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Review Article

ANTIBIOTICS 1956—1961

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INTRODUCTION

ANTIBIOTIC is a young word in terms of the history of our language. It stems from antibiosis, a term coined in 1889 by Vuillemin (1) to describe a broad philosophic concept of a biologic relationship often observed but not previously specifically named. The term existed in the scientific vocabulary only a little more than 50 years before it was applied in the health professions in its present very restricted sense, and less than a decade later became a household word known to most well-informed laymen.

Rational application of the phenomenon of antibiosis for treatment of diseases according to scientific principles is a development of the last 20 years, but crude antibiotic therapy was practiced empirically at least 2,500 years ago and there was a prolonged flurry of laboratory experiments and less well-controlled clinical experimentation which started more than 80 years ago with the work of Pasteur and his students on anthrax (2) and was pursued enthusiastically, if not always carefully, by others in a wide array of infectious diseases for nearly 40 years. An antibiotic product, pyocyanase, derived from *Pseudomonas pyocyanea* (*aeruginosa*) was marketed in Europe before World War I. These early historical developments have been reviewed (3, 4).

The current era of antibiotic therapy began in 1929 with the now celebrated observation of Fleming and his coining the term "*penicillin*" to designate the then unidentified antibacterial metabolic product of the mold (5). This was followed by a lag period of a little more than a decade before the logarithmic period in which we now are emerged.

The antibiotics discussed below have been selected from those discovered or first marketed since 1955. Thus, the subjects reviewed represent some of the developments of approximately the most recent quarter of the active period of the current era of antibiotic therapy.

The discussion of specific antibiotics is preceded by some general comments on topics of current interest and import in antibiotic therapy. In general, these are topics the significance of which has been widely recognized only since 1955, although a few voices in the wilderness were warning about some of them prior to that time.

GENERAL CONSIDERATIONS

Blood Levels.—It is convenient and it has become conventional to compare different antibiotics or different formulations of a given antibiotic in terms of the concentration of the drug demonstrable in the serum at different times after a single oral dose or during a course of multiple doses. Unfortunately, there is a natural tendency to assume that such comparisons afford a valid basis for predicting therapeutic efficacy of the products, and much of the advertising of antibiotic products has taken advantage of this tendency. While comparison of blood levels produced may lead to valid conclusions regarding the relative therapeutic efficacy of different formulations of the same antibiotic, it is obvious that it is not equally justifiable to use such figures in the same way for evaluating different antibiotics. The data may provide useful physiologic and pharmacologic information about the drugs, e.g., absorption, resorption, excretion, metabolic degradation, etc., but they do not necessarily indicate anything about relative therapeutic efficacy.

First, there is the matter of bacterial sensitivity. A low concentration of a drug to which the infecting pathogen is very sensitive may be expected to be more effective than a considerably higher concentration of one to which it is only moderately sensitive.

Second, bacteria are more common in other tissues than in blood. Therefore, since the important point for therapeutics is the activity of the drug against the specific organism and the concentration of the drug at the site of infection where the organisms are, the blood level *per se* has little real significance in evaluation of therapeutic potential unless the gradient between the blood and the different tissues is known so that the concentration of the drug at the actual sites of infection can be calculated. Indeed, a study of "distribution volumes" of several antibiotics, including penicillins G and V; the tetracyclines; the macrolides, erythromycin and oleandomycin; streptomycin; and novobiocin, indicated that "high blood levels result in a low distribution volume, and vice versa" (6).

Probably more useful than serum concentration for evaluating potential therapeutic value of an antibiotic is the serum dilution factor, i.e., the extent to which the serum can be diluted and still remain bactericidal or bacteriostatic. Although this factor does not indicate anything about the concentration of the drug at the site of infection, it does at least take cognizance of the sensitivity of the organism to the specific antibiotic under investigation.

Enhancement of Blood and/or Lymph Levels.—Following the general line of fallacious reasoning referred to above, there has been considerable emphasis on preparations

formulated with agents reputed to provide higher blood levels of the antibiotic than can normally be achieved with equivalent doses of similar preparations lacking the adjuvant. The emphasis has been greatest in the tetracycline field where, it has been stated, "economic competition among pharmaceutical manufacturers for markets and over-zealous advertising agencies have led to some extravagant claims regarding enhancement of blood levels attainable with various formulations of tetracyclines" (7).

Careful experimentation and objectively critical appraisal of published results indicate that many of the claims of enhanced blood levels achieved with tetracyclines formulated with glucosamine, sodium hexametaphosphate, and other "potentiating" agents cannot be statistically supported and that for all practical purposes the various formulations are comparable in clinical efficacy whether or not they contain the alleged blood-level-enhancing factors. Indeed, most of the work reported prior to October 1957 on enhancement can be ignored as erroneous and irrelevant because results produced with the new formulations were compared to those produced with the then regularly available commercial formulations which contained calcium and/or magnesium salts that chelate tetracyclines, thus reducing their availability for absorption. Therefore, most of the results before late 1957 showing improved blood levels with the new formulations were due more to the depressing action of additives in the control products than to enhancing agents in the experimental ones. Moreover, many reports were based on studies employing single oral doses given to fasting, normal, healthy volunteers, an experimental situation bearing little resemblance to actual clinical practice with patients who receive multiple doses at different times without regard to time elapsed since the last meal. Subsequent work has failed to reveal significant, if any, advantages of the "enhanced" products under practical clinical conditions. For further discussion of the controversy over blood levels of tetracyclines and other antibiotic preparations, see reference 7 (pp. 62 and 214) and references 8–15.

Related to the blood-level *vs.* tissue-level controversy and the emphasis on agents to enhance blood levels is the matter of antibiotic concentrations in the lymph. The importance of the lymphatic system in bacterial invasion suggests the desirability of supporting this protective mechanism by providing significant concentrations of appropriate antibiotics in the lymph during bacterial attack. Macromolecular salts of various antibiotic bases with appropriate polysaccharides or certain polycarboxyl acids are stated to have increased affinity for the lymph system (16). The products have been designated antibiolymphins. In comparison with the corresponding streptomycin and neomycin sulfates, streptolymphin and neolymphin produce lower but more sustained blood levels and much higher and more prolonged lymph levels. Acute toxicity (mouse) is said to be less for "lymphins" than for the corresponding sulfates. The products have been prepared and tested in Czechoslovakia and are not available commercially in the United States.

Antibiotic Prophylaxis.—Apart from the now well-substantiated prophylactic use of benzathine penicillin in cases of known or suspected past history of rheumatic fever, there are few, if any, well-documented indications for antibiotic prophylaxis. Indeed, there is a mounting body of evidence that useless prophylaxis may not only contribute to gradual diminution of the therapeutic efficacy of antibiotics by sensitizing the patient and engendering emergence of resistant strains of bacteria, but may actually delay recovery (17, 18), predispose the patient to virus infections (17, 19), and encourage implantation in the respiratory and urinary tracts of organisms ordinarily uncommon in those regions except as transients or parasites (20).

The high incidence of respiratory disease and resultant loss of time from work or other regular activities has been a major factor in the (unfortunately) common practice of using antibiotics (mainly penicillin and tetracyclines) prophylactically in treating upper respiratory infections. The earliest study of mass prophylaxis (with penicillin) involving 1,486 subjects in the experimental group and 1,451 control subjects for a total of 9,264 patient-months disclosed no significant difference in incidence of respiratory or of nonrespiratory illness in the two groups (21). The argument that antibiotics are given in treating the common cold in pediatric patients to prevent secondary complications has been completely demolished with the demonstration in a series of 217 children that the incidence of secondary complications is the same (about 15%) whether or not antibiotics are given, that complications in untreated patients generally are of bacterial etiology and can be treated effectively with antibiotics if and when they arise while complications in subjects treated prophylactically often are viral and are not amenable to antibiotics, and that the average recovery time for uncom-

plicated cases was 5.8 days for the controls, 7.2 days for the penicillin group, and 8 days for patients treated with one of the tetracyclines (17). Specific treatment of complications at their inception is preferable to prophylaxis at the beginning of fever. Pure cultures of colon bacilli have been found in the throats of children given penicillin prophylactically for the common cold (22). Instances of routine antibiotic prophylaxis appearing to increase rather than decrease infection are numerous (17–26). A sharp increase in death rate (from 29 per 1,000 in 1954 to 144 per 1,000 in 1958) among infants born after premature rupture of fetal membranes has been correlated with prophylactic use of antibiotics (27). The subject of prophylaxis in its various ramifications, including indications and contraindications in various aspects of surgery (26, 28–31) where it was concluded in a survey of many top-ranking hospitals by the Medical Audit Program (32) that in a series of 1,536 patients there was a 74% needless misuse of antibiotics in inguinal herniorrhaphies, has been reviewed (ref. 7, p. 13).

Mixed Therapy.—*Combinations of Antibiotics.*—There are very few clearly substantiated indications for mixed therapy, despite the prevalence of this form of treatment. Outstanding examples that have been well established empirically are concurrent administration of penicillin and streptomycin in some cases of subacute bacterial endocarditis, and joint use of streptomycin and PAS and isoniazid in tuberculosis. In brucellosis, streptomycin or tetracycline given with an appropriate sulfonamide usually produces better clinical results than any one of the agents alone. But apart from these few instances, it is extremely difficult to find objectively critical reports to support administration of many of the antibiotic combinations that have been proposed for general use in other infections. Jones and Finland (33–40) in neither their own laboratory and clinical observations nor in thorough review of the literature could find experimentally demonstrable evidence to support mixed therapy as a general practice. As pointed out in the "New Drugs Development" volume of the 1960 edition of the U.S.D., "their conclusions lend support to the editorial view (41) that 'most often the only justification for including one or another of the components is the fact that it is being produced by a particular manufacturer or that pressure is brought to bear on the management by the sales force to provide some combination to meet effective competition.' "

Except in the three disease entities mentioned above, administration, in adequate dosage, of the more active member of any combination usually produces therapeutic results equal or superior to those produced by the combination. Carefully controlled studies have failed to confirm the subjective claim, vigorously promoted in some advertising, that simultaneous use of two or more antibiotics suppresses emergence of resistant strains of organisms from initially sensitive ones. While it is possible that in some instances emergence of such strains may be slightly delayed, careful studies have shown that it is not completely suppressed and that after repeated exposure to the mixtures, organisms become resistant to both components just as if they had been exposed to the single entities (36, 38, 42). For a possible exception to the general rule about joint use of two antibiotics, see the discussion of novobiocin (below).

Antibiotic-Corticosteroid Mixtures.—Simultaneous use of antibiotics and of corticosteroids, whether for treatment of a single disease entity or of different involvements, is a highly controversial matter. Only a few years ago, a general dictum was that when antibiotics are being employed, corticosteroid hormones are contraindicated. However, successful use of cortisone, to provide subjective improvement, during chloramphenicol treatment of typhoid and typhus fevers was demonstrated in 1954, although the numerous pitfalls, including the false sense of security that the dramatic subjective improvement of the patient may give the physician, were pointed out (43). In general, systemic use of antibiotic-steroid combinations or of the separate entities simultaneously is seldom warranted, and it should usually be looked upon with disfavor. A critical appraisal of the rationale and physiologic complications and of the rare indications and numerous contraindications for such use has been published (44).

While recognizing the inherent danger of systemic antibiotic-corticoid therapy, a number of investigators have recommended such mixtures for local use. Representative proposals are use of antibiotic-steroid mixtures in nebulized sprays for treatment of laryngeal conditions (45), and in ointments for cutaneous infections (46, 47), especially when pyogenic (48). Although the corticosteroids may alleviate irritation and inflammation and may thereby produce a subjective feeling of improvement on the part of the patient and, it is sometimes contended, accelerate healing processes, their use in any infection is potentially hazardous. The inflammatory process is an important first line of defense to localize infection. If this natural process is eliminated or

minimized before the antibiotic takes effect, the possibility of rapid spread of the infection, which may become systemic, is increased. Moreover, there is the possibility that some of the steroid may be absorbed and induce systemic effects of its own, and care must be exercised in selection of the appropriate compound. It has been reported that hydrocortisone decreases resistance to infection and depresses immune responses, while corticosterone, in the same dosage, does not (49).

Antibiotics and Gamma Globulin.—In another category of mixed therapy, joint use of gamma globulin and antibiotics, particularly chloramphenicol, has been reported to produce better response than antibiotics alone (50, 51).

The pros and cons of mixed antibiotic therapy have been reviewed and editorialized extensively (52–59).

Antibiotics as Growth Stimulants.—Certain antibiotics were shown as early as 1946 to have a pronounced growth-promoting effect on the young of some animals (60), and today fortification of feeds, especially for poultry and swine, is routine in animal husbandry and poultry raising. The subject of antibiotics in animal nutrition has been reviewed thoroughly (61).

Following demonstration of the beneficial effect of antibiotic-supplemented feeds on growth of young animals, it was natural that similar studies should be conducted with humans. Chlortetracycline, used in small amounts as a diet-supplement, has been reported to stimulate growth of grossly undernourished children (62), and tetracycline used similarly has been reported to produce marked improvement in growth of dystrophic infants (63).

ANTIFUNGAL ANTIBIOTICS

Amphotericin B

Trade Name: Fungizone (Squibb).
Biologic Origin: Streptomyces sp. (M-4575 in Squibb culture collection) isolated from soil collected in Orinoco River region of Venezuela.
First Reported: 1956 (64).
Clinical Use: antifungal in systemic and superficial mycoses; also to reduce or prevent yeast or monilial overgrowth during tetracycline therapy.
Usual Dose; Intravenous Infusion: 1 mg./Kg./day, the daily dose being dissolved in 1 L. of 5% glucose and infused over a minimum time of 6 hours. This route is more reliable than the oral route. *Oral:* 2 Gm./day for adults has been effective for coccidioidomycosis and other disseminated fungus diseases. Doses up to 5 Gm./day have been used. Daily dose is divided when drug is given orally.
Toxicity: Appears to be low, but nausea, diarrhea, anorexia, and mild reversible azotemia have been reported.

Chemistry.—Amphotericin consists of two closely related compounds, amphotericins A and B. The complete molecular structures are unknown, but the empirical formula, $C_{46}H_{73}NO_{20}$, has been determined for the B fraction. The chemical literature has been reviewed (65, 66).

Amphotericin B, which is several times more active than the A fraction against fungi *in vivo* (67) and is the form that is regularly available, is a lactone-containing polyene structure (68), a type of configuration that has particular interest because of its presence in a number of antibiotics that exhibit antimycotic activity. The aminodesoxyhexose, mycosamine, which occurs among the degradation products of nystatin, is also an important moiety of amphotericin B. Chemical evidence suggests varying degrees of relationship between amphotericin and nystatin and some of the less well-known antifungal antibiotics, e.g., candicidin, filipin, fungichromin, pimaricin, rimocidin, and trichomycin.

The amphotericins are soluble in lower alcohols and in water-saturated butanol, but are less soluble in anhydrous butanol and in water (69). Both amphotericins are stable at moderate temperatures when dry and protected from light and air. In solution, stability is closely related to temperature and pH, maximum stability being in the neutral range.

The biologic activity of amphotericins is markedly affected by pH. The MIC of amphotericin B against several test organisms decreases from about 2.5 mcg./ml. at pH 4.5 to about 0.02 mcg./ml. at pH 8.0 (70), but there is a plateau of more or less uniform activity between pH 6.0 and 7.5. Therefore, assays are generally performed in media adjusted to a value within that range. Various methods for assaying amphotericins biologically, using *Saccharomyces cerevisiae* and *Candida albicans* as test organisms, have been developed (64, 70–72), including techniques for specifically determining amphotericin B activity in serum (70) and other body fluids (72) and in mixtures of the A and B fractions (72).

Absorption and Excretion.—Although amphotericin is poorly absorbed from the gastrointestinal tract, concentrations several times those required to inhibit several species of pathogenic fungi *in vitro* can be achieved in the blood following oral administration (71). The drug disappears from the blood slowly, and concentrations from 30 to 80% of the maximum achieved may still be present 12 or more hours after the last dose. However, continued dosage does not lead to significantly increased concentration. Once a daily dose of 2 Gm. has been reached, very little

increase in blood level is achieved by increasing the daily intake.

Higher blood levels are produced by intravenous infusion than by oral administration, and even 20 hours after infusion blood levels above the maximum achieved by oral administration may be expected. Significant amounts of the drug are absorbed from the plasma by the red cells (73) and small amounts appear in the spinal fluid after infusion of 0.37 to 1.0 Gm. daily.

Absorption of intramuscular doses is insignificant (70).

Total 24-hour urinary output of amphotericin B usually ranges from about 100 to 300 mcg. when patients are given a 5-Gm. oral dose daily; slightly larger amounts being eliminated if a mixture of the A and B components is given. On an intravenous infusion regimen, about 5% of the total daily dose appears in the total 24-hour urine. However, excretion continues for several days after the last dose is administered, and as much as 40% of the total dose may ultimately be recovered. Blood levels up to 20% of the maximum achieved during therapy may still be present a week after treatment is discontinued.

Clinical Uses.—The most significant use of amphotericin is in treatment of the systemic mycoses, prognosis of which, until the advent of this drug, was usually poor and always entailed, at best, a long period of illness and of convalescence. Among such infections successfully treated with amphotericin are cryptococcosis (74–76), histoplasmosis (76, 78, 79), blastomycosis (76, 77), coccidioidomycosis (76, 80–84), *Candida albicans* peritonitis (85), and American leishmaniasis (86). See also the proceedings of the New York Academy of Science's "Second Conference on Medical Mycology" (86a).

The duration of infection before amphotericin was started has ranged in a number of cases from 1 month to 10 years (74, 80). Among six cases of cryptococcal meningitis clinically rated "poor," "critical," "paraplegic," or "moribund" at the start of treatment with 50 to 100 mg., intravenously infused in 1 L. of 5% glucose over a period of 6 hours daily for total doses of 1.4 to 3.7 Gm., 5 survived (74) and, of these, 3 were considered "normal" on the basis of clinical criteria and spinal fluid findings, 1 was considered improved, and the paraplegic case was rated "improved" in terms of spinal fluid findings, although "unchanged" in clinical status. Other investigators have administered oral doses (4 to 5 Gm./day) in treating cryptococcosis, histoplasmosis, blastomycosis, and coccidioidomycosis. A number of patients who failed to respond satisfactorily to oral therapy have been rated "apparent recovery" or "objectively improved" after intravenous therapy, and, in general the intravenous route is preferred because of its greater reliability.

In all cases, treatment is a prolonged affair, ranging in the instances cited from about 1 to 14 months. Unequivocal evaluation of antimycotic agents for systemic infections is a slow process because of the long follow-up periods necessary to establish cure indisputably rather than mere arrest. Results of studies of experimental infections in animals have suggested that in nontoxic doses amphotericin is fungistatic rather than fungicidal (87, 88). However, there is much interspecies variation in sensitivity to drugs and, pending more information, it cannot justifiably be assumed that the same conclusion applies to treatment of human infections.

Amphotericin B, formulated in an ointment, has been considered curative in infants with cutaneous candidiasis in the diaper area and in guinea pigs experimentally infected with *Trichophyton mentagrophytes* (67).

Given orally, the antiobiotic has been reported effective in checking and depressing tetracycline-induced overgrowth by *Candida albicans* in the gastrointestinal tract (89–94). A tetracycline-amphotericin ratio of 5:1 has been recommended (92). Mysteclin F (Squibb) is a preparation incorporating the two antibiotics in a single preparation in this ratio. Despite the reduction induced in the fecal load of *Candida*, amphotericin seems to have little or no effect on the number of *Candida* organisms recoverable from throat swabs and sputum (90).

Unessential use of any antibiotic is undesirable for many reasons, several of which were mentioned above in the discussion of prophylaxis and of mixed therapy. Therefore, since not all patients receiving tetracycline develop candidial or monilial overgrowth, prophylactic use of amphotericin during tetracycline thrapy should be limited to classes of patients experience has shown to be particularly prone to such side effects, i.e., the elderly, the debilitated and/or undernourished, the pregnant, and the menstruating.

A mixture of amphotericin B (50 mg.) and neomycin (1 Gm.), given on an appropriate schedule, has been rated "one of the better drugs for preoperative preparation of the colon" in elective surgery (95).

Amphotericin may become an important adjunct in tissue culture for virus propagation.

At a concentration of 20 mcg./ml., it suppresses growth of species of *Candida, Rhodotorula, and Aspergillus* as effectively as 100 mcg. of nystatin (96).

Resistance.—No evidence of increased resistance of initially sensitive fungi ascribable to exposure to amphotericin in clinical practice has been reported. However, varying degrees of resistance can be induced *in vivo*, depending on the species and strain of fungus studied, the culture medium employed, the frequency of transfer, and other experimental variables (97–99). *In vitro*, there is cross resistance between amphotericin and nystatin (97). This is not surprising in view of the chemical relationship of the two drugs.

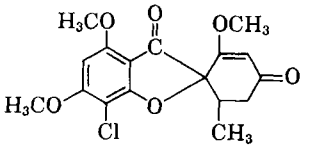
Toxicity.—Incidence of untoward side effects, either acute or residual, during clinical use of oral amphotericin appears to be low. However, experience with the drug is still relatively limited, in comparison with older antibacterial antibiotics. A dose of 2 Gm. daily given orally for 14 months has been used "with no subjective or objective side effects" and "no evidence of hepatic, renal, or hematopoietic reactions" (100). However, in a few instances "mild and reversible" azotemia, nausea, diarrhea, anorexia (76), and "transitory impairment of renal function, ... suggestive of nephrotoxicity" (101) have been reported. In general, the early impression of low clinical toxicity has been supported by more recent studies (86a) and is consistent with the results of pharmacologic and toxicologic studies on animals (102). Incidence of untoward effects may be considerably higher via the intravenous route.

Griseofulvin

Trade Name: Fulvicin (Schering), Grifulvin (McNeil). Also available as Griseofulvin (Ayerst).
Biologic Origin: Penicillium griseofulvin, P. janczewskii, P. nigricans.
First Reported: 1939 (98).
First Clinical Use: 1958 (116).
Clinical Use: Systemic treatment of superficial dermatomycoses. Nocardiosis, sporotrichosis, chromoblastomycosis, and deep infections with *Actinomyces bovis* have been reported to respond occasionally to griseofulvin, but generally it is not considered effective in systemic or deep mycoses.
Usual Dose; Adult: 250 mg. 4 times daily by mouth. In severe infections, 2 Gm. daily, in four divided doses, until clinical response is evident, then reduced to 1 Gm./day. *Children:* If over 50 lb., 250 mg. 3 times daily. *Infants* (Under 2 years): 125 mg. twice daily or, alternatively, 10 mg./lb. of body weight/day.
Toxicity: Apparently low, but gastrointestinal upset, diarrhea, headache, and occasional confusion, exhiliration, and insomnia have been reported. Decreased systolic blood pressure and lymphocytosis also have been reported. Side effects generally have not been severe enough to warrant discontinuance of the drug.

Historical Comment.—Griseofulvin, an antifungal compound originally discovered in 1939 among the metabolic products of *Penicillium griseofulvin* (103) and subsequently of other species of *Penicillium* (104, 105) was studied intensively by phytopathologists for nearly 20 years, as a potentially useful agent for systemic control of fungi pathogenic on plants, before it was investigated for possible usefulness in treatment of mycotic infections of animals. The antibiotic, also known as the "curling factor" because of the characteristic morphologic aberrations it causes in the germ tubes of sensitive fungi (106), is stated to affect only those species that have chitinous cell walls and to be ineffective against species that have cell walls of cellulose or of other nonchitinous material (107), but this view has been questioned. The uses of griseofulvin in plant pathology have been reviewed (108).

Chemistry.—The empirical formula, $C_{17}H_{17}ClO_6$, originally proposed (103) has been confirmed (109, 110), and the following structure has been developed (111).



Griseofulvin

Griseofulvin is only slightly soluble in water (0.001% at pH 7.0). It is soluble to the extent of 0.1% in methanol and ethanol (107). It is thermostable over the pH range 3.0 to 8.8 at 25° for at least 4 weeks and at pH 7.0 withstands autoclaving at 250° F. for 30 minutes. When dry, it is stable at 100° F. for at least 20 months (112).

Absorption and Excretion.—Orally administered griseofulvin is absorbed rapidly and excreted slowly. In experiments with dogs and guinea pigs, little or no antibiotic was found in urine collected within 24 hours after first administration of doses ranging from 0.1 to 2.0 Gm./Kg./day, and there was no significant cumulative excretion (113). During the third week of a continuous feeding experiment, there was little difference in total daily excretion of drug whether animals were dosed at 0.1, 0.2, or 0.5 Gm./Kg.

In another experiment, excretion continued for 1 week after the last of 3 days of dosing with 1 Gm./Kg. About 0.5% of a single oral dose of 50 mg./Kg. given to guinea pigs was recovered from feces collected during the first 24 hours, but

none was found in feces excreted during the next 24 hours. The doses used for these studies were well above those (15 mg./Kg./day for 5 to 15 days) that were curative for *Trichophyton mentagrophytes* infections in guinea pigs.

Data similarly indicating that only a small fraction of griseofulvin ingested by human beings is excreted in the urine have been reported (114), although others have reported recovery from urine of 50% or more of an orally administered dose (115).

Clinical Use.—Clinical interest in griseofulvin and its use in medicine stemmed from the demonstration in 1958, nearly 20 years after discovery of the drug, that, administered orally in adequate dosage, it provided effective and safe systemic protection for guinea pigs experimentally infected with ringworm (*Microsporum canis*) or with *Trichophyton mentagrophytes* and that it was curative even when the onset of treatment was delayed until the infection was well established (116). After a few days of treatment of ringworm, the fungus can be found only in the upper portions of the hair shaft; the lower portion, formed after initiation of treatment, grows out free of infection, presumably because of the presence of griseofulvin in the hair. That the antibiotic is actually present in hair after oral administration can be demonstrated by extraction with hot methanol (117). The drug proved to be effective in curing spontaneous *Trichophyton verrucosum* infections in calves (118), *Microsporum canis* infections in cats (119), and *M. canis* and *T. mentagrophytes* infections in dogs (120), and this led to the first trials in human medication.

The efficacy of griseofulvin as a systemic antidermatophyte in humans has been well established (114, 115, 120 136a). The following dermatophytes that commonly infect hair, nails, and skin are susceptible to it: *Trichophyton interdigitale*, *T. mentagrophytes*, *T. rubrum*, *T. schoenleini*, *T. sulfureum*, *T. tonsurans*, *Microsporum audouini*, *M. canis*, *M. gypseum*, and *Epidermophyton floccosum* (112), and it has been stated that the antibiotic " is active in low concentration against all the common dermatophytes" (116).

Infections caused by any of the above fungi in adults generally yield to an oral dosage of 250 mg. every 6 hours. In children, the total daily dose (but not the number of doses per day) may be reduced. (See capsule summary above.) Improvement and incipient clearing may occur soon after initiation of treatment, but cure may require several weeks or even months of continued dosage.

The rapidity of response to griseofulvin depends on the site and duration of infection. Tinea capitis may be "clinically cured" in 3 to 7 weeks, but if there is follicular involvement response generally is slower, and 3 to 4 months of daily dosage may be required (129). Infections of the glabrous skin may be cleared in 2 to 4 weeks, infections of the soles and palms in 2 to 8 weeks, when fingernails are involved 10 or more weeks may be needed to effect "clinical cure," and when toenails are infected an even longer time (123). Evidence of improvement in onychomycosis usually is seen within 2 weeks in fingernails and in 3 to 4 weeks in toenails, although complete cure may require several months, due to the relatively slow growth of nails, estimated at about 1 mm./wk. for fingernails and about 0.5 mm./wk. for toenails (112).

Topical application of griseofulvin may effect some improvement in dermatophytic infections, but generally the results are not as satisfactory as when the drug is given orally (137–139). This may be because griseofulvin probably does not eradicate the dermatophyte from infected hair, skin, or nails but rather, by its presence in the new tissue as it forms, renders the new tissue resistant to invasion. When the drug is applied topically, little, if any, occurs in the tissues. However, one group reported erratic response of tinea pedis to oral griseofulvin but good response to topical application (140). This is contrary to the majority of reports.

Although markedly effective against the superficial mycoses affecting skin, hair, and nails, griseofulvin generally is ineffective in deep mycoses in humans, when given orally in doses of 0.5 to 2.0 Gm. daily (138), a conclusion which is supported by the results found in experimental blastomycosis, histoplasmosis, cryptococcosis, and coccidioidomycosis (141). However, others have claimed "some therapeutic effect" in nocardiosis and sporotrichosis (142, 142a), in chromoblastomycosis (143), and perhaps in actinomycosis (128).

Resistance.—There are natural variations in susceptibility of fungus species to griseofulvin, even among the genera of dermatophytes that are most sensitive. However, although resistance can be induced *in vitro*, there is no evidence to date of emergence of strains with increased resistance from initially sensitive strains as a result of clinical use of the antibiotic. Occasionally, *C. albicans* has seemed to become dominant and to cause intertriginous dermatitis of groins and/or toe webs as the original griseofulvin-sensitive dermatophyte has been inhibited (129).

Toxicity.—Experiments on animals and clinical observation on humans indicate that toxicity of griseofulvin is low, both in incidence and severity. Mice survive oral doses of 50 mg./Kg. (112), but massive intraperitoneal doses (2 Gm./Kg.) cause severe damage to the seminal epithelium in rats, and intravenous doses of 100 to 200 mg./Kg. (about 10 times the therapeutic dose) cause arrest of mitosis at metaphase (144).

The comment "the incidence of toxicity and side reactions (with therapeutic doses in humans) is extremely low" (130) is typical of the majority of clinical reports. In most patients on whom laboratory studies have been performed, there has been no change in urinalysis, hemoglobin levels, differential counts, hepatic function, and no constant trend in variability of sperm counts. Occasionally, a transient decrease in total leukocyte count has been noted but frequently this has returned to normal despite continued medication.

However, in some instances, about 30% according to one author (145), a relative lymphocytosis has appeared and in some there has been mild albuminuria, although kidney function tests revealed no abnormality. Other side effects occasionally reported have been elevation of icteric index without visible evidence of jaundice, diarrhea, fatigue, vague feelings of gastrointestinal uneasiness after the first 3 to 7 days of treatment, a feeling of "fullness" in the head, confusion, exhiliration, and insomia. Incidence of these effects has been less than 2% and generally they have disappeared even though medication was continued. It has been suggested that some of the effects may have been psychogenic. Imbibing of alcohol while under griseofulvin treatment has been reported to lead to "tachycardia and a flush" (112), and morbilliform eruptions with temperature elevation have been noted (145).

An effect which probably is not of psychogenic origin is the reported 10 to 15% decrease in systolic blood pressure without any subjective symptoms. In the only report on this effect, it was observed in 26 of 52 patients studied (112).

There is no question of the tremendous value of griseofulvin in treating dermatomycoses. It fills a long-standing desire of dermatologists for a relatively safe drug for systemic treatment of such infections from within outwards to replace, or at least complement, the older and sometimes disappointing attack from the outside via topical medication. However, as the drug becomes more widely used, side effects and details not yet recognized may be revealed. The reported decrease in systolic pressure would be an interesting facet to pursue.

Nicetin

Nicetin is the Parke, Davis & Company trade name for the antifungal substance formerly designated CMA-37. This intensely bitter, light yellowish crystalline compound, which is soluble in water or in physiologic saline only to the extent of 0.125%, is closely related to chloramphenicol in chemical structure, but differs markedly from it in antibiotic activity.

Chemically, Nicetin differs from chloramphenicol only in having a ketone group substituted for the secondary alcohol group of the latter. But, in contrast to chloramphenicol, which is a potent antibacterial and antirickettsial drug with no significant action against fungi, Nicetin is effective in moniliasis and in some infections of skin and nails, but has little activity against bacteria.

In the first clinical trial, a case of bronchopulmonary moniliasis which had failed to respond to all other treatments and in which the patient was failing rapidly was given 1 Gm. of Nicetin on the first day and then 250 to 500 mg. 3 times daily. Favorable response occurred almost at once, and after 6 weeks of treatment the patient was free of cough and other symptoms and had gained weight (146). A 2% ointment of Nicetin and a 2% solution of the drug in propylene glycol were found far superior to ammoniated mercury and other common agents in treating 75 cases of various fungus infections of the skin, scalp, and nails (147). Desirable features of the drug are that it is fungicidal, not merely fungistatic, and that it is keratolytic, which facilitates its penetration in the deeper layers of infected skin.

Since Nicetin is so closely related chemically to chloramphenicol which has been alleged although not proved to induce aplastic anemia and other blood dyscrasias, systemic use, except in extreme situations unresponsive to other treatment, would be ill advised. Urinary frequency, dysuria, and hematuria have been observed when doses of 250 to 500 mg. of Nicetin have been given thrice daily. These effects have been ascribed to a chemically-induced hemorrhagic cystitis (147).

Two of 75 patients treated topically with 2% Nicetin (in ointment or in solution in propylene glycol) developed allergic reactions consisting of "intense edema" and marked erythema of the treated limb within 4 to 8 hours (147). The symptoms were controlled with antihistamines or with prednisone. It was not determined whether the reaction was a direct allergic response

to the drug or resulted from the sudden release of large amounts of allergenic lysis products from the invading fungus. Nonetheless, it was recommended that topical treatment not be continued for more than 10 days.

Note: Nicetin is not currently available commercially.

ANTIBACTERIAL ANTIBIOTICS

It is not difficult to find microorganisms that produce compounds antagonistic to bacteria. Despite the literally hundreds of antibacterial antibiotics that have been isolated and described, it still is not too difficult to discover a new one. But to find one that is clinically useful enough to warrant industrial production is becoming extremely difficult. To warrant production in the face of competition from the established agents, such as penicillin, the tetracyclines, chloramphenicol, etc., a new antibiotic must have some special characteristic that the others lack. One such characteristic is the ability to inhibit organisms, especially so-called "hospital" staphylococci, that have become resistant to the older antibiotics that have been prescribed with such abandon for a decade or more.

Kanamycin, novobiocin, ristocetin, and vancomycin all came into production during the period covered in this survey and were developed for marketing primarily because of their activity against penicillin- and tetracycline-resistant staphylococci. Frequently these antibiotics are no more active than the older ones against the common strains of *Staphylococcus*, and in some instances they are less active. It is to be hoped that restraint and responsibility will be exercised in prescribing these newer drugs, limiting their use to situations individually demonstrated to be unresponsive to the older agents, so that their effectiveness can be preserved. Many hospitals, through their own self-policing, have restricted use of novobiocin, ristocetin, vancomycin, and others to such situations.

Colistin attracts attention primarily because of its activity against recalcitrant Gram-negative bacteria and paromomycin, discovered in 1959, has been developed to the production stage primarily because of its special value in amebiasis, shigellosis, and salmonellosis.

Finally, several modifications of other antibiotics, e.g., semisynthetic penicillins and various derivatives of tetracyclines, have been developed during the past five years.

Colistin

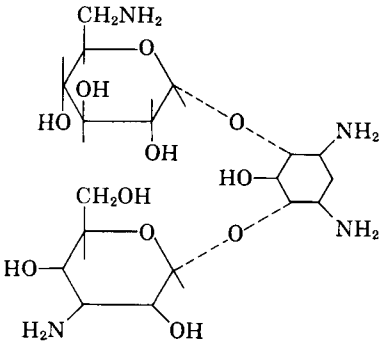
Trade Name: Coly-Mycin (Warner-Chilcott).
Biologic Source: *Bacillus (Aerobacillus) colistinus*, isolated from soil collected in Fukuchima prefecture, Japan.
First Reported: 1950 (147a).
Clinical Use: Urinary tract infections due to Gram-negative organisms, including especially species of *Pseudomonas* but, in general, excluding species of *Proteus*. Also useful in respiratory, surgical, wound, burn, or other infections caused by sensitive organisms.
Usual Dose: 2.5 mg./Kg./day (1.1 mg./lb./day) in 2 to 4 divided intramuscular doses. In severe infections, a large total daily dose may be needed but the total daily dose should not exceed 5 mg./Kg. (2.3 mg./lb.).
Note: Orally administered colistin is poorly absorbed. Therefore, for systemic treatment, intramuscular injection is indicated.
Toxicity: No major toxic effects have been reported in patients receiving normal therapeutic doses. Occasional mild nausea, dermatitis, drug fever, and circumoral paresthesia have been noted but have subsided spontaneously upon withdrawal or reduction of the dosage. Mild pain at the injection site also has been reported.
Comment: Colistin, originally discovered in Japan in 1950, had been extensively studied and used clinically in Japan, Italy, and France before being introduced in the United States in 1959. Extensive references in the Japanese and European clinical literature are cited (147b). Colistin is similar to (and may be identical with) polymyxin E (147c, 147d).

Kanamycin

Trade Name: Kantrex (Bristol).
Biologic Origin: *Streptomyces kanamyceticus*, isolated from soil collected in Nagano prefecture, Japan.
First Reported: 1957 (148, 149).
Clinical Uses: Staphylococcal infections resistant to commonly used antibiotics. Refractory urinary tract infections, especially those due to staphylococci, *E. coli*, or gonococci. May be beneficial in *Proteus* infections but not indicated when *Pseudomonas* or enterococci are the etiologic agents. Tuberculosis may respond in favorable cases, but the drug is not impressive in advanced cases, especially those that have failed to respond to other drugs. *p*-Aminosalicylic acid should be used jointly with kanamycin.
Usual Dose: In acute or chronic infections, 1 to 2 Gm. daily, injected in 2 to 4 equally divided doses, or a dose of 15 mg./Kg./day may be used in divided doses. Total dose in any one course of treatment should, if possible, not exceed 40 Gm. For extended use in chronic illness, dose should not exceed 6 Gm./wk. In tuberculosis, a schedule of 1 Gm. twice a day, 2 days a week with PAS, 10 Gm. daily, has been recommended.
Note: For systemic medication, kanamycin must be given by injection, since it is not absorbed well from the gastrointestinal tract. Pain or irritation at the injection site is fairly frequent.
Toxicity: Acute toxicity is low in short-term therapy. Nephrotoxicity is the major untoward effect. Symptoms usually are mild and subside spontaneously when the therapy is terminated. However, upon continued use, as in chronic illness, eighth cranial nerve damage, involving auditory or vestibular function or both may occur.

Comment: Kanamycin was discovered in the laboratories of the Japanese National Institute of Health (Tokyo) in 1957 and was marketed in the United States in 1958. Results of some of the microbiologic and chemical research that preceded release of the drug have been recorded in a single symposium publication (150).

Chemistry.—Kanamycin, related chemically to neomycin and streptomycin, shares some of their antibacterial and pharmacologic characteristics. The molecule contains 2 amino sugars linked glycosidally to 2-desoxystreptamine. The latter is also a moiety of neamine (Neomycin A)



Kanamycin

The chemical research leading to characterization of the compound and elucidation of the above structure has been reviewed (66). A second antibiotic, kanamycin B, occurs in crude preparations (151–153) but is present only in minor amounts, if at all, in medicinal kanamycin.

Kanamycin is stable and water-soluble. It is resistant to acid and alkaline hydrolysis and is thermostable in the pH range of 2.0 to 11.0. At pH 6.0 to 8.0, it withstands boiling for 30 minutes. Detailed data on stability, solubility, and other pharmaceutically important properties have been published (154).

Absorption and Excretion.—Kanamycin is poorly absorbed following oral administration, and this route is not suitable for systemic medication. The drug is well absorbed following intramuscular or subcutaneous (155) or intraperitoneal injection (156).

In humans, kanamycin appears in the blood about 15 minutes after intramuscular injection, and peak levels are attained in about 1 hour on either a single- or multiple-dose schedule. The concentrations fall rapidly after the peak is achieved (157), and blood taken 24 hours after a single injection of 1 Gm. or less generally does not contain assayable amounts of the antibiotic.

In infants and children, intramuscular injection of 5 mg./Kg. produces blood levels roughly comparable to those obtained with 250 mg. in adults, but the concentration tends to fall more rapidly (158).

A progressive build-up of concentration is not seen on a multiple-injection schedule, and after successive doses of 0.25, 0.5, or 1.0 Gm. the blood levels are seldom more than a fraction of the levels achieved 1 hour after the initial injection.

Primary excretion of kanamycin is renal, although some also appears in the bile. The renal elimination appears to be simple glomerular filtration (155, 159). Total 24-hour urinary recovery from human subjects with normal kidney function has been reported as 40% of the total dose for bed patients (160), ranging up to 100% for healthy volunteers (161). These differences may not be as great as appears on the surface (157).

Normally, in patients with good kidney function, there is no build-up of concentration. But on a multiple-dosage schedule, the plasma level may increase abruptly when there is renal impairment, and with the elevated levels toxic manifestations, especially ototoxicity, may be anticipated. Since the drug is also nephrotoxic, it should be used only with extreme caution in subjects with functional renal problems (162). The serum half-life of kanamycin in normal subjects has been estimated to be about 4 hours, but in uremic patients the drug was detectable in the blood for 4 to 5 days after a single dose of 1 Gm. (162). Severe hepatic disease may also cause accumulation of kanamycin, even when the drug is administered orally, despite poor absorption from the gastrointestinal tract (162, 163).

Clinical Uses.—The *in vitro* antibiotic spectrum of kanamycin embraces several Gram-negative and some Gram-positive species of bacteria and various acid-fast organisms, including *Mycobacterium tuberculosis* H 37 Rv. In general, the clinically useful spectrum is similar, provided the drug is administered in adequate dosage for an adequate length of time.

Staphylococcal Infections.—Kanamycin in divided intramuscular doses of 10 to 100 mg./Kg./day was rated "good" in treating mild and moderately severe cases and as "effective" in about 50% of children severely ill with hospital-acquired straphylococcal infections during the 1958 epidemic outbreak in a maternity service in Houston, Texas (165). Most of the strains were resistant to penicillin, streptomycin, and the tetracyclines, but all were inhibited *in vitro* by less than 6.25 mcg. kanamycin/ml. The infections had existed for from 6 hours up to 40 days before initiation of kanamycin, and about two-thirds of the patients had already failed to respond to at

least one, and most of them to two or three, other antibiotics. Other studies, both *in vitro* and clinical, have confirmed the sensitivity of the majority of clinically isolated staphylococci to kanamycin (166–168).

From purely microbiologic considerations, such as sensitivity of organisms, static *vs.* cidal concentrations of drug, etc., neomycin is a more effective antistaphylococcal agent than kanamycin (166, 169), but kanamycin probably is not as toxic as neomycin and, therefore, is preferable to it for systemic medication (170, 171). Kanamycin, ristocetin, and vancomycin can be considered as essentially in the same category for treatment of staphylococcal infections recalcitrant to safer antibiotics.

Urinary Tract.—The rapid appearance of kanamycin, and the relatively high concentrations it reaches, in the urine make it a useful drug for treating susceptible infections of the urinary tract. Good results generally can be achieved when infections are due to *Staphylococcus, Escherichia coli,* or *Gonococcus* (172). *Proteus* infections respond moderately (172) and infections due to *Aerobacter* or to *Klebsiella* can be treated satisfactorily, if the dosage is adequate (173). Others have confirmed these conclusions (174–176). Tuberculous infections of the urinary tract also may respond, if sufficient dosage is provided for an adequate time and PAS is used jointly with kanamycin (172).

Pulmonary Tuberculosis.—Reports from Japan have rated kanamycin "almost equivalent to the streptomycin-PAS combination" and "an excellent antituberculosis drug" (177), but investigators in the United States have not been so enthusiastic (178–180) because of the "toxic potentialities for the eighth cranial nerve and the kidneys, and hypersensitivity reactions," and because of the rapid development of resistance by the tubercle bacilli to the drug.

Other Infections.—Reports are conflicting regarding the effectiveness of kanamycin in *Salmonella* and *Shigella* infections (181–184). Certainly it is not as effective as chloramphenicol in typhoid fever, although it may have some effect in reducing the typhoid-carrier state (183). Administered orally in adequate dosage, it is useful as a preoperative intestinal "antiseptic," giving adequate control of streptococci, coliforms, and *Clostridia* (185–187).

Kanamycin has been reported to be effective in reducing the "cerebral manifestations" resulting from absorption of bacterially formed protein metabolites in patients with cirrhosis of the liver (188), but others have found that the beneficial "blood ammonia changes may not be sustained or reproducible with repeated courses" of treatment (189).

In ointments or creams (0.5%) the antibiotic has been effective in the treatment of cutaneous bacterial infections and has not appeared to be irritant or allergenic (190). However, such use probably should be discouraged as long as effective agents not suitable for systemic administration are available.

Resistance.—There is little or no cross resistance between kanamycin and most of the antibiotics commonly used systemically. However, there is almost complete cross resistance between kanamycin and neomycin and, among strains of *Staphylococcus aureus* and of *Enterobacteriaceae,* there may be complete cross resistance among kanamycin, neomycin, and paromomycin (168) and moderate cross resistance to streptomycin (168). Some cross resistance between kanamycin and streptomycin also appears in *Salmonella typhimurium* (191). Upon repeated exposure, other organisms, including *Mycobacterium tuberculosis,* also develop resistance to kanamycin (192–195).

Views are conflicting regarding emergence of resistant strains under clinical conditions (172, 183, 185, 196–198). The consensus seems to be that microorganisms, other than tubercle bacilli, do not readily develop resistance to kanamycin under clinical circumstances as long as the drug is used cautiously. However, highly resistant staphylococci have been isolated from clinical material (199).

Toxicity.—Results of acute and chronic toxicity studies in animals suggest that kanamycin is somewhat less toxic than streptomycin (155, 200).

Opinions differ concerning chronic toxicity upon extended use in humans, although there is agreement that the drug is potentially nephrotoxic and ototoxic. Donomae (201) considered auditory loss through eighth nerve damage "extremely improbable if the dosage is maintained below 6 Gm. per week," and he stated "there is no other side effect of considerable significance." However, others consider the drug "essentially nontoxic" in doses of 1.5 to 2.0 Gm. daily for less than a total amount of 40 Gm., but caution that when the total exceeds 40 Gm. "there is a clear chance" of permanent eighth nerve damage and deafness (161) and that even with lower total doses signs of renal impairment may be expected during or after treatment (202). Others also have commented on the need for vigilance to detect renal and auditory damage before irreparable

impairment occurs (171, 178, 198, 203, 204). It is clear that toxic effects will occur sooner and be more extensive in patients who have impaired renal function before therapy is started (205, 206) and that eighth nerve damage may continue to progress even after treatment with kanamycin has been discontinued (207).

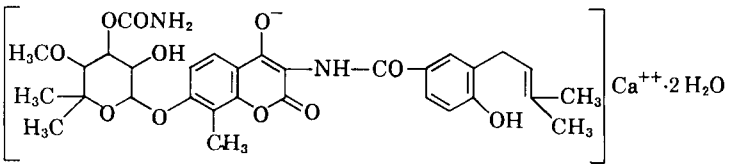
The monopantothenate of kanamycin has been reported less acutely toxic than the sulfate in animals (208), but no studies were made of possible delayed effects.

Irritation, stinging, and pain at the injection site are common (161,198), the incidence possibly being as high as 50 to 60% (198).

Novobiocin

Trade Names: Albamycin (Upjohn); Cardelmycin (Pfizer); Cathomycin (Merck).
Biologic Origin: Streptomyces niveus (*S. spheroides*) isolated from soil collected in Queens Village, N. Y.
First Reported: 1956 (209, 210).
Clinical Use: Staphylococcal infections resistant to the antibiotics in common use. Limited applications in other infections (see text).
Dose; Adult: average 250 mg. orally every 6 hours or 500 mg. every 12 hours; in severe infections, 500 mg. every 6 hours or 1 Gm. every 12 hours. *Children:* 15 mg./Kg. daily in 4 divided doses or, in severe infections, 30 to 45 mg./Kg. daily in 4 divided doses. Prolonged treatment with high doses may lead to skin rashes and other complications.
Note 1: There is considerable variation from patient to patient in serum binding of the drug. Therefore, dosage should be determined on an individual basis.
Note 2: Because of dermatologic side effects induced if the drug is used in too large a dose or for too long a time, the minimal dose capable of coping with the infection should be employed. To be weighed against allergenic potential of the drug is its potential for inducing emergence of resistant strains of bacteria if used in sub-bactericidal amounts. Five hundred milligrams every 12 hours has been recommended as a safe middle course for adults.
Toxicity: Principal manifestations are skin rash, urticaria, and drug fever. Leucopenia also has been reported. Very high doses may induce liver damage. When properly used, the incidence of skin eruptions is relatively low, but precautions are necessary. (See Note above and also text which follows.)

Chemistry.—Novobiocin forms both acid and neutral salts. The calcium salt has the following structure



Calcium Novobiocin

The extensive literature on the chemistry of novobiocin has been reviewed (66).

Absorption and Excretion.—Novobiocin is not readily absorbed via the oral route (211), but the calcium and sodium salts are, as is dihydronovobiocin. The drug is absorbed following intramuscular injection also, but frequently causes appreciable reaction in muscle or subcutaneous tissue when so administered. In the discussion that follows novobiocin refers to the sodium or calcium salt.

Maximum serum levels are achieved within 2 to 4 hours after oral administration. According to Finland and Nichols (212) higher blood levels are achievable with novobiocin than with any other antibiotic given in comparable dosage. However, there is much patient to patient variability, and after a single dose of 250 mg., maximum serum levels may range from less than 3 to about 20 mcg./ml.

On a multiple-dosage schedule, there is some accumulation during the period of the first few doses, but the serum levels soon stabilize and only minor fluctuations occur when 500 mg. of the sodium salt are given at 6 to 8-hour intervals. Peak concentrations are higher and are reached earlier when the drug is given on an empty stomach. Following absoprtion, it quickly appears in almost all body tissues and fluids except the cerebrospinal fluid.

Novobiocin is excreted in the bile, urine, and feces. Major excretion is via the feces. Much of the fecal novobiocin probably arrives there via the bile, following hepatic concentration and elimination, but some probably represents unabsorbed drug. The sustained high blood levels novobiocin reaches may be brought about by reabsorption from the intestine of drug which, having been previously absorbed, has passed through the liver and again into the intestine via the bile.

Urinary excretion accounts for about 3% of the total administered dose (213). Concentrations are usually higher in the urine than in the serum, but the range of concentrations is not so great.

Clinical Uses.—Generally, the monobasic sodium salt is prescribed for adults; the neutral

calcium salt, suspended in syrup, often is preferred in pediatric practice.

Novobiocin is active principally against staphylococci and other Gram-positive organisms, although it has limited activity against some strains of selected Gram-negative species. Certainly, its major usefulness is in treating staphylococcal pyodermas insensitive to other antibiotics (214–218), and some investigators believe use of the drug should be restricted to such situations (215).

Novobiocin has been reported effective also in pneumococcic pneumonia (219), undulant fever (220), and in genitourinary infections due to *Bacillus proteus*, *Micrococcus aureus*, or *Streptococcus faecalis* (221). However, others have not been impressed with its performance in nonvenereal urinary infections by a variety of organisms (222, 223), and it has been declared ineffective in acute gonorrhea (224) and in primary and secondary syphilis (225).

In ophthalmology, novobiocin in a concentration of 1% is not irritating and does not inhibit corneal regeneration (226), and it is useful in treating ocular infections caused by susceptible organisms, especially staphylococci (227, 228).

Resistance.—There is a "definite tendency" for bacterial strains of "increased resistance" to appear, when novobiocin is used alone in clinical practice (229, 232). Laboratory data indicate that a one-step increase of ten-to thirtyfold may occur (230) but that a "synergistic" action and marked retardation of emergence of resistant bacterial strains occur when neomycin or bacitracin (230) or chloramphenicol, chlortetracycline, oxytetracycline, streptomycin, or penicillin (209), and novobiocin are used concomitantly. Synergism between novobiocin and tetracycline and novobiocin and nystatin in treating amebiasis also has been noted (231). These observations have led to the recommendation that another antibiotic to which the etiologic agent is initially sensitive should always be used jointly with novobiocin, especially when a prolonged period of treatment is anticipated, e.g., suppurating lesions or staphylococcal abscesses.

It should be cautioned that in selecting drugs for combined use, attention must be given to a third component, namely, the organism, in the complex system. Two antibiotics which act synergistically against one species or strain of microorganism might be indifferent, or even act antagonistically, against a different species with different metabolic requirements and pathways. Moreover, experience with other mixtures indicates that although emergence of resistant forms may be retarded, it is seldom eliminated and there is, therefore, always the possibility of producing organisms resistant to both drugs instead of just one.

Toxicity.—Novobiocin probably has a low order of toxicity when properly used, although the incidence of untoward complications has been reported to be as high as 12.7% in one study (232) and 18.7% in another (233). High doses were used in both series of patients. The most common side effect is transient skin rash or other dermatologic manifestation which disappears when the drug is withdrawn or therapy is terminated. A more serious effect is leucopenia, the incidence of which is said to be about 1% (234). An elevated icteric index also may arise, although it is not clear whether this is due to bile or to a yellow pigment metabolite of the drug (235). But in any event, the pigment may interfere with determination of serum bilirubin. Thrombocytopenia also has been reported (236).

That these are not constant features of novobiocin therapy is indicated by the report of a patient who received 250 mg. daily for 6 months after a priming dose of 250 mg. four times daily for 3 days and whose icteric index and blood cell count remained normal throughout the treatment (237). Despite a history of allergy to other drugs, the patient showed no evidence of allergy to novobiocin.

Animal experiments have demonstrated severe degenerative changes in liver and kidneys when high doses (300 mg./Kg./day) have been given.

While some authors have reported "remarkable freedom from side effects" (238), others have commented that eruptions were of "such alarming proportions to the patient and . . . physician . . . that the resultant effect was to curtail the number of patients available for study" (239).

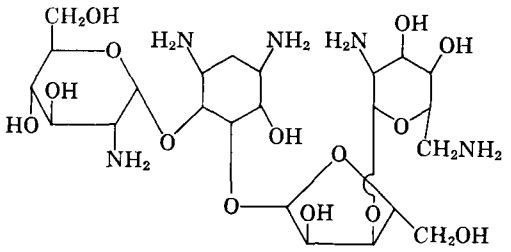
In a comprehensive survey, involving 10 groups of clinicians and aimed at resolving the differences of opinion regarding toxicity of novobiocin, the overall incidence of skin rash was found to be 8.9%, and it was concluded that eruption is likely to occur only when the drug is given in a dose of 2 Gm. or more/day for 6 days or more (240). It was recommended that, in view of the therapeutic effectiveness of the drug in low concentrations against susceptible organisms and the ease with which high blood levels are obtained, 0.5 Gm. of novobiocin given orally twice daily should be an adequate dosage. It was estimated that on such a regimen the incidence of skin rash should be less than 1%.

Paromomycin

Trade Name: Humatin (Parke, Davis).
Biologic Origin: Streptomyces rimosus forma *paro-*

momycinus, isolated from soil collected in Colombia.
First Reported: 1959 (241).
Clinical Uses: Amebiasis, salmonellosis, and shigellosis. Also useful in management of hepatic coma and possibly clearing of typhoid and shigella carriers.
Usual Dose; Amebiasis: Adults 0.75 to 1.0 Gm. (or children 22 mg./Kg.) orally in divided doses for 5 days. Larger doses have been used for both children and adults with no ill effects. *Hepatic Coma:* 4 Gm. or more daily, in divided doses for 5 to 6 days. *Bacillary Dysentery and Nonspecific Diarrhea in Children:* Doses of 50 mg./Kg./day, in divided doses for 5 to 7 days.
Note: Paromomycin is not absorbed significantly from the gastrointestinal tract. Hence, in the conditions cited, the full effect of oral doses is exerted on organisms in that system.
Toxicity: Toxicity is low. In normal subjects and in patients free of diarrhea or dysentery, loose stools generally develop if the daily oral dose exceeds 2 Gm. for more than 3 days. On a dose of 4 to 10 Gm. daily, moderately severe diarrhea occurs, but other untoward effects have not been noted.

Chemistry.—Paromomycin is a stable, amorphous, white substance that is very soluble in water, moderately soluble in methanol, and sparingly soluble in absolute ethanol. It is optically acitve (242). Chemical studies (243, 244) leading to formulation of the following structure have been published.



Paromomycin

Paromomycin is basic and readily forms salts with acids, especially mineral acids. For clinical use, it is prepared as the sulfate.

Absorption and Excretion.—The drug is poorly absorbed following oral administration. In monkeys 1 to 3.5% of an oral dose can be recovered from the 24-hour urine, but up to 17% of an intravenous dose can be recovered in the same period (241). Rapid and efficient absorption follows subcutaneous administration in hamsters, and the drug persists in the plasma for about 4 hours (243). In humans, it is used only via the oral route.

Clinical Uses.—Paromomycin is active *in vitro* against a wide array of Gram-positive, Gram-negative, and acid-fast bacteria, including human type tubercle bacilli (241, 245). It is amebicidal also, either in the presence or absence of bacteria. By use of drug-resistant bacteria in the same culture with amebae, it has been shown that the drug is a direct-acting amebicide (243).

Since paromomycin is not absorbed from the intestinal tract, it is useful via the oral route for treatment of diseases that are caused by susceptible organisms and require vigorous antimicrobial action in the gut. It has been successful in amebiasis (246–252) and in bacillary and nonspecific diarrhea in children. It has proved effective, in a 5-day course of treatment (40 mg./lb.), not only in curing the primary infection in shigellosis and salmonellosis, but also in clearing carriers of the organisms (253). Typhoid carriers, previously treated with chloramphenicol, have been cleared by use of paromomycin (253).

A dose of 500 mg. given 4 times daily for 4 days is effective in the preoperative preparation of the colon for elective surgery.

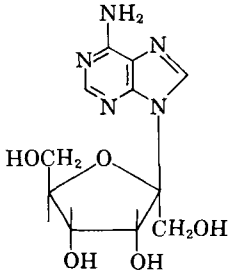
Paromomycin is effective also in management of hepatic coma, unless some liver failure or massive uncontrolled gastrointestinal bleeding is underlying (254), and it may be possible to restore protein safely to the diet sooner when this drug is used than under the conventional treatments (255, 256).

Toxicity.—Toxicity is low. See capsule sketch at the beginning of this section and, for details, reference 241.

Psicofuranine

Psicofuranine, discovered in 1959 in culture filtrates of *Streptomyces hygroscopicus* var. *decoyicus* in the Upjohn Laboratories (257–259), is unusual in three respects: (*a*) it is a nucleoside antibiotic, (*b*) it has both antibacterial and antitumor activity, and (*c*) it has a low order of antibacterial activity *in vitro*, as tested in common laboratory media, but is highly effective in protecting experimentally infected animals. It is not commercially available at present.

The compound is very soluble in dimethylformamide, dimethylsulfoxide, and hot water. At room temperature, water solubility and methanol solubility are about 8 mg./ml. (257). It has been identified as 6-amino-9-D-psicofuranosylpurine (257) having the following structure



Psicofuranine

which is the same as that proposed for angustimycin (260), discovered in Japan. Psicofuranine is least stable in acid (half-life 18 hours at pH 2.0 and temperature 30°) and most stable at a neutral pH (261). Studies of the kinetics of degradation and of the products formed (261–263) have yielded information of potential value for estimating stability of the compound in aqueous media and for determining conditions suitable for bioassay (264, 265). The lack of activity of psicofuranine when conventional *in vitro* bioassays are attempted has been circumvented by development of special procedures which permit determination of concentrations down to 10 mcg./ml. in water or 3 mcg./ml. in blood or serum by a plate technique or as low as 0.5 mcg./ml. turbidimetrically (264). A physical method, involving paper chromatography and ultraviolet absorption characteristics (266), and a chemical method (267) also have been published, the latter being usefully applied in studies of absorption and excretion of the antibiotic (263, 268).

Dogs excrete a fraction of orally or parenterally administered psicofuranine in the urine (263), indicating absorption. There is evidence of irreversible binding of the drug, and a tendency for it to concentrate in the kidney (269), a factor that would need to be considered in patients with renal impairment. Determination of urine levels might be a useful criterion of absorption when low doses, following which it is difficult to determine blood levels, have been administered (269).

Despite the low order of *in vitro* activity, psicofuranine "compares favorably with commercially available antibiotics" in chemotherapeutic effectiveness in animals experimentally infected with *Staphylococcus aureus* (including strains resistant to other antibiotics), *Streptococcus hemolyticus*, and *Escherichia coli* (270). There appears to be no cross resistance between psicofuranine and antibiotics in common use (270).

Oral or intraperitoneal doses of 100 mg./Kg./day effectively increase the number of survivors and tumor regressions in rats with several types of tumors (271).

Acute LD_{50} values in the mouse and rat and subacute toxicity studies in the rat and the dog indicate that psicofuranine has a relatively low order of activity for these species (271). In the latter study, there was a tendency toward leucopenia at high doses, 300 or more mg./Kg./day. At very high dosage, there may be some liver damage and loss of weight.

Ristocetin

Trade Name: Spontin (Abbott).
Biologic Origin: *Nocardia lurida*, isolated from soil collected at the Garden of the Gods, Colo.
First Reported: 1957 (272, 273).
Clinical Uses: Staphylococcal or other Gram-positive infections resistant to other antibiotics.
Dose; Adult: 2 Gm./day, in 2 or 3 divided doses, intravenously in 5% dextrose containing 250 mg. of ristocetin per 125 ml. For severe or relatively insensitive infections, doses as high as 50 mg./Kg./day (4 Gm. for 165 to 175 pound adult) or higher may be used, but maximum dose should not exceed 6 Gm./day. *Children:* Doses should be proportionately less, according to weight.
Note 1: Administration of a single normal dose by the intravenous drip should be completed in 35 to 40 minutes. If rate is slower, adequate blood and tissue levels may not be achieved; if more rapid, irritation and other undesirable effects may occur.
Note 2: Concentration of ristocetin in dextrose solution infused should not exceed 1.25%, and when use of such a concentration is necessary rate of administration should not exceed 2 ml./min.
Toxicity: Hematologic complications (anemia, neutropenia, leucopenia), rash, fever, and diarrhea have been noted. Irritation and chemical thrombophlebitis may occur if too high a concentration of drug is used.

Chemistry.—Ristocetin, as available for medicinal use, consists of a mixture of two related amphoteric compounds, ristocetins A and B. Both contain amino and phenolic groups and sugars. The molecular weights of ristocetins A and B are probably about 4,000 (273). The compounds have been isolated as free bases and crystallized as sulfates. They are soluble in acidic aqueous media, but are only slightly soluble at neutrality. They are insoluble in most organic solvents.

Ristocetin is stable in acidic aqueous solutions but is rapidly inactivated above pH 7.0. Sterile solutions at an appropriate pH can be stored in a refrigerator for at least 1 month without significant loss of potency (273).

A plate assay techique, employing an appropriate strain of *Bacillus subtilis*, and a tube-dilution assay, using a saprophytic strain of *Corynebacterium* have been developed (272). Pure ristocetin A is used as the reference standard. This fraction, which is about one-fourth to one-third as antibacterially active as ristocetin B, has been assigned an arbitrary unit value of 1,000 units/mg.

Absorption and Excretion.—No ristocetin can be detected in either the blood or the urine after oral administration, but it is absorbed after intramuscular injection and can be detected in the blood (rabbit experiments) up to 8 hours after administration of 15 mg./Kg. or for about 3 hours after an intravenous dose. Similar values have been recorded in pediatric practice (274). Currently, only the intravenous route is

employed in medicine. About 30% of an intravenous dose (in dogs) can be recovered from urine passed in the first 4 hours and an additional 15% during the next 4 hours. Glomerular filtration appears to be the principal excretion mechanism. This may be followed by some tubular reabsorption (275).

Clinical Uses.—In general, ristocetin is not as active as penicillin and some other common antibiotics against the common cocci. Therefore, and also because it must be given intravenously, it is not suitable for treatment of routine infections. However, it is of value against infections caused by some strains of *Staphylococcus* and other cocci (streptococci, enterococci, pneumococci, etc.) that are insensitive to the conventional antibiotics (276–285), although failures also have been recorded (285, 286).

Ristocetin is active against the tuberculosis organism *in vitro*, but mouse protection studies and a few clinical trials in human patients have been disappointing.

Resistance.—Initially sensitive strains of staphylococci exposed to sublethal concentrations of ristocetin *in vitro* give rise to resistant variants in a slow, stepwise pattern (272, 287), but no cases of resistance arising during clinical use have been reported. Cross resistance with other commonly used antibiotics does not seem to occur.

Toxicity.—Untoward side effects are not uncommon in patients receiving ristocetin, although if the daily dose does not exceed 25 mg./Kg. and is given in two or three equally spaced and divided doses, complications generally are minimal. Much of the effect probably depends on the concentration of the solution used and the rate at which it is infused.

The most serious effects are hematologic. Eight in a series of 10 consecutive patients given from 750 mg. in 5 days to 57 Gm. in 11 days displayed such reactions (288) including neutropenia, acute anemia and platelet depression, and leucopenia. Studies with larger numbers of patients, numbering several hundred in the aggregate, indicate neutropenia in about 4 to 7.5% of patients recieving the drug (278, 280, 289).

Ototoxicity and nephrotoxicity also have been reported to occur occasionally (285), as well as rash, and local myositis (290) and, when doses are high, diarrhea.

Thrombophlebitis and irritation are induced at the injection site if too high a concentration is used. Care must be exercised to prevent spillage into adjacent extravascular tissue to avoid irritation and, generally, intramuscular injection should be avoided. It has been reported that the inflammatory response can be decreased by addition of 5 mg. of cortisone to intramuscular doses of 25 mg./Kg. (274). This seems to be a dubious procedure, in view of the possible complications produced by steroids in infection. It seems preferable to control (or minimize incidence of) such reactions by limiting the concentration of ristocetin solution that is employed. Usually a 0.2% solution in 5% dextrose is reasonably safe if it is infused at the appropriate rate (see capsule sketch at beginning of this section). In no instance should the concentration exceed 1.25%.

Vancomycin

Trade Name: Vancocin (Lilly).
Biologic Origin: *Streptomyces orientalis* isolated from soil samples collected in Indonesia and India.
First Reported: 1956 (291, 292).
Clinical Uses: Infections due to strains of staphylococci and other Gram-positive organisms resistant to the common antibiotics.
Usual Dose; Adults: 1 to 2 Gm. daily, administered intravenously in four equally spaced and divided injections, the drug being dissolved in 5% glucose.
Children: 20 mg./lb./day, administered as above.
Toxicity: Toxicity is relatively low. Most common untoward effect is phlebitis, following repeated injections. A rash sometimes occurs and, in prolonged treatment with high doses, ototoxicity and nephrotoxicity may appear.

Chemistry.—Elemental analysis of vancomycin base indicates at least 7% nitrogen, 16 to 17% carbohydrate, and 4.37% organic chlorine. Ultraviolet and infrared analyses indicate the presence of substituted phenolic groups and hydroxyl or amino, amide, and aromatic groups. Glucose and aspartic acid moieties also are known to be present, but the complete structure has not been determined. Estimates of molecular weight have ranged from 785 to 3,500, suggesting the possibility of macromolecular aggregation. Titration data indicate a value between 3,200 to 3,500 ± 200.

The free amphoteric base is insoluble in water, but is soluble (up to about 25%) in several polar, water-miscible solvents, e.g., dimethyl sulfoxide and dimethyl acetamide. The hydrochloride and the sulfate are water soluble, the solubility of the former being in excess of 100 mg./ml. The hydrochloride is moderately soluble in aqueous methanol also. It is precipitated from aqueous solutions by heavy metals and from acidic solutions by ammonium sulfate and sodium chloride (291, 293). It is the hydrochloride which is distributed for medicinal use under the name Vancocin (Lilly).

Stability decreases abruptly below pH 3.0

and above pH 7.0 at 37°. Within that range, loss of activity is about 10% in 6 days at 37°.

At 5°, there is about 10% loss in 6 months at pH values between 1 and 9 but about the same loss in 9 days at pH 10.0.

Absorption and Excretion.—For systemic action, vancomycin must be administered parenterally. The intravenous route is preferred because of mild to moderate pain commonly accompanying intramuscular injection.

The drug permeates most body fluids quickly, following injection, but only small amounts appear in the bile and, in the absence of meningeal damage, none in the spinal fluid. After a single dose of 500 mg., in healthy adults, antibacterial concentrations of vancomycin are soon present in the blood, and assayable amounts still remain after 24 hours (294). There is some accumulation of vancomycin following multiple repeated doses, and concentrations two to three times those required to kill most strains of micrococci *in vitro* generally are achieved.

Excretion is primarily renal, and high concentrations of vancomycin occur in the urine, even after a single dose of 500 mg. Values as high as 2,400 mcg./ml. have been found 1 hour after such a dose. The average concentration after 6 hours is about 300 mcg./ml., and after 24 hours is about 100 mcg./ml. (294).

After oral administration, very little vancomycin appears in the urine and, normally, none can be found in the blood of healthy subjects but large amounts, ranging from 400 to 24,000 mcg./Gm., depending on time elapsed since administration, are found in the stools. From none to about 110 mcg./Gm. may appear in the stools after intravenous doses.

It has been concluded from experiments with animals and observations on humans that "vancomycin is not metabolized to a great extent in the body" (295).

Clinical Uses.—Vancomycin is particularly active against Gram-positive cocci, especially staphylococci (291, 296–300). Indications for its use are pneumococcal bronchopneumonia, pharyngitis, erysipelas (301), acute micrococcal endocarditis (302), and, in general, infections due to staphylococci resistant to other antibiotics (300, 303–305). Vancomycin has been called the drug of choice for oral administration in micrococcal ileocolitis ". . . because of its bactericidal effect and because of the large quantities that are excreted in the stool . . ." (306).

Impaired renal function probably is a valid contraindication for use of vancomycin systemically.

In a study of eight antistaphylococcal antibiotics, including erythromycin and novobiocin, vancomycin was rated best with respect to bactericidal action and low resistance-inducing potential (307), and in another study of 1,350 organisms, more than one-third of which were strains of *Staphylococcus aureus* resistant to penicillin, all were found to be sensitive to vancomycin (296). Others have considered vancomycin, ristocetin, and kanamycin all about equally effective clinically (285).

Resistance.—In laboratory exposure, cultures of vancomycin-sensitive bacteria may lose some sensitivity to the drug, but usually only slowly and to a small extent (294, 297, 301). Clinical experience with the drug has been excellent with respect to its low resistance-inducing potential (301, 308, 309). However, strains rendered resistant to novobiocin or to ristocetin often are resistant to vancomycin also, indicating a high degree of cross resistance among the three drugs (285).

Toxicity.—Mild to severe phlebitis may occur in 10 to 25% of patients receiving several intravenous injections of vancomycin, and a skin rash has been noted in about 4 to 5%. These are the most common side reactions. However, when high doses and/or prolonged treatment are employed (or even with normal doses if there is uremia or kidney malfunction) high blood levels may occur and under these circumstances ototoxicity has been noted (285, 302, 303, 305). But, if proper precautions are used to guard against building up too high a serum level of vancomycin, even the patient with poor renal function need not be denied this antibiotic (310).

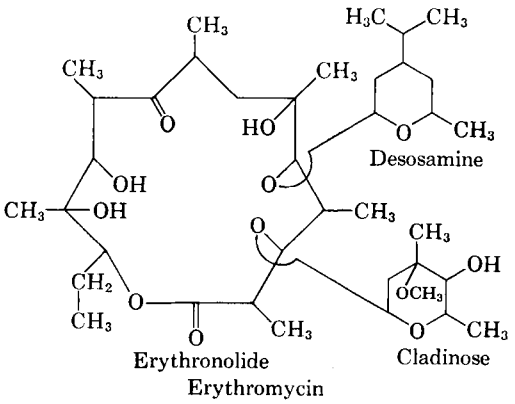
Evidence of eighth nerve damage, exhibited by auditory deficiency, generally has not been observed when serum levels have been within the limits usually provided by a total daily dose of 2 Gm. (range 7.6 to 20 mcg./ml. and average about 13 mcg./ml.) if there is no renal impairment. However, when prolonged treatment with vancomycin is indicated, serum levels of the antibiotic and renal function should be determined and audiometric tests should be made periodically in order to guard against possible deafness and nephrotoxicity.

The Erythromycin-Oleandomycin Controversy

Erythromycin, marketed by Abbott as Erythrocin and by Lilly as Ilotycin and, in the form of the propionyl ester, as Ilosone, was discovered in 1952 in culture filtrates of *Streptomyces erythreus* which had been isolated from soil collected near Iloilio City on the island of Panay in the Philippine Archipelago (311). Oleandomycin

(Matromycin, Pfizer) a metabolic product of *Streptomyces antibioticus* (312), is also available as triacetyloleandomycin (Cyclamycin, Pfizer) and as triacetyloleandomycin combined with glucosamine (Cyclamen, Wyeth; and Tao, Roerig). A 1:2 mixture of oleandomycin and tetracycline is marketed as Signemycin (Pfizer) and, phosphate-buffered, as Signemycin V (Pfizer).

Erythromycin and oleandomycin are macrolide antibiotics. Among the dozen or so other members of the macrolide group of antibiotics thus far recognized are carbomycin, griseomycin, marbomycin, methymycin, picromycin, and spiramycin. The macrolides are characterized by a highly substituted many-membered lactone system to which is attached, glycosidally, an amino sugar. Other sugar and nonsugar moieties also may be present. The chemistry of these and other antibiotics with complex ring structures, notably peptide lactones and polyene macrolides, has been reviewed (65, 66, 313). The structure of erythromycin has been determined (314), but the complete structure of oleandomycin is still unknown.



Erythromycin

Although they are active against a number of Gram-positive organisms and against selected Gram-negative species, both antibiotics are valuable primarily because of their antistaphylococcal activity, especially against strains resistant to penicillin and the tetracyclines. Both drugs are readily absorbed from the intestinal tract and are administered orally for systemic action Certain derivatives, such as the propionyl ester of erythromycin and the triacetyl-derivative of oleandomycin, are more rapidly and uniformly absorbed than the respective parent compounds and produce higher serum levels. Neither drug is significantly toxic when properly used (see reviews in the "United States Dispensatory," 25th ed., 1955 and the 1960 Supplement thereto), although rare instances of jaundiec have been attributed to prolonged use of propionyl erythromycin ester lauryl sulfate but not of other forms of erythromycin. At the clinical level, erythromycin and oleandomycin preparations may be considered strictly competitive items.

The value of erythromycin in controlling and preventing spread of epidemic penicillin-resistant staphylococcal infection in hospital nurseries has been demonstrated (315, 316), and it has been considered a satisfactory substitute for penicillin prophylaxis of group A β-hemolytic streptococcal disease (317). A study of more than 12,000 newborn infants demonstrated that erythromycin ointment (5 mg./Gm.) is equally as satisfactory as silver nitrate as a prophylactic against ophthalmia neonatorum and is much less irritating (318). Numerous reports dealing with substantial numbers of patients comment favorably also on the clinical value of oleandomycin in treating infections caused by Gram-positive organisms (319–323). There is no question that both are useful drugs. However, they cannot be considered equivalent.

Many claims for the superiority of triacetyloleandomycin have been made on the basis of the relatively high blood levels it produces, reportedly from 80 to 140% higher than oleandomycin (324) and greatly in excess of those produced by erythromycin. However, evaluations made in terms of antibacterial activity of serum rather than actual content of antibiotic reveal "no significant difference (between triacetyloleandomycin and erythromycin) . . . in the ability to inhibit bacterial growth" (325) and that the "somewhat greater (four- to fivefold) blood levels of oleandomycin did not compensate for the marked difference in antibacterial activity" (326). Erythromycin is from 4 to 10 times more active than oleandomycin against strains of pneumococci, streptococci, and staphylococci sensitive to both (326), a view supported by others (34, 42, 327). It has been pointed out that the propionyl ester of erythromycin is absorbed more consistently than the base, and that the serum levels achieved with the ester compare favorably with those attained with triacetyloleandomycin and that "in terms of antibacterial activity of the subjects' serum . . . . propionyl erythromycin gave effective serum levels that were more than 10 times as high" (326). Objective studies have led to the conclusion that "oleandomycin and spiramycin are sufficiently inferior to erythromycin to indicate that their adoption for general use in the treatment of infections is unwarranted and should be discouraged" (36, 37). Entirely apart from the question of efficacy is the matter

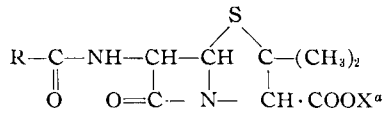
of resistance. Since cross resistance occurs between oleandomycin and erythromycin, one is faced with the situation that extensive "use of an inferior drug (oleandomycin) can deprive a better one of its usefulness" (328).

Many claims of synergism between oleandomycin and other antibiotics have been made, the most enthusiastic claims being made for a 2:1 mixture of tetracycline and oleandomycin (Signemycin, Pfizer). The view has been expressed editorially that the combination is essentially a "nostrum" foisted on an unsuspecting medical public to create a market for an "inferior agent," oleandomycin, by using it to dilute or contaminate "an established antibiotic, tetracycline," and that much of the advertising copy "exaggerates the truth and all too often only distorts it and renders it meaningless when inferior wares are being peddled" (329). When the claim for synergistic action of the mixture was shown to be misleading, advertising emphasis shifted to the claim that use of the mixture tended to retard emergence of resistant bacterial strains from initially sensitive ones. However, carefully controlled studies have failed to substantiate this claim and have shown that, after exposure to the mixture, organisms, often are resistant not only to oleandomycin but to tetracycline also, just as if they had been exposed to it alone (36, 37, 42).

Modified Penicillins

Any procedure which violates the integrity of the β-lactam or thiazolidine rings of the accompanying structure destroys the antibacterial activity of the molecule. Therefore, since the molecule is unstable under the conditions of many chemical procedures, efforts to produce biologically active new types of penicillin were seriously hampered and, until recently, the only successful attempts were those in which various precursors were added to the culture substrate with the hope that the mold would incorporate the compound or a moiety of it into the R group and produce a new form of penicillin. More than three dozen "unnatural" penicillins were biosynthesized in this way, and the structures and antibacterial activity of many of them have been summarized (4).

Synnematin B.—Synnematin, a product isolated in 1951 from *Cephalosporium salmosynnematin* (330, 331), is a mixture of two compounds (332), one of which, synnematin B, was later shown to be identical with cephalosporin N and to be a previously unknown biosynthetic penicillin, namely, D-4-amino-4-carboxy-*n*-butyl penicillin (333). Another source of synnematin B is



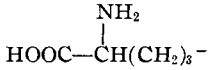
Type of Penicillin	R
Benzyl (penicillin G)	$-CH_2-C_6H_5$
D-4-Amino-4-carboxy-*n*-butyl (synnematin B, cephalosporin N)	$HOOC-CH(NH_2)(CH_2)_3-$
Phenoxymethyl (penicillin V)	$-OCH_2-C_6H_5$
Phenoxyethyl (phenethicillin)	$-O(CH_2)_2-C_6H_5$
2,6-Dimethoxyphenyl (methicillin, BRL-1241)	$-C_6H_3(OCH_3)_2$
Phenylmercaptomethyl	$-S-CH_2-C_6H_5$

[a] Depending on the type of penicillin, X in the commercially available products may be H, K, Na, or procaine.

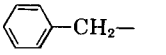
Emericellopsis terricola var. *glabra*, a member of the *Aspergillaceae* (334, 335).

Synnematin B is unique among other known penicillins because of its effectiveness against the *Salmonella* and *Proteus* groups of organisms (336–343), including typhoid and paratyphoid A infections in man (343). This activity against important Gram-negative pathogens, the low degree of toxicity (comparable to conventional penicillins), and other pharmacologically favorable characteristics (344) should engender considerable interest in this antibiotic. In a single trial, it was found curative in primary syphilis (345).

Chemical studies (330, 346, 347) and assay techniques (334, 344) have been recorded. The fact that such a pronounced change in antibacterial spectrum is produced when the



group found in synnematin B is substituted for the



group of penicillin G suggests interesting leads for further study, now that techniques for producing semisynthetic penicillins have been developed.

Semisynthetic Penicillins.—In 1957, after many years of unsuccessful attempts, Sheehan

succeeded in total synthesis of an antibacterially active penicillin (348). The product was phenoxymethyl penicillin, a compound which had been produced experimentally in the United States about ten years before by the biosynthetic technique of adding precursors to the culture substrate of the mold but had been passed over as an item for industrial production until investigators in Europe demonstrated that its acid-stability made it particularly useful for oral administration (349–353). Phenoxymethyl penicillin, better known as penicillin V, is distributed as Pen-Vee-Oral (Wyeth) and V-Cillin (Lilly).

Sheehan's work was a chemical triumph and stands as a monument to man's pertinacity in his attempts to understand and duplicate the products of nature and then to improve on them. The methods developed permitted synthesis of penicillins not previously known, but the techniques were complicated, the yields were low, and none of the compounds produced proved sufficiently superior to biosynthetic penicillins to warrant industrial production. However, it was recognized that if one of the intermediates, 6-aminopenicillanic acid, could be obtained in sufficient quantity, synthesis from that point on should not be prohibitively expensive and that the possibilities for synthesis of new compounds should be almost limitless.

British workers observed that 6-aminopenicillanic acid occurs in crude penicillin fermentation media, presumably the mold also uses this as a building block in biosynthesizing penicillin, and they found a means of stopping the fermentation at that point and removing the moiety in reasonable yield by ion exchange (354). Synthetic methods can be employed to attach various acidic and other side chains to the naturally produced 6-aminopenicillanic acid, and more than 500 such semisynthetic penicillins have now been produced (355). The first of these to be exploited commercially was the potassium salt of a mixture of the D and the L isomers of 6-(α-phenoxypropionamido)penicillinate, commonly known as phenoxyethyl penicillin and assigned the generic name phenethicillin. Others that are being studied are sodium 6-(2,6-dimethoxybenzamido)penicillinate monohydrate and phenylmercaptomethyl penicillin.

Phenethicillin is available as Alpen (Schering), Broxil (Beecham), Chemipen (Squibb), Darcil (Wyeth), Dramcillin S (White), Maxipen (Roerig), and Syncillin (Bristol). It has appeared in the literature also as PA 228, BRL 152, P 152 DL, and as penicillin 152. The dimethoxybenzamido derivative is available as Celbenin (Beecham) and Staphcillin (Bristol) and has appeared in the literature also as BRL-1241 and, more recently, as methicillin.

Synthesis and physical comparison of the diastereoisomers of phenethicillin have been reported (356). The diastereoisometric mixture is more active than the individual components against sensitive organisms and has an antibacterial spectrum similar to that of penicillin V (357). Pharmacologically (358) and in clinical practice (359, 360), the two analogs of phenethicillin appear similar. Presence of food in the upper gastrointestinal tract when phenethicillin is administered orally markedly interferes with absorption—may reduce subsequent serum levels 30 to 60%—and to ensure maximum absorption the drug should be given on a fasting stomach or at least 1 hour before meals (360), although there is some evidence that, despite the lower serum levels achieved when the drug is given after a meal, the levels that are achieved are better sustained (361).

A major advertising claim made for phenethicillin is that, when given orally, it produces much higher blood levels than penicillin V given by the same route and than penicillin G given intramuscularly. It is also claimed that phenethicillin is excreted into the urine in greater amounts than penicillin V. The advertisements fail to point out that the "greater amounts found in the urine . . . produce appreciably less antibacterial activity when assayed against the same standard," that the "higher serum levels are also less active against hemolytic streptococcus and pneumococcus and no more active against *Staph. aureus* than those produced by orally administered penicillin V" (361). Nor is it pointed out that, although the peak concentration attained with oral phenethicillin is greater than that produced by intramuscular penicillin G when assayed in terms of drug given, "the peak is much lower when compared against the same standard and expressed in terms of units of penicillin G" or that the high serum levels attained with the new product are very brief while intramuscular penicillin G produces much more sustained levels and substantially greater urinary recovery (361). It has been pointed out that "thus, although the claim of better absorption and excretion and higher serum level of phenethicillin may be partly correct, . . . this is true in a very restricted sense and is therapeutically meaningless" and actually "misleading since it clearly implies greater antibacterial and presumably curative activity, which, in fact, the drug does not possess, at least in any broad sense of the various

uses to which penicillin is put" (361). In the same study it was shown that phenylmercaptomethyl penicillin, which produces lower serum levels and is excreted in a smaller proportion in the urine provides "substantially and significantly greater antibacterial activity from equivalent doses given by mouth, fasting or after a meal, by any of the standards of comparison that were used" (361). In comparison with phenethicillin and penicillin V, phenylmercaptomethyl penicillin, whether given before or after a meal, produced the highest levels of antibacterial activity despite the fact that the concentrations in the serum were lower and a smaller proportion was recovered from the urine. As pointed out earlier in this paper, the concentration of an antibiotic in the serum is not alone a significant statistic for evaluation of its clinical value. Of equal importance is the activity of the drug against the specific pathogen to be controlled and, unless some uniform standards are applied, comparisons based on serum level, amount recovered in the urine, etc., are quite meaningless in any practical or therapeutic sense.

Others, in a comparative study of penicillins G, V, phenethicillin, and 6-(2,6-dimethoxybenzamido)penicillinate monohydrate (henceforth designated methicillin), also have drawn attention to "the quite large differences in the range of their antibacterial activity" and to the fact that "it is . . . no longer possible to treat all penicillins as though they were equivalent" (362). This situation is going to become more complicated and more confusing as the number of semisynthetic penicillins on the market multiplies unless some uniform standards of reference are adopted.

Methicillin attracts special interest because of its uniformly (to date) good performance against staphylococci resistant to penicillin G and the other penicillins currently available (362–370). It is "both stable and active in the presence of staphylococcal penicillinase" (368), although it may act as a penicillinase inducer and after 24 to 48 hours of growth of the organisms suffer some loss of activity (365). However, attempts to induce strains of *Staphylococcus aureus* to become resistant to methicillin have failed and *Staph.* strains reisolated from patients receiving the drug showed no change in the minimum inhibitory concentration (365). A novel proposal for controlling staphylococcal cross infection in hospitals is the spraying of methicillin into the air to produce droplets which circulate much as the staphylococci do. It has been reported that such a procedure prevented the usual acquisition of pyogenic staphylococci by newborn infants (371).

Methicillin is unstable in acid media; therefore, the oral route is not suitable for systemic medication. Given slowly by deep intramuscular injection, it is no more painful than penicillin G and no local reactions at the injection site have been noted. A dose of 1 Gm. of the sodium salt at 4- to 6-hour intervals has been recommended for adults (368).

A good comparative appraisal of the newer penicillins has been published by Dowling (371a).

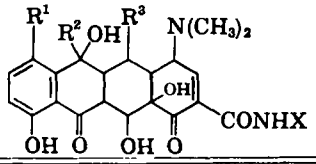
Modified Tetracyclines

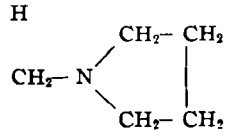
Despite the similarity of structure of members of the tetracycline family listed in the accompanying generalized formula, they are not quantitatively identical antimicrobially or pharmacologically. For example, frequently CTC is more active against staphylococci and pneumococci, OTC against *Pseudomonas pyocyanea*, and TC against *Proteus* (372, 373). The differences may be slight or relatively large, depending on the species and strain of organism involved. However, there is complete cross resistance among the three compounds, whether the organisms are resistant when isolated from clinical material (344) or are induced by exposure to any one of them in the laboratory (375).

Pharmacologic differences are somewhat greater, especially with respect to absorption and excretion and to gastrointestinal side effects. TC generally produces higher and more prolonged antibiotic activity than OTC and CTC in the blood (376), and it is less likely to induce severe gastrointestinal disturbance, especially the acute, and sometimes fatal, staphylococcal enterocolitis which may follow treatment with CTC or especially OTC (377–380). Principally because of its supposed pharmacologic advantages, TC has largely replaced CTC and OTC in clinical practice.

Demethylchlortetracycline (Declomycin, Lederle).—The demethylchlortetracyclines, originally produced in 1957 by chemical manipulation of TC (381–383), constitute a group of derivatives with greater acid- and alkali-stability than the corresponding methylated compounds. Subsequently, 6-demethyltetracycline and 7-chloro-6-demethyltetracycline (DMCT) were found among the metabolic products of a mutant strain of *Streptomyces auriofaciens*, the organism from which CTC was obtained.

One of these, DMCT (available as Declomycin, Lederle), was found to be, on the average, about twice as active as TC against many pathogens and rarely less active, to be readily absorbed following oral administration, and to be excreted



Drug	Symbol	R^1	R^2	R^3	X	Year Discovered
Chlortetracycline	CTC	Cl	CH_3	H	H	1948
Oxytetracycline	OTC	H	CH_3	OH	H	1950
Tetracycline	TC	H	CH_3	H	H	1953
Demethylchlortetracycline	DMCT	Cl	H	H	H	1957
N-(Pyrrolidinomethyl)tetracycline	PMT	H	CH_3	H		1958

about half as rapidly as TC. A thorough and authoritative review of the bacteriologic, clinical, and pharmacologic position of DMCT has been published (384).

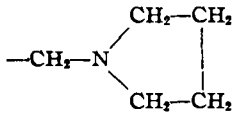
Against some strains of streptococci, DMCT may be almost 5 times as active as TC, although the average figure is about 2.3 (385). Against some resistant staphylococci, it may show somewhat lesser relative activity (386).

Presumably because of the lower rate of excretion of DMCT, therapeutic serum levels are maintained for a longer time after administration of this antibiotic than after an equivalent dose of TC. Therefore, satisfactory therapy can be achieved with DMCT given in smaller, or in less frequent, doses. DMCT, like its analogs, is concentrated in the liver and excreted into the bile, where the concentration may be up to 30 times that occurring in the serum at the same time (387).

Toxicity of DMCT appears to be no greater than that of its earlier analogs. In moderate doses, its tendency to cause diarrhea is minimal, and in therapeutically equivalent doses DMCT probably tends to produce slightly less, certainly not more, gastrointestinal side effect than TC.

A curious phototoxicity not observed in patients receiving other TC analogs has occurred in some subjects exposed to bright sunlight while under treatment with DMCT. The reaction manifests itself as an exaggerated sunburn with high fever, eosinophilia, and increased blood platelets. The effect occurs only on skin exposed to rays in the 2,700 to 3,200 Å. range, which is filtered out by ordinary window glass, and appears to be a true phototoxicity, not merely a hypersensitivity phenomenon (384, 388-390). The incidence is not definitely established. One observer noted 4 cases among 27 ambulatory patients (389) while another reported 40 instances among 2,682 patients (390).

Pyrrolidinomethyltetracycline (Syntetrin, Bristol). — N - Pyrrolidinomethyltetracycline (PMT) is prepared, by partial synthesis, from TC by replacing one hydrogen of the NH_2 group of the latter with



The dry compound is reasonably stable—loss of potency 15% in 4 months at 56°, 18% in 1 year at 37°, and 7% in 18 months at 25° (391). In solution, it may retain full potency for 1 or 2 days at room temperature. The compound is soluble in water, and concentrations of 1 Gm./ml. can be prepared over the pH range 1.5 to 8.5. Its chemical and physical properties have been reviewed (392) and abstracted (393).

Qualitatively and quantitatively, PMT is similar to TC in antibiotic spectrum. The principal advantage claimed for the derivative over the parent compound is greater solubility in the physiologic pH range (394). For systemic use it is given parenterally, and it may be a useful addition to the armamentarium for the patient who needs tetracycline therapy but cannot (or will not) accept oral medication. It is claimed to provide higher blood levels than TC and to cause less pain and irritation at the injection site (391), and to provide better therapeutic effects and fewer side effects, especially gastrointestinal, than oral TC (395–397). It is interesting that, despite the claim of less pain at the injection site, in preparing an intramuscular dosage form of PMT a local anesthetic (lidocaine, 40 mg./350 mg. PMT) has been added. Both the intramuscular and the intravenous dosage forms contain ascorbic acid.

One intravenous injection of 275 mg. per day for 4 to 7 days is normally adequate for average

infections due to sensitive organisms. Severe infections or those caused by less sensitive strains may require 2 to 4 injections daily. In children, 5 to 25 mg./Kg. is the normal dose range.

Intramuscular doses are given by deep injection into the upper outer quadrant of the gluteal muscle. Injection into dermal or subcutaneous tissue must be avoided. Injection in such tissues may cause unnecessary pain and tissue reaction. In intravenous administration, extravenous spillage must be avoided for the same reason.

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Research Articles

Synthesis and Pharmacological Studies of Some Aliphatic Hemicholinium Analogs

By MARVIN F. POWERS, STEFAN KRUGER, and FRED W. SCHUELER

The aromatic nucleus of hemicholinium-3 was replaced with an aliphatic hexamethylene chain without loss of the characteristic pharmacological activity. The toxic dose was elevated in mice about tenfold by the change. Choline chloride was a very effective antidote to intoxication from the aliphatic hemicholinium. Another compound synthesized, a pyridine analog, had marked anticholinesterase activity, but caused a flaccid paralysis in avian musculature. A third compound, a trimethylamine analog, elicited neuromuscular blockade typical of decamethonium.

HEMICHOLINIUM-3, hereafter called HC-3, was synthesized in 1954 during an investigation of a series of bis-quaternary ammonium compounds (1). The general pharmacology of HC-3 has recently been reviewed by Schueler (2). Marshall and Long (3) synthesized and investigated pharmacologically some hemicholinium analogs and found none to be more potent than HC-3. The analogs studied by these workers contained structural changes at the cationic heads and in the biphenyl nucleus. It appears that the crucial moiety is at the cationic head rather than at the biphenyl grouping, since the introduction of an ether or a methylene linkage between the two phenyls elicited only a slight decrease in potency, whereas certain relatively minor changes at the cationic heads either markedly reduced or abolished HC-3-like activity (3). Considering the alterations already studied, one may question whether the aromatic nucleus is an essential feature in the HC-3 molecule. The introduction of a methylene chain would not only incorporate an aliphatic moiety, but would also provide an approach for a systematic study of the effect on pharmacologic activity

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