Antimicrobial Activity of Neomycin C Against
*Staphylococcus epidermidis*

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In vitro studies indicate that neomycin C is 50% as active as neomycin B against *Staphylococcus epidermidis* (ATCC 12228).

Neomycin, a group of aminoglycoside antibiotics produced by *Streptomyces fradiae* (10), is composed of approximately five components of varying antimicrobial activity (4). Neomycin B, the major component, is the most active, with antimicrobial activity decreasing in order of neomycin C to neamine (5). Low potency B and C (LPB and LPC) are mono-acetyl derivatives of neomycins B and C which show essentially no activity (W. S. Chilton, Ph.D. Thesis, University of Illinois, Urbana, 1963). Since the bioactivity of neamine, LPB, and LPC is extremely low or nonexistent, they are not considered in the calculation of biological potency by gas-liquid chromatography (8). Neomycins D, E, and F may also be present in commercially produced samples of neomycin (3). However, because of their extremely low percentage, they also are eliminated from the potency calculation by gas chromatography.

The ratio of antimicrobial activity of neomycin B to C varies with the species of organism tested (2) and the conditions of the microbial assay system (6). Bioassay results for production neomycin sulfate powders, which contain various amounts of neomycins B and C, give low values for total neomycin content since they are compared to the USP Neomycin Reference Standard, Issue I, which is 99% neomycin B. This difference in response, however, is not seen with the gas-liquid chromatographic method (7) which gives equal response to both isomers.

For comparison of gas chromatographic results with microbiological potency determinations, it is necessary to use a conversion factor for neomycin C.

The response of neomycin C against *Staphylococcus aureus* (ATCC 6538P) was previously reported by Tsuji et al. (8) to be one-third that of neomycin B. When the one-third response factor was used to calculate the drug content of neomycin sulfate powders assayed by gas-liquid chromatography, the calculated potency correlated well with values obtained microbiologically.

The Code of Federal Regulations (1) lists *S. epidermidis* (ATCC 12228) as the preferred assay organism for neomycin, and therefore the appropriate response factor of neomycin C against this microorganism had to be determined if the gas chromatographic assay method was be used for the routine determination of neomycin in pharmaceutical products (9).

Neomycin sulfate USP lot I (assumed to be 100% pure neomycin B) and neomycin C sulfate 8246-DNR-58 (90.5% pure, with no neomycin B present, as determined by gas chromatography) were dried in a vacuum oven at 60°C at <5 mm
of Hg for 3 hr. After drying, the samples were immediately weighed into 10-ml volumetric flasks in various ratios of neomycin B to C at a final concentration of 4 mg of neomycin sulfate per ml. The samples were brought to volume with 0.1 M (pH 8.0) potassium phosphate buffer. Appropriate dilutions with 0.1 M (pH 8.0) potassium phosphate buffer were then made of these solutions, and the samples were assayed with the method described in the Code of Federal Regulations, Title 21, for the assay of neomycin sulfate (1).

Three individual assays performed on each of two different days were used to calculate the microbiological response of neomycins B and C. The total potency was calculated assuming a 50% microbiological response with a corrected potency of 90.5% for neomycin C.

The calculated microbiological response (micrograms per milligram) against S. epidermidis (ATCC 12228) was shown to be approximately linear over the range of 0 to 100% neomycin C (Fig. 1). The slope and intercept (1.05 and -0.03) were found not to be significantly different from 1.0 and 0.0, respectively. For a 50% response, a slope of 1.0 is needed. Essentially all of the neomycin was recovered, since an intercept of 0.0 would indicate complete recovery.

On the basis of the data presented, we have assigned a response factor of 50% to neomycin C for use in the calculation of microbiological equivalency of potencies determined by gas chromatography.

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LITERATURE CITED