STUDIES ON EXOGENOUS CHOLESTEROL METABOLISM IN HUMAN ATHEROSCLEROSIS WITH THE AID OF ISOTOPES

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I. Introduction

Human studies are particularly indicated in atherosclerosis research in view of the uncertainties in translating data collected in atherosclerotic animals to man. The types of experiments which can be performed are of course limited by considerations of safety; however, some studies of the dynamics of metabolism can be made directly in man with complete safety utilizing the various labeling isotopes of biological interest. Studies of the dynamics of metabolism in health and disease directly in man promise much toward clarifying the pathological physiology of atherosclerosis.

In spite of the enormous research effort which has been directed toward the effective control of human atherosclerosis, it remains the principal cause of death in the United States (34). No hypothesis as to the

etiology of this disease has been presented with sufficient experimental and clinical evidence to immunize it from vigorous and more or less effective attack. One point is certain, however, that the metabolism of tissue and serum lipids and lipoproteins is intimately involved in the pathogenesis of this disease; and at this time the study of atherosclerosis as a disease incident to abnormal lipid metabolism would appear to offer the best approach to its ultimate control. The final understanding of the pathological physiology of atherosclerosis must contain answers to many fundamental problems of lipid and cholesterol metabolism.

The incrimination of deranged lipid metabolism as a promising approach to the pathological physiology of atherosclerosis arises from a variety of evidence. There is no doubt that atherosclerotic plaques contain increased deposits of lipids; that cholesterol feedings in suitable animals produce experimental atherosclerosis; that certain diseases characterized by deranged lipid metabolism, i.e., xanthoma tuberosum, xanthoma tendinosum, diabetes, hypothyroidism, and nephrosis, show an increase in atherosclerosis; and also that certain evidence would indicate that the incidence of atherosclerosis can be influenced to a degree by alterations in the lipid content of the diet. However, on closer examination of this evidence it becomes obvious that the morbid anatomy of atheromatous plaques tells us little or nothing of the metabolic sequences leading to their formation; that many features of experimental atherosclerosis differ rather strikingly from the human disease; that the vast majority of patients having atherosclerosis show no obvious derangement in lipid metabolism; and finally that attempts to control the disease by dietary means have been disappointing. Indeed many phases of lipid metabolism as it applies to human atherosclerosis remain controversial. In order to correctly integrate the various conflicting ideas which are current in atherosclerosis research more information is needed on the fundamentals of lipid absorption, the lipid transport system of the blood, blood and tissue lipid intermetabolism, hepatic function, and bile acid metabolism. The intensely dynamic state of all phases of lipid and lipoprotein metabolism is slowly unfolding to the attack of modern isotopic techniques; however, the picture is far from complete.

The present paper deals with a variety of experiments on the fundamentals of cholesterol metabolism performed with cholesterol labeled with tritium. Wherever possible human experiments have been done; however, in attempting to explain the results seen in the human studies it has been necessary to resort to animal experiments. This step, from animals to man, is obviously a difficult one to take with certainty in metabolic research, but in many areas of fundamental lipoprotein metabolism animal studies would appear to be the only possible approach. In

this paper the metabolism of exogenous or dietary cholesterol is emphasized; however, the intricate intermetabolism of exogenous and endogenous cholesterol metabolism in all *in vivo* animal and human studies is such that exogenous and endogenous cholesterol metabolism cannot be described wholly independently. Our understanding of exogenous cholesterol metabolism is not complete, and in some sections of this paper data have been presented which lack sufficient corollary information to allow exact interpretation. In the main, however, some interpretation of the data is possible, and a description of some aspects of our knowledge of the fundamentals of exogenous cholesterol metabolism is presented.

II. Experimental Methods

1. *Tritium Labeled Cholesterol* (Cholesterol-H^3)

Cholesterol-H^3 was prepared according to the method of Bloch and Rittenberg (8) with a few minor changes (35). This catalytic exchange method of labeling provides a compound entirely suitable for the study of exogenous cholesterol metabolism. Biggs and Kritchevsky (4) and Hellman *et al.* (28) compared cholesterol-C^{14} and cholesterol-H^3 metabolism in *in vivo* studies. Results of studies of the metabolic fate of exogenous cholesterol were identical whether carbon-14 labeled or tritium labeled cholesterol was used. The label in the cholesterol-H^3 so prepared is divided approximately equally between the phenanthrene ring structure and the side chain, i.e., 46% in the vicinity of the Δ^5-3-hydroxy system and 54% in the isopropyl group of the side chain (21, 22).

Tritium decays with a half-life of 12.5 years emitting a β-particle of only 18 kev maximum energy. These physical characteristics make it particularly suitable for human studies for a practical dose, i.e., 1 mc of cholesterol-H^3, can be given to a subject without approaching the maximum permissible amount of this isotope in the human body as set by the National Committee on Radiation Protection.

2. *Assay of Tritium Radioactivity*

Both ionization chambers and active-gas filled counting tubes operated in the proportional region have been used for measuring tritium activities in this paper. Tritium-hydrogen gas was generated from a water sample prepared from the biological sample under investigation. The ionization chambers were filled with tritium-hydrogen to one atmosphere pressure and assayed on a vibrating reed electrometer with a suitable potentiometer (6, 23). The active-gas filled counters were filled to partial pressures of 8 cm of mercury with tritium-hydrogen and 20 cm of mercury with isopentane. A suitable amplifier-scaler was used.

3. Chemical Procedures

a. Cholesterol Specific Activity Measurements. An alcohol-acetone (1:1) solution of the serum, plasma, or tissue lipids was prepared and made up to a known volume. The concentrations of total and free cholesterol were determined colorimetrically as described below. The cholesterol-H^3 in a suitable aliquot together with a known amount of carrier cholesterol was precipitated with digitonin. The cholesterol-digitonide was ignited in oxygen in a suitable combustion train to provide the water sample used for hydrogen generation. The specific activity was calculated using a cholesterol-digitonide empirical formula of $C_{56}H_{108}O_{28}$.

For total cholesterol specific activities, the lipid extract was saponified with potassium hydroxide prior to digitonin precipitation. For free cholesterol specific activity measurements, this saponification step was omitted. Esterified cholesterol specific activities when desired were calculated from the free and total cholesterol specific activities and the free and total cholesterol concentrations.

The standard deviation of the cholesterol specific activity measurements in the range of activities of this paper is $\pm 6\%$.

b. Cholesterol Determinations. Cholesterol determinations on serum or on aliquots of the alcohol-acetone extracts were done according to the method of Schoenheimer and Sperry (47) as modified by Sobel and Mayer (49) and Colman and McPhee (13).

4. Collection of Rat Lymph

A polyethylene tube, outside diameter 0.060 in., was placed in the abdominal portion of the thoracic duct according to the technique described by Bloom *et al.* (9). A second polyethylene tube was fixed in the stomach to facilitate the administration of cholesterol-H^3. After recovery from the ether anesthesia the animals were maintained in restraining cages without anesthesia throughout the lymph collections. Approximately 6 hours after surgery if the lymph flow was good each donor animal was given 5 ml of whole milk plus 30 mg of cholesterol-H^3 dissolved in 1 ml of cottonseed oil. The lymph subsequently collected was delivered to a flask containing acid citrate dextrose anticoagulant to prevent clotting. Each 100 ml of this solution contains sodium citrate USP 2.06 gm, citric acid 0.75 gm, and dextrose 2.29 gm. The lymph used for the injection experiments was collected as soon after the cholesterol-H^3 administration as the lymph became pure white and chylous. The lymph was stored at 4°C and was used within 48 hours of collection.

5. Ultracentrifugal Analysis of Serum Lipoproteins

The method of ultracentrifugal analysis of the low density lipoproteins of the serum as developed by Gofman and his associates has been utilized

this study (10). The method classifies the serum lipoproteins in terms of their flotation rates in the ultracentrifuge under carefully defined conditions of temperature, solution density, and centrifugal force. Gofman and associates classify the low density lipoproteins in terms of S_f units.* The S_f^0 values in this paper correspond to the "Standard Flotation Class" of Gofman and have been corrected for Johnston-Ogston concentration effects (32).

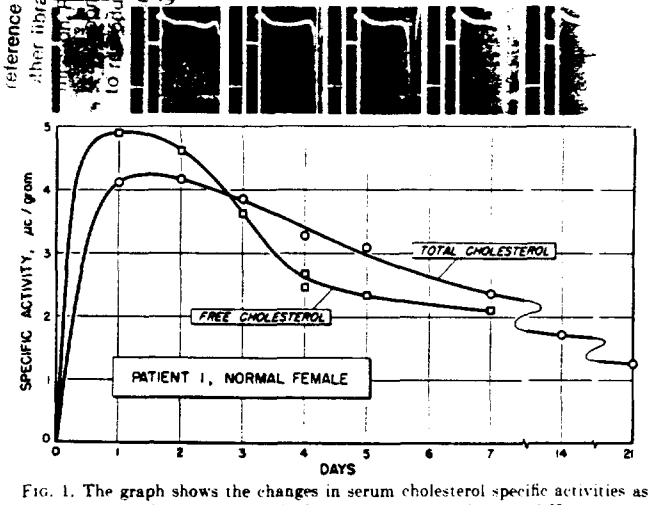

FIG. 1. The graph shows the changes in serum cholesterol specific activities as a function of time following a single feeding of 0.63 gm of cholesterol-H^3 (sp. act. = 1.07 μc/mg) in a fatty meal. The lipoprotein spectrum shows: $S_f^0$0-12 = 259 mg%, $S_f^0$12-400 = 74 mg%. Serum cholesterol values: free = 42 mg%, total = 193 mg%. (From Biggs, M. W., and Colman, D., *Circulation* **7**, 393, 1953.)

III. Human Studies—An Apparent Metabolic Defect in Exogenous Cholesterol Metabolism in Atherosclerosis

In the course of the last several years cholesterol-H^3 has been fed to some twenty patients and the dynamics of the appearance and disappearance of labeled cholesterol in the plasma as a function of time have been determined. This group includes a variety of different clinical entities. Many can be classified as "healthy" individuals, several had xanthoma tuberosum, several xanthoma tendinosum, one nephritis, one biliary cir-

* 1 S_f unit = Svedberg of flotation = a flotation rate of 10^{-13} cm/sec/dyne/gm at 26°C in a medium of sodium chloride of solution density 1.063.

tions, and several had previously experienced a myocardial infarction. Ultracentrifugal analyses of the serum lipoproteins were done.

Each patient received a single dose of cholesterol-H^3 dissolved in cottonseed oil and emulsified into a mixture of 250 ml of whole milk and 45 gm of ice cream. The total and free cholesterol specific activities of the serum were determined as a function of time. Typical results to be

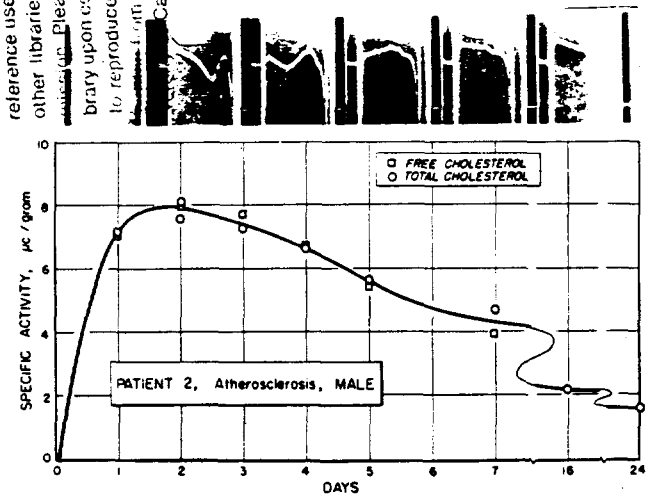

FIG. 2. The graph shows the changes in serum cholesterol specific activity as a function of time following a single feeding of 0.54 gm of cholesterol-H^3 (sp. act. = 1.07 μc mg) in a fatty meal. The lipoprotein spectrum shows: S_f0-12 = 432 mg%, S_f12-400 = 429 mg%. Serum cholesterol values: free = 66 mg%, total = 266 mg%. (From Biggs, M. W., and Colman, D., *Circulation* **7**, 393, 1953.)

found in "normal" individuals are exemplified in Fig. 1. Figure 2 illustrates the results found in a patient who had one year previously experienced a myocardial infarction. Typical results seen in xanthoma tendinosum are illustrated in Fig. 3, in xanthoma tuberosum in Fig. 4.

In the usual patient the peak serum cholesterol specific activity is not reached until between 24 and 48 hours following the cholesterol-H^3 meal and usually between 8 and 15% of the fed cholesterol-H^3 exists in the circulating plasma at this time. The delayed maximum serum cholesterol specific activity is of course a function of the rate of cholesterol-H^3 absorption and the metabolic fate of dietary cholesterol during the first day or two following its aborption. The rate of cholesterol absorption in

means difficult to establish. In rats with thoracic duct fistulae, absorption continues for approximately 24 hours following a single cholesterol meal (10–20). It is reasonable to assume that absorption continues for as long or longer in man, and that the rapid rise in serum cholesterol specific activity during the first 24 hours is predominantly a function of

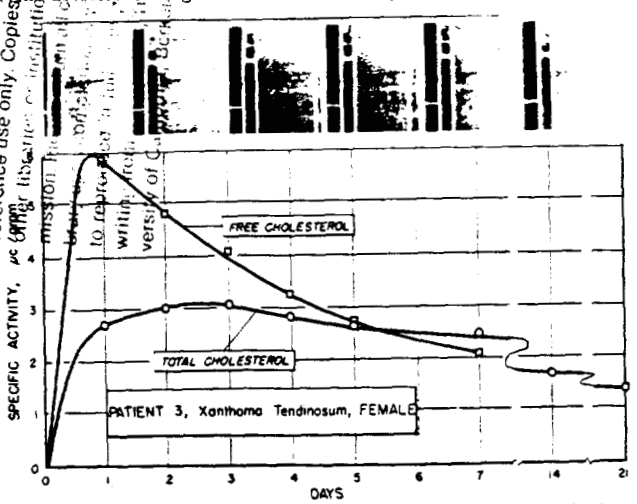

FIG. 3. The graph shows the changes in serum cholesterol specific activities as a function of time following a single feeding of 0.60 gm of cholesterol-H^3 (sp. act. = 1.07 μc mg) in a fatty meal. The lipoprotein spectrum shows: S$_f$0-12 = 974 mg%, S$_f$12-400 = 193 mg%. Serum cholesterol values: free = 143 mg%, total = 519 mg%. (From Biggs, M. W., and Colman, D., *Circulation* **7**, 393, 1953.)

absorption rate. Integrated into the rate of cholesterol absorption to determine the final shape of the specific activity curves is the rate of hepatic removal of chyle cholesterol and the rate of its subsequent return to the systemic circulation. This metabolic pathway will be discussed in more detail in a later section of this paper.

The existing evidence would indicate that appreciable amounts of dietary cholesterol are excreted in the feces without absorption. Perhaps the strongest supporting evidence that the absorption of dietary cholesterol is not efficient arises from animal studies with thoracic duct fistulae. Appreciably less cholesterol is recovered in the collected lymph than is administered orally. In man large amounts of tritium label fed as cholesterol-H^3 appear in the feces over a period of 3 to 4 days following

a single feeding. There seems no doubt that some of the excreted tritium label represents cholesterol which has been absorbed and returned to the intestine as bile acid or as cholesterol: however, the bulk of the tritium label in the stools following a cholesterol-H^3 meal must represent unabsorbed cholesterol. After the fourth day the content of label excreted in the stools falls to very small amounts although the serum cholesterol specific activity remains high. This speaks against an efficient absorption of dietary cholesterol with resecretion of a large portion back into the

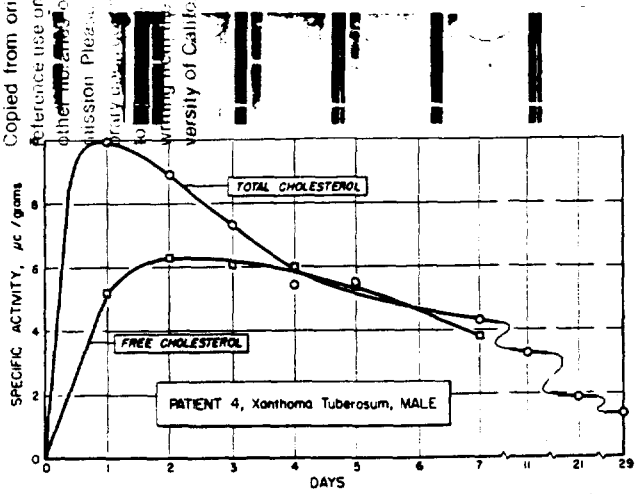

FIG. 4. The graph shows the changes in serum cholesterol specific activities as a function of time following a single feeding of 0.72 gm of cholesterol-H^3 (sp. act. = 1.07 μc mg) in a fatty meal. The lipoprotein spectrum shows: S_f0-12 = 124 mg%, S_f12-400 = 537 mg%. Serum cholesterol values: free = 73 mg%, total = 259 mg%. (From Biggs, M. W., and Colman, D., *Circulation* **7**, 393, 1953.)

gut. It would appear that on the average something around 50% of ingested dietary cholesterol is absorbed. In two patients on whom stool examinations were done in our laboratory, 72% and 52% of the ingested label fed as cholesterol-H^3 appeared in the feces in 4 days (5). These patients received 0.86 and 0.69 gm of cholesterol respectively. Three patients reported by Hellman and co-workers (28) excreted 11, 14, and 27% of a small 10 mg dose of labeled cholesterol in 4 days; while a 4th patient excreted 48% of the cholesterol dose following a feeding of 1 gm.

The relationships between the free and total cholesterol specific activities in the serum during the first few days following a cholesterol-H^3 meal are of particular interest. In the normal patient the free cholesterol specific activity is higher than the esterified cholesterol specific activity for a period of approximately 3 days (Fig. 1). In the patient with xanthoma tendinosum these relationships are retained but exaggerated. The free cholesterol specific activity here rises to inordinately high levels and may continue above the esterified cholesterol level for 4 or 5 days (Fig. 3). In a patient with xanthoma tuberosum the reverse situation exists. Here the esterified cholesterol has the higher specific activity for 3 or 4 days. (Fig. 4). Patients with intermediate specific activity curves between the extremes are easily found (Fig. 2). Figure 2 illustrates the findings in a patient in whom the free and esterified cholesterol specific activities are the same within experimental error.

It can be demonstrated that the type of low density lipoprotein spectrum as determined in the ultracentrifuge accurately predicts the free and esterified cholesterol specific activity relationships to be expected 24 hours following a cholesterol-H^3 feeding. When the spectrum shows predominantly S_f0-12 lipoproteins in the serum, the free cholesterol specific activity is higher; when the S_f12-400 lipoprotein molecules predominate the esterified cholesterol specific activity is higher. This interrelationship is illustrated in Table I. Twelve patients are listed together with the ratios of free cholesterol specific activity to total cholesterol specific activity at the end of day one and the ratio of the concentration of S_f0-12 molecules to S_f12-400 molecules in the lipoprotein spectrum. Calculation of Pearson's product-moment coefficient of correlation between these two parameters gives a value of 0.92. The serum cholesterol values are included but these data do not give a significant positive correlation between the type of specific activity curve seen and the serum cholesterol.

Extensive correlation studies have been done to establish the relationship between the serum lipoprotein spectrum and the development of atherosclerosis. The "Cooperative Study of Lipoproteins and Atherosclerosis" demonstrated an elevation of both the serum S_f20-100 lipoproteins and of the serum cholesterol as an antecedent finding in atherosclerosis as manifest by definite evidence of coronary artery disease (13a). Gofman and co-workers reported in this same study their use of the "atherogenic index" calculated from the standard serum lipoprotein concentrations and discussed its predictive capacity for myocardial infarctions* (13a, 24, 38). This is the best predictive index of atherosclerosis

* This index, α is calculated as follows:

$$\alpha = 0.1\ (S_f0\text{-}12) + 0.16\ (S_f12\text{-}400)$$

TABLE I

*Correlation Study of Cholesterol Specific Activity Absorption Curves, Lipoprotein Molecular Spectra, and the Total Serum Cholesterol**

Diagnosis	S_f0-12 (mg %)	S_f12-100 (mg %)	Free Chol. S.A. (μc/mg)	Total Chol. S.A. (μc/mg)	Free chol. / Total chol. S.A.	S_f0-12 / S_f12-100	Total serum chol. (mg %)
1. Xanthoma tendinosum	974	175	5.90	2.70	2.19	5.57	515
2. Healthy male	604	294	2.25	1.52	1.48	[illegible]	[illegible]
3. Xanthoma tendinosum	966	146	7.83	5.83	1.31	2.17	565
4. Healthy male	274	140	8.16	6.20	1.32	1.97	226
5. Healthy male	446	237	9.26	7.11	1.30	1.89	352
6. Healthy female	259	74	4.90	4.11	1.20	3.50	193
7. Nephritis	441	365	6.95	6.94	1.00	1.21	266
8. Healed myocardial infarction	432	429	7.03	7.16	1.00	1.01	266
9. Xanthoma tuberosum	211	827	3.70	4.84	0.77	0.26	529
10. Healthy male	178	455	4.51	8.00	0.56	0.39	290
11. Xanthoma tuberosum	124	537	5.15	9.95	0.52	0.23	259
12. Xanthoma tuberosum	115	1164	0.61	1.36	0.48	0.14	666

* From Biggs, M. W., and Colman, D., *Circulation* **7**, 398 (1953).

development yet devised for man. The reader is referred to the papers of these authors for details of their correlation studies.

A normal low density lipoprotein spectrum as exemplified by a healthy 26-year-old female is illustrated in Fig. 1. The predominant lipoprotein species is the $S_f{}^0$0-12 class of molecules. Patients with xanthoma tuberosum do not show this normal pattern but rather have high concentrations of $S_f{}^0$12-400 lipoprotein molecules (Fig. 4). Many patients can be readily found who have lipoprotein spectra which lie intermediate between the normal and xanthoma tuberosum patterns (Fig. 2). From clinical observations of patients with xanthoma tuberosum it is a known fact that they show an incidence of atherosclerosis higher than that of the normal population. It should be noted, too, that all types of experimental atherosclerosis show elevated serum values for $S_f{}^0$12-400 molecules. It is of particular interest and not surprising then that Gofman and co-workers found the serum $S_f{}^0$12-400 lipoprotein concentration should be weighted in their atherogenic index calculations: that the concentration of these molecules proves of particular value in predicting atherogenesis. It is of course true that patients with xanthoma tendinosum who do not have increased $S_f{}^0$12-400 lipoprotein concentrations also have an increased incidence of atherosclerosis. They show increased concentrations of $S_f{}^0$0-12 molecules; thus $S_f{}^0$0-12 molecules, too, appear to be associated with developing atherosclerosis if present in the serum in sufficient concentrations. It remains to be seen whether the serum $S_f{}^0$0-12 molecules in xanthoma tendinosum are chemically identical with those in the normal patient.

IV. Animal Studies—the Hepatic Uptake of Chyle Cholesterol

A series of animal experiments have been done with the prime objective of clarifying the dynamics of exogenous cholesterol metabolism in man.

It has been firmly established that dietary cholesterol is absorbed in the chyle and enters the systemic circulation via the thoracic duct lymph (3, 12). There is no evidence that any exogenous cholesterol enters directly into the portal venous system. Further it is known that the newly absorbed dietary cholesterol in chyle is contained in a lipoprotein molecule floating with an $S_f{}^0$ rate greater than 400 $S_f{}^0$ units (7, 11). All the evidence would indicate that the newly absorbed cholesterol is contained within the so called chylomicrons. Tracer cholesterol studies show that newly absorbed cholesterol exists in both the free and esterified forms in chyle, the larger amount being in the esterified fraction (7, 10, 12).

1. Experimental

Chyle containing cholesterol-H^3 was collected as described under methods. The donor animal was given 30 mg of cholesterol-H^3 (sp. act. = 1.81 μc/mg) dissolved in 1 ml of cottonseed oil plus 5 ml of whole milk by stomach tube. An experimental group of fifteen rats, Wistar-Purdue strain, female, weighing 200–225 gm, each received 0.5 ml of the chyle containing cholesterol-H^3 intravenously by tail vein. The 0.5 ml of chyle injected contained 28 mg % free cholesterol (sp. act. = 0.43 μc/mg) and 88 mg % total cholesterol (sp. act. = 0.54 μc/mg). These animals had been allowed Purina Rat Chow *ad lib* up to the experimental period. The animals were killed by exsanguination under ether anesthesia in groups of three at 30 min, 45 min, 1 hr, 2 hr, and 4 hr after the chyle injection. The blood was collected in a syringe containing a drop of heparin solution, and the plasma was separated from the red blood cells immediately by centrifugation. Immediately following exsanguination the liver was excised and lyophilized. The free and total cholesterol content and specific activities were determined for the plasma and liver cholesterol.

Chyle containing stearic-H^3-acid was collected from a donor animal given 2.0 mg of stearic-H^3-acid (sp. act. = 83.3 μc/mg) and 30 mg of unlabeled cholesterol dissolved in 1 ml of cottonseed oil together with 5 ml of whole milk by stomach tube. Twelve animals similar to those used in the cholesterol-H^3 experiment above were each given 0.5 ml of chyle containing stearic-H^3-acid intravenously (sp. act. = 3.56 μc/ml of chyle). These animals were also killed in groups of 3 at 15 min, 30 min, 45 min, and 1 hr. The blood was collected in a heparinized tube to prevent clotting. The livers were removed immediately and frozen. Aliquots of whole blood and of wet liver were combusted directly in a combustion tube and the water obtained used for tritium assay. Thus the total tritium content of a known aliquot of blood or liver was obtained.

2. Results

At 30 minutes following the cholesterol-H^3 chyle injection, over 90% of the chyle cholesterol has been removed from the circulating plasma. At this time over 80% of the injected chyle cholesterol is to be found in the liver. The percentages of injected chyle cholesterol found in the plasma and liver at each time interval are recorded in Table II. For these calculations the plasma volume for each rat has been estimated at 10 ml.

During the period between 30 minutes and 1 hour following the chyle injection, the specific activities of the liver cholesterol rose to levels greater than 5 times that of the corresponding plasma cholesterol specific activities. The plasma total cholesterol specific activities and the liver

total cholesterol specific activities are recorded in Table III. This table also gives the free, total, and esterified cholesterol concentrations for the whole liver as well as the specific activities for these metabolic pools. The average values for free, total, and esterified cholesterol specific activities in the liver have been plotted in Fig. 5, and this gives a qualitative picture of the changes in free and esterified cholesterol specific activities as a function of time.

TABLE II

*Percentage of Chyle-Cholesterol-H³ and Chyle-Stearic-H³ Acid in the Liver and Blood Following Intravenous Injection**

Animal number	Time after chyle injection	Per cent of injected chol.-H³ in plasma	Per cent of injected chol.-H³ in liver	Animal number	Per cent of injected stearic-H³-acid in whole blood	Per cent of injected stearic-H³-acid in liver
	15 min	—	—	16†	8	30
	15 min	—	—	17	7	18
	15 min	—	—	18	—	28
1	30 min	4	89	19	9	24
2	30 min	9	81	20	10	21
3	30 min	7	85	21	7	19
4	45 min	8	81	22	8	15
5	45 min	7	76	23	7	24
6	45 min	4	81	24	7	18
7	1 hour	7	77	25	6	21
8	1 hour	7	78	26	7	22
9	1 hour	6	76	27	7	16
10	2 hours	7	37			
11	2 hours	—	—			
12	2 hours	8	46			
13	4 hours	6	34			
14	4 hours	8	36			
15	4 hours	8	33			

* In part from Biggs, M. W., *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva*, 1955, p. 529.

† Time intervals after chyle injection given in column 2 apply to animals 16 to 27.

The results obtained in the stearic-H³-acid injected animals are recorded in Table II as the percentage of the total tritium injected in the chyle found in the liver and whole blood.

3. Discussion

During the period 30 minutes to 1 hour following the chyle-cholesterol-H³ injection the liver total cholesterol specific activity has risen to

some 6 times the specific activity of the plasma total cholesterol. Such a finding shows unequivocally that dietary cholesterol following its entrance into the systemic circulation is in a separate metabolic pool; that it does not normally mix in a metabolic sense with the rest of the serum cholesterol but is specifically and rapidly removed from the circulating blood into the liver. The literature of the life sciences is full

TABLE III

*Plasma and Liver Cholesterol Specific Activities Following the Intravenous Injection of Chyle Containing Cholesterol-H³**

Rat number	Time after chyle injection	Plasma total cholesterol specific activity (×10⁻³ μc/mg)	Liver cholesterol content			Liver cholesterol specific activity (×10⁻³ μc/mg)		
			Free (mg)	Total (mg)	Ester (mg)	Free	Total	Ester
[illegible]	30 min	1.4	12.3	15.6	3.3	6.9	12.9	35
2	30 min	2.6	12.2	14.3	2.1	6.6	12.8	49
3	30 min	1.9	18.4	21.1	2.7	4.6	9.1	40
4	45 min	2.2	12.1	15.1	3.0	8.5	12.1	27
5	45 min	2.3	13.3	15.6	2.3	7.2	10.9	32
6	45 min	1.5	13.4	16.5	3.1	8.4	11.0	22
7	1 hour	1.9	12.1	16.6	4.5	7.9	10.5	17
8	1 hour	1.9	14.4	16.8	2.4	8.2	10.4	24
9	1 hour	2.4	13.7	16.0	2.3	9.9	10.7	15
10	2 hours	1.8	13.6	16.8	3.2	3.7	4.9	10
11	2 hours	—	—	—	—	—	—	—
12	2 hours	3.5	12.3	15.1	2.8	7.7	6.9	3
13	4 hours	2.5	16.1	19.4	3.3	3.8	4.0	—
14	4 hours	2.9	17.0	20.0	3.0	4.1	4.1	—
15	4 hours	3.1	13.8	15.0	1.2	4.5	4.9	—

* From Biggs, M. W., *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva,* 1955, p. 529.

of papers indicating that the liver is of prime importance in the metabolism of both exogenous and endogenous cholesterol. It is not surprising then to find the liver actively participating in chyle cholesterol metabolism. The feature of particular interest when viewed in light of the specific activity absorption curves seen in man and in light of current ideas of the serum lipid transport system is the fact that exogenous cholesterol follows this specific pathway to the liver and does not initially mix with the other serum lipids. The implications of this observation will be discussed further in Section V.

Following the chyle cholesterol-H³ injection the specific activity of the liver esterified cholesterol pool rises quickly and falls precipitously

during the 30 minutes to 1 hour period (Fig. 5). The relative height of the specific activity observed in esterified cholesterol is resultant from the relatively small size of the esterified cholesterol pool in the liver; however, a simple calculation shows that the esterified cholesterol pool in the liver at 30 minutes contains approximately 0.111 μc of tritium. The tritium content in the 0.5 ml of chyle injected existent in the esterified

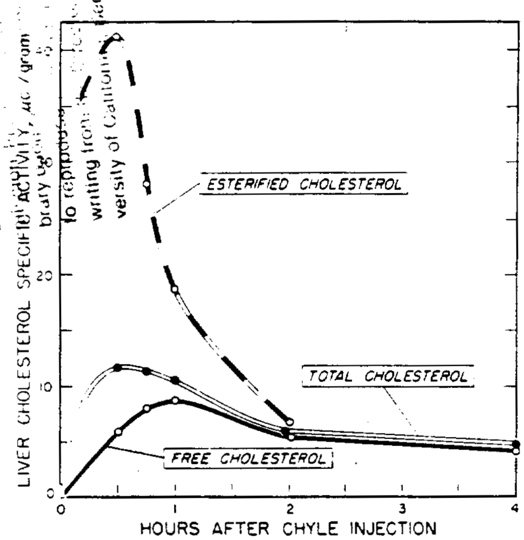

FIG. 5. Free and esterified liver cholesterol specific activities following the intravenous injection of chyle-cholesterol-H³.

cholesterol fraction was 0.178 μc. Thus about 60% of the injected esterified cholesterol is present in the liver at 30 minutes. Since we are observing a dynamic state and the esterified cholesterol specific activity as seen in Fig. 5 is falling rapidly, appreciably more than 60% of the esterified cholesterol injected probably entered the liver still esterified.

The evidence is good that the fall of esterified cholesterol specific activity in the liver during the period of 30 minutes to 1 hour is due to hydrolysis of cholesterol esters and not due to return of these esters intact to the blood. In the original chyle injected there was 0.060 μc of tritium in free cholesterol. At 30 minutes following the chyle cholesterol injection there was on the average 0.083 μc of tritium in free cholesterol in the liver; at 45 minutes this had increased to 0.104 μc in free cholesterol,

and at 1 hour there was 0.116 μc in free cholesterol in the liver. Such evidence speaks strongly in support of a "clearing" mechanism for the removal of dietary cholesterol into the liver without appreciable cholesterase activity as a serum event prior to hepatic removal.

The details of the hepatic clearing of chyle cholesterol are poorly understood. The presence of lipid material in the reticulo-endothelial system of the liver following cholesterol-fat feedings has been reinvestigated and extended by Friedman and co-workers (18, 19). These authors point to the reticulo-endothelial system as performing the initial important step of removing the exogenous cholesterol from the serum into the liver. The specificity of the hepatic uptake of chyle must certainly be a function of the morphology of the lipoproteins in which the exogenous cholesterol exists in the serum.

The data presented in Table II following the injection of chyle containing stearic-H^3-acid is of interest when viewed against a simple concept of "phagocytosis" of chylomicrons by the hepatic reticulo-endothelial system. The data show an effective divergence of the metabolic fate for neutral fat and cholesterol early in their metabolism following absorption. If the chylomicrons are removed *in toto* into the hepatic reticulo-endothelial system the findings would seem to require a rapid resecretion of fat back into the circulation in some form for distribution elsewhere. An alternative explanation of the data is that the neutral fat content of chylomicrons can be reduced at various tissue sites other than the liver prior to hepatic uptake of chyle cholesterol.

V. Integration of Human and Animal Studies

In the human studies it was observed that the forms of the cholesterol specific activity absorption curves are a function of the $S_f^0$12-400 lipoprotein molecules in the serum. When the $S_f^0$12-400 molecules predominate the esterified cholesterol of the serum has the higher specific activity at 24 hours following a single cholesterol-H^3 meal. When the $S_f^0$0-12 molecules predominate the free cholesterol has the higher specific activity.

The animal studies reported here and in the literature afford a number of observations on fundamental exogenous cholesterol metabolism which appear pertinent to the interpretation of the human data. In summary these observations are:

1. Dietary cholesterol is absorbed in the chyle of the thoracic duct (3, 12) in lipoprotein molecules floating in the ultracentrifuge with a flotation rate of greater than 400 S_f^0 units (7). Presumably the exogenous cholesterol is in the chylomicrons (11) which contain over 90% neutral fat.

2. Exogenous cholesterol in the chyle is in both free and esterified forms, and in animals the greater amount is esterified (10).

5. In the rat, exogenous cholesterol upon entering the systemic circulation in chyle is rapidly removed into the liver without mixing metabolically with the other cholesterol pools of the plasma.

6. The clearing of chyle cholesterol into the liver does not involve appreciable hydrolysis of exogenous cholesterol esters as a plasma event; however, once within the liver the exogenous cholesterol esters are hydrolyzed rapidly. Once hydrolyzed, exogenous cholesterol undoubtedly loses its identity and mixes with the hepatic cholesterol of endogenous origin (28).

7. Studies of the binding forces within the lipoprotein molecules of the serum show that free cholesterol exchanges freely between the various lipoprotein molecules and red blood cell cholesterol (25). Esterified cholesterol exchange occurs but at a much less rapid rate (2).

On examination of the different human specific activity absorption curves, two possible explanations suggest themselves. First, the composition of chyle might differ in various subjects; or, secondly, if the chyle cholesterol composition is essentially the same in all, the metabolic fate of chyle cholesterol upon entrance into the systemic circulation must differ from individual to individual. These possibilities are considered below and it appears most likely that the rate of hepatic chyle cholesterol uptake and the rate of cholesterol ester hydrolysis determines the shape of the specific activity curves observed.

If exogenous cholesterol entered in the chyle of the normal patient predominantly free, while entering in the chyle of the xanthoma-tuberosum-like patient predominantly esterified, the observed specific activity absorption curves would be at least partially explained. The composition of human chyle with respect to cholesterol is not accurately known. Lindgren (36) found the chylomicron fraction separated centrifugally from the serum of two normal patients to contain 3 and 6% cholesterol respectively. The ratio of ester to free cholesterol found was approximately 2 in both cases. Peters and Man (39) examined patients with chyluria and chylothorax and found ester to free cholesterol ratios in the fluid so obtained "of the same order of magnitude as those in the sera." Rat chyle during cholesterol absorption shows an ester to free cholesterol ratio between 1 and 4. The evidence would suggest that more exogenous cholesterol enters normally in the esterified form than free. If one estimates the serum cholesterol specific activities to be expected in the normal patient, conservatively estimating that equal amounts of free and esterified exogenous cholesterol-H^3 enter the systemic circulation in chyle, the esterified serum cholesterol should have the higher specific activity during the period 24 to 48 hours following a cholesterol-H^3 meal if simple dilution into the free and ester cholesterol pools occurs. There is little doubt that the free and ester cholesterol pools of both

the blood and liver would be involved (27, 28). An estimation of the size of the blood cholesterol pools in patient number 1 (Fig. 1) gives a free cholesterol pool of approximately 4.2 gm and an esterified cholesterol pool of 3.4 gm.* The cholesterol pool in the liver is less well known; however it is entirely reasonable to assume that the free hepatic cholesterol pool is appreciably larger than the esterified hepatic cholesterol pool in this healthy patient. Thus the specific activity curves seen in the normal patient appear to require a metabolic hydrolysis of exogenous cholesterol esters early in dietary cholesterol metabolism. Without such hydrolysis the specific activity curves seen in xanthoma tuberosum would be expected. Such reasoning does not eliminate differences in the composition of chyle as a possible factor; however, in the absence of any positive evidence to support such an hypothesis I believe that the rate of *in vivo* hydrolysis of newly absorbed cholesterol esters determines the shape of the specific activity curves observed.

The possibility that *in vivo* hydrolysis of exogenous cholesterol esters might occur in the circulating plasma has received attention in our laboratory. That cholesterase exists in the plasma has been unequivocally shown by Sperry and Stoyanoff (50, 51); however, these workers observed an esterification of free cholesterol rather than a hydrolysis of esters on incubating serum for prolonged periods of time. Studying the *in vivo* clearing effect of serum obtained following intravenous heparin injections, we have been unable to demonstrate that cholesterol esters serve as a substrate for the enzymes involves (7). Although cholesterase activity is present in the serum, the animal studies would indicate that the site of exogenous cholesterol ester hydrolysis is within the liver.

In light of our present knowledge of exogenous cholesterol metabolism from the animal studies and human experiments the best hypothesis regarding the normal metabolic fate of chyle cholesterol in man can be summarized as follows: When the S_f12-400 lipoprotein concentration of the plasma is low, chyle cholesterol enters a small metabolic pool, and removal into the liver is relatively rapid and efficient. Within the liver the chyle cholesterol esters are hydrolyzed, and the resultant free cholesterol becomes indistinguishable from endogenous hepatic cholesterol. The hepatic free cholesterol and serum free cholesterol equilibrate rapidly. The S_f0-12 lipoprotein molecules, since they are the major cholesterol carriers of the serum in normal patients, participate actively in this equilibrium. The net result is a shift of exogenous cholesterol-H^3 from the esterified pool in the serum into the serum free cholesterol pool via the liver. The introduction of cholesterol esters of hepatic synthesis

* Estimated using a body wt = 60 kg; red cell volume = 27 cc/kg; red cell free cholesterol concentration = 200 mg %; plasma volume = 37 cc/kg.

into the serum is known to be a much slower process than the equilibration of free cholesterol between the liver and plasma lipoproteins (29).

Figure 6 is a schematic representation of the normal fate of exogenous cholesterol-H^3 as interpreted from the present data.

If the concentration of $S_f{}^0$12-400 molecules is large, chyle cholesterol enters a relatively large metabolic pool and the effective rate of hepatic uptake of chyle cholesterol is slow. Exchange of cholesterol-H^3 esters between all of the lipoprotein species in the plasma will occur, and the specific activity absorption curves seen in xanthoma tuberosum will be the result.

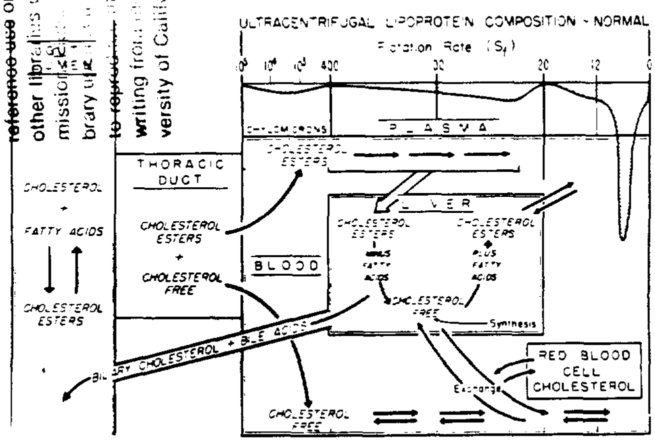

FIG. 6. Schematic representation of exogenous cholesterol metabolism in the normal patient. The $S_f{}^0$20-400 lipoprotein pool is small and hepatic uptake is rapid.

The esterified cholesterol specific activity will be higher than the free cholesterol specific activity for the effective shift of cholesterol-H^3 esters of serum to free cholesterol-H^3 does not occur as efficiently as in the normal patient. A schematic representation of the fate of exogenous cholesterol esters of chyle in xanthoma tuberosum is presented in Fig. 7.

Thus the human cholesterol specific activity curves appear to be the net result of two dynamic metabolic processes: the rate of hepatic uptake of chyle cholesterol esters and the relative rates of return of free and esterified cholesterol of hepatic origin to the plasma.

In the course of the last several years a theory of how the lipid transport system of the serum functions has been developed by Gofman, Lindgren, Pierce, Nichols, and Freeman (36, 37, 43). These authors have

been able to demonstrate in various ways a steplike conversion of one series of lipoproteins into another of higher density, i.e., $S_f^0400 \rightarrow S_f^0100$. Chemical tests on the various step-wise conversions show a gradual reduction in glyceride content. The observations with stearic-H^3-acid and cholesterol-H^3 in Section IV of this paper showing an effective

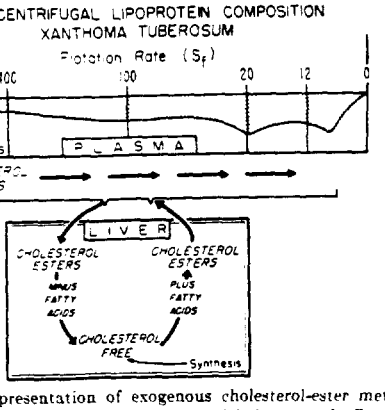

FIG. 7. Schematic representation of exogenous cholesterol-ester metabolism in xanthoma tuberosum. The $S_f^0$20–400 lipoprotein pool is large, and effective hepatic uptake is slow.

divergence of metabolic pathways early in chyle metabolism are consistent with their ideas.

VI. Inhibition of Dietary Cholesterol Absorption with Sitosterol

Regulation of the serum cholesterol levels and the lipoprotein distribution within the various lipoprotein species of the serum can be effected to a degree by alterations in the fat and carbohydrate content of the diet without caloric restrictions. The usual patient shows changes toward an improved serum lipoprotein picture on a diet wherein the animal fats have been replaced by vegetable fats. This improvement, however, usually ceases short of the desired clinical effect. Whether or not the cholesterol content of the diet is of any consequence in determining the serum cholesterol level continues to be debated. The debate is complicated by the obvious fact that although the cholesterol content of the diet be kept low cholesterol continues to be secreted into the intestinal lumen in bile in amounts variously estimated at around 1.25 gm per day, and there is some evidence for cholesterol secretion into the gut lumen by

the intestinal mucosa. Too, there is some evidence in both animals and man to suggest that the efficiency of dietary cholesterol absorption decreases as the ingested dose increases.

In order to determine what portion of the serum cholesterol concentration is effectively a function of the cholesterol entering from the intestine in chyle it is necessary to employ a measure which inhibits cholesterol absorption but permits the absorption of other lipids. Sitosterol would appear to provide such a research tool. The following experiment was performed to see how efficiently sitosterol inhibits the absorption of dietary cholesterol in man and to attempt an evaluation of what fraction of the serum cholesterol concentration is a function of the quantity of cholesterol entering the circulation in thoracic duct chyle.

Peterson in 1951 published his observations that a soybean-sterol mixture included in the diet of cholesterol fed chicks prevented the serum hypercholesterolemia and the cholesterol deposits in the aorta and liver which would normally occur when cholesterol was fed alone (40, 42). Pollak reported similar results in rabbits and observed a reduction in serum cholesterol in man (44, 45). Since these early observations there have been numerous observations in man and in the main some lowering of serum cholesterol values have been observed following the addition of sitosterols to the diet (1, 33, 41, 48). Nearly all reports indicate that the best results are obtained in patients with a hypercholesterolemia; less significant changes occurring in normocholesterolemic individuals. Some reports have failed to observe a definite lowering of serum cholesterol values (17, 53). Peterson in his original reports believed the effects observed were due to a reduction in exogenous cholesterol absorption. This has been shown to be true in rats using tracer cholesterol (30). The mechanism of this reduction in cholesterol absorption is not known, but a mixed crystal complex of cholesterol and sitosterol interfering with the colloidal dispersion of cholesterol in the absorptive processes has been proposed (15). Since sitosterols undergo esterification in the intestinal lumen a competitive inhibition of the esterification of cholesterol has been postulated. It was originally believed that the sitosterols were not absorbed (46, 52), however, it has now been conclusively demonstrated that a small percentage, perhaps 10%, is absorbed (26, 31). Indeed the soy sterols have been shown to be atherogenic in rabbits (14).

As indicated above the results in man utilizing soy sterols have been variable. It seems unlikely that the soy sterol preparation or experimental design explains all of the divergent results. Rather these results seem to point up the fact that the serum cholesterol value in man is the integrated sum of several metabolic variables involving both exogenous

and endogenous cholesterol, and the fraction of the serum cholesterol level which is effectively a function of exogenous cholesterol absorption may vary from individual to individual. It has been shown in the initial sections of this paper how differently exogenous cholesterol is processed in various individuals.

Cholesterol-H^3 was fed to two patients with divergent lipoprotein spectra in an attempt to discover how efficient the soy sterols are in inhibiting cholesterol absorption and to get some information about the size of the effective fraction of the serum cholesterol concentration arising from exogenous cholesterol sources.

1. Experimental

The two patients were given an oral feeding of cholesterol-H^3 in a fatty meal, and the dynamics of cholesterol absorption were then observed for a period of 10 days. The rise and fall of free and esterified cholesterol specific activities in the serum were measured. At the completion of this 10-day control period both subjects were placed on sitosterol,* 18 gm per day in 3 divided doses before meals. After a suitable period the cholesterol-H^3 meal was repeated, and the dynamics of cholesterol metabolism again measured and compared with the control results.

Patient number one was a 38-year-old male, weight 214 lb, who was clinically well with a negative past history. He was part of a larger study being conducted to evaluate the effects of soy sterols on the serum cholesterol and was just completing a period on placebo medication when the control study was done. His diet and weight remained unchanged during the period of study. This patient initially ingested 0.115 gm of cholesterol-H^3 with a specific activity of 1.81 μc/mg. This cholesterol was dissolved in 5.0 ml of Wesson oil and emulsified in 45 gm of ice cream and 250 ml of whole milk. The free and total serum cholesterol specific activities were determined for 10 days. At the end of the 10-day period the patient was shifted from the placebo to 18 gm of sitosterol per day. After the medication had been taken for 32 days the study with labeled cholesterol was repeated. The subject received 0.132 gm of the cholesterol-H^3, specific activity equal to 1.81 μc/mg as before but with the addition of 6 gm of sitosterol. The specific activity measurements were repeated. A blood sample was taken at the time of the second cholesterol-H^3 dose to determine how much cholesterol-H^3 remained in the serum from the first dose.

Patient number two was a 41-year-old male, weight 200 lb, who had a clinical diagnosis of xanthoma tuberosum. He had not received prior

* The sitosterol preparation used in this study was "Cytellin" kindly provided by Dr. Robert Shipley of the Eli Lilly Company.

treatment of any sort in spite of a past history of a bout of chest pain suspected but not proven to be of myocardial origin. He sought care primarily because of the xanthomatous growths on his skin and was cared for by a dermatologist. The initial cholesterol-H^3 feeding contained 0.210 gm of cholesterol with a specific activity of 0.871 μc/mg. At the end of 10 days he was started on sitosterol, 18 gm per day. After a period of 32 days on the medication, the study with cholesterol-H^3 was repeated. It is to be noted that no diet instructions were given; however, the patient lost 12 lb in body weight during the 52 days of sitosterol medication. The second dose of cholesterol also contained 0.210 gm of cholesterol with a higher specific activity of 1.81 μc/mg; 6 gm of the sitosterol medication was included in the ingested fatty meal.

2. Results

In patient number one the average of 7 separate total serum cholesterol determinations, each done in duplicate, during the 10-day control period was 291 ± 5 mg%. After 32 days on 18 gm of sitosterol per day the total serum cholesterol as measured in 7 separate serum samples during the second cholesterol-H^3 study was 273 ± 3 mg%. The serum lipoprotein spectrum at the beginning of the experiment showed: S_f0-12 = 604 mg%; S_f12-20 = 117 mg%; S_f20-100 = 125 mg%; and S_f100-400 = 52 mg%. The results of the specific activity determinations are given in Fig. 8. The results of the control period are as expected. The free cholesterol specific activity was higher than the total cholesterol specific activity for a period of 2 days. The peak total cholesterol specific activity was reached between 1 and 2 days. The total cholesterol specific activity at the time the free and total cholesterol specific activities are identical represents approximately 7.5% of the ingested cholesterol in the serum at this time. The results following the ingestion of sitosterol are also included in Fig. 8. Here there is a general flattening of the curve and the free and total cholesterol specific activity values are the same within experimental error. At the maximum total cholesterol specific activity obtained on day 3 approximately 3.3% of the ingested cholesterol-H^3 was in the serum. The serum total cholesterol specific activity at the time of the second cholesterol-H^3 feeding was 0.16×10^{-3} μc/mg.

In patient number two the average of 7 serum cholesterol determinations in the control period was 566 ± 40 mg%. After 52 days on 18 gm of sitosterol per day the average serum cholesterol was 276 ± 13 mg%. Initially the serum low density lipoprotein spectrum showed S_f0-12 = 230 mg%; S_f12-20 = 131 mg%; S_f20-100 = 726 mg%; and S_f100-400 = 785 mg%. After treatment the values were S_f0-12 = 115 mg%; S_f12-20 = 89 mg%; S_f20-100 = 553 mg%; S_f100-400 = 522 mg%. The

results are recorded in Fig. 9. In the control and sitosterol experimental periods an equivalent amount of cholesterol was fed; however, the specific activity of the initial feeding was lower than in the experimental feeding. In the graph the curves for the control period have been corrected to the same specific activity dosage scale used in the experimental period. Again the results of the control period are as expected. The total cholesterol

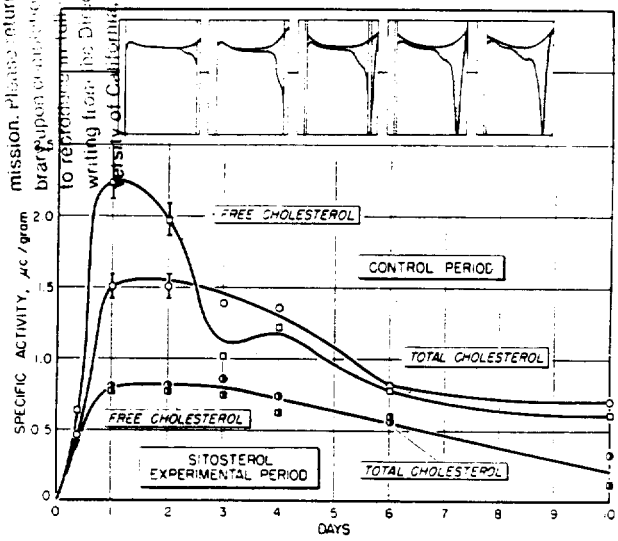

FIG. 8. The inhibition of exogenous cholesterol-H^3 absorption in patient 1. At the top of the figure is a line tracing of the serum lipoprotein spectrum as determined in the ultracentrifuge.

specific activity continued higher than the free cholesterol specific activity for a period of 3–4 days. The cholesterol-H^3 in the total serum cholesterol at the end of day 4, when the free and total cholesterol values are approximately the same, represents approximately 8.1% of the ingested dose. The experimental period is also graphically demonstrated in Fig. 9. The rise in serum cholesterol specific activity values was not nearly as high as in the control period. The maximum specific activity at day one represents only approximately 1.4% of the ingested dose. The serum total cholesterol specific activity at the time of the second cholesterol-H^3 feeding was 0.12×10^{-3} μc/mg.

3. Discussion

Sitosterol in the dosage schedule used appears to be quite effective in reducing cholesterol absorption in man. Absorption was not completely prevented but was reduced in the experimental period to approximately 44% of the control absorption in patient one and to approximately 17% of the control absorption in patient number two. These changes in cholesterol absorption efficiency have been estimated from the amount of cholesterol-H^3 in the serum at the point of equilibrium of free and

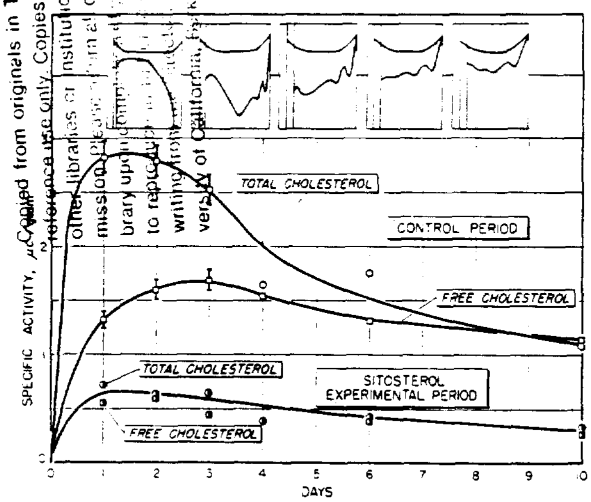

FIG. 9. The inhibition of exogenous cholesterol-H^3 absorption in patient 2. At the top of the figure is a line tracing of the serum lipoprotein spectrum as determined in the ultracentrifuge.

total cholesterol specific activities. There is no evidence to indicate that these differences so calculated in amounts of tracer cholesterol appearing in the serum in the experimental versus the control period are not primarily determined by quantitative differences in the amount of exogenous cholesterol absorbed. The total exogenous cholesterol absorbed is of course greater than the amount appearing in the serum for the liver cholesterol pool, and possibly other tissue cholesterol pools are also involved.

In patient one, whose lipoprotein spectrum shows predominantly lipoprotein molecules in the S_f 0-12 class, it is of particular interest that

a reduction of over 50% in the amount of chyle cholesterol entering the circulating blood over a period of 32 days produced only a small reduction, about 6%, in the serum cholesterol values. Thus in this patient the "effective" fraction of the serum cholesterol value which can be attributed to the amount of chyle cholesterol entering the circulation is quite small.

The results in patient two show a dramatic reduction in both the serum cholesterol values and in the amount of cholesterol-H^3 appearing in the serum. Interpretation of the data is seriously complicated by the fact that the patient lost 12 pounds of body weight during the experimental period. A reduction of total calories in the diet of such a patient would be expected to produce a dramatic fall in serum cholesterol without the addition of any other form of medication. There seems little question however that the absorption of dietary cholesterol was strikingly reduced in this patient. Further studies with better dietary control will be necessary to evaluate what effective fraction of the serum cholesterol value arises directly from exogenous sources in such patients.

The dynamics of exogenous cholesterol metabolism as viewed in the serum cholesterol pools are altered under the influence of sitosterol medication. The free and total cholesterol specific activities are essentially the same through the experimental period of observation. The tentative explanation for these findings is that they result solely from a general slowing and depression of cholesterol absorption from the gut.

The data do not provide an accurate measure of the turnover rate of serum cholesterol under sitosterol therapy; however, the descending values of the total cholesterol specific activities in the control and experimental periods do not indicate any gross alteration in serum cholesterol turnover under sitosterol medication. In patient one, in whom the serum cholesterol values varied only slightly during the period of sitosterol administration, the serum cholesterol specific activity on day 10 of the control period was 0.75×10^{-3} μc/mg. On this day he was started on 18 gm of sitosterol per day. Thirty-two days later the serum cholesterol specific activity was 0.16×10^{-3} μc/mg, which would indicate a serum cholesterol half-time of approximately 14 days under the influence of sitosterol medication.

From these observations there appears to be no doubt that cholesterol absorption can be appreciably reduced in man by the administration of sitosterol. The magnitude of this reduction would appear to be sizeable; more than 50% of the exogenous cholesterol-H^3 which would normally be absorbed fails to appear in the circulating blood when administered in association with sitosterol. However the numerous clinical observations on the effects of sitosterol medication seem to clearly

indicate that only a relatively small fraction of the serum cholesterol level in normocholesterolemic patients is directly a function of the amount of chyle cholesterol entering the blood. In view of the known fact that the endogenous hepatic cholesterol synthesis rate is a function of the exogenous cholesterol intake these results are to be expected. Further dietary and sitosterol studies are necessary to establish what fraction of the serum cholesterol level is directly a function of the chyle cholesterol concentration in various pathological conditions. The clinical trials thus far reported in the literature indicate that sitosterol effects a greater reduction in serum cholesterol values in various hypercholesterolemic states.

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