The sterol substrate specificity of acyl CoA:cholesterol acyltransferase from rat liver

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Abstract Rat liver microsomes were incubated with various sterols suspended in Triton WR-1339, and the extent of esterification of these sterols by acyl CoA:cholesterol acyltransferase was determined. A 3β-hydroxyl group was required for esterification to occur. Furthermore, the rate of ester formation of campesterol was only 20% that of cholesterol, and the rates for sitosterol and stigmasterol were below detectable limits indicating that the structure of the alkyl side chain plays an important role in the interaction between substrate and enzyme. Additional evidence concerning the importance of the side chain was obtained by following the esterification of a series of linear side chain analogues of cholesterol. Maximal ester formation was obtained when the longest chain on C-20 had five carbons (the same as cholesterol) and either an increase or decrease in the number of carbons reduced the amount of ester formed. Sterols containing a 4-*gem*-dimethyl group were not esterified, while 4α-methylcholest-7-en-3β-ol showed significant esterification. Lathosterol, cholestanol, and desmosterol were esterified 41%, 70%, and 62%, respectively, as well as was cholesterol. The relationship between the specificity of acyl CoA:cholesterol acyltransferase and the occurrence of sterol esters in tissues is discussed.—**Tavani, D. M., W. R. Nes, and J. T. Billheimer.** The sterol substrate specificity of acyl CoA:acyltransferase from rat liver. *J. Lipid Res.* 1982. **23:** 774–781.

Supplementary key words sterol esters • esterification • 24-alkylsterols • lanosterol • lathosterol • desmosterol • cholestanol

The functional integrity of the mammalian membrane appears to require cholesterol. Although this sterol is the principal sterol biosynthesized by mammals, sterols with longer or shorter side chains or with a different extent of branching in the side chain exist in organisms which can form a part of the mammalian diet (1). In addition, the diet can provide Δ^7 and $\Delta^{5,24}$ sterols and these, along with sterols methylated at C-4 and C-14, are present as precursors of cholesterol (2). It has been estimated that, in man, about 5% of the daily intake of phytosterols may be absorbed, and in rat 1% of sterol in the liver can be from plant origin (3, 4). About 16% of total sterol in rat is thought to be cholesterol precursors although most of this is present in the skin (5). In addition, the presence of cholestanol and sitosterol have been associated with two disease states, cerebrotendinous xanthomatosis and β-sitosterolemia, respectively (6, 7). Because of the normal presence of low amounts of sterols other than cholesterol and the apparent morbidity associated with high concentrations of these sterols, several investigations have been carried out with mammals concerning the possible metabolism of these sterols by pathways which metabolize cholesterol, namely bile acid synthesis, steroid hormone synthesis, and cholesteryl ester formation. The first enzyme in bile acid synthsis, 7α-cholesterol hydroxylase, is very specific for cholesterol as a substrate; neither sitosterol nor cholesterol analogues differing in the length of their alkyl side chain are oxidized (8–10). On the other hand, the side chain cleavage enzymes that convert cholesterol to pregnenolone can utilize sitosterol, the side chain analogues of cholesterol, and some oxygenated cholesterol derivatives as substrates (10–12). The enzyme responsible for the intracellular esterification of cholesterol is acyl CoA:cholesterol acyltransferase (ACAT; EC 2.3.1.26). ACAT is a membrane-bound enzyme which is found in the microsomal fraction of a cellular homogenate (13). Investigations concerning the substrate specificity of ACAT have been hampered by the fact that the cholesterol in the membrane can be utilized as a substrate and is preferred over exogenous cholesterol added in an organic solvent (14). Recently, in this laboratory, an ACAT assay has been developed in which the cholesterol is dispersed with the aid of the detergent, Triton WR-1339 (15). The addition of cholesterol in this manner results in a 3-fold increase in ACAT activity due to the utilization of exogenous cholesterol. Utilizing the same assay procedure, we have examined the ability of ACAT to form esters of sterols other than cholesterol.

MATERIALS AND METHODS

Cholesterol was obtained from J. T. Baker and purified through the dibromide. Sitosterol, desmosterol, cholestanol, and campesterol were purchased from Applied Science Laboratories; ergosterol was from ICN;

Abbreviations: ACAT, acyl CoA:cholesterol acyltransferase.

stigmasterol was from Sigma Chemical Co.; lanosterol, lathosterol, epicholesterol, and cholest-4-en-3β-ol were from Steraloids. 4,4-Dimethylcholest-7-en-3β-ol and 4α-methyl-cholest-7-en-3β-ol were prepared as described earlier (16, 17). The side chain analogues of cholesterol (see Fig. 2) were prepared in this laboratory and their structures were identified as described by Nes et al. (18). All sterols were shown to be greater than 98% pure by TLC and HPLC except for the following: after chromatographic purification lanosterol contained about 4% dihydrolanosterol, 4,4-dimethylcholest-7-en-3β-ol contained about 5% of the 3α-ol isomer, and the C_{25}-side chain analogue of cholesterol about 5% of an unidentified impurity. [4-^{14}C]Cholesterol, [1-^{14}C]oleoyl coenzyme A, and [1-^{14}C]oleic acid were obtained from New England Nuclear Corporation, and [4-^{14}C]sitosterol was from the Amersham Corporation. Cholesteryl oleate was purchased from Sigma Chemical Co. Cholesteryl stearate, desmosteryl stearate, lanosteryl stearate, campesteryl stearate, epicholesteryl stearate, and sitosteryl stearate were synthesized according to the procedure of Pinter, Hamilton, and Muldrey (19).

Gas–liquid chromatography was performed on a Hewlett Packard 5840 Gas Chromatograph using a column of 1% XE-60 at 235°C. Separation of sterol esters by high performance liquid chromatography (HPLC) was performed on a Zorbax ODS (C-18) column at 60°C using a mobile phase of acetonitrile–isopropanol 60:40 (v/v). The sterol esters were detected using a LDC Spectromonitor III set at 205 nm. The expected retention time of desmosteryl oleate was obtained by adjustment of the k′ for desmosteryl stearate with a factor for the relative contribution which the Δ^9-double bond in the fatty acid moiety had on mobility. This contribution (0.75) was equal to the k′ of cholesteryl oleate divided by the k′ for cholesteryl stearate. The expected retention time of sitosteryl oleate was determined in the same manner.

The livers were obtained from male Sprague-Dawley rats (200–300 g) and the microsomal fraction was prepared as described previously (15).

The assay of ACAT activity has been described in detail by Billheimer, Tavani, and Nes (15). The standard assay medium contained 100 μg of microsomal protein, 1 mg of fatty acid-free bovine serum albumin in 0.1 M potassium phosphate buffer (pH 7.4), 2 mM glutathione, 0.3% (600 μg) of Triton WR-1339, and 100 μM [1-^{14}C]oleoyl CoA (10,000 dpm/nmol) in a total volume of 0.2 ml. Sterols were added as an aqueous dispersion in Triton WR-1339; the final amount in the assay medium was 20 μg. In competition experiments, cholesterol and a second sterol were each present in the amount of 10 μg. All components except oleoyl CoA were preincubated for 30 min. The reaction was initiated by the addition of [^{14}C]oleoyl CoA and incubated for 15 min. The reaction was stopped by the addition of 4 ml of chloroform–methanol 2:1 (v/v); 10 μg of cholesteryl oleate and [4-^{14}C]cholesterol (20,000 dpm) were added as a marker and internal standard, respectively. After separation into two layers by the addition of 0.8 ml of water, the chloroform layer was removed and the sterol esters were isolated by thin-layer chromatography on Gelman ITLC-SA polysilicic acid gel-impregnated sheets using petroleum ether–diethyl ether–acetic acid 85:15:0.5 (v/v). This system separated cholesteryl oleate (R_f 0.90) from other lipids which could be labeled during the reaction, e.g., triglycerides (R_f 0.74), oleic acid (R_f 0.55), and cholesterol (R_f 0.28). The common 4-desmethylsterols other than cholesterol had the same relative migration as cholesterol except for the 4-methylsterols which were slightly less polar due to shielding of the 3β-hydroxyl group, and epicholesterol which contains a 3α-hydroxyl group. Sterol esters as a lipid class have the same relative migration on silica gel irrespective of chain lengths and degrees of unsaturation (20). In agreement with this the stearate esters of cholesterol, desmosterol, epicholesterol, lanosterol, sitosterol, and campesterol were found to have the same relative mobility as cholesteryl oleate. The spots corresponding to cholesterol and sterol ester were cut out after visualization by spraying with a solution of 10% phosphomolybdic acid in 90% ethanol, placed directly into scintillation vials, and counted using Omnifluor scintillation cocktail. The recoveries of both cholesterol and cholesteryl oleate were about 70% under the above conditions. An assay using boiled microsomes was used as a control. The average specific enzymatic activity in the presence of detergent alone was 1.0 nmol/15 min/mg, and in the presence of 20 μg of cholesterol it was 11.3 nmol/15 min/mg. Protein was determined by the method of Lowry et al. (21).

RESULTS

The sterol substrate specificity of ACAT has not previously been analyzed because the microsomal-bound enzyme is capable of utilizing endogenous membrane cholesterol as a source of substrate and prefers the endogenous cholesterol over exogenous cholesterol added in acetone (14). This inability to esterify exogenous cholesterol appears to be due to an inability of the suspended cholesterol to traverse the aqueous surroundings and reach the membrane in a form utilizable by the enzyme. Investigators in this laboratory have shown that if one disperses the cholesterol in the non-ionic detergent, Triton WR-1339, the exogenous cholesterol is esterified by ACAT and about a 300% increase in activity over the non-supplemented reaction is obtained (15). The in-

crease was shown to be primarily due to esterification of exogenous cholesterol by following the reaction using [^{14}C]cholesterol in the place of [^{14}C]oleoyl CoA. Therefore, the esterification of other sterols can be followed by adding them to the reaction medium in a detergent suspension. The investigation is complicated, however, by the fact that a certain amount of endogenous cholesterol is esterified even in the presence of exogenous sterol. This endogenous esterification, defined as the formation of cholesteryl oleate in the absence of exogenous sterol, was relatively constant at about 1.0 nmol/15 min per mg protein. The esterification of sterols other than cholesterol was obtained by subtracting the esterification due to endogenous cholesterol and is presented as percent esterification versus cholesterol according to the following equation:

$$\% \text{ sterol esterified relative to cholesterol} = 100 \times \frac{(\text{E } \bar{c} \text{ sterol} - \text{E } \bar{s} \text{ sterol})}{(\text{E } \bar{c} \text{ cholesterol} - \text{E } \bar{s} \text{ cholesterol})}.$$

E $\bar{c}$ sterol = esterification in presence of 20 μg of exogenous sterol.

E $\bar{s}$ sterol = esterification in absence of added sterol.

E $\bar{c}$ cholesterol = esterification in presence of 20 μg of exogenous cholesterol.

E $\bar{s}$ cholesterol = esterification in absence of added cholesterol.

With this method sterols that are esterified at a rate at least 5% of that of cholesterol can be measured. Sterols which may be esterified at a lesser rate cannot be detected due to the variability in the assay (about 10%) in which the total ester formed will not differ significantly from the ester formed from endogenous cholesterol in the absence of added sterol.

Data obtained for the esterification of several sterols are shown in **Table 1.** For esterification to occur, the hydroxyl group at C-3 must be in the β-configuration since the α-isomer, epicholesterol, was not esterified.

Campesterol is the only one of the four 24-alkylsterols that is esterified by ACAT, and then only 20% as well as cholesterol. This suggests that the structure of the alkyl side chain is critical in binding to the active site of the enzyme, since campesterol differs from cholesterol only by the addition of a methyl group at C-24. Increasing the size of the substituent at C-24 further to an ethyl group (sitosterol) reduced esterification to below detectable limits. (The lack of esterification of sitosterol was also shown in experiments utilizing [^{14}C]sitosterol as a substrate; data not given).

Sterols that contain a *gem*-dimethyl group at C-4 (e.g., lanosterol) were not esterified (Table 1). The lack of esterification is probably the result of steric hindrance

TABLE 1. The percent esterification of various sterols relative to cholesterol by ACAT in rat liver microsomes[a]

Sterol	Unsaturation in B Ring	Substituent at C-24	Additional Feature	% Activity Relative to Cholesterol[b]
Cholesterol	Δ^5	H		100
3-Epicholesterol	Δ^5	H	3α-ol	<5[c]
Sitosterol	Δ^5	α-Ethyl		<5
Stigmasterol	Δ^5	α-Ethyl	Δ^{22}	<5
Campesterol	Δ^5	α-Methyl		23.4 (16.2, 26.4, 27.6)
Ergosterol	$\Delta^{5,7}$	β-Methyl	Δ^{22}	<5
Lanosterol	Δ^8	H	Δ^{24} 4,4-Dimethyl 14α-Methyl	<5
Cycloartenol	None	H	Δ^{24} 4,4-Dimethyl 14α-Methyl 9,19-Cyclopropane	<5
4,4-Dimethyl-cholest-7-en-3β-ol	Δ^7	H	4,4-Dimethyl	<5
4α-Methyl-cholest-7-en-3β-ol	Δ^7	H	4α-Methyl	42.9 (51.1, 34.6)
Cholestanol	None	H		69.6 (66.5, 72.7)
Desmosterol	Δ^5	H	Δ^{24}	62.0 (57.5, 66.5)
Lathosterol	Δ^7	H		41.4 (38.1, 44.7)
Cholest-4-en-3β-ol	None	H	Δ^4	8.0 (6.4, 9.6)

[a] Microsomes (100 μg) were preincubated for 30 min with 1 mg of fatty acid-free bovine serum albumin, 600 μg of Triton WR-1339, and, when present, 20 μg of the appropriate sterol in a final volume of 200 μl. The reaction was initiated by the addition of 20 nmol [^{14}C]oleoyl-CoA and incubated for 15 min.

[b] Each value represents the mean of at least two experiments in which assays were carried out in triplicate. Means of individual experiments in parentheses. Within each experiment triplicate values agreed within 15% of the mean.

[c] Less than 5 means there was no detectable increase between the amount of esterification in the presence of added sterol and that in the absence of additional sterol.

of the methyl groups at C-4 on the reactivity of the 3β-hydroxyl group. In agreement with this, the sterol 4α-methylcholest-7-en-3β-ol, which contains only one methyl substituent at C-4 is esterified at an appreciable rate (45%). Similar results were obtained by Brady and Gaylor (22) who found about 7-fold more ^{14}C-labeled monomethyl sterol than [^{14}C]dimethyl sterol in the ester fraction when these sterols were incubated with an extract from rat liver.

Within the nucleus of the sterol neither the presence of unsaturation in the B-ring nor, when present, the positioning of the double bond at C-5 (as in cholesterol) appears to be critical for esterification by ACAT. Both cholestanol, the saturated analog of cholesterol, and lathosterol, the Δ^7-isomer of cholesterol, showed significant esterification. In contrast, cholest-4-en-3β-ol, an isomer of cholesterol which has an unsaturated A ring, shows very little activity. This reduced activity may be a result of an electronic effect, since the double bond is adjacent to the hydroxyl group, or the unsaturation may hinder the A ring from attaining a particular conformation (e.g., chair) required for reactivity. Similarly, Aringer (8) showed that cholest-4-en-3β-ol was a poor substrate for cholesterol 7α-hyroxylase. The presence of unsaturation in the side chain at C-24 (desmosterol) reduced activity by about 40%.

The esterification of desmosterol was dependent upon the concentration of desmosterol in the assay (**Table 2**).

The esterification of the sterols was followed by the incorporation of [^{14}C]oleoyl CoA into the sterol ester fraction and not by the incorporation of labeled sterol. Therefore, it is possible (though unlikely) that those sterols, which when added to the reaction medium brought about increased ester synthesis, were not themselves esterified but in some undetermined way increased the rate of endogenous cholesterol esterification by ACAT. To test this possibility, utilizing desmosterol as a substrate, all components of the reaction medium were increased 10-fold, and after separation by thin-layer chromatography, the sterol ester fraction was hydrolyzed in ethanolic KOH and the resulting free sterols were isolated and analyzed by gas–liquid chromatography. When the assay was carried out in the absence of exogenous sterol only one major sterol, cholesterol, was found in the ester fraction. Upon the addition of desmosterol to the assay medium a second sterol, with a retention time of 1.10 relative to cholesterol corresponding to that of desmosterol, was present in the ester fraction. Quantitation of the desmosterol ester by a comparison of peak area versus the peak area of desmosterol standards led to a value of 65 nmol desmosteryl oleate formed during the reaction. This compares favorably with the amount (55.3 nmol) obtained by the incorporation of [^{14}C]oleoyl CoA. The data demonstrate that desmosterol was being esterified by ACAT and that the amount of desmosteryl ester formed could be determined by the incorporation of [^{14}C]oleoyl CoA into the ester fraction.

TABLE 2. Increase in esterification by ACAT upon addition of exogenous desmosterol[a]

Addition	ACAT Activity[b]
	nmol/15 min/mg protein
None	1.16 (1.25, 1.08)
Desmosterol, 5 μg	3.2 (3.2, 3.2)
Desmosterol, 10 μg	4.53 (4.95, 4.15)
Desmosterol, 15 μg	7.23 (7.15, 7.32)
Desmosterol, 20 μg	8.64 (9.33, 8.0)
Cholesterol, 20 μg	11.35 (11.93, 10.78)

[a] Microsomes were preincubated for 30 min in the presence of the above additions. Incubations were initiated by the addition of [^{14}C]oleoyl CoA.

[b] Each value represents the mean of two experiments in which assays were carried out in duplicate. Means of two experiments in parentheses. Within each experiment duplicate values agreed within 10% of the mean.

Similarly, another set of assays was performed which were identical to the ones just described except that the sterol ester fraction was separated by HPLC. When no exogenous sterol was present in the assay medium, only the fraction corresponding to cholesteryl oleate was labeled (**Fig. 1-B**). Upon addition of 20 μg of desmosterol to the assay medium, radioactivity was found in a second fraction which corresponded to desmosteryl oleate (Fig. 1-C). The labeling of cholesteryl oleate was not significantly influenced by whether desmosterol was present in or absent from the incubation system, demonstrating that the observed increase in esterification was entirely due to the esterification of desmosterol.

Since microsomes are known to contain cholesteryl ester hydrolase activity, it is possible that the esterification of desmosterol could have been due to a reversal of this reaction instead of being the result of a transesterification reaction catalyzed by ACAT. However, no esterification occurred when the [^{14}C]oleoyl CoA in the incubation was replaced by [^{14}C]oleic acid.

Since the introduction of even one methyl group into the side chain of cholesterol resulted in an 80% reduction of esterification, the effect of the deletion of a portion of the side chain of cholesterol upon esterification was investigated. Accordingly, pregn-5-en-3β-ol (side chain consisting of C-20 and C-21), and derivatives of pregn-5-en-3β-ol, which differ from the parent compound only in the addition of a linear array of 4, 5, 6, 7, or 9 carbons at C-20 (**Fig. 2**), were analyzed for esterification by ACAT. Maximal activity was observed when the alkyl group attached to C-20 was 5 carbons in length, which is the same length as in cholesterol (**Fig. 3**). The ability of the sterols to be esterified by ACAT declined sharply with either the reduction or extension of the length of

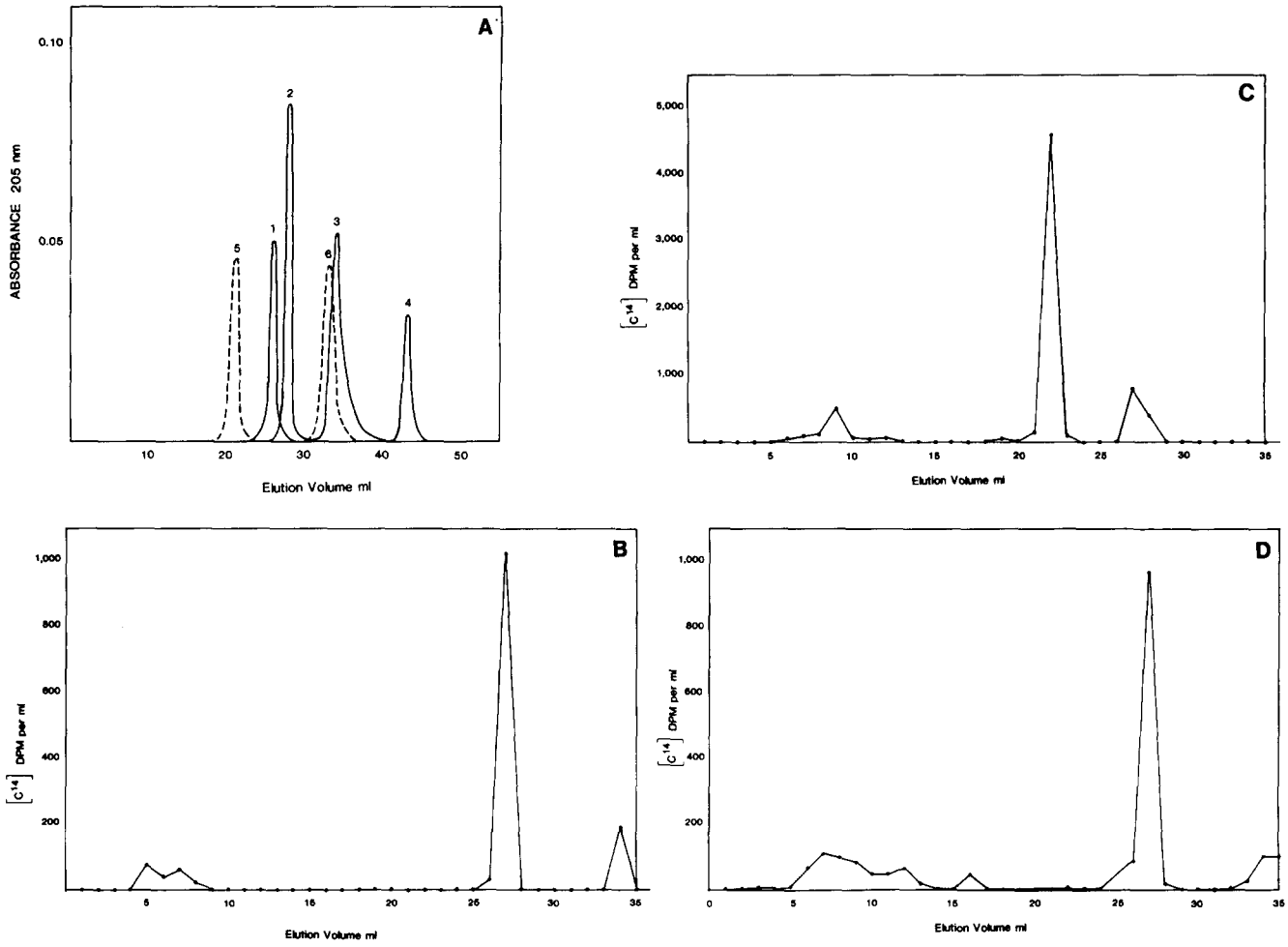


Fig. 1. Identification of HPLC of sterol esters synthesized by ACAT. A, Elution profile of various sterol esters (20 μg) dissolved in isopropanol obtained on a Zorbax ODS column. Peak identification: 1) cholesteryl oleate, 2) desmosteryl stearate, 3) cholesteryl stearate, 4) sitosteryl stearate; predicted elution profile (see Materials and Methods) of 5) desmosteryl oleate and 6) sitosteryl oleate. B, Incubation for synthesis of sterol ester(s) in the absence of exogenous sterol was carried out as described in the text. The sterol ester fraction was separated from other labeled lipids by TLC and extracted with diethyl ether. After evaporation the sterol esters were suspended in 100 μl isopropanol and 20 μl was separated by HPLC. Fractions (1 ml) were collected in scintillation vials and counted. C, Same procedure as B except that 200 μg desmosterol was added to the incubation medium. D, Same procedure as B except that 200 μg sitosterol was added to the incubation medium.

the side chain from that observed in cholesterol. The fact that the sterol with an *n*-alkyl group on C-20, the same length (C_5) as in cholesterol, is esterified as well as cholesterol is (Fig. 3) suggests that the enzyme does not recognize the terminal branching of cholesterol. The effect of chain length closely paralleled what has been reported for the dehydrogenation at C-7 and C-22 by protozoan enzymes (18) and the 7α-hydroxylation in rat liver (10).

Those sterols that were not esterified were also studied to ascertain if they were capable of inhibiting the esterification of exogenous cholesterol by ACAT (**Table 3**). In these experiments 10 μg of exogenous cholesterol and 10 μg of an inactive sterol were added to the incubation medium and the esterification of cholesterol was compared to reactions containing only cholesterol (10 μg). The contribution of endogenous cholesterol to the ester fraction was subtracted in both cases. Epicholesterol, which was not esterified, did not inhibit the esterification of cholesterol. Thus, a 3β-hydroxyl group seems to be required for any interaction with the enzyme. The 24-alkyl sterols and those sterols containing a *gem*-dimethyl group at C-4 were capable of inhibiting exogenous cholesterol esterification by 25–45%. This inhibition is presumably competitive though no kinetic analysis has been performed. The ability of the linear side chain analogs of cholesterol to inhibit esterification was dependent on the length of the side chain. The C_{30}-analogue (9 carbon atoms on C-20, excluding C-21) was not inhibitory and again suggests a hydrophobic binding pocket in the active site of ACAT that is specific for the cholesterol side chain and cannot accommodate a side chain which is increased

by four carbon atoms over that of cholesterol as in the case of C_{30} analogue. In contrast, pregn-5-en-3β-ol with a side chain consisting of only two carbons was inhibitory.

Although several sterols were capable of inhibiting the esterification of exogenous cholesterol, these sterols had little effect on the esterification of the inherent cholesterol under the conditions employed. The amount of cholesteryl oleate formed in the presence or absence of exogenous sitosterol was essentially the same (Fig. 2). Similarly, esterification, as measured by the incorporation of radioactivity into the ester fraction, was unaffected by the addition of 20 μg of the other sterols (Table 3), with the exception of pregn-5-en-3β-ol (C_{21}). The C_{21}-sterol caused about a 50% decrease in esterification from 0.95 nmol/15 min per mg protein to 0.44 nmol/15 min per mg protein. This inhibition may be common to steroids with a shortened side chain, since androst-5-en-3β-ol also inhibits ACAT (23).

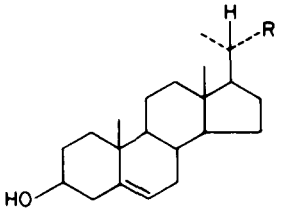
DISCUSSION

The enzyme, ACAT, in the studies reported here is very specific as to the structure of the sterol which will act as a substrate, and maximal esterification is obtained with its normal substrate cholesterol. A 3β-hydroxyl group is required for the sterol to be a substrate. The enzyme is very sensitive toward even minor changes of the structure of the side chain of a sterol from that of cholesterol, which suggests that the side chain is very important in the binding of the substrate in the active site of ACAT. Such a hydrophobic pocket may freeze the side chain of cholesterol into a particular conformation that aligns the A ring of the planar nucleus so that the 3β-hydroxyl group is in position to be esterified.

The specificity of ACAT compares well with information obtained by other investigators concerning the occurrence (or lack of occurrence) of sterol esters in animals. In β-sitosterolemia, a disease in humans in which plant sterols in the diet are absorbed in abnormally high amounts (26% versus <5%), sitosterol, in the resulting tissue lipomas, was found to be entirely in the free form (7) which would be predicted by our in vitro studies. In addition, it has been observed that sitosterol does not seem to be esterified in the intestinal wall before incorporation into chylomicrons, whereas cholesterol is preferentially in the esterified form and campesterol was intermediate (24, 25). Nes, Thampi, and Lin (4) also found a faster turnover rate for sitosterol than cholesterol in rat. This could be explained by the difference in the ability of the two sterols to be esterified. Cholesterol can be stored within the cells as cholesteryl esters; however, inasmuch as sitosterol is not esterified it should spend less time within the tissues and a greater portion of time in the plasma, which would increase the likelihood of its being secreted in the bile.

In contrast to the 24-alkyl sterols, in vitro studies suggest that cholestanol is esterified by ACAT at almost the same rate as cholesterol. In the disease cerebrotendinous xanthomatosis, cholestanol is present in the brain in large quantities and about 40% of the cholestanol found is in the esterified form (6). Within the rat in all tissues, the primary precursor sterols found in the ester fraction are the desmethylsterols, again agreeing with the in vitro data and suggesting that, within the cell, ACAT is the predominant if not the only enzyme responsible for sterol esterification (5).

Several oxygenated analogues of cholesterol, including 25-hydroxycholesterol nd 7-ketocholesterol, have been shown to inhibit cholesterol synthesis in vivo and in cell culture and most of these compounds also stimulate cholesteryl ester synthesis (26). Recently, 25-hydroxycholesterol has been shown to stimulate ACAT activity in vitro (14). Some of these compounds have been shown



R=H, PREGN-5-EN-3β-OL (C_{21})
R=$(CH_2)_3CH_3$, 20(R)-n-BUTYLPREGN-5-EN-3β-OL (C_{25})
R=$(CH_2)_4CH_3$, 20(R)-n-PENTYLPREGN-5-EN-3β-OL (C_{26})
R=$(CH_2)_5CH_3$, 20(R)-n-HEXYLPREGN-5-EN-3β-OL (C_{27})
R=$(CH_2)_6CH_3$, 20(R)-n-HEPTYLPREGN-5-EN-3β-OL (C_{28})
R=$(CH_2)_8CH_3$, 20(R)-n-NONYLPREGN-5-EN-3β-OL (C_{30})

Fig. 2. Structures of linear side chain analogues of cholesterol.

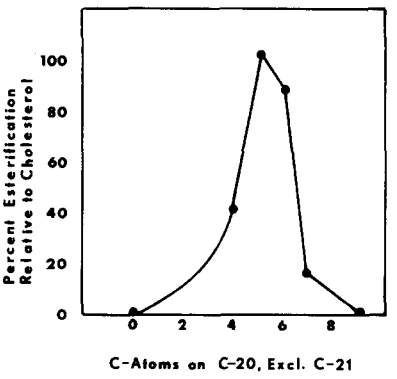


Fig. 3. Graph of percent esterification of terminally unbranched side chain analogues (Fig. 3) of cholesterol relative to cholesterol versus the number of carbons (other than C-21) that are attached to C-20 in the side chain. Incubations were performed as described in Materials and Methods. Each value represents the average of triplicate results from two separate experiments.

TABLE 3. Effect of various sterols on the esterification of exogenous or endogenous cholesterol by ACAT

Sterol Other Than Cholesterol	% Esterification Exogenous Cholesterol[a]	% Esterification Endogenous Cholesterol[b]
None	100	100
3-Epicholesterol	96 (100, 93)[c]	110 (105, 114)[c]
Sitosterol	61 (60, 62)	100 (106, 104, 89)
Stigmasterol	63 (69, 56)	102 (88, 117)
Ergosterol	56 (52, 60)	106 (87, 125)
Lanosterol	54 (48, 51, 63)	115 (100, 130)
4,4-Dimethylcholest-7-en-3β-ol	69 (78, 60)	109 (129, 89)
C_{21} (pregn-5-en-3β-ol)	49 (46, 53)	45 (35, 55)
C_{30}(20(R)-*n*-nonylpregn-5-en-3β-ol)	98 (92, 104)	87 (99, 74)

[a] Microsomes were preincubated for 30 min in the absence of exogenous sterol, the presence of 10 μg cholesterol, or in the presence of 10 μg of cholesterol and 10 μg of a second sterol. Incubations were initiated by the addition of [^{14}C]oleoyl CoA.

[b] Incubations carried out as above in the presence or absence of 20 μg sterol.

[c] Each value represents the mean of at least two experiments in which assays were carried out in triplicate. Means of individual experiments in parentheses. Within each experiment triplicate values agreed within 15% of the mean.

to be metabolized to ester or diester and it is of interest if the esterification of these compounds is carried out by ACAT (27, 28). Because of the specificity of the enzyme toward the side-chain of cholesterol, it does not seem likely that sterols containing an oxygenated side chain (e.g., 25-hydroxycholesterol) would be esterified at an appreciable rate by ACAT. Presently, studies are being carried out concerning the esterification of oxygenated analogues of cholesterol.

In addition to the intracellular formation of cholesteryl esters catalyzed by ACAT, cholesteryl esters are also formed in the plasma by a separate enzyme, lecithin:cholesterol acyltransferase. The sterol substrate specificity of lecithin:cholesterol acyltransferase is similar to that of ACAT except that it is capable of esterifying sitosterol and in the plasma sitosterol is primarily present in the esterified form (29–31). The physiological significance of this difference is presently unknown.

This work was supported in part by Grant AM-12172 from the National Institutes of Health, USPHS Grant SO 7 RR-07129, and American Heart Association (Southeastern Pennsylvania Chapter) #1009. We would like to express our thanks to Dr. J. M. Joseph who synthesized the linear side chain analogues of cholesterol and to Mr. Stewart Avart who synthesized the sterol stearates.

Manuscript received 3 August 1981, in revised form 10 December 1981, and in re-revised form 5 February 1982.

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