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-a Recent Advance in Aminoglycoside Therapy

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The Aminoglycosides: A Resumé of their Development

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Antibiotics have two family trees. The roots of the first lie in the moulds and, although the background of this family is quite fascinating (Selwyn, 1980, 1982), I shall not pursue it further. My concern here is with the second family which has its roots firmly among the bacteria (Fig. 1). The higher bacteria—the *Streptomyces*

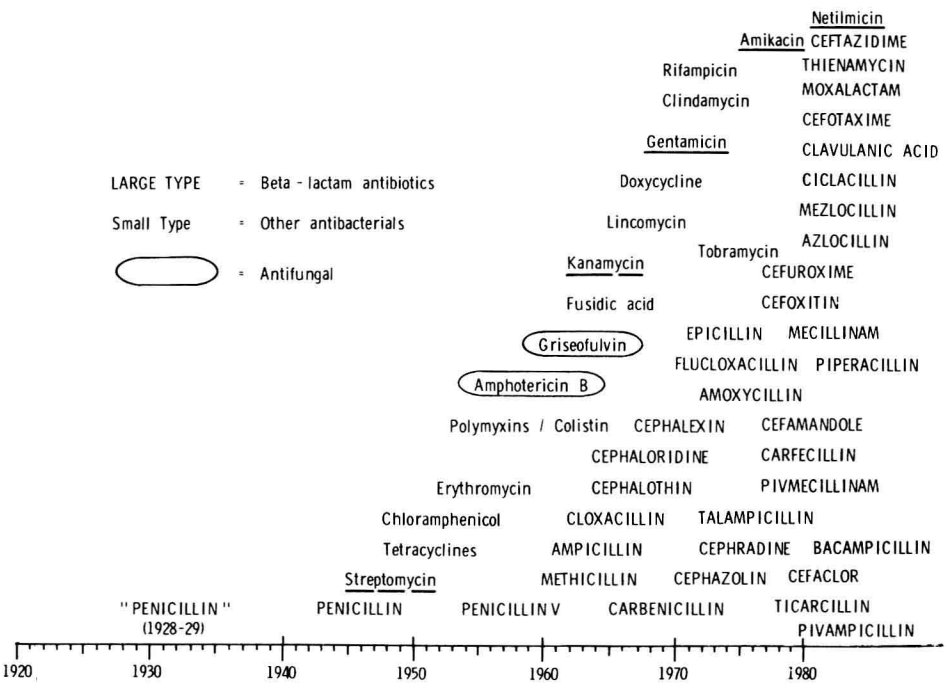

Figure 1. The evolution of our antibiotics.

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group of actinomycetes—have produced a remarkably wide range of antibiotics, quietly and with very little publicity.

The story began over 65 years ago with the work of Selman Waksman (Waksman and Curtis, 1916), a Russian émigré, at Rutgers University in New Jersey. He was aware, as he subsequently reported in a number of papers in obscure soil journals, that antibiotics were produced by bacteria in the soil. He often proposed that these could be used clinically, but he was a soil bacteriologist and the message fell on deaf ears.

By 1939 a French Canadian, René Dubos, inspired by the work of Waksman, his teacher, rather than that of Fleming, was deliberately looking for antibiotics from soil bacteria. By chance, he discovered a species of aerobic spore-bearing bacillus from which he produced a number of antibiotics that are still used topically—notably gramicidin and tyrocidine. The first of these became commercially available in 1941, but these antibiotics were far too toxic to be given systemically.

Waksman himself had meanwhile isolated the first of a series of antibiotics from his beloved actinomycetes. At the outset he obtained, in 1940, a toxic agent, actinomycin, which was quite effective against Gram-positive bacteria and fungi. This was followed in 1942 by the isolation of streptothricin, which extended the spectrum of activity to Gram-negative bacteria; but this antibiotic also was too toxic for systemic use.

Unfortunately for Waksman and for the world, the species of *Micromonospora* he was studying at that time in parallel with the actinomycetes produced only very low activity antibiotics; had fate decreed otherwise, gentamicin and many other important drugs might have appeared sooner, as they all come from that very genus. And this in itself is curious—not being a *Streptomyces* species, the organism is an outsider.

Streptomycin

By 1944 Waksman had isolated streptomycin and shown it to be active against a wide range of Gram-positive and Gram-negative bacteria (Schatz *et al.*, 1944; Fig. 2). Shortly afterwards the main use for streptomycin was proved to be in the treatment of tuberculosis. By 1947 the structure of the new antibiotic had been worked out. It was an aminosugar at one end and an aminocyclitol at the other. Thus "aminoglycoside" is incorrect terminology; the antibiotics should strictly be called "aminoglycosidic aminocyclitols" since all are mixtures, apart from spectinomycin (a pure aminocyclitol) and a therapeutically unimportant antibiotic, kasugamycin (a pure aminoglycoside).

The fact that resistance to streptomycin rapidly developed and was clinically important was shown by Feldman and others within a few years. Fortunately, para-aminosalicyclic acid (PAS) had already been discovered in 1946 by Lehmann, and in 1952 the Squibb company introduced isoniazid. These two drugs saved the day for streptomycin.

Neomycin and Kanamycin

Five years after the isolation of streptomycin, Waksman and Lechevalier (1949) published a paper on neomycin which they found to be active against streptomycin-

Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria.*†

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With the exception of streptothricin,[1] most of the antibiotic substances known at the present time, including penicillin and other mold products as well as gramicidin and actinomycin, act largely upon gram-positive bacteria. The activity of these substances upon gram-negative organisms is highly selective, as in the case of penicillin, which affects the *Neisseria* group and has little activity upon *Escherichia coli* and other gram-negative bacteria,[2] or else much larger quantities are required to bring about the inhibition of these bacteria, as in the case of actinomycin.[3] Among the antibiotic agents that act selectively alike against both gram-positive and gram-negative bacteria, streptothricin occupies a prominent place; since this substance is water-soluble and possesses limited toxicity to animals, it is of particular interest from a chemotherapeutic point of view. Unfortunately, streptothricin has very little activity against a number of bacteria found among both the gram-negative (*Pseudomonas fluorescens, Ps. aeruginosa*) and the gram-positive (*Bacillus mycoides* and *B. cereus*) groups.

In a search for antagonistic organisms that are active against gram-negative bacteria, and from which antibiotic substances could be isolated, the actinomycetes were found[4] to offer extensive potentialities. Although most of the antibacterial agents produced by these organisms are also active against gram-positive bacteria, certain few of them exert a marked selective activity against many of the gram-negative types of bacteria. *Actinomyces lavendulæ*, which produces streptothricin, is such an organism. After detailed examination of a large number of cultures, either isolated at random from different natural and enriched soils and composts, or selected from the culture collection, another organism was found that produces an antibiotic substance which apparently combines many of the desirable antibacterial properties. This organism is similar, in most of its cultural characteristics as well as in its morphology,

* Journal Series paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

† With partial support from a grant made by the Commonwealth Fund of New York.

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2 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A., Heatley, D., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **241**, 177; *Nature*, 1942, **148**, 758; **149**, 356.

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Figure 2. The 1944 paper which announced the original isolation and features of streptomycin (Schatz et al., *1944).*

resistant organisms. However, not only was it nephrotoxic, but it was also more ototoxic than streptomycin. Consequently, its systemic administration was regarded as dangerous. Nevertheless, 10 years later, C. W. H. Havard, Professor Paul Garrod and Pamela Waterworth (Havard *et al.*, 1959) were faced with a therapeutic dilemma that is still familiar, even today—though fortunately not in such extreme terms as is evident from the title of their paper—"Deaf or dead?" (Fig. 3). A patient with infective endocarditis was dying and they put this question to him: would he rather face certain death or take the chance of living though he would almost inevitably become deaf. He chose to live but did indeed become stone deaf. The tragedy of this case is that 2 years previously kanamycin had been isolated from a new species of *Streptomyces* in Japan (Umezawa *et al.*, 1957), but did not become available until the following year, 1960. This would have treated the endocarditis very satisfactorily and although there might have been a minor degree of ototoxicity in view of the large doses required, the deafness would not have been complete.

DEAF OR DEAD?

A CASE OF SUBACUTE BACTERIAL ENDOCARDITIS TREATED WITH PENICILLIN AND NEOMYCIN

BY

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The following is the history of a patient who had to be warned that the treatment necessary to save his life would probably cause severe deafness. He accepted this consequence, and suffered it, but he is alive and well nearly a year later. The history also illustrates the value of tests of combined antibiotic bactericidal action in difficult cases of bacterial endocarditis.

Figure 3. The dilemma of choosing between toxicity and efficacy with the earlier aminoglycosides (Havard et al., *1959).*

Gentamicin

In 1963 Marvin Weinstein, another of Waksman's colleagues, who was now working in the Schering Laboratories, announced the discovery of gentamicin (Weinstein *et al.*, 1963), describing it as a drug produced by *Micromonospora purpurea*—a close relative of the species that had produced the worst yield for Waksman. The "micin" part of its name (and subsequently that of sisomicin and netilmicin) indicates its origin from an organism outside the strepto*myces* group. Interestingly, the original proprietary name Garamycin (with its confusing "y") was given by Weinstein in honour of his son Gary. What was most important was gentamicin's activity against *Pseudomonas aeruginosa;* and minimal inhibitory concentrations (MICs) of less than 1 μg/ml were reported, as compared with the much higher levels required of the other aminoglycosides. Thus, although weight-for-weight gentamicin was more toxic than many other drugs, it could still be used because of its greater activity. Weinstein also showed that the effect of serum on the activity of gentamicin was minimal.

The molecular formula of gentamicin was rapidly worked out and the drug was shown to be comprised of at least three components, C_1, C_2 and C_{1a} being the essential ones. By 1967, when it was released for free use, gentamicin was able to provide valuable protection against Gram-negative organisms as well as many other bacteria, such as *Staphylococcus aureus*—but resistance was a constant threat.

Amikacin and Tobramycin

Considerable attention was paid to finding new drugs. Amikacin, a modification of kanamycin, was announced in 1972, again from Japan (Kawaguchi *et al.*, 1972). Work in America and elsewhere rapidly vindicated this drug as a very useful addition to the aminoglycosides. Its value lies in the fact that the inactivating enzymes that destroy the gentamicin and tobramycin molecules do not always damage the amikacin molecule.

Tobramycin was originally isolated as "factor 6" of the nebramycin groups of drugs (Wick and Welles, 1968). Except for a modest increase in activity against pseudomonads, tobramycin has nothing to offer over gentamicin; and because of the problem of cross-resistance, I do not regard it as an important advance in aminoglycoside therapy.

Sisomicin and Netilmicin

Sisomicin is a naturally occurring analogue of gentamicin C_{1a} isolated from a separate species of *Micromonospora* (Weinstein *et al.*, 1970). Weinstein had originally intended naming the new antibiotic "Ricamicin" after Richard, a brother of Gary! When this was not approved the name "sisomicin" was derived from the last vowel of the species title *Micromonospora imyoensis*. It appears to have no consistent advantage over gentamicin, but owes its importance to the fact that its N-alkyl derivative is netilmicin—whose name is a contraction of N-ethylsisomicin. The first paper on netilmicin (Fig. 4) came from the Schering Corporation in March

Synthesis of 1-*N*-Ethylsisomicin: A Broad-spectrum Semisynthetic Aminoglycoside Antibiotic

By J. J. Wright

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Summary Site-selective reductive *N*-alkylation of aminoglycoside antibiotics related to gentamicin is described and the structure of the products and of related compounds is unambiguously established using circular dichroism and mass spectrometry.

The aminoglycoside antibiotics are important therapeutic agents because of their activity against gram-negative bacteria not readily susceptible to other antibiotics. Their widespread clinical use has led to a growing number of resistant bacterial strains, whose resistance usually results from enzymic modification of the antibiotic. In the case of sisomicin (**1**),[1] such modes of inactivation include acetylation of one of the amine groups attached to carbons 2′, 6′ and 3, and adenylylation of the hydroxy group attached to carbon 2″.[2] There is thus considerable importance attached to the preparation of aminoglycoside antibiotics which are active against a high proportion of these resistant organisms. Amikacin, the 1-*N*-(*S*)-4-amino-2-hydroxybutyryl derivative of kanamycin A has been demonstrated to possess such desirable properties.[3] We now report the novel synthesis, from the gentamicin–sisomicin class of antibiotics, of 1-*N*-alkyl derivatives which also possess an improved resistance spectrum.

We have found that the relative reactivity of the amine groups of the gentamicin antibiotics towards reductive alkylation in the presence of an aldehyde and a hydride-donor reducing agent is pH-dependent. Although the C-6′

Figure 4. The first announcement of the semisynthetic production of netilmicin (Wright, 1976).

1976 and showed that the drug had a number of important advantages over sisomicin and other aminoglycosides (Wright, 1976). The next month another paper was published describing the antibacterial activity of netilmicin (Rahal *et al.*, 1976). Against 171 isolates of Enterobacteriaceae, *Staph. aureus* and *Ps. aeruginosa*—including those resistant to gentamicin—it showed markedly greater activity than either gentamicin or amikacin. No hint was given of anything special in terms of clinical or toxicological effects. Then in November 1976 Weinstein and his colleagues described these other aspects (Miller *et al.*, 1976). We shall consider these different features of the new antibiotic during this conference. We may then be able to decide whether or not netilmicin is a worthy culmination of almost 40 years of aminoglycoside research (see Fig. 1).