CHOLESTERYL ESTER METABOLISM IN LIVER AND BLOOD PLASMA OF VARIOUS ANIMAL SPECIES

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SUMMARY

In eight different animal species the activities of the following enzymes were determined: (1) the plasma lecithin:cholesterol acyltransferase (LCAT); (2) the liver microsomal acyl-CoA:cholesterol acyltransferase; and (3) the liver lysosomal acid cholesterol esterase. In addition, the plasma and liver concentrations of free and esterified cholesterol were determined.

The activities of the three enzymes differed considerably in the various animal species. The highest LCAT activity was observed in monkey and man, whereas calf displayed a rather low activity. In human no acyl-CoA:cholesterol acyltransferase could be demonstrated, and in liver from guinea pig a very low activity was observed. This is in contrast to the high activity found in rat liver. The highest cholesteryl ester hydrolyzing activity (at pH 4.5) was observed in rabbit and calf liver, whereas the hydrolytic activity in liver from rat, guinea pig, dog and swine was rather low.

Key words: Atherosclerosis - Blood plasma - Cholesterol - Cholesterol esterification - Cholesteryl ester hydrolysis - Lecithin:cholesterol acyltransferase (LCAT) - Liver - Mammals

INTRODUCTION

Major differences exist in the concentration of plasma lipoproteins in different animal species. The tendency to develop hypercholesterolaemia and atherosclerosis, either spontaneously or after the feeding of special diets, also differs markedly.

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Little is, however, known of the metabolic basis for these differences observed in various animal species.

The present investigation has been undertaken on man, monkey, pig, calf, dog, rabbit, guinea pig and rat. The aim of the study is to see whether there is any relation between the tendency to develop hypercholesterolaemia and atherosclerosis, and the activity of three enzymes responsible for formation and breakdown of cholesteryl esters (CE), and to get a better understanding of the metabolic role of these enzymes. The enzymes investigated are: (1) the plasma lecithin:cholesterol acyltransferase (LCAT), which catalyzes the formation of most plasma CE in man; (2) the lysosomal acid cholesterol esterase in liver, which may play a role in the hydrolysis of CE in liver and thus participate in the elimination of cholesterol from the body; and (3) the microsomal acyl-CoA:cholesterol acyltransferase in liver, an enzyme thought to be responsible for the formation of the CE of the plasma very low density lipoproteins (VLDL) in some animal species.

MATERIALS AND METHODS

Chemicals

[7α-3H]Cholesterol (spec. act. 9.4 Ci/m mole) was obtained from The Radiochemical Centre, Amersham, England, and Triton X-100 from Rohm and Haas Company, Philadelphia, Pa., U.S.A. Crystalline human serum albumin (containing 1.7 moles of free fatty acids per mole of albumin) and human serum albumin (20% solution for intravenous use, containing 3.8 moles of fatty acids per mole of albumin) were obtained from AB Kabi, Stockholm, Sweden. Ellman reagent (5,5-dithiobis(2-nitrobenzoic acid)) and ATP were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A., CoA from C. F. Boehringer und Soehne, Mannheim, Germany, and mercaptoethanol from Eastman Organic Chemicals, Rochester, N.Y., U.S.A.

Tissue sources

Each group consisted of 5 animals. Male rats of the Wistar (Möl) strain (mean weight 270 g) were given slight ether anaesthesia, and blood was drawn by aortic puncture. Male guinea pigs (mean weight 300 g) were bled shortly after exposure to carbon dioxide gas. The male rabbits (mean weight 1.9 kg) were killed by the injection of 2 ml of 10% mebunal intracardially and then bled. Both guinea pigs and rabbits were of local strains bred at the National Institute of Public Health, Oslo, Norway. Biopsies from male mongrel dogs (weighing about 15 kg) were obtained in connection with experimental abdominal operations under general anaesthesia (mebunal). Calf blood and liver were obtained from male animals about 2 weeks old at the time of slaughter. Blood and liver from adult pigs (sex not known) were obtained in the same way as from the calves. Four monkeys of the Cercopithecus strain (3 female and 1 male) and 1 of the Macaca irus strain (female) were used. The monkeys weighed between 3 and 5 kg, and blood samples and liver biopsies were obtained under mebunal anaesthesia. Biopsies and blood samples from 5 patients (2 female and 3
male, age 18–71 years) were obtained in connection with abdominal operations under general anaesthesia (aloferin and $N_2O$). None of the patients suffered from diseases known to influence cholesterol metabolism, and the liver function tests and the microscopic appearance of the livers were normal.

Except for heparin-plasma being obtained in the case of the rats, serum was obtained from all other animal species. To ensure that initial LCAT activity could be measured, the blood samples were always cooled on ice immediately after sampling. The liver tissue was chilled on ice immediately after removal. Homogenization was performed in 0.25 $M$ sucrose, and a cytoplasmic extract was prepared by removing nuclei, unbroken cells and debris by centrifugation at 6000 $g \times$ min. The homogenates and blood samples were kept frozen (—20 °C) until used.

**Diets**

From man, monkey and dog biopsies were taken under general anaesthesia after overnight fasting; all other animals had free access to food and water until liver tissue and blood samples were obtained. No attempt was made to give all animals a standardized diet, and they all therefore were fed their usual diets. The rats, monkeys, guinea pigs, rabbits and dogs were fed stock diets consisting of 20–24 % (w/w) of crude protein, 51–53 % of crude carbohydrate, 3.4–3.5 % of crude lipids and 6 % ash. The cholesterol content of these stock diets varied between 26 mg per 100 g (guinea pig and rabbit) and 84 mg per 100 g (rat and monkey). The calves were milk-fed. As for man and swine no specifications can be given.

**Assay procedures**

**Lecithin:cholesterol acyltransferase (LCAT) activity.** Two methods for determining the LCAT activity were used. Method I was a slight modification of the procedure described by Glomset and Wright\textsuperscript{10}: a pooled serum (or plasma) sample from most or all of the animals constituting a group was heated at 56–58 °C for 1 h and centrifuged if precipitates had formed. Radioactive free cholesterol (FC) was added in a small volume of acetone (usually 25 $\mu$l of acetone to 5 ml of serum) and was allowed to equilibrate with the endogenous, unlabelled cholesterol for 4 h at 37 °C. The solution was stored at —20 °C until used. LCAT activity was then assayed by incubating 1 vol. of serum (or plasma) with 8 vol. of the heat-inactivated homologous substrate source, and incubations were conducted for 1 h or for 4 h. The reaction was rectilinear for at least 4 h in human, dog and calf serum, but only for about 1 h in serum from rat, guinea pig, rabbit, pig and monkey. The reactions were stopped by chloroform/methanol, and total lipids were separated by thin-layer chromatography and the fractions comprising free and esterified cholesterol were scraped off and counted by liquid scintillation. (The separation procedure is described in more detail later.)

Method II has been described elsewhere\textsuperscript{11}: trace amounts of labelled cholesterol were added to serum (or plasma). During a preincubation period of 4 h in which the LCAT was inhibited by a disulphide (5,5-dithiobi(2-nitrobenzoic acid)), the tracer
equilibrated with endogenous lipoprotein cholesterol. The enzyme was reactivated by excess of thiol (mercaptoethanol), and the activated samples were incubated for 1 h. Determination of the amount of labelled FC esterified during incubation was done as in Method I.

**Acyl-CoA:cholesterol acyltransferase activity in liver.** The acyl-CoA:cholesterol acyltransferase activity in the liver homogenates was assayed principally according to the method of Goodman et al.\(^\text{12}\) with minor modifications as described previously.\(^\text{13}\) The incubation mixtures contained in a total volume of 0.6 ml: tissue homogenate (cytoplasmic extract) corresponding to 10 mg of whole liver, 0.3 µmole of CoA, 7.5 µmoles of ATP, 50 µmoles of sodium phosphate and \([\text{7a-}^{3}\text{H}]\text{cholesterol}\) (ca. \(1 \cdot 10^5\) c.p.m.) which was added in 15 µl of acetone. An optimal amount of human serum albumin (containing fatty acids) was added (the amount is given in the legend to Table 2). Final pH was 7.3, and the incubation time was 1 h. The further separation of free and esterified cholesterol is described below.

**Cholesterol esterase activity in liver. Hydrolysis of cholesteryl esters.** Cholesterol esterase activity was measured using serum with radioactive lipoproteins as substrate source. \([\text{7a-}^{3}\text{H}]\text{Cholesterol}\) was added to pooled serum samples from the animals constituting a group and during incubation at 37 °C for 24 h labelled CE were formed. During the incubation the radioactive cholesterol became esterified to a variable degree, ranging from 13% in dog serum, 18% in calf serum, rabbit 22%, guinea pig 37%, pig 48%, human 55%, monkey 57%, to 58% in serum from rat. The preparation of lipoproteins containing labelled CE has been described in more detail elsewhere.\(^\text{5}\) The concentrations of CE in these substrate preparations, together with the percentage decrease in labelled CE during the incubation, form the basis for calculation of CE hydrolysis at pH 4.5.

Liver homogenates were tested both with homologous labelled lipoproteins and with labelled human plasma lipoproteins as substrate, the latter thus serving as a standard substrate for all animal species. As the liver homogenates contain endogenous CE, care was taken to ensure that the exogenous, labelled CE were always added in such an excess that the amount of unlabelled endogenous CE became relatively unimportant in the assay system. The hydrolytic activity was measured as follows: to 250 µl of McIlvaine citrate–phosphate buffer\(^\text{14}\) (pH 4.4) liver homogenate from 1 mg of liver was added. Serum with labelled lipoproteins served as substrate source in an amount corresponding to 0.9 µl of serum. Triton X-100 was always added to the incubation mixtures in optimal amounts, as stated in Table 2. Incubation time was 30 min.

**Cholesterol esterase. Esterification of cholesterol at pH 4.0.** The esterification of cholesterol at pH 4 was assayed as follows: to 250 µl of McIlvaine citrate–phosphate buffer\(^\text{14}\) (pH 3.8) were added: homogenate corresponding to 2.5 mg of whole liver, and 1.5 mg of human serum albumin defatted according to the method of Chen.\(^\text{15}\) \([\text{7a-}^{3}\text{H}]\text{Cholesterol}\) was added in 15 µl of acetone and esterification was measured after 1 h incubation.

Incubations for all assays on enzyme activities were stopped by the addition
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of 20 vol. of chloroform/methanol (2:1, v/v), and lipids were extracted according to Folch et al.\textsuperscript{16}. After separation of the lipids by thin-layer chromatography, the gel containing labelled FC and labelled CE was scraped off and counted in a liquid scintillation spectrometer. This procedure has been described in more detail elsewhere\textsuperscript{5}.

The serum concentration of FC was determined by the method of Schoenheimer and Sperry\textsuperscript{17}. Total cholesterol was measured by the method of Levine and Zak adapted for Auto-Analyzer\textsuperscript{18}. Free and esterified cholesterol in the liver homogenates were determined by a modification of the Auto-Analyzer method after previous separation of the lipids by thin-layer chromatography.

The protein content of the liver homogenates was determined by the method of Lowry et al.\textsuperscript{19}.

RESULTS

Lecithin:cholesterol acyltransferase (LCAT) activity

LCAT activity as determined by the method of Glomset and Wright\textsuperscript{10} (Method I) generally revealed a somewhat lower activity as compared to the activity determined according to Stokke and Norum\textsuperscript{11} (Method II). In the first method heat-inactivated substrate from a common pool is added in such an excess that differences in substrate composition in the various serum samples become negligible. The variation in the rate of esterification observed by this method within one group of animals is therefore thought to reflect differences in the concentration of the LCAT enzyme alone. As the esterification as measured by this method can be greatly influenced by the heat-inactivated substrate source\textsuperscript{20}, homologous serum lipoproteins were always used in these assays. In Method II esterification is not only influenced by the concentration of enzyme, but also by the concentration of substrate lipoproteins in the individual blood samples.

Table 1 and Fig. 2 show that there is a wide variation in LCAT activity, and that by Method II the highest activity was found in monkey and man (105 and 79 \( \mu \)moles of CE formed per litre serum per h, respectively), whereas calf serum displayed the lowest activity (9 \( \mu \)moles of CE formed per litre per h).

As for some animal species marked discrepancies between the results obtained by the two methods were observed, the difference being most conspicuous in monkey and dog in which Method I revealed a LCAT activity which was only about half that observed by Method II. There is no explanation for this, but it may be due to the heat-inactivated substrate source used in Method I not being optimal for the esterification in these animals.

In Table 1 are also given the data on free and esterified cholesterol in serum from the animals included in this study. It can be seen that despite wide variations in the concentration of total cholesterol, the percentage of cholesterol present as esters is fairly constant. The two points of extremity are represented by serum from rabbit and from calf, in which 66.9\% and 83.3\%, respectively, of total cholesterol is present as esters. The reason for the relatively constant percentage of cholesterol
TABLE 1

CHOLESTEROL ESTERIFYING ACTIVITY AND THE CONCENTRATION OF FREE AND ESTERIFIED CHOLESTEROL IN SERUM OF VARIOUS ANIMAL SPECIES

Serum lecithin:cholesterol acyltransferase activity was determined by two different methods (see text). The activities are expressed as µmoles of CE formed per l serum per h. In addition the per cent of labelled cholesterol esterified during 1 h incubation is given. Values are given as means ± S.D.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Conc. of serum cholesterol (mM)</th>
<th>Lecithin:cholesterol acyltransferase activity</th>
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<tbody>
<tr>
<td></td>
<td>total chol.</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>1.14 ± 0.23</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>1.19 ± 0.23</td>
<td>0.98 ± 0.19</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.42 ± 0.36</td>
<td>0.95 ± 0.22</td>
</tr>
<tr>
<td>Dog</td>
<td>3.44 ± 0.71</td>
<td>2.43 ± 0.62</td>
</tr>
<tr>
<td>Calf</td>
<td>2.06 ± 0.88</td>
<td>1.81 ± 0.77</td>
</tr>
<tr>
<td>Pig</td>
<td>2.87 ± 0.42</td>
<td>2.15 ± 0.33</td>
</tr>
<tr>
<td>Monkey</td>
<td>2.45 ± 0.47</td>
<td>1.73 ± 0.36</td>
</tr>
<tr>
<td>Man</td>
<td>4.73 ± 1.66</td>
<td>3.35 ± 1.17</td>
</tr>
</tbody>
</table>
present as esters is not known. The data in Table 1 do not suggest any correlation between LCAT activity and the percentage of total cholesterol present as esters in the sera from different animal species. The data presented here on the total cholesterol and on the CE concentration in serum from the different animal species are in agreement with those reported by others\textsuperscript{21,22}.

In Fig. 1 the correlation between the rate of esterification (as determined by both methods for determination of lecithin:cholesterol acyltransfer) and the concentration of FC in serum from the different animal species is given. It appears that such a relation may exist, although rat and monkey (by Method II) display higher LCAT activity, and calf a lower activity, than would be expected from the concentration of FC in serum.

**Acyl-CoA:cholesterol acyltransferase activity in liver**

Table 2 and Fig. 2 show that the acyl-CoA:cholesterol acyltransferase activity in liver varies considerably. In rat a rather high activity was found (14.6\% of labelled FC esterified per h). In dog, swine and monkey, in which significant esterification was also observed, the values obtained were 3.4\%, 3.6\% and 1.6\%, respectively. In human liver no activity could be detected. The very small activity observed in liver from guinea pig does not deviate significantly from zero.

The acyl-CoA:cholesterol acyltransferase activity might also have been expressed as the absolute amount of CE synthetized per g liver per min. However, as the equilibration between the isotope and the endogenous, unlabelled FC is uncertain\textsuperscript{13,23}, and since no complete equilibration between exogenous and endogenous FC takes
TABLE 2
FORMATION AND BREAKDOWN OF CHOLESTERYL ESTERS IN LIVER OF VARIOUS ANIMAL SPECIES

The table shows the activity of the microsomal acyl-CoA:cholesterol acyltransferase and of the lysosomal acid cholesterol esterase in liver of various animal species, and in addition the concentration of total and free cholesterol in the liver homogenates are given. Acyl-CoA:cholesterol acyltransferase activity was measured in the presence of albumin, the amount added ranging from 20 mg in assays with rat, dog, pig and monkey liver, 10 mg in assays on human liver, rabbit 5 mg, to 2 mg when assaying the esterifying activity in liver from guinea pig and calf. Hydrolysis of CE by the acid cholesterol esterase in liver from the various animal species was determined in the presence of optimal amounts of Triton X-100. Triton was added in amounts varying between 0.05 and 0.07% (final concn.). Values are given as means ± S.D.

| Animal species | Conc. of cholesterol in liver (μmoles/g liver) | Acyl-CoA : cholesterol acyltransferase activity | Acid cholesterol esterase
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>total chol.</td>
<td>FC</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>5.8 ± 0.7</td>
<td>4.9 ± 0.4</td>
<td>14.6 ± 2.4</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3.1 ± 1.3</td>
<td>2.8 ± 1.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4.9 ± 0.9</td>
<td>4.0 ± 0.7</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Dog</td>
<td>4.7 ± 2.0</td>
<td>4.3 ± 1.8</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>Calf</td>
<td>6.7 ± 1.1</td>
<td>5.4 ± 0.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Pig</td>
<td>6.6 ± 1.0</td>
<td>5.5 ± 0.6</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>Monkey</td>
<td>4.5 ± 1.0</td>
<td>3.3 ± 0.7</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Man</td>
<td>4.6 ± 0.9</td>
<td>4.0 ± 0.8</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

a Values are given as per cent of added [7α-3H]cholesterol esterified in 1 h.
b The concentration of CE in this human serum preparation was 3.62 mM.
Fig. 2. Comparison of serum lecithin:cholesterol acyltransferase (LCAT) activity, liver acyl-CoA:cholesterol acyltransferase activity and the activity of the liver lysosomal acid cholesterol esterase in various animal species. LCAT activity was determined by two different methods, the light and dark columns representing results obtained by Method I and Method II, respectively. For further details, see text.

place\textsuperscript{13}, exact calculations on enzyme activities are difficult to perform. Therefore, only percentage values are given.

\textit{Acid cholesterol esterase activity in liver. Hydrolysis of CE at pH 4.5}

Lipoproteins with labelled CE served as substrate, and the activity was tested both against human plasma lipoproteins, thus serving as a standard substrate, and against homologous lipoproteins. Table 2 and Fig. 2 show that the cholesterol esterase activity differed markedly from one group of animals to another. The highest activity was observed in rabbit and calf liver, whereas low activity was found in liver from dog, pig, rat and guinea pig. In human and monkey liver the activity was somewhere in between. Irrespective of the addition of human plasma lipoproteins, or homologous lipoproteins, the same percentage decrease in labelled CE was observed. The explanation of this is that despite marked differences in the concentration of CE in the substrate sources, all concentrations fit the initial rectilinear part of the substrate concentration curve\textsuperscript{6}. In this study enzyme activities were not determined at substrate concentrations giving maximal velocities of the reactions, as these measurements are most time-consuming and difficult to perform\textsuperscript{6}. Thus, instead of being data on maximal velocity of hydrolysis, the values given indicate the rate with which a given amount
of CE fitting the initial, rectilinear part of the substrate concentration curve, may be hydrolyzed by the liver of various animal species.

Acid cholesterol esterase. Cholesterol esterification at pH 4.0

Table 2 shows that liver homogenates from all animals displayed some cholesterol esterifying activity at low pH, high values generally being observed in animals with high hydrolytic activity. This supports the assumption that both esterification of FC and hydrolysis of CE at low pH are catalyzed by the same enzyme, namely a reversible cholesterol esterase.

If the same degree of isotope exchange between the added labelled FC and the unlabelled endogenous FC of the homogenates takes place in these experiments as in the experiments on the acyl-CoA:cholesterol acyltransferase reaction, the capacities of the two esterification reactions show significant differences in various groups of animals. The highest esterifying activity at pH 4.0 is found in liver from calf, which, on the other hand, shows very low acyl-CoA:cholesterol acyltransferase activity, whereas rat, in which acyl-CoA:cholesterol acyltransferase activity was most prominent, displayed a very small degree of esterification at pH 4.0. However, as hydrolysis is thought to be the main direction at low pH, the physiological importance of the esterification at pH 4 is uncertain, and the data are difficult to interpret.

The concentrations of free and esterified cholesterol in the liver homogenates are given in Table 2. As with serum the total amount of cholesterol and the amount present as esters, are remarkably constant. The data are in fairly good agreement with those of Gould.

The concentrations of liver lipids, and of the enzyme activities in liver described above, were also calculated according to the tissue content of protein. As the relative values did not differ from those referred to wet weight tissue, they were omitted in this presentation.

In Fig. 2 column diagrams visualize the plasma LCAT activity and the activity of the liver enzymes acyl-CoA:cholesterol acyltransferase and acid cholesterol esterase in all groups of animals investigated. The wide variation in enzyme activities is then easily seen. However, no pattern unveiling any correlation between enzyme activities and known peculiarities in cholesterol metabolism can be detected.

DISCUSSION

Our data on the LCAT activity in serum from different animal species reveal a wide variation in activity. This variation is not in agreement with the results obtained by Felt who found that the esterifying activity in man, guinea pig, rat and rabbit did not differ substantially, and who concluded that it seemed improbable that there was any relation between the LCAT reaction and the susceptibility of different species to atherosclerosis. Our present investigation does not exclude that such a relation may exist. However, as our experimental conditions differ considerably from those of
Felt, the results are not directly comparable. On the other hand, the activity observed in human blood serum in our experiments is in good agreement with that observed by others\textsuperscript{4,20}. In rabbit an activity very similar to that observed by Rose\textsuperscript{25}, but quite different from that found by Stefanovich\textsuperscript{26} was observed, whereas rat serum displayed a lower activity than that reported by Sugano and Portman\textsuperscript{27} (42–53 versus 80 µmoles of FC esterified per litre serum per h). The lower LCAT activity in calf serum observed by us as compared to that reported by others in bovine plasma\textsuperscript{28} cannot be explained, but may be due to our use of very young, milk-fed animals. When comparing data obtained by different authors the fact that the esterification rate for any species can be greatly influenced by the heat-inactivated substrate source\textsuperscript{29}, and by the heat-inactivation itself\textsuperscript{29}, should also be taken into consideration.

In human plasma a relationship between the concentration of FC and the esterification rate has been demonstrated\textsuperscript{30}. From Fig. 1 it seems to be apparent that there is a correlation between the rate of cholesterol esterification and the concentration of FC in serum from the different animal species. The most marked deviation is shown by monkey and rat serum in which the LCAT activity (as determined by Method II) is higher than would be expected if a strict correlation should exist. Calf, on the other hand, display a relatively low activity.

In human blood plasma the LCAT reaction is thought to be responsible for the formation of all plasma CE, except for the small fraction derived from the intestinal tract. In rat the plasma LCAT is probably responsible for the formation of the CE of high density lipoproteins (HDL), whereas the CE of very low density lipoproteins (VLDL) in rat plasma are supposed to be synthetized in the liver by the microsomal acyl-CoA:cholesterol acyltransferase\textsuperscript{9}. The basis for this assumption is that the microsomal esterifying enzyme in rat liver displays a strong relative specificity for the formation of cholesteryl oleate\textsuperscript{12}, which also is the predominant CE in VLDL in rat\textsuperscript{31,32}. Moreover, Mahley \textit{et al.}\textsuperscript{33} have shown that the VLDL-like particles present in the Golgi apparatus of rat liver contain CE. Finally, the composition of the CE of VLDL differs appreciably from that of the low density lipoproteins (LDL) and HDL, and is not compatible with the known specificity of the plasma acyltransferase\textsuperscript{4}. These considerations are in agreement with our finding of acyl-CoA:cholesterol acyltransferase activity in liver from rat. Since we have not been able to detect any acyl-CoA:cholesterol acyltransferase activity in liver from man (see also ref.\textsuperscript{5}) and guinea pig, and have only found traces of activity in calf liver (Table 2 and Fig. 2), the CE of the plasma VLDL in these animals might seem to be derived from other sources than the microsomal esterifying system.

As is apparent from Table 2 and Fig. 2 the acyl-CoA:cholesterol acyltransferase activity in rabbit liver appears to be small. However, after feeding rabbits with a cholesterol-containing diet, Rose\textsuperscript{25} found that the serum cholesterol esterifying activity then observed could not account for the great elevation of serum CE. His data were compatible with the liver being the major source of blood esters in cholesterol-fed rabbits. However, the data of Rose do not exclude that CE from the intestinal mucosa may make an important contribution to the serum CE in these feeding experi-
ments. The possibility seems to exist that the esterifying activity in liver may become an important contributary factor to the serum CE, even in this animal which under normal circumstances did not reveal any considerable esterifying activity in liver. Chapman et al. demonstrated VLDL-like particles in the Golgi apparatus in livers from guinea pigs fed a diet rich in lipids. The amount of CE in these particles was some 20-fold higher than in animals fed a normal diet. Moreover, as cholesterol oleate has been shown to accumulate in the liver of rats on experimental diets, influence on acyl-CoA:cholesterol acyltransferase activity by dietary manipulations seems possible.

The function of the lysosomal cholesterol esterase is not clear. Stein et al. have found that in rat, chylomicron CE are hydrolyzed at the plasma membrane surface of liver parenchymal cells by a cholesterol esterase found to be most active at neutral pH. Recently, Rachmilewitz et al., by a radioautographic study, showed uptake of intact HDL in rat liver cells, the labelling being predominantly over the lysosomes. It therefore seems possible that different pathways may exist for the hydrolysis of CE of the various classes of plasma lipoproteins, and that the chylomicron CE are hydrolyzed at the plasma membrane surface, while the CE of one or more of the other lipoprotein classes are hydrolyzed in the liver lysosomes by the acid cholesterol esterase. The further metabolic fate of the FC resulting from the cholesterol esterase reaction is not known, and further investigations will also be necessary to elucidate the significance of the different esterase activities observed in the various animal species.

Some of the animal species included in this study possess certain characteristics with respect to the development of hypercholesterolaemia and atherosclerosis. In rabbit marked hypercholesterolaemia and advanced atherosclerosis develop rapidly as a response to the addition of cholesterol to the diet, and in guinea pig abnormalities resembling those of plasma LCAT deficiency have been shown to develop as a response to dietary cholesterol. Rat and dog, on the other hand remain relatively resistant to dietary induced hypercholesterolaemia and atherosclerosis as these animals compensate the increased intake of cholesterol by increasing the biliary excretion of neutral sterols and bile acids. In swine and monkey the pathological characteristics of both spontaneous and aggravate atherosclerosis bear a striking resemblance to human atherosclerosis, as do the lesions in rabbits fed cholesterol intermittently.

Whether the differences in enzyme activities and in the concentrations of lipids observed in this study, are in any relation to the susceptibility of various animal species to develop hypercholesterolaemia and atherosclerosis, is not clear. The observed differences do, however, cast doubt on the direct applicability to man of results obtained in studies of cholesterol metabolism in other animal species. And, moreover, some of the observations presented here may form a basis from which dietary manipulations may induce changes, which then in turn can throw more light on some of the obscure aspects of the metabolism of CE.
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