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Research Article

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BODY CHOLESTEROL METABOLISM IN MAN. I. THE EQUILIBRATION OF SERUM AND TISSUE CHOLESTEROL * †

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This study was undertaken to investigate the differential rates of exchange and the extent of the equilibration between serum and tissue cholesterol in man over prolonged periods of time. Previous studies in animals have pointed to species differences in the exchange of cholesterol between the serum and tissue compartments. Gould has reported that tissue cholesterol in dogs appears to be in a dynamic state of equilibrium with serum cholesterol and that equilibration of serum and tissue cholesterol can be demonstrated within 14 days after the administration of C^{14}-cholesterol (2). Studies in rats by Avigan and Steinberg, however, have suggested that complete equilibration between serum and tissue cholesterol is not present even after periods ranging up to 7 weeks (3).

The present studies in man indicate that the cholesterol in all body tissues other than nervous tissue constitutes a "pool" which appears to exchange with the serum cholesterol. The studies also have demonstrated that complete equilibration of serum and arterial intimal cholesterol does occur in normal human blood vessels, although at rates that are generally slower than those observed in other body tissues.

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MATERIAL AND METHODS

Seven hospitalized patients terminally ill with neoplastic disease, one hospitalized patient with an acute cerebral thrombosis, and one ambulatory patient who died suddenly from a pulmonary embolism were included in the study. None of the patients had evidence of hypercholesterolemia, diabetes mellitus, thyroid dysfunction, or the nephrotic syndrome. Five of the patients with malignancies showed signs of tissue wasting, but the remaining individuals appeared to be in a good nutritional state.

All patients were given a tracer dose of 4-C^{14}-cholesterol at intervals ranging from 1 to 226 days prior to death. The labeled cholesterol used for injection was obtained commercially (New England Nuclear Corp.) and had a specific activity of 37.7 μc per mg cholesterol. It was dissolved in ethanol and passed through a sintered glass filter. The ethanol solution was diluted with saline and mixed with approximately 30 ml of the patient's own plasma which had been obtained on the day of cholesterol administration. This mixture, representing 20 to 30 μc of C^{14}-cholesterol, was injected into an antecubital vein through an indwelling needle.

Specimens of liver, kidney, spleen, lung, adrenal gland, intestine, fat, muscle, vena cava, pulmonary artery, coronary artery, femoral artery, aorta, and brain were removed at autopsy 4 to 10 hours after the patient's death and immediately frozen. Blood specimens were obtained either antemortem on the day of the patient's death or during the post-mortem examination. Because of post-mortem hemolysis, specific activities of whole blood cholesterol, rather than separate serum and red cell specific activities, were determined in those blood samples obtained at autopsy. All adventitial material was stripped away from the tissues to be analyzed. The tissues were minced, ground, and extracted into boiling acetone and alcohol. Aliquots of the extract were used for the determination of free and total cholesterol content by the method of Schoenheimer and Sperry (4). Duplicate aliquots were also analyzed for free and total C^{14}-cholesterol radioactivity. To measure C^{14} radioactivity in the free cholesterol fraction, the acetone-alcohol extract was acidified, and the cholesterol was precipitated with digitonin. The samples were washed once with acetone and ether and twice with ether and were then dried. The digitonide precipitate was dissolved in 2 ml methanol

TABLE I

The equilibration of serum and tissue cholesterol in nine human subjects

Patient Sex Age	Wt	Diagnosis	Time†	Tissue cholesterol-4-C^{14} specific activity *											
				Serum		RBC		Liver		Spleen		Kidney		Lung	
				Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
	kg		*days*												
H.C. ♀ 56	62.0	Carcinoma of breast	1	320‡		247		648		132		68		99	
R.D. ♂ 43	64.7	Monocytic leukemia	4	560‡				620		584		382		326	
A.W. ♂ 75	60.9	Carcinoma of lung	6	501		544		561		346		201		256	
R.N. ♂ 69	83.5	Cerebral thrombosis	15	376‡	370			359	364	375	400	392	358	409	432
E.M. ♀ 77	53.6	Carcinoma of stomach	17	226	214	239	230	208	230	224	230	206	228	244	229
M.L. ♀ 42	59.8	Pulmonary embolism	20	308	329	313	332	307	294	313	321	334	350	338	362
R.L. ♀ 74	71.3	Cerebral thrombosis	30	129‡	138			127	125	137	133	123	140	146	135
A.M. ♂ 19	68.6	Monocytic leukemia	125	38	42	36	37	38	41	41	41	42	39	34	38
J.G. ♀ 48	44.5	Carcinoma of breast	226	21‡	22			20	21	24	21	21	19	18	21

Patient Sex Age	Wt	Diagnosis	Time†	Tissue cholesterol-4-C^{14} specific activity *											
				Adrenal		Intestine		Muscle		Fat		Abdominal aorta		Brain	
				Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
	kg		*days*												
H.C. ♀ 56	62.0	Carcinoma of breast	1	33		19		13		10		3		0	
R.D. ♂ 43	64.7	Monocytic leukemia	4	204		110		54		56		39		0	
A.W. ♂ 75	60.9	Carcinoma of lung	6	153		71		79		56		35		0	
R.N. ♂ 69	83.5	Cerebral thrombosis	15	322	344	314	301	282	253	76	62	53	32	7	5
E.M. ♀ 77	53.6	Carcinoma of stomach	17	180	187	201	206	134	148	109	120	20	23	3	3
M.L. ♀ 42	59.8	Pulmonary embolism	20	308	284	260	282	337	320	226	216	138	130	8	10
R.L. ♀ 74	71.3	Cerebral thrombosis	30	111	120	115	113	122	130	101	117	32	30	5	3
A.M. ♂ 19	68.6	Monocytic leukemia	125	43	41	37	39	44	46	40	43	36	38	2	1
J.G. ♀ 48	44.5	Carcinoma of breast	226			23	20	19	20	23	23	24	26	1	1

* Cholesterol-4-C^{14} specific activity = cpm C^{14}-cholesterol (digitonide precipitable material)/mg cholesterol.
† Time = interval between C^{14}-cholesterol administration and death of patients.
‡ Whole blood cholesterol-4-C^{14} specific activity.

and counted in a Tri-Carb liquid scintillation spectrometer after the addition of 18 ml of scintillation solution.[1] For determinations of total cholesterol radioactivity, duplicate aliquots of the acetone-alcohol extract were saponified with KOH, acidified, and then handled in the manner described for measurement of free cholesterol radioactivity. All radioactive samples were counted to a statistical accuracy of at least 5 per cent (5). Variations in counting between duplicate samples were not greater than 4 per cent.

In the blood vessels that were analyzed, the intima and adventitia were stripped from each other without an attempt to separate the media. Whenever possible, atherosclerotic segments of the arteries were separated from relatively normal areas and analyses of the separated portions for cholesterol specific activity were made. The degree of atherosclerosis was estimated grossly on a scale of 0 to 4+, where 0 represented absence of visible atherosclerosis; 1+ minimal but visible fatty streaking; 2+ few plaques but little or no vessel narrowing; 3+ numerous but discrete plaques with some vessel narrowing but generally no ulceration or calcification; and 4+ severe atherosclerosis with numerous plaques, frequently confluent, calcified, ulcerating, and causing vessel narrowing. Segments of arterial tissue were dried to a constant weight, and the cholesterol content and cholesterol specific activity were determined as described above.

In Patient M.L. the cholesterol in aliquots of the acetone-alcohol extracts of serum, liver, kidney, spleen, and muscle were purified by the bromination method of Schwenk and Werthessen (6), and the cholesterol specific activity of the purified material was determined as previously described.

In Patients A.M. and J.G. a lipid extract of serum,

[1] Prepared as follows: 4.0 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-di-2-(5-phenyloxazolyl)-benzene (POPOP) were made up to 1 L with toluene.

TABLE II

The equilibration of serum and blood vessel cholesterol in nine human patients

Patient	Time	Serum Sp. act.*	Vena cava Sp. act.	Pulmonary artery Sp. act.	Coronary artery		Abdominal aorta		Femoral artery	
					Degree of atheroscl. 0–4+	Sp. act.	Degree of atheroscl. 0–4+	Sp. act.	Degree of atheroscl. 0–4+	Sp. act.
	days									
H.C.	1	320	32	30	4+	3	4+	3	4+	4
R.D.	4	560	109	93	1+	56	1+	39	2+	59
A.W.	6	501	108	73	4+	26	3+	35	3+	34
R.N.	15	376	176	81	3+	56	4+	53	3+	76
E.M.	17	226	193	79	4+	23	4+	20	3+	30
M.L.	20	308	341	231	2+	148	1+	138	1+	137
R.L.	30	129	106	80	3+	33	2+	32	2+	36
A.M.	125	38	44	45	0	37	0	36	0	36
J.G.	226	21	19	20	1+	23	1+	24	0	22

* Sp. act. =cpm C^{14}-free cholesterol/mg free cholesterol.

liver, spleen, intestine, kidney, and fat was made according to the method of Folch, Lees and Stanley (7) and the total C^{14} radioactivity of duplicate aliquots of the lipid extract was measured after addition of the previously described scintillation solution. Duplicate aliquots of the same lipid extract were also treated with digitonin and analyzed for C^{14}-cholesterol radioactivity as described above.

Equilibration of cholesterol between the serum and tissues

1. *Liver and red blood cells.* The cholesterol in the liver and red cells showed the most rapid rate of equilibration with serum cholesterol as compared with the other tissues studied (Table I). Essentially complete equilibration was noted between these tissues in all patients studied except the one who died 14 hours after receiving C^{14}-cholesterol; this patient had specific activities of liver and red cell cholesterol that were 202 and 77 per cent, respectively, of the serum specific activity. During the 4 to 226 days after C^{14}-cholesterol administration, free and total cholesterol specific activities in the liver ranged from 92 to 112 per cent of the serum specific activity, whereas the red cell specific activities ranged from 88 to 108 per cent of the serum specific activity. The specific activities of free and total cholesterol in these tissues were generally comparable to each other.

2. *Kidney, spleen, lung, adrenal, and intestine.* Isotopic equilibrium between the serum cholesterol and the cholesterol in the kidney, spleen, lung, adrenal gland, and intestine was generally reached within 20 days after the administration of labeled cholesterol. The relative tissue specific activities from day 30 onward ranged from 86 to 110 per cent of the serum specific activity for the kidney, 95 to 114 per cent for spleen, 86 to 113 per cent for lung, 86 to 113 per cent for adrenal, and 83 to 110 per cent for intestine. No consistent differences between the specific activities of free and total cholesterol were observed.

3. *Fat and muscle.* Almost complete equilibration of fat and muscle cholesterol with serum cholesterol was reached in 30 days, and the equilibrium state appeared to persist at 125 and 226 days. The specific activity of the cholesterol in fat tissue ranged from 78 to 110 per cent of the serum specific activity, whereas that in the muscle ranged from 90 to 116 per cent of the serum specific activity between days 30 and 226.

4. *Blood vessels.* Inferior vena cava—Of the blood vessels examined (Table II), the exchange of tissue cholesterol with serum cholesterol was most rapid in the vena cava, where essentially complete equilibration with the serum cholesterol occurred at 20 to 30 days and persisted during the remainder of the study.

Pulmonary artery—The rate of equilibration of serum cholesterol with that in the pulmonary artery was intermediate between those observed in the vena cava and systemic arteries. During the first month the maximum cholesterol specific activity in the pulmonary artery was 75 per cent of the serum specific activity. At 125 and 226 days, equilibration was essentially complete, and cholesterol specific activities in the pulmonary artery were

TABLE III

Comparison of the degree of atherosclerosis, intimal cholesterol content, and the equilibration between serum and aortic intimal cholesterol

Patient	Time	Degree atheroscl. 0–4+	Aortic $intima_{chol}$	Degree equilibration*
	days		*mg chol/g dry wt*	%
H.C.	1	4+	86.4	1
R.D.	4	1+	26.8	7
		3+	44.3	2
A.W.	6	3+	38.2	7
R.N.	15	4+	104.1	14
E.M.	17	2+	27.4	13
		4+	59.7	9
M.L.	20	1+	7.9	45
		3+	36.4	24
R.L.	30	2+	48.5	25
		3+	51.0	17
A.M.	125	0	7.1	95
J.G.	226	0	13.4	108
		1+	19.5	114

* Degree equilibration of serum and aortic intimal cholesterol (%) = (C^{14}-cholesterol sp. act. of aortic intima/C^{14}-cholesterol sp. act. of serum) ×100.

118 and 95 per cent of the serum cholesterol specific activities.

Systemic arteries—As compared with the other tissues, the rate of equilibration of serum cholesterol with that in the arterial intima appeared to be a relatively slow process. At the end of 30 days the maximum arterial intimal specific activity was 45 per cent of the serum specific activity. By 125 and 226 days after C^{14}-cholesterol administration, the arterial intimal specific activities in two patients without vascular disease ranged between 93 and 118 per cent of the serum specific activities.

Analyses of different areas of intima in the same patient, and comparison of intimal cholesterol specific activities in different patients who died at generally similar intervals after labeled cholesterol administration (Table III), indicate that the rate of exchange of serum and intimal cholesterol is inversely related to the degree of atherosclerosis and the cholesterol content of the vessels examined. In Patient M.L. a relatively normal segment of aorta with a cholesterol content of 7.9 mg per g dry tissue had a C^{14}-cholesterol specific activity of 45 per cent as compared with a neighboring atherosclerotic segment where there was a cholesterol concentration of 36.4 mg per g and a cholesterol specific activity which was 24 per cent of that in the serum. Likewise, in Patient R.L. an aortic segment with grade 2 atherosclerosis had a specific activity which was 25 per cent of that in the serum in contrast to the cholesterol specific activity of 17 per cent in an adjacent severely atherosclerotic area. In Patients R.N., E.M., and M.L., who died at roughly comparable intervals of 15, 17, and 20 days, respectively, after receiving labeled cholesterol, different relative aortic intimal specific activities were present. Patients R.N. and E.M., with severe atherosclerosis, had aortic specific activities of only 14 and 9 per cent of their serum specific activities, whereas Patient M.L., with a relatively normal aorta, had an aortic intimal specific activity which was 45 per cent of that in the serum.

In the patients studied, the cholesterol specific activities of the abdominal aorta, coronary artery, and femoral artery were generally comparable to one another when the degree of atherosclerosis of the segments examined was taken into account.

5. *Central nervous system.* The brain specific activity in all pataients studied was low and ranged from 0 to 5 per cent of the serum specific activity levels.

Noncholesterol C^{14} tissue radioactivity—The specific activities of the digitonin-precipitable material of the tissues were comparable before and after dibromide purification. No measureable C^{14} radioactivity other than that in the digitonin-precipitable material was found in the lipid-soluble extract of the tissues.

DISCUSSION

The rate of exchange of serum and tissue cholesterol appears to vary among the different tissues of the body. Similarly to previous reports (8–11), the present investigation indicates that the liver and red blood cell cholesterol exchanges rapidly with serum cholesterol and that in these tissues complete equilibration is reached within 4 days. In most of the other tissues studied the equilibration with serum cholesterol is more or less complete within 1 month. Over longer periods (ranging up to 7 months), a state of equilibrium appears to persist, tissue cholesterol specific activities remaining generally similar to the serum specific activities.

The rate at which serum cholesterol exchanges with cholesterol in the arteries is slower than in the other tissue studied except the brain. However, as has been reported previously (8, 12, 13), significant exchange may take place in the arteries. The present studies indicate that in normal arterial intima complete equilibration with serum cholesterol does occur. Significant amounts of cholesterol radioactivity were demonstrated in atherosclerotic plaques, although within the time period of these studies, complete exchange was not observed. The observation that cholesterol specific activity in atherosclerotic segments was lower than in less diseased areas suggests that the presence of atherosclerosis delays the rate at which complete equilibration between serum and intimal cholesterol takes place. The lower specific cholesterol activities in the plaques does not necessarily mean that less total cholesterol is exchanging in the plaques, since most of the atherosclerotic segments analyzed had significantly higher cholesterol concentrations than had less diseased areas. The demonstration of complete equilibration of cholesterol between the serum and normal intima indicates that the cholesterol of normal arterial intima is in a dynamic state of equilibrium with serum cholesterol. These observations lend some rationale to the use of hypocholesterolemic measures in the prevention of atherosclerosis.

The equilibration of serum and intimal cholesterol suggests that at least some of the cholesterol deposited in the vessel wall had its origin in the serum cholesterol. Recent studies in rabbits have indicated that the entrance of cholesterol into the intima from the serum accounts for practically all of the cholesterol being deposited in the vessel wall, thus suggesting that *in situ* arterial cholesterol synthesis does not play a significant role in the accumulation of cholesterol in arteries (14). The present study does not yield informa-

tion regarding the relative importance of these factors in man, but it has been demonstrated *in vitro* that *in situ* sterol synthesis does occur in human arterial tissue (15, 16).

The similar specific activities of cholesterol in the coronary artery, thoracic and abdominal aorta, and femoral artery of each patient indicates that similar rates of exchange with serum cholesterol were present in these areas. These results are unlike those reported recently with *in vitro* preparations in which differential rates of penetration of labeled cholesterol into different segments of the aorta were observed (17).

The brain and other nervous tissue were the only tissues that showed negligible degrees of exchange with the serum cholesterol. These findings, along with the observation that the brain synthesis of cholesterol appears almost to cease after completion of myelinization (18), suggest that the turnover of brain cholesterol in adults is an extremely slow process. They also indicate that most of the cholesterol in the central nervous system is outside the cholesterol miscible pool that can be measured by isotope dilution techniques (19).

Segments of skin were not analyzed in the present investigation, but collateral studies have indicated that the cholesterol in the skin is also a part of the cholesterol miscible pool (19).

The measurement of absolute rates of exchange of cholesterol between the blood and tissues could not be determined from these studies in which the tissues were analyzed at varying intervals after the administration of a single dose of labeled cholesterol. The rate at which the specific activities of the serum and tissue compartments approach each other depends not only on the rate of transfer of labeled cholesterol from the serum to the tissues but also on the rate of decay of labeled cholesterol in the serum compartment. Initially, the blood levels of C^{14}-cholesterol specific activity decrease rapidly by a series of decreasing exponential rates (13, 19, 20) and, during the periods in which the blood cholesterol radioactivity is greatest, more labeled cholesterol enters the tissues than at later stages when lesser concentrations of labeled cholesterol are present in the blood. Nevertheless, these studies are useful in comparing relative rates of exchange of serum and tissue cholesterol and, when they are conducted over intervals long enough to allow complete equilibration to occur, they do yield information needed to identify the tissue compartments comprising the miscible pool of cholesterol.

SUMMARY AND CONCLUSIONS

1. Post-mortem analyses of tissues for specific activity of C^{14}-cholesterol have been performed in nine patients who died 1 to 226 days after receiving tracer doses of C^{14}-labeled cholesterol.

2. The rate of equilibration of serum and tissue cholesterol varied among the different body tissues.

3. With the exception of the cholesterol in the brain and blood vessels, all tissues studied attained complete equilibration with serum cholesterol within 1 month and maintained it throughout the remainder of the 7-month study.

4. The brain cholesterol showed negligible degrees of equilibration with serum cholesterol throughout the period of study.

5. Complete equilibration of arterial intimal cholesterol with serum cholesterol was demonstrated after prolonged periods of study.

6. Significant C^{14}-cholesterol radioactivity was present in atherosclerotic plaques of the patients studied, but the presence of atherosclerosis appeared to retard the rate at which equilibration with serum cholesterol occurred.

7. The miscible body pool of cholesterol in man includes the cholesterol in all the body tissues outside the nervous system.

8. The cholesterol in normal human blood vessels is in a dynamic state of equilibrium with serum cholesterol.

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