a course of treatment. No more serious side effect has been observed.

Gentamicin.—Gentamicin is a new antibiotic, the first clinically useful antibiotic produced by a species of *Micromonospora* of the actinomycetes group (*Micromonospora purpurea*). It is a combination of two isomeric pseudo-oligosaccharides and is related to the streptomycin-neomycin-kanamycin group of antibiotics (100–102). It is bactericidal against susceptible organisms at concentrations two to three times bacteristatic concentrations.

Gentamicin is of particular interest because of its activity against both gram-positive and gram-negative bacteria, the latter including proteus and pseudomonas (101, 103–110). Figure 6, taken from the most recent study (110), summarizes the results of minimum inhibitory concentration determinations made against organisms isolated from clinical cases. In addition, 13 strains of *Neisseria gonorrhoeae,* 23 *Haemophilus influenzae,* 2 *Pasteurella* sp., and 12 *Salmonella* sp. were sensitive to concentrations ranging from 0.8 to 6.3 μg/ml. Hence, gentamicin combines the activity of polymyxin and colimycin against pseudomonas with the activity of neomycin and related antibiotics against proteus and all strains of staphylococci. In addition, there is some evidence that gentamicin may potentiate the action of ampicillin against certain strains of *Proteus mirabilis* (105).

Resistance to gentamicin can be developed in the laboratory and occurs

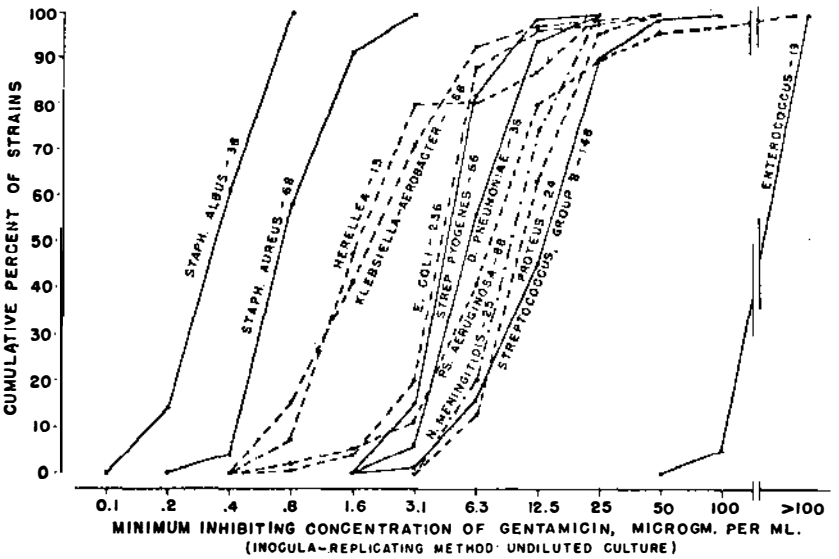

FIG. 6. Antibacterial spectrum of gentamicin. (Published with the permission of Dr. Klein and his co-workers and the *American Journal of Medical Sciences.*)

slowly in a step-wise fashion (101, 104, 110). Such resistant strains are cross-resistant to other members of the streptomycin-neomycin group of antibiotics. Clinically isolated strains which appear insensitive to this group, however, seem to retain sensitivity toward gentamicin (101, 103, 110).

There is little direct evidence on the mode of action of gentamicin. However, concentrations having no effect on coccal forms of staphylococci completely inhibit the growth of L-forms. It has been suggested, therefore, that its primary site of action is not in the cell wall (90).

Gentamicin is not well absorbed when given orally. After a single intramuscular administration of the recommended dose, 0.4 mg/kg, peak concentrations of about 2 μg/ml have been obtained in the serum within one half to one hour. This concentration decreased to about one third of a microgram per millilitre within four to six hours (111). Data on serum concentrations obtained after larger doses have also been reported (109–111). Twofold increase in dose results in an approximate doubling of peak concentrations.

Very much higher concentrations have been found in urine. Gentamicin/creatinine clearance ratios of one or nearly one have been found in dogs. Using large doses, 1.6 and 3.2 mg/kg, almost complete recovery of unchanged antibiotic has been obtained in 24 hours; however, at lower doses, recovery was slower initially, increasing in rate as drug administration was continued (109, 111). Only small amounts are cleared in the bile. The ac-

tivity of gentamicin *in vitro* is reduced in the presence of serum. Various estimates have been made of the degree of serum binding; all fall within the range of 25 to 30 per cent (101, 104, 110, 112).

Gentamicin has received clinical trial in gram-negative urinary tract infections, several of which had responded unsatisfactorily to other agents. Results of treatment were favourable in most cases considering the types and sites of infection, and were similar to those obtained with other appropriate antibiotics. Proteus infections, on the whole, responded less well than did infections caused by more susceptible organisms. Where an unsatisfactory response to treatment occurred, development of resistance by the offending organism was rarely the cause. In general, gram-negative bacteremia and nonurinary infections responded poorly to gentamicin in the doses used (105, 108–110, 113). There were, however, notable exceptions. Two septicaemias caused by pseudomonas in severely burned patients were controlled with gentamicin (106); pulmonary infections in ten patients responded to treatment with gentamicin and showed clinical and radiographic resolution of consolidation, disappearance of symptoms, and reduction in sputum (108); ventricular fluid was sterilized in an infant with pseudomonas meningitis after polymyxin had failed to elicit a response (110).

Toxic doses of gentamicin in laboratory animals have caused damage to renal and vestibular function. In patients with normal kidney function, slight increases in serum BUN were common during treatment. Transient elevation of SGOT has also been noted. In some patients with impaired renal function and hence decreased ability to excrete the drug, disturbance of vestibular function—in some cases irreversible loss—has occurred. Complete bilateral loss of labyrinthine function has been associated usually with high peak serum concentrations of gentamicin of the order of 12 to 13 μg/ml.

Naphthyridine Derivative

Nalidixic acid.—Nalidixic acid is a new synthetic antibacterial agent, the most active in a series of 1,8-naphthyridine derivatives (114). Its structure is shown in Figure 7.

This substance is bactericidal *in vitro* against a wide range of gram-negative organisms. Concentrations of 10 to 30 μg/ml inhibit the growth of

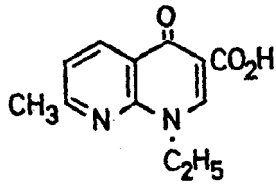

Fig. 7. Nalidixic acid.

80 per cent, or more, of strains of *E. coli, Aerobacter aerogenes, Proteus,* and *Paracolobactrum.* A few strains of *Pseudomonas aeruginosa* are sensitive to concentrations between 60 and 120 μg/ml (115). In a survey of 630 strains of gram-negative pathogens isolated from urine, the activity of nalidixic acid was surpassed only by kanamycin (116). Other studies have confirmed its effectiveness against clinically important gram-negative bacteria. Most gram-positive bacteria are sensitive only to high concentrations (117–121).

The development of resistance to nalidixic acid has been studied and the results are not encouraging. Single-step, eightfold increases in resistance have been observed in gram-negative bacilli. Twenty-four-fold increases occurred within four passages. Other strains have been found to develop resistance of the multiple-step type (117). The ready development of resistance by gram-negative bacilli after a few passages in drug-containing medium has been confirmed in other studies (115, 118). Fortunately, there appears to be no cross-resistance between nalidixic acid and other antibacterial agents (118).

Goss, Dietz & Cook have investigated the mode of action of nalidixic acid on *E. coli* (122, 123). It was found that the incorporation of precursors into DNA was inhibited, prior to loss of viability, by concentrations of nalidixic acid approximately the same as minimal bactericidal concentrations. Because inhibition occurred before there was a loss of viability and because the concentrations required for both events were approximately the same, it would appear that the effect observed was the primary effect. RNA synthesis continued in the presence of concentrations of the drug, which inhibited DNA synthesis, thus excluding the formation of the purine and pyrimidine bases and their conversion to the corresponding ribonucleotides as primary sites of action. This and other evidence strongly suggest that these deficiencies in nuclear synthesis in the presence of competent cytoplasmic synthesis result in imbalance of metabolism and death of the cell. It was also shown that cells exposed to nalidixic acid for as long as 75 minutes remained viable after the drug was removed, indicating that it was not firmly bound to the cell.

Nalidixic acid is rapidly absorbed from the gastrointestinal tract. About 80 per cent is recoverable from the urine as definitely characterized products (124). The conversion of the parent substance to conjugated and hydroxylated forms seems to begin almost immediately upon absorption. The degradation scheme is outlined in Figure 8.

Most of the material in the urine appears as the monoglucuronide of the parent substance, but a considerable fraction is excreted as the unconjugated 7-hydroxymethyl metabolite. Smaller amounts of the parent substance, the glucuronide of the hydroxymethyl metabolite and the 3,7-dicarboxylic acid, also appear in the urine. Interestingly, the hydroxymethyl metabolite has an *in vitro* spectrum of antibacterial activity very similar to that of nalidixic acid.

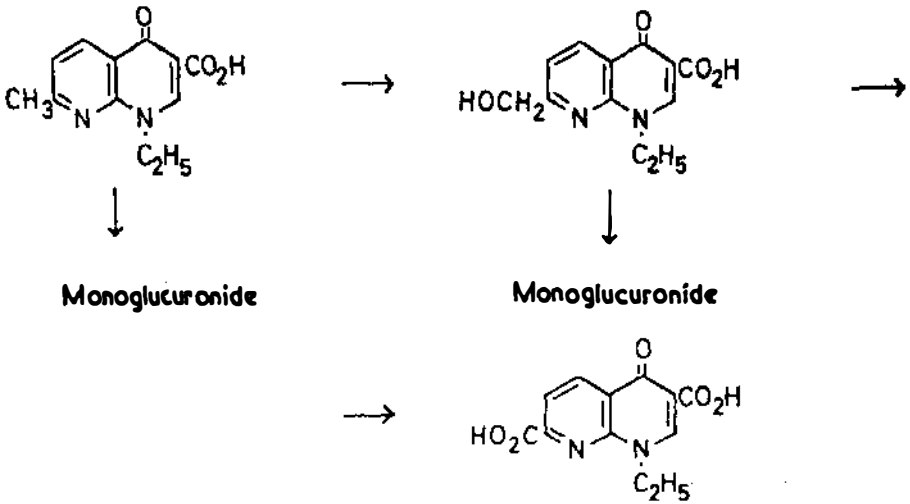

FIG. 8. *In vivo* degradation of nalidixic acid.

Concentrations found in the serum have been low in relation to the *in vitro* sensitivity of gram-negative bacteria. This will probably limit its routine use in systemic infections. Minimum inhibitory concentrations were increased 4- to 32-fold in the presence of 50 per cent serum, indicating a high degree of binding to serum proteins (117). Such binding was found, however, to be readily reversible (118).

Clinically, nalidixic acid has proved useful in the treatment of urinary tract infection (116, 117, 125–127). In many of the cases, other agents had been used unsuccessfully. However, relapses were not uncommon and, in some cases, resistant organisms were recovered after a period of therapy (128).

Toxicity of this compound at recommended doses seems to be minimal and limited to occasional gastrointestinal upset and a few dermatological reactions which disappear after completion of treatment or cessation of therapy (116, 125–128). One case of photosensitization has been reported (129).

LITERATURE CITED

1. Rosenman, S. B., and Warren, G. H., *Antimicrobial Agents Chemotherapy—1961*, 611–19 (1962)
2. Barber, M., and Waterworth, P. M., *Brit. Med. J.*, **II**, 344–49 (1964)
3. Klein, J. O., and Finland, M., *New Engl. J. Med.*, **269**, 1019–25 (1963)
4. Hopper, M. W., Yurchenco, J. A., Rosenman, S. B., Gillen, A. L., and Warren, G. H., *Antimicrobial Agents Chemotherapy—1963*, 305–10 (1964)
5. Martin, C. M., Kushnick, T. H., Nuccio, P. A., Gray, D. F., Bernstein, I., and Webb, N. C., Jr., *Antimicrobial Agents Chemotherapy—1963*, 290–98 (1964)
6. Martin, C. M., Nuccio, P. A., Gray, D. F., Bernstein, I., Webb, N. C., Jr., Rosenman, S. B., and Warren, G. H., *Antimicrobial*

Agents Chemotherapy—1963, 299–304 (1964)
7. Warren, G. H., *Brit. Med. J.*, **II**, 940–41 (1964)
8. Glassman, J. M., Warren, G. H., Rosenman, S. B., and Agersborg, H. P. K., *Toxicol. Appl. Pharmacol.*, **6**, 220–31 (1964)
9. Warren, G. H., and Gray, J., *Proc. Soc. Exptl. Biol. Med.*, **116**, 317–23 (1964)
10. Aldrich, K. M., and Sword, C. P., *J. Bacteriol.*, **87**, 690–95 (1964)
11. Richards, H. C., Housley, J. R., and Spooner, D. F., *Nature*, **199**, 354–56 (1963)
12. Pollock, M. R., in Resistance of bacteria to the penicillins, *Ciba Found. Study Group*, **13**, 56–70 (1962)
13. Spooner, D. F., and Spicer, A. B., *J. Gen. Microbiol.*, **33**, VII (1963)
14. Smith, J. T., Hamilton-Miller, J. M. T., and Knox, R., *Nature*, **203**, 1148–50 (1964)
15. Novick, R. P., *Biochem. J.*, **83**, 229–35 (1962)
16. Hugo, W. B., and Stretton, R. G., *Nature*, **202**, 1217 (1964)
17. Hale, L. J., Morton, E. V. B., Spooner, D. F., and Woolfe, G., *Brit. Med. J.*, **II**, 940 (1964)
18. Gloxhuber, Von C., Offe, H. A., Rauenbusch, E., Scholtan, W., and Schmid, J., *Arzneimittel-Forsch.*, **15**, 322–30 (1965)
19. Nauman, Von P., and Kempf, B., *Arzneimittel-Forsch.*, **15**, 139–45 (1965)
20. Knolt, Von T., Lange, A., and Volkening, R., *Arzneimittel-Forsch.*, **15**, 331–40 (1965)
21. Mossner, Von G., Mawer, H., and Meisel, C., *Arzneimittel-Forsch.*, **15**, 344–48 (1965)
22. Marget, Von W., *Arzneimittel-Forsch.*, **15**, 341–44 (1965)
23. Gangerosa, E. J., and Cary, S. G., *J. Am. Med. Assoc.*, **173**, 1808–10 (1960)
24. Svihus, R. H., Lucerd, E. M., Mikolajczy, K. R. J., and Carter, E. E., *J. Am. Med. Assoc.*, **177**, 121–24 (1961)
25. Sanders, A. C., Pelczar, M. J., and Hoefling, A. F., *Antibiot. Chemotherapy*, **12**, 10–16 (1962)
26. Gentele, H., Lagerholm, B., and Lodin, A., *Acta Dermato-Venereol.* **40**, 256–59 (1960)
27. Sokoloff, B., and Goldstein, H., *J. Am. Med. Assoc.*, **184**, 197–200 (1963)
28. Lanigan-O'Keefe, F. M., *Brit. J. Venereal Diseases*, **39**, 241 (1963)
29. Barber, M., *J. Gen. Microbiol.*, **35**, 183–90 (1964)
30. Knox, R., and Smith, J. T., *Brit. Med. J.*, **I**, 396 (1963)
31. Ayliffe, G. A. J., Barber, M., and Waterworth, P. M., *Brit. Med. J.*, **I**, 396 (1963)
32. Ayliffe, G. A. J., and Barber, M., *Brit. Med. J.*, **II**, 202–5 (1963)
33. Knox, R., and Smith, J. T., *Brit. Med. J.*, **II**, 205–7 (1963)
34. Eriksen, K. R., and Erichsen, I., *Acta Pathol. Microbiol. Scand.*, **62**, 255–75 (1964)
35. Eriksen, K. R., and Erichsen, I., *ibid.*, 399–408
36. Kjellander, J. O., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **113**, 1031–37 (1963)
37. Kjellander, J. O., Klein, J. O., and Finland, M., *Proc. Soc. Exptl. Biol. Med.*, **113**, 1023–31 (1963)
38. Jevons, M. P., *Brit. Med. J.*, **I**, 124–25 (1961)
39. Sutherland, R., and Rolinson, G. N., *J. Bacteriol.*, **87**, 887–99 (1964)
40. Colley, E. W., McNicol, M. W., and Bracken, P. M., *Lancet*, **I**, 595–97 (1965)
41. Jevons, M. P., Coe, A. W., and Parker, M. T., *Lancet*, **I**, 904–7 (1963)
42. Borowski, J., Kamienska, K., and Rutecka, I., *Brit. Med. J.*, **I**, 983 (1964)
43. Eriksen, K. R., *Acta Pathol. Microbiol. Scand.*, **61**, 154–55 (1964)
44. Feinberg, S. M., *J. Am. Med. Assoc.*, **178**, 815–18 (1961)
45. Van Arsdel, P. P., Jr., *J. Am. Med. Assoc.*, **191**, 238–39 (1965)
46. Cohen, S. B., *J. Am. Med. Assoc.*, **186**, 899–902 (1963)
47. Luton, E. F., *J. Am. Med. Assoc.*, **190**, 39–40 (1964)
48. Levine, B. B., *Federation Proc.*, **24**, 45–50 (1965)
49. Parker, C. W., *Federation Proc.*, **24**, 51–54 (1965)
50. Batchelor, F. R., Dewdney, J. M., and Gazzard, D., *Nature*, **206**, 362–64 (1965)
51. Tuft, L., *Am. J. Med. Sci.*, **230**, 370–79 (1955)
52. Idsoe, O., and Wary, P. N. *Bull. World Health Organ.*, **18**, 323–44 (1958)

53. Parker, C. W., DeWeck, A. L., Kern, M., and Eisen, H. N., *J. Exptl. Med.*, **115,** 803–19 (1962)
54. DeWeck, A. L., *Intern. Arch. Allergy,* **22,** 245–54 (1963)
55. Parker, C. W., Shapiro, J., Kern, M., and Eisen, H. N., *J. Exptl. Med.*, **115,** 821–38 (1962)
56. Levine, B., and Price, V. H., *Immunology,* **7,** 527–41 (1964)
57. Siegel, B. B., and Levine, B. B., *J. Allergy,* **35,** 488–98 (1964)
58. Ley, A. B., Harris, J. P. Brinkley, M., Liles, B., Jack, J. A., and Cahan, A., *Science,* **127,** 1118–19 (1958)
59. DeWeck, A. L., *Nature,* **202,** 975–77 (1964)
60. Shelley, W. B., *J. Am. Med. Assoc.,* **184,** 171–78 (1963)
61. Katz, H. I., Gill, K. A., Baxter, D. L., and Moschella, S. L., *J. Am. Med. Assoc.,* **188,** 351–54 (1964)
62. Shelley, W. B., and Comaish, J. S., *J. Am. Med. Assoc.,* **192,** 122–26 (1965)
63. Chauvette, R. R., Flynn, E. H., Jackson, B. G., Lavignino, E. R., Morin, R. B., Mueller, R. A., Pioch, R. P., Roeske, R. W., Ryan, C. W., Spencer, J. L., and Van Heyningen, E., *J. Am. Chem. Soc.,* **84,** 3401–2 (1962)
64. Boniece, W. S., Wick, W. E., Holmes, D. H., and Redman, C. E., *J. Bacteriol.,* **84,** 1292–96 (1962)
65. Muggleton, P. W., O'Callaghan, C. H., and Stevens, W. K., *Brit. Med. J.,* **II,** 1234–37 (1964)
66. Wick, W. E., and Boniece, W. C., *Appl. Microbiol.,* **13,** 248–53 (1965)
67. Jago, M., *Brit. J. Pharmacol.,* **22,** 22–23 (1964)
68. Frost, B. M., and Valiant, M. E., *J. Pathol. Bacteriol.,* **88,** 125–36 (1964)
69. Barber, M., and Chain, E. B., *Ann. Rev. Pharmacol.,* **4,** 115–38 (1964)
70. Burchall, J. J., Ferone, R., and Hitchings, G. H., *Ann. Rev. Pharmacol.,* **5,** 53–76 (1965)
71. Griffith, R. S., and Black, H. R., *J. Am. Med. Assoc.,* **189,** 823–28 (1964)
72. Weinstein, L., Kaplan, K., and Chang, T., *J. Am. Med. Assoc.,* **189,** 829–34 (1964)
73. Klein, J. O., Eickhoff, T. C., Tilles, J. G., and Finland, M., *Am. J. Med. Sci.,* **248,** 640–56 (1964)
74. Kabins, S. A., Eisenstein, B., and Cohen, S., *J. Am. Med. Assoc.,* **193,** 165–66 (1965)
75. Murdoch, J. M., Spiers, C. F., Geddes, A. M., and Wallace, E. T., *Brit. Med. J.,* **II,** 1238–40 (1964)
76. Stewart, G. T., and Holt, R. J., *Lancet,* **II,** 1305–9 (1964)
77. O'Callaghan, C. H., and Muggleton, P. W., *Biochem. J.,* **89,** 304–8 (1963)
78. Mason, D. J., Dietz, A., and Deboer, C., *Antimicrobial Agents Chemotherapy—1962,* 554–59 (1963)
79. Herr, R. R., and Bergy, M. E., *Antimicrobial Agents Chemotherapy—1962,* 560–64 (1963)
80. Hanka, L. J., Mason, D. J., Bioch, M. R., and Treick, R. W., *Antimicrobial Agents Chemotherapy—1962,* 565–69 (1963)
81. Lewis, C., Clapp, H. W., and Grady, J. E., *Antimicrobial Agents Chemotherapy—1962,* 570–82 (1963)
82. Daikos, G. K., Kontomichalou, P., Papadoyannakis, N., and Tourkantonis, A., *Antimicrobial Agents Chemotherapy—1963,* 197–99 (1964)
83. Holloway, W. J., Kahlbaugh, R. A., and Scott, E. G., *Antimicrobial Agents Chemotherapy—1963,* 200–3 (1964)
84. Harnecker, J., Contreras, J., Gilabert, B., and Ubilla, V., *Antimicrobial Agents Chemotherapy—1963,* 204–9 (1964)
85. Barber, M., and Waterworth, P. M., *Brit. Med. J.,* **II,** 603–6 (1964)
86. Clapper, W. E., Meade, G. H., and Stewart, D. B., *Am. J. Med. Sci.,* **247,** 274–77 (1964)
87. Haight, T. H., and Finland, M., *Proc. Soc. Exptl. Biol. Med.,* **81,** 175–83 (1962)
88. Garrod, L. P., *Brit. Med. J.,* **II,** 57–63 (1957)
89. Macleod, A. J., Ross, H. B., Ozere, R. L., Digout, G., and Van Rooyen, C. E., *Can. Med. Assoc. J.,* **91,** 1056–60 (1964)
90. Kagan, B. M., Zolla, S., Busser, R., and Liepnicks, S., *J. Bacteriol.,* **88,** 630–32 (1964)
91. Guze, L. B., and Kalmanson, G. M., *Science,* **143,** 1340–41 (1964)
92. Josten, J. J., and Allen, P. M., *Biochem. Biophys. Res. Commun.,* **14,** 241–44 (1964)

93. Meyer, C. E., and Lewis, C., *Antimicrobial Agents Chemotherapy—1963,* 169–75 (1964)
94. Vavra, J. J., Sokolski, W. T., and Lawson, J. B., *Antimicrobial Agents Chemotherapy—1963,* 176–82 (1964)
95. Ma, P., Lim, M., and Nodine, J. H., *Antimicrobial Agents Chemotherapy—1963,* 183–88 (1964)
96. Medina, A., Fiske, N., Hjelt-Harvey, I., Brown, C. D., and Prigot, A., *Antimicrobial Agents Chemotherapy—1963,* 189–96 (1964)
97. Walters, E. W., Romansky, M. J., and Johnson, A. C., *Antimicrobial Agents Chemotherapy—1963,* 210–15 (1964)
98. Geddes, A. M., Sleet, R. A., and Murdoch, J. M., *Brit. Med. J.,* **II, 670–72 (1964)**
99. Gray, J. E., Purmalis, A., and Feenstra, E. S., *Toxicol. Appl. Pharmacol.,* **6,** 476–96 (1964)
100. Weinstein, M. J., Luedemann, G. M., Oden, E. M., Wagman, G. H., Rosselet, J. P., Marquez, J. A., Coniglio, C. T., Charney, W., Herzog, H. L., and Black, J., *J. Med. Chem.,* **6,** 463-64 (1963)
101. Weinstein, M. J., Luedemann, G. M., Oden, E. M., and Wagman, G. H., *Antimicrobial Agents Chemotherapy—1963,* 1–7 (1964)
102. Rosselet, J. P., Marquez, J., Meseck, E., Murawski, A., Hamdan, A., Joyner, C. I., Schmidt, R., Migliore, D., and Herzog, H. L., *Antimicrobial Agents Chemotherapy—1963,* 14–16 (1964)
103. White, A., *Antimicrobial Agents Chemotherapy—1963,* 17–19 (1964)
104. Rubenis, M., Kozij, V. M., and Jackson, G. G., *Antimicrobial Agents Chemotherapy—1963,* 153–56 (1964)
105. Sweedler, D. R., Gravenhemper, C. F., Bulger, R. J., Brodie, J. L., and Kirby, W. M. M., *Antimicrobial Agents Chemotherapy—1963,* 157–60 (1964)
106. Graber, C. D., Stone, H. H., Kolb, L., and Martin, J. D., Jr., *Antimicrobial Agents Chemotherapy—1963,* 161–63 (1964)
107. Rabinovitch, S., Snyder, I. S., and Smith, I. M., *Antimicrobial Agents Chemotherapy—1963,* 164–68 (1964)
108. Brayton, R. G., and Louria, D. B., *Arch. Internal Med.,* **114,** 205–12 (1964)
109. Jao, R. L., and Jackson, G. G., *J. Am. Med. Assoc.,* **189,** 817–22 (1964)
110. Klein, J. O., Eickhoff, T. C., and Finland, M., *Am. J. Med. Sci.,* **248,** 528–43 (1964)
111. Black, J., Calesnick, B., Williams, D., and Weinstein, M. J., *Antimicrobial Agents Chemotherapy—1963,* 138–47 (1964)
112. Bulger, R. J., Sidell, S., and Kirby, W. M. M., *Ann. Internal Med.,* **59,** 593–604 (1963)
113. Jao, R. L., and Jackson, G. G., *Antimicrobial Agents Chemotherapy—1963,* 148–52 (1964)
114. Lesher, G. Y., Froelich, E. J., Gruett, M. D., and Bailey, J. H., *J. Med. Pharm. Chem.,* **5,** 1063–65 (1962)
115. Gandelman, A. L., and Mann, P. H., *Current Therap. Res.,* **6,** 424–30 (1964)
116. Reese, L., *Can. Med. Assoc. J.,* **92,** 394–97 (1965)
117. Buchbinder, M., Webb, J. C., Anderson, La V., and McCabe, W. R., *Antimicrobial Agents Chemotherapy—1962,* 308–17 (1963)
118. Deitz, W. H., Bailey, J. H., and Froelich, E. J., *Antimicrobial Agents Chemotherapy—1963,* 583–87 (1964)
119. Lishman, I. V., and Swinney, J., *Brit. J. Urol.,* **35,** 116–21 (1963)
120. Slade, D. A., *Brit. J. Urol.,* **35,** 125–28 (1963)
121. Carroll, G., *J. Urol.,* **90,** 476–78 (1963)
122. Goss, W. A., Deitz, W. H., and Cook, T. M., *J. Bacteriol.,* **88,** 1112–18 (1964)
123. Goss, W. A., Dietz, W. H., and Cook, T. M., *J. Bacteriol.,* **89,** 1068–74 (1965)
124. McChesney, E. W., Froelich, E. J., Lesher, G. Y., Crain, A. V. R., and Rosi, D., *Toxicol. Appl. Pharmacol.,* **6,** 292–309 (1964)
125. Swinney, J., *Practitioner,* **192,** 701–6 (1964)
126. Harper, J. M., and Howse, R. M., *Current Therap. Res.,* **6,** 265–70 (1964)
127. Holland, J. T., and Montgomerie, J. Z., *New Zealand J. Med.,* **63,** 498–501 (1964)
128. Sleet, R. A., Gray, W., Calder, M., and Murdoch, J. M., *Chemotherapia,* **8,** 137–41 (1964)
129. Zelickson, A. S., *J. Am. Med. Assoc.,* **190,** 556–57 (1964)

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