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Binding of neomycin and analogues by fatty acids *in vitro*

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SYNOPSIS Neomycin forms insoluble complexes with long-chain fatty acids *in vitro*. This reduces the diffusion of neomycin but does not directly neutralize its antibiotic activity. Possible effects *in vivo* are discussed.

The activity of antibiotics *in vivo* may be reduced by their interaction with constituents of blood or tissues, for example, the protein binding of penicillin and the binding of neomycin to mucus (Saggers and Lawson, 1966). During a study of the action of antibiotics on cutaneous bacteria *in vitro*, neomycin was found to precipitate long-chain fatty acids of the type present in skin. As neomycin and its analogues are used mainly topically and orally, the reaction with fatty acids could occur in both these sites. The purpose of this paper is to describe an investigation of reactions *in vitro* between fatty acids and several aminoglucoside antibiotics.

METHODS AND RESULTS

As long-chain fatty acids are almost insoluble in water, the more soluble sodium salts were used. These were added in aqueous solution to a solution of neomycin sulphate (Boots Pure Drug Co.) in phosphate buffer. The final concentration of acid was 0·3 g/100 ml, of neomycin base 0·1 g/100 ml, and buffer M/10, *p*H 7·4. After incubation at 37°C for 60 minutes, the mixtures were vigorously shaken and 0·10 ml aliquots were assayed for neomycin activity. A cup diffusion method, based on that of Grove and Randall (1955), was used, with the Oxford staphylococcus as the test organism. A standard curve was obtained by assaying neomycin sulphate in the buffer without fatty acids.

On adding neomycin to solutions containing fatty acids with 12 or more carbon atoms, there was an immediate precipitate, followed within 60 minutes by coarse flocculation. Short-chain acids did not precipitate. Assay of the antibiotic in mixtures showed marked loss of neomycin activity in those in which a precipitate had formed, but not in the others (Table I).

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TABLE I

EFFECT OF INCREASING CHAIN LENGTH ON FATTY ACID INACTIVATION OF NEOMYCIN SULPHATE, IN M/10 PHOSPHATE BUFFER, *p*H 7·4

Fatty Acid (0·3 g/100 ml) and No. of Carbon Atoms	*Precipitation with Neomycin Sulphate*	*Percentage Activity of Neomycin Sulphate (0·1 g/100 ml) in Presence of Various Fatty Acids*
Control (no fatty acid added)	—	100
Acetate (2)	—	100
Propionate (3)	—	100
Butyrate (4)	—	100
Hexoate (8)	—	100
Octoate (10)	—	100
Laurate (12)	++	4
Myristate (14)	++	7
Palmitate (16)	++	10
Stearate (18)	++	20
Oleate (18)	++	24
Linoleate (18)	++	18
Linolenate (18)	++	28

To determine whether the loss of neomycin activity was due to impaired diffusion of the antibiotic or to actual inactivation of it, sets of nutrient agar plates were prepared containing a range of neomycin concentrations (0·1 to 6·4 μg/ml) and a constant concentration of one or other of three fatty acids. As a control, a fourth set of plates was prepared without fatty acid. Segments of the plates were inoculated with six strains of *Staphylococcus aureus* and the minimum inhibitory concentration (MIC) of neomycin was read after incubation for 20 hours at 37°C. Similar experiments were performed with other aminoglucosides. The results are summarized in Table II. The presence of fatty acid in the medium had no significant effect on the MIC of the antibiotics. The loss of activity in the previous diffusion experiments was therefore probably due to binding without inactivation of the drug.

To compare the behaviour of aminoglucosides

TABLE II

RANGE OF MINIMAL INHIBITORY CONCENTRATIONS OF AMINOGLUCOSIDE ANTIBIOTICS FOR SIX SENSITIVE STRAINS OF STAPHYLOCOCCUS AUREUS[1]

Antibiotic (as Sulphate)	*Control (no fatty acid added)*	*Palmitate + 0·2% (w/v)*	*Oleate + 0·2% (w/v)*	*Stearate + 0·2% (w/v)*
Neomycin	0·8-1·6	0·4-1·6	0·4-1·6	0·4-1·6
Kanamycin	1·6-6·4	1·6-6·4	3·2-6·4	1·6-3·2
Gentamicin	0·1-0·4	0·1-0·4	0·1-0·4	0·1-0·8
Framycetin	0·4-0·8	0·4-0·8	0·4-0·8	0·4-1·6
Paromomycin	0·4-0·8	0·8-1·6	0·4-0·8	0·2-0·8

[1]Antibiotics dispersed in agar, with and without fatty acids; results in μg/ml.

with some other antibiotics in the presence of fatty acids, nutrient agar plates were prepared containing fatty acids (0·5 g/100 ml), adjusted to *p*H 7·4 and seeded with *Staph. aureus*. Antibiotic discs (Mast) were placed on the plates, and after incubation for 20 hours at 37°C, zones of inhibition were measured (Table III). Experiments were performed with 10 strains of *Staph. aureus*, each of which was sensitive

TABLE III

EFFECT OF INCORPORATION OF FATTY ACIDS IN PLAIN AGAR ON ANTIBIOTIC INHIBITORY ZONES AT *p*H 7·4[1]

Antibiotic Disc	*Radius (mm) of Inhibition around Antibiotic Discs*			
	Plain Agar	*Oleate + 0·5% (w/v)*	*Palmitate + 0·5% (w/v)*	*Stearate + 0·5% (w/v)*
Penicillin G 1·5 U	10·0	11·5	12·0	12·0
Tetracycline 50 μg	8·0	9·5	8·5	9·0
Streptomycin 25 μg	8·0	8·0	7·5	7·5
Neomycin 10 μg	7·5	2·0	1·5	1·5
Kanamycin 30 μg	9·0	6·0	5·0	5·0
Gentamicin 10 μg	8·5	2·5	2·5	3·0
Framycetin 50 μg	10·0	3·5	3·0	3·5
Paromomycin 10 μg	8·0	3·0	3·0	2·5

[1]Each figure is mean for 10 sensitive strains of *Staph. aureus*.

to all the antibiotics. Plates without fatty acids were included as controls. The growth of bacteria was completely inhibited by several acids—hexoate, myristate, laurate, linoleate, and linolenate. Acetate, propionate, butyrate, and octoate had little effect on growth or zone size. Palmitate, stearate, and oleate markedly reduced the size of zones around the aminoglucoside discs. By contrast the zones of inhibition around penicillin and tetracycline were slightly larger than in the control plates, probably because the growth of the organism was slowed by the fatty acid (Cooper and Gillespie, 1952). In similar experiments, it was found that fatty acids in a concentration as low as 0·1 g/100 ml reduced the size of the zone around aminoglucoside discs. In experiments in which the *p*H of the medium was adjusted to 6·0, 7·0, and 8·0 by means of hydrochloric acid and sodium hydroxide, the results were similar, although the size of zones in the control plates was different from those at *p*H 7·4.

DISCUSSION

The above experiments indicate that the loss of activity of these antibiotics in the presence of fatty acids is due to the reduced diffusion of the complex. The union of fatty acids and neomycin *in vivo* could alter the activity of either fatty acids or neomycin; the former may be relevant to neomycin-induced steatorrhoea, and the latter to topical therapy with these drugs.

It is well established that oral neomycin causes steatorrhoea (Jacobson, Chodos, and Faloon, 1960; Hvidt and Kjeldsen, 1963; Rothfeld and Osborne, 1963; Asatoor, Chamberlain, Emmerson, Johnson, Levi and Milne, 1967), which is manifested by increased faecal fat, nitrogen, sodium, and calcium, with reduced carotene and xylose absorption. It has been suggested that bile salts, in particular sodium glycocholate, are precipitated by neomycin (Faloon, Paes, Woolfolk, Nankin, Wallace, and Haro, 1966) but these authors found that oral supplements of bile salts did not correct the steatorrhoea. Moreover, kanamycin caused little precipitation of bile salts at the *p*H of the small bowel contents, yet certainly caused substantial steatorrhoea. The precipitation of bile salts by the antibiotics does not therefore fully explain the steatorrhoea. The ability of the antibiotics to precipitate stearic, palmitic, and some long-chain unsaturated fatty acids, the main components of dietary fat, may also be a causative factor. Faloon *et al* (1966) showed that when neomycin was administered through a tube directly to the ileum, there was less steatorrhoea than when the drug was given by mouth. However, this result might also be explained by the fact that most of the dietary fatty acids are absorbed before they reach the ileum and so would not be available to unite with neomycin delivered there through a tube. The oral administration of 4 g of neomycin daily, on the basis of the *in vitro* assay, could bind about 12 to 15 g of long-chain fatty acids. In six normal subjects, Asatoor *et al* (1967) found that this dose of neomycin increased the faecal fat by about 10 g daily, a figure certainly of the same order as the prediction *in vitro*.

The application of aminoglucoside antibiotics to the skin might result in their union with free fatty acids which make up about 40% of skin surface lipid (Ricketts, Squire, and Topley, 1951). It is

possible that this binding could prolong exposure of the skin to these antibiotics and therefore increase the risk of allergy to them. Clinical evidence of this is seen after topical neomycin therapy. Kirton and Munro-Ashman (1965) claimed that neomycin allergy was due to the prolonged retention of the drug in the skin. The combination between skin neomycin and skin fats might also increase the tendency of neomycin-resistant staphylococci to proliferate. Such resistant strains were first seen in 1960 (Quie, Collin, and Cardle, 1960; Finegold and Gaylor, 1960) and have since become world wide (*Lancet*, 1965; Jevons, John, and Parker, 1966). Topical use of the antibiotics has often been quickly followed by the proliferation and spread of neomycin-resistant strains (Lowbury, Babb, Brown, and Collins, 1964; Rountree and Beard, 1965; Alder and Gillespie, 1967). The persistence of neomycin in the skin after treatment might well be a factor in encouraging the appearance of these strains.

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