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Research Article

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Effects of Neomycin Alone and in Combination with Cholestyramine on Serum Cholesterol and Fecal Steroids in Hypercholesterolemic Subjects

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ABSTRACT Effects of neomycin were studied on serum cholesterol and fecal steroids in hypercholesterolemic patients during a short treatment period (4 wk) and a long treatment period (16 mo), using small (1.5 g/d) and large (up to 6 g/d) doses alone and in combination with cholestyramine. In the short-term low-dose study the decrease in serum cholesterol by 21% was associated with a proportionate increase in fecal cholesterol elimination as neutral sterols through impaired cholesterol absorption. Serum cholesterol remained low and fecal steroid excretion remained elevated in the long-term neomycin study. Increasing the dosage from 1.5 to 6 g/d at the end of the 16-mo period brought about a further slight decrease in serum cholesterol and a small further increase in fecal neutral and acidic steroids. The increases in fecal bile acids and fat but not in neutral sterols were positively correlated with the increases in the neomycin dosage. Thus, large neomycin doses can also cause bile acid malabsorption. In another series of patients, a decrease (25%) in serum cholesterol by cholestyramine was associated with a proportional increase in the fecal elimination of cholesterol (2.5-fold) as bile acids. The inclusion of neomycin in cholestyramine therapy further increased fecal steroid output (solely as neutral sterols) by only about one-fifth of that due to cholestyramine, but further decreased serum cholesterol almost to the same extent (−17%) as cholestyramine alone. The overall decrease was 38%, no side effects occurred, and the patients found combination therapy convenient. Neomycin decreased serum cholesterol in different studies by 10±2, 17±4, and 12±4% per 100 mg/d of the increment in fecal steroids, the respective decrease for cholestyramine being only 2.2±0.5%. Thus, neomycin effectively reduced serum cholesterol by a relatively small increase in cholesterol elimination (via cholesterol malabsorption) compared with cholestyramine-induced bile acid malabsorption.

This study was presented in part at the 7th Annual Meeting of the European Society for Clinical Investigation, April 1973 (abstracted in *Eur. J. Clin. Invest.* 1973. **3**: 356), and at a Symposium on Lipid Metabolism and Atherosclerosis, Amsterdam, 1973 (abstracted in 1973. *Excerpta Med. Int. Congr. Ser. NB.* **283**: 83).

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INTRODUCTION

Neomycin, an almost unabsorbable polybasic antibiotic, reduces serum cholesterol in doses of 1–2 g/d by ≅20% without major side effects even in long-term use (1, 2). Cholestyramine is an unabsorbable ion exchanger that binds bile acids in the intestinal lumen so that cholesterol elimination into the stools as bile acids is enhanced, subsequent average reductions of serum cholesterol being in the range of 20 to 30% (3). Because neomycin precipitates dihydroxy bile acids in vitro (4–8), its cholesterol-lowering effect was also assumed to be a result of enhanced bile acid excretion into feces, particularly because earlier studies, employing relatively large doses of neomycin or *N*-methylated neomycin derivative without antibiotic properties, actually demonstrated an increase in both fecal bile acids and neutral sterols (6, 9, 10). However, analysis of intestinal contents after administration of neomycin with a test meal revealed that the drug precipitated fatty acids and cholesterol, and only to a small extent bile acids from intraluminal micelles in man (11). Small neomycin doses actually reduced serum cholesterol by enhancing cholesterol elimination into feces as neutral sterols, having no consistent effect on fecal bile acids (12, 13). It is not, however, known whether large neomycin doses could also increase fecal bile acid output; whether the neomycin-induced increase in fecal steroids is long-standing, as the serum cholesterol lowering is; whether the decrease in serum cholesterol is related to changes in cholesterol

elimination; and whether cholesterol synthesis is compensatorily increased by the drug. Cholesterol turnover studies off and on neomycin did not reveal any significant changes in the half time of the serum cholesterol radioactivity decay curve (14), and the unchanged slope of this curve during the neomycin-induced fall in serum cholesterol was interpreted to be a result of the combined action of reduced absorption, enhanced mobilization of tissue cholesterol and stimulated cholesterol synthesis (13).

In the present study emphasis is placed on the effect of small and large neomycin doses on fecal steroids and serum cholesterol in short- and long-term treatment, and to neomycin-induced alterations in these parameters under conditions when cholesterol elimination is increased by cholestyramine in the form of bile acids.

METHODS

Patients. The studies reported here were conducted on 17 subjects (Table I) in the metabolic ward. Most of the patients had a clear xanthomatotic, familial type II hypercholesterolemia and severe to moderate atheromatotic arterial disease. In patients 3, 7, 8, and 14 the family history was less clear, the degree of hypercholesterolemia was less severe and xanthomata were absent. Before the present investigation most of the patients had been on a low-cholesterol diet. Patient 8 had idiopathic hypercalciuria associated with enhanced intestinal calcium absorption and slight renal tubular insufficiency but normal plasma creatinine and creatinine clearance. There was no evidence of hyperparathyroidism. The purpose and design of the study were explained to the patients, and they agreed to act as volunteers. The protocol was approved by the ethics committee of the hospital.

Studies. The general plan of the study was first to record base-line values during a control period, then treat patients 1–8 with neomycin alone, and patients 9–17 with cholestyramine followed by a combination of cholestyramine and neomycin. For these purposes all the subjects were placed on a solid food low-cholesterol diet containing 80 g fat (a mixture of lard and soya oil)/2,400 kcal. Analysis of the diet revealed that the average cholesterol content was 120 mg/2,400 kcal. Chromic oxide (Cr_2O_3) and β-sitosterol (200 mg of each three times a day) were given so that the respective corrections for fecal flow and any degradation of cholesterol during intestinal transit could be made (15). After 7–10 d on the diet, Cr_2O_3 and β-sitosterol 2- or 3-d stool collections were started. After one to three collections either neomycin (1.5 g/d) or cholestyramine (Cuemid; 32 g/d) therapy was started. Fecal collections were performed during the last few days of each treatment period. After 10–12 d of cholestyramine, neomycin was also given to patients 9–17 for an additional 10–12 d. In patients 2 and 5 fecal collection was performed during rehospitalization after the patients had been on neomycin for 4.5 and 1.5 mo, respectively. Patient 12 was first on cholestyramine for 35 d and then on combined cholestyramine-neomycin therapy for 12 d. Rehospitalization to obtain the control values of this case took place 2 mo later.

Even though maximal blood cholesterol reduction was achieved during the 1st wk on the drugs, any mobilization of tissue cholesterol may have continued so that sterol metabolism of the patients was still most likely in a nonsteady state at the end of each short-term treatment period. Thus, the sterol balance value, the difference between cholesterol intake and fecal excretion of acidic and neutral steroids of cholesterol origin obtained at the end of each short-term drug period, might have exceeded the actual cholesterol synthesis by an

TABLE I
Clinical Data of Patients

Patient number	Age	Sex	Height	Weight	Plasma lipids*		Clinical remarks
					Cholesterol	Triglycerides	
	yr		*cm*	*kg*	*mg/100 ml*	*mmol/liter*	
1	48	F	160	68	645	1.80	FHC, Tx, OMI, OCVA, DL‡
2	62	F	156	64	852	2.04	FHC, Tx, OMI, cholecystectomy, DL
3	42	F	163	60	387	2.68	Occlusion of right subcl. artery
4	40	M	172	78	484	3.11	FHC, DL
5	47	M	176	72	611	1.89	FHC, CAD, Tx, hypercalciuria
6	33	M	174	65	556	1.97	FHC, Tx
7	64	F	161	74	359	4.94	OMI, diabetes, hypertension
8	35	M	189	98	301	1.98	Hypercalciuria
9	24	M	165	53	538	2.22	FHC, Tx, CAD
10	30	M	166	61	563	2.01	FHC, CAD
11	26	M	164	61	639	2.08	FHC, OMI, Tx
12	31	M	169	67	666	2.30	FHC, Tx, CAD
13	49	F	167	65	706	1.83	FHC, OCVA
14	47	M	171	64	347	2.45	OMI, intermittent claudication, DL
15	34	M	173	69	487	2.64	FHC, Tx, CAD
16	50	F	154	62	628	4.32	FHC, Tx, OMI, OCVA
17	44	M	173	71	445	2.50	FHC, Tx

* The highest lipid level ever recorded before any treatment.
‡ FHC, familial hypercholesterolemia; OMI, old myocardial infarction; CAD, coronary artery disease. Tx, tendon xanthomata; OCVA, old cerebrovascular accident; DL, latent diabetes.

unknown amount of the cholesterol mobilized from tissues. Therefore, to eliminate any nonsteady state of the short-term experiments, sterol balance studies were repeated after the patients had been on neomycin for 1 yr or more. Five subjects volunteered (patients 1, 4, 5, 10, and 13) to undergo long-term studies and were rehospitalized after they had been on neomycin for 390-660 d. Patients 10 and 13 had been on combined cholestyramine-neomycin treatment for 2 mo after the first studies but since then had been on neomycin alone. After the base-line reinvestigations had been performed, the neomycin dose was increased from 1.5 to 2 g in patient 10, to 3 g in patient 13, and to 6 g in patients 1, 4, and 5. Fecal collections were repeated at the end of the 10- to 14-d period on the large neomycin dose.

Chemical analysis. Total serum cholesterol was determined by the method of Pearson et al. (16) from the petroleum ether extract of nonsaponifiable material. Triglycerides were measured according to Kessler and Lederer (17). Fecal and acidic steroids were isolated separately and their masses were quantitated by the thin-layer chromatography–gas-liquid chromatography method as presented previously (18, 19). This method permits a distinct separation between neutral sterols of cholesterol and plant sterol origin provided unchanged parent sterols are isolated from the bacterial conversion products with thin-layer chromatography before gas-liquid chromatography quantitation. Thin-layer chromatography purification is not necessary for bile acids (20). Chromic oxide was determined according to Bolin et al. (21). It was used as a marker for corrections of day-to-day variations in the fecal flow. Because the recovery of β-sitosterol was complete from feces, the fecal flow corrected with chromic oxide was used for fecal steroid, fat, and mass calculations.

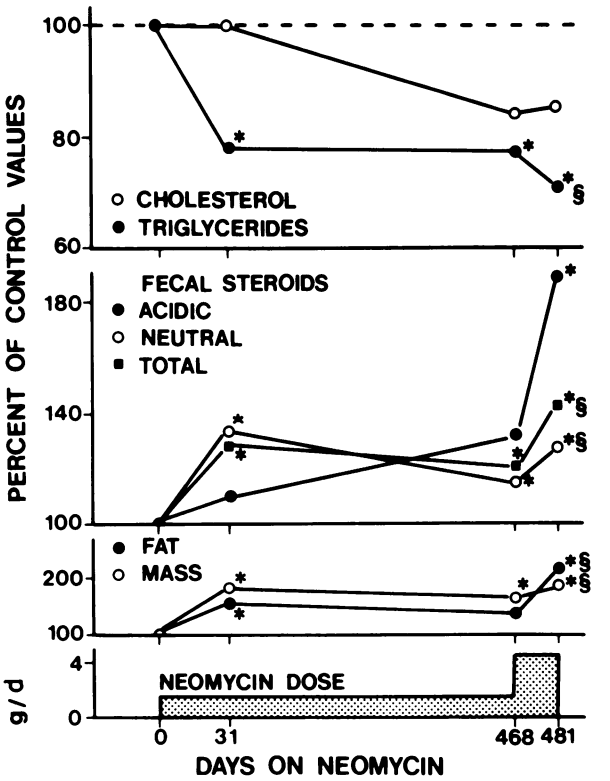

FIGURE 1 Relative mean changes in serum lipids (upper panel), fecal steroids, and fecal fat and mass by neomycin. Calculated from results in Table II. *, $P < 0.05$ from control values. §, Significant change ($P < 0.05$) caused by high dose.

RESULTS

Short-term effects of neomycin. Neomycin, 1.5 g/d, reduced serum cholesterol by 21% (Table II, Fig. 1), this reduction being reached during the 1st wk of neomycin treatment. Serum triglycerides remained unaffected. Fecal mass was doubled and fecal fat was increased by 2.7 g/d. The drug exerted no consistent effect on fecal bile acids but did increase fecal neutral steroid elimination on the average by 228 mg/d. Because the dietary cholesterol intake was low (mean 105 mg/d), most of this increase was a result of enhanced excretion of endogenous neutral sterols. The increment in total fecal steroids was 261 mg/d (30% increase). The change in the total fecal steroid output was significantly correlated ($r = 0.736$) with the change in serum cholesterol, indicating that the greater the increase in cholesterol elimination, the greater the decrease in the serum cholesterol level (Fig. 2).

Long-term effects of neomycin. As shown in Table II and Fig. 1, after 1 yr or more on neomycin, serum cholesterol was still 23% lower than before treatment, although fecal fat was no longer consistently elevated. Fecal neutral and total steroid excretions, and fecal mass were still significantly increased. Expressed as per kilogram of body weight, fecal bile acids (3.91 mg/kg per d) were slightly ($P < 0.05$) above the pretreatment level (3.05 mg/kg per d). The mean daily cholesterol elimination was 846 mg without neomycin treatment and 1,008 mg after 16 mo with neomycin treatment, the correlation ($r = 0.706$) between the decrease in serum cholesterol and elimination being not quite statistically significant (Fig. 2).

High-dosage effects of neomycin. The increase in the neomycin dose (see Table II) from 1.5 to 2–3 g/d after long-term treatment had no further effect on serum cholesterol, but at a dose of 6 g/d an additional drop was recorded in the serum total cholesterol level of patients 4 and 5. The further mean reduction of the whole group was 9% and the overall fall 29%. The fall in cholesterol was significantly correlated with the neomycin dose ($r = 0.690$; $P < 0.01$; the differences from the control values at all neomycin doses were correlated with the respective neomycin doses shown in the footnote to Table II). The increase in the neomycin dose was associated with slightly impaired fat absorption and with an increase in fecal steroid elimination (195 mg/d; 16%) that took place not only in the neutral steroid fraction but also in the acidic sterol fraction. The largest increase in fecal steroids was found on the highest dose in patients 4 and 5 in whom the fall in serum cholesterol was also largest. The correlation between the changes in serum cholesterol and fecal steroids did not quite reach a significant level,

TABLE II

Serum Lipids and Fecal Steroids in Patients Treated with Neomycin

Patient number	Serum lipids*		Stool collections		Fecal steroids		
	Cholesterol	Triglycerides			Acidic	Neutral	Total
	mg/100 ml		*no.*	*d*	*mg/24 h*		
Control							
1	436	144	1	3	209	736	945
2	445	115	1	3	100	777	877
3	314	229	1	3	187	563	750
4	346	227	1	3	296	841	1,137
5	466	143	1	3	209	548	757
6	402	128	1	3	187	304	491
7	236	220	1	3	318	667	985
8	208	147	1	3	135	704	839
10	351	147	1	3	147	422	569
13	307	190	1	3	196	626	822
Mean±SE‡	351±34	170±17			205±26	643±60	848±68
Neomycin, low dose for 10–146 d (mean 31 d)§							
1	332	103	1	3	204	940	1,144
2	386	205	1	3	118	1,092	1,210
3	251	127	1	3	114	737	851
4	229	245	1	3	424	1,156	1,580
5	360	123	1	3	186	1,115	1,201
6	375	157	1	3	190	390	580
7	154	174	1	3	532	718	1,250
8	185	149	1	3	138	821	959
Mean±SE	284±32‖	160±17			238±55	871±92‖	1,109±108‖
Neomycin, low dose for 403–660 d (mean 468 d)							
1	337	137	3	6	251±10	978±31	1,229±39
4	243	113	3	6	351±51	929±4	1,280±34
5	329	127	3	6	387±24	576±12	963±48
10	294	123	2	4	198	500	698
13	262	193	2	6	196	674	870
Mean±SE	293±18‖	139±14			277±39	731±95‖	1,008±110‖
Neomycin, high dose for 10–14 d¶							
1	327	157	3	6	298±13**	1,109±52	1,407±58
4	205	106	3	6	723±83**	991±91	1,714±125**
5	275	143	3	6	492±32	711±6**	1,203±34**
10	283	122	2	4	197	516	713
13	252	198	2	6	220	760	980
Mean±SE	268±20‖	145±16			386±99	817±105‖**	1,203±172‖**

* Means of two to three determinations at the end of each period.

‡ Calculated for patients 1–8.

§ 1.5 g/d for 10–12 d except in patient 2 for 136 d and in patient 5 for 46 d.

‖ Significant change ($P < 0.05$ at least) from control values.

¶ Dose increased from 1.5 g/d after 403–660 d to 6 g/d in patients 1–5, to 2 g/d in patient 10, and to 3 g/d in patient 13.

** Significant change caused by high dose of neomycin.

however (Fig. 2). The plot of the changes in the neomycin dose against the respective changes in fecal lipids revealed a positive correlation for bile acids and fecal fat but not for neutral sterols (Fig. 3).

Effects of cholestyramine. Cholestyramine had no effect on serum triglycerides but it did decrease total cholesterol by 25% (Table III and Fig. 4). This was associated with an increase in fecal bile acids from 203 to 1,497 mg/d, whereas fecal neutral steroids were not consistently changed. Total cholesterol elimination was enhanced from 838 to 2,205 mg/d, the increase being significantly correlated with the reduction in serum cholesterol ($r = 0.850$; $P < 0.01$).

Effects of neomycin in combination with cholestyramine. The administration of neomycin to patients on cholestyramine (Table III, Fig. 4) further reduced

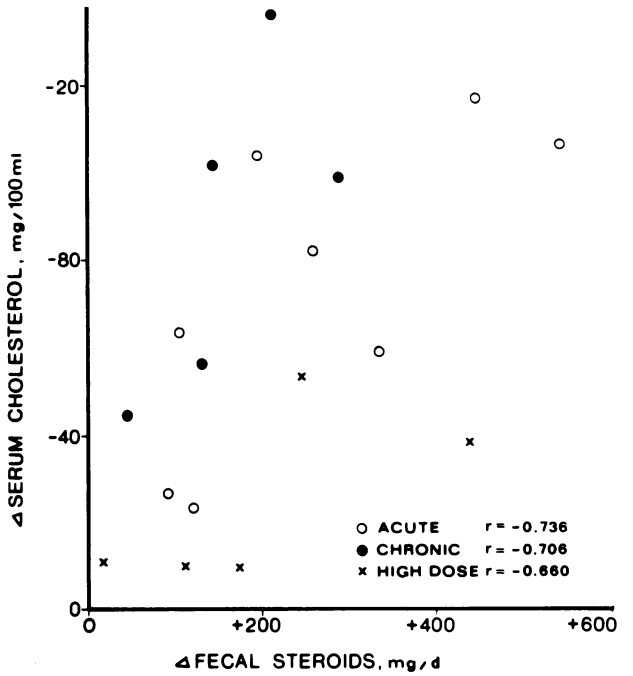

FIGURE 2 Correlation between the neomycin-induced decrease in serum cholesterol and the corresponding increase in fecal steroids. Changes calculated between control and 31 d (acute), control and 468 d (chronic), low dose (468 d) and high dose of Table II; $r = -0.736$, $P < 0.05$. Other correlation did not reach a statistically significant level.

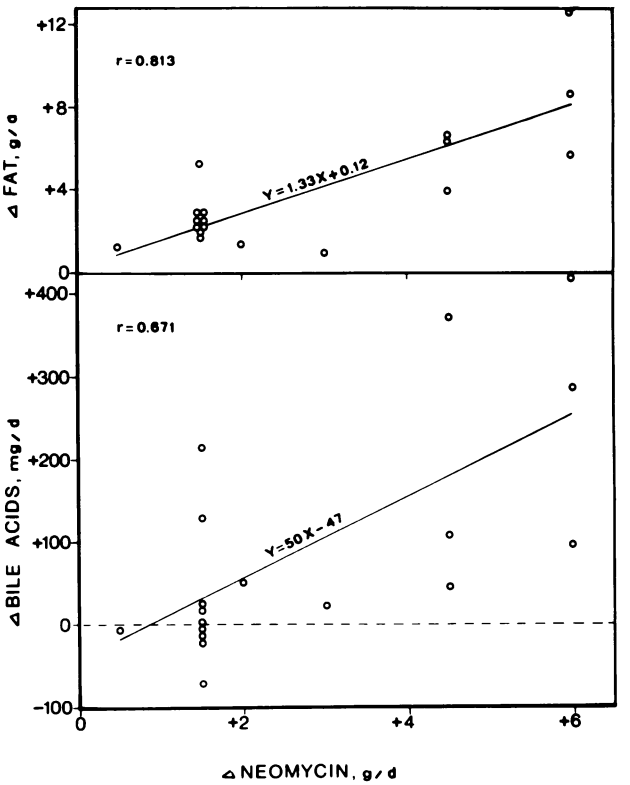

FIGURE 3 Plot of changes in fecal fat (upper panel) and fecal bile acids (lower panel) against changes in neomycin dose (P values <0.001 and <0.01, respectively). Differences between periods of low dose (31 d) and control, high dose and low dose (468 d), and high dose and control of Table II are included. Correlation for fecal neutral sterols was not statistically significant ($r = -0.077$).

total serum cholesterol by 17%, a decrement only slightly lower than that caused by neomycin (21%) and cholestyramine (25%). Serum triglycerides were significantly decreased. The overall reduction was 38% for total cholesterol and 25% for triglycerides. Body weight was unchanged.

Fecal weight was increased during cholestyramine treatment by 81 g/d and during combined treatment, by 110 g/d. Fecal fat increased correspondingly by 1.41 and 1.76 g/d, the overall increase for fecal weight being 191 g/d and for fecal fat 3.17 g/d. Neomycin reversed cholestyramine-induced constipation but did not cause diarrhea. The patients thus found combination therapy preferable to cholestyramine alone.

Neomycin further increased fecal total steroids from 2,205 to 2,583 mg/d (17%) in the subjects treated with cholestyramine. Combined therapy seemed to be effective as long as the drugs were administered because in patients 10 and 13 the additional changes in serum lipids and fecal steroids were detectable for at least 72 d (Table III). The increase in fecal steroids was mostly a result of enhanced neutral steroid excretion (+253±29 mg/d), of the same order of magnitude as in the series treated with neomycin alone (+229±59 mg/d). The additional change in fecal steroids was not correlated with that in serum cholesterol. However, the overall decrease in serum cholesterol by combined cholestyramine-neomycin treatment was significantly correlated with the corresponding increase in fecal steroids ($r = 0.743$, $P < 0.01$).

Calculation from Tables II and III revealed that neomycin reduced serum cholesterol by 10±2, 17±4, and 13±4% per 100 mg/d of the increase of fecal steroids in the acute, chronic, and cholestyramine studies, respectively. For cholestyramine alone this value was only 2.2±0.5%, indicating a marked difference between the mechanisms by which the two drugs lower serum cholesterol.

Effect of neomycin on bacterial conversion of fecal sterols. The relative amount of intact (Δ^5-sterols) fecal plant sterols was greater than that of cholesterol (Table IV) probably on account of the different physical state of dietary sterols. Neomycin significantly reduced bacterial conversion of cholesterol and plant sterols (Δ^5-sterols) to the secondary products (5-β-sterols) in short-term therapy whether neomycin was used alone or in combination with cholestyramine. In contrast to earlier observations (13), the secondary products disappeared completely in only one case. Possibly because

TABLE III

Effects of Cholestyramine Alone and in Combination with Neomycin on Plasma and Fecal Lipids

Patient number	Plasma lipids*		Fecal steroids‡			Fecal fat	Fecal mass
	Cholesterol	Triglycerides	Acidic	Neutral	Total		
	mg/100 ml	*mg/100 ml*		*mg/d*		*g/d*	*g/d*
Control values							
9	413	126	153	496	649	3.45	146
10	351	162	147	422	569	2.49	223
11	392	140	139	454	593	3.04	120
12	460	201	296	929	1,225	3.91	186
13	307	185	196	626	822	3.16	94
14	265	194	179	914	1,093	4.18	224
15	445	188	226	468	694	3.60	195
16	379	171	194	573	767	2.80	168
17	615	290	297	831	1,128	2.40	202
Mean±SE	403±34	184±16	203±20	635±68	838±83	3.23±0.21	173±15
Cholestyramine for 10–35 d							
9	280	140	1,681	505	2,186	3.61	292
10	272	146	1,267	518	1,785	6.26	358
11	316	122	1,160	539	1,699	5.71	282
12	264	178	2,426	1,396	3,822	7.09	258
	270	170	2,311	1,452	3,763	6.95	244
13	271	191	945	745	1,690	4.15	170
14	180	130	753	838	1,591	4.50	263
15	341	190	1,695	424	2,119	3.90	165
16	259	145	1,609	672	2,281	3.20	248
17	522	386	2,049	683	2,732	3.50	263
Mean±SE	301±31§	180±27	1,497±170§	708±103	2,205±230§	4.64±0.45§	254±20§
Cholestyramine plus neomycin for 10–72 d							
9	203	114	1,495	771	2,266	5.80	404
10	220	112	1,055	798	1,853	6.84	430
	222	118	1,110	846	1,956	5.40	395
11	277	112	1,315	807	2,122	5.63	406
12	211	143	3,150	1,568	4,718	8.65	339
13	227	138	1,310	1,065	2,375	4.88	272
	221	131	1,220	1,178	2,398	3.25	290
14	155	109	403	1,252	1,655	4.09	205
15	286	156	1,600	666	2,266	7.40	375
16	213	85	2,285	871	3,156	9.60	591
17	480	289	1,980	853	2,833	4.70	252
Mean±SE	252±31§‖	140±20‖	1,641±261§	961±96§‖	2,583±307§‖	6.40±0.62§‖	364±38§‖

Cholestyramine (32 g/d) was first given for 10–12 d except to patient 12 for 35 d followed by inclusion of neomycin. The dose for patient 16 was 3 g/d, otherwise 1.5 g/d, and it was given for 10–12 d except to patients 10 and 13 for up to 72 d.

* Mean of two to three determinations at the end of each period.

‡ One 3-d collection at the end of each period. In patient 12 measurements were performed after 10–12 d and 33–35 d on cholestyramine therapy and in patients 10 and 13 after 10–12 d and 70–72 d on combined therapy.

§ Statistically significant change ($P < 0.05$ at least) from control values.

‖ Statistically significant change ($P < 0.05$ at least) from cholestyramine values.

of the development of bacterial resistance to neomycin (22), in long-term treatment reduced bacterial conversion of cholesterol was no longer detectable, and the increase in the neomycin dose to 6 g/d after long-term treatment had no significant effect on this conversion. These findings suggest that the persistent decrease in serum cholesterol and increase in fecal neutral sterols are not associated with the antibacterial action of neomycin.

DISCUSSION

Normalization of serum cholesterol in severe hypercholesterolemia by any means is a difficult task and in

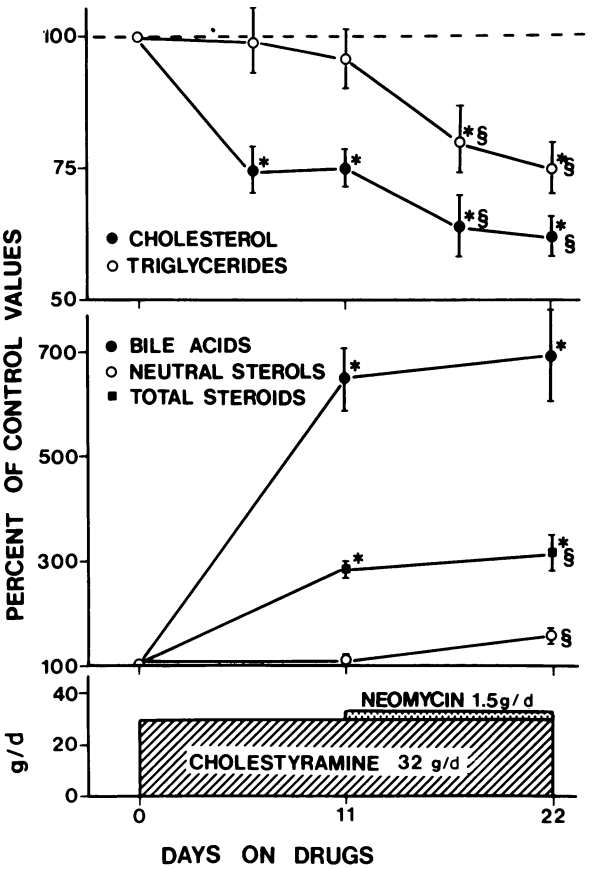

FIGURE 4 Effects of cholestyramine and neomycin on serum lipids and fecal steroids. Relative changes (mean±SE) calculated from studies in Table III. Serum lipid values at 7 and 19 d are included, and demonstrate virtually maximal effect during the 1st wk on each regimen (no significant change between days 7 and 12, and 19 and 24). *, $P < 0.05$ from control values. §, Significant change by inclusion of neomycin.

the long term only occasional cases with heterozygous familial hypercholesterolemia became normocholesterolemic even after an ileal bypass operation (23, 24). Of the suitable hypolipidemic drugs, clofibrate, nicotinic acid, ion exchangers, and probucol frequently fail for one reason or another. Neomycin is an effective alternative to the drug arsenal for the management of severe forms of hypercholesterolemia. Occasional ototoxic reports (25) and the antibiotic nature of neomycin have greatly limited its use as a lipid-lowering agent. No serious side effects have been reported during hypolipidemic therapy despite total consumption of several kilograms by many hypercholesterolemic subjects. The combination of neomycin with cholestyramine links two different hypocholesterolemic mechanisms—cholesterol malabsorption and bile acid malabsorption—and resulted, in the present study, in a marked decrease in serum cholesterol (38%) even in severe hypercholesterolemia. In addition, by combining two

TABLE IV
Effects of Neomycin on Bacterial Conversion Products of Fecal Sterols in Man

Treatment	Percentage of intact sterols	
	Cholesterol	β-sitosterol
Neomycin study		
None	10±3	35±3
Neomycin, acute	39±21*	63±18*
Neomycin, chronic	10±3*	48±3*
Neomycin, high dose	32±23*	69±11*
Cholestyramine-neomycin study		
None	26±13	49±9
Cholestyramine	32±13	46±12
Cholestyramine + neomycin	48±12*	62±9*

Treatments as in Tables II and III; cholesterol contains cholestanol and β-sitosterol contains β-sitostanol as major additional sterol; the remainder of cholesterol had been changed by bacteria to coprostanol and coprostanone, and that of β-sitosterol to ethyl coprostanol and ethyl coprostanone.

* Statistically significant change ($P < 0.05$) from previous period.

opposite side effects the patients found combination treatment convenient.

The changes in serum cholesterol and fecal sterols were quantitatively similar whether neomycin was used alone or in combination with cholestyramine. Thus, the enhanced elimination of cholesterol as bile acid and subsequent increase in cholesterol synthesis caused by cholestyramine did not modify the mechanism by which neomycin lowers serum cholesterol. Enhanced cholesterol elimination as fecal neutral sterols because of impaired cholesterol absorption seems to be a primary phenomenon wherein small neomycin doses decrease the serum cholesterol level (12, 13). This action is based on the disruption of micelles by neomycin in the intestinal lumen (11). Comparative studies in vitro showed that neomycin precipitated sterols, phospholipids, and bile acids more effectively from the micellar phase of intestinal contents, obtained after a fat meal, than from the duodenal bile and that almost total sterol precipitation was obtained at fairly low neomycin additions (26). Cholesterol is only absorbed in the upper small intestine, and micellar solubilization is obligatory for its absorption (27, 28). Thus, once cholesterol is precipitated by neomycin it is hardly resolubilized in the sterol-absorbing area of the gut and is not absorbed. Accordingly, the increase in fecal neutral sterols resulting from administration of neomycin should equal that precipitated in the upper gut lumen. In view of the finding that the total daily flux of cholesterol into the gut lumen is usually 1 g or more (29), the fecal increase was fairly small (≅200 mg/d)

and, in addition, it was not related to the neomycin dose. The drug, even when administered three times a day, acts for only a few hours in the upper small intestine, leaving most of the daily intestinal influx of cholesterol unexposed and absorption unaffected. At the time of the exposure the precipitation may be relatively high (26) so that a further impairment of absorption should be obtained with a higher dose, mainly by more frequent daily administrations.

Fecal fat and bile acids were only transiently or inconsistently increased by the small neomycin doses. Thus, fatty acids and bile acids precipitated by neomycin in the upper small intestine are mostly absorbed when passing down in the gut. This is possible because fatty acids can be absorbed by diffusion without micellar solubilization from the whole length of the small intestine (30), and the reabsorption of conjugated bile acids is effective in the terminal ileum (31, 32). If the precipitate were large, as it can be in the presence of high neomycin concentrations (26), the absorption capacity would be exceeded, and increased amounts of fatty acids and bile acids would escape to the colon and feces. In fact, the change in neomycin dosage was correlated (Fig. 2) with the change in both fecal bile acids and fat. Even if the correlation on high dosage remains linear at doses of 10–12 g/d a substantial increase in fecal bile acids could take place. Mucosal lesions and subsequent malabsorption (33, 34) would actually facilitate the effect. Thus, though the earlier observations on marked increases of fecal bile acids on 6–12 g/d could partly be caused by nonspecific analytic methods (9, 10), large neomycin doses actually appear to enhance cholesterol elimination as both cholesterol and bile acids. A transient increase in fecal bile acids was found by Sedaghat et al. (13) on small neomycin doses and a small but consistent increase has been found by others on 6 g/d of neomycin (35, 36). Because the drug effectively precipitates dihydroxy bile acids in vitro (8, 11, 26), large neomycin doses would mostly increase fecal loss of chenodeoxycholate. Accordingly, the biliary bile acid composition should also be changed. In fact, 6 g/d appear to reduce the pool size of chenodeoxycholate (37).

The leveling-off of serum cholesterol could be caused by (*a*) decrease in biliary secretion of cholesterol, (*b*) restoration of absorption, (*c*) increased tissue mobilization, and (*d*) enhanced synthesis. Though some change might have occurred in the first two conditions after the transition period, the persistant increase in neutral sterol elimination via reduced absorption and the data by Sedaghat et al. (13) indicate that intestinal flux is unchanged exclude these alternatives. The significant correlation found between the fall in serum cholesterol and the increase in fecal steroids indicated that a small fall in cholesterol is a result of a small increase in elimination and not of extensively increased synthesis and mobilization. On the other hand, an extensive fall in cholesterol is caused by a greatly increased elimination and not by a negligible increase in synthesis and mobilization. Because the present subjects lost up to 190 g of cholesterol in the long-term neomycin treatment, it can be inferred that this could not be balanced solely by increased tissue mobilization but that synthesis is also increased. Earlier studies have shown a small decrease in the tissue cholesterol pool but no actual change in half times or courses of the decay curve of serum cholesterol specific activity (13, 14).

Neomycin alone or in combination with cholestyramine increased fecal steroid output by ≅250 mg/d, whereas cholestyramine caused an increase that was about five times higher. Thus, despite an almost equal decrease in serum cholesterol, the requirement for the compensatory increase in cholesterol synthesis caused by neomycin should be only about one-fifth that caused by cholestyramine. This suggests that stimulation of synthesis is mediated by different mechanism(s). Cholestyramine enhances the removal of cholesterol solely as bile acids and depletes hepatocyte cholesterol in man (24), a change considered to be the major factor in stimulating hepatic cholesterol synthesis (38). In addition, the drug may stimulate intestinal cholesterol synthesis to some extent in man (39).

Inhibition of cholesterol absorption by neomycin, on the other hand, decreases the supply of absorbed cholesterol to the hepatocyte and should stimulate hepatic cholesterol synthesis (38). However, in the rat neomycin actually had no effect on hepatic cholesterolgenesis but almost doubled intestinal cholesterol synthesis from acetate and increased conversion of mevalonate to nonsaponifiable lipids other than cholesterol (8). The few analyses of intestinal biopsies[1] did not reveal any gross differences in the mucosal cholesterol, squalene, and methyl sterol concentrations, but the synthesis of all these components was increased from both acetate and mevalonate in vitro during long-term neomycin treatment of hypercholesterolemic patients. Because the effective decrease in serum cholesterol by neomycin depends mainly on the sluggish increase in synthesis to balance cholesterol malabsorption, the mechanism and site of synthesis stimulation clearly need further exploration.

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