Action of Neomycin on the Intraluminal Phase of Lipid Absorption

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ABSTRACT Administration of a single 1 g dose of neomycin sulfate to five healthy subjects simultaneously with a test meal caused a marked increase in the proportion of fatty acid and bile acid in the ultracentrifuged deposit of aspirated intestinal contents. Labeled cholesterol was precipitated in a similar manner in two hypercholesterolemic patients. Neomycin had no effect on the pancreatic lipase concentration or on the pH of intestinal contents. These results confirm that the ability of neomycin to precipitate micellar lipids is due to interaction between the polybasic neomycin molecule and ionized fatty acids and bile acids. This mechanism provides an explanation for both the steatorrhea and hypocholesterolemia induced by this compound.

INTRODUCTION

Oral administration of neomycin, an aminoglucoside antibiotic, induces steatorrhea (1) and lowers the serum cholesterol (2) in man. This has led to its use as a hypocholesterolemic agent (3). The exact mechanism underlying these actions of neomycin remains uncertain but the two most plausible explanations are a toxic effect on the intestinal mucosa (4, 5) and precipitation of bile acids (6, 7) and fatty acid soaps (8) from solution. Theoretically, either of these mechanisms could impair lipid absorption and thus explain both the steatorrhea and the increased fecal excretion of neutral sterols and bile acids observed in subjects receiving this drug (9, 10). In addition, inhibition of pancreatic lipase has been suggested as an alternative explanation for the steatorrhea (11, 12).

Recently, Thompson, MacMahon, and Claes (13) have shown that neomycin and other polybasic compounds precipitate fatty acid and cholesterol from mixed micellar solutions both in vitro and within the intestinal lumen of the rat. Their results suggested that interaction between fatty acid and bile acid anions and the cationic amino groups of neomycin led to precipitation of the whole micellar complex, including unionized components such as monoglyceride and cholesterol. The importance of micelle formation in solubilizing dietary fat and cholesterol during the intraluminal phase of lipid absorption is now well established (14). The disruptive effect of neomycin on this process in control subjects and patients with hypercholesterolemia forms the subject of this paper.

METHODS

Five healthy male physicians were fasted overnight. Each was then intubated by the oral route with a Dreiling double lumen tube, the tip of which was located fluoroscopically at the duodeno-jejunal flexure. A test meal of 15 g of Casilan[1] (90% calcium caseinate), 45 g of glucose, and 25 ml of olive oil, previously homogenized with 250 ml of water in a Waring blendor, was then introduced into the stomach via the gastric lumen of the tube and flushed in with 50 ml of water. After a 10 min equilibration period, the intestinal contents were aspirated through the distal lumen of the Dreiling tube by continuous mechanical suction for three consecutive ½ hr periods. Each ½ hr period was subdivided into a 20 min collection, taken into a flask kept at 70°C to inactivate lipase (15), followed by a 10 min collection, taken into a flask kept at 4°C to preserve lipase activity. The volume and pH of all collections were measured. The study was repeated on another occasion, at least 1 wk later, when each subject was given a similar test meal together with 1 g of neomycin sulfate British Pharmocopoeia[2] dissolved in 20 ml of water and administered simultaneously into the stomach.

Hypercholesterolemic subjects. Two patients, one male (J.F.) and one female (A.W.) both with familial type II

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[1] Glaxo Laboratories Ltd., Greenford, Essex, England.

[2] Roussel Laboratories, London, England.

TABLE I

The Mean Concentration of Total Saponifiable Fatty Acid, Bile Acid, and Lipase and the pH of Intestinal Contents Aspirated from the Duodeno-Jejunal Flexure of Five Healthy Subjects during a Test Meal, Given without and with 1 g of Neomycin Sulfate

	Fatty acid*	Bile salt*	Lipase*	pH‡
	mEq/liter	*mmole/liter*	*U/ml*	
Control	40.8 ±13.0	7.3 ±1.5	490 ±248	5.9 (4.4–7.6)
Neomycin	27.9 ±9.7	6.1 ±1.5	442 ±246	6.1 (4.2–7.0)

* Mean ±1 SD.

‡ Mean and observed range.

hypercholesterolemia (16), were also studied. One patient (J.F.) had been treated with 2 g of neomycin sulfate daily for the preceding 12 months, and the other (A.W.) had never previously received this drug. Both patients were studied on two occasions as above but each of their test meals contained an additional 250 mg of crystalline cholesterol, dissolved in the olive oil and labeled with 3–6 μCi of cholesterol-^{3}H on the first occasion and cholesterol-^{14}C on the second. Although collections were divided into three ½ hr periods as before, suction was limited to only 2 out of every 10 min with the resultant aspirates being pooled and collected at 70°C as above. This modification to the experimental protocol was designed to reduce depletion of the bile salt pool to a minimum.

Ultracentrifugal studies. The heat-inactivated samples of intestinal contents (10 or 20 ml) were centrifuged at 100,000 *g* for 3 hr at 37°C in duplicate (17), using a modified M.S.E. Superspeed 40 ultracentrifuge with a swing-out rotor (Measuring & Scientific Equipment Ltd., London, England). The supernatant oil phase was aspirated with a wide-bore needle and a syringe and then discarded. The remaining infranatant micellar phase was aspirated in a similar manner and retained. The residual precipitate was also kept for analysis.

Analytical studies. The total saponifiable fatty acid content of whole samples collected at 70°C and of the micellar phase and precipitate after ultracentrifugation was determined (18). In the hypercholesterolemic subjects aliquots of the fatty acid extract were also assayed for radioactivity using a Beckman LS-250 Liquid Scintillation Counter with an external standard ratio method of quench correction. The total bile acid content of the corresponding duplicate samples was also determined. After preliminary alkalinization to pH 10, each sample was diluted with methanol, centrifuged, and the precipitate washed twice with methanol. This was pooled with the original supernatant and assayed enzymatically (19). The lipase concentration of the two 4°C collections from each of the paired studies in healthy subjects was measured at pH 9 by the method of Worning and Mullertz (20).

Calculations. The concentrations of fatty acid, bile acid, and labeled cholesterol in each period were measured directly in samples of whole intestinal contents, micellar phase, and precipitate; the concentration of fatty acid in the oil phase was calculated by subtracting the sum of the concentrations in micellar phase and precipitate from the whole. The actual quantity of fatty acid and bile acid aspirated in each period was calculated by multiplying concentration by volume. In the case of the micellar phase, this was done by assuming that the micellar phase volume was the same as that of the whole sample; this overestimated micellar phase content by about 5% on the average. The overall mean concentrations of fatty acid and bile acid in each phase of intestinal content were calculated by summing the individual amounts aspirated in each of the periods and dividing by the total volume of the three 70°C collections. The overall mean concentration of lipase was calculated in a similar manner but we used the total volume of the two 4°C collections. The statistical significance of differences between paired samples was assessed by Student's *t* test.

RESULTS

The mean concentrations of total saponifiable fatty acid, bile acid, lipase, and hydrogen ions in the intestinal contents of five healthy subjects are shown in Table I. There was no significant difference between the lipase values during the control and neomycin studies nor was the pH significantly altered. However, there was a slight reduction in the mean fatty acid and bile acid concentrations during the neomycin study.

The mean total volume of intestinal contents aspirated per hour was 389 ml during the control study and 355 ml after neoycin. This difference was not significant and, taken in conjunction with the data in Table I, suggests that recoveries during both studies were comparable.

Phase distribution of fatty acid and bile acid. The mean total saponifiable fatty acid concentration of ultracentrifuged intestinal contents from the same five subjects is shown in Fig. 1. Administration of neomycin increased the mean concentration of fatty acid in the precipitate from 2.8 to 9.4 mEq/liter of whole intestinal contents ($P < 0.01$) with a corresponding reduction in the micellar phase from 18.1 to 9.8 mEq/liter of intestinal

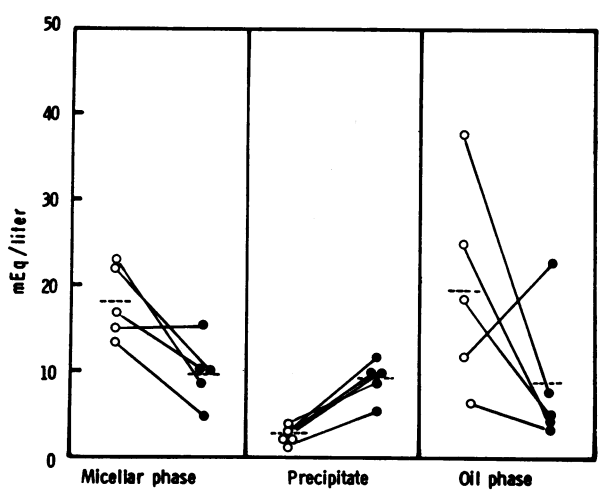

FIGURE 1 Effect of neomycin on the phase distribution of total saponifiable fatty acid in intestinal contents from five healthy subjects. The mean of each group is shown by a broken line. The results are expressed as milliequivalents of fatty acid in each phase per liter of whole intestinal contents. Control, ○; neomycin, ●.

contents ($P < 0.05$). Neomycin also appeared to decrease the concentration of fatty acid in the oil phase but the individual variations were so wide that these differences were not significant.

The mean bile acid concentration of ultracentrifuged intestinal contents from the same subjects is shown in Fig. 2. Neomycin caused an increase in the mean concentration of bile acid in the precipitate from 0.25 to 1.7 mmoles/liter of intestinal contents ($P < 0.01$) and a corresponding reduction in the micellar phase bile acid concentration from 7.1 to 4.4 mmoles/liter of contents ($P < 0.01$).

The distribution of fatty acid and bile acid in intestinal contents after ultracentrifugation can also be expressed as a percentage of the total amount in the whole sample, as shown in Fig. 3. In the control study only 6.6% of the fatty acid was in the precipitate with 43.5% in the micellar phase and 49.9% in the oil phase. The corresponding values for the neomycin study were 34, 31, and 37%, respectively.

During the control study, only 3.3% of the bile acid was in the precipitate, the remainder being in the micellar phase. However, neomycin increased the proportion of bile acid in the precipitate to 26.8%.

Hypercholesterolemic subjects. The percentage distribution of fatty acid, bile acid, and labeled cholesterol in the two hypercholesterolemic patients is shown in Fig. 4. In one (J.F.) neomycin increased the proportion of fatty acid in the precipitate from 12.6 to 33.9% without, however, causing any increased precipitation of bile acid. Nevertheless, the proportion of cholesterol in the precipitate increased from 9.1% during the control study to 57.0% after neomycin. At the same time, the proportion of cholesterol in the micellar phase decreased from 52.3 to 33.0%.

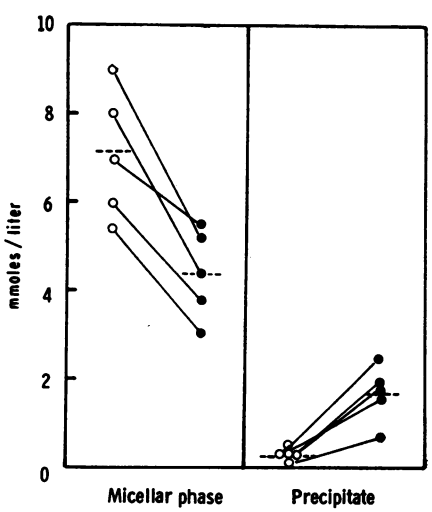

FIGURE 2 Effect of neomycin on the phase distribution of bile acid in intestinal contents from five healthy subjects. The mean of each group is shown by a broken line. The results are expressed as millimoles of bile acid in each phase per liter of whole intestinal contents. Control, ○; neomycin, ●.

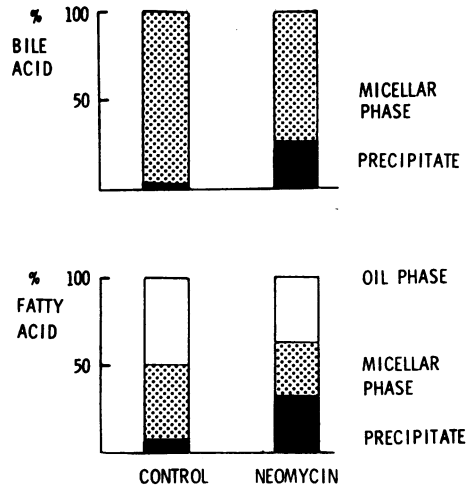

FIGURE 3 Effect of neomycin on the percentage distribution of bile acid and fatty acid in ultracentrifuged intestinal contents. Results represent the means of five healthy subjects.

In the other patient (A.W.) neomycin increased the precipitation of both fatty acid (from 9.1 to 46.4%) and bile acid (from 4.5 to 27.3%). Neomycin also increased the percentage of cholesterol in the precipitate from 3.2% during the control study to 51.5%, and reduced the micellar cholesterol from 47.5 to 5.9%. This patient, who had never previously had neomycin, underwent a second neomycin study after receiving 2 g daily for 1 month. The object of this was to determine whether chronic administration of the antibiotic, which might alter the bacterial flora of the gastrointestinal tract, could have been responsible for its failure to induce bile acid precipitation in patient J.F. The results in A.W. did not support this explanation since 46.8% of the fatty acid and 27.8% of the bile acid were in the precipitate during the second neomycin study, as compared with 46.4 and 27.3% respectively on the previous occasion.

DISCUSSION

These results show that a single 1 g dose of neomycin, when given with a test meal, induced marked precipitation of both fatty acids and bile acids within the intestinal lumen of healthy subjects. In a similar study, Hardison and Rosenberg (21) failed to detect any effect of neomycin on the intraluminal phase of fat absorption, but they did not state whether they gave neomycin simultaneously with their test meal nor whether they examined the deposit of ultracentrifuged intestinal contents. During the present study administration of neomycin to the two hypercholesterolemic patients caused

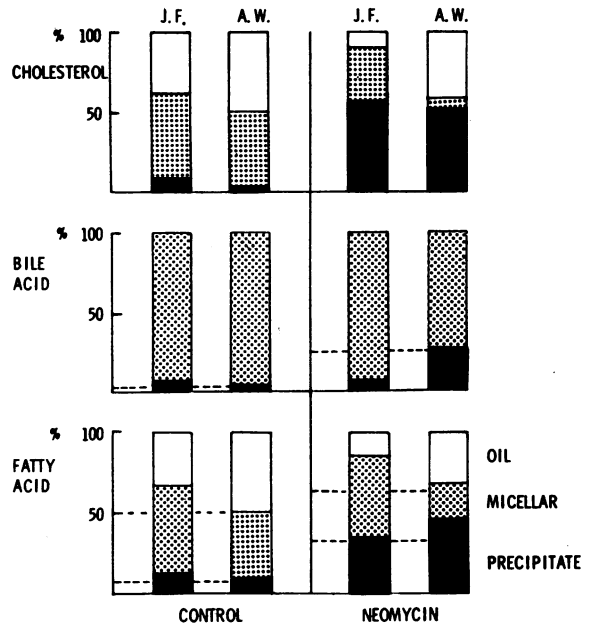

FIGURE 4 Effect of neomycin on the percentage distribution of bile acid, fatty acid, and labeled cholesterol in ultracentrifuged intestinal contents from two hypercholesterolemic patients (J.F. and A.W.). The broken lines represent the corresponding mean values in five normal subjects (see Fig. 3).

increased precipitation of both fatty acid and labeled cholesterol. In one of these patients neomycin did not cause any increased precipitation of bile acid. This unexpected finding is compatible with previous evidence that precipitation of micellar cholesterol is primarily dependent upon ionic interaction between neomycin and fatty acid (13). However, precipitation of bile acids by neomycin, which occurred in all the other subjects, would undoubtedly accentuate cholesterol precipitation within the intestinal lumen. The absorption of a nonpolar lipid, such as cholesterol, is markedly dependent upon its prior incorporation into a micellar phase within the intestinal lumen. In contrast, micelle formation is not an absolute requirement for the absorption of more polar lipids, such as fatty acids (22), although under normal circumstances they participate actively in this process. Cholesterol is normally absorbed less efficiently than fatty acid and has been shown to precipitate within the intestinal lumen after absorption of the polar lipid components of a mixed micellar solution has occurred (23). The greater vulnerability of cholesterol to factors which adversely influence micelle formation may explain why small doses of neomycin can apparently lower the serum cholesterol without causing overt steatorrhea (24).

The hypocholesterolemic action of neomycin has been shown to be accompanied by a marked increase in the fecal excretion of neutral sterols and, to a lesser extent, of bile acid (10). Cholestyramine, a polybasic resin, lowers the serum cholesterol in a similar manner but differs from neomycin by its insolubility and more marked effect on bile acid excretion (25). Neomycin in moderate doses of 3–6 g daily is also known to induce malabsorption of dietary fat (26, 27), and it has been suggested that this is due to inhibition of lipolysis (11, 12). However, the normal lipase values obtained during the present study render this explanation most unlikely. On the contrary, the action of pancreatic lipase may even be enhanced by neomycin (28) which presumably acts in an analogous manner to calcium by binding fatty acid released during hydrolysis of triglyceride, thus preventing end-product inhibition of the reaction (29). The role of mucosal damage in neomycin-induced malabsorption is somewhat more difficult to assess, especially since some of the evidence is conflicting (4, 5, 21). However, it is of interest that the steatorrhea induced by colchicine, which acts solely through its deleterious effect on the intestinal mucosa, is less marked than that induced by neomycin (30).

Samuel, Holtzman, Mailman, and Sekowski have shown that neomycin is an effective agent for treating patients with hypercholesterolemia, especially when used in conjunction with clofibrate (31). The present results lend support to the suggestion that the hypocholesterolemic effect of neomycin is related more to its polybasic molecular structure than to its antibiotic action (32). The latter exerts a quite separate influence on cholesterol metabolism by preventing bacterial conversion of primary to secondary bile acids in the colon (21). Further elucidation of the relative importance of these mechanisms may provide a basis for the development of other polybasic compounds as hypocholesterolemic agents.

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