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The Effect of Chlorophenoxyisobutyric Acid and Ethinyl Estradiol on Cholesterol Turnover *

PAUL J. NESTEL,† EUGENE Z. HIRSCH,‡ AND ELIZABETH A. COUZENS

(From the University of Melbourne, Department of Medicine, the Royal Melbourne Hospital, Melbourne, Victoria, Australia)

Measurements of cholesterol turnover in man have been reported previously (1–3), but a relationship between turnover and cholesterol concentration has not been demonstrated (3). This may be due, in part, to the complex distribution of cholesterol in the body that renders the interpretation of isotopic data difficult.

Cholesterol exists in the body in a number of separate pools that appear to turn over at differing rates (4). However, Chobanian and Hollander (5) and Avigan, Steinberg, and Berman (6) have shown that when labeled cholesterol is injected into man or into the rat, equilibration of the total exchangeable cholesterol in the body is eventually achieved and that the subsequent removal of labeled cholesterol from the body represents the turnover of the slowly miscible pool of cholesterol.

Since it seemed probable that cholesterol lowering drugs would affect the rate at which cholesterol is synthesized or excreted, we have made measurements of cholesterol turnover before and after treatment with cholesterol lowering drugs. We have studied the effects of chlorophenoxyisobutyric acid and ethinyl estradiol and have demonstrated that such studies may provide an indication of the mechanism of action of such drugs.

Methods

Ten subjects with coronary heart disease, aged 37 to 62, were studied. Eight had had a myocardial infarction approximately 1 year previously, and two suffered from angina pectoris. Three patients were being treated with anticoagulants. The dietary intake was not restricted, but daily records of all food eaten showed that each subject consumed an acceptably uniform diet throughout the study.

Varying doses, averaging approximately 50 μc of 4-C^{14}-cholesterol,[1] were administered to each subject. The cholesterol was dissolved in 0.5 ml ethanol and slowly added to 20 ml of the subject's plasma that had been collected on that day. The plasma was agitated in a water bath at 37° C for 1 hour and then injected intravenously.

Samples of venous blood were collected before breakfast for 4 days and then at weekly intervals for 10 to 14 weeks. The plasma was separated at 4° C and extracted in 20 vol of chloroform: methanol 2:1 (vol/vol). Samples were evaporated under nitrogen and separated on silicic acid columns into a fraction containing cholesterol esters, which was eluted with 25 ml 1% diethyl ether in heptane, and a fraction containing free cholesterol, which was eluted with 50 ml chloroform. Contamination of one fraction by the other was less than 1%. Radioactivity in the eluates was measured in a Tri-Carb liquid scintillation spectrometer using 0.3% diphenyloxazole in toluene as scintillator solvent. Free and esterified cholesterol was measured by the method of Sperry and Webb (7).

The decline in specific activities of free and esterified cholesterol was plotted semilogarithmically. Specific activity was measured at weekly intervals for at least 6 weeks after isotopic equilibration was reached. The half-time disappearance of the 4-C^{14}-cholesterol was obtained from the linear portion of the specific activity time curve, and the fractional turnover rate was calculated from the equation $K = 1/1.44 \times t_{\frac{1}{2}}$.

After the cholesterol turnover rate had been determined, cholesterol lowering agents were administered. Ethyl chlorophenoxyisobutyrate (CPIB) [2] was taken orally by six subjects in a dose of 0.5 g four times a day. Ethinyl estradiol was taken orally by three subjects in a daily dose of 150 μg. The concentration and specific activity of free and esterified cholesterol were measured at approximately weekly intervals for a further 4 to 6 weeks.

Results

Isotopic equilibration of free and esterified cholesterol. The plasma free cholesterol specific ac-

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† Address requests for reprints to Dr. Paul J. Nestel, Royal Melbourne Hospital, Melbourne, Victoria, Australia.

‡ Fellow, Lakeside Hospital, Cleveland, Ohio.

[1] Radiochemical Centre, Amersham, England.

[2] CPIB or Atromid-S was kindly provided by Mr. Stott of Imperial Chemical Industries.

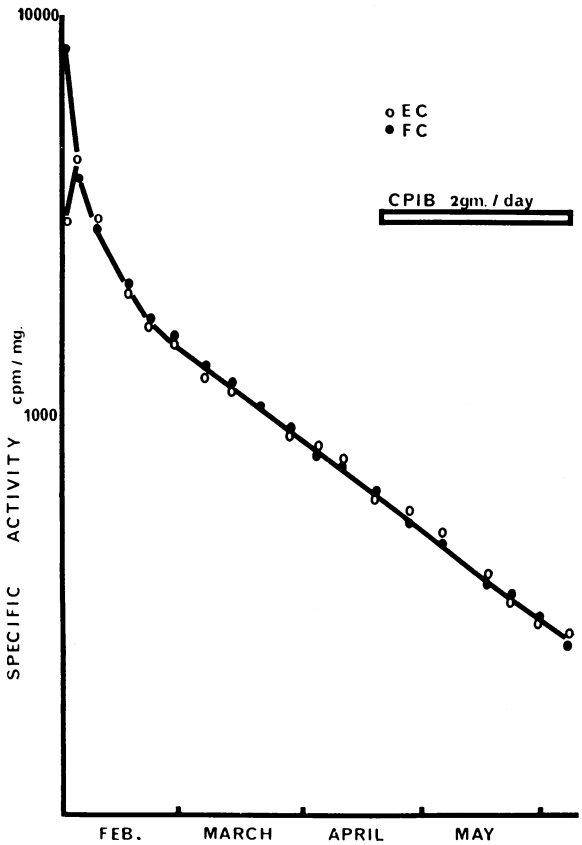

FIG. 1. SUBJECT HOV: C^{14}-CHOLESTEROL SPECIFIC ACTIVITY TIME CURVE BEFORE AND DURING TREATMENT WITH ETHYL CHLOROPHENOXYISOBUTYRATE (CPIB) IN THE SOLITARY SUBJECT WHO FAILED TO RESPOND TO THE DRUG. EC = esterified cholesterol; FC = free cholesterol.

tivity fell rapidly during the first few days and subsequently declined at decreasing rates. A constant exponential rate of fall was achieved in all studies by the end of the fourth week (Figures 1 to 4). In the subject who did not receive CPIB or estrogens, and in the subject in whom CPIB failed to lower the serum cholesterol (Figure 1), the rate of fall in specific activity remained constant for up to 18 weeks, when the studies were terminated.

The plasma cholesterol ester specific activity was initially lower than that of free cholesterol and rose for several days. In general the cholesterol ester specific activity curve crossed the free cholesterol curve at its peak. This peak was reached on the third day in eight studies and between the third and seventh day in the remaining two. The specific activities of free and esterified cholesterol were virtually identical after the first week.

Fractional turnover rates and serum cholesterol. The serum cholesterol levels, the specific activities on day 30, and the fractional turnover rates are shown in Table I. The calculations of fractional turnover rates were made from the linear portion of the curve, generally from the end of week 4 to the end of week 9.

The fractional turnover rates varied from 0.015 to 0.025 per day. The correlation betwen fractional turnover rate and serum cholesterol concentration was found to be significant ($p = < 0.05$).

The specific activities on day 30 have been corrected to a dose of 10^6 cpm per kg. (Isotopic equilibration of the exchangeable pool of cholesterol appeared to have been achieved in every study by day 30.) There was no correlation between specific activity on day 30 and serum cholesterol concentration.

Effect of CPIB. CPIB produced a fall in cholesterol concentration that exceeded 5% in five of six subjects (Table II). The figures represent the means of the determinations made during the measurements of fractional turnover rate before and during treatment.

The decline in esterified cholesterol in the five subjects who responded varied from 19 to 33 (mean 25) % and that of free cholesterol from 6 to 22 (mean 15) %. The fall in esterified cholesterol was invariably greater than that in free cholesterol.

In the patient who did not respond to CPIB

TABLE I

Relationship between serum cholesterol concentration, specific activity after isotopic equilibration, and fractional turnover rate

Subject	Serum cholesterol concentration	Cholesterol specific activity on day 30*	Cholesterol fractional turnover rate
	mg/100 ml	*cpm/mg*	*per day*
McQ	230	410	0.017
Led	255	528	0.015
Wis	260	481	0.015
Tir	280	408	0.015
Win	318	694	0.017
Hov	319	747	0.017
Whe	350	379	0.025
Kir	361	379	0.016
Tul	390	619	0.020
Cad	442	397	0.020

* Corrected to an injected dose of 10^6 cpm per kg. Isotopic equilibration had been achieved in all subjects on day 30.

TABLE II

Effect of ethyl chlorophenoxyisobutyrate (CPIB) on mean serum concentration and fractional turnover rate of esterified and free cholesterol

Subject	Esterified cholesterol				Free cholesterol			
	Concentration		Fractional turnover rate		Concentration		Fractional turnover rate	
	Before	After	Before	After	Before	After	Before	After
	mg/100 ml		*per day*		*mg/100 ml*		*per day*	
Hov	224	216	0.017	0.017	95	94	0.017	0.017
Win	220	178	0.017	0.012	98	85	0.017	0.014
Kir	260	200	0.016	0.012	101	95	0.016	0.014
Tul	280	190	0.020	0.016	110	86	0.020	0.016
Cad	312	250	0.020	0.013	130	115	0.020	0.016
Whe	250	170	0.025	0.011	100	80	0.025	0.015

the fractional turnover rate remained unchanged (Figure 1, Table II). In the other five subjects there was a decrease in the rate of fall in specific activity, and this effect was more pronounced on cholesterol esters (Table II, Figures 2 and 3). These studies may not have been continued until a new steady state was established with certainty. The rates of change in specific activity have, however, been documented as changes in fractional

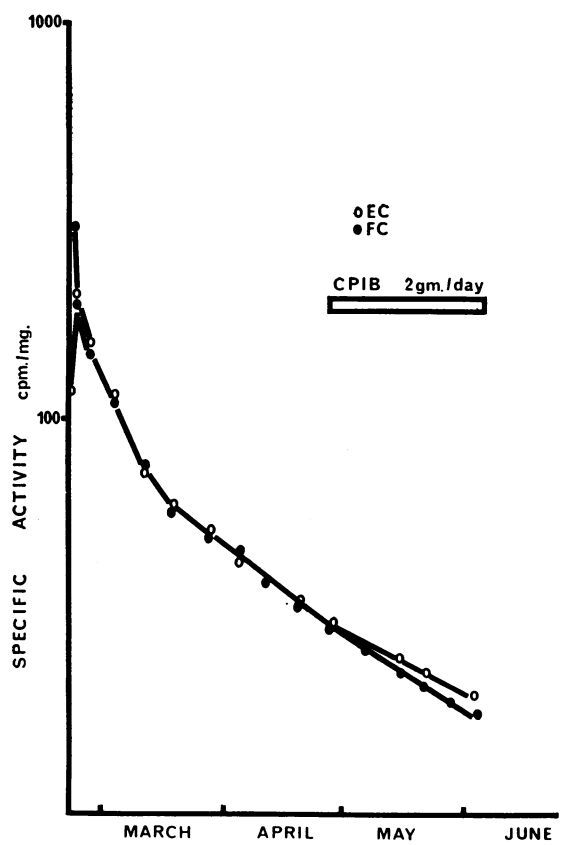

FIG. 2. SUBJECT WIN: C^{14}-CHOLESTEROL SPECIFIC ACTIVITY TIME CURVE BEFORE AND DURING TREATMENT WITH CPIB. EC = esterified cholesterol; FC = free cholesterol.

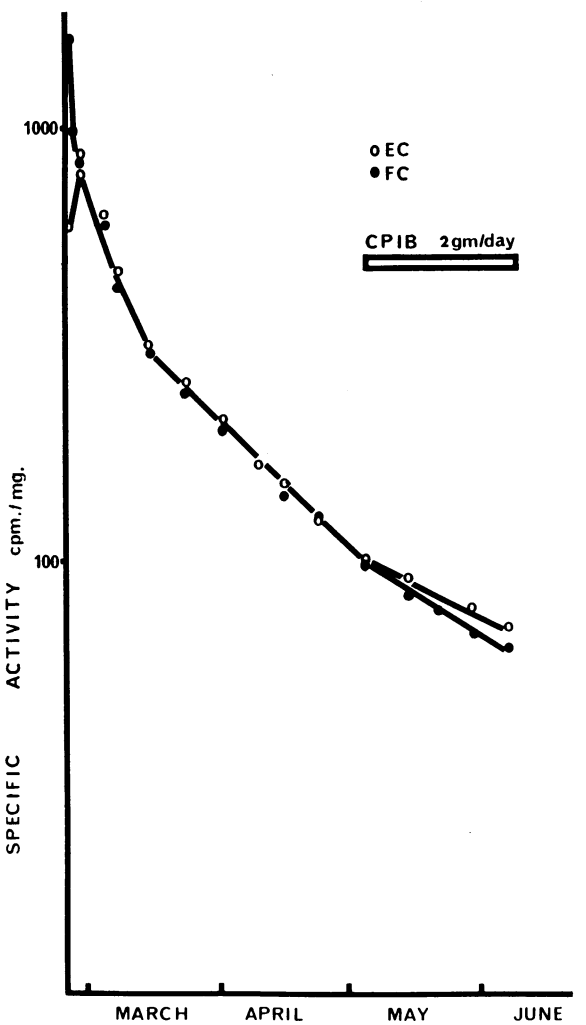

FIG. 3. SUBJECT WHE: C^{14}-CHOLESTEROL SPECIFIC ACTIVITY TIME CURVE BEFORE AND DURING CPIB TREATMENT. EC = esterified cholesterol; FC = free cholesterol.

TABLE III

Effect of ethinyl estradiol on mean serum concentration and fractional turnover rate of esterified and free cholesterol

	Esterified cholesterol				Free cholesterol			
	Concentration		Fractional turnover rate		Concentration		Fractional turnover rate	
Subject	Before	After	Before	After	Before	After	Before	After
	mg/100 ml		*per day*		*mg/100 ml*		*per day*	
Led	180	150	0.015	0.029	75	60	0.015	0.029
McQ	165	130	0.017	0.029	65	50	0.017	0.029
Tir	190	165	0.015	0.027	90	76	0.015	0.027

turnover rate for the purpose of illustrating the direction of change in turnover.

Effect of ethinyl estradiol. The administration of this drug to three subjects produced decrements in plasma free and esterified cholesterol concentrations (Table III, Figure 4). The falls in esterified cholesterol were 16, 21, and 13% and those in free cholesterol 20, 23, and 16%, respectively.

There was a marked increase in the rates of fall in specific activity that was of similar magnitude in all three subjects (Table III, Figure 4). These affected free and esterified cholesterol to a similar degree.

Discussion

These studies confirm previous observations that cholesterol turnover represents the sum of the turnovers of a number of pools but that isotopic equilibration in man is generally reached by the end of the first month (3). Chobanian and Hollander (5) have demonstrated this more directly by measuring the specific activities of cholesterol in many tissues after the injection of C^{14}-cholesterol. They were able to show that the miscible pool of cholesterol in man includes the cholesterol in all the body tissues outside the nervous system and that this pool is in isotopic equilibrium by the end of the first month. We have therefore assumed that the rate of disappearance of C^{14}-cholesterol from the plasma from week 4 to week 9 represents the turnover of the miscible pool of cholesterol. The fractional turnover rates of C^{14}-cholesterol derived from the equilibrated portion of the curves varied from 0.015 to 0.025 per day. However, in nine of the ten studies the range of fractional turnover rates was between 0.015 and 0.020 per day indicating much less variation than in the study of Chobanian, Burrows, and Hollander (3). This may be due to the greater homogeneity of our group of subjects. The constant rate of fall in specific activity in all our subjects, which was followed in some for as long as 18 weeks, excludes the possibility that equilibration had not been reached at the time when these calculations were made.

Our finding of a significant direct relationship between serum cholesterol concentration and fractional turnover rate is at variance with that reported by Chobanian and associates (3) and may be due to the heterogeneity of their group of subjects. Our finding cannot be interpreted adequately without an accurate measurement of pool sizes and turnover rates. Since the fractional

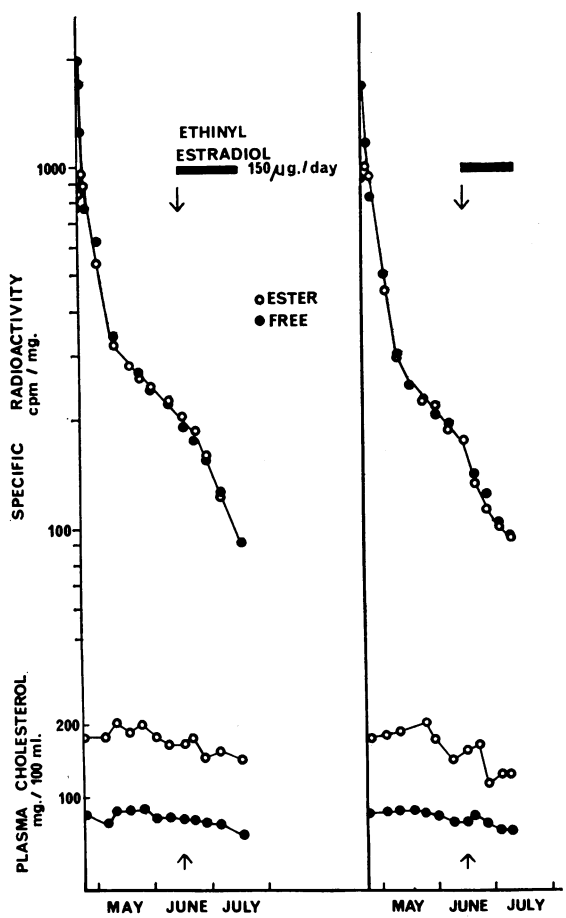

FIG. 4. SUBJECTS LED AND MCQ: C^{14}-CHOLESTEROL SPECIFIC ACTIVITY TIME CURVES AND CHOLESTEROL CONCENTRATIONS BEFORE AND DURING ETHINYL ESTRADIOL TREATMENT.

turnover rate bears a direct relationship to turnover rate and an inverse relationship to pool size, it would be interesting to know whether a relationship also existed between serum cholesterol and either total exchangeable cholesterol or turnover rate. The lack of any correlation between serum cholesterol concentration and the specific activity observed on the day 30 soon after isotopic equilibration argues against such a simple relationship, since the specific activity reflects both turnover rate and pool size.

Ethyl chlorophenoxyisobutyrate (CPIB) (8) and ethinyl estradiol (9) are potent cholesterol lowering drugs, and in our studies, falls in plasma cholesterol concentration were associated with characteristic changes in the rates of fall in cholesterol specific activity.

After CPIB administration there was a mean fall of 25% and 15% in the plasma concentration of esterified and free cholesterol, respectively, in the five of the six subjects who responded. These falls in cholesterol concentration were accompanied by a decrease in the rates of fall of specific activity. The greater falls in esterified cholesterol concentration were accompanied by correspondingly slower rates of fall in specific activity. The model that would most readily account for a sudden decrease in the rate of fall of specific radioactivity is one in which synthesis of new unlabeled material is inhibited. Under these circumstances the dilution of the labeled pool will be reduced. Although other mechanisms of action of CPIB, such as increased excretion, have not been excluded, it is apparent that a major increase in excretion would have produced an increase in the rate of fall in specific radioactivity. This does not necessarily mean that the synthesis of free cholesterol alone is inhibited. Since plasma triglycerides are also reduced by CPIB, it is possible that the synthesis of other lipids, of protein, or of lipoprotein is being inhibited.

The somewhat greater depression of esterified cholesterol suggests that esterification was also altered. We have confirmed previous findings (10, 11) that the administration of CPIB results in an increase in the proportion of oleate and palmitoleate and a decrease in the proportion of linoleate in the cholesterol esters of whole plasma (12). These changes were not confined to cholesterol esters, and both triglycerides and phospholipids were affected to a lesser extent. The significance of this is obscure.

Since this study was completed, abstracts of two other studies have been published that are consistent with our observations. Gould, Avoy, and Swyryd (13) have demonstrated that synthesis of cholesterol from acetate was diminished in livers of CPIB fed rats. Duncan, Best, and Despopoulos (14) have found a decrease in the rate of lipoprotein secretion by livers of CPIB-fed rats.

The mode of action of estrogens appears to be different. The falls in cholesterol concentration were accompanied by increased rates of fall in specific radioactivity. This is most readily explained by an increase in the total turnover rate. These findings are consistent with the studies of Kritchevsky, Staple, Rabinowitz, and Whitehouse (15), who showed that cholesterol oxidation was greater with liver mitochondrial preparations of female than of male rats. Moreover, the administration of estrogens to male rats increased the rate of oxidation. Since synthesis of cholesterol by liver homogenates was also greater in female rats, it would appear that, in the rat, estrogens increase the turnover rate of cholesterol.

Summary

The fractional turnover rates of cholesterol were measured in ten subjects with coronary heart disease. C^{14}-cholesterol was complexed with plasma lipoproteins *in vitro* and injected intravenously. Isotopic equilibration was reached after 4 weeks, and measurement of the rate of removal of labeled cholesterol was then continued for 2 to 3 months.

A significant relationship was found between fractional turnover rate and serum cholesterol concentration.

When determinations of initial fractional turnover rates were completed, six subjects were treated with chlorophenoxyisobutyric acid and three others with ethinyl estradiol. Both drugs produced falls in plasma cholesterol concentration. The administration of ethyl chlorophenoxyisobutyrate (CPIB) produced a decrease in the rate of fall of cholesterol specific activity indicating a probable inhibition of cholesterol or lipoprotein synthesis. On the other hand, treatment with ethinyl estradiol resulted in an increase in the rate of fall of cholesterol specific activity indicating increased turnover or catabolism.

Acknowledgment

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