Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia

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Abstract The influence of clofibrate on cholesterol metabolism in patients with hyperlipidemia was studied by means of sterol balance and isotope kinetic techniques and by measurements of flow rates of cholesterol through the biliary tract. Long-term balance studies were carried out on a metabolic ward in 24 patients with all currently recognized types of hyperlipidemia; in five other patients with hypercholesterolemia, pool sizes and turnover rates of cholesterol were defined by compartmental analysis before and after three years' daily administration of the drug.

Except in fat-induced hypertriglyceridemia (two patients), clofibrate caused reduced plasma levels of triglycerides and cholesterol in all categories of hyperlipidemia. As a general rule, excretion of cholesterol into bile and feces was significantly increased and fecal bile acid excretion was decreased, regardless of the type of lipoprotein abnormality. Despite a net increase in steroid excretion in most patients with hyperlipidemia, cholesterol synthesis was not increased; indeed, in many patients synthesis appeared to be decreased. While the data obtained in 29 patients were not always consistent, the bulk of the evidence suggests that, in all forms of hyperlipidemia except fat-induced hyperglyceridemia, the drug causes an increased output of cholesterol while simultaneously inhibiting any compensatory increase in cholesterol synthesis. Therefore, it appeared that the increased excretion of steroids was most likely derived from cholesterol stored in tissues. This conclusion was strengthened by finding that long-term administration of the drug can cause marked reduction in body pools of cholesterol.

These findings are reflected clinically by resolution of skin and tendon xanthomatosis. However, it is not yet known whether the accumulation of cholesterol in arterial walls that is part of the process of atherogenesis can be inhibited or reversed by the drug.

Supplementary key words synthesis · absorption · excretion · tissue flux · biliary flow rates · pool sizes

In recent years the drug clofibrate (Atromid-S, Ayerst Laboratories, New York) has gained widespread attention as an effective and relatively nontoxic agent for lowering plasma levels of cholesterol and triglycerides in man (1–10). In 1964, recognizing the existence of various incompletely defined hyperlipidemic states, we initiated a double-blind outpatient study of the effects of this drug in some 70 patients with elevated plasma lipid levels. (The types of hyperlipidemia in these patients were classified by the paper-strip electrophoresis methods and criteria of Fredrickson, Levy, and Lees [11]; the results of this trial are being reported separately [12].) Simultaneously we were developing techniques for the study of cholesterol balance in man (13–18), and thus, soon after recognizing the potential usefulness of this drug in almost all kinds of hyperlipidemia, we were able to apply these balance methods to the elucidation of the mode of action of clofibrate on cholesterol metabolism.

It is the purpose of this report to describe the effects in man of clofibrate on the key parameters of regulation of cholesterol metabolism—absorption, excretion, synthesis, and mobilization from tissues. The data presented here were derived from several kinds of long-term studies in 29 patients: sterol balance data, analyses of isotope kinetic studies, and measurements of hourly flow rates of cholesterol through the biliary tract (19). In most patients clofibrate caused a striking increase in excretion of neutral steroids of endogenous origin. We will show our reasons for concluding that this excess neutral

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steroid output was due to a flux of cholesterol from tissue pools. The changes we will describe occurred without any direct relationship to preexisting levels of plasma cholesterol; they were seen in all currently recognized forms of hyperlipidemia except the rare disorder, fat-induced hypertriglyceridemia (20).

METHODS

Patients

The studies reported here were carried out on 24 patients on the metabolic ward (nos. 1–24) and on 5 outpatients (nos. 25–29) at the Rockefeller University Hospital. The age, sex, habitus, caloric intake, and clinical diagnosis of each patient are given in Table 1; in those patients who received radioactive cholesterol, the amount and type of isotope given are shown. Cholesterol balance studies in a few of these patients have been reported elsewhere (22); the objectives were different from those of the present study.

Diets

All inpatients except patient 24 were maintained at constant body weight on liquid formula feedings throughout the study, as described previously (23, 24); patient 24 ate a solid food diet containing a mixture of fats constituting about 40% of total calories. Table 2 presents the composition of the liquid formula diets used in this study; the types of fat and carbohydrate and their percentages of total calories are given. In all formulas except diets G, J, and K, 15% of total calories was supplied as milk protein (RI-5, Ross Laboratories, Columbus, Ohio); diets G, J, and K contained 20% of total calories as this same protein. Cholesterol was a component of all formulas, a very small amount as a component of the milk protein and a larger amount inherent in two of the fats (butter oil and lard); plant sterols were inherent constituents of the corn and cottonseed oils. During the preparation of formulas, an additional quantity of cholesterol was added to formulas A and E, and of plant sterols to formulas A, D, and E; these added sterols were dissolved in the fat of each formula before it was mixed with other constituents at the time of large-scale homogenization (40-kg batches). The homogeneity of each batch was verified by analyses of its cholesterol and plant sterol contents (14). Patients 3 and 4, who were fed diets G, J, and K, received 300 mg of a β-sitosterol preparation twice daily in capsule form (see footnote in Table 2 describing the source of purified β-sitosterol).

Plasma lipids

In the earlier studies, plasma cholesterol was measured by the method of Abell, Levy, Brodie, and Kendall (25), and plasma triglyceride by a microgravimetric procedure (26). In later studies, measurements were made on a Technicon AutoAnalyzer (model I) (27, 28). Cholesterol analyses by the two procedures agreed within ± 7%, but the earlier triglyceride measurements were usually higher in every case by about 100 mg/100 ml plasma. The methods used in any one patient were the same throughout his study; the procedure employed is shown in the appropriate tables.

Isotopic sterols

A measured quantity of [4-^{14}C]cholesterol (50–100 μCi) was administered intravenously in a single dose to 16 patients (Table 1). 1 ml of ethanol containing the radioactive tracer was dispersed in 150 ml of physiologic saline; the mixture was immediately administered intravenously, and any residual radioactivity remaining in the infusion set was determined after ethanol extraction.

Patient 21 was fed a small amount of radioactive cholesterol daily in all formula feedings throughout his study (32 wk). [1,2-^{3}H]Cholesterol dissolved in 10 ml of ethanol was added to 40-kg batches of formula during homogenization in order to hold constant the isotopic sterol content of all formulas administered to this patient (homogeneity of all formulas in regard to radioactivity was verified by specific activity measurements).

Both forms of radioactive cholesterol were obtained from New England Nuclear Corp., Boston, Mass. In every case more than 95% of radioactivity chromatographed with cholesterol on Florisil thin-layer plates in ethyl ether–heptane 55:45. The cholesterol was administered without further purification.

Concentration and specific activity of total plasma cholesterol were determined biweekly; radioactivity was measured on an aliquot of the same extract made for determination of concentration, with the radioactivity being measured in a Packard Tri-Carb scintillation counter (model 3003) as previously described (14).

Fecal steroid analysis and measurement of cholesterol absorption

Fecal neutral and acidic steroids were isolated separately, and their masses and specific activities were measured by methods presented previously (13, 14). These procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit (5α,3β-OH and 5α,3-keto compounds).

In all patients in whom balance studies were carried out (except nos. 12, 15, and 19), amounts of neutral steroids excreted were corrected for losses occurring during intestinal transit and for variations in fecal flow

TABLE 1. Clinical data

Patient	Age/Sex	Weight	Height	% of Ideal Weight[a]	Caloric Intake	Radioactive Cholesterol (isotope/dose)[b]	Clinical Diagnosis and Type of Lipoproteinemia
		kg	*cm*		*kcal/day*	*μCi*	
1 IG	46/F	56	155	112	2062	^{14}C/100	IHD,[c] normolipoproteinemia
2 CZ	63/M	94	181	122	2563	^{14}C/100	IHD, PVD,[c] normolipoproteinemia
3 RI	11/M	23	131		2175		Hypertriglyceridemia (type I)[d]
4 SI	11/M	28	139		2100		Hypertriglyceridemia (type I)
5 JT	69/M	72	166	119	2374	^{14}C/100(A) ^{14}C/160(B)	Senility, hypercholesterolemia (type II)
6 JW	72/F	54	143	124	1672	^{14}C/100	IHD, xanthomatosis, hypercholesterolemia (type II)
7 AB	70/F	49	147	113	1590	^{14}C/100	IHD, PVD, hypercholesterolemia (type II)
8 HT	57/M	46	162	84	1672	^{14}C/100	Hypercholesterolemia (type II)
9 JM	61/M	70	176	104	2500		IHD, xanthomatosis, hypercholesterolemia (type II)
10 JO	41/M	83	170	103	3000	^{14}C/160	IHD, hypercholesterolemia (type II), xanthelasma
11 GW	48/M	81	173	114	2840	^{14}C/160	Xanthomatosis, hypercholesterolemia (type II)
12 EK	68/M	72	165	119	2250	^{14}C/100	IHD, hypertriglyceridemia (type III)
13 ES	20/M	70	178	101	2875		Xanthomatosis, hypertriglyceridemia (type III)
14 DB	47/M	63	175	93	2375	^{14}C/100	PVD, hypertriglyceridemia (type III)
15 AM	60/F	66	164	122	2150		IHD, PVD, CLL,[c] hypertriglyceridemia (type IV)
16 AG	48/M	95	186	126	3200	^{14}C/100	IHD, hypertriglyceridemia (type IV)
17 TK	48/M	61	175	96	2375		Hypertriglyceridemia (type IV)
18 AGo	50/M	70	169	110	2500	^{14}C/100	IHD, PVD, xanthomatosis, hypertriglyceridemia (type IV)
19 DR	59/F	54	157	105	1940	^{3}H/100	Hypertriglyceridemia (type V)
20 RT	48/M	65	164	108	2190	^{14}C/100	IHD, hypertriglyceridemia (type V)
21 DA	55/M	64	168	104	2140	^{3}H/277	IHD, hypertriglyceridemia (type V)
22 JJ	39/F	71	162	131	2185	^{14}C/100	Hypertriglyceridemia (type V)
23 MR	60/M	67	167	109	2375	^{14}C/100	Hypertriglyceridemia (type V), xanthomatosis
24 BL	40/M	82	186	109	2870		IHD, hypertriglyceridemia (type V)
25 MB	66/M	75	170	113	solid food diet ad lib.	^{3}H/100 ^{14}C/88	Hypercholesterolemia (type II)
26 JL	57/M	91	188	110	"	^{3}H/100 ^{14}C/80	IHD, hypercholesterolemia (type II)
27 JJ	46/M	96	173	139	"	^{14}C/100 ^{3}H/80	Gout, hypertension, alcoholism, hypercholesterolemia, hypertriglyceridemia (type IIb)
28 RL	52/M	79	173	111	"	^{3}H/125 ^{14}C/87	Hypercholesterolemia, hypertriglyceridemia (type IIb)
29 BH	54/M	85	174	114	"	^{3}H/118 ^{14}C/102	IHD, PVD, xanthomatosis, hypercholesterolemia (type II), gout

[a] According to life insurance tables of weight for height, age, and sex (21).
[b] In all patients who received isotopes (except no. 21), the radioactive cholesterol was given as a single dose intravenously. Patient 21 was given an intravenous priming dose of 50 μCi of [1,2-^{3}H]cholesterol and 1 μCi/day in divided doses in formula feedings for 227 days.
[c] IHD, ischemic heart disease; PVD, peripheral vascular disease; CLL, chronic lymphocytic leukemia.
[d] Type of lipoprotein pattern according to Fredrickson et al. (11).

rates, with dietary β-sitosterol as an internal standard (16, 29). The three exceptional patients received fat-free diets, and corrections were made only for variations in fecal flow, with the use of chromic oxide as an internal standard. Chromic oxide was always used as a marker for correction of day-to-day variations in the fecal flow of acidic steroids (15).

Daily absorption of exogenous cholesterol was measured by methods recently described (17, 18). Method I (equations 10 and 11 of Ref. 17) makes use of data obtained after administration of a single intravenous dose of radiocholesterol, while method II (equations 11, 15, and 16 of Ref. 17) utilizes data obtained after continuous oral labeling.

Measurement of biliary flow of cholesterol

Hourly flow rates of biliary cholesterol were determined as described recently (19). The procedure con-

TABLE 2. Formula diets and their sterol contents

Diet	Dietary Fat (percentage and type)	Dietary Carbohydrate (percentage and type)	Cholesterol	β-Sitosterol
			mg/500 kcal	*mg/500 kcal*
A	40% Lard	45% Dextrose	119[a]	72[b]
B	40% Cottonseed oil	45% Dextrose	10[c]	71[d]
C	40% Corn oil	45% Dextrose	6[c]	148[d]
D	40% Butter oil	45% Dextrose	66[e]	45[b]
E	20% Butter oil	65% Dextrose	130[e]	37[b]
F	None	85% Dextrose	6[c]	None
G	None	80% Dextrose	8[c]	None
H	None	40% Dextrose 45% Corn starch	6[c]	None
J	30% Butter oil	55% Dextrose	53	None
K	40% Butter oil	45% Dextrose	68	None

[a] 30 mg of cholesterol was inherent in the protein and lard; 89 mg of additional cholesterol was incorporated into the formula.

[b] β-Sitosterol (Mann Research Laboratories, New York) was purified and prepared in microcrystalline form by Dr. Erold R. Diller, Eli Lilly and Co., Indianapolis, Ind.; the final product contained 90% β-sitosterol and 10% campesterol.

[c] This amount of cholesterol was inherent in the protein and fat used in formulas.

[d] This amount of β-sitosterol was inherent in the fat used in the formulas.

[e] 36 mg of cholesterol was inherent in the protein and butter oil; 94 mg of additional cholesterol was incorporated into the formula.

sisted of intubation with a double-lumen tube through which a liquid formula containing 40% of calories as cottonseed oil was infused at a constant rate into the second portion of the duodenum, along with [1,2-^{3}H]-cholesterol as an internal standard. The total amount of formula infused per 24 hr was the same as that previously shown to maintain constant body weight in the weeks preceding the test. A small sample of the mixture of infusate plus intestinal contents was withdrawn at a constant rate (approximately 10 ml/hr) from a site 10 cm distally; less than 5% of the total flow of any constituent passing this point was removed from its enterohepatic circulation. The hourly flow of biliary cholesterol was determined according to the following equation: biliary cholesterol (mg/hr) = rate of infusion of [1,2-^{3}H]-cholesterol (dpm/hr) ÷ specific activity of withdrawn cholesterol (dpm/mg). Since the infusate contained no cholesterol, any cholesterol withdrawn distally was entirely of endogenous origin; its mass was measured by gas–liquid chromatography and its radioactivity was determined as described above. A more detailed description of these procedures will be published elsewhere.

Measurement of specific activity of biliary and intestinal mucosal cholesterol

Simultaneous determinations of specific activities of cholesterol in bile, intestinal mucosa, and plasma were carried out as described recently (22). Samples of jejunal mucosa were obtained with a hydraulic biopsy tube (model 4.7 mm, Quinton Instruments, Seattle, Wash.). The tube was passed under fluoroscopic guidance into the upper jejunum (generally 15 cm beyond the ligament of Treitz); about six mucosal biopsies, with a total weight of approximately 40 mg (wet wt), were then obtained. During the same intubation, a sample of intestinal contents rich in bile was obtained after intravenous administration of cholecystokinin (generously supplied by Prof. E. Jorpes, Karolinska Inst., Stockholm); venous blood was withdrawn at the same time.

Experimental design

This investigation of the effects of clofibrate on the metabolism of cholesterol in various hyperlipidemic states will be presented in four parts. *Study I:* The major study of this report involved 20 inpatients. Cholesterol balance studies were carried out in order to determine the influence of the drug on different parameters of cholesterol metabolism: plasma lipid levels, excretion of neutral and acidic steroids, absorption of dietary cholesterol, and total body synthesis of cholesterol. *Study II:* The effects of clofibrate on the rates of biliary secretion of cholesterol by the liver were examined in three patients (nos. 9, 17, and 24). This study was carried out in an attempt to determine the source of the increase in excretion in the feces of endogenous neutral steroids noted in study I. *Study III:* The effects of clofibrate on intestinal mucosal synthesis of cholesterol was studied in four patients: in three the rate was purposely accelerated by simultaneous administration of cholestyramine (nos. 5, 10, and 11), while in patient 18 cholestyramine stimulation was not required. *Study IV:* The long-term effects of clofibrate administration on cholesterol synthesis rates and pool sizes were studied in five outpatients (nos. 25–29). This study was undertaken in order to test the conclusion drawn from studies I–III, namely, that clofibrate caused a flux of stored cholesterol out of tissue pools.

Study I. Effects of clofibrate on cholesterol balance. Cholesterol balance was measured in two (and occasionally in three) periods. In period I, patients received two placebo capsules twice daily; these capsules were identical in appearance to capsules containing clofibrate, and each contained 500 mg of corn oil. In period II, two capsules of clofibrate (500 mg each) were given twice daily. Patients 6 and 18 were studies in period III when clofibrate was replaced by placebo capsules. Throughout all periods the intakes of cholesterol, plant sterols, and total calories were held constant, and body weights remained unchanged.

Continuous collections of stools were made for measurement of fecal neutral and acidic steroids. In 10 patients who had been labeled with radioactive cholesterol for measurements of cholesterol absorption, exogenous

cholesterol was ingested in amounts ranging from 276 to 781 mg/day. In one patient (no. 18) simultaneous estimations of specific activity of cholesterol in plasma and intestinal mucosa were made several times during periods I, II, and III.

Study II. Effects of clofibrate on biliary output of cholesterol. The hourly output of biliary cholesterol was measured in three patients (nos. 9, 17, and 24) during placebo and clofibrate periods (labeled periods I and II, as in study I). In patient 9, one study was made during the control period and one during the drug treatment period; sterol balance was measured throughout both 24-day periods. In patient 17, two pairs of studies of biliary output of cholesterol were carried out, each pair being completed over a 14-day period. Patient 24 also underwent two pairs of flow studies. Over this 2-month period he was maintained on a solid food diet; however, during the period of study of biliary output he received no feedings except the infused formula. Sterol balance studies were not performed in patients 17 and 24; in both, plasma lipid concentrations decreased markedly on clofibrate.

Study III. Effects of clofibrate on intestinal synthesis of cholesterol. Patients 5B, 10, and 11 were investigated by sterol balance techniques as follows. In period A, control values were obtained when the diet was free of cholesterol; in period B, cholestyramine was added to the regimen in amounts ranging from 10 to 20 g/day; and in period C, cholestyramine was continued and clofibrate was added at a level of 2 g/day. These three patients had previously been labeled by intravenous administration of a single dose of radioactive cholesterol. At least three determinations of the specific activities of biliary, intestinal mucosal, and plasma cholesterol were made at weekly intervals in each period; intubations were not carried out until the decay in specific activity of plasma cholesterol had become log-linear, in order to allow time for equilibration of radioactive cholesterol between plasma, bile, and mucosa. In the fourth patient of study III (patient 18), it was not necessary to stimulate intestinal cholesterol synthesis with cholestyramine; the design was therefore similar to that in study I (periods I and III, placebo; period II, clofibrate).

Study IV. Effects of long-term administration of clofibrate on body pools of cholesterol. A long-term study of more than three years' duration was performed in five outpatients (nos. 25–29) to determine the effects of clofibrate on sizes of body pools of cholesterol. Before initiation of drug treatment, radioactive cholesterol was given intravenously; turnover rates and pool sizes for cholesterol were estimated from specific activity–time curves of radioactive cholesterol according to a two-pool model (30). On completion of these measurements, 2 g of clofibrate was given daily for periods of 3 or more yr, and plasma cholesterol and triglyceride concentrations were determined at about 3-month intervals. Then the drug was discontinued, and 6–10 wk later turnovers and pool sizes were again estimated in the same manner by isotope kinetics in an attempt to determine whether these parameters had been altered by long intervening periods of clofibrate administration. We considered it important to the design of the study that the decay curves be determined when the patients were not being given clofibrate, so as to avoid drug-induced changes in rate constants and pool sizes.

RESULTS

Effects of clofibrate on cholesterol balance (study I)

Plasma lipids. Concentrations of total plasma cholesterol and triglycerides during placebo and drug treatment periods in 20 patients are presented in Table 3. The values shown include consecutive samples taken during that portion of each period when patients had attained a steady state with respect to plasma lipid concentrations. These results are part of a larger study, being reported elsewhere (12), of the effects of clofibrate on plasma lipids in 70 patients. The data on the 20 patients in this study are shown in Table 3 in order to permit comparison with changes induced by the drug in fecal steroid excretion in each of these patients (see below).

In these 20 patients a statistically significant lowering of plasma concentrations was noted for cholesterol in 16 and for triglycerides in 14; these reductions occurred without regard to type of hyperlipidemia or degree of saturation of the dietary fat. The main exceptions to the rule were four patients (nos. 16, 19, 20, and 22) with lipoprotein patterns of types IV and V in whom cholesterol concentrations rose slightly even while triglyceride concentrations fell; three of four of these observations were made in patients fed fat-free or lard diets. In two patients with type I hyperlipoproteinemia (nos. 3 and 4) on a fat-free diet, plasma cholesterol concentrations fell slightly but significantly on clofibrate, while triglycerides showed no change. On fat-containing diets, however, these two patients experienced marked chylomicronemia, along with elevated levels of cholesterol as well as triglycerides, but clofibrate caused no significant changes in plasma lipid levels (12).

Fecal endogenous neutral steroids. Values for excretion of endogenous neutral, acidic, and total fecal steroids for study I are presented in Table 4; in Figs. 1–5 these data are depicted graphically in relation to specific activity–time curves for plasma cholesterol after administration of a single dose of radioactive cholesterol intravenously.

In regard to cholesterol balance, the most striking effect seen in patients treated with clofibrate was an in-

TABLE 3. Plasma lipid changes in placebo and clofibrate treatment periods (study I)

Patient	Lipoprotein Pattern	Diet	Plasma Cholesterol[a]			Plasma Triglycerides[b]		
			Period I	Period II	Difference	Period I	Period II	Difference
			mg/100 ml ± SD (*n*)		*II − I (or III)*	*mg/100 ml* ± SD (*n*)		*II − I (or III)*
1	N	A	234 ± 14 (19)	195 ± 10 (14)	−39 $P < 0.001$[c]	131 ± 19 (19)	66 ± 6 (14)	−65 $P < 0.001$[c]
2	N	A	227 ± 17 (18)	195 ± 10 (13)	−32 $P < 0.001$	120 ± 20 (18)	77 ± 12 (13)	−43 $P < 0.001$
3	I	G	95 ± 14 (10)	82 ± 7 (9)	−13 $P < 0.05$	257 ± 50 (10)	219 ± 33 (9)	−38 NS
		K	240 ± 23 (13)	265 ± 11 (7)	+25 NS	2253 ± 288 (13)	2459 ± 376 (7)	+206 NS
4	I	G	87 ± 6 (9)	77 ± 9 (11)	−10 $P < 0.01$	264 ± 43 (9)	266 ± 55 (11)	+2 NS
		J	210 ± 11 (8)	228 ± 26 (13)	+18 NS	1870 ± 394 (8)	2012 ± 471 (13)	+142 NS
5A	II	A	261 ± 8 (4)	233 ± 14 (21)	−28 $P < 0.001$	139 ± 18 (4)	119 ± 14 (21)	−20 NS
6	II	A	325 ± 14 (9)	222 ± 9 (12)	−103 $P < 0.001$	96 ± 20 (9)	66 ± 12 (12)	−30 $P < 0.001$
			293 ± 9 (8)[d]		−71 $P < 0.001$	78 ± 13 (8)[d]		−12 $P < 0.05$
7	II	A	287 ± 14 (12)	219 ± 7 (7)	−68 $P < 0.001$	152 ± 29 (12)	133 ± 9 (7)	−19 NS
			266 ± 6 (6)[d]		−47 $P < 0.001$	164 ± 19 (6)[d]		−31 $P < 0.005$
8	II	A	307 ± 14 (16)	258 ± 16 (9)	−49 $P < 0.001$	148 ± 14 (16)	85 ± 13 (9)	−63 $P < 0.001$
9	II	B	286 ± 12 (6)	260 ± 14 (6)	−26 $P < 0.05$	94 ± 9 (6)	62 ± 3 (6)	−32 $P < 0.001$
12	III	H	640 ± 47 (5)	295 ± 26 (3)	−345 $P < 0.001$	1055 ± 223 (5)	400 ± 45 (3)	−655 $P < 0.001$
13	III	A	362 ± 17 (7)	239 ± 11 (9)	−123 $P < 0.001$	236 ± 28 (7)	264 ± 25 (9)	+28 NS
14	III	C	170 ± 7 (9)	139 ± 6 (10)	−31 $P < 0.001$	190 ± 13 (9)	108 ± 18 (10)	−82 $P < 0.001$
15	IV	F	544 ± 24 (9)	275 ± 5 (5)	−269 $P < 0.001$	1101 ± 71 (9)	451 ± 64 (5)	−650 $P < 0.001$
16	IV	A	199 ± 15 (16)	213 ± 6 (12)	+14 $P < 0.001$	239 ± 26 (16)	182 ± 17 (12)	−57 NS
18	IV	B	201 ± 9 (8)	195 ± 8 (11)	−6 NS	325 ± 25 (8)	284 ± 23 (11)	−41 $P < 0.005$
			235 ± 14 (10)[d]		−40 $P < 0.01$	403 ± 44 (10)[d]		−119 $P < 0.001$
19	V	F	150 ± 6 (5)	179 ± 9 (4)	+29 $P < 0.001$	739 ± 61 (5)	751 ± 101 (4)	+12 NS
20	V	C	237 ± 9 (5)	245 ± 10 (7)	+8 NS	1269 ± 224 (5)	773 ± 62 (7)	−496 $P < 0.001$
21	V	D	403 ± 18 (8)	287 ± 17 (34)	−116 $P < 0.001$	2089 ± 266 (8)	1000 ± 137 (34)	−1089 $P < 0.001$
22	V	E	197 ± 19 (4)	237 ± 11 (7)	+40 NS	600 ± 99 (4)	371 ± 74 (7)	−229 $P < 0.001$
23	V	A	310 ± 8 (11)	196 ± 8 (11)	−114 $P < 0.001$	546 ± 68 (11)	239 ± 20 (11)	−307 $P < 0.001$

[a] Plasma cholesterol concentrations by the method of Abell et al. (25) in patients 12, 15, and 19–22, and by AutoAnalyzer (27) in patients 1–11, 13, 14, 16–18, 23, and 24.

[b] Plasma triglyceride concentrations by microgravimetry (26) in patients 12, 15, and 19–22, and by AutoAnalyzer (28) in patients 1–11, 13, 14, 16–18, 23, and 24.

[c] Significance of difference determined by Student's *t* test (31); NS, not significant.

[d] Period III.

crease in excretion of neutral steroids of endogenous origin: significant increases were found in 17 of 20 patients (all except nos. 2, 14, and 16). However, the magnitude of this increase varied considerably from patient to patient and with type of plasma lipoprotein abnormality, but there was no apparent correlation with the type of dietary fat. In one normocholesterolemic adult an increase in endogenous neutral steroids was transitory;

in two children with type I hyperlipoproteinemia, there were no significant changes when fat-free formulas were fed, but in both there were significant increases when fat-containing diets were being fed; in all five patients with primary hypercholesterolemia (type II lipoprotein pattern), the drug produced an increase in fecal neutral steroids that in most cases persisted throughout the period of drug treatment; and in 9 of 11 patients with primary hypertriglyceridemia (types III, IV, and V lipoprotein patterns), clofibrate caused a still greater output of endogenous neutral steroids. The increases were highly variable from patient to patient, and they were unrelated to the magnitude of neutral steroid excretion in the placebo period.

Fecal acidic steroids. The excretion of acidic steroids decreased significantly in 6 of 20 patients (nos. 4, 6, 8, 15, 18, and 19, Table 4) during clofibrate administration; in five of these cases the diets were fat-free or contained a saturated fat. In 17 of 20 patients the mean values for acidic steroid excretion were lower on clofibrate than in the control period; according to the sign test (31) this finding reflects a significant decrease for the group as a whole ($P < 0.01$). In some patients the reduction in acidic steroid excretion was striking, as shown in Figs. 1–5; in no patient did a significant increase occur.

Decreased outputs were noted in hypercholesterolemic patients (type II lipoprotein patterns) and in those with hypertriglyceridemia (type III, IV, and V patterns). It is also worthy of note that the average output of acidic steroids in five patients with type II lipoprotein patterns (201 ± 85 mg/day) was significantly lower than that found in eight patients with types IV and V (489 ± 289 mg/day) ($P < 0.05$).

Total fecal endogenous steroids. In 11 of 20 patients, excretions of total endogenous (endogenous neutral + acidic) steroids were increased significantly during treatment with clofibrate. In all cases the increases were the result of an enhanced excretion of endogenous neutral steroids. However, in five patients (nos. 1, 8, 18, 19, and 22) who had significant increases in fecal endogenous neutral steroids, the excretions of total endogenous steroids were not significantly raised because of simultaneous decreases in output of acidic steroids. Nevertheless, in the majority of patients who showed a greater output of neutral steroids on clofibrate, this increment exceeded any decrement in acidic steroid excretion. Patient 18 was unique in showing a decrease in acidic steroid excretion that was considerably larger than the increase in neutral steroid excretion; as a consequence, the output of total fecal endogenous steroids was significantly reduced on clofibrate.

Cholesterol absorption. Table 5 shows the absorption of dietary cholesterol during control and clofibrate treatment periods in 10 patients whose diets contained measurable amounts of cholesterol (276–781 mg/day); in all cases the diets contained saturated fats. In 5 of these 10 patients, absorption of cholesterol was not significantly altered when clofibrate was introduced. However, in three patients significant decreases occurred on clofibrate (nos. 16, 21, and 23), and in two others the changes were equivocal (in patients 6 and 7 the absorption during clofibrate treatment [period II] was significantly lower than in the preliminary control periods [period I] but was not reduced when results in period II were compared with control period III). When the daily decrement in cholesterol absorption is compared with the daily increment in excretion of fecal neutral steroids of endogenous origin (last column of Table 5), it is seen that the latter exceeded the former very strikingly in four of the five patients in whom cholesterol absorption appeared to be diminished by clofibrate.

Cholesterol balance and turnover. In order to estimate cholesterol turnover from sterol balance data it is necessary that the patient be in a metabolic steady state. In previous studies (17, 32, 33) we have emphasized that it is not possible to determine with certainty at which point the steady state with respect to body cholesterol is reached, since present methods do not permit the direct assessment of the total body pool of cholesterol. In those studies we assumed that the steady state was present after any prolonged period during which body weight, plasma lipid concentrations, and fecal steroid excretion were constant. In the present investigation, body weight and plasma lipids rapidly approached steady levels after introduction of clofibrate, but even after periods of drug administration that ranged from 24 to 112 days it was often impossible to determine with confidence when the excretion of fecal steroids became constant (Figs. 1–5). For instance, in two patients (nos. 1 and 23), increases in excretion of fecal neutral steroids of endogenous origin appeared to be transitory; in these patients it might be concluded that a steady state had been reached toward the latter part of the drug treatment period. But in 13 others (nos. 5–9, 12, 13, 15, 18–22) the increases in neutral steroid excretion appeared to persist throughout period II; thus, in this larger group it could not be ascertained whether a new steady state had been reached or not. In view of this uncertainty over attainment of a new metabolic steady state on clofibrate, we believe that sterol balance data alone are not adequate for estimation of cholesterol turnover in this investigative situation.

Comparison of plasma cholesterol decrement with fecal steroid change. Since clofibrate usually causes a significant lowering of plasma cholesterol, we can ask whether the decrement of cholesterol in the plasma compartment could account for the increase in total fecal steroid excretion found in many patients. In Table 6 a comparison

TABLE 4. Cholesterol balance data in placebo and clofibrate treatment periods (study I)

Patient	Diet	Cholesterol Intake	Fecal Endogenous Neutral Steroids			Fecal Acidic Steroids			Total Fecal Endogenous Steroids		
			Period I	Period II	Difference II − I (or III)	Period I	Period II	Difference II − I (or III)	Period I	Period II	Difference II − I (or III)
		mg/day	*mg/day* ± SD		*mg/day*	*mg/day* ± SD		*mg/day*	*mg/day* ± SD		*mg/day*
Type N											
1 (IG)	A	490	687 ± 14 (56:14)[a]	818 ± 149 (56:14)[a]	+131 $P < 0.005$[b]	216 ± 80	165 ± 84	−51 NS	903 ± 137	983 ± 186	+81 NS
2 (CZ)	A	609	1003 ± 152 (28:7)	1081 ± 125 (40:10)	+78 NS	380 ± 106	313 ± 70	−67 NS	1383 ± 243	1394 ± 115	+9 NS
Type I											
3 (RI)	G	35	242 ± 19 (56:7)	203 ± 69 (48:6)	−39 NS	49 ± 8	39 ± 10	−10 NS	291 ± 29	242 ± 73	−49 NS
	K	307	900 ± 54 (24:6)	1186 ± 113 (24:6)	+286 $P < 0.01$	67 ± 14	57 ± 18	−10 NS	952 ± 50	1243 ± 98	+291 $P < 0.01$
4 (SI)	G	34	209 ± 47 (56:7)	188 ± 54 (56:7)	−21 NS	116 ± 31	64 ± 27	−52 $P < 0.005$	325 ± 59	252 ± 61	−73 $P < 0.05$
	J	219	581 ± 72 (24:6)	661 ± 42 (24:6)	+80 $P = 0.05$	114 ± 48	110 ± 26	−4 NS	710 ± 97	771 ± 62	+61 NS
Type II											
5A (JT)	A	565	548 ± 61 (40:10)	916 ± 194 (76:19)	+368 $P < 0.001$	343 ± 116	279 ± 193	−64 NS	891 ± 179	1213 ± 289	+322 $P < 0.001$
6 (JW)	A	398	448 ± 48 (40:10)	873 ± 160 (72:18)	+425 $P < 0.001$	200 ± 34	113 ± 69	−87 $P < 0.001$	648 ± 56	986 ± 181	+338 $P < 0.001$
			669 ± 97[c] (48:12)		+204 $P < 0.001$	130 ± 39[c]		−17 NS	799 ± 95[c]		+187 NS
7 (AB)	A	379	551 ± 131 (40:10)	704 ± 98 (56:14)	+153 $P < 0.005$	163 ± 52	131 ± 52	−32 NS	714 ± 90	835 ± 113	+121 $P < 0.025$
			519 ± 81[c] (32:8)		+185 $P < 0.005$	129 ± 47[c]		+2 NS	648 ± 99[c]		+187 $P < 0.001$
8 (HT)	A	398	275 ± 29 (44:11)	437 ± 90 (36:9)	+162 $P < 0.001$	220 ± 48	114 ± 51	−106 $P < 0.001$	496 ± 60	551 ± 89	+55 NS
9 (JM)	B	50	577 ± 68 (24:6)	785 ± 55 (24:6)	+208 $P < 0.005$	133 ± 36	137 ± 37	+4 NS	710 ± 62	922 ± 59	+212 $P < 0.005$
Type III											
12 (EK)	H	27	285 ± 29[d] (36:9)	545 ± 146[d] (32:8)	+260 $P < 0.001$	144 ± 19	139 ± 51	−5 NS	429 ± 22	684 ± 180	+255 $P < 0.005$
13 (ES)	A	679	1279 ± 65[c] (24:6)	1619 ± 218 (54:14)	+340 $P < 0.001$	324 ± 173	261 ± 82	−63 NS	1604 ± 170	1873 ± 232	+269 $P < 0.01$
14 (DB)	C	29	692 ± 48 (48:6)	641 ± 81 (40:10)	−51 NS	196 ± 48	199 ± 48	+3 NS	888 ± 79	838 ± 95	−50 NS
Type IV											
15 (AM)	F	26	187 ± 23[d] (32:8)	504 ± 152[d] (39:12)	+317 $P < 0.001$	297 ± 90	201 ± 82	−96 $P < 0.05$	484 ± 111	705 ± 106	+221 $P < 0.001$
16 (AGa)	A	755	972 ± 246 (24:6)	1198 ± 295 (60:15)	+226 NS	1132 ± 379	867 ± 446	−265 NS	2104 ± 439	2065 ± 502	−39 NS
18 (AGo)	B	50	608 ± 60 (32:8)	705 ± 49 (56:14)	+97 $P < 0.001$	441 ± 96	247 ± 76	−194 $P < 0.001$	1049 ± 69	952 ± 96	−97 $P < 0.01$
			581 ± 57[c] (32:8)		+124 $P < 0.001$	605 ± 147[c]		−358 $P < 0.001$	1186 ± 154[c]		−234 $P < 0.001$

Type V												
19	(DR)	F	23	366 ± 51[d] (21:3)	546 ± 75[d] (37:4)	+180 $P < 0.02$	415 ± 33	265 ± 78	−150 $P < 0.02$	781 ± 38	811 ± 104	+30 NS
20	(RT)	C	26	974 ± 183 (32:4)	1522 ± 386 (40:5)	+548 $P < 0.025$	421 ± 28	393 ± 95	−28 NS	1393 ± 174	1915 ± 334	+522 $P < 0.02$
21	(DA)	D	282	918 ± 147 (21:3)	1399 ± 273 (112:16)	+481 $P < 0.001$	324 ± 127	259 ± 142	−65 NS	1242 ± 227	1658 ± 256	+416 $P < 0.02$
22	(JJ)	E	682	774 ± 210 (65:4)	1195 ± 232 (92:6)	+421 $P < 0.02$	314 ± 70	264 ± 97	−50 NS	1088 ± 271	1459 ± 290	+371 NS
23	(MR)	A	565	513 ± 90 (44:10)	971 ± 232 (56:14)	+458 $P < 0.001$	249 ± 75	306 ± 99	+20 NS	762 ± 144	1277 ± 201	+515 $P < 0.001$

[a] Duration of balance study (days) for the period given and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this column gives the average stool collection period in days.
[b] Significance of difference of periods II − I (or III) (Student's *t*-test, Ref. 31).
[c] Period III.
[d] In patients 12, 15, and 19, corrections for possible losses of neutral steroids with the use of β-sitosterol were not made. Therefore, the values shown may be underestimated.

TABLE 5. Changes in cholesterol absorption during administration of placebo and clofibrate

		Period I				Period II				Difference in Absorption (period II − I)		Increment in Fecal Endogenous Neutral Steroids (period II − I)
Patient	Diet	Days: No. of Determinations[a]	Cholesterol Intake	Cholesterol Absorption[b]		Days: No. of Determinations[a]	Cholesterol Intake	Cholesterol Absorption[b]				
			mg/day	*mg/day*	%		*mg/day*	*mg/day*	%	*mg/day*	*significance*[c]	*mg/day*
1	A	56:14	490	189 ± 45	39	56:14	483	179 ± 64	37	−10	NS	+131
2	A	28:7	607	291 ± 39	48	40:10	614	228 ± 83	37	−63	NS	+78
5A	A	40:10	565	131 ± 36	23	76:19	565	122 ± 98	22	−9	NS	+368
6	A	40:10	387	161 ± 38	42	72:18	402	116 ± 74	29	−45	$P < 0.05$	+425
		48:12[d]	405	130 ± 50	32					−14	NS	+204
7	A	40:10	379	200 ± 63	53	56:14	379	131 ± 47	35	−69	$P < 0.01$	+153
		32:8[d]	379	117 ± 20	31					+14	NS	+185
8	A	44:11	402	204 ± 40	51	36:9	394	191 ± 62	48	−13	NS	+162
16	A	24:6	781	283 ± 72	36	56:14	766	190 ± 83	25	−93	$P < 0.02$	+97
21	D	21:3	276	144 ± 18	52	121:16	289	79 ± 44	27	−65	$P < 0.01$	+481
22	E	65:4	682	334 ± 50	49	92:6	682	313 ± 99	46	−21	NS	+421
23	A	44:10	565	307 ± 64	54	56:14	565	212 ± 57	38	−95	$P < 0.001$	+458

[a] Duration of balance study (days) for the period given and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this column gives the average stool collection period in days.
[b] All absorption data were calculated by method I (17) except in patient 23, in whom method II (17) was used.
[c] Significance of differences between periods II and I by Student's *t* test (31); NS, not significant.
[d] Period III.

TABLE 6. Comparison of decrement in plasma cholesterol with increment in fecal endogenous steroid excretion caused by clofibrate[a]

Patient	Xantho-matosis	Duration of Period II	Decrement[b] in Plasma Cholesterol Content	Increment[c] in Total Fecal Endogenous Steroids
		days	*g/period II*	*g/period II*
5A	−	76	−0.91	+24.47
6	+	72	−2.50	+24.33
7	−	56	−1.56	+5.93
9	+	24	−0.82	+11.34
13	+	54	−3.87	+15.52
20	−	40	+0.23	+20.84
21	−	112	−3.34	+46.59
23	+	56	−4.34	+28.84

[a] All decrements in plasma cholesterol were statistically significant except in patient 20 (Table 3). All increments in total fecal endogenous steroid excretion were statistically significant (Table 4).

[b] The change of cholesterol in the plasma compartment for period II was calculated as follows: average change in plasma cholesterol concentration (period II − period I) (mg%) × 10 × estimated plasma volume (in liters). The plasma volume was calculated as 4.5% of body weight (34).

[c] The increment in total fecal endogenous steroids during period II was calculated as follows: average change in fecal total endogenous steroids (period II − period I) (mg/day) × number of days in period II.

is presented of the decrease in plasma cholesterol content and in total increments in fecal steroid excretion during period II in those eight patients who experienced a significant increase in excretion of total endogenous steroids together with a significant decrease in plasma cholesterol content. (The sterol balance data for patients 12 and 15 are omitted in this tabulation because the totals shown in Table 4 for these two patients may be underestimates [see footnotes to Table 4].)

As shown in Table 6, in all cases the increments in fecal steroid excretion were considerably greater than the decrements in plasma cholesterol content. Thus, in period II, not only was a portion of plasma cholesterol excreted, but additional fecal steroids must have been derived from either enhanced cholesterol synthesis or mobilization of cholesterol from preexisting tissue pools.

Specific activity–time curves of plasma cholesterol. Figs. 1–5 show specific activity–time curves of plasma cholesterol in the 14 patients who received radioactive cholesterol intravenously. In these patients specific activities were determined biweekly until log-linear curves were clearly established. Clofibrate was then substituted for its placebo. In eight patients (nos. 2, 5A, 7, 8, 12, 16, 18, and 20) the slopes were decreased as long as clofibrate was given; in one patient (no. 14) there was a temporary reduction in slope; and in three patients (nos. 1, 6, and 22) the slopes were not altered. In two (nos. 21 and 23) the slopes were increased.

It seems of interest to correlate these changes in specific activity curves with alterations in fecal steroid excretions.

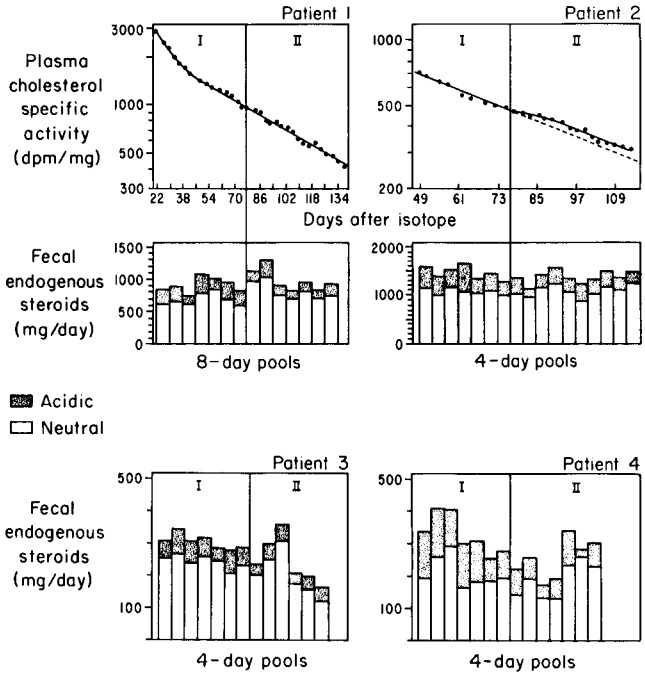

FIG. 1. Cholesterol balance data in patients 1–4 (period I, placebo; II, clofibrate). In patients 1 and 2, specific activity–time curves of plasma cholesterol are compared with excretion of total, acidic (stippled bars), and neutral (open bars) fecal steroids; these two patients had normal plasma lipids. In patient 1 no change occurred in the slope of the specific activity curve when clofibrate was introduced; a transitory increase in fecal endogenous neutral steroids was observed, but after 16 days this returned to control values. In patient 2 the slope of the specific activity curve was reduced on clofibrate, but fecal steroid excretion remained unchanged. Patients 3 and 4 with type I hyperlipoproteinemia were studied on fat-free diets. In patient 3, excretion of fecal endogenous neutral steroids decreased towards the end of period II, and in patient 4 no significant changes were found in steroid excretion.

In five patients (nos. 5A, 7, 12, 15, and 20) slopes of specific activity were decreased when clofibrate was administered despite significant increases in excretion of total steroids, and in two others (nos. 6 and 22) fecal excretions were increased without any change in the slope. In three patients (nos. 2, 14, and 18) there were significant decreases in the rate of specific activity decay while fecal steroid excretions were either unchanged or decreased. Finally, in only two patients (nos. 21 and 23) were there increases in slopes associated with an increased fecal steroid excretion.

Thus, 7 of 14 patients who had increases in total steroid excretion showed no increases in decline of specific activity decay. These results are strikingly different from our previous studies, in which increments in fecal steroid excretion were consistently associated with increased slopes of specific activity–time curves. In these previous studies that involved interruption of the enterohepatic circulation of cholesterol (32) and bile acids (22), we postulated that the combined increase in fecal steroids and increased rate of specific activity decay indicated

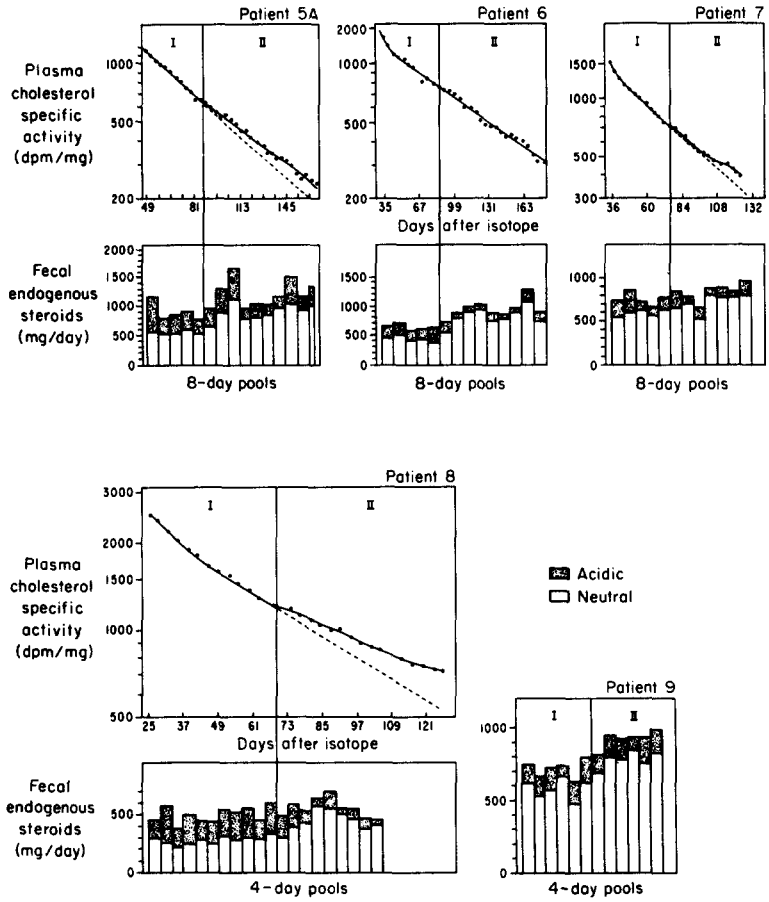

FIG. 2. Cholesterol balance data in patients 5–9 (period I, placebo; II, clofibrate). All of these patients had primary hypercholesterolemia (type II lipoprotein patterns). In every patient, excretion of endogenous neutral steroids was significantly increased on clofibrate (see Table 4), and in three of four patients the slopes of specific activity–time curves of plasma cholesterol were reduced. Acidic steroid excretions were significantly reduced in patients 6 and 8.

an increased cholesterol turnover; by contrast, the presence of this association in only 2 of 14 patients has led us to question whether the increased excretion of fecal steroids as a result of clofibrate treatment represented accelerated turnover.

Effects of clofibrate on biliary output of cholesterol (study II)

In studies of the hourly output of biliary cholesterol into the duodenum, direct evidence was obtained in three patients (nos. 9, 17, and 24) that this output was significantly increased by clofibrate (Table 7). In patient 9, the average increase in biliary cholesterol was 12 mg/hr, or 288 mg/day; this increase correlated well with an average daily increase in excretion of fecal endogenous neutral steroids of 208 mg/day (Table 4). In patients 17 and 24, measurements of output of biliary cholesterol were made twice during placebo administration and twice during clofibrate treatment; in both patients the hourly flow was consistently greater on clofibrate. Thus, it would appear that a significant portion of the increase in fecal endogenous neutral steroids found during treatment with clofibrate could have been due to an increase in the secretion of cholesterol into bile.

Effects of clofibrate on intestinal cholesterol synthesis (study III)

Concentrations of total plasma cholesterol and triglycerides during placebo, cholestyramine, and cholestyramine + clofibrate treatment periods are shown for three patients in Table 8. Cholestyramine lowered plasma cholesterol concentrations in all three patients, and further addition of clofibrate had little or no added effect. In patients 10 and 11 the administration of cholestyramine caused triglycerides to increase, as we have previously noted (22); the further addition of clofibrate to the regimen caused triglycerides to decrease to control values. Patient 5B was anomalous in that triglycerides rose during clofibrate therapy.

The sterol balance data for these patients are presented in Table 8. The results for the control and cholestyramine periods (A and B, respectively) have been reported

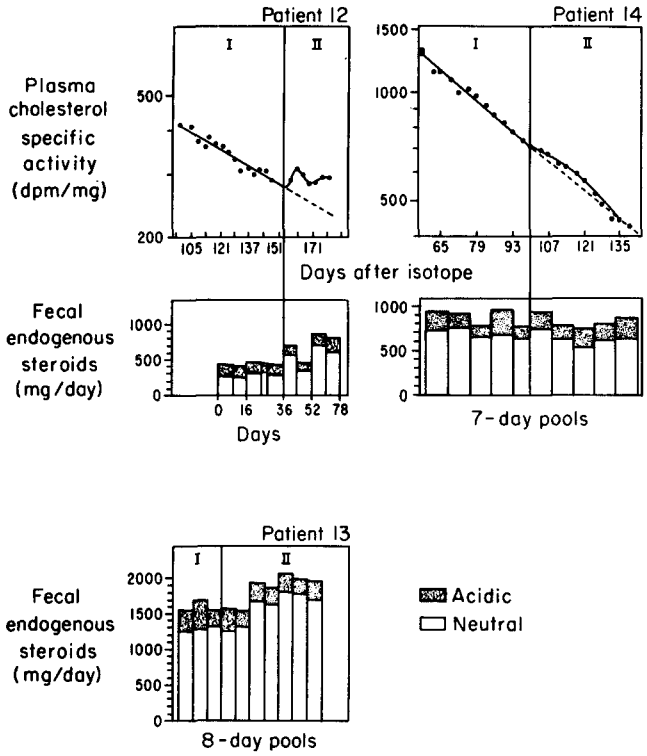

FIG. 3. Cholesterol balance data in patients 12–14 (period I, placebo; II, clofibrate). In these patients with type III lipoprotein patterns the excretion of fecal endogenous neutral steroids was significantly increased in two (nos. 12 and 13) and was unchanged in one (no. 14). In patient 12 the increased excretion of neutral steroids was accompanied by a sudden marked decrease in decline of specific activity; in patient 14 the slope of the specific activity curve decreased temporarily over a 28-day period.

separately (22); cholestyramine caused a marked increase in excretion of acidic steroids in all patients and a significant but lesser increase in neutral steroid excretion in two patients. The addition of clofibrate caused a further enhancement in neutral steroid in all three patients; the increments in neutral steroids on the addition of clofibrate ranged from 273 to 522 mg/day. In the case of acidic steroids, the effects of clofibrate were variable. In one patient, acidic steroid excretion was decreased on clofibrate, in one it was increased, and in one it was unchanged. Thus, the high output of bile acids caused by cholestyramine apparently did not prevent enhancement in neutral steroid excretion produced by clofibrate, or vice versa.

We examined the effects of cholestyramine with and without clofibrate on intestinal synthesis of cholesterol in these same patients. The results are presented in Fig. 6 along with the findings in a fourth patient (no. 18), who was studied under somewhat different conditions (see below). After administration of a single dose of radioactive cholesterol intravenously, the specific activities of cholesterol in intestinal mucosa of patients 5B, 10, and 11 closely approximated those of cholesterol in bile and plasma in period A (3–8 wk after intravenous labeling). These values provided base lines for comparisons with subsequent periods. When cholestyramine was introduced to bind bile acids within the intestinal lumen (period B), specific activities in mucosa were significantly lower than those of the plasma and bile in patients 5B and 10, suggesting enhanced cholesterol synthesis by the

TABLE 7. Rates of biliary flow of cholesterol during administration of placebo (period I) and clofibrate (period II)

Patient	Biliary Cholesterol Flow Rates				
	Period I	Period II	Comparison	Difference	
	mg/hr ± SD	*mg/hr* ± SD		*mg/hr*	*significance*
9[a]	28 ± 5 (18:9)[d]	40 ± 3 (24:12)	II − I	+12	$P < 0.001$
17A[a,b]	33 ± 7 (20:20)	40 ± 10 (20:20) 6d[e]	IIA − IA IIA − IB	7 5	$P < 0.02$ NS
17B[a,b]	35 ± 6 (20:20)	50 ± 19 (20:20) 13d[e]	IIB − IA IIB − IB	17 15	$P < 0.001$ $P < 0.001$
24A[b,c]	33 ± 10 (24:24)	56 ± 7 (24:6) 4d[e]	IIA − IA IIA − IB	23 21	$P < 0.001$ $P < 0.001$
24B[b,c]	35 ± 7 (24:24)	51 ± 8 (24:12) 19d[e]	IIB − IA IIB − IB	18 16	$P < 0.001$ $P < 0.001$

[a] Patients 9 and 17 were fed a liquid formula (diet B) throughout their hospitalization. The same liquid formula was infused during the biliary flow study.

[b] In Patients 17 and 24, two biliary flow studies (A and B) were carried out in period I (placebo), then two (A and B) in period II (clofibrate).

[c] Patient 24 normally received a solid food diet containing mixed fat; during the biliary flow study, diet B was infused.

[d] Duration (hours) of steady state bile flow and number of pooled bile samples of equal length, e.g., 18 hr steady state and 9 samples each of 2 hr length.

[e] Number of days after starting clofibrate.

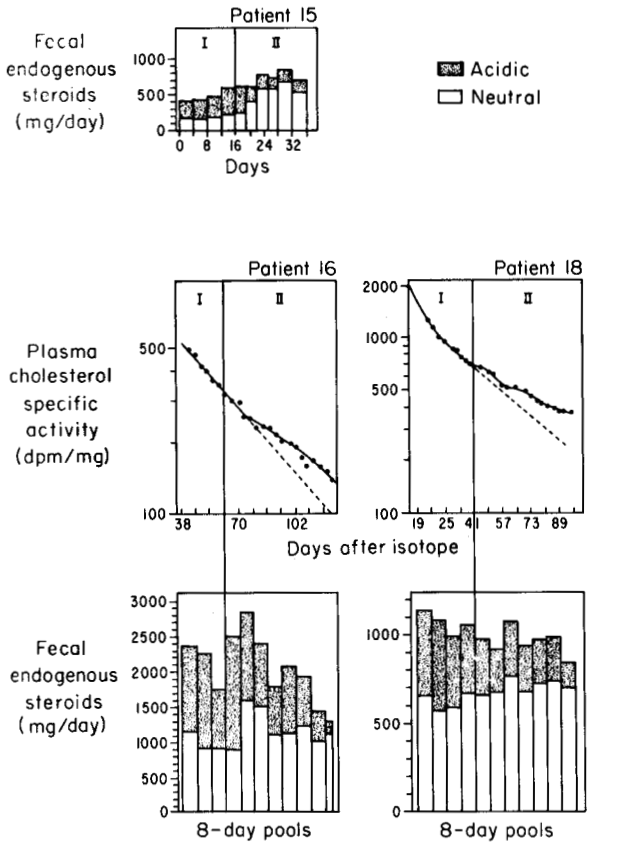

FIG. 4. Cholesterol balance data in patients 15, 16, and 18 (period I, placebo; II, clofibrate). These patients with type IV lipoprotein patterns had a variable response to clofibrate, judged in terms of fecal steroid excretion. Patient 15 showed a clear increase in neutral steroid output on the drug. Patient 16 had a transitory increase in neutral steroid excretion in period II followed by a return to control values, and late in period II acidic steroid output decreased markedly. In patient 18, neutral steroid output increased on clofibrate, and acidic steroids decreased; thus, there was no net increase in steroid excretion in period II. Slopes of specific activity curves decreased when clofibrate was administered in patients 16 and 18.

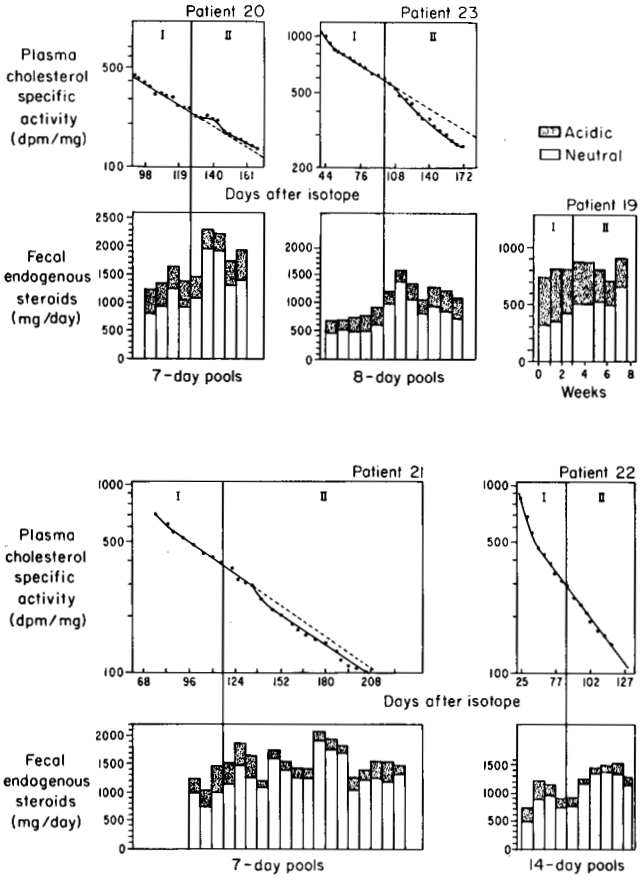

FIG. 5. Cholesterol balance data in patients 19–23 (period I, placebo; II, clofibrate). In these five patients with type V lipoprotein patterns, excretions of endogenous neutral steroids were increased when clofibrate was instituted. One patient (no. 20) showed a decrease in the slope of the specific activity–time curve of plasma cholesterol during period II, another (no. 22) had no change, and two (nos. 21 and 23) had increased slopes.

mucosa. In period C, clofibrate was given along with cholestyramine, and in 2 wk or less the previous differences in specific activities between plasma and mucosa were obliterated. We conclude from these latter findings in period C that clofibrate abolished the increase in cholesterol synthesis that had been induced in mucosa by cholestyramine. All changes in ratios of mucosa/plasma specific activities between periods A vs. B and B vs. C were statistically significant at $P < 0.01$ (Table 8). In patient 11, cholestyramine induction of cholesterol synthesis in the mucosa was not demonstrable in period B; hence, in period C the clofibrate effect shown in the other patients could not be expected.

All three patients discussed above showed specific activities of cholesterol in mucosa and plasma that were almost identical during the control period A; all three had familial hypercholesterolemia (type II lipoprotein pattern). In the fourth patient (no. 18), who had hypertriglyceridemia (type IV), quite different results were obtained. In this patient the specific activities of cholesterol in mucosa were distinctly and consistently lower than those of plasma cholesterol in control period I; we conclude that in this patient some significant degree of mucosal synthesis of cholesterol was demonstrable even in the presence of an intact enterohepatic circulation of bile acids. Thus, there was no need in this patient to enhance intestinal cholesterol synthesis by binding bile acids with cholestyramine, as was done in patients 5B and 11. The introduction of clofibrate in period II produced a distinct change: specific activities of mucosal cholesterol became the same as those of plasma cholesterol, suggesting that the drug had inhibited cholesterol synthesis in the mucosa. This conclusion is supported by results obtained in period III when clofibrate was discontinued: specific activities in mucosa again dropped below those of plasma, indicating that the inhibition of

TABLE 8. Combined study of cholestyramine plus clofibrate (study III)

Patient	Plasma Cholesterol[a] Period[c] A	B	C
	mg/100 ml ± SD (n)		
5B	244 ± 9 (8)	202 ± 7 (7)[d]	211 ± 13 (7)[e]
10	209 ± 12 (7)	172 ± 13 (19)[d]	158 ± 10 (7)[d]
11	502 ± 53 (4)	368 ± 21 (8)[d]	383 ± 11 (6)[e]
	Plasma Triglycerides[b] Period[c] A	B	C
	mg/100 ml ± SD (n)		
5B	153 ± 21 (8)	136 ± 11 (7)[d]	335 ± 37 (7)[f]
10	180 ± 33 (7)	251 ± 39 (19)[f]	122 ± 13 (7)[d]
11	126 ± 18 (6)	282 ± 34 (8)[f]	153 ± 28 (6)[d]
	Endogenous Neutral Fecal Steroids Period[c] A	B	C
	mg/day		
5B	463 ± 51 (36:9)[g]	551 ± 64[f] (36:9)	839 ± 167[f] (60:15)
10	1009 ± 229 (44:11)	735 ± 101[d] (100:25)	1257 ± 213[f] (32:8)
11	474 ± 39 (32:8)	824 ± 89[f] (40:10)	1097 ± 226[f] (32:8)
	Acidic Fecal Steroids Period[c] A	B	C
	mg/day		
5B	210 ± 48	620 ± 143[f]	767 ± 202[f]
10	646 ± 102	2344 ± 784[f]	1724 ± 600[d]
11	234 ± 58	1239 ± 430[f]	1050 ± 117[e]
	Specific Activity Ratios (mucosa/plasma) Period[c] A	B	C
	mean ± SD (n)		
5B	1.00 ± 0.05 (4)	0.75 ± 0.21 (6)[d]	1.16 ± 0.18 (3)[f]
10	1.10 ± 0.10 (4)	0.71 ± 0.13 (8)[d]	1.01 ± 0.23 (5)[f]
11	1.04 ± 0.02 (2)	0.93 ± 0.04 (4)[d]	0.89 ± 0.03 (4)[e]

All patients had hypercholesteremia (type II lipoprotein patterns) and were fed diet B.

[a] See footnote *a*, Table 3.

[b] See footnote *b*, Table 3.

[c] Period A, control period on cholesterol-free diet; period B, addition of cholestyramine (10–20 g/day); period C, further addition of clofibrate (2 g/day).

[d] Decrease in comparison with preceding period significant at $P < 0.05$ level (31).

[e] No significant difference from preceding period.

[f] Increase in comparison with preceding period significant at $P < 0.05$ level (31).

[g] Duration of balance study (days) for the period given and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this column gives the average stool collection period in days.

mucosal synthesis of cholesterol caused by clofibrate was released when the drug was discontinued. The changes in specific activity ratios (mucosa/plasma) between periods I vs. II and II vs. III were highly significant ($P < 0.01$).

Effects of long-term administration of clofibrate on body pools of cholesterol (study IV)

Table 9 presents results of isotope kinetic studies in five patients with type II lipoprotein patterns who were studied as outpatients before, during, and after long-term clofibrate administration. All patients showed a significant decrease in plasma cholesterol levels during drug administration (mean, 18%; range, 6–25%); body weights remained unchanged throughout the 4-yr period except in patient 26, who gained 6 kg. In four of these patients (nos. 25, 26, 27, and 29) who received clofibrate for approximately 3 yr, turnover rates and pool sizes of cholesterol were determined before and not less than 6 wk after discontinuing drug treatment. In three out of four patients the second study showed a reduction in the half-life of the slower exponential of the specific activity decay curve; in patient 26 this half-life was reduced from 46 to 20 days. Despite this decrease in half-life, the turnover of cholesterol was not increased after 3 yr of drug therapy; indeed, in patient 26 cholesterol turnover (PR) was reduced significantly. Therefore, the changes in half-life in the second studies must have been due to decreased pool sizes. Indeed, in three out of four patients the sizes of pools A and B were reduced after long-term drug administration, with the greatest reductions occurring in the slowly miscible pool B.

In the fifth patient (no. 28) the specific activity curve obtained before beginning clofibrate treatment was not satisfactory, and it is not reported in Table 9. The first satisfactory isotope study in this patient was carried out near the end of the long-term treatment with clofibrate and again 6 wk after termination of treatment. This patient had a marked increase in cholesterol turnover after the drug was discontinued, a finding that implies that cholesterol synthesis had been partially inhibited by clofibrate. A particularly striking finding was the great increase in the sizes of cholesterol pools only 6 wk after the drug was discontinued; indeed, if the calculations are reliable, the patient accumulated 42 g of cholesterol in this period. It should be noted that, if cholesterol synthesis had truly been increased by 2 g/day during this period, he would have had to retain only half of the cholesterol produced by this increased synthesis to account for an accumulation of 42 g.

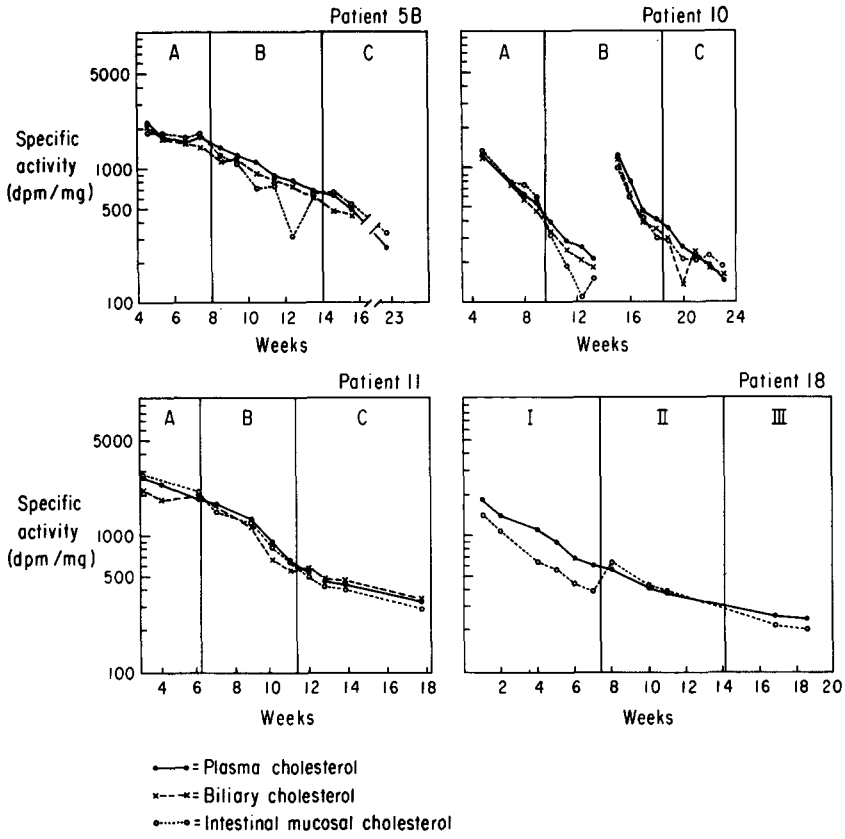

FIG. 6. Specific activity–time curves for cholesterol in plasma, bile, and intestinal mucosa in patients 5B, 10, 11, and 18. In patients 5B, 10, and 11 (type II lipoprotein patterns), specific activities of cholesterol were similar in plasma, bile, and intestinal mucosa during the control period (A). In period B (cholestyramine), specific activities in mucosa dropped below those in plasma and bile in patients 5B and 10, while patient 11 showed little change. When clofibrate was administered in addition to cholestyramine (period C), specific activities of cholesterol in mucosa again rose above those of plasma and bile in patients 5B and 10. Patient 18 (type IV lipoprotein pattern) showed a lower specific activity of cholesterol in mucosa than in plasma in the control period (I). When clofibrate was given (period II), specific activities in mucosa and plasma became identical; when the drug was withdrawn (period III), a difference in activities between plasma and mucosa was again demonstrated. All differences in ratios (mucosa/plasma specific activity) between periods A vs. B, B vs. C, I vs. II, and II vs. III were significant ($P < 0.01$), except in patient 11 (see Table 8).

DISCUSSION

Changes caused by clofibrate

The results presented in this report can be listed as follows. (*a*) Plasma cholesterol concentrations are lowered by clofibrate in all forms of hyperlipidemia except familial fat-induced hyperglyceridemia (type I lipoprotein pattern). (*b*) The excretion of neutral steroids in feces is almost always increased during drug administration, regardless of the form of hyperlipidemia. (*c*) Rates of cholesterol synthesis are not increased by clofibrate; indeed, they may be decreased in the intestinal mucosa (and presumably in other tissues, such as liver, as well). (*d*) The combination of a decreased slope of the time curve of cholesterol specific activity when clofibrate treatment is instituted together with an increased excretion of fecal neutral steroids is a unique finding in our experience, suggesting an accelerated mobilization of labeled cholesterol out of tissue storage sites. (*e*) The presumption of an increased flux of cholesterol out of the tissues is supported by the finding of an increased rate of flow of cholesterol through the biliary tract into the duodenum. (*f*) A reduction in tissue pools of cholesterol is further supported by studies of compartmental analysis carried out before and after administration of clofibrate for 3 yr.

Thus, clofibrate appears to have at least two major effects on the regulation of cholesterol metabolism in man: reduction of synthesis and increased efflux from tissues. Whether these two actions are related or independent is not yet clear; and whether the effects of the drug on triglyceride metabolism, a problem that has not been studied in this laboratory as to mode of action, are related to those on cholesterol can only be speculated upon.

Plasma lipids and lipoproteins. Numerous studies (1–10) have demonstrated that clofibrate is effective in lowering

TABLE 9. Alterations in body cholesterol pools during 3 yr of clofibrate therapy (defined by two-pool isotope kinetic studies [30, 35] before and after drug administration)

Patient	Cholesterol[a]	α[b]	β[b]	PR[b]	M_A[b]	$\overline{M}_B$[b]	$-K_{AA}$[b]	$-K_{BB}$[b]	γ_{ab}[b]
	mg/100 ml	*days*	*days*	*g/day*	*g*	*g*			*g/day*
25									
B[c]	275	4.9	57.0	1.35	20.1	52.2	0.115	0.038	1.41
A[d]	234	5.7	54.9	1.01	19.6	35.2	0.090	0.044	1.10
26									
B	275	6.1	46.0	3.22	36.4	71.6	0.089	0.038	1.60
A	234	6.2	20.0	2.04	25.0	34.4	0.087	0.058	0.90
27									
B	278	4.5	58.0	2.08	39.5	97.4	0.127	0.039	3.16
A	260	4.5	42.0	1.87	33.6	52.5	0.115	0.055	2.35
28[e]									
B	291								
D	240	5.5	54.0	1.06	29.0	50.0	0.101	0.038	1.22
A	255	4.8	33.6	3.02	52.0	69.7	0.104	0.061	2.87
29									
B	319	7.0	63.0	1.25	40.3	66.7	0.076	0.035	1.81
A	288	5.0	58.0	1.37	32.4	72.1	0.111	0.040	2.30

[a] Mean value of weekly plasma determinations during period of isotope study while patients were eating ad lib. and were not taking clofibrate.

[b] α = half-life of cholesterol in pool A; β = half-life of cholesterol in pool B; PR = production rate or turnover of new cholesterol into pool A; M_A = size of pool A; $\overline{M}_B$ = size of pool B, mean of minimum and maximum values assuming cholesterol absorption of 0.2 g/day; $-K_{AA}$ = rate constant for total removal of cholesterol from pool A; $-K_{BB}$ = rate constant for total removal of cholesterol from pool B; γ_{ab} = transfer rate from A to B.

[c] B = before clofibrate therapy of 3 yr duration.

[d] A = 40–63 days after discontinuation of clofibrate.

[e] Isotopic studies were carried out during (D) the final 3 months of a 3-yr clofibrate trial and again 2 wk after (A) discontinuing the drug.

the plasma lipids in man. In studies initiated 7 yr ago in this laboratory (12), we observed that the greatest lowering of plasma lipids occurred in patients with lipoprotein patterns of types III, IV, and V; that patients with type II patterns had significant but lesser reductions of lipids; and that there was no response to the drug in patients with fat-induced hyperglyceridemia (type I hyperlipoproteinemia) on fat-containing diets. The drug usually caused decreases in plasma cholesterol as well as triglyceride concentrations; decreases in pre-β- were usually more striking than in β-lipoprotein levels.

The present studies have been limited to mechanisms of action of clofibrate on cholesterol metabolism in man. The lowered cholesterol levels in the plasma could have been caused by any of the following actions: (*a*) clofibrate might decrease the absorption and enhance the excretion of cholesterol; (*b*) it might promote the conversion of cholesterol into bile acids; (*c*) it might inhibit cholesterol synthesis; or (*d*) it might cause a redistribution of cholesterol between plasma and tissue compartments. In previous studies we have found that other hypocholesterolemic agents act at one or more of these control points. For example, plant sterols inhibit the absorption of cholesterol (32); cholestyramine inhibits bile acid reabsorption, which in turn increases the conversion of cholesterol into bile acids (22); diets rich in unsaturated fats act primarily to cause redistribution of cholesterol from plasma into tissue pools (36). In the case of clofibrate our findings suggest that the drug affects both excretion and synthesis of cholesterol; the following paragraphs will attempt to dissect the several causes for these two effects.

Mechanism of action of clofibrate

Cholesterol excretion. An increased excretion of endogenous neutral steroids in feces was seen in 15 of 20 patients. The magnitude and duration of the effect leave little doubt of the results (although they are not in complete agreement with those of Horlick, Kudchodkar, and Sodhi [37], who found no changes in four patients with type IV lipoprotein patterns). Average daily increments in endogenous neutral steroids (compared with control periods) were found to range from 97 to 481 mg over drug periods ranging from 24 to 122 days.

An increased output of endogenous neutral steroids can be due to reduced absorption of cholesterol or to greater secretion of cholesterol into the intestinal tract. Although the absorption of exogenous cholesterol was significantly reduced in 3 of 10 patients, in the majority of those who showed an increased output of endogenous neutral steroids there was no significant change in cholesterol absorption. On the other hand, we have obtained direct evidence that the drug enhances the output of cholesterol through the biliary tract: in five studies carried out in three patients with a new intubation tech-

nique (19) we showed that the hourly output of cholesterol was very much larger during administration of clofibrate. Indeed, this increased biliary efflux may have caused the occasional decrease in absorption of exogenous cholesterol noted above; as we have shown previously (18), only about 30–50% of the cholesterol that enters the intestine is absorbed, and hence the secretion of more endogenous cholesterol into the intestine can reduce the absorption of exogenous cholesterol through competitive inhibition (32).

We can visualize three possible origins for an increase in the amount of cholesterol secreted into bile and excreted in feces. First, the conversion of cholesterol into bile acids might be inhibited: if cholesterol synthesis remained unchanged, a greater fraction of cholesterol would necessarily be excreted as endogenous neutral steroids. Second, cholesterol synthesis might be enhanced: the excess cholesterol could be excreted as neutral rather than as acidic steroids. Third, cholesterol might be mobilized from preexisting tissue pools and excreted as neutral sterols through the liver. These possibilities will now be examined.

Bile acid synthesis. Clofibrate caused a significant reduction in fecal excretion of bile acids in 6 of 20 patients. (In contrast to the findings of Horlick et al. [37], who noted a doubling of bile acid excretion in 3 patients with type II lipoprotein patterns, we noted no significant increases in bile acid excretion in any of 20 patients, 5 of whom had type II lipoprotein patterns.) This reduced excretion could have been due to decreased bile acid synthesis, and this in turn might have led to an increased output of endogenous neutral steroids. There are two reasons why we do not favor this as an explanation for the increased neutral steroid excretion caused by the drug. First, in many patients the increase in neutral steroid excretion substantially exceeded the decrease in bile acid excretion, and thus the output of total steroids (endogenous neutral + acidic) was greater in half the patients on clofibrate. Second, clofibrate did not reverse the increased bile acid synthesis induced by cholestyramine; the excretion of bile acids during treatment with cholestyramine plus clofibrate was no lower than when cholestyramine was given alone, yet the increased excretion of neutral steroids caused by clofibrate was found even during simultaneous administration of cholestyramine.

Cholesterol synthesis. We must now consider whether the increased excretion of endogenous neutral steroids during clofibrate administration was due to enhanced cholesterol synthesis. In previous studies we have been able to answer this question through use of the sterol balance method. For instance, we concluded that cholesterol synthesis was accelerated following interruption of the enterohepatic circulation of cholesterol (32) and bile acids (22); in both of those studies the conclusions drawn from sterol balance data were valid because the data were obtained in metabolic steady states. However, in the present study we were never convinced that our drug-treated patients achieved a new steady state during the relatively short balance periods described here (24–122 days), and hence we have concluded that the question of whether cholesterol synthesis was increased by the drug (to explain the increased excretion of fecal neutral steroids) cannot be answered through use of sterol balance data. Reasons for indecision in regard to achieving a new steady state on clofibrate were: (*a*) we noted a lack of constancy in neutral steroid excretion after the drug was instituted, and (*b*) we were accumulating independent evidence that tissue pools of cholesterol were being continually depleted. By definition, the metabolic steady state pertains only when tissue pools of cholesterol remain fixed; if there are reasons to think that tissue stores are changing, a steady state cannot be assumed. Therefore, as discussed previously (17), the sterol balance method cannot be relied on to give reliable synthesis rates under such conditions.

Nor, on theoretical grounds, can compartmental analysis be used to measure turnover rates in the unsteady state (22). We have presented the specific activity–time curves obtained in 14 patients after administration of a single dose of radioactive cholesterol in Figs. 1–5 not because they have quantitative usefulness, nor even because the changes in slope observed on clofibrate have conclusive qualitative meaning. Rather, we have included these data because, *when considered in conjunction with other evidence* (an increased fecal excretion of neutral steroids, a diminished plasma pool of cholesterol, and lack of a systematic change in absorption of exogenous [unlabeled] cholesterol), the decreases in slope that were often observed appeared to indicate that in many patients cholesterol synthesis could not have been increased but, if anything, was probably decreased. This conclusion is supported by the findings in study III that cholesterol synthesis in the intestinal mucosa was diminished when the drug was given.

Studies of specific activity–time curves of plasma cholesterol in man under the influence of clofibrate were first reported by Nestel, Hirsch, and Couzens (38); they found that slopes of specific activity curves were consistently flattened when clofibrate was introduced. This observation that seems incompatible with increased cholesterol synthesis has been confirmed by Horlick et al. (37) and by the present study: in 7 of 14 of our patients, curves were flattened or unchanged despite marked increases in excretion of endogenous neutral steroids. However, another explanation for the change in decay with clofibrate has been given (37), namely, mobilization of cholesterol from tissue pools. If specific activities of cholesterol in slowly miscible pools were even slightly

higher than that of plasma cholesterol when clofibrate was introduced, any mobilization of tissue cholesterol into the plasma compartment should slow the rate of decline of the specific activity of plasma cholesterol. Thus, we cannot be certain whether the decreased slopes of specific activity of plasma cholesterol were due to decreased synthesis (for which we presented other evidence) or to mobilization from tissues (for which we have also presented evidence), or both. On the other hand, these curves seem incompatible with an increased synthesis of cholesterol; therefore, we have concluded that the flux of cholesterol into feces cannot be explained by increased cholesterol production.

Since specific activity–time curves of plasma cholesterol are the resultant of interactions of several parameters of cholesterol metabolism (synthesis, pool sizes, absorption, and compartmental transfers), it was necessary to obtain direct evidence for the action of clofibrate on cholesterol synthesis in a specific tissue. In the present study we obtained some evidence on this point by studying intestinal mucosa; we employed the approach used previously in our studies of the effects of cholestyramine on mucosal synthesis of cholesterol (22). When cholestyramine was administered to three patients with hypercholesterolemia, the specific activity of cholesterol in mucosa fell clearly below that of plasma in two of the three, indicating increased synthesis (22) and confirming the findings of Dietschy and coworkers (39–42) that bile acids inhibit the mucosal synthesis of cholesterol in man as well as in lower animals. When, in those two patients, clofibrate was added to the regimen containing cholestryamine, the specific activity of mucosal cholesterol became the same as or higher than that of plasma cholesterol. The only reasonable explanation for this observation is that clofibrate reduced the rate of cholesterol synthesis that had been increased by cholestyramine. This inhibitory effect of clofibrate was seen also in a patient with hypertriglyceridemia in whom cholesterol synthesis in the mucosa was clearly demonstrable without cholestyramine stimulation; administration of clofibrate to this patient caused the specific activities in mucosal and plasma cholesterol to become identical. These findings suggested that clofibrate can inhibit cholesterol synthesis in the intestinal mucosa of man; there is strong evidence from studies in lower animals that the drug inhibits cholesterol synthesis in the liver (43).

In view of all these findings, there is little evidence to support the concept that the increased output of neutral steroids induced by clofibrate can be attributed to accelerated cholesterol synthesis. Indeed, in certain cases the drug may actually retard synthesis. Therefore, the increased excretion of endogenous cholesterol must be explained in another way: there seems to be only one plausible explanation, namely, that in hyperlipidemic states clofibrate causes mobilization of cholesterol from body pools. Evidence for this will now be examined.

Body pools of cholesterol. If cholesterol excretion is increased while at the same time synthesis and absorption are not changed, it is necessary to conclude that a net flux of cholesterol out of body pools is occurring. The pools thus depleted could be plasma or tissue pools, or both.

In the present study clofibrate caused a reduction in plasma cholesterol in almost all patients. The largest decreases were found in those patients whose hypercholesterolemia was associated with hypertriglyceridemia; however, cholesterol reductions were seen also in patients with type II lipoprotein patterns (hypercholesterolemia alone), in whom the average decrease in plasma cholesterol was about 15%. Somewhat greater reductions of hypercholesterolemia have been reported with other approaches, with unsaturated fat diets (36), nicotinic acid (44), cholestyramine (22, 45–47), and the ileal exclusion operation (22, 48–50), but the changes we have observed on clofibrate cannot be dismissed as being trivial.

Our balance results seem to indicate that the increased excretion of fecal steroids cannot be explained simply in terms of a reduction in size of the plasma (or plasma–liver) pool of cholesterol. We have compared the increment in total fecal steroid excretion with the decrement in plasma cholesterol content in eight patients who had significant increases in fecal steroid output (Table 6). The largest decrement in total plasma cholesterol content during the period of clofibrate treatment was 4.34 g, while the increments in total fecal steroids ranged from 5.93 to 46.59 g (average 22 g). It is clear, then, that much of the increase in fecal steroids was derived from pools other than plasma or even plasma plus liver.

Data obtained in study IV, the long-term outpatient study, support the conclusion that cholesterol can be mobilized from sites of storage in tissues. Studies were carried out by compartmental analysis (35) before and after 3 yr of continual administration of clofibrate in five patients. The results indicated that the drug usually caused large decreases in the size of pool B. It must be borne in mind that such estimates of pool size by compartmental analysis have not yet been verified in man. However, Wilson (51) carried out kinetic analyses in living baboons, and then, after killing them, verified his calculations by "chemical dissection" of the entire body (as well as of specific tissues); he found reasonably close agreement between the data obtained by the two approaches.

Kinetic analysis of isotope data gives no information about the amount of cholesterol in specific tissue pools; indeed, there is no way at present to estimate changes in the cholesterol content of individual organs or tissues in

man during life. Nevertheless, it has been observed repeatedly that the size of xanthomas in skin and tendons can be reduced by clofibrate (3, 7, 9, 52); these clinical observations support our conclusion that cholesterol can be mobilized from tissues by this drug. Indeed, our sterol balance data indicate that large amounts of cholesterol were fluxed out of tissue stores even in patients who were free of xanthomatosis (Table 6). Although the origin of this excess cholesterol is not clear, it is relevant to note that Crouse, Grundy, and Ahrens (53, 54) reported recently that significant quantities of cholesterol accumulate in tendinous tissues as a function of aging in man; this accumulation occurred in the absence of any evidence of xanthomatosis or antemortem hypercholesterolemia. Although it is possible that the cholesterol mobilized from patients with hyperlipidemia by clofibrate may have originated in connective tissues, it remains to be shown whether these fluxes may not also have involved the internal organs (liver, spleen, kidneys, etc.) or, alternatively, such bulk tissues as muscle and adipose tissue.

We may question whether the rapidity with which cholesterol is fluxed from tissue pools might exceed the liver's capacity to excrete it; the result of such an imbalance would be to cause plasma cholesterol levels to rise even while the excretion of fecal neutral steroids increased. This sequence of events could explain the findings in patients 16 and 19, in whom plasma cholesterol levels were significantly elevated on clofibrate while fecal steroid excretion was increased. Indeed, we have learned of occasional patients treated for years with hypocholesterolemic agents in whom plasma cholesterol levels failed to decrease until the xanthomata resolved.[1]

The duration of cholesterol flux must clearly be related to the amount previously stored in excess of critical body needs that is available for excretion. In other words, if our explanation for the increased excretion of fecal neutral steroids is correct, the period of increased sterol output will be limited by the amount available to be fluxed. The average increase in neutral steroid excretion was about 300 mg/day (Table 4); had this continued for a year, about 100 g of cholesterol would have been fluxed from tissue stores. In hyperlipidemic patients, many of whom had xanthomatosis and atheromatosis, it is entirely conceivable that as much or more than 100 g of cholesterol had been accumulated in tissue storage sites, but definitive data on the amount of cholesterol in the tissues of such patients are still lacking. Since the total pool of exchangeable cholesterol in normolipidemic men can amount to 60–100 g (55), it is not unlikely that in hyperlipidemic adults several hundred grams of cholesterol can be accumulated over a lifetime. It is not surprising, then, that in the drug periods described here (24–112 days) we failed to see the excretion of fecal neutral steroids on clofibrate return to pretreatment base lines.

At the molecular level, we do not understand how cholesterol is mobilized from tissue stores by clofibrate, nor do we know whether this is a primary or secondary drug effect. Indeed, the various changes in lipid metabolism that have been observed in association with clofibrate administration are difficult to explain by any single mechanism. For example, the lowering of plasma triglycerides as well as of cholesterol, the inhibition of intestinal synthesis of cholesterol, and the increased excretion of endogenous cholesterol are not necessarily integrated. Additional studies will be required to determine whether this drug is active at only one or at multiple control points in regulation of lipid metabolism.

Indications for clinical use of clofibrate

It is now clear that clofibrate is effective in lowering the plasma lipid levels in most patients with hypertriglyceridemia who have lipoprotein patterns of types III, IV, and V. In addition to the dramatic lowering of plasma triglycerides in these patients, cholesterol levels are usually reduced. Thus, the choice of clofibrate for treatment of patients with hypertriglyceridemia would appear to be justified at this time, since side effects are rare. Trials in a small number of patients here have shown a significant additive effect of clofibrate given together with diets rich in unsaturated fats.

The value of clofibrate in patients with hypercholesterolemia and type II lipoprotein patterns is less obvious: the agent causes a significant lowering of plasma cholesterol levels, but the lowering is often less than can be achieved by other means. However, the present study appears to show that clofibrate promotes the mobilization of tissue cholesterol in this group of patients just as in those with hypertriglyceridemia. Hence, we believe that its long-term use in these patients can be justified, even though the immediate effects on plasma cholesterol levels are not particularly impressive.

It is rational to propose that the management of hyperlipidemia can be facilitated by combinations of drugs. Indeed, Howard and Hyams (56) have reported better results (in terms of plasma lipid levels only) with clofibrate plus a bile acid sequestrant (DEAE-Sephadex) than with clofibrate alone. This is a rational combination of agents; a bile acid sequestrant reduces bile acid pool sizes and impedes cholesterol absorption (22), but it also enhances cholesterol synthesis. Clofibrate, on the other hand, appears to inhibit cholesterol synthesis, but it enhances tissue cholesterol mobilization and excretion and reduces tissue cholesterol pool sizes. Furthermore, in those patients who manifest elevated triglyceride levels on administration of cholestyramine (22), this phenom-

[1] Oliver, M. F., A. Khachadurian, and D. Berkowitz. Personal communications.

enon would presumably be prevented by clofibrate. Thus, the combined use of agents having these complementary actions can be expected to reduce tissue cholesterol pools as well as plasma lipid levels.

It remains to be demonstrated whether such reductions in tissue pools of cholesterol are germane to the issue of whether the risk of premature arteriosclerosis can be lowered. Large-scale intervention trials with clofibrate, both primary and secondary, have recently been reported (57–60), and the several investigators have reached the same conclusion, namely, that the drug confers a protective effect against new events of coronary heart disease without appearing to do so through the mediation of lowered plasma cholesterol levels. Although even more convincing answers to this "drug–heart question" are still needed, it is also true that through mechanistic studies with drugs affecting lipid metabolism we can expect to describe the critical determinants that regulate the metabolism of compounds like cholesterol that are essential in health as well as in disease.

We are grateful to Mrs. Stephanie Tonner, Mrs. Eleanor Mathusek, Miss Susan Turner, Mrs. Norma Deering, Miss Truus Willems, Mrs. Roberta Morganstern, Mr. Fred Rubel, and Mr. Albert Angevin for their excellent technical assistance.

This study was supported in part by U.S. Public Health Service grant HE-06222 from the National Heart and Lung Institute, and by U.S. Public Health Service grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Resources.

Manuscript received 12 January 1972; accepted 4 April 1972.

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