

DOCUMENT SOURCE	
University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE	Committees, Speeches, Reports, Harden B. Jones 1940s
BANCROFT/UARC ID NO.	77/112c
CARTON NO.	3
FOLDER NAME	Reports, Observations on the Fate of Ingested Cholesterol in Man
NOTES	21/2
FOUND BY/DATE FOUND	Simon Storch 11/15/94

COPY

722170

BEST COPY AVAILABLE

Reported from *Circulation*
Vol. VI, No. 3, September 1952
P. 339-345, U.S.A.

Observations on the Fate of Ingested Cholesterol in Man

By M. W. BIGGS, M.D., D. KRITCHEVSKY, Ph.D., D. COLMAN, A.B., J. W. GOFMAN, M.D., Ph.D.,
H. B. JONES, Ph.D., F. T. LINDGREN, B.S., G. HYDE, M.D., AND T. P. LYON, M.D.

Tritium-labeled cholesterol has been used to study several aspects of exogenous cholesterol metabolism in man. The rate and magnitude of the appearance of ingested cholesterol in the various blood compartments has been followed. Fecal excretion of the labeling material was measured. Cholesterol of dietary origin was demonstrated in a human atherosclerotic aorta.

THE PART played by the lipid content of the diet in the development of atherosclerosis is a controversial point among investigators of atherosclerosis and associated diseases. Some investigators would implicate the fat and cholesterol content of the diet as a major contributing factor while others deem it of little or no importance. The rightful positioning of dietary factors in the pathologic sequence of events leading to the development of atherosclerosis must await a more complete knowledge of some of the fundamental aspects of lipid metabolism. The present report deals primarily with the absorption and turnover of ingested cholesterol in the various compartments of the blood in four patients, all of whom undoubtedly had some degree of atherosclerosis. One patient had previously had a typical myocardial infarction, the second had chronic glomerulonephritis with hypertension, the third had xanthoma tendinosum of many years' duration, and the fourth experienced a cerebrovascular accident and came to autopsy.

Each of these patients was given a single oral dose of tritium-labeled cholesterol by mouth. The appearance of this labeled compound in the total serum cholesterol, free serum cholesterol, and red blood cell cholesterol was followed for from two to six weeks. Examination of the feces were done in two cases.

METHODS

Tritium (H^3) labeled cholesterol has been used as the tracer compound in these studies. This choice

From the Division of Medical Physics, Donner Laboratory of Medical Physics and the Radiation Laboratory, University of California, Berkeley 4, California.

of labeling material stems primarily from the fact that tritium in the doses required does not involve an appreciable radiation hazard, hence is safe for human experimentation. The tritium-labeled cholesterol was prepared by a catalyzed exchange reaction according to the method of Bloch and Rittenberg,¹ and the specific activity of the tritium-cholesterol so prepared varied from 0.48 to 1.07 mc. of tritium per gram of cholesterol in different preparations. This compound so prepared contains 46 per cent of the label in the vicinity of the Δ^3 -hydroxyl system and 54 per cent in the isopropyl group of the side chain according to Fukushima and Gallagher.² It has been shown repeatedly that this tritium label occupies stable positions in that there is no refection of specific activity on refluxing this tritium-cholesterol in 30 per cent potassium hydroxide in 50 per cent alcohol for 12 hours. Recent work in this laboratory employing cholesterol doubly labeled with carbon-14 and tritium for feeding experiments in rabbits indicates that tritium-labeled cholesterol is a suitable tracer compound for the investigation of many aspects of *in vivo* cholesterol metabolism.³

The patients reported here each received an oral dose of tritium-cholesterol dissolved in warm Wesson oil emulsified into whole milk, excepting patient 2, who received the tritium-cholesterol in the crystalline form dispersed in milk.

The various blood and tissue cholesterol samples were all isolated as the digitonide; these samples in turn were combusted and the water obtained allowed to react with lithium aluminum hydride to generate hydrogen. The hydrogen-tritium mixture was assayed in an ionization chamber.⁴ The total tritium content of the feces was determined by burning a sample of whole, wet feces and assaying the water obtained as above.

EXPERIMENTAL DATA

Patient 1 was a 69 year old woman who five years previously had experienced a sudden collapse associated with crushing chest pain. Electrocardiograms showed changes of a posterior myocardial infarction. Since this episode the patient has been on

Copied from reference in file 77/112c

1161216

DOCUMENT SOURCE	
University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE	
Hester B Jones Committees... Papers	
BANCROFT/UARC ID NO.	
79/112c	
CARTON NO.	
3	
FOLDER NAME	
Observation on the Fate of Ingested Cholesterol	
NOTES	
p 212	
FOUND BY/DATE FOUND	
Susan Strach 11/15/94	

COPY

limited activity and has felt fairly well except for occasional episodes of chest pain and dyspnea usually associated with excitement or overexertion. At the time of this study the patient felt well, her appetite was good, her weight was stationary, and bowel function was regarded as normal.

Fluoroscopic examination of the chest showed no evidence of cardiac enlargement. The pulse was regular at 76 and the heart sounds were not remarkable. The blood pressure was 140/95. The abdomen was negative and there was no edema.

Laboratory examination of the urine was negative. The red blood count was 5.0 million, hemoglobin was 14.0 Gm., white blood count was 6,000. The electrocardiogram showed signs of a healed posterior myocardial infarction.

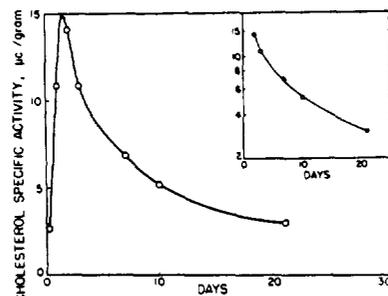
This patient was given 1.55 Gm. of tritium-labeled cholesterol (specific activity equal to 0.480 mc. of tritium per gram of cholesterol) by mouth. The cholesterol was dissolved in 10 cc. of warm Wesson oil and emulsified into a glass of whole milk. Studies on the specific activity of the total serum cholesterol were done over a period of three weeks. The results are tabulated in figure 1.

An ultracentrifugal analysis of this patient's serum lipoproteins at the beginning of the experiment according to the method of Gofman⁷ showed the following concentrations*: S_1 12-20 = 114 mg. per 100 cc.; S_1 20-100 = 428 mg. per 100 cc. The analytic ultracentrifuge plate is included in figure 5.

A serum sample obtained 24 hours after the administration of the tritium-cholesterol dose was submitted to ultracentrifugal partition according to the method of Lindgren.⁸ In this way the lipoproteins of the serum were separated into four fractions according to their Svedberg flotation rates, that is, S_1 3-7, S_1 10-13, S_1 17 and S_1 20+.† The specific activity of the total cholesterol of each fraction was determined. The results follow: S_1 3-7 cholesterol S. A. = 11.0 μ c. per gram; S_1 10-13

cholesterol S. A. = 11.0 μ c. per gram; S_1 17 cholesterol S. A. = 10.3 μ c. per gram; and S_1 20+ cholesterol S. A. = 10.9 μ c. per gram. The specific activity of the total serum cholesterol determined prior to ultracentrifugal treatment was 10.9 μ c. per gram.

A body water sample obtained on day two by freeze drying a serum sample did not show measurable activity. The sensitivity of the counting equipment is such that 1.0×10^{-3}



Sample	Serum cholesterol values, mg. %		Serum total cholesterol specific activities, μ c./gram	Percent of T-cholesterol found in total blood*
	free	total		
6 hours	64	225	2.7	2.4
1 day	57	199	10.9	9.7
2 days	54	183	14.1	12.6
3 days	53	181	10.9	9.7
7 days	60	179	6.9	6.2
10 days	58	199	9.2	8.6
21 days	54	207	3.0	2.6

FIG. 1.—The appearance of tritium-cholesterol in the blood of patient 1 following ingestion of 1.55 Gm. of cholesterol, containing 0.744 mc. of tritium. The insert is a semilog plot of the descending specific activity values.

* Approximated using a total blood volume equal to 67 cc. per kilogram of body weight.

μ c. of tritium per gram of water can be detected.

Patient 2 was a 41 year old male who had chronic glomerulonephritis with hypertension of unknown duration. Two years prior to this study he first noticed backache and was found to have albuminuria and an elevated blood pressure. These initial signs were soon followed by cramping of the muscles of the hands and subjective swelling of the face. Within three months frank edema of the hands and ankles developed associated with nocturia.

At the time of this study the patient was ambulatory and manifested no objective edema. There was marked weakness on moderate exertion. Occipital headaches were frequent and subjective edema of the face was present each morning on awakening.

* The molecules are identified by their characteristic flotation rates under specified ultracentrifugal conditions. Units are Svedbergs of flotation (S_1). One S_1 unit equals a migration rate of 10^{-10} cm. sec./dyne. Gm. at 26 C. in a medium of NaCl solution of density 1.063.

† The fraction designated S_1 20+ includes all the lipoprotein molecules with Svedberg flotation rates of 20 units (S_1 20) and greater, up to and including chylomicrons.

DOCUMENT SOURCE University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE Harris B. Jones, Committee on Papers	
BANCROFT/UARC ID NO. 79/1120	COPY
CARTON NO. 3	
FOLDER NAME Characterization of the Fate of Ingested Cholesterol	
NOTES p. 3/18	
FOUND BY/DATE FOUND Susan Steinhilber 11/15/94	

Vision was failing steadily. The appetite was good and bowel function was regarded as normal.

Fluoroscopic examination of the heart showed some left ventricular enlargement. The pulse was regular at 75 and the blood pressure was 230/135. There was grade III hypertensive retinopathy. There was no objective edema or ascites, and examination of the abdomen was negative.

Clinical laboratory studies showed a red cell count of 2.80 million, hemoglobin of 9.3 Gm., and a white cell count of 9,700. The urine had a specific gravity of 1.015 and showed 4+ albumin, an occasional white blood cell, a rare red blood cell and an occasional coarse granular and hyaline cast. The nonprotein nitrogen was 79 mg per cent; blood creatin 6.7 mg. per 100 cc. The total serum proteins were 7.7 mg. per 100 cc. with an albumin-globulin ratio of 0.35. The basal metabolic rate was -3. A fasting serum calcium was 4.7 mEq. per liter. An electrocardiogram was interpreted as a normal tracing.

This patient ingested 0.856 Gm. of tritium-cholesterol (specific activity equal to 0.853 mc. per gram) emulsified as crystalline cholesterol into 250 cc. of whole milk in a Waring blender. Following this single meal of 730 μ c. of tritium-cholesterol in crystalline form the patient was placed on a 2400 calorie diet which contained 100 Gm. of protein and 80 to 90 Gm. of fat. Salt intake was limited to 2 to 3 Gm. per day.

The results of studies on the blood cholesterol are tabulated in figure 2. Studies on the total tritium content of the feces are contained in table 1.

The feces obtained on day 2 containing 169 μ c. of tritium was lyophilized and the dried residue extracted first with 95 per cent alcohol and then with ether. The extracts were combined and saponified with 20 per cent potassium hydroxide in 50 per cent alcohol. The crude nonsaponifiable material (1.34 Gm.) contained 147 μ c. of tritium and had a specific activity of 110 μ c. per gram.

An ultracentrifugal analysis of this patient's serum lipoprotein structure showed: S₁ 12.20 = 97 mg. per 100 cc.; S₂ 20-100 = 168 mg. per 100 cc. The analytic ultracentrifuge plate is included in figure 5.

Patient 3 was a 51 year old woman who has had known xanthomata in the tendons at the ankles and elbows since the age of 20. Two years prior to this study she first began to experience postprandial distress and tightening across the anterior chest and

exertion. Eighteen months ago there had been a frank myocardial infarction requiring one month's hospitalization. Since this episode she supposedly had been on a "low fat" diet but continued to experience angina pectoris with pain radiating into the

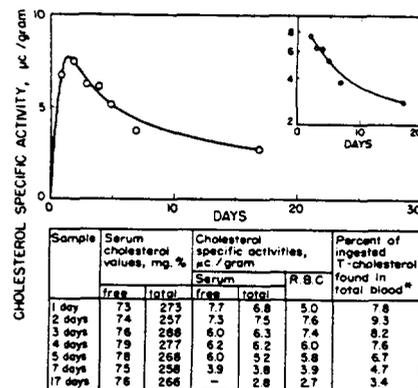


FIG. 2.—The appearance of tritium-cholesterol in the blood of patient 2 following the ingestion of 0.856 Gm. of cholesterol containing 0.730 mc. of tritium. The insert is a semilog plot of descending specific activity values.

* Approximated using a total plasma volume of 39 cc. per kilogram body weight, and a total red cell volume of 28 cc. per kilogram body weight.

TABLE 1.—Tritium Content of the Feces Following the Oral Administration of 730 μ c. of Crystalline Tritium-Cholesterol in Patient 2

Sample	Stool Wet Weight	Tritium Content		Administered Tritium-Cholesterol
		Gm.	μ c.	
Day 1	132	261	25.8	
Day 2	148	169	23.2	
Day 3	207	123	16.8	
Day 4	184	46	6.3	
Day 5	129	23	3.2	
Day 6	234	10	1.4	
Day 7	147	5	0.7	
Totals		637	87.4	
Day 17	100	1.5	0.21	

left arm and neck on moderate exertion. At the time of this experiment the patient was ambulatory and able to support herself doing sedentary work.

Examination showed numerous tendinous xanthomata at the ankles, elbows and on the feet and hands. There were also xanthelasmata. The heart

DOCUMENT SOURCE University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE <i>Harden B. Jones Committee Reports</i>	
BANCROFT/UARC ID NO. <i>79/112c</i>	COPY
CARTON NO. <i>3</i>	
FOLDER NAME <i>Observation on the Fate of Ingested Cholesterol</i>	
NOTES <i>p 4/8</i>	
FOUND BY/DATE FOUND <i>Susan Steach 11/15/94</i>	

sounds were normal and the pulse was regular at 70. The blood pressure was 110/70. Funduscopic examination showed some tortuosity and silver wiring of the retinal vessels. The rest of the examination was negative.

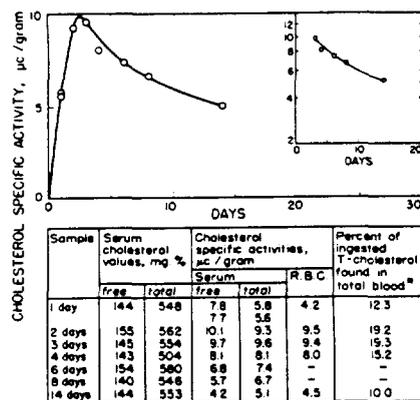


FIG. 3.—The appearance of tritium-cholesterol in the blood of patient 3 following the ingestion of 0.688 Gm. of cholesterol containing 0.736 mc. of tritium. The insert is a semilog plot of descending specific activity values.

^a Approximated using a total plasma volume of 39 cc. per kilogram body weight, and a total red cell volume of 28 cc. per kilogram body weight.

TABLE 2.—Tritium Content of the Feces Following the Oral Administration of 736 µc. of Tritium-Cholesterol in Patient 3

Sample	Stool Wet Weight	Tritium Content	Administered Tritium-cholesterol
	Gm.	µc.	%
Day 1	111	0.5	0.1
Day 2	99	26	3.5
Day 3	122	291	39.5
Day 4	94	67	9.1
Day 5	105	30	4.1
Day 6	none	—	—
Day 7	116	4	0.6
Totals		418.5	56.9

Laboratory studies showed a red cell count of 3.10 million, a hemoglobin of 10.0 Gm., and a white blood count of 8,500. The urine examination was normal. The electrocardiogram showed evidence of past myocardial damage.

The patient was given 0.688 Gm. of tritium-labeled cholesterol—specific activity equal to 1.07 mc. per gram—by mouth. This 0.736 mc. of tritium-cholesterol was dissolved in 10 cc. of warm Wesson oil and emulsified into milk. Following this fatty meal the patient was placed on a hospital diet adequate in calories and vitamins but limited to 20 Gm. of fat. The patient continued on this diet throughout the study.

The results of the studies on the blood are given in figure 3. The stool data are contained in table 2.

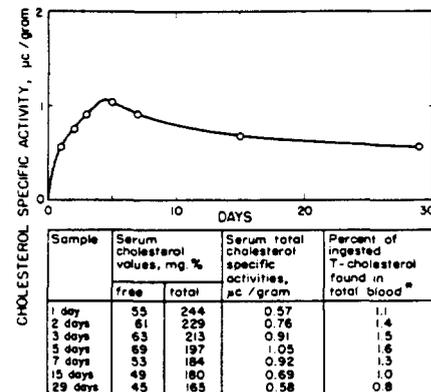


FIG. 4.—The appearance of tritium-cholesterol in the blood of patient 4 following ingestion of 0.650 Gm. of cholesterol containing 0.696 mc. of tritium.

^a Approximated using a total blood volume equal to 67 cc. per kilogram of body weight.

An ultracentrifugal analysis of this patient's lipoprotein structure showed: S_f 5-10 = 870 mg. per 100 cc.; S_f 12-20 = 187 mg. per 100 cc.; S_f 20-100 = 112 mg. per 100 cc. The analytic ultracentrifuge plate is included in figure 5.

Patient 4 was a 62 year old man who experienced a massive cerebrovascular accident and was brought to the hospital unconscious. Details of his history prior to this fatal episode are lacking. At the time the tritium-cholesterol was given the patient was comatose. The neck was stiff and there was a specific paralysis of the right arm and leg. Examination of the lungs revealed inspiratory rales at both lung bases. The blood pressure was 170/90. The cardiac rhythm was regular at 80, and there was a stable

DOCUMENT SOURCE	University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA
RECORDS SERIES TITLE	Harden B. Jones Committee Papers
BANCROFT/UAAC ID NO.	74/1120
CARTON NO.	3
FOLDER NAME	Observations on the Fate of Ingested Cholesterol
NOTES	p 5/8
FOUND BY/DATE FOUND	James Storch 11/15/94

COPY

murmur in the aortic region. The patient was incontinent of urine and feces.

For eight days prior to the beginning of this study the patient had received nothing by mouth; saline and glucose dlyses had been

transient period beginning at about three weeks small oral liquid feedings were given. Forty-three days after the tritium-cholesterol was given the patient died and an autopsy was done.

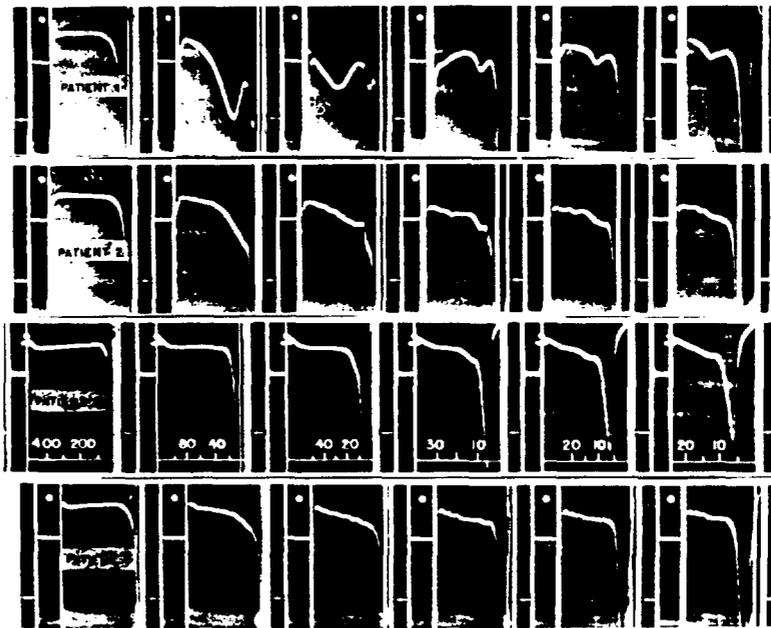


FIG. 5.—Ultracentrifugal flotation patterns showing the low density lipoprotein spectrum present in the four patients studied for response to oral ingestion of H^3 -labeled cholesterol. Runs are made at 52,640 revolutions per minute with photographs taken at 0, 6, 12, 22, 30, 38 minutes after full rotor speed is reached. The plate of patient 3 is ruled with an S_2 rate grid, so that the flotation rate of any lipoprotein appearing in each frame can be immediately determined. This grid also applies to corresponding frames in the plates of patients 1, 2, 4.

All sera are concentrated five-fold prior to analytic ultracentrifugation, except for that of patient 3 which is one-fold.

The S_2 12-20 lipoprotein is elevated in all patients. The S_2 20-100 lipoprotein level is elevated in all patients except patient 3.

administered daily. The patient received 0.650 Gm. of tritium-cholesterol (specific activity equal to 1.07 mc. of tritium per gram) by stomach tube. This 0.696 mc. of tritium-cholesterol was dissolved in 15 cc. of Wesson oil and emulsified into 250 cc. of whole milk. Following this cholesterol feeding the patient received nothing by mouth for three weeks, all nourishment being administered by injection. For a

The immediate cause of death was an extensive bilateral hypostatic bronchopneumonia. Examination of the cerebral vessels showed marked atherosclerotic changes and in the left parietal region there was a large area of encephalomalacia secondary to thrombosis of the left middle cerebral artery. The aorta which was saved for chemical examination was so extensively involved with atherosclerosis that

by the University of California at Berkeley, The Bancroft Library, The University Archives, Berkeley, California 94720

DOCUMENT SOURCE	University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE	Harden B. Jones Committee Papers	
BANCROFT/UAARC ID NO.	79/112c	COPY
CARTON NO.	3	
FOLDER NAME	Observations on the Fate of Ingested Cholesterol	
NOTES	p. 6/8	
FOUND BY/DATE FOUND	Susan Stark 11/15/94	

no areas of "normal" aorta were visible. Many of the plaques were ulcerated and calcified. The coronary vessels showed calcification and numerous areas of atherosclerotic stenosis.

The total serum cholesterol specific activities are recorded in figure 4. The total liver cholesterol was found to have a specific activity of 0.30 μc . per gram. The thickened intima of the aorta was carefully separated from the adventitia and much of the media and consisted of 36.0 Gm. of tissue. From this source, 1.31 Gm. of cholesterol were separated. This cholesterol separated as the digitonide had a specific activity of 0.03 μc . per gram.

An ultracentrifugal analysis of this patient's serum lipoprotein structure eight days following the cerebrovascular accident showed: S_1 12-20 = 65.4 mg. per 100 cc.; S_1 20-100 = 82.3 mg. per 100 cc. The analytic ultracentrifuge plate is included in figure 5.

DISCUSSION

Following a single tracer cholesterol meal to man the serum cholesterol specific activity increased rapidly during the first day and reached a maximum specific activity in from 36 to 72 hours as in figures 1 to 3. Thus cholesterol absorption in man appears to be a slow process normally continuing over a period of approximately two to three days. Examination of the feces of patients 2 and 3 showed continued excretion of large amounts of tracer material over a period of several days entirely consistent with such a period of cholesterol absorption.

There can be no doubt that dietary cholesterol plays a role in serum cholesterol metabolism for appreciable amounts of dietary tracer cholesterol were readily demonstrable in the serum of patients 1, 2, and 3 throughout the study periods. Two days after the tritium-cholesterol meal approximately 12.5 per cent of the 1.55 Gm. of cholesterol fed to patient 1 existed in the circulating blood; about 9.3 per cent of the administered dose was demonstrable in patient 2; and about 19.2 per cent of the dose was found in the blood of patient 3.

It has been observed repeatedly in hypercholesterolemic rabbits that during the first 72 hours following a single tritium-cholesterol

meal the specific activity of esterified serum cholesterol is greater than the free cholesterol specific activity. In man this was not the case; indeed the reverse was demonstrated, the free cholesterol initially having a higher specific activity than the esterified fraction. The explanation of this difference remains to be clarified. Whether this represents a fundamental species difference in metabolic pathways or whether the observed difference is a quantitative one of "defective processing" in the case of the rabbit remains to be seen.

Correct analysis of the blood cholesterol specific activity curves presented in figures 1 to 3 is extremely difficult, for the curves most assuredly reflect the integration of a large number of metabolic processes continuing at various rates. For the first 48 to 72 hours, during the initial rising portion of the curves, intestinal absorption and transport of the absorbed cholesterol to the systemic circulation via the lymph of the thoracic duct would appear to be paramount in determining the shape of the curve. It has been demonstrated previously that liver cholesterol and serum cholesterol are in rapid equilibrium^{8, 9} and the initial steep descending portion of the curves occurring at two to five days probably represents a period during which serum cholesterol is exchanging with various tissue cholesterol pools, particularly liver. The terminal flattened portion of the curves would appear to approach the turnover rate of body cholesterol as a whole.

The observation that red blood cell cholesterol, following an initial lag period, reaches an equilibrium state with the free cholesterol of the serum *in vivo* confirms in man the previous observations of Hazerman and Gould.^{10, 11} The presence of tritium-cholesterol in all of the various lipoprotein species after a period of 24 hours following the cholesterol meal in patient 1 is of interest. It has been shown in rats that absorbed cholesterol is transported via the lymph of the thoracic duct to the systemic circulation.^{12, 13} Ultracentrifugal studies on such lymph would indicate that newly absorbed cholesterol is in both the esterified and free states and exists in or is associated with a lipoprotein structure with an S_1 rate

DOCUMENT SOURCE University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE <i>Hardin B Jones Committee - Papers</i>	
BANCROFT/UARC ID NO. <i>77/112c</i>	COPY
CARTON NO. <i>3</i>	
FOLDER NAME <i>Observations on the Fate of Ingested Cholesterol</i>	
NOTES <i>p 7/8</i>	
FOUND BY/DATE FOUND <i>Simon Stead 11/15/94</i>	

greater than 100. The equilibrium state found after 24 hours in patient 1, with cholesterol from all of the lipoprotein species having the same specific activity, requires that the in vivo metabolism of serum lipoproteins involves a fairly rapid interchange of cholesterol moieties whether esterified or free. The mechanism for this remains to be elucidated. Preliminary in vitro studies of the interchange of cholesterol between various isolated lipoprotein species of different S_f rates indicate that free cholesterol exchanges rapidly but that esterified cholesterol does not.

At this stage in our knowledge of fecal steroid metabolism certain limitations on the interpretation of the total tritium content of the feces in patients 2 and 3 must be realized. On good and varied evidence¹⁴⁻¹⁶ it has been held that the conversion of cholesterol to coprosterol goes via cholestenone and occurs within the intestinal lumen of the large bowel. Gallagher¹ has demonstrated that the in vitro conversion of deuterium cholesterol to cholestenone may result in the loss of 46 per cent of the deuterium label. Thus the possibility exists that during the formation of coprosterol from cholesterol some tritium label might exchange; however, at present the evidence would indicate that this does not occur. The in vivo formation of cholic acid¹⁷ and pregnanediol¹⁸ from cholesterol presumably via cholestenone does not result in loss of the label at C-3. Of course if tritium exchange did occur with a substance that was absorbed, estimations of the amount of cholesterol absorbed by a measure of the amount of label disappearing from the feces would be in error. Experiments in which tritium-cholesterol and C¹⁴-labeled cholesterol have been fed to rats do not show a greater disappearance of the tritium-label from the gut above that of the C¹⁴ label.¹⁹

At present it is permissible to say that the 87.4 per cent of the ingested tritium in patient 2 and 56.9 per cent in patient 3 found in the feces represents minimum values for the percentage of unabsorbed cholesterol. The 12.6 per cent and 42.1 per cent respectively which disappeared from the gut probably was absorbed as tritium-cholesterol. It is important here to point out that patient 2 received the

tracer cholesterol in the crystalline form while patient 3 received hers dissolved in warm Weston oil. An experiment to evaluate some of the factors important in man in determining the efficiency of cholesterol absorption, such as the physical state of the ingested cholesterol, is at present under way. The possibility, of course, exists that patients with hypercholesterolemia and xanthoma tuberosum absorb cholesterol more completely than patients with "normal" serum cholesterol values. This point of course requires more study.

Patient 4 requires a brief comment. The lipid metabolism of a dying, fasting man as might be expected appears to be grossly altered from that found in a normal, feeding person. Two other comatose patients with cerebrovascular accidents were studied simultaneously with patient 4. Each of these patients showed only trace amounts of the ingested cholesterol in the blood. Fecal collections were not possible here because of irregularity of stools and fecal incontinence. Presumably the very low serum cholesterol specific activities indicate very poor cholesterol absorption.

It was possible, however, in patient 4 to make a qualitative demonstration of exogenous cholesterol entering into the metabolism of the human atherosclerotic aorta. The amount of cholesterol isolated from the aorta (1.31 Gm.) and the specific activity found (0.03 $\mu\text{c. per gram}$) preclude the possibility that the activity found in the aorta could have been in blood or lymph cholesterol contained in the processed sample. To label 1.31 Gm. of cholesterol to a specific activity of 0.03 $\mu\text{c. per gram}$ would require the inclusion of all the cholesterol from approximately 29 cc. of blood with a specific activity equal to 0.58 $\mu\text{c. per gram}$ of cholesterol, the value present in serum cholesterol on day 29. The aortic sample processed weighed only 360 Gm.

If an approximated average serum cholesterol specific activity value of 0.6 $\mu\text{c. per gram}$ is assumed for the period of the experiment, it can be estimated that an appreciable amount (5.65 $\mu\text{g.}$) of the 1.31 Gm. of cholesterol isolated from the aortic sample arose from the serum as the result of the intermetabolism of

DOCUMENT SOURCE	
University of California at Berkeley The Bancroft Library/The University Archives, Berkeley CA	
RECORDS SERIES TITLE	
Herschel B. Jones Committee Reports	
BANCROFT/UARC ID NO.	
77/1120	
CARTON NO.	
3	
FOLDER NAME	
Observations on the Fate of Ingested Cholesterol	
NOTES	
p. 2/3	
FOUND BY/DATE FOUND	
Susan Storch 11/15/94	

COPY

aortic cholesterol and serum cholesterol during the 43 day period.

SUMMARY

Tritium-labeled cholesterol was used to investigate certain aspects of the metabolism of ingested cholesterol in four human subjects, all of whom presumably had atherosclerosis.

1. Cholesterol absorption is not efficient in man; however, approximately 12.6 per cent, 9.3 per cent and 19.2 per cent of the administered tritium-cholesterol could be demonstrated in the circulating blood two days after the tritium-cholesterol feeding in patients 1, 2 and 3 respectively.

2. Cholesterol absorption in man is slow and a peak serum cholesterol specific activity following a single labeled cholesterol feeding occurs in from 36 to 72 hours.

3. Serum free cholesterol has a higher specific activity than esterified cholesterol for 24 to 48 hours after a single tracer cholesterol meal. This is the reverse of that previously found in cholesterol-fed rabbits where the specific activity of the esterified cholesterol was the greater.

4. The specific activities of cholesterol isolated from the various lipoprotein fractions of the serum separated ultracentrifugally were identical 24 hours after the tritium-cholesterol feeding.

5. Free serum cholesterol and red blood cell cholesterol are in equilibrium in vivo.

6. Quantitative determinations of the tritium content of the feces for seven days following a tritium-cholesterol meal were made in two patients.

7. The qualitative demonstration of exogenous cholesterol entering into the metabolism of the human atherosclerotic aorta was made.

REFERENCE

¹ BLOCH, K., AND RITTENBERG, D.: The preparation of deuterio-cholesterol. *J. Biol. Chem.* **149**: 505, 1943.

² KRITCHEVSKY, D., BIGGS, M. W., AND FRIEDMAN, N. K.: Preparation of tritiated cholesterol. Berkeley, Univ. of California Radiation Laboratory Publication #644, 1950.

³ FUKUSHIMA, D. K., AND GALLAGHER, T. F.: Platinum catalyzed hydrogen deuterium exchange with steroids. *Federation Proc.* **9**: 19, 1950.

⁴ GALLAGHER, T. F.: Studies in the metabolism

oids. Isotopes in biochemistry. Ciba Foundation Conference, Philadelphia, Blakiston, 1951. Pp. 28-40.

⁵ BIGGS, M. W., AND KRITCHEVSKY, D.: Observations on the suitability of tritium labeled cholesterol for the study of cholesterol metabolism. *Arch. Biochem.* **36**: 430, 1952.

⁶ —, AND KIRK, M. R.: Assay of samples doubly labeled with radioactive hydrogen and carbon. *Analytical Chem.* **24**: 223, 1952.

⁷ GOFMAN, J. W., LINDGREN, F. T., ELLIOTT, H. A., MANIZ, W., HEWITT, J., STRISOWER, B., AND HERRING, V.: The role of lipids and lipoproteins in atherosclerosis. *Science* **3**: 166, 1950.

⁸ —, JONES, H. B., LINDGREN, F. T., LYON, T. P., ELLIOTT, H. A., AND STRISOWER, B.: Blood lipids and human atherosclerosis. *Circulation* **2**: 161, 1950.

⁹ LINDGREN, F. T., ELLIOTT, H. A., AND GOFMAN, J. W.: The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins, with applications to the study of atherosclerosis. *J. Physical and Colloid Chem.* **55**: 80, 1951.

¹⁰ BIGGS, M. W., AND KRITCHEVSKY, D.: Observations with radioactive hydrogen (H³) in experimental atherosclerosis. *Circulation* **4**: 34, 1951.

¹¹ GOULD, R. G.: Lipid metabolism and atherosclerosis. *Am. J. Med.* **11**: 209, 1951.

¹² HAZERMAN, J. S., AND GOULD, R. G.: The *in vitro* interchange of cholesterol between plasma and red cells. *Proc. Soc. Exper. Biol. & Med.* **78**: 329, 1951.

¹³ BIGGS, M. W., FRIEDMAN, M., AND BYERS, S. O.: Intestinal lymphatic transport of absorbed cholesterol. *Proc. Soc. Exper. Biol. & Med.* **78**: 641, 1951.

¹⁴ CHAIKOFF, I. H., BLOOM, B., SPIERSTEIN, M. D., KIYASU, J. M., REINHARDT, W. O., DAUBEN, W. G., AND EASTHAM, J. F.: C-14 cholesterol I. Lymphatic transport of absorbed cholesterol-4-C-14. *J. Biol. Chem.* **194**: 407, 1952.

¹⁵ SCHOENHEIMER, R., RITTENBERG, D., AND GRAFF, M.: Deuterium as an indicator in the study of intermediary metabolism. IV. The mechanism of coprosterol formation. *J. Biol. Chem.* **111**: 183, 1935.

¹⁶ ROSENHEIM, O., AND WEBSTER, T. A.: A dietary factor concerned in coprosterol formation. *Biochem. J.* **35**: 920, 1941.

¹⁷ —, AND —: The mechanism of coprosterol formation *in vivo*. Cholestenone as an intermediate. *Biochem. J.* **37**: 513, 1943.

¹⁸ BLOCH, K., BERG, B. N., AND RITTENBERG, D.: The biological conversion of cholesterol to cholic acid. *J. Biol. Chem.* **149**: 511, 1943.

¹⁹ —: The biological conversion of cholesterol to pregnanediol. *J. Biol. Chem.* **157**: 661, 1945.

²⁰ KRITCHEVSKY, D.: Data to be published.

Copyright in this reference publication is held by the University of California, Berkeley, California, 94720.