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Research Article

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Effects of Neomycin on Absorption, Synthesis, and/or Flux of Cholesterol in Man

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ABSTRACT The mode of action of the hypocholesteremic drug neomycin (2 g/day) was studied in four patients. All showed a significant reduction in plasma cholesterol concentrations (mean 25%, range 18–31%), and in one of three patients with hyperglyceridemia there was a decrease of plasma triglycerides of 26%. Cholesterol absorption was measured in three of four patients: there was a marked decrease. Sterol balance studies in four patients showed an unabating increase in fecal neutral steroid excretion (mean increase 345 mg/day, range 323–361) for 3–5 wk after plasma cholesterol levels had reached a new and lower plateau. Fecal acidic steroid excretion increased temporarily in two patients, with a sustained increase of 93 mg/day in only one. Daily stool weights increased significantly in three of four patients, though none had steatorrhea; there was a significant reduction in excretion of secondary bile acids; neutral sterol degradation rates were not affected by the drug. Slopes of plasma cholesterol-specific activity time curves did not change.

These results fail to support the suggestion that neomycin acts as a bile acid precipitant. The finding of increased fecal neutral steroid excretion is consistent with decreased cholesterol absorption, but also with increased cholesterol synthesis (secondary to release of negative feedback control), with increased flux of cholesterol from tissues, or with a combination of all three actions.

INTRODUCTION

The effectiveness of orally administered neomycin in decreasing plasma cholesterol levels in man was first observed in 1958 (1), and since then it has been demonstrated that a few other antibacterial drugs have similar properties. Of these kanamycin (2) and paromomycin (3) are similar in structure to neomycin, but chlortetracycline (2), chloramphenicol (4), and para-aminosalicylic acid (2) have markedly different molecular configurations. The administration of large oral doses of neomycin (12 g daily) has been shown to cause malabsorption (5, 6); small doses (2 g daily), however, reduce plasma cholesterol levels without inducing steatorrhea (7).

De Somer, Vanderhaeghe, and Eyssen (8) originally suggested that neomycin might act as a bile acid sequestrant, and subsequently Thompson, MacMahon, and Claes, and Thompson, Barrowman, Gutierrez, and Dowling reported in vitro and in vivo (9, 10) data supporting the concept that neomycin precipitates fatty acids, bile acids, and cholesterol out of micellar suspension in small-intestinal contents. Two previous studies have indicated that neomycin causes increased fecal excretion of neutral and acidic steroids (11, 12), whereas another observed increased bile acid excretion only (13). In 1968 Samuel, Holtzman, Meilman, and Perl (14), using isotope kinetic techniques, showed that the administration of neomycin resulted in a decrease in the size of tissue pools of cholesterol.

The present study represents an effort to clarify some of the remaining uncertainties regarding the mode of action of neomycin as a hypocholesteremic drug. Cholesterol absorption was shown to be markedly reduced in the three patients tested, and in four patients sterol balance studies showed a marked increase in fecal neutral steroid excretion. However, it was not possible to distinguish whether this increment was due solely to decreased absorption: increased cholesterol synthesis and increased cholesterol flux from tissues are alternative and additional explanations for the findings. Failure to find a consistent increase in fecal acidic steroids appears to weigh against the idea that neomycin is a bile acid sequestrant in vivo.

METHODS

Patients. Studies were carried out on four patients during hospitalization on a metabolic ward, where balance studies lasting 13–31 wk were carried out. The age, sex, body build, caloric intake, and clinical diagnosis of each patient are presented in Table I. There were three men and one woman aged 52–72 yr. All patients had elevated plasma lipids: patients 1 and 4 had ischemic heart disease.

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TABLE I
Clinical Data

Patient	Initials	Age	Sex	Height	Weight		Calories‡	Diagnosis
		yr		*cm*	*kg*	*% ideal**		
1	N. A.	55	F	158	55	105	1,900	Hypercholesteremia, IHD§
2	A. J.	72	M	172	63	96	1,700	Hyperglyceridemia, COPD‖
3	S. B.	52	M	171	86	121	3,030	Hyperglyceridemia
4	J. L.	52	M	159	63	117	2,300	Hyperglyceridemia, IHD

* According to life insurance tables (15).
‡ Needed to maintain constant body weight.
§ IHD, ischemic heart disease.
‖ COPD, chronic obstructive pulmonary disease.

Diets. Metabolic studies were carried out continuously in all patients. Body weights were maintained constant by orally administered liquid formula feedings as previously described (16, 17); dietary fat contributed 40%, protein 15%, and carbohydrate 45% of total caloric intake. The formula contained 119 mg cholesterol and 72 mg β-sitosterol/500 cal, and the fat content (in the form of lard) simulated the quality of fat in the average American diet. Minerals and vitamins were supplemented as described previously (17).

Plasma lipids. Concentrations of plasma cholesterol and triglycerides were determined twice weekly by the methods of Block, Jarrett, and Levine (18) and Kessler and Lederer (19), respectively, on the AutoAnalyzer (Model I) (Technicon Instruments Corp., Tarrytown, N. Y.).

Isotopic sterols. [1,2-^{3}H]Cholesterol and [4-^{14}C]cholesterol were obtained from New England Nuclear Corp., Boston, Mass., and were purified by thin-layer chromatography on Florisil (Floridin Co., Tallahassee, Fla.) with ethyl ether-heptane 45:55 (vol/vol). Only that material that chromatographed with the same R_f value as a pure sterol standard was administered to patients. For intravenous administration the labeled sterol dissolved in ethanol was suspended in 250 ml saline and immediately infused intravenously. Residual radioactivity in the infusion set was determined after ethanol extraction to determine the exact dose administered. For oral administration, [4-^{14}C]cholesterol in ethanol was mixed with liquid formula and given at 8:00 a.m. Radioactivity was measured in a Packard Tri-Carb Scintillation Counter (Model 3380, Packard Instrument Co., Inc., Downers Grove, Ill.) as previously described (20) on aliquots of the same plasma extracts used for cholesterol determinations.

Absorption. Cholesterol absorption was estimated by two methods: both (described and discussed as methods I and IV in ref. 21) measure unabsorbed dietary cholesterol by analysis of fecal neutral steroids, and absorbed cholesterol is determined by difference.

Analysis of fecal steroids. Fecal neutral and acidic steroids were isolated separately from 4-day pools: their mass and radioactivity were measured by methods developed in this laboratory (20, 22). Gas-liquid chromatography columns containing HiEff-8B 1% on Gas Chrom Q (Applied Science Labs, Inc., State College, Pa.) were used to determine the individual bile acid components of the fecal bile acids. β-Sitosterol was used as an internal standard to correct for losses of cholesterol during intestinal transit as well as for variations in fecal flow (23, 24). Chromic oxide was employed as an internal standard to correct for fecal flow variations in bile acid excretion (25).

Other laboratory data. To monitor possible toxic effects of neomycin on the kidneys and auditory nerves, urinalyses were carried out weekly and audiograms twice weekly. Blood urea nitrogen, serum creatinine, sodium, potassium, bilirubin, serum glutamic oxaloacetic transaminase (SGOT),[1] serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, lactic dehydrogenase, plasma proteins, hemoglobin, and white cell counts were measured at frequent intervals. Fecal fat excretion (26) was determined in each inpatient during control and neomycin periods.

Experimental design. Patients were maintained on liquid formula feedings throughout their studies on the metabolic ward. Control periods lasted a minimum of 6 wk and were followed by neomycin periods lasting at least 5 wk; neomycin was administered 1 g twice daily. Patients 2, 3, and 4 received a single dose of [1,2-^{3}H]cholesterol intravenously (100–150 μCi) approximately a week after admission, and oral doses of [4-^{14}C]cholesterol (1–11 μCi) on several occasions at widely spaced intervals for measurements of cholesterol absorption by method IV.

Informed written consent was obtained in all cases for the use of neomycin and radioactive sterols.

RESULTS

Clinical effects of neomycin. None of the four patients reported any undesirable side effects during drug therapy except patient 3, in whom diarrhea persisted throughout the neomycin period of 5 wk. All patients reported increased fecal bulk and bowel frequency, especially in the first 2 wk of drug usage. Mean bowel frequencies were 0.8, 0.6, 1.2, and 1.0/day during control periods in the four patients, and during neomycin administration 0.9, 2.3, 2.8, and 1.9, respectively. In three of the four patients daily stool weights increased 2–4-fold (Table II). In another experimental series, measurements of the dry weight of stool in patient 2 indicated that the increased stool weight during neomycin admini-

[1] *Abbreviations used in this paper:* SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

TABLE II

Effects of Neomycin on Intestinal Function and Activity of Bacterial Flora

Patient	1			2			3			4		
Period	C*	T*	N*	C	T	N	C	T	N	C	T	N
No. of stool collections‡	10	2	7	9	2	7	14	2	7	7	2	6
Daily stool weight, $g \pm SD$	50±44	74±51	77±75	64±30	312±44	262±31	83±29	229±131	252±51	64±17	165±55	145±16
Difference (N-C)§		27 NS			198 $P < 0.001$			169 $P < 0.001$			81 $P < 0.001$	
Fecal fat, *g/day*	3.5		4.5	3.5		2.3	2.2		2.3	4.7		4.5
"Secondary" fecal neutral steroids (3β-OH, 5β- and 3-keto, 5β-cpds.) *mg/day*±*SD*	672±81		0	7±6		0	469±159		0	488±187		0
β-Sitosterol recovery %‖	67		65	62		56	54		52	56		54
Fecal bile acids, *mg/day*±*SD*												
Total	160±69	141±56	253±115	259±37	2,093±1,670	224±48	328±75	336±68	277±72	202±37	506±109	178±63
Cholic	19		170	23		146	9		56	10		91
Deoxycholic	53		0	55		0	154		19	59		9
Chenodeoxycholic	34		83	54		78	52		141	38		32
Lithocholic	45		0	39		0	88		61	53		46
Isodeoxycholic¶	9		0	88		0	25		0	42		0

* C, control period; T, transitional period of 1st 8 days on neomycin; N, neomycin period after T.

‡ Collection periods of 4 days each.

§ Significance by t test between periods N and C.

‖ Relative to chromic oxide.

¶ Isodeoxycholic acid, 3β, 12α-di-OH-5β-cholanic acid.

stration was entirely due to an increase in water content from 72 to 85%, the dry weight of stool remaining stable. There was no steatorrhea in any of these four patients; however, to maintain body weights at constant levels (control level±1 kg), it was necessary to increase caloric intakes by 2, 7, and 4% in patients 1, 2, and 4. In patient 3, who complained of persistent watery stools, no significant increase in total daily caloric intake was needed.

There were no abnormalities in any of the weekly urinalyses or in the frequent measurements of blood urea nitrogen, creatinine, sodium, potassium, bilirubin, lactate dehydrogenase, SGOT, SGPT, alkaline phosphatase, and plasma proteins, or in hemoglobin, white cell counts, or differentials. Audiograms carried out twice weekly showed no changes.

Intestinal effects of neomycin. Table II shows that during neomycin administration there was complete disappearance of the "secondary" fecal neutral steroids, coprostanol and coprostanone, suggesting that the drug abolished those species of intestinal bacteria responsible for effecting the conversion of cholesterol to these 5β-products. Furthermore, there was an almost complete disappearance of deoxy- and isodeoxycholic (3β,12α-diOH-5β-cholanic) acids from the fecal acidic steroid fractions in all four patients; lithocholic acid disappeared in two patients and was reduced in the other two. The percent and absolute amounts of cholic acid increased in all four patients; chenodeoxycholic acid increased strikingly only in patient 3.

On the other hand, neomycin had no effect on neutral sterol degradation, as indicated by lack of change in the recoveries of β-sitosterol. Thus, it would appear that whatever intestinal bacteria are responsible for the degradation of neutral steroids in the course of their transit through the intestinal canal (23, 24) were not affected by neomycin in these patients, whereas those bacteria that converted cholesterol to coprostanol, and cholic to deoxycholic, were strikingly altered (either in numbers or in enzyme activities).

Table II also shows that in patients 2 and 4 there was a marked increase in daily excretion of fecal bile acids in the transitional period (the first 8 days after institution of neomycin). This effect was only temporary: bile acid excretion returned to control levels subsequently. Only in patient 1 was there a persistent small increase in fecal bile acid excretion (93 mg/day) on neomycin; it was this patient who showed the smallest increase in daily stool weight on the drug.

Thus, despite quantitative changes in neutral and acidic steroid excretion patterns caused by neomycin, there appeared to be no consistent relationship between increased stool bulk, bowel frequency, and total fecal bile acid excretion.

Effects on plasma lipids and sterol balance data. Fig. 1 illustrates the changes observed on administering neomycin to the four patients. The solid horizontal bars in the four graphs designate those consecutive weeks during which there appeared to be the greatest constancy in body weight, in clinical status, in plasma cholesterol lev-

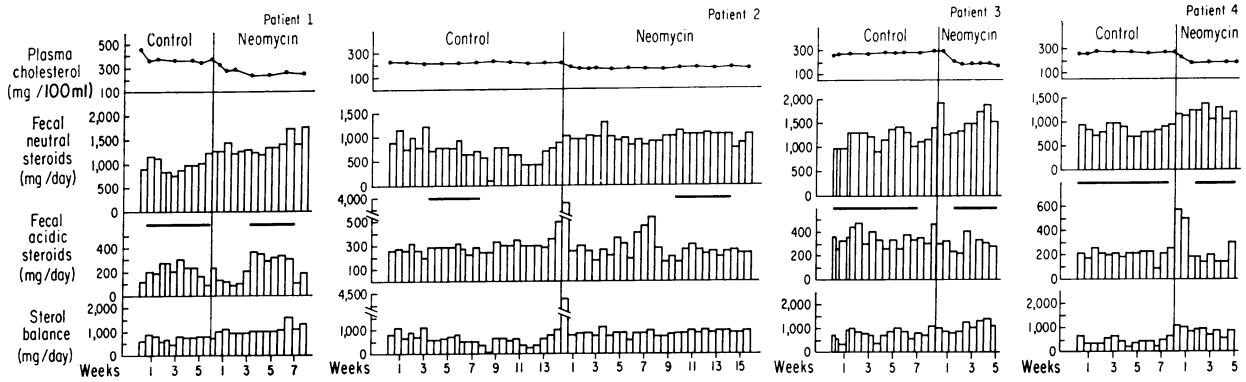

FIGURE 1 Sterol balance studies in patients 1–4. The solid horizontal bars depict steady state periods when there was the greatest constancy in body weight, clinical status, plasma cholesterol levels, and fecal steroid excretion. For instance, patient 2 developed influenza during the last week of neomycin therapy; the steady state excludes this period. There was some difficulty in maintaining constant body weight in patient 3; the steady state periods represent times of constancy in this parameter.

els, and in fecal steroid excretion; the data obtained during those weeks are shown in numerical form in Table III. Transitional periods immediately after the institution of neomycin dosage, during which plasma cholesterol levels fell to new and lower plateaus, were uniformly excluded in these calculations.

Fig. 1 shows that plasma cholesterol levels decreased rapidly in all four inpatients. The mean decrease was 25% with a range of 18–31%, and the maximum decrease was achieved within 2 wk. As indicated in Table III, plasma glycerides decreased significantly only in patient 3, one of three patients in this series who had hyperglyceridemia; in him there was a 26% decrease in glycerides.

The temporary increase in bile acid excretion in patients 2 and 4 is seen in Fig. 1, as well as the return to control levels after 1 wk. On the other hand, neutral sterol excretion increased significantly in all four patients, and the higher excretion levels were maintained throughout the neomycin period. The mean increase was 345 mg/day, with a range of 323–361 mg/day.

Changes induced by neomycin in sterol balance are shown in Fig. 1 and in Table III. Sterol balances, representing the difference between cholesterol intake and excretion, were uniformly negative in the control periods, with a mean of 618 mg/day (range 426–741). Since we assume that all patients had attained the metabolic steady state after a few weeks on the control regimen, the sterol balance figures for each patient in the control period reflect their daily cholesterol synthesis rates. However, we cannot assume that during neomycin administration a new metabolic steady state is reached in as short a time as represented by these experiments (5–15 wk), especially if the likelihood exists that the drug causes an efflux of cholesterol from tissue stores (14). Thus, in the neomycin period, the cholesterol balance represents the difference between intake and the sum of cholesterol synthesis plus cholesterol efflux from tissue stores. During neomycin administration the sterol balance became significantly more negative in all four patients, reaching a new mean of 960 mg/day (range 762–1,179).

Cholesterol absorption. Table IV presents the several measurements of cholesterol absorption made during control and neomycin periods in three patients. It is evident that cholesterol absorption decreased in each patient, and in patient 3 very markedly, during neomycin dosage.

DISCUSSION

Effect of diarrhea

Since fecal mass and bowel frequency increased in each of our patients during neomycin therapy, the problem had to be considered of the effect of diarrhea on sterol metabolism in general and plasma cholesterol levels in particular. Although the administration of Metamucil has been reported to reduce serum cholesterol levels by causing an increase in fecal bile acid excretion (27, 28), induction of diarrhea by magnesium sulfate failed to change serum cholesterol levels (29). Meihoff and Kern (30) induced diarrhea by giving oral mannitol to patients. Although the turnover of [^{14}C]cholic acid was greatly increased, they commented that "rapid transit alone was not a major cause of either rapid bile salt excretion or steatorrhea in normal subjects."

We have noted on a number of occasions that bile acid excretion rises suddenly and strikingly during an unexpected bout of diarrhea. However, we have not

TABLE III

Effects of Neomycin on Plasma Lipid Concentrations and Sterol Balance Data

Patient	1		2		3		4	
Period	C	N	C	N	C	N	C	N
Plasma lipids*								
Cholesterol	379±48 (16)	261±25 (19)	210±14 (57)	158±9 (36)	253±9 (38)	208±20 (11)	255±14 (33)	189±10 (12)
	$P < 0.0005$		$P < 0.0005$		$P < 0.0005$		$P < 0.0005$	
Triglycerides	131±20 (16)	128±24 (19)	206±32 (44)	200±27 (30)	520±170 (30)	384±89 (11)	230±33 (22)	249±66 (12)
	NS		NS		$P < 0.005$		NS	
Balance data‡								
Time in each period, *days*	42	31	21	27	47	25	27	24
No. of stool collections analyzed	10	7	9	7	14	7	12	6
Chromic oxide recovery, %	102	86	89	91	90	86	90	81
β-Sitosterol recovery, %	67	65	62	56	54	52	56	54
Cholesterol intake, *mg/day*	461	449	396	468	760	709	595	602
Total fecal neutral steroids‖								
mg/day±SD	1,028±146	1,381±175	739±92	1,062±30	1,173±168	1,515±204	825±104	1,186±119
Difference (N-C)¶	+353 $P < 0.005$		+323 $P < 0.005$		+342 $P < 0.005$		+361 $P < 0.0005$	
Total fecal acidic steroids, *mg/day±SD*	160±69	253±115	259±37	224±42	328±75	277±72	196±54	178±63
Difference (N-C)¶	+93 $P < 0.05$		−35 NS		−51 NS		−18 NS	
Total fecal steroids,‖								
mg/day±SD	1,188±133	1,634±204	998±104	1,286±39	1,501±217	1,792±244	1,021±108	1,364±124
Difference¶	+446 $P < 0.005$		+288 $P < 0.005$		+291 $P < 0.01$		+343 $P < 0.005$	
Total neutral steroids of endogenous origin,**	—	—	468±33	582±15	663±153	891±139	628±16	732±74
mg/day±SD			(7)	(2)	(4)	(5)	(3)	(4)
Difference¶			+114 $P < 0.005$		+228 $P < 0.05$		+104 $P < 0.05$	
Daily cholesterol turnover, *mg/day±SD*‡‡	—	—	718±50 (7)	849±70 (2)	1,012±173 (4)	1,188±149 (5)	821±16 (3)	922±86 (4)
Difference¶			+131 $P < 0.025$		−176 $P < 0.05$		+101 $P < 0.05$	
Cholesterol balance (excretion-intake), *(mg/day±SD*	701±133	1,179±209	602±87	818±39	741±226	1,079±226	426±114	762±124
Difference¶	+478 $P < 0.005$		+216 $P < 0.005$		+338 $P < 0.005$		+336 $P < 0.005$	

* Mg/100 ml plasma ±SD (*n* determinations).

‡ During metabolic steady state.

§ Relative to recovery of Cr_2O_3.

‖ mg/day ±SD (*n*), exclusive of plant sterols.

¶ Significance by *t* test between periods N and C.

** Neutral steroids determined isotopically (Eq. 4, Ref. 48).

‡‡ Sum of neutral steroids determined isotopically plus acidic steroids determined by GLC (Eq. 7, Ref. 48).

seen an increase in neutral sterol excretion during such episodes, and thus the persistent increase in neutral steroid excretion that characterized the response to neomycin appears to be unrelated to diarrhea per se. However, to test this point, patient 2, on completion of the present study, was given lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) (30 ml three times daily). During a control period of 6 wk the average number of daily bowel movements was 0.72, and during 2 wk of lactulose administration the frequency increased to 2.71 bowel movements daily. However, plasma cholesterol levels remained unchanged. Other antibiotic drugs tested by us in previous years (such as tetracycline, ampicillin, etc.) have usually induced diarrhea but they failed to alter plasma cholesterol levels (2, 4). We therefore conclude that diarrhea per se has little or no persistent effect on sterol metabolism or on plasma cholesterol levels.

Mechanism of decrease in cholesterol absorption

Our results show that oral neomycin results in a significant decrease in cholesterol absorption. Any of three mechanisms can be invoked.

Neomycin as sequestrant. DeSomer and colleagues (8) were the first to observe that in the test tube, neomycin precipitates bile acids out of solution. Eyssen, Evrard, and Vanderhaeghe (31) suggested that the hypocholesteremic effect of neomycin might be due to the formation of insoluble aggregations of bile acids with the polybasic antibiotics in the intestinal lumen, and that neomycin was acting like cholestyramine as a bile acid sequestrant. Van den Bosch and Claes (32) demonstrated in vitro precipitation of deoxycholate by neomycin at a pH of 6.3, and these observations were confirmed and extended by Thompson and colleagues (9),

who did in vitro studies with bile acid micelles. Faloon et al. (33) showed that neomycin precipitated bile acids out of human bile in vitro, and Thompson et al. (10) demonstrated that neomycin decreased the fatty acid and bile acid content of micelles in the human intestinal lumen. However, Hardison and Rosenberg (34) obtained different results: they found that during neomycin administration in three patients, bile salt and fatty acid concentrations in the duodenal micellar phase were not reduced.

If the hypocholesteremic action of neomycin is due to decreased cholesterol absorption secondary to bile acid sequestration, daily fecal bile acid excretion might be expected to increase during neomycin therapy. Indeed, Goldsmith, Hamilton, and Miller (13), Powell, Nunes, Harding, and Vacca (11), and Faloon, Rubulis, and Rubert (12) reported that fecal bile acid excretion was increased in patients treated with neomycin. Our studies do not agree with the above: in three of four patients given neomycin for 5–15 wk there was no difference in bile acid excretion between neomycin and control periods, and in the remaining patient the increase in bile acid output was only 97 mg/day. However, in two patients there was a striking rise in bile acid excretion during the 1st wk of neomycin dosage; thereafter, the excretion rates returned to base-line control levels. Miettinen (35) very recently reported results similar to our own: decreased cholesterol absorption and increased fecal neutral steroid excretion.

These findings appear to indicate that neomycin, given orally in a dose of 2 g daily, is not an effective bile acid-sequestering agent and that its mode of action as a hypocholesteremic drug is different from that of cholestyramine. However, we cannot exclude the possibility that neomycin sequesters bile acids and hinders normal micelle formation in the upper small intestine where cholesterol absorption is maximal, but that the aggregates dissociate in the ileum, thus allowing bile acids to remain in the enterohepatic circulation and to escape excretion in feces. Indeed, Thompson et al. (9) found that neomycin binding of micellar bile acids was reversible and that precipitated taurocholate readily redissolved in buffer solution. We know of no direct evidence on this occurrence in vivo.

Toxic action on mucosa. Large doses of oral neomycin (12 g daily) cause steatorrhea (5) with diminished absorption of glucose, xylose, iron, vitamin B_{12}, and carotene, as well as fats. Histological studies in jejunal mucosa obtained by suction biopsy from subjects on 8–12 g neomycin daily showed changes similar to those seen in idiopathic steatorrhea and celiac disease (6). Indeed, even on doses as low as 3 g daily, patients have been reported to show malabsorption of fats and *d*-xylose (36). Even though our previous studies of fat absorption in seven patients on 2 g/day doses failed to demonstrate steatorrhea in a single case (7) and even though we showed in the present study that none of our four inpatients had steatorrhea, it was clearly evident that bowel habits were affected: three of four patients had large increases in daily stool weights even after an 8-day adaptation period, and stool frequency increased regularly and persistently in these formula-fed patients. Since we did not perform intestinal mucosa biopsies, we cannot deny that morphological changes might have occurred. However, the absence of such findings would not necessarily rule out the possibility that the minute amounts of neomycin absorbed by the intestine (2) may interfere with one or another biochemical step in the transfer of cholesterol from the brush border (as free cholesterol) to the lymphatics largely as the oleate ester [37]).

TABLE IV
Data on Cholesterol Absorption

	Cholesterol absorption			
	Control period		Neomycin period	
Patient	Method I*	Method IV‡	Method I*	Method IV‡
		% ±SD		
2	40±12 (7)	56±10 (2)	26±10 (3)	24±11 (2)
3	39±5 (3)	49±13 (2)	6±6 (4)	3
4	52±9 (3)	49	32±8 (4)	20±5 (2)

* Numbers in parentheses represent the number of stool collections analyzed (21).
† Numbers in parentheses represent the number of method IV tests carried out, each an analysis of 8 days' stool collections after oral administration of labeled cholesterol (21).

Change in bacterial flora. The administration of oral neomycin has profound effects on the bacterial flora of the intestine (11, 38), and it is well known that this agent is hypocholesteremic only when administered by mouth (2). Is it possible that the drug decreases cholesterol absorption by some effect on the intestinal flora?

The oral administration of 2 g neomycin daily resulted in the virtual disappearance of the secondary bile acid, deoxycholic acid, from the feces of the four inpatients, and in two there was a significant reduction in excretion of lithocholic acid. We presume that the bacteria that elaborate 7α-dehydroxylase were inhibited in their growth: indeed, Samuel, Holtzman, Meilman, and Sekowski (4) have shown a direct relationship between plasma cholesterol levels and the in vitro 7α-dehydroxylation of bile acids by fecal bacteria in patients treated with neomycin (and other antibiotics). In those pa-

tients who had no decrease in plasma cholesterol on these antibiotics, fecal 7α-dehydroxylation was not inhibited.

Whether the degree of enrichment of intestinal luminal contents with deoxycholate has a direct effect on cholesterol absorption or an indirect effect through some action of its own on mucosal cell function remains conjectural; some of the numerous possibilities are discussed in Heaton's monograph (39) and in a report on bile acid diarrhea by Hofmann and Poley (40).

Interpretation of sterol balance data

During neomycin therapy there was a significant increase in the total fecal sterol excretion in all four patients. In the paragraphs below we will seek to distinguish whether this increased excretion was due to redistribution of cholesterol from plasma to feces, decreased absorption of cholesterol, increased synthesis, efflux from tissue stores, or some combination of these factors.

Plasma cholesterol decrement. One possible source of an increment in fecal sterol excretion is the cholesterol circulating in the plasma, since in all four patients the plasma concentrations decreased on neomycin administration. This possibility can be ruled out as follows. During the transitional periods when plasma cholesterol concentrations decreased to reach a new plateau, the decrements in plasma cholesterol contents (assuming plasma volume to equal 4.5% of total body weight [41]) were 2.9, 1.5, 1.7, and 1.9 g in patients 1, 2, 3, and 4, respectively. During the same time period the increments in fecal steroid output were 3, 18, 3.5, and 5 g, respectively; these data reflect the large but temporary increase in bile acid excretion in patients 2 and 4. However, despite the lack of change in plasma cholesterol content in any of these patients during several *subsequent* weeks of neomycin administration, there was a continuing mean daily increment of total fecal steroid excretion, compared to control periods, of 478, 216, 338, and 336 mg/day.

Thus, although some part of the excretion increment in the transitional period can be ascribed to redistribution of plasma cholesterol, this explanation cannot explain the continued increments after plasma levels had reached new plateaus.

Decreased cholesterol absorption. Combining the sterol balance data shown in Table III with the changes in percent absorption of dietary cholesterol listed in Table IV, we can calculate that patient 2 absorbed 145 mg less in the neomycin period than in the control period; this factor alone would have led to a 145 mg increment in fecal neutral steroid output per day. However in this patient the actual increment in fecal neutral steroids on neomycin was 323 mg/day, which leaves 178 mg unaccounted for. Similar calculations for patients 3 and 4 show the following comparisons: patient 3, 241 mg absorption decrement, 342 mg output increment, 101 mg unaccounted for; patient 4, 150 vs. 361, and 211 mg unaccounted for.

Thus, although all three patients absorbed significantly less dietary cholesterol during neomycin dosage, it is evident that the increments in fecal neutral steroid outputs cannot be explained solely on this basis. However, these calculations neglect the probability that the absorption of cholesterol of endogenous origin was also interfered with by neomycin. Indeed, that part of the increment in fecal neutral steroid excretion referred to as "unaccounted for" in the preceding paragraph could conceivably represent increases in unabsorbed endogenous cholesterol. However, at present we have no way to compare the absorption of endogenous vs. exogenous cholesterol, nor do we measure the rate of input of endogenous cholesterol into the intestinal lumen. Thus, it is impossible (with balance data alone) to resolve the question whether the entire increment in fecal neutral steroid excretion during neomycin administration is due solely to decreased absorption of cholesterol (exogenous plus endogenous). However, some light is shed on the matter by consideration of specific activity-decay curves after intravenous administration of labeled cholesterol; such consideration leads us to discard cholesterol malabsorption as a *single* factor explaining the experimental results, and to question whether the unaccounted-for increments can be ascribed to increased cholesterol synthesis, or to flux of cholesterol from tissue stores, or both.

Increased synthesis or flux, or both. Resolutions of the dilemma we have posed have been sought, in the past, through interpretations of changes in slope of specific activity-time curves after intravenous administration of labeled cholesterol: a steepening of slope on institution of a new regimen has been considered to reflect increased synthesis, a flattening to represent decreased synthesis. Although workers in this laboratory have placed some reliance on these slope-changes in published studies of the mechanism of action of unsaturated dietary fats (42), β-sitosterol (43), cholestyramine (44), and clofibrate (45), we have always taken the position that this practice is defensible only in those situations when independent evidence can be produced to support the interpretation.

In patients 2, 3, and 4 we found no change in slope of plasma cholesterol decay curves on instituting neomycin (Fig. 2), either with curve fitting by eye or by computer; yet independent tests showed decreased absorption of dietary cholesterol (and presumably also of endogenous cholesterol). Decreased absorption of unlabeled cholesterol would be expected to cause a flattened slope, and this was not observed. This would seem to rule out the possibility that the entire increase in fecal

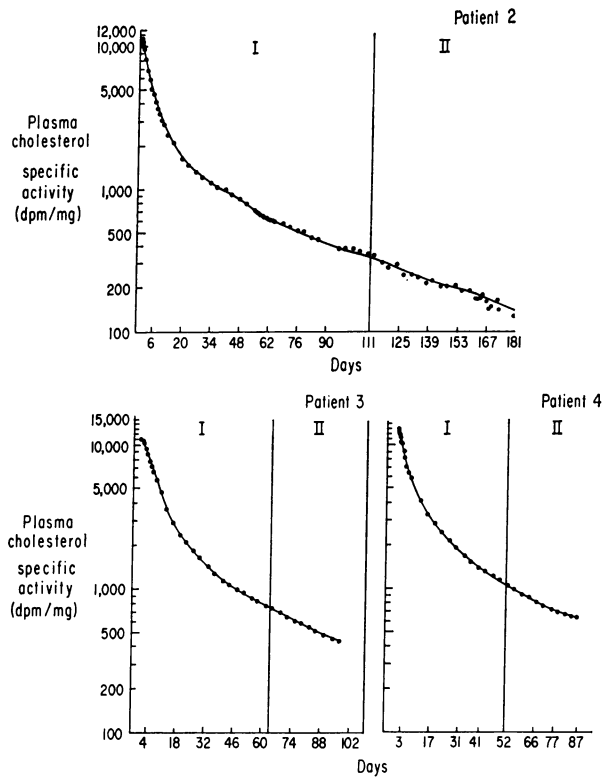

FIGURE 2 Plasma cholesterol specific activity-time curves in patients 2–4 during the control period (I) and the administration of neomycin (II). There was no change in the slope of the curves on instituting neomycin.

neutral steroid excretion on neomycin was due solely to decreased cholesterol absorption. However, release of feedback control of cholesterol synthesis secondary to decreased absorption would cause a counterbalancing increase in slope. To what extent one effect can precisely balance another we do not know.

Likewise, if cholesterol more richly labeled in tissues than in plasma were mobilized into the plasma compartment, a flattening of slope would be expected (46). But this change would be hidden if simultaneously there were increased synthesis secondary to decreased absorption.

The possibility could be considered that some damage to mucosal cells occurs with the low doses we have been studying (though the damage has not been recognized), and that this leads to an increased turnover of mucosal cells and exudation of cholesterol newly synthesized in these cells into the gut lumen. This possibility was examined in patient 2, subsequent to the completion of the studies reported in this paper. While ingesting a cholesterol-free diet, he was relabeled with a single intravenous dose of radioactive cholesterol, and the specific activities of plasma cholesterol were compared to those of the fecal neutral steroids (exclusive of plant sterols). The two sets of specific activity data compared extremely closely before and after the reinstitution of neomycin therapy. This suggests that there was no significant input of unlabeled cholesterol into the gut lumen from the hypothetically damaged mucosa, for in that case (as we have previously demonstrated [44] when cholestyramine is administered) the specific activities of fecal neutral steroids would have been significantly lower than that of plasma cholesterol. (These data do not rule out an increased transudation of labeled plasma cholesterol through the hypothetically damaged mucosa into the gut lumen).

These uncertainties prevent our reaching a firm conclusion from the present data whether the increment in fecal steroid output on neomycin was due to increased synthesis, to flux of cholesterol from tissues, to decreased absorption, or to all three factors. What is needed to resolve this dilemma is a measure of cholesterol synthesis that is valid in the unsteady state; although work is in progress in this laboratory on such a method (47), we were not prepared to apply this new approach to the above problem at the time the present studies were undertaken.

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