Cholesterol synthesis and esterification in experimental xanthoma tissues

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Abstract We studied cholesterol metabolism in experimental xanthoma tissues which were induced by injection of high molecular weight sodium dextran sulfate into the dermis of hypercholesterolemic rabbits. Control studies were performed on dermal specimens of the dextran sulfate-injected site of normolipemic rabbits. Cholesterol accumulation was much greater in the hypercholesterolemic rabbit tissues than in the normolipemic rabbit tissues. Histiocytes and foam cells in such lesions had an ability to synthesize cholesterol. However, cholesterol synthesis was suppressed in the cholesterol-rich tissues of hypercholesterolemic rabbits. This suppression was obviously caused by the accumulation of cholesterol in the tissues which take up lipoprotein in hypercholesterolemic rabbits. On the other hand, esterification of cholesterol was greater in the hypercholesterolemic rabbit tissues than in the normolipemic rabbit tissues. Cholesterol was esterified more selectively with oleic acid than with palmitic acid. Therefore, cholesteryl oleate increased in the tissues concomitantly with the accumulation of cholesteryl esters. Fatty acids of serum origin rather than those synthesized in situ were more important in the esterification process. It was suggested that cholesterol esterification was mediated by acyl-coenzyme A:cholesterol acyltransferase and that lecithin:cholesterol acyltransferase contributed little to the process.—**Kodama, H., Y. Nagao, K. Arakawa, J. Tada, and N. Nohara.** Cholesterol synthesis and esterification in experimental xanthoma tissues. *J. Lipid Res.* 1981. **22:** 1033–1041.

Supplementary key words foam cells · dextran sulfate · acyl-coenzyme A:cholesterol acyltransferase · lecithin:cholesterol acyltransferase

Xanthoma is one of the diseases of cholesterol accumulation in peripheral tissues and it may develop as a skin manifestation of hyperlipoproteinemia. Foam cells that contain cholesterol, especially cholesteryl esters, in their cytoplasm infiltrate into xanthoma tissues.

Previous experimental xanthomas have been induced by feeding animals a diet supplemented with cholesterol or lanolin. In such animals, however, individual differences are observed in the period from the beginning of the feeding to the spontaneous development of xanthomas and in the severity of the xanthomas. We (1) have reported that intradermal injection with high molecular weight sodium dextran sulfate (DS) induces infiltration of histiocytes into the dermis of both normolipemic (NLR) and hypercholesterolemic rabbits (HCR). The histiocytes contain DS in their cytoplasm and transform into foam cells. Sudan III staining shows that lipid granules accumulate in the foam cells of HCR after repeated DS injections at the same site. On the other hand, lipid accumulation in the histiocytes of NLR is minimal even if the injection is repeated at the same site. According to the lipid analytical studies, cholesteryl ester contents in the lesions of HCR increase remarkably, concomitantly with the injection frequency, and accumulation of free cholesterol is less prominent than that of cholesteryl esters. Cholesterol in the lesions of NLR shows little increase after repeated injections. These observations indicate that the intradermal DS injection induces a useful experimental xanthoma of maturation (degree of cholesterol accumulation) at a desired site of HCR.

We (1) have postulated that foam cells incorporate plasma lipoproteins and that cholesterol accumulates in the xanthomatous lesions of HCR induced by the intradermal DS injection. One possible explanation is that cholesterol-fed animals have peculiar lipoproteins that have high affinity to histiocytes. It has been observed that cultured arterial smooth muscle cells (2, 3) and cultured aortic medial cells (4, 5) accumulate more cholesteryl esters in the presence of serum or LDL from hypercholesterolemic animals. Recently, Goldstein and co-workers (6) have reported that the synthesis and accumulation of cholesteryl esters by mouse peritoneal macrophages is stimulated by incubation with β-VLDL (very low density lipo-

Abbreviations: NLR, normolipemic rabbit; HCR, hypercholesterolemic rabbit; DS, sodium dextran sulfate; MEM, minimum essential medium; FBS, fetal bovine serum; TLC, thin-layer chromatography; LDL, low density lipoprotein; β-VLDL, very low density lipoproteins with β mobility on electrophoresis; HDL_c, cholesterol-induced high density lipoprotein; ACAT, acyl-coenzyme A:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase.

proteins with β electrophoretic mobility) isolated from the plasma of cholesterol-fed dogs, and that LDL, HDL_c (cholesterol-induced high density lipoproteins), and apoprotein-E HDL_c of the same hypercholesterolemic dogs have little or no stimulatory effect on the accumulation of cholesteryl esters. A second possibility is that histiocytes readily incorporate an LDL-DS complex, and cholesteryl esters accumulate in these cells. It is well known that LDL is able to complex with DS (7, 8). Basu et al. (9) have demonstrated by in vitro studies that mouse peritoneal macrophages ingest large amounts of LDL-DS complex.

In the present study, we have observed cholesterol metabolism in experimental xanthoma tissues of HCR, with untreated skin and DS-injected dermis of NLR as controls.

MATERIALS AND METHODS

Sodium [2-^{14}C]acetate (58 mCi/mmol, 66,495 dpm/nmol) and [D-U-^{14}C]glucose (295 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. [1-^{14}C]Oleic acid (45–55 mCi/mmol) and [1-^{14}C]palmitic acid (45–55 mCi/mmol) were purchased from Commissariat a L'Energie Atomique. Eagle's minimum essential medium (MEM), purchased from Nissui Seikagaku Co., Ltd., was added with glutamine and $NaHCO_3$ as indicated. Fetal bovine serum (FBS), obtained from Grand Island Biochemical Co., was heat-inactivated (56°C, 30 min) before use. DS (Sodium Dextran Sulphate 500, from dextran with molecular weight 500,000) was purchased from Pharmacia Fine Chemicals.

Preparation of dermal specimens

Eleven New Zealand white rabbits were purchased. Six rabbits were fed commercial chow pellets without added cholesterol. The other five were rendered hypercholesterolemic by consuming a diet supplemented with 2% cholesterol. The fasting serum cholesterol level of each of the normolipemic rabbits (NLR 1 through 6), was 31 mg/dl, 24 mg/dl, 26 mg/dl, 58 mg/dl, 38 mg/dl, and 39 mg/dl, respectively. The serum cholesterol level of each of the hypercholesterolemic rabbits (HCR 1 through 5) was 997 mg/dl, 1,280 mg/dl, 623 mg/dl, 1,189 mg/dl, and 485 mg/dl, respectively. Papular xanthomas were disseminated on the dorsum and buttocks of HCR 2. Histological examination revealed foam cell infiltration in the xanthoma tissues. Spontaneously developed xanthomas were not observed on the other hypercholesterolemic rabbits by visual inspection. Intradermal injection with 0.2 ml of 2% DS solution was repeated at the same site of the dorsum two or three times at an interval of 5 days. The DS solution was sterilized by filtration through a 0.45-μm Millipore filter before use.

Rabbits were anesthetized by intravenous ketamine injection and skin specimens were obtained by excision from the DS-injected site and the untreated site without local anesthesia to prevent the contamination with anesthetics. After removal of epidermis and subcutaneous tissues, dermal specimens were cut into pieces 1.5 mm in thickness at most. Each specimen of about 100 mg (wet weight) was prepared and was dispensed in a Petri dish.

Cholesterol synthesis and cholesterol esterification with in situ synthesized fatty acids in dermal specimens

Cholesterol synthesis in experimental xanthoma tissues was assayed by counting the radioactivity of free cholesterol and the cholesterol moiety of cholesteryl esters in the tissues which were incubated with [^{14}C]-acetate. Cholesterol esterification with fatty acids synthesized in situ was analyzed by counting the radioactivity of the fatty acid moiety of cholesteryl esters.

For the preliminary study, we investigated the incubation time. Two rabbits, NLR 1 and HCR 1, were intradermally injected with the DS solution at the same site three times at intervals of 5 days. The dermal specimens were obtained 5 days after the third injection. They were dispensed in four Petri dishes and were incubated for 6, 12, 24, and 36 hr with 30 μCi [^{14}C]acetate in 3 ml of Eagle's MEM without serum in a humidified incubator (5% CO_2 and 95% air) at 37°C. Triplicate batches of dermal specimens of NLR 2 and HCR 2 were obtained from an untreated site and the DS-injected site. In this instance, there were two injections, 5 days apart, and the skin specimens were obtained 10 days after the second injection. Each sample was incubated for 24 hr with 30 μCi [^{14}C]acetate in 3 ml of Eagle's MEM with and without 10% FBS.

After the incubation, lipids were extracted from the skin specimens with chloroform–methanol 2:1. Cholesteryl esters, triglycerides, free fatty acids, free cholesterol, and polar lipids were separated by thin-layer chromatography on silica gel with 5% binder. The plates were developed in petroleum ether–ethyl ether–acetic acid 82:18:1. Each lipid class was extracted from the corresponding band after visualization in iodine vapor.

Half of the free cholesterol and cholesteryl ester sample was analyzed quantitatively. The remainder of the free cholesterol was dissolved in 1 ml of ethanol and mixed with 1 ml of 1% digitonin solution in 50% ethanol. After standing overnight, the solution was

centrifuged at 1,500 *g* for 10 min. The cholesterol digitonide was dissolved in Aquasol 2 (New England Nuclear) and was assayed for radioactivity in a liquid scintillation spectrometer (Aloka LSC-653, Aloka Co.). The remainder of the cholesteryl esters in each specimen was saponified with 2% KOH in 95% ethanol according to the method described by Newman, Gray, and Zilversmit (10). Both the cholesterol and fatty acids were dissolved in the scintillator and counted.

Cholesterol synthesis in dermal specimens of NLR in the presence of either NLR serum or HCR serum

Dermal specimens of NLR 3 were obtained from a site 10 days after the second injection. Triplicate samples were incubated for 24 hr with 20 μCi of [^{14}C]-acetate in 2 ml of Eagle's MEM that was supplemented with either 15% NLR 3 serum or 15% HCR 3 serum. After the incubation, free cholesterol and cholesteryl esters in the specimens were quantitatively analyzed. Radioactivity in free cholesterol and the cholesterol fraction of cholesteryl esters was also assayed.

Incorporation of in situ synthesized fatty acids into cholesteryl esters, triglycerides, and lecithin in experimental xanthoma tissues

Triplicate dermal specimens of NLR 4 and HCR 3 were obtained from the twice-injected site and were incubated for 24 hr with 30 μCi of [^{14}C]acetate in 3 ml of Eagle's MEM. Fatty acids from cholesteryl esters were isolated as described above. Lecithin was obtained from the polar lipid fraction by TLC according to the method of Skipski, Peterson, and Barclay (11). Triglycerides and lecithin were saponified in 15 ml of 2% NaOH in 95% ethanol for 2 hr at 60°C. After acidification with 0.2 N H_2SO_4, the fatty acids were extracted with petroleum ether. Each fatty acid fraction was assayed for radioactivity.

Incorporation of in situ synthesized fatty acids into cholesteryl esters separated according to the degree of unsaturation

Triplicate dermal specimens of the twice-injected site of NLR 3 and HCR 3 were incubated for 24 hr with 20 μCi of [^{14}C]acetate in 3 ml of Eagle's MEM. Cholesteryl esters were separated according to degree of unsaturation by TLC on silica gel plates impregnated with silver nitrate (12). The radioactivity in saturated fatty acids, monoenoic acids, dienoic acids, and the other fractions was assayed.

Incorporation of [^{14}C]palmitate and [^{14}C]oleate into cholesteryl esters, polar lipids, and triglycerides

The sodium salts of [^{14}C]palmitic acid and [^{14}C]oleic acid were combined with bovine serum albumin by the method described by St. Clair, Smith, and Wood (3). The specific activities of palmitate and oleate were 21,201 dpm/nmol and 19,323 dpm/nmol, respectively. Either [^{14}C]palmitate or [^{14}C]oleate was added to Eagle's MEM with and without 10% FBS in a final concentration of 2 μCi/ml. Dermal specimens of NLR 5 and HCR 4 were obtained from the twice-injected site 10 days after the second injection. Triplicate samples of each specimen were incubated for 24 hr in 2 ml of each medium. This experiment was planned to investigate whether cholesterol is esterified with palmitate and oleate of serum origin and to determine which of the two fatty acids plays a dominant role in cholesterol esterification.

Incubation of specimens with [^{14}C]glucose

Duplicate dermal specimens from the twice-injected site of NLR 2 and HCR 3 were incubated for 24 hr in Eagle's MEM that contained 9 μCi of [^{14}C]glucose and co-factors (20 μM ATP, 80 μM CoA, 10 μM NADH, 10 μM NADPH, 20 μM Mg^{2+}, and 20 μM Mn^{2+}). This experiment was performed both in the presence and absence of 10% FBS.

Fatty acid composition of cholesteryl esters

NLR 6 and HCR 5 were intradermally injected with the DS solution at dorsal sites one to three times at an interval of 5 days. Duplicate specimens were obtained from an uninjected site and from each injected site 5 days after the last injection. Cholesteryl esters were saponified by the method described above. The fatty acid fraction was esterified with diazomethane and the methyl esters were analyzed by gas–liquid chromatography using a gas chromatograph (Yanaco G-80 FP, Yanaco Ltd.) equipped with a flame ionization detector. A glass column (0.3 × 225 cm) packed with 10% Silicone EGSS-X, Chromosorb W (AW) DMCS (60–80 mesh, Yanaco Ltd.) was used. Injection temperature was 210°C and oven temperature was maintained at 190°C. The carrier gas was nitrogen at flow rate of 17.5 ml/min.

RESULTS

Cholesterol contents and cholesterol synthesis in dermal specimens

Fig. 1 shows the time course of radioactivity in free cholesterol and cholesteryl esters of both HCR 1 specimens and NLR 1 specimens that were incubated with [^{14}C]acetate. The radioactivity of HCR specimens increased markedly during the 24-hr incubation and then reached a steady-state plateau. Radioactivity of the NLR specimens continued to rise for at least 36 hr.

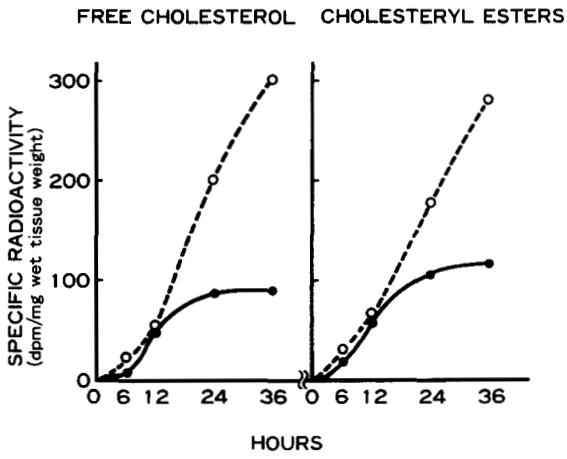

Fig. 1. Time course of radioactivity of free cholesterol and cholesteryl esters in the dermal specimens from a normolipemic rabbit (○) and a hypercholesterolemic rabbit (●) according to the incubation time. The skin specimens were obtained 5 days after the third injection from the site where 0.2 ml of 2% sodium dextran sulfate solution was intradermally injected three times at an interval of 5 days. They were incubated with 30 μCi of [^{14}C]acetate in 3 ml of Eagle's minimum essential medium without serum for 6, 12, 24, and 36 hr. Each value represents a single incubation.

Therefore, 24-hr was used as the suitable incubation time in further investigations.

The free and esterified cholesterol contents in the specimens of NLR 2 and HCR 2 are shown in **Fig. 2.** Cholesterol in the untreated NLR specimens was minimal and a slight increase was observed in the DS-injected NLR specimens. Both free cholesterol and cholesteryl esters accumulated in the untreated HCR specimens. This accumulation was apparently caused by the presence of spontaneously developed foam cells in the dermis of HCR 2. The most striking finding was a large increase in cholesteryl esters in the DS-injected HCR specimens. Fig. 2 also shows the radioactivity of free cholesterol and the cholesterol moiety of cholesteryl esters in the NLR 2 and HCR 2 specimens that have been incubated with [^{14}C]acetate in Eagle's MEM with and without FBS. In both NLR specimens and HCR specimens, the radioactivity of free cholesterol and the cholesterol moiety of cholesteryl esters was larger in the DS-injected site than in the untreated site. An important observation was that the radioactivity was smaller in HCR specimens than in NLR specimens, especially in free cholesterol of the DS-injected site. FBS in the medium had slight effects on cholesterol synthesis in both NLR specimens and HCR specimens. Apparently histiocytes and foam cells in the specimens have an ability to synthesize cholesterol from acetate and large amounts of cholesterol are synthesized in NLR tissues which contain histiocytes with little cholesterol accumulation. In the HCR specimens with cholesterol-rich foam cells, however, cholesterol synthesis was greatly suppressed.

Data on cholesterol synthesis in the DS-injected NLR 3 specimens, in the presence of NLR 3 serum or in the presence of HCR 3 serum, are shown in **Fig. 3.** A large increase in cholesteryl ester and a moderate increase in free cholesterol were observed in the specimens that had been incubated in the medium with HCR serum. The radioactivity of free

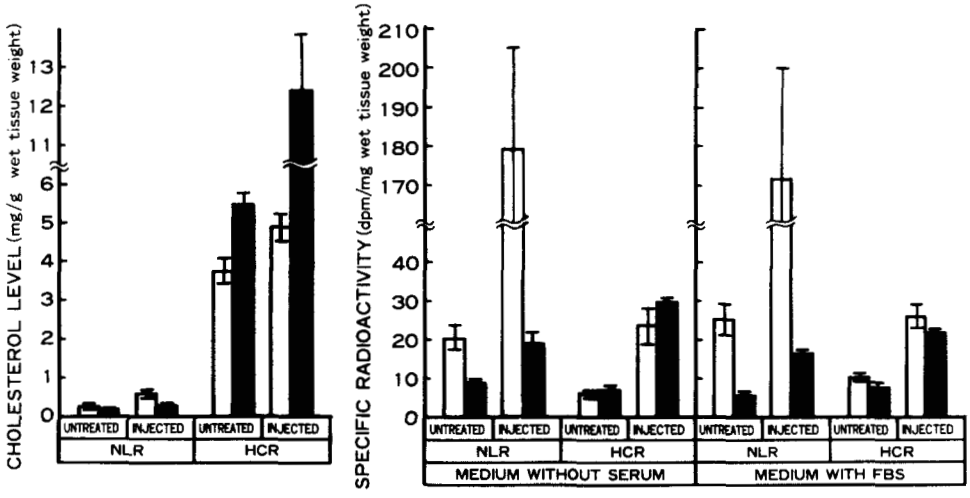

Fig. 2. Amount (left panel) and radioactivity (right panel) of free cholesterol (□) and the cholesterol moiety of cholesteryl esters (■) in dermal specimens from a normolipemic rabbit (NLR) and a hypercholesterolemic rabbit (HCR). The dermal specimens were obtained from the untreated site and, 10 days after the second injection, from the site where 0.2 ml of 2% sodium dextran sulfate solution was intradermally injected twice at an interval of 5 days. They were incubated for 24 hr with 30 μCi of [^{14}C]acetate in 3 ml of Eagle's minimum essential medium both with and without 10% fetal bovine serum. Each value represents the mean ± SD of triplicate incubations.

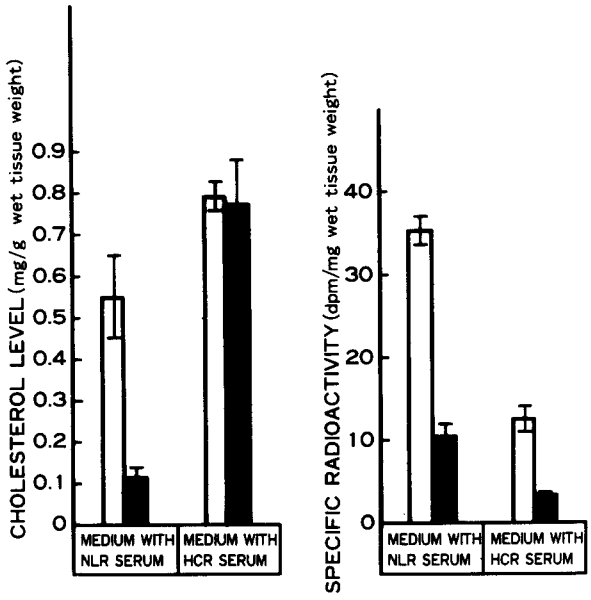

Fig. 3. Amount (left panel) and radioactivity (right panel) of free cholesterol (□) and cholesterol moiety of cholesteryl esters (■) in the dermal specimens from a normolipemic rabbit which were incubated for 24 hours with 20 μCi of [^{14}C]acetate in 2 ml of Eagle's minimum essential medium either with 15% normolipemic rabbit serum or 15% hypercholesterolemic rabbit serum. The dermal specimens were obtained 10 days after the second injection from the site where 0.2 ml of 2% sodium dextran sulfate solution was intradermally injected twice at an interval of 5 days. Each value represents the mean ± SD of triplicate incubations.

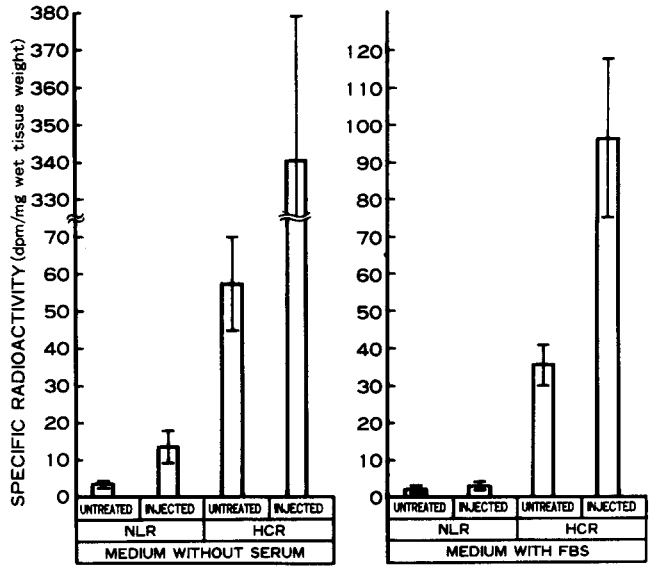

Fig. 4. Radioactivity of fatty acids of cholesteryl esters of the same specimens as shown in Fig. 2.

cholesterol and the cholesterol moiety of cholesteryl esters was much smaller in the specimens that had been incubated with HCR serum. This finding indicates that cholesterol synthesis in the tissues is more reduced in the presence of HCR serum than in the presence of NLR serum. The suppression of cholesterol synthesis is apparently caused by the accumulation of cholesteryl esters in such tissues.

Cholesterol esterification with in situ synthesized fatty acids in dermal specimens

Although the incorporation of [^{14}C]acetate was larger in free cholesterol than in esterified cholesterol of the NLR 2 specimens, most of the synthesized cholesterol was esterified in the HCR 2 specimens (Fig. 2). On the other hand, incorporation into the fatty acids of cholesteryl esters was slight in the NLR 2 specimens and very high in the HCR 2 specimens, especially in the DS-injected site (**Fig. 4**). Apparently, in the cholesterol-rich HCR specimens, cholesterol is readily esterified with the fatty acids that are synthesized in situ.

Source of fatty acids used in cholesterol esterification

Esterification of cholesterol with in situ synthesized fatty acids was reduced in the presence of FBS, especially in the cholesterol-rich HCR 2 specimens (Fig. 4). Total fatty acid synthesis was not suppressed in the presence of FBS (data not shown). When NLR and HCR specimens were incubated with [^{14}C]palmitate or [^{14}C]oleate, incorporation of radioactivity was more pronounced in the HCR specimens (**Fig. 5**) and the incorporation was suppressed in the presence of FBS (data not shown). It is suggested that not only synthesized fatty acids but the fatty acids in the circulation play a role in cholesterol esterification.

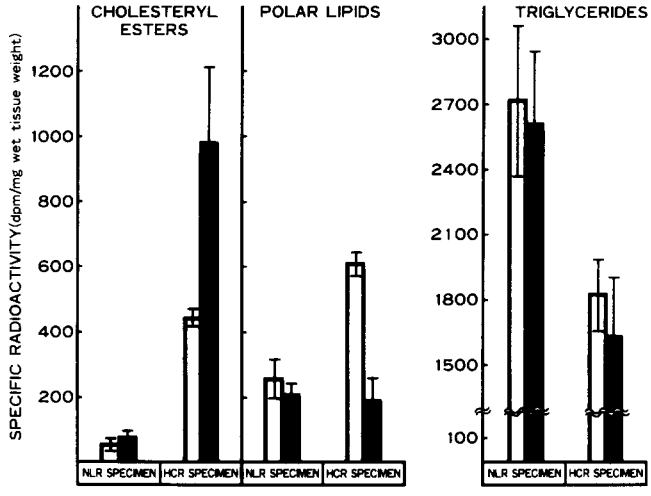

Fig. 5. Incorporation of [^{14}C]palmitate (□) and [^{14}C]oleate (■) into cholesteryl esters, polar lipids, and triglycerides in dermal specimens from a normolipemic rabbit (NLR) and a hypercholesterolemic rabbit (HCR). The dermal specimens were obtained 10 days after the second injection from the site where 0.2 ml of 2% sodium dextran sulfate solution was intradermally injected twice at an interval of 5 days. Each value represents the mean ± SD of triplicate incubations.

The fatty acid composition of cholesteryl esters in the DS-injected specimens showed characteristic findings according to the frequency of injection (**Table 1**). The most characteristic findings were observed for oleic and palmitic acids. Oleic acid was the major component of cholesteryl ester fatty acids in the HCR 5 serum. On the other hand, palmitic acid was the major component of cholesteryl esters of NLR 6 serum. Similarity of the fatty acid pattern of cholesteryl esters was not observed between the serum and the dermal specimens. Cholesteryl oleate was low in the HCR specimens of the untreated site and the once-injected site. It increased dramatically at the sites where the DS was injected two or three times. In the latter instance, oleic acid comprised about 60% of total cholesteryl ester fatty acids. On the other hand, palmitic acid was the major component in the specimens from the untreated site and from the site with only one injection and it was reduced considerably in specimens from sites of two or three injections. Myristic acid and stearic acid decreased and palmitoleic acid and linoleic acid increased concomitantly with the injection frequency. The increase in oleic acid and the decrease in palmitic acid with the injection frequency were not extreme in the NLR specimens. The changes in composition of the other fatty acids in the NLR specimens were similar to those in the HCR specimens.

When the dermal specimens from the twice-injected sites of NLR 3 and HCR 3 were incubated with [^{14}C]-acetate, most of the isotope was incorporated into cholesteryl esters of saturated fatty acids and the incorporation of [^{14}C]acetate into cholesteryl esters of monoenoic acids was minimal (**Table 2**). It was concluded that fatty acids synthesized in situ do not play an important role in cholesterol esterification.

Fatty acids that are preferably esterified with cholesterol

The radioactivity of [^{14}C]palmitate and [^{14}C]oleate that was incorporated into various lipid classes of the NLR 5 specimens and the HCR 4 specimens in the absence of FBS is shown in Fig. 5. Incorporation of ^{14}C from the two labeled acids into the cholesterol moiety of cholesteryl esters and into the glycerol moiety of triglycerides and polar lipids was negligible (data not shown). When the DS-injected skin specimens of NLR and HCR were incubated with [^{14}C]palmitate or [^{14}C]-oleate, the amount of the incorporated palmitate and oleate into triglycerides was apparently similar. More palmitate than oleate was incorporated into polar lipids. On the other hand, a larger amount of [^{14}C]-oleate than [^{14}C]palmitate was incorporated into cholesteryl esters of HCR specimens. These findings indicate that cholesterol of cholesterol-rich xanthoma tissues is more actively esterified with oleate than with palmitate of serum origin.

Pathway of cholesterol esterification

Incorporation of [^{14}C]acetate into fatty acids of cholesteryl esters, lecithin, and triglycerides was compared in the NLR 4 specimens and in the HCR 3 specimens of the DS-injected site (**Fig. 6**). Although cholesterol esterification with in situ synthesized fatty acids was greater in the HCR specimens than in the NLR specimens, there was much more incorporation of [^{14}C]acetate into fatty acids of lecithin in the NLR

TABLE 1. Cholesterol (total or ester) level and fatty acid composition of cholesteryl esters in dermal specimens and serum of a normolipemic rabbit (NLR) and a hypercholesterolemic rabbit (HCR)

		Fatty Acid Composition of Cholesteryl Esters												Cholesteryl Esters	
		NLR						HCR							
	Injections	14:0	16:0	16:1	18:0	18:1	18:2	14:0	16:0	16:1	18:0	18:1	18:2	NLR	HCR
		%						%						*mg/g wet weight*	
Dermal specimens[a]	0	13.5	54.7	2.7	23.6	4.4	1.1	11.4	61.6	3.3	16.2	5.1	2.4	1.8	2.2
	1	11.2	60.4	3.2	16.4	7.3	1.5	8.4	61.9	4.1	14.6	8.1	2.9	0.9	2.5
	2	8.2	60.7	4.9	11.5	10.2	4.5	2.4	21.8	8.6	3.5	47.0	16.7	0.8	7.5
	3	7.9	41.0	10.4	9.1	24.6	7.0	1.5	12.5	8.1	2.1	60.9	14.9	0.8	8.7
														Total Cholesterol *mg/dl*	
Serum		5.2	62.9	2.6	11.1	13.8	4.4	0.3	29.5	8.3	5.7	49.9	6.3	39	485

[a] NLR and HCR were intradermally injected with 0.2 ml of 2% sodium dextran sulfate solution one to three times at the same site at an interval of 5 days. Dermal specimens were obtained from untreated sites and from each injected site 5 days after the last injection. Each value, except for total cholesterol level of serum, represents the average of values obtained from duplicate specimens of a rabbit. The value of serum total cholesterol level represents a single assay.

TABLE 2. Incorporation of [^{14}C]acetate into cholesteryl esters in dermal specimens of a normolipemic rabbit (NLR) and a hypercholesterolemic rabbit (HCR)

Fatty Acid Class[b]	NLR Specimens[a]	HCR Specimens[a]
	dpm/mg wet weight	
Saturated	15.3 ± 5.2	89.6 ± 32.0
Monoenoic	3.1 ± 1.2	4.1 ± 1.0
Dienoic	4.8 ± 1.3	9.7 ± 2.1
Others	32.8 ± 6.7	44.6 ± 6.0

[a] NLR and HCR were intradermally injected with 0.2 ml of 2% sodium dextran sulfate solution twice at the same site at an interval of 5 days. The dermal specimens were obtained from the injected site 10 days after the second injection and were incubated for 24 hr with 20 μCi of [^{14}C]acetate in 3 ml of Eagle's minimum essential medium without serum.

[b] Cholesteryl esters were separated according to the degree of unsaturation of the fatty acid moiety.

Data are means ± SD of triplicate incubations.

specimens than in the HCR specimens. As shown in Fig. 5, when the HCR 4 specimens of the DS-injected site were incubated with [^{14}C]palmitate or [^{14}C]oleate, cholesterol was esterified with more oleate than palmitate and polar lipids incorporated more palmitate than oleate. These findings indicate that cholesterol esterification with the 2-fatty acids of lecithin is not the main pathway. It is suggested that acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity is more important than lecithin:cholesterol acyltransferase (LCAT) activity in cholesterol esterification.

Glucose as a substrate of cholesterol synthesis and cholesterol esterification

Using [^{14}C]glucose as a substrate, there was only a little radioactivity detected in free cholesterol, the cholesterol moiety of cholesteryl esters, and the fatty acids of cholesteryl esters in every specimen of NLR 2 and HCR 3, both in the presence and absence of FBS. However, there was considerable radioactivity in triglycerides (data not shown). Apparently glucose does not play a dominant role in cholesterol synthesis and cholesterol esterification in the experimental xanthoma tissues.

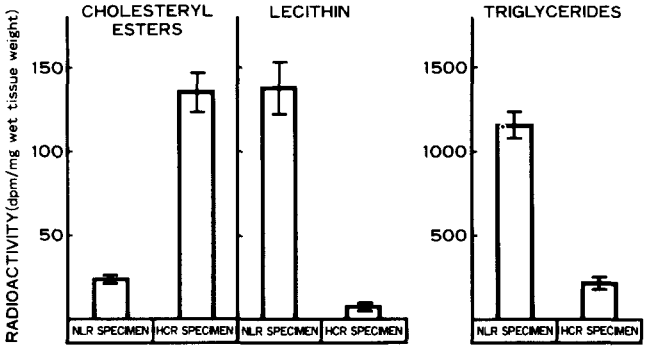

Fig. 6. Radioactivity in fatty acids of cholesteryl esters, lecithin, and triglycerides in dermal specimens from a normolipemic rabbit (NLR) and a hypercholesterolemic rabbit (HCR), after a 24-hr incubation with 30 μCi of [^{14}C]acetate in 3 ml of Eagle's minimum essential medium without serum. The dermal specimens were obtained 10 days after the second injection from the site where 0.2 ml of 2% sodium dextran sulfate solution was intradermally injected twice at an interval of 5 days. Each value represents the mean ± SD of triplicate incubations.

DISCUSSION

It has been shown that plasma lipoproteins contribute to the cholesterol accumulation in foam cells of xanthoma by the following findings: *1*) hyperlipemic xanthoma disappears spontaneously as the plasma lipid level is normalized; *2*) the rate of cholesterol synthesis is very low in human xanthoma and experimental rabbit xanthoma (13); *3*) human xanthoma tissue incorporates intravenously administered isotopically-labeled LDL (14) or cholesterol (15, 16); *4*) enzyme histochemical study of human xanthoma has revealed that foam cells have a rather high activity of lysosomal enzymes and they show no evidence of an increased lipid synthetic activity (17); and *5*) immunohistochemical study of experimental rabbit xanthoma (18) and human xanthoma (19) has revealed the presence of β-lipoproteins in the cytoplasm of foam cells. However, dynamic studies on cholesterol metabolism in xanthoma tissues have not been done.

Goldstein and Brown (20) have shown that most of the plasma LDL is degraded by the LDL pathway that is mediated by LDL-specific receptors on extrahepatic parenchymal cells. In these cells, incoming cholesteryl esters are hydrolyzed in lysosomes and suppress the cholesterol synthesis in the cells by the reduction of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The excess cholesterol is reesterified and stored in the cytoplasm. Goldstein and Brown (21) have also postulated that one-third of the LDL in normal man is degraded through the scavenger pathway that is mediated by macrophage-like cells including dermal histiocytes. According to the scavenger pathway concept, scavenger cells take up LDL and the endocytosis of LDL is not controlled by the LDL receptors. In the patients with homozygous familial hypercholesterolemia, these cells overaccumulate cholesterol and transform into foam cells. Brown and his colleagues (22) have demonstrated the hydrolysis-reesterification mechanism of cholesteryl esters in the mouse peritoneal macrophages that have incorporated and degraded acetylated LDL and subsequently accumulated cholesteryl esters. This mechanism indicates that the incoming cholesteryl esters of acetylated LDL are hydrolyzed in lysosomes and the resultant free cholesterol is reesterified by

ACAT in the cytosol where the newly formed esters are stored as lipid droplets. Foam cells of xanthoma are derived from dermal histiocytes (23) and are thought to be a model of scavenger cells that have injested large amounts of LDL cholesterol (21).

Although Wilson (13) has reported that cholesterol synthesis in xanthoma tissues is minimal, histiocytes and foam cells of the present experimental xanthoma are shown to have an ability to synthesize cholesterol. However, cholesterol synthesis was suppressed in the cholesterol-rich tissues of HCR. This suppression is obviously caused by the accumulation of cholesterol in the tissues that have incorporated hypercholesterolemic rabbit serum lipoproteins.

Baes, van Gent, and Pries (24) have pointed out that more cholesteryl esters accumulate in the older human xanthoma tissues and the major fatty acid component of cholesteryl esters is oleic acid. Parker, Peterson, and Odland (25) have observed that cholesteryl esters increase gradually in the nuchal skin of hypercholesterolemic rabbits after consuming a cholesterol-supplemented diet. In the present study, the rate of cholesterol esterification increased in the tissues that contained large amounts of cholesterol.

Even though foam cells of HCR accumulate cholesterol of serum origin, the fatty acid composition of cholesteryl esters in the experimental xanthoma tissues of early stages is not similar to that of the serum cholesteryl esters. This finding indicates that cholesteryl esters in the tissues do not reflect the serum cholesteryl esters and that resynthesis of cholesteryl esters occurs in the tissues. In all probability, hydrolysis of cholesteryl esters and reesterification of the residual free cholesterol takes place in such tissues. According to former studies on atherosclerosis, esterification of cholesterol is thought to be mediated by LCAT (26, 27) or by ACAT (28). Hashimoto et al. (28) have reported that cholesterol is esterified selectively with oleic acid by ACAT in atherosclerotic lesions. Our study has also revealed that cholesterol is esterified with oleic acid rather than with palmitic acid in the experimental xanthoma tissues. Furthermore, it is suggested that ACAT is more important than LCAT in the cholesterol esterification. Thus, oleic acid increases and palmitic acid decreases in the fatty acids of cholesteryl esters as the xanthomatous lesions mature with accumulation of cholesteryl esters. Although the rate of cholesterol esterification with fatty acids synthesized in situ increases in the tissues that contain large amounts of cholesterol, serum free fatty acids are the main source of fatty acids that contribute to cholesterol esterification.

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