

The Effects of Colestipol Resin and of Colestipol Plus Clofibrate on the Turnover of Plasma Cholesterol in Man

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ABSTRACT Studies were conducted to determine the effects of colestipol hydrochloride, a new bile acid-sequestrant resin, on some of the parameters of cholesterol turnover and metabolism in man. Three normal volunteers and eight hyperlipidemic patients participated in three sets of cholesterol turnover studies carried out at intervals of approximately 1 yr. The effects of colestipol were assessed by comparing the results obtained before therapy with those obtained on repeat study after several months of resin therapy. Colestipol treatment significantly reduced the serum cholesterol concentration (mean reduction 21%), and produced a large increase in the production rate of cholesterol (mean 86%) and in the turnover rate of cholesterol in pool 1 (mean 46%). The values of the intercompartmental rate constants and of the size of the rapidly exchangeable pool were unchanged with therapy.

The turnover studies were carried out for 12–13 wk, and were analyzed according to a two-pool model. Although long-term studies of cholesterol turnover conform to a three-pool, rather than a two-pool model, the present studies probably provide a valid estimate of the effects of therapy on certain parameters, namely the production rate, the size, and the turnover rate of pool 1.

Repeated studies in four untreated subjects showed a striking constancy with time for the kinetic parameters for each subject. The production rate was particularly constant from year to year for a given subject, and showed a pooled standard deviation of only 3%. The findings suggest that the total body turnover of cholesterol is under close homeostatic control in an integrated manner.

Combined drug therapy with colestipol plus clofibrate further reduced the serum cholesterol level in three of

four patients, and reduced the triglyceride level in all four patients. Addition of clofibrate to the treatment program produced only small decreases in the production rate, which were not significantly different from the small decreases seen in two patients who were continued (and restudied) on colestipol alone. The findings do not support the suggestion that clofibrate can block the increased rate of cholesterol synthesis and turnover resulting from bile acid-sequestrant treatment. The effects on serum lipids, however, make the combined drug therapy potentially quite useful.

INTRODUCTION

Considerable information is now available about the turnover of plasma cholesterol in normal and in hyperlipidemic humans. When kinetic studies are conducted for periods of about 10–12 wk, the turnover of cholesterol can be described satisfactorily by a simple two-pool model (1–4). This model has been used to examine the relationships between some parameters of cholesterol metabolism and other clinical features such as body weight (3). The kinetic approach has also been employed to assess the effects of drugs that lower serum lipids, such as cholestyramine resin (1, 5, 6), neomycin (2), and clofibrate (7). The effects of ileal bypass surgery on body cholesterol metabolism have been examined by the turnover technique (6, 8).

More recent studies have demonstrated that much longer studies of cholesterol turnover in man do not fit a two-pool model closely, but require a three-pool model (9) or an alternative approach, input-output analysis, (10, 11) for satisfactory analysis of the data. These studies indicate that certain parameters of cholesterol metabolism, particularly the production rate and the size of the rapidly exchangeable compartment, can be estimated accurately by both medium- and long-term studies, whereas other parameters, such as total body exchangeable cholesterol, can be estimated only poorly.

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Received for publication 7 March 1973 and in revised form 11 June 1973.

TABLE I
Characteristics of the Subjects Studied

Subject	Age*	Sex	Height	Weight			% Ideal body weight†
				Study I	Study II	Study III	
			cm			kg	
F. C.	51	M	175	76.2	77.6	—§	112
R. M.	53	M	183	76.2	72.6	74.4	102
R. N.	54	M	183	79.4	—	79.8	108
J. B.	61	M	145	63.5	64.4	64.9	100
E. C.	55	F	163	57.6	58.1	56.7	105
H. L.	40	M	173	79.4	76.0	77.1	115
L. Z.	53	M	180	77.1	69.9	—	100
J. G.	51	F	168	66.5	66.5	67.4	110
H. G.	60	M	180	78.9	79.1	79.2	98
A. S.	51	F	160	53.1	50.6	50.3	98
G. W.	45	F	160	49.9	52.6	54.0	96

* At the time of study I.

† For study I body weight, from *Stat. Bull. Metrop. Life Insur. Co.* 1959.

§ The symbol (—) means that the study was not carried out in that subject.

In recent years increasing clinical attention has been focused on drugs that lower serum lipids in hyperlipidemic patients. One effective agent is cholestyramine, an anion-exchange resin that binds bile acids in the intestine and prevents their reabsorption (1, 6, 12–14). This drug results in a greatly increased fecal excretion of acidic steroids (6, 14), and secondarily in an increased catabolism and synthesis of cholesterol and a reduced plasma cholesterol level.

Colestipol hydrochloride (Colestid, U-26,597A, The Upjohn Co., Kalamazoo, Mich.), a new bile acid-sequestering resin, is a high-molecular weight, insoluble polyethylenepolyamine polymer with 1-chloro-2,3-epoxypropane, which effectively lowers serum cholesterol in experimental animals and in humans (15–18). We now report the results of studies carried out to determine the effects of colestipol resin on some of the parameters of cholesterol turnover and metabolism in man. A small number of studies were also conducted to examine the effects of combined drug therapy with colestipol plus clofibrate

METHODS

11 volunteer subjects, whose ages and sexes are listed in Table I, participated in these studies. Three of the subjects, F. C., R. M., and R. N., served as normal controls. The other eight subjects had mild to moderate degrees of hyperlipidemia; their plasma lipid levels, and the types of lipoprotein patterns (19, 20), seen on lipoprotein electrophoresis (21) at the start of the first study, are given in Table II. Two of the hyperlipidemic patients, G. W. and L. Z., had clinical coronary heart disease, as indicated by previously documented myocardial infarctions. Two of the patients, E. C. and J. G., had asymptomatic hyper-

tension; the other patients were clinically well. All subjects ate their usual diets during each of the studies, and were specifically asked not to modify their diets during the course of these studies. None of the subjects lost or gained significant amounts of weight during a given study. Weight changes exceeding 5% of body weight were, however, seen between studies in three of the subjects, L. Z., A. S., and G. W.: see Table I.

Three sets of turnover studies, designated studies I, II, and III, were carried out at intervals of approximately 1 yr. In each study [$4\text{-}^{14}\text{C}$]cholesterol complexed with serum lipoprotein was injected intravenously, and the specific radioactivity of serum total cholesterol was determined on blood samples collected three times during the first week, and at approximately weekly intervals thereafter for a total of 12–13 wk. In each study, the first two samples were collected 1 day and 3 days, respectively, after isotope injection. The methods used have been described fully previously (1, 9).

The data from each study were analyzed by digital computer, as described elsewhere (9), in order to determine the parameters of a two-pool model which would provide the best fit. No improvement in fit was obtained when the data were analyzed according to a three-pool model. We have reported recently (9) that long-term (e.g., 32–41 wk duration) turnover curves conform to a three-pool model, whereas medium-term data (e.g., 12 wk) are satisfactorily described by a two-pool model.

The model used is illustrated in Fig. 1. The notation is identical with that described elsewhere (9). The pools are denoted by arabic numbers (pools 1 and 2), rate constants are denoted by k , the rate of transfer of cholesterol mass (in g/day) into or out of a pool is denoted by R , cholesterol production rate (PR)¹ is given by R_0 , pool

¹ Abbreviations used in this paper: k , rate constant; M , pool size; PR, cholesterol production rate; R , rate of transfer of cholesterol mass.

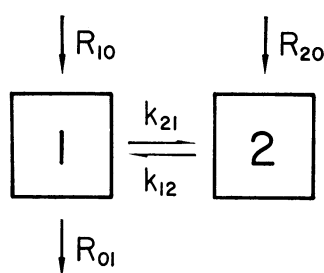


FIGURE 1 Two-pool model of cholesterol turnover. (See text for explanation of symbols and discussion.)

sizes are designated M_1 and M_2 . For this model the equation for the specific activity of pool 1 (sp act₁) is: $\text{sp act}_1 = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t}$. The constants α_1 and α_2 are the rate constants of the two exponentials that describe the turnover curve. The half-times of the exponential terms are derived from the α values, since $t_{1/2} = \ln 2/\alpha$. The constants A_1 and A_2 were previously (1) referred to as C_A and C_B , respectively.

At the time of study I (begun January 1970) no subject was taking a lipid-lowering drug, and all subjects had been in an apparent steady state, with reasonably constant plasma lipid concentrations, for several months. Serum containing approximately 22 μCi of ^{14}C -labeled cholesterol was injected into each subject. After the completion of study I, seven of the eight hyperlipidemic subjects were treated with colestipol hydrochloride at a dose of 15 g/day (5 g three times a day). In all of these subjects, resin treatment was continued without interruption for at least 2 yr.

Study II was begun after treatment with colestipol resin had been continued for at least 5 mo (range, 5–9 mo; mean 8 mo) in the subjects under investigation. All subjects had stable plasma lipid levels for several weeks before the start of the study. Each subject was given approximately 24 μCi [^{14}C]cholesterol. Several months later four of the patients being treated with colestipol resin were also begun on treatment with clofibrate (Atromid-S, Ayerst Laboratories, Div. of American Home Products Corp., New York) at a dose of 2 g/day (1 g twice daily). A new apparent steady state with regard to plasma lipid levels was reached in less than 4 wk. Study III was begun approximately 8 wk after the start of combined drug therapy (with resin plus clofibrate) in these patients. Each subject was injected with approximately 17 μCi of [^{14}C]cholesterol. The data for all three sets of studies were later adjusted to an injected dose of 25 μCi for each study in each subject, for final data analysis.

The residual radioactivity in the plasma just before the start of study II was very low (specific radioactivity less than 0.3% of that found in the 1-day sample). At the start of study III the residual radioactivity (from the previous two studies) ranged from 0.2 to 0.7% (mean 0.5%) of the level of ^{14}C found in the 1-day sample. In one subject (J. B.) the residual radioactivity was somewhat higher (2% of that of the 1-day sample). The data were corrected for the observed low levels of residual radioactivity; the corrections were extremely small in every instance.

Statistical analysis of the results obtained in the three series of studies was carried out by comparing the change in each of the parameters of the model (PR, k , and M , see Fig. 1) to the changes observed for these parameters in the untreated subjects by the t test for uncorrelated samples. The significance of the change in parameters seen when resin was continued alone in study III was assessed by an analysis of variance (22).

TABLE II
Plasma Lipid Levels during the Periods of the Studies*

Subject	LP pattern† (type)	Study I		Rx§	Study II		Rx§	Study III	
		Cholesterol	TG		Cholesterol	TG		Cholesterol	TG
F. C.	NL	220±2	80±5	0	215±3	76±4	0	—	—
R. M.	NL	237±3	105±4	0	218±3	101±4	0	243±4**	128±5 **
R. N.	NL	211±2	62±4	0	—	—	0	207±3	71±5
J. B.	IV	223±3	293±13	0	224±4	266±25	0	218±4	257±14
E. C.	IIB	314±6	178±9	C	238±3	139±9	C	260±3 **	175±6**
H. L.	IV	295±5	301±50	C	187±4	152±11	C	233±8 **	195±12¶
L. Z.	IV	238±4	346±21	C	165±4	112±9	—	—	—
J. G.	IIA	286±5	154±7	C	242±5	199±8	C + A	197±3 **	96±5 **
H. G.	IIA	312±4	144±10	C	272±4	180±9¶	C + A	229±3 **	95±5 **
A. S.	IIB	311±5	178±10	C	241±3	196±10	C + A	188±3 **	91±5 **
G. W.	IIB	334±6	172±7	C	315±7¶	139±9	C + A	311±7¶	107±8 ‡‡

* Plasma cholesterol and triglyceride (TG) concentrations are listed as the mean±SEM values (in mg/100 ml) during the period of each study. n , 14 or 15 in all instances.

† Lipoprotein pattern at the time of study I (no treatment); NL, normal.

§ 0, No drug treatment; C, resin (colestipol); A, clofibrate (Atromid-S).

|| Significantly different from corresponding lipid level in study I, with $P < 0.01$.

¶ $P < 0.05$ (as cf. study I).

** Significantly different from corresponding lipid level in study II, with $P < 0.01$.

‡‡ $P < 0.05$ (as cf. study II).

TABLE III
Reproducibility of the Derived Kinetic Parameters in Untreated Subjects

Subject	Study	PR*	$k_{11}\ddagger$	$k_{22}\S$	k_{21}	M_1	Minimum $M_2\parallel$	Maximum $M_2\parallel$
		<i>g/day</i>	<i>day⁻¹</i>	<i>day⁻¹</i>	<i>day⁻¹</i>	<i>g</i>	<i>g</i>	<i>g</i>
F. C.	I	1.17	-0.1133	-0.0492	0.0665	25.0	33.8	53.6
	II	1.17	-0.1118	-0.0486	0.0680	26.8	37.4	57.5
R. M.	I	1.38	-0.1179	-0.0445	0.0626	24.9	35.0	61.5
	II	1.43	-0.1305	-0.0472	0.0700	23.6	35.0	60.9
	III	1.42	-0.1273	-0.0506	0.0692	24.4	33.3	57.4
R. N.	¶	1.18	-0.1024	-0.0385	0.0609	28.4	44.9	70.1
	I	1.12	-0.1115	-0.0531	0.0697	26.7	35.0	52.3
	III	1.09	-0.1145	-0.0499	0.0743	27.1	40.3	58.1
J. B.	I	0.91	-0.1067	-0.0518	0.0654	22.0	27.8	41.4
	II	0.89	-0.1061	-0.0680	0.0671	22.7	22.4	32.4
	III	0.88	-0.1053	-0.0503	0.0639	21.2	26.9	40.4
Pooled SD		0.032	0.00445	0.00623	0.00360	0.97	3.38	5.64

* PR, production rate in pool 1.

‡ k_{11} = the rate constant for the total rate of removal of cholesterol from pool 1.

§ k_{22} = k_{12} for the model shown in Fig. 1.

|| Minimum M_2 and Maximum M_2 are the lower and upper limiting values for the size of pool 2, calculated as described previously (3, 9).

¶ This turnover study in subject R. N. was carried out for 9 wk in 1966.

RESULTS

Effects of treatment on serum lipid levels. The four untreated subjects (three normal controls plus hypertriglyceridemic subject J. B.) maintained fairly constant serum cholesterol and triglyceride levels during the nearly 3 yr of these studies. For these subjects, the mean serum cholesterol values during study II and study III were an average of 96.6% and 99.5%, respectively, of the values observed during study I (see Table II).

Treatment with colestipol resin resulted in a highly significant reduction of the serum cholesterol concentration in every treated subject (see Table II). The average reduction (study II as compared to study I) was 21.1% (range 36.6%–5.7%). Statistically significant changes were also seen in the serum triglyceride concentrations of six of the seven treated subjects. The direction of the change in triglyceride level was, however, not consistent: four subjects showed significant decreases in triglyceride levels, whereas two subjects showed significant increases (Table II).

Addition of clofibrate to treatment with colestipol resin resulted in a highly significant further reduction in both the serum cholesterol and triglyceride concentrations in three of the four patients so treated. In these three subjects (J. G., H. G., and A. S.) the

mean cholesterol concentrations during Study III were 68.9%, 73.4%, and 60.5% of those observed during study I (see Table II). In the fourth patient (G. W.) the mean serum cholesterol level during study III did not differ from that during study II, although the combined drug treatment did produce a lower triglyceride level than that seen with resin alone.

Two subjects, E. C. and H. L., were continued on colestipol treatment alone, without the addition of clofibrate. Serum cholesterol and triglyceride concentrations rose between studies II and III in both of these subjects. The serum cholesterol concentrations remained, however, highly significantly reduced (by 17% and 24%, respectively) when compared to the values seen in study I, before treatment (see Table II).

Untreated control subjects. The reproducibility of the kinetic parameters for any given subject was examined by repeat studies in untreated subjects. The results are shown in Table III. Two subjects were studied in all three sets of studies. Two subjects were studied in two of the three sets of studies; in addition, data were available for one of these latter subjects from a turnover study carried out in 1966. Inspection of the results (Table III) shows that there was a remarkable constancy with time for the kinetic parameters for each subject. The pooled standard deviations, calculated by an analysis of variance (two-way classification) ranged from 3 to 13% of the mean for the

TABLE IV
Effect of Colestipol Resin Therapy

Subject	$A_{11}\S$		$A_{22}\S$		$t\frac{1}{2}$ of 1st exponential		$t\frac{1}{2}$ of 2nd exponential		PR		k_{11}	
	I‡	II‡	I	II	I	II	I	II	I	II	I	II
	dpm/mg	dpm/mg	dpm/mg	dpm/mg	days	days	days	days	g/day	g/day	day ⁻¹	day ⁻¹
E. C.	2,605	3,043	640	431	5.27	3.59	62.6	33.3	0.71	1.52	-0.1078	-0.1716
H. L.	1,718	1,896	440	286	4.25	3.37	41.4	28.5	1.51	2.65	-0.1332	-0.1822
L. Z.	1,692	1,984	619	382	4.31	3.24	39.6	23.2	1.21	2.51	-0.1225	-0.1839
J. G.	1,573	2,255	638	320	4.09	3.58	38.3	32.5	1.25	2.09	-0.1258	-0.1724
H. G.	1,428	1,691	469	268	5.07	3.93	38.8	27.2	1.50	2.76	-0.1081	-0.1556
A. S.	1,939	2,619	754	287	5.96	3.89	44.9	30.0	0.85	2.05	-0.0881	-0.1630
G. W.	1,834	1,814	534	527	5.22	4.36	38.9	24.3	1.27	1.86	-0.1069	-0.1297
Mean	1,835	2,185	585	357	4.88	3.71	43.5	28.4	1.19	2.21	-0.1132	-0.1655

* See text and footnotes to Table III for definition of symbols and comments on the parameters.

‡ I, study I, without treatment; II, study II, on colestipol resin treatment.

§ All data were normalized to an injected dose of 25 μ Ci (5.5×10^7 dpm) for each study in each subject for final data analysis.

parameters listed. The estimates of PR in pool 1 were the most constant, and those of the limiting values of M_2 and of pool 2 turnover rate (k_{22}) were the least constant from study to study for a given subject.

Effects of colestipol therapy. The effects of colestipol therapy were assessed by comparing the results obtained without therapy (study I) with those obtained during resin therapy (study II) in the seven subjects who were studied under both conditions. The results are presented in Table IV. The most substantial effects of colestipol therapy were: (a) a mean increase of 86% in the production rate in pool 1; (b) a mean increase of 46% in k_{11} , the turnover rate in compartment 1; and (c) a mean decrease of 35% in the half-time of the second exponential for the equation describing the turnover curve. The statistical significance of the changes observed with therapy was assessed by comparing the mean difference due to resin treatment with the mean difference seen in the control subjects (Table III). The control differences were drawn randomly from the untreated group so that each subject contributed only once to the differences. The increases in the production rate and in k_{11} were both highly significant ($P < 0.01$). With this rigorous statistical analysis, none of the other parameters of the two-pool model (Fig. 1) changed significantly.

Two of the subjects were continued on colestipol resin only, and were studied again one year later (study III) while on this continued therapy. The results obtained for these two subjects (E. C. and H. L.) are shown in the top portion of Table V. There was a tendency for the production rate to return toward the control values (of study I, see Table IV), although the change was not statistically significant due to the small

number of observations. Changes in the other parameters were too variable to even establish a trend.

Effects of colestipol plus clofibrate. In the bottom portion of Table V are shown the results obtained with the three subjects, J. G., H. G., and A. S., who received a combination of resin plus clofibrate for the third study. (Subject G. W. is not included in this analysis because her dosage of colestipol was increased between studies II and III, as well as starting on clofibrate, and hence the data are not comparable to the data on the other patients whose dosage of colestipol did not change). The production rate decreased (by 0.22 and 0.46 g/day) on combined drug therapy (study III as compared to study II) in two of the patients, J. G. and A. S., but did not change in the third subject, H. G. All three subjects showed significant reductions in serum cholesterol levels with combined drug therapy (as compared to resin treatment alone, Table II). The decrease in production rate in the patients on combined drug therapy was not significantly different from the decrease in PR (mean 0.28 g/day) seen in the two patients who had been continued on resin alone. With combined drug therapy there was a consistent small decrease in the size of pool 1, but the significance of this was difficult to assess since the two subjects who continued on resin alone showed such a variable response. Changes in the rate parameters observed with the combination therapy were not significantly different from the changes in the patients who continued on resin alone.

In order to obtain more information about the effects of clofibrate in the subjects treated with resin plus clofibrate, clofibrate administration was discontinued in subjects J. G. and A. S. 14 wk after the injection of

k ₂₂		k ₂₁		M ₁		Minimum M ₂		Maximum M ₂	
I	II	I	II	I	II	I	II	I	II
day ⁻¹		day ⁻¹		g		g		g	
-0.0348	-0.0422	0.0660	0.0764	17.1	16.0	32.4	28.9	47.2	60.3
-0.0466	-0.0482	0.0746	0.0781	25.7	25.4	41.1	41.2	69.2	92.0
-0.0559	-0.0596	0.0722	0.0767	24.0	23.4	31.0	30.2	49.1	69.0
-0.0618	-0.0427	0.0762	0.0757	25.1	21.6	30.9	38.2	47.9	82.3
-0.0464	-0.0461	0.0555	0.0582	28.5	28.3	34.0	35.7	62.0	91.2
-0.0437	-0.0384	0.0470	0.0557	20.6	19.1	22.2	27.7	37.0	75.8
-0.0438	-0.0579	0.0528	0.0512	23.4	23.7	28.3	21.0	52.7	49.6
-0.0476	-0.0479	0.0635	0.0674	23.5	22.5	31.4	31.8	52.2	74.3

[4-¹⁴C]cholesterol (study III). Blood samples were collected for an additional 6–7 wk, in order to determine the corresponding data points for the turnover curve. On discontinuing clofibrate the serum cholesterol levels rose within 1 wk, and remained fairly stable thereafter, at mean levels which were 16% and 9% higher than the mean levels observed during the first 13 wk of study. The effects on the turnover curve of subject J. G. are shown in Fig. 2. Similar results were obtained with the other subject. Along with the rise in serum cholesterol level, on stopping clofibrate there was a decrease in the slope of the turnover curve in both of the subjects.

DISCUSSION

These studies were designed to examine the effects of treatment with colestipol resin on some aspects of cholesterol metabolism and turnover in hyperlipidemic

humans. Colestipol was well tolerated, without significant side effects, in all seven subjects during the 2-yr period of drug administration. The effects of colestipol were assessed by comparing the results of cholesterol turnover studies carried out without (before) therapy with those obtained on repeat study after several months of resin therapy. It was assumed that a new steady state had been achieved by the time the second study was carried out. It is recognized, however, that this assumption may not be completely valid. Colestipol treatment resulted in a significant reduction in serum cholesterol concentration (mean reduction 21%, range 36%–6%). This was observed in every subject, regardless of lipoprotein phenotype, since the series included patients with the patterns of Types IIA, IIB, and IV. A number of the kinetic parameters, of the turnover curve and the two-pool model, were altered by therapy. The largest effects seen were increases in the production rate

TABLE V
Comparison of Continuation of Resin Therapy Alone with Combination Therapy of Resin and Clofibrate*

Subject	PR		k ₁₁		k ₂₂		k ₂₁		M ₁		Minimum M ₂		Maximum M ₂	
	g/day		day ⁻¹		day ⁻¹		day ⁻¹		g		g		g	
	II	III	II	III	II	III	II	III	II	III	II	III	II	III
	C†	C†	C	C	C	C	C	C	C	C	C	C	C	C
E. C.	1.52	1.27	-0.1716	-0.1364	-0.0422	-0.0408	0.0764	0.0667	16.0	18.3	28.9	29.9	60.3	56.2
H. L.	2.65	2.35	-0.1822	-0.1835	-0.0482	-0.0522	0.0781	0.0848	25.4	23.8	41.2	38.6	92.0	79.7
	II	III	II	III	II	III	II	III	II	III	II	III	II	III
	C†	C + A†	C	C + A	C	C + A	C	C + A	C	C + A	C	C + A	C	C + A
J. G.	2.09	1.87	-0.1724	-0.1754	-0.0427	-0.0433	0.0757	0.0859	21.6	20.9	38.2	41.4	82.3	79.9
H. G.	2.76	2.76	-0.1556	-0.1670	-0.0461	-0.0501	0.0582	0.0685	28.3	28.0	35.7	38.3	91.2	89.4
A. S.	2.05	1.59	-0.1630	-0.1515	-0.0384	-0.0388	0.0557	0.0575	19.1	16.9	27.7	25.0	75.8	60.7

* See text and footnotes to Table III for definition of symbols and comments on the parameters.

† II, study II; III, study III; C, colestipol resin treatment; A, clofibrate (Atromid-S).

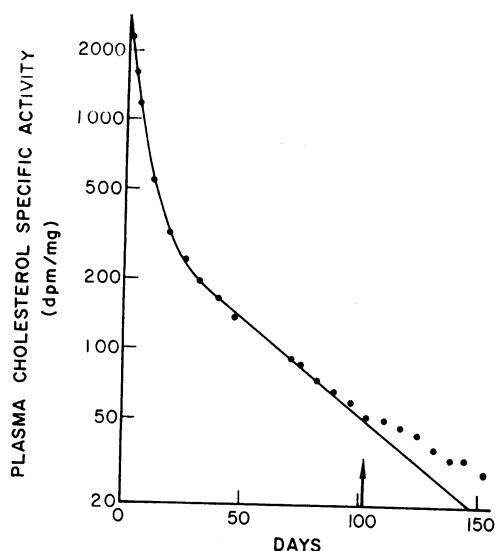


FIGURE 2 The turnover of plasma cholesterol in subject J. G. during study III, while on combined drug treatment with colestipol plus clofibrate. Clofibrate was discontinued on day 102 (arrow). The curve drawn represents the best fit obtained with the two-pool model for the data collected during the first 13 wk of the study.

(mean 86%), and in the turnover rate of pool 1 (k_1 , mean 46%). These effects are comparable to the effects of cholestyramine resin treatment, previously reported for a smaller series of subjects (1). Treatment with colestipol did not alter the values of M_1 , the size of the rapidly exchangeable pool, or of the intercompartmental rate constants. These results thus provide a quantitative description of the effects of colestipol on a number of parameters of cholesterol metabolism.

The estimates of the lower and upper limiting values of M_2 were also not significantly changed with therapy. It should be noted, however, that the interpretation of the limiting values of M_2 is insecure, since the values considerably underestimate the size of the slowly exchangeable cholesterol body pool as estimated from long-term studies and a three-pool model.

The turnover studies reported here were carried out for 12–13 wk, and were analyzed according to the two-pool model. We have recently reported that much longer studies of cholesterol turnover conform to a three-pool rather than a two-pool model (9). The long-term studies (9) included data collected during a continuation of study I in five of the subjects reported here (the untreated control subjects F. C., R. M., R. N., and J. B., and patient H. L.). In the previous report (9), parameters for a three-pool model calculated from long-term data were compared to parameters for a two-pool model calculated from medium-term data. It was demonstrated that medium-term data provided a valid estimate for M_1 , a slightly (8–9%) elevated value for the pro-

duction rate, and a quantitatively unreliable (low) estimate of total exchangeable body cholesterol, as compared to long-term data. We concluded that estimates of the production rate from studies of 10–12 wk duration can be considered valid if corrected by reduction by 8–9% (9).

Since the three sets of studies reported here were all carried out for the same medium-term duration, it is likely that the studies can be compared with each other, and that they provide a quantitatively valid estimate of the effects of therapy on certain parameters, namely the production rate, the size, and the turnover rate of pool 1. This conclusion involves the assumption that a similar model would be appropriate for long-term studies both during and before drug therapy. Evidence in support of this assumption is available from recent cholesterol turnover studies carried out in rats (23). The same three-pool model was found satisfactory for the description of long-term turnover studies in untreated rats and in rats treated with either colestipol resin or with neomycin (23). Until these findings are replicated in humans, however, quantitative conclusions about the effects of therapy on physiologically interpreted parameters should be considered as tentative. Caution in interpretation is also indicated in view of the suggestion of Grundy, Ahrens, and Salen (6) that, in patients treated with cholestyramine resin, intestinal mucosal cholesterol may not exchange completely with plasma cholesterol before being secreted into the intestinal lumen. To the extent that this phenomenon occurs during resin treatment, the value of the production rate in pool 1 will underestimate the total body turnover rate of cholesterol.

The mechanisms by which colestipol (and other bile acid sequestrants) alters the metabolism of cholesterol warrant consideration. By binding bile acids in the intestinal lumen, the resin results in an increased fecal excretion of acidic steroids and a decreased reabsorption of bile acids. These changes lead directly to two well-characterized effects on cholesterol metabolism: first, an increase in the rate of cholesterol biosynthesis in the intestinal mucosa, since this process is regulated in vivo by intraluminal concentration of bile acids (24, 25); and second, an increase in the rate of conversion of cholesterol to bile acids, since the rate-limiting reaction, the 7 α -hydroxylation of cholesterol, is under negative feedback control by the rate of bile acid reabsorption and return to the liver (26, 27). Resin treatment also results in an increased rate of cholesterol synthesis in the liver (28, 29) although the mechanism for this effect is less clear. The rate of cholesterol synthesis in the liver appears to be regulated by cholesterol influx to the liver after absorption by the intestine (30, 31). If the resin results in decreased absorption of

cholesterol, therefore, an increased rate of hepatic biosynthesis would be anticipated. Decreased absorption of cholesterol, reflected in an increased excretion of neutral sterol, has, however, been seen in some but not in all patients studied (6, 14). Accordingly, other effects of the resin on hepatic synthesis of cholesterol are probably also involved. These effects remain to be defined, but may relate to the increased rate of cholesterol catabolism to bile acids, and the increased rate of turnover and presumably decreased hepatic pool size of bile acids, which occur with resin therapy.

The increased rates of cholesterol synthesis in intestinal mucosa and liver, and of cholesterol conversion to bile acids result in the development of a new steady state during drug therapy, characterized by an increased rate of turnover of cholesterol. The extent of this increase has been quantitatively defined in this study by determining the effect of colestipol resin on the production rate. Along with these changes there also occurs a decrease in the level of plasma cholesterol. The mechanisms responsible for the reduction in plasma cholesterol concentration are not understood. In the present series, there was no correlation between the extent of the increase in production rate with resin therapy and the decrease in serum cholesterol level, when the parameter changes were examined in either relative or absolute terms. Moreover, resin treatment did not affect the size of the entire rapidly exchangeable pool, but only of that small portion of it represented by plasma cholesterol. It is likely that the metabolic changes induced by resin treatment in some way affect the metabolism of plasma lipoproteins, resulting in a consistent lowering in the plasma cholesterol level. This effect provides the basis for the therapeutic usefulness of sequestrant resin in the treatment of hypercholesterolemia.

Repeated studies in four untreated subjects demonstrated that there was a striking constancy with time for the kinetic parameters for each subject. The production rate was particularly constant from year to year for a given subject, and showed a pooled standard deviation of only 0.03 g/day (a coefficient of variation of less than 3%), despite the fact that the values from subject to subject differed by as much as 60% (subject R. M. as compared to J. B., Table III). Reproducibility of kinetic parameters in a single subject studied on two occasions was previously observed by Samuel, Holtzman, Meilman, and Perl (2). These findings suggest that the total body turnover of cholesterol is under close homeostatic control in an integrated manner. The nature of this control is obscure, since the two major cholesterol-synthesizing tissues, mucosa and liver, appear to be regulated by different mechanisms (31, also see above), and since cholesterol synthesis can occur in a wide range of other tissues. More

information is needed about factors that can integrate the control of cholesterol turnover in different tissues: a variety of factors, including genetic, dietary, and metabolic ones, are probably involved.

Increased plasma triglyceride levels in some patients have been reported previously during cholestyramine (6, 32) and during colestipol therapy (16-18). In the present study four subjects showed significant decreases and two showed significant increases (Table II) in triglyceride concentration during resin treatment. Since hypertriglyceridemia appears to be a significant coronary risk factor (33), as is hypercholesterolemia, a rise in triglyceride level should be considered as an undesirable side effect when it occurs during resin treatment. In each of the small number of subjects studied here, the rise in triglyceride level was more than fully reversed by the addition of clofibrate to the treatment regimen.

Combined drug therapy, with colestipol plus clofibrate, significantly reduced the plasma cholesterol level in three of four patients, and reduced the triglyceride level in all four patients, as compared to treatment with resin alone. The effectiveness of the combination of clofibrate plus a bile acid-binding resin (cholestyramine or DEAE-Sephadex) has been reported by others (7, 34). Clofibrate is known to have a number of effects on cholesterol metabolism. These include the inhibition of cholesterol biosynthesis in the liver (35-37), and the enhancement of cholesterol excretion in bile and feces in patients on sterol balance study (7). It has been suggested (7) that clofibrate can also inhibit cholesterol synthesis in the intestinal mucosa of man and, furthermore, can reduce the rate of mucosal cholesterol synthesis that had been increased by cholestyramine; this suggestion was based on data on the specific radioactivity of mucosal cholesterol. Studies of the effect of clofibrate on the turnover of plasma cholesterol have demonstrated a flattening of the slope of the turnover curve when clofibrate was administered (7, 38, 39). The interpretation of this finding is uncertain, although it has been considered to reflect inhibition of cholesterol synthesis or mobilization of cholesterol from tissue pools. In addition, clofibrate has significant and important effects on plasma triglyceride and very low-density lipoprotein metabolism (40, 41).

The combination of bile acid sequestrant plus clofibrate would seem to be rational since, from the published reports cited, clofibrate could be expected to inhibit partly the increased rate of cholesterol synthesis and turnover induced by the resin sequestrant. Such an inhibition would presumably result in a period of negative sterol balance and a reduction in body pools of cholesterol. It was thus anticipated in the present study that combined drug therapy would result in a significant

reduction in the production rate, as compared to the results obtained with colestipol resin alone. Surprisingly, however, only small decreases in production rate were observed in two patients (0.22 and 0.46 g/day, Table V), and the production rate did not change in the third patient studied. The change in production rate bore no apparent relationship to the change in serum cholesterol level (mean decrease of 45, 43, and 53 mg/100 ml in the three patients, Table II) produced by addition of clofibrate to therapy. The change in the production rate in these patients was not significantly different from the small decreases in production rate (0.25 and 0.30 g/day, Table V) seen in the two patients who had been continued (and restudied) on resin alone. Although the number of patients studied was small, the statistical analysis employed, comparison of a group of two vs. three patients with the observed SEM of parameter values, could have demonstrated a statistically significant effect of clofibrate if the patients on combined drug treatment had shown a mean decrease in production rate of 0.52 g/day more than did the patients on resin alone. These findings are not consistent with the interpretation that clofibrate was blocking the increased rate of cholesterol synthesis and turnover resulting from colestipol treatment. Because of the small number of studies, however, these results should not be considered as disproving the earlier interpretation, and further studies will be required to resolve this question.

On discontinuing clofibrate there was a decrease in the slope of the turnover curves (Fig. 2) in both of the two patients so studied. If clofibrate had been inhibiting cholesterol synthesis, one might anticipate that the removal of this inhibition would result in an increase in the slope of the turnover curve. The interpretation of changes in slope of turnover curves is, however, insecure, since various changes in production rate, intercompartmental rate constants, and pool sizes can have similar net effects.

These results indicate that the effects of clofibrate on cholesterol metabolism in man, at least in patients also receiving bile acid sequestrant therapy, warrant further investigation and evaluation. The potential clinical usefulness, however, of the combination of bile acid sequestrant resin plus clofibrate in the treatment of hyperlipidemia seems clear.

ACKNOWLEDGMENTS

We thank Ms. G. Stanton, Ms. A. Campbell, Ms. B. Adams, and Mr. R. Sciacca for expert assistance.

This work was supported by Grants HL-14236 (SCR), AM-05968, and HD-03993 from the National Institutes of Health, Bethesda, Maryland, and by grants from the Sharon Research Institute, the William Lightfoot Schultz Foundation, and The Upjohn Company.

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