Reduction of Serum Cholesterol Concentrations by Neomycin, Para-aminosalicylic Acid, and Other Antibacterial Drugs in Man

By Paul Samuel, M.D., and William I. Waithe, M.S.

It was previously reported that neomycin in daily oral doses of 1.5 to 2 Gm. reduced serum cholesterol levels in man.\textsuperscript{1, 2} An extension of these observations, the effect of other antibacterial drugs, and studies on the mechanisms of action are reported here.

Effect of Antibacterial Drugs on Serum Cholesterol Concentrations

Material and Methods

Fifty-six patients were studied in 112 experimental periods of 2 to 37 weeks. Twenty-seven patients were male and 29 were female, with an age range of 21 to 76 years. Forty patients were hospitalized and 16 were outpatients. Hospitalized patients were maintained on regular hospital diets in which 40 to 45 per cent of the calories were derived from fat. The food intake of the 16 outpatients was uncontrolled, but they were instructed to adhere to their customary diets. Medications known to influence serum cholesterol concentrations or other antibacterial drugs were not given. Patients were weighed weekly, and blood counts, tests of urine, blood urea nitrogen, serum bilirubin, and cephalin flocculation were carried out periodically.

Serum cholesterol concentrations were determined once a week in the fasting state by the method of Zak et al.,\textsuperscript{3} after the precipitation of serum with an alcohol-acetone mixture. Serum phospholipid determinations were carried out by the method of Simonsen et al.,\textsuperscript{4} and the proportion of cholesterol in the alpha- and beta-lipoproteins was measured by the method of Langan et al.\textsuperscript{5} The control serum cholesterol levels, prior to the administration of the drugs, were observed for periods of 6 weeks or longer. When a patient was given different drugs consecutively, an interval of 3 to 12 weeks was interposed, during which cholesterol concentrations returned to control levels, before the next drug was given, except when indicated otherwise. Serum cholesterol concentrations were determined in 48 patients for 3 to 12 weeks after all experimental medications were discontinued.

A preparation containing 70 per cent neomycin base (Myelisulfad* was given orally to 30 patients at daily doses of 1.5 to 2 Gm. in two daily doses for periods varying between 4 and 37 weeks. The doses of medication in this study, referred to as neomycin, represent the weight of Myelisulfad.

The concentrations of esterified cholesterol, phospholipids, and alpha- and beta-cholesterol were determined serially in the serum of 10 subjects during the control period and during oral administration of neomycin. Ten patients were given 60 mg. of neomycin intramuscularly once daily for a period of 3 weeks.

Three patients were given 500 mg. of neamine+ (neomycin A) daily for 5 to 6 weeks. The neomycin molecule can be split into two constituents: neamine and neobiosamine. The neamine moiety retains definite antibacterial properties although the range and potency of its activity is somewhat different from that of commercial neomycin.\textsuperscript{9}

Para-aminosalicylic acid (PAS) was given to 15 patients in 30 experimental periods for 5 to 23 weeks at daily dose levels varying between 2 and 12 Gm. Six subjects were given 2 Gm. of kanamycin§ per day orally, for periods of 3 to 8 weeks. Chlorotetracycline$ (Aureomycin) was given to 6 patients by mouth in daily doses of 1 to 1.5 Gm. for 3 to 6 weeks. A variety of other antibacterial drugs was administered to small numbers of patients (table 1).

Results

The results of the oral administration of neomycin are shown in table 2. All 30 cases

\textsuperscript{*}Supplied by The Upjohn Company, Kalamazoo, Michigan.
\textsuperscript{+}Obtained by courtesy of Dr. Selman A. Waksman and Merck Sharp & Dohme, West Point, Pennsylvania.
\textsuperscript{§}Supplied by the Bristol Laboratories, Syracuse, New York.
\textsuperscript{$}Supplied by the Lederle Laboratories, Pearl River, New York.
showed a fall in serum cholesterol, the mean decrease being 21 per cent. Serum cholesterol level reached a low point after 1 to 3 weeks of neomycin administration and remained there as long as neomycin was administered, returning to control levels 1 to 8 weeks later (figs. 1, 2, and 3).

More complete studies of the serum lipids were made in 10 patients, eight of whom had clinical vascular disease or diabetes mellitus. The concentration of esterified cholesterol and of phospholipids decreased in the serum in proportion to the fall of total cholesterol. The average of the esterified fraction of cholesterol of the 10 patients was 78 per cent of the total during the control period and 77 per cent during the administration of neomycin. The average cholesterol-phospholipid ratio was 1.2, both before and during neomycin administration. The average concentration of beta-lipoprotein cholesterol in the serum was 83 per cent of the total cholesterol during the control period and 82 per cent after the reduction of serum cholesterol by neomycin.

Oral administration of neomycin was combined with other antibacterial drugs in seven instances. The second drug was added after the concentration of serum cholesterol was reduced by neomycin to its low point. Two grams of mandelamine for 2 weeks, 15 Gm. of phthalylsulfathiazole for 5 weeks, 300 mg. of isoniazid for 7 weeks, and 1 Gm. of oxytetracycline for 3 weeks were added daily to the neomycin regimen without influencing the effect of neomycin (fig. 3).

The results of daily intramuscular administration of 60 mg. of neomycin are summarized in table 3. In the 10 patients studied, the daily intramuscular injection of the drug failed to alter the concentration of serum cholesterol (figs. 1 and 3).

No major side effects occurred during the administration of neomycin. Ten of the 30 patients who were given neomycin orally developed temporary, mild diarrhea that was usually controlled easily with tincture of par-goric; in one case neomycin had to be discontinued at the end of 6 weeks. The weight of the patients did not vary more than 2 lbs. in a period of 6 months. The hematopoietic, hepatic, and renal functions were not altered.

Neamine, given by mouth, also reduced serum cholesterol in three patients (table 4 and fig. 2). The results of PAS administration at different dose levels are shown in table 5. Eleven of the 15 subjects had pulmonary tuberculosis. These patients were given 300 mg. of isoniazid

---

**Table 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Daily dose</th>
<th>Number of patients</th>
<th>Weeks of medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalylsulfathiazole*</td>
<td>6 and 12 Gm.</td>
<td>2</td>
<td>3 and 5</td>
</tr>
<tr>
<td>Isoniazid†</td>
<td>300 mg.</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Penicillin‡</td>
<td>1 million units</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dihydrostreptomycin§</td>
<td>2 to 3 Gm.</td>
<td>3</td>
<td>2 to 3</td>
</tr>
<tr>
<td>Oxytetracycline§</td>
<td>1 Gm.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
<td></td>
<td>1 Gm.</td>
</tr>
<tr>
<td>Polymyxin§</td>
<td>150 mg.</td>
<td>2</td>
<td>2 and 3</td>
</tr>
<tr>
<td>Erythromycin‡</td>
<td>1 Gm.</td>
<td>2</td>
<td>3 and 4</td>
</tr>
<tr>
<td>Novobiocin*</td>
<td>1 Gm.</td>
<td>2</td>
<td>3 and 4</td>
</tr>
<tr>
<td>Carbomycin§</td>
<td>1 Gm.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bacitracin§</td>
<td>20,000 units</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Viomycin§</td>
<td>2 to 3 Gm.</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Supplied by the Merck Sharp and Dohme Laboratories.
†Supplied by Eli Lilly and Co.
‡Supplied by Abbott Laboratories.
§Supplied by Pfizer Laboratories.
||Supplied by Parke, Davis and Co.
daily and 1 Gm. of streptomycin intramuscularly twice weekly, in addition to the experimental PAS medication. The administration of isoniazid and streptomycin was maintained during the entire study, including the control periods, and was ultimately demonstrated not to influence serum cholesterol concentrations. At lower dose levels of 2 to 6 Gm., PAS reduced cholesterol levels in one half of 14 experiments; at doses of 8 to 12 Gm., serum cholesterol fell in each of 16 experiments. The concentration of serum cholesterol was lowered 1 to 4 weeks after PAS was started and remained low as long as the drug was given, returning to control levels 1 to 3 weeks after discontinuance of PAS (fig. 4).

In two patients cholesterol levels were measured during the administration of PAS, neomycin and PAS, and neomycin alone (fig. 5). There was no significant difference during these three study periods.

Mild nausea was noted periodically, pri-
Table 3

Effect of Intramuscular Administration of Neomycin (60 mg. Daily) for Three Weeks on Average Total Serum Cholesterol Level (mg./100 ml.) in Ten Patients

<table>
<thead>
<tr>
<th>Patient, age, sex</th>
<th>Diagnosis</th>
<th>Average total cholesterol</th>
<th>Control</th>
<th>Neomycin</th>
<th>p&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.G. 43 M</td>
<td>Cerebrovascular accident</td>
<td>242 ± 26*</td>
<td>260 ± 14*</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>J.M. 35 M</td>
<td>Multiple sclerosis</td>
<td>158 ± 11</td>
<td>168 ± 0</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>M.L. 56 F</td>
<td>Cerebrovascular accident</td>
<td>321 ± 23</td>
<td>303 ± 21</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>R.R. 52 F</td>
<td>Diabetes mellitus</td>
<td>339 ± 26</td>
<td>322 ± 19</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>R.O. 46 F</td>
<td>Cerebrovascular accident, diabetes mellitus</td>
<td>304 ± 27</td>
<td>306 ± 25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>J.G. 44 M</td>
<td>Paraplegia of undetermined cause</td>
<td>264 ± 14</td>
<td>269 ± 20</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>T.D. 58 M</td>
<td>Tabes dorsalis</td>
<td>235 ± 18</td>
<td>237 ± 6</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>M.S. 73 F</td>
<td>Peripheral vascular disease</td>
<td>210 ± 15</td>
<td>207 ± 7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>A.S. 65 F</td>
<td>Paraplegia of undetermined cause</td>
<td>242 ± 13</td>
<td>249 ± 28</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>J.D. 21 F</td>
<td>Paraplegia of undetermined cause</td>
<td>194 ± 12</td>
<td>196 ± 0</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

*Standard deviation.

marily during the use of higher doses of PAS, but no patient’s weight varied more than 2 lbs. during the study. A mild diarrhea, similar in character to that following the use of neomycin occurred in four of 15 patients. This, however, did not influence the reduction of serum cholesterol, and was rapidly controlled. No other side effects were observed.

Kanamycin reduced serum cholesterol levels in three of six patients (table 4). The daily administration of 1 to 1.5 Gm. of chlortetracycline (table 4) resulted in a reduction of average serum cholesterol in four of six patients. An easily controlled diarrhea of the previously described type occurred in one patient (D.F.) during the administration of kanamycin, and another in the chlortetracycline group (R.J.), without affecting the lowering of serum cholesterol. A variety of other antibacterial drugs, administered to a small number of patients (table 1), failed to alter serum cholesterol concentrations.

Serum Concentrations of Neomycin after Oral and Intramuscular Administration

Material and Methods

Ten hospitalized patients were studied, five of them from the previous experimental group. None of the patients had evidence of kidney or liver disease. No other antibacterial drugs were given prior to or during the experiment. One gram of neomycin was administered orally, twice daily at 8-hour intervals, to three patients. A single dose of 2 Gm. of neomycin was given to five subjects. In three of these patients the 24-hour urinary output of neomycin was measured. Finally, five patients were given a single injection of 60 mg. of neomycin intramuscularly. Urine was collected from each subject, and the 24-hour excretion of neomycin was measured. Serum specimens were obtained before and serially during 12 to 24 hours after administration of the drug.

Serum and urinary concentrations of neomycin were determined by the cylinder-plate method of Grove and Randall, with Staphylococcus aureus ATCC 6538 as a test organism. Specimens were tested undiluted. Standard curves of neomycin, diluted in phosphate buffer pH 8, were established with antibiotic-free, aseptically collected, normal human serum and urine.

Figure 1

Effect of intramuscular and oral administration of neomycin on serum cholesterol concentrations.
Results
In the three patients who received 1 Gm. of neomycin orally twice in 8-hour intervals, only traces of the drug could be found in the serum (table 6). When a single dose of 2 Gm. of neomycin was given orally, the average serum concentration of the drug reached its peak value after 2 hours (table 7A) of 0.28 μg. per milliliter. When a single intramuscular dose of 60 mg. of neomycin was injected, the average peak concentration (3.4 μg. per milliliter) in the serum was reached after 1
hour (table 7B). This medication did not alter serum cholesterol levels (table 3), although the average serum concentrations of the drug were 12-fold higher after the injection of 60 mg. than after an oral dose of 2 Gm.

Concentration of Neomycin in the Bile after
Oral and Intramuscular Administration

Material and Methods

Four hospitalized patients (table 8) were studied who underwent cholecystectomy because of cholelithiasis and cholecystitis. A T-tube was placed in the common hepatic duct by the surgeon, through which drained 250 to 600 ml. of bile daily prior to and during the experiment. The tests were performed 4 to 17 days after the operation. The patients were afebrile, out of bed, had normal kidney function, were orally fed, and were not given other antibacterial drugs for 6 days or longer at the time of the experiment. A single oral dose of 2 Gm. of neomycin was given to four patients. Subsequently, (in 2- to 4-day intervals) a single intramuscular injection of 60 mg. was administered to three of these subjects. Serum, bile, and urine specimens were collected before and serially during the 24 hours that followed administration of the drug.

Serum and urinary concentrations of neomycin were determined as described. Urine was diluted with phosphate buffer pH 8, whenever necessary. Studies on the bile blanks and standard curves for neomycin were established by diluting neomycin with antibiotic-free bile, aseptically collected individually from each patient prior to the tests in each of the four subjects, by the technical procedure described above. Each individual's bile was also used for the standard reference points in his assay.

Results

The concentration of neomycin in the bile, serum, and urine, following the oral administration of 2 Gm. and the intramuscular injection of 60 mg. of the drug, are shown in table 8. Following the oral administration of neomycin, small amounts appeared in the serum and were found erratically in the bile. Significant amounts, however, appeared in the urine. Following the intramuscular injection of neomycin, appreciable serum levels were maintained in the blood for at least 8 hours. During this time its presence in the bile could be demonstrated, and its concentration in the
Table 4
Effect of Oral Administration of Neamine, Kanamycin, and Chlorotetracycline (Aureomycin) on Average Total Serum Cholesterol Concentrations

<table>
<thead>
<tr>
<th>Patient, age, sex</th>
<th>Diagnosis</th>
<th>Drug and daily dose</th>
<th>Weeks on medication</th>
<th>Control</th>
<th>Average total cholesterol</th>
<th>Per cent fall</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.L. 44 F</td>
<td>Cerebrovascular accident</td>
<td>Neamine, 500 mg.</td>
<td>6</td>
<td>292 ± 13*</td>
<td>245 ± 9*</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C.B. 43 F</td>
<td>Cerebrovascular accident, diabetes mellitus</td>
<td>Neamine, 500 mg.</td>
<td>6</td>
<td>256 ± 12</td>
<td>236 ± 16</td>
<td>8</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>A.W. 53 M</td>
<td>Cerebrovascular accident</td>
<td>Neamine, 500 mg.</td>
<td>5</td>
<td>229 ± 12</td>
<td>195 ± 19</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L.D. 44 M</td>
<td>Peripheral vascular disease</td>
<td>Kanamycin, 2 Gm.</td>
<td>8</td>
<td>230 ± 16</td>
<td>200 ± 7</td>
<td>15</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Z.A. 47 F</td>
<td>Familial hypercholesteremia</td>
<td>Kanamycin, 2 Gm.</td>
<td>7</td>
<td>545 ± 25</td>
<td>499 ± 16</td>
<td>8</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>B.A. 62 M</td>
<td>Coronary artery disease</td>
<td>Kanamycin, 2 Gm.</td>
<td>5</td>
<td>357 ± 10</td>
<td>321 ± 25</td>
<td>10</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>A.W. 53 M</td>
<td>Cerebrovascular accident</td>
<td>Kanamycin, 2 Gm.</td>
<td>5</td>
<td>229 ± 18</td>
<td>218 ± 7</td>
<td>8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>D.F. 55 M</td>
<td>No clinical disease</td>
<td>Kanamycin, 2 Gm.</td>
<td>5</td>
<td>260 ± 16</td>
<td>250 ± 25</td>
<td>8</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>L.I. 50 M</td>
<td>Cerebrovascular accident</td>
<td>Kanamycin, 2 Gm.</td>
<td>3</td>
<td>224 ± 16</td>
<td>222 ± 16</td>
<td>8</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>B.A. 62 M</td>
<td>Coronary artery disease</td>
<td>Chlorotetracycline, 1.5 Gm.</td>
<td>6</td>
<td>357 ± 10</td>
<td>289 ± 29</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N.K. 62 M</td>
<td>Coronary artery disease</td>
<td>Chlorotetracycline, 1.5 Gm.</td>
<td>5</td>
<td>383 ± 17</td>
<td>355 ± 36</td>
<td>7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>M.J. 34 M</td>
<td>Spinal cord lesion</td>
<td>Chlorotetracycline, 1 Gm.</td>
<td>5</td>
<td>204 ± 12</td>
<td>183 ± 10</td>
<td>10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A.W. 53 M</td>
<td>Cerebrovascular accident</td>
<td>Chlorotetracycline, 1 Gm.</td>
<td>4</td>
<td>292 ± 13</td>
<td>251 ± 10</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Z.A. 47 F</td>
<td>Familial hypercholesteremia</td>
<td>Chlorotetracycline, 1 Gm.</td>
<td>3</td>
<td>545 ± 25</td>
<td>545 ± 46</td>
<td>8</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

*Standard deviation.

Material and Methods

Radioactive Fat Absorption Studies

50 μg per milliliter.

Three patients were studied, all of whom were included in the study group and one was an outpatient. Two were hospitalized, and one was an outpatient. Three were hospitalized, and one was an outpatient. Two radioactive fat absorption tests were performed after the administration of 131I-neomycin (2 Gm. daily) on the 2nd, 10th, 12th, and 18th days, respectively. The second test was performed after the administration of 131I-neomycin (2 Gm. daily) on the 2nd, 10th, 12th, and 18th days, respectively.

The patients were given 10 drops of Lugol's solution, TISP on the day preceding the test, and were then returned to control levels in each patient. The time of the initial test, the test, and the amount given during the 12 hours following the test.

Benefits

On the day of the initial test, serum cholesterol levels of the four patients studied, when pretreatment by the second test, were carried out the respective serum cholesterol levels of the four patients studied, were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent. In three of the four patients (fig 6A, B, and D) there were 291, 293, and 271 mg per cent.
the shape of the absorption curve was the same during neomycin and control periods. Total stool radioactivity ranged from 0 to 2.4 per cent, with no significant difference between the neomycin and control periods. One patient (fig. 6C) showed a somewhat higher absorption during the control period, but even in this case the absence of radioactivity in the stools indicated a complete but late absorption. The persistence of a flat absorption curve, despite the rise in serum cholesterol in the fourth patient (fig. 6D), may indicate that delayed absorption was independent of the presence of neomycin. If it was a consequence of the administration of the drug, the lowering of the serum cholesterol level was not related to delayed fat absorption.

**Discussion**

Neomycin is widely used orally to disinfect the intestinal tract. The rapid decrease of the total bacterial count in the feces during the first few days of administration is followed by overgrowth of nonsusceptible microorganisms. Although the short-term changes of the intestinal flora have been repeatedly described, only scanty data are available following its long-term use. The long-term oral
administration of the drug alters significantly the character of the intestinal bacterial flora. It is generally accepted that neomycin is poorly absorbed from the gastrointestinal tract, whereas rapid diffusion and distribution of the drug are observed after its parenteral administration. In the present study the intramuscular administration of 60 mg. of neomycin resulted in 12-fold higher average serum concentrations than oral doses of 2 Gm. Since oral neomycin lowered serum cholesterol concentrations significantly, whereas the above intramuscular doses failed to alter the level of serum cholesterol, it is suggested that the effect of the drug is dependent upon its activity in the gastrointestinal tract. Furthermore, the experiments on the biliary excretion of neomycin suggest that the biliary excretion of the drug is probably derived from the general circulation, and that the existence of an enterohepatic circulation of the drug, and thus its direct action upon the liver cell when administered orally, is unlikely.

In contrast to data on man neomycin appears to be ineffective in lowering the serum cholesterol level in normal and cholesterol-fed rats, and in cholesterol-fed rabbits. In fact, in rats fed cholesterol and cholic acid, neomycin appeared to increase serum cholesterol concentrations and aortic sudanophilia.

In the present study, the oral use of neomycin seemed to be well tolerated over long periods of time in patients without kidney impairment. The only side effect was a temporary mild diarrhea in about one third of the subjects treated orally. Any relation of the cholesterol-lowering activity of the drug to diarrhea is highly unlikely, since the duration of diarrhea was insignificant compared to the length of the experiments, and patients without diarrhea exhibited similar reduction in serum cholesterol. Furthermore, the administration of other antibiotics, which failed to lower serum cholesterol levels, caused diarrhea as frequently as neomycin. Finally no patient lost more than 2 lbs. of weight in the group treated with neomycin. Stormont et al. reported severe diarrhea in three of 68 cirrhotic patients treated for hepatic coma with oral doses of 4 to 8 Gm. of neomycin daily. Monilia was cultured in the stools of Perin and associates, who described an "experimental malabsorption syndrome" associated with steatorrhea and morphologic changes of jejunal mucosa following daily oral doses of 12 Gm. of neomycin. This syndrome had to be considered in searching for the mechanism of the cholesterol-lowering action of the drug, although in the present study serum cholesterol concentrations were reduced with considerably lower doses of neomycin. Radioactive fat-absorption tests, in the present study, have indicated that the long-term oral administration of 2 Gm. of neomycin per day, accompanied by significant reduction of serum cholesterol concentrations, resulted in no alteration in the normal fat-absorption patterns in two subjects, and in a delayed but complete absorp-
Among the diverse antibacterial drugs used in this study, the antibacterial activity and molecular structure of neamine and kanamycin resemble that of neomycin. The mechanism of their moderate cholesterol-lowering effect is possibly similar to that of neomycin. The oral use of PAS may cause irritation of the gastrointestinal mucosa and was reported to result in hepatic toxicity. Tygstrup et al. published a preliminary report on the cholesterol-lowering effect of PAS, comparable to the results of this study. Chlortetracycline was reported to cause liver toxicity in patients, and Nelson et al. reported that the addition of chlortetracycline to the regimen of cholesterol-fed rabbits augmented the serum cholesterol concentrations and vascular atheromatosis of the animals.

There is no evidence available to explain the serum cholesterol-lowering effect of these diverse antibacterial drugs. Although PAS and chlortetracycline are readily absorbed, one cannot rule out the possibility that their action would be mediated through changes in the gastrointestinal tract. There is no close chemical resemblance between the molecular structures of PAS or chlortetracycline and that of neomycin. The only common denominator between these substances is their antibacterial activity. It should be pointed out that, although a variety of other antibacterial drugs failed to lower serum cholesterol concentration of fats in two other patients. These data suggest that the cholesterol-reducing activity of neomycin is not dependent for its effect on absorption of dietary fats.

Figure 6
Results of radioactive fat-absorption tests in four patients after long-term administration of neomycin and during control periods.
centrations in this study, these drugs were each used only in a relatively small number of patients for comparatively short periods.

At present, the exact mechanism of the cholesterol-lowering action of neomycin is not understood. The effect of the drug may be due to modifications of the character of the intestinal bacterial flora or inhibition of intestinal enzyme systems involved in the specific absorption of cholesterol.

The possible role of intestinal bacteria in cholesterol metabolism was mentioned as early as 1896. More recently Rosenheim and Webster\(^2\) found that the administration of succinyl sulfathiazole to cholesterol-fed rats inhibited completely the formation of coprosterol. This was accompanied by a decrease of the number of Escherichia coli in the intestine of the animals. Curran and Brewster\(^2\) obtained from the duodenum of patients with chronic cholecystitis a strain of Esch. coli, capable of liberating carbon dioxide from labeled cholesterol "in vitro." Wainfan et al.\(^2\) demonstrated that the administration of succinyl sulfathiazole and streptomycin to cholesterol-fed mice inhibited the destruction of cholesterol in the carcass of the animals, concomitant with a striking decrease in the number of intestinal bacteria. In a later series of experiments the same authors isolated 15 bacterial species from the feces and intestinal contents of cholesterol-fed rats, which destroyed or modified cholesterol "in vitro." The addition of dinitrophenol, nicotinamide, or thyroxine and related compounds to the media increased significantly the activity of the bacteria upon cholesterol. Abell, Mosbach, and Kendall\(^2\) suggested that the action of thiouracil on the cholesterol and bile acid metabolism of the dog might be related to the effect of this substance upon the intestinal bacterial flora. Ahrens\(^2\) has also emphasized the possible importance of intestinal bacteria in the metabolism of cholesterol.

Danielsson and Gustafsson\(^2\) reported that germ-free rats had significantly higher serum cholesterol values than controls, and that cholesterol was not reduced to coprostanol in the

---

**Table 7A**

* Serum Concentrations of Neomycin (µg./ml.) Following the Administration of a Single Oral Dose of 2 Gm.

<table>
<thead>
<tr>
<th>Patient age, sex</th>
<th>Weeks*</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.W. 53 M</td>
<td>20</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.C. 57 F</td>
<td>11</td>
<td>---</td>
<td>0</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>9.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.M. 54 M</td>
<td>8</td>
<td>0</td>
<td>Tr</td>
<td>0.5</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.F. 42 M</td>
<td>---</td>
<td>0.4</td>
<td>0.2</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.P. 51 M</td>
<td>---</td>
<td>0</td>
<td>Tr</td>
<td>0.3</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0</td>
<td>0.24</td>
<td>0.28</td>
<td>Tr</td>
<td>Tr</td>
<td>0</td>
<td>8.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Weeks on neomycin at time of test.

†Traces (less than 0.2 µg./ml.).

---

**Table 7B**

* Serum Concentrations of Neomycin (µg./ml.) Following the Administration of a Single Intramuscular Dose of 60 mg.

<table>
<thead>
<tr>
<th>Patient age, sex</th>
<th>Weeks*</th>
<th>½</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.B. 58 F</td>
<td>3</td>
<td>2.1</td>
<td>4.3</td>
<td>3.3</td>
<td>1.7</td>
<td>1.6</td>
<td>0.5</td>
<td>0</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.D. 62 M</td>
<td>3</td>
<td>---</td>
<td>4.1</td>
<td>3.2</td>
<td>1.6</td>
<td>1.1</td>
<td>0.6</td>
<td>0</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.F. 42 M</td>
<td>3</td>
<td>3.2</td>
<td>2.8</td>
<td>2.0</td>
<td>1.5</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.F. 48 M</td>
<td>3</td>
<td>1.5</td>
<td>1.8</td>
<td>1.3</td>
<td>0.7</td>
<td>0.5</td>
<td>Tr</td>
<td>0</td>
<td>19.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.P. 51 M</td>
<td>---</td>
<td>3.0</td>
<td>4.0</td>
<td>2.0</td>
<td>1.1</td>
<td>1.1</td>
<td>0.2</td>
<td>0</td>
<td>14.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.5</td>
<td>3.4</td>
<td>2.4</td>
<td>1.3</td>
<td>1.0</td>
<td>0.3</td>
<td>0</td>
<td>22.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Weeks on neomycin at time of test.
gastrointestinal tract of the germ-free animals. In contrast, another group of investigators found lower serum cholesterol levels in germ-free rats than in the conventionally reared animals.\textsuperscript{31,32} Forbes et al.,\textsuperscript{32} however, found no significant difference between the serum cholesterol concentration of the germ-free and conventional chickens. Gustafsson et al.\textsuperscript{33} demonstrated that after the administration of labeled cholic acid to germ-free rats, only taurocholic acid was present in the feces, instead of a variety of compounds, and they obtained similar results in conventional animals by the feeding of antibacterial drugs. The half-life of cholic acid was considerably increased in the germ-free rats, with a larger pool than in conventional animals.\textsuperscript{34} Goldsmith et al.\textsuperscript{35} have confirmed the cholesterol-lowering effect of orally administered neomycin in man, and reported a three- to four-fold increase in fecal excretion of bile acids.

These series of findings suggest that changes in the intestinal bacterial flora might influence sterol metabolism and the turnover of bile acids, which in turn may exert an effect upon the metabolism of cholesterol. Several factors may influence the concentration of serum cholesterol in man. If the character of the intestinal bacterial flora is one of them, the well-known epidemiologic data, concerning differences in serum cholesterol levels among different populations of various parts of the world, may be due, in part, to differences in the intestinal flora. Environmental factors, such as diet, water supply, general hygienic conditions etc., are known to cause modifications in the character of intestinal bacteria, and thus would influence the concentration of serum cholesterol.

Summary

The serum cholesterol concentrations of 56 patients were studied in 112 experimental periods following the administration of neomycin and a variety of antibacterial drugs. Additional studies on the mechanism of the serum cholesterol-lowering effect of neomycin were performed.

The oral administration of neomycin for 4 to 37 weeks, in daily doses of 1.5 or 2 Gm., significantly reduced the mean serum cholesterol concentration in each of 30 patients by 14 to 29 per cent (average, 21 per cent). Serum cholesterol concentrations remained low for the duration of neomycin administration, and returned to control levels after the drug was discontinued. In 10 patients the

\textit{Circulation, Volume XXIV, September 1961}
esterified fraction of cholesterol and the concentration of phospholipids decreased in the serum in proportion to total cholesterol and the distribution of cholesterol between the alpha- and beta-lipoproteins remained unchanged during neomycin administration.

Combination of oral neomycin with mandelamine, phthalylsulfathiazole, isoniazid, oxytetracycline, and tetracycline did not influence the lowering effect of neomycin on serum cholesterol levels.

Serum levels of neomycin after intramuscular injections of 60 mg. in 10 subjects averaged 12 times higher concentrations than was produced by the oral administration of 2 Gm. of neomycin. The intramuscular administration of 60 mg. of neomycin daily to 10 patients for 3 weeks failed to alter serum cholesterol concentrations. This suggests that the cholesterol-lowering effect of neomycin depends upon its action in the gastrointestinal tract.

The concentration of neomycin was serially determined in the bile, serum, and urine of four patients. Biliary concentrations were higher, or of the same order of magnitude, after intramuscular than after oral administration of the drug. Therefore enterohepatic circulation of neomycin is unlikely.

Radioactive fat-absorption tests following the reduction of serum cholesterol by long-term oral administration of neomycin showed that the absorption of dietary fats was normal in two patients and delayed but complete in two other subjects, suggesting that the cholesterol-reducing activity of neomycin is not dependent on its effect on absorption of dietary fats.

The daily oral administration for 5 to 23 weeks of 8 to 12 Gm. of para-aminosalicylic acid to 15 patients lowered serum cholesterol concentrations significantly in each experiment by 14 to 44 per cent (average: 26 per cent), whereas daily doses varying between 2 and 6 Gm. reduced cholesterol less markedly. The daily addition of 2 Gm. of oral neomycin to high doses of para-aminosalicylic acid did not result in further lowering of serum cholesterol concentrations.

The administration of neamine (500 mg. daily), kanamycin (1 Gm. daily) and chlorotetracycline (1 or 1.5 Gm. daily) lowered serum cholesterol concentrations, although less markedly than neomycin or para-aminosalicylic acid.

The oral administration of phthalylsulfathiazole, isoniazid, penicillin, dihydrostreptomycin, oxytetracycline, chloramphenicol, polymyxin, erythromycin, novobiocin, bacitracin, and viomycin failed to alter serum cholesterol concentrations.

The possible role of the intestinal bacterial flora in the cholesterol-lowering effect of neomycin and other antibacterial drugs, and the general relationship of intestinal bacteria to cholesterol metabolism, were discussed.

Acknowledgment

The authors are indebted to Drs. Charles F. Wilkinson, Jr., Lewis Thomas, Currier McEwen, Forrest E. Kendall, Edward Melman, Quentin B. Deming, and Gabor Kaley, for their interest and advice, and to Drs. Michael M. Daeso, Menard M. Gertler (Department of Physical Medicine and Rehabilitation, New York University, Goldwater Memorial Hospital), Harry H. Epstein (Triboro Hospital), and Henry Doubleit (Department of Surgery, New York University-Bellevue Medical Center), who kindly made available patients for study. The cooperation of Dr. Alan W. Bernheimer and Miss Jeanette Winter, of the Department of Microbiology, and Mr. Carl Collica and Miss Elsie Testa, of the Radiosotope Laboratories, New York University-Bellevue Medical Center, is gratefully acknowledged.

References


Circulation, Volume XXIV, September 1961
31. Wostmann, B. S.: Personal communication.
Reduction of Serum Cholesterol Concentrations by Neomycin, Para-aminosalicylic Acid, and Other Antibacterial Drugs in Man

PAUL SAMUEL and WILLIAM I. WAITHE

*Circulation*. 1961;24:578-591
doi: 10.1161/01.CIR.24.3.578

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1961 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/24/3/578

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org/subscriptions/