Tissue and Species Differences in Bile Salt–Dependent Neutral Cholesteryl Ester Hydrolase Activity and Gene Expression

Reza Zolfaghari, Earl H. Harrison, Jang H. Han, William J. Rutter, and Edward A. Fisher

Enzymatic activity and mRNA abundance for neutral bile salt–dependent cholesteryl ester hydrolase (CEH) were determined in rat and rabbit tissues. In rat liver and intestine, enzyme activity and mRNA levels varied independently. Particularly striking in most tissue samples was the absence of detectable CEH mRNA in the presence of enzymatic activity, suggesting that there was an exogenous source of enzyme. Rabbits differed from rats in four ways. First, neither CEH activity nor mRNA was present in any liver sample. Second, CEH mRNA was present in nearly all intestinal samples, and its abundance tended to correlate with enzymatic activity. Third, rabbit CEH mRNA was approximately 250 bases shorter than the rat message. Fourth, we have previously shown that rat plasma contains CEH activity, whereas in the present studies, rabbit plasma did not contain such activity. Overall, our studies indicate that CEH activity in rat liver, intestine, and plasma can be derived exogenously, most likely from the uptake and transport of pancreatic enzyme. In contrast, in rabbit the lack of CEH activity in plasma and liver and the capacity of the intestine for in situ synthesis of CEH suggest that this animal does not have the same ability to distribute pancreatic CEH. These species differences in CEH metabolism may partly explain the greater susceptibility of rabbit tissues to accumulate cholesteryl esters. (Arteriosclerosis and Thrombosis 1992;12:295–301)

KEY WORDS • cholesteryl ester hydrolase • rats • rabbits • gene expression

The liver and intestine have central functions in mammalian cholesteryl ester metabolism. The regulation of this process is not well defined. For example, only one enzyme present in these organs and having significant hydrolytic activity against cholesteryl esters at neutral pH, bile salt–dependent cholesteryl ester hydrolase (CEH), has been characterized at the molecular level.1-3

In previous studies, we have reported that CEH activity in the rat liver and intestine is quite variable among individual animals. Moreover, the properties of the enzymatic activity in both tissues were remarkably similar to purified pancreatic CEH (pCEH) and were, in fact, specifically and completely inhibited by an antibody to pCEH.4 It has been known for some time that pCEH is secreted into the intestinal lumen, where it participates in the processing of dietary lipid. In addition, there is evidence that pCEH is internalized by the intestinal mucosal cells and participates in the intracellular metabolism of cholesteryl esters (for example, see References 5–7). We speculated that the pancreas may also be a source of hepatic activity, as CEH activity has been found in rat intestinal plasma and lymph.4,5 Thus, secreted pCEH could be taken up by enterocytes, transported by lymph to plasma, and distributed to the liver as well as to other tissues. In many ways, this model resembles the metabolic pathway of lipoprotein lipase (LPL). This enzyme is made in one site (for example, adipose tissue), is transported across a layer of cells (the endothelium), appears in plasma, and binds to and is internalized by hepatic cells.6-11

In subsequent studies, however, we showed that RNA extracted from a pool of rat livers contained CEH mRNA, indicating at least in some cases, a capacity for hepatic synthesis of the enzyme.12 These findings were confirmed and extended by Hui, Brockman, and their colleagues (Kissel et al12 and Camulli et al13), who reported that the rat liver can contain both mRNA and protein species indistinguishable from pCEH message and protein. However, Schotz and his colleagues (Reue et al14), who reported that the rat liver can contain both mRNA and protein species indistinguishable from pCEH message and protein, have recently reported that rat liver does not contain CEH mRNA. In this report, we will reconcile these discrepant results.

Most studies of CEH have focused on the rat. We were interested in examining CEH metabolism in the rabbit. These two species vary dramatically in their tendency to accumulate cholesteryl esters in their tissues, as reflected in the rabbit's susceptibility and the rat's resistance to dietary-induced atherosclerosis. Besides readily storing cholesteryl esters, rabbit liver produces a cholesterol-poor bile compared with that of the
rat, which may also be related to a decreased ability to hydrolyze cholesterol esters.

This report summarizes our continued investigation of the noted variability of rat liver CEH activity. Specifically, we have examined whether this variability is regulated at the level of CEH mRNA. We also address the controversial issue of the origin—exogenous versus endogenous—of this activity. Finally, we have extended our examination of CEH activity and gene expression to the rat intestine and have compared our overall results for the rat to those obtained for the rabbit.

**Methods**

**General Procedures**

All animal procedures were approved by the institutional animal care and use committee. Adult male Lewis rats weighing 200–400 g were obtained from Charles River Laboratories (Wilmington, Mass.), and adult male New Zealand White rabbits weighing 2.5–3.5 kg were purchased from Ace Animals (Hazelton, Pa.). Both rats and rabbits were housed individually and were fed commercial chow ad libitum. Rats were killed by inhalation of carbon dioxide and rabbits by phenobarbital injection. The animals were immediately dissected.

Livers were removed, washed with cold (4°C) distilled water, and then minced into small pieces. A segment of the jejunal portion of the small intestine was dissected and trimmed of any adherant material. The intestinal contents were removed by irrigation with cold distilled water. After slitting open the intestinal segment, the mucosal cells were collected by gentle scraping with a glass slide. Liver and intestinal samples were divided into two aliquots and were stored at -70°C until further use.

Total RNA was extracted from tissue samples by homogenization in 5 M guanidine thiocyanate (10 ml/g tissue) followed by precipitation in the presence of 6 M guanidine hydrochloride as previously described. The total RNA so obtained was precipitated by 2.5 volumes of cold (−20°C) ethanol after addition of 0.1 volume of 2.5 M sodium acetate (pH 5.0). The precipitated total RNA was first centrifuged at 15,000g for 10 minutes at −10°C, washed with 80% cold ethanol, and centrifuged as described previously. The pellet was then dissolved in autoclaved water and stored at −70°C until further use.

To isolate mRNA, total RNA was first dissolved in 0.01 M tris(hydroxymethyl)aminomethane (Tris) HCl (pH 7.5) and 1 mM EDTA and then subjected to affinity chromatography on oligo (dT)-cellulose (Boehringer Mannheim, Indianapolis, Ind.) as described elsewhere. The mRNA so obtained was precipitated in cold ethanol as described previously. The precipitate was then washed with cold 80% ethanol, dried under vacuum, dissolved in autoclaved water, and stored at −70°C until analyzed. Both total RNA and mRNA were quantified by absorbance at 260 nm, and the purity was assessed by the 260 nm/280 nm ratio. The ratios were in the range 1.8–2.2.

For Northern blot analysis, mRNA samples were prepared in a buffer containing 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS) (pH 7.0), 5 mM sodium acetate, 1 mM EDTA, 1.23 M formaldehyde, 50% formamide, 7% (vol/vol) glycerol, and 5 mg/ml bromophenol blue. After heating at 65°C for 15 minutes, mRNA (1–3 μg) was subjected to electrophoresis at 80 V for 4–5 hours in a 1.5% agarose gel containing 2.2 M formaldehyde using a running buffer containing 20 mM MOPS, 5 mM sodium acetate, and 1 mM EDTA. After electrophoresis, the RNA samples were transferred overnight to Nytran membranes (Schleicher & Schuell, Inc., Keene, N.H.) in 10× saline–sodium citrate buffer (SSC) (20× SSC is 3 M NaCl and 0.3 M sodium citrate; pH 7.0). The filter membrane was then cross-linked in an ultraviolet Stratalinker (Stratagene, La Jolla, Calif.). For slot-blot analysis, a manifold (Bio-Rad, Richmond, Calif.) was used to apply to the Nytran membranes 5-μg samples of total RNA per slot, following the directions of the manufacturers. The membranes were then cross-linked by ultraviolet radiation.

Rat pCEH cDNA was labeled with deoxycytidine 5′-[α-32P]triphosphate with a specific activity of 3,000 Ci/mmol (New England Nuclear, Boston, Mass.), using the random-primer method. Northern blot analysis (see below and Figure 4) has demonstrated that this probe detects a single species of mRNA in either rat or rabbit tissues. The filters were prehybridized at 42°C for at least 2 hours and then hybridized at 42°C overnight in a solution containing 50% formamide, 3× SSC, 1 mM Na2PO4, 1× Denhardt’s solution, 100 μg/ml salmon sperm DNA, and 7% dextran sulfate. For hybridization, typically 5–10×10⁶ cpm of a probe with a specific activity >10⁶ cpm/μg DNA was added.

After hybridization, the membranes were washed twice at room temperature in 2× SSC and 0.1% sodium dodecyl sulfate (SDS) for 10 minutes each and then twice at 55°C in 0.1× SSC and 1% SDS for 40 minutes each. Autoradiograms were developed after exposure of the washed membranes to x-ray film (XAR-5, Eastman Kodak Co., Rochester, N.Y.) at −70°C for up to 4 days. Signals were quantified by scanning with a laser densitometer (Hoefer Scientific Instruments, San Francisco, Calif.).

After hybridization with labeled CEH cDNA, membranes were stripped of probe, following the manufacturer’s protocol, and exposed to x-ray film to confirm that the signal had been removed. To measure the abundance of actin mRNA, rehybridization was performed as previously, but a genomic fragment of the mouse β-actin gene (kindly provided by D. Friedman, University of Texas, San Antonio) was used as the control probe.

**Preparation and Microinjection of Xenopus laevis Oocytes**

The procedures were performed essentially as described. Briefly, for each RNA sample, 25–50 oocytes collected from Xenopus laevis were injected with 50 nl of a solution of either mRNA (2 mg/ml in water) or total RNA (3 mg/ml). Oocytes were incubated at 19°C for 3–5 days. After incubation, the oocytes were washed and homogenized in 0.01 M Tris maleate (pH 7.5) containing 0.25 M sucrose. After centrifugation at 1,000g for 15 minutes at 4°C, the supernatant was collected for CEH activity measurement.

**Enzyme Assays**

CEH activity was determined based on procedures previously described. The reaction mixture in a final volume of 0.2 ml contained 0.05 M Tris maleate (pH
7.0), sodium cholate (10 mM for oocytes and 20–80 mM for rat and rabbit tissues), and appropriately diluted homogenate. Reactions were initiated by addition of 2 nmol cholesteryl [1-14C]oleate with a specific activity of 25 µCi/µmol (Amersham, Arlington Heights, Ill.) in 0.01 ml ethanol, and the tubes were incubated in a water bath at 37°C for various times, up to 24 hours for oocytes and 30 minutes for the rat and rabbit tissues. The released [14C]oleate was assayed as described.* All the activity determinations were done in duplicate or triplicate, and the coefficient of variability was less than 10%. One unit of CEH activity is defined as 1 pmol [14C]oleate released per 24 hours for oocytes and 1 nmol [14C]oleate released per hour for rat and rabbit tissues.

To assay CEH activity in rabbit plasma, the aforementioned protocol was used with slight modifications. The cholate concentration varied from 0 to 100 mM, the incubation time was up to 4 hours, and the volume of plasma sample used was 50 µL. The dilution of the plasma in the 50–µl sample ranged from none to 1:400.

For antibody neutralization experiments, to an appropriate amount of tissue homogenate was added 5 µg of either rabbit anti-rat pCEH immunoglobulin G (IgG) (generously provided by Linda L. Gallo, George Washington University, Washington, D.C.) or rabbit preimmune IgG. After incubation at 4°C overnight, the samples were assayed for CEH activity as described previously.

Nonspecific esterase activity was determined by measuring at 420 nm the production of o-nitrophenol from o-nitrophenyl acetate as previously described. Appropriately diluted liver homogenate in a final volume of 0.25 ml was added to 2.7 ml of a solution containing 20 mM NaH2PO4 buffer (pH 7.4), 1 mM EDTA, and 0.1% Triton X-100. After a 10-minute incubation at 25°C, the reaction was initiated by the addition of 0.05 ml 0.18 M o-nitrophenyl acetate dissolved in ice-cold methanol. The change in absorbance at 420 nm the production of o-nitrophenol from [14C]oleate released per 24 hours for oocytes and 1 nmol [14C]oleate released per hour for rat and rabbit tissues. The released [14C]oleate was assayed as described.*

To increase the sensitivity of the assay to confirm the lack of detectable CEH mRNA, we enriched the mRNA content of four selected samples of total RNA by oligo (dT) column chromatography. Three micrograms of each poly-A+ mRNA sample so obtained was analyzed by Northern blotting. As shown in Figure 1, mRNA from a total RNA sample previously positive for CEH mRNA produced a strong hybridization signal (lane 2, upper panel). In contrast, no hybridization bands corresponding to CEH mRNA were observed in the three other mRNA samples (lanes 1, 3, and 4). These three samples had been isolated from total RNA negative for CEH mRNA by the previous Northern analysis. Note that the 3 µg poly-A+ mRNA used for each sample corresponds to the mRNA contained in approximately 300 µg total RNA. Thus, the blot shown in Figure 1 contains at least 10 times more mRNA in each lane compared with the previous Northern analysis using total RNA.

In contrast to the results with a probe for CEH, when all four poly-A+ samples were hybridized to a probe for β-actin, there was an easily detectable message in all lanes (Figure 1, lower panel), indicating that the results for CEH were not due to general decreases in mRNA abundance or blotting artifacts. Therefore, we conclude that there is great variability in the abundance of CEH mRNA among rat livers. This result reconciles the findings in two recent reports that concluded that rat liver does and does not contain CEH mRNA.

However, there was no clear relation between the variability of CEH enzymatic activity and message level in liver homogenates. Table 2 summarizes results from the four rat liver samples used in Figure 1, as well as from the other rat liver (from the original group of 10 animals) that contained a detectable level of CEH activity.

![FIGURE 1. Northern blot analysis of rat liver RNA. Samples of purified poly-A+ RNA (3 µg from each liver per lane) were separated on denaturing agarose gels and transferred to nylon membranes. Hybridization was then performed with a 32P-labeled DNA probe for cholesteryl ester hydrolase (CEH) (upper panel). After stripping and reexposing the blot to confirm that the CEH probe had been removed, the membrane was rehybridized to a labeled DNA probe for β-actin (lower panel).](http://atvb.ahajournals.org/)

| Table 1. Cholesteryl Ester Hydrolase Activity of Tissue Homogenates From Rats and Rabbits* |
|------------------|---|---|
| Animal          | Liver | Intestine |
| Rat (n=10)      | 1,345±1,324 | 28,988±5,121 |
|                 | (5–3,350) | (23,555–36,503) |
| Rabbit (n=15)   | 3±1    | 11,180±11,924 |
|                 | (2–5)  | (730–35,757) |

Data are mean±SD with range in parentheses.

*Cholesteryl ester hydrolase activity units are per gram of tissue. One unit=1 nmol oleic acid released from cholesteryl oleate per hour.

†Final cholate concentration was 20 mM for liver and 80 mM for intestine.

Results

Rat and Rabbit Hepatic Studies

CEH activity was highly variable among the liver homogenates of 10 rats of the Lewis strain, which was similar to previous results in other strains.* CEH activity ranged from almost zero to >3,000 nmol/hr/g liver tissue and was extremely variable, as indicated by the standard deviations of the mean values (Table 1). To determine whether the variability of CEH activity in rat liver was related to changes in mRNA abundance, total RNA samples from individual rat livers were isolated, and 25 µg of each sample was analyzed by Northern blotting. Among 10 samples, two contained demonstrable CEH mRNA (data not shown).

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mRNA (animal No. 5; the other rat numbers correspond to the lane numbering in Figure 1). Note that the mRNA levels vary from essentially absent (rats 1, 3, and 4) to intermediate (rat 5) and high (rat 2) but that there is no direct or inverse correlation with enzyme activity. To ensure that the variation in enzyme activity was not due to an artifact related to sample degradation, we measured in the homogenates an unrelated enzymatic activity, nonspecific esterase. All five samples had significant levels of this activity within a relatively narrow range, 246±65 units (mean±SD). There was no correlation between nonspecific esterase and CEH activities, as indicated by the finding that the two samples with the highest nonspecific esterase activity (rats 2 and 3) had the lowest and highest levels, respectively, of CEH activity.

Another artifact leading to the wide range of CEH activity that we and others have observed would be accidental contamination of our samples by variable amounts of pancreatic tissue taken at the time of dissection. To assess this possibility, the activity of amylase, another pancreatic enzyme, was measured. The variation in CEH activity in 10 liver samples was 640-fold, while the small amount of amylase activity (<1% of the pancreatic activity per gram of tissue) was found to vary only sixfold. If both enzymes were derived from gross contamination of liver by pancreatic tissue, there would be similar variability in both activities. The variation in CEH activity in 10 liver samples was consistent with the findings by others of amylase activity from gross contamination of liver by pancreatic tissue, there would be similar variability in both activities. The variation in CEH activity in 10 liver samples was consistent with the findings by others of amylase activity from gross contamination of liver by pancreatic tissue, there would be similar variability in both activities.

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We next turned to the Xenopus oocyte expression system to address two questions: 1) Did the negligible amount of CEH activity in the liver of rat 2, despite abundant message, indicate anything about the translatability of the CEH mRNA of this sample? and 2) Was the CEH activity measured in the livers of rats 1, 3, and 4 encoded by mRNAs expressible in the oocyte but not hybridizable to the CEH cDNA probe? That is, were other protein species besides CEH responsible for the finding of abundant CEH activity in these liver homogenates in the absence of detectable CEH message?

The results are summarized in Table 2. Note that CEH activity was expressed only in oocytes injected with those RNA samples that were shown to have CEH mRNA by Northern analysis. The expressed activity was also inhibited (>95%) by the monospecific antibody to pCEH. The level of expression correlated with the abundance of CEH mRNA (compare results in rats 2 and 5), indicating that the levels of homogenate CEH activity in rats 2 and 5 (5 and 1,623 nmol/hr/g, respectively) could not be explained by differences in the translatability of the CEH messages contained in each sample.

The lack of CEH expression after injection of RNA samples from rats 1, 3, and 4 implies that the livers of these rats did not contain other unrelated mRNA species that encode enzymatic activities similar to those of CEH. Further evidence that the CEH activity of these homogenates was derived from authentic CEH, despite the absence of CEH message, came from antibody inhibition studies. Greater than 95% of the CEH activity in the liver homogenates of rats 1, 3, and 4 was inhibited by the antibody to pCEH.

Because the rabbit has a greater tendency than the rat to accumulate cholesteryl esters, we examined potential species differences in hepatic CEH activity and gene expression. Essentially no enzymatic activity was observed in any of the 15 animals whose data are summarized in Table 1, as well as in another group of 15 animals. The concentration of cholate required for maximal activity of CEH in vitro varies in homogenates of different tissues. To ensure that the results in the rabbit were not due to use of a suboptimal concentration of cholate in the assay, we measured the CEH activity in our samples, using a range of cholate from 0 to 50 mM. No stimulation of CEH activity occurred at any level.

Because we observed in the rat that CEH mRNA can be abundant in the absence of significant enzyme activity (rat 2, Figure 1 and Table 2), we subjected the RNA isolated from the rabbit livers to blotting analysis. As shown in Figure 2B, none of the 15 samples contained detectable CEH mRNA, even after 4 days of exposure of the blot to x-ray film. In contrast, each sample contained abundant β-actin mRNA (Figure 2A). Because we noted in the rat that CEH activity was expressed in oocytes only with RNA shown to be positive by blotting (Table 2), it was not surprising that injection of any of the rabbit RNA samples did not result in expression of enzyme activity.

**Rat and Rabbit Intestinal Studies**

As noted in the introduction, there is a large and variable amount of CEH activity in rat intestinal homogenates. To determine whether the enterocyte has the capacity to synthesize CEH, we performed blotting analysis using RNA isolated from intestinal mucosal cells. Significant levels of CEH message were found in only one of six samples (Figure 3B, column 1, row 3). As in the rat liver, there was no correlation in selected samples between the tissue homogenate activity and

<table>
<thead>
<tr>
<th>Rat</th>
<th>CEH activity*</th>
<th>Relative level†</th>
<th>Oocyte expression‡</th>
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</thead>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>5</td>
<td>++ ++</td>
<td>555</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
<td>580</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
<td>1,623</td>
<td>+</td>
<td>133</td>
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*Cholesteryl ester hydrolyase (CEH) activity units are per gram of liver tissue. One unit=1 nmol oleic acid released from cholesteryl oleate per hour.
†Assessed by densitometry of autoradiograms. None detected.
‡CEH activity units are per oocyte. One unit=1 nmol oleic acid released from cholesteryl oleate per 24 hours.
mRNA abundance (Table 3), and all samples contained β-actin mRNA (Figure 3A, column 1).

To ensure that the CEH activity in samples without detectable CEH message was not associated with other unrelated protein species, we performed oocyte injection and antibody inhibition experiments. Greater than 95% of the enzyme activity in the tissue homogenates was inhibited in the presence of the monospecific antibody to rat pCEH. CEH activity in the oocyte was expressed only with the RNA sample that was positive by blotting (Table 3), and this expressed activity was also inhibited (>95%) by the antibody. Thus, as in rat liver, we can conclude that in rat intestine, there can be significant levels of authentic CEH activity in the tissue homogenate in the absence of CEH mRNA in the tissue. Furthermore, the variation in homogenate activity cannot be explained by changes in CEH gene expression in the intestine.

We also examined the CEH activity and gene expression in rabbit intestine. Unlike rabbit liver, the intestine contains significant levels of CEH activity (Table 1). Compared with rat intestine, the average CEH level was lower and more variable in the rabbit. However, six of seven rabbit intestinal RNA samples contained detectable CEH message (Figure 3B, column 2), indicating that the capability of rabbit enterocytes to synthesize CEH was quite frequent, in contrast to the rat.

When *Xenopus* oocytes were injected with selected samples of rabbit intestinal RNA, there was an excellent correlation (r²=0.99) between the level of expressed enzyme activity and mRNA abundance (Table 4; rabbit numbers correspond to the row numbering in Figure 3). We also noted that except for one sample (rabbit 4, Table 4), the rank orders of CEH mRNA abundance, expression in oocytes, and homogenate activity were identical.

Finally, to assess species variation in CEH mRNA length, samples of poly-A+ mRNA were subjected to Northern blot analysis (Figure 4). Rat liver and intestine contained a single species of CEH mRNA of approximately 2,200 bases (lanes 1 and 3, respectively). Consistent with the results in Figure 2, rabbit liver did not contain CEH mRNA (lane 2). In rabbit intestine, CEH mRNA was approximately 1,950 bases (lane 4). There was no evidence of RNA degradation in any lane.

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**Table 3. Cholesteryl Ester Hydrolase Activity in Homogenates of Rat Intestine and Injected *Xenopus* Oocytes**

<table>
<thead>
<tr>
<th>Rat</th>
<th>CEH activity*</th>
<th>Relative level†</th>
<th>Oocyte expression‡</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>23,555</td>
<td>+</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>36,503</td>
<td>−</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>28,878</td>
<td>−</td>
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<tr>
<td>4</td>
<td>24,398</td>
<td>−</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

*Cholesteryl ester hydrolase (CEH) activity units are per gram of intestinal mucosal cells. One unit=1 nmol oleic acid released from cholesteryl oleate per hour.
†Assessed by densitometry of autoradiograms. −, None detected.
‡CEH activity units are per oocyte. One unit=1 pmol oleic acid released from cholesteryl oleate per 24 hours.

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**Table 4. Cholesteryl Ester Hydrolase Activity in Homogenates of Rabbit Intestine and Injected *Xenopus* Oocytes**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>CEH activity*</th>
<th>Relative level†</th>
<th>Oocyte expression‡</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>19,563</td>
<td>++++</td>
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<td>2</td>
<td>730</td>
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<td>4</td>
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<td>185</td>
</tr>
<tr>
<td>6</td>
<td>12,850</td>
<td>+</td>
<td>38</td>
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Although CEH mRNA is shorter in the rabbit than in the rat, this would not result in a smaller protein product if the variation were in the untranslated region. However, that the length polymorphism involves the translated region is suggested by the apparent molecular weight of 55,000–59,000 d on SDS-acrylamide gels of a partially purified rabbit bile salt–dependent CEH. Most estimates of the molecular weight of the rat enzyme are in the 67,000–72,000-d range (for example, see Reference 2).

Rabbit Plasma Cholesteryl Ester Hydrolase Activity

We previously reported CEH activity in rat plasma with characteristics identical to the pancreatic enzyme. To determine whether rabbit plasma also contained a bile salt–dependent CEH activity, we assayed samples from eight animals. Despite varying the cholate concentration and dilution of the plasma sample (see "Methods"), no activity above background was detected in any sample.

Discussion

In these studies, we initially explored the possibility that the highly variable expression of CEH activity in rat liver and intestine was the consequence of the differential expression of the mRNA for the enzyme. Our approach was to compare the tissue activity levels with mRNA abundance. The presence of CEH mRNA was assessed by two methods: 1) detection of the message by Northern blotting analysis, using a full-length cDNA probe for the rat pancreatic enzyme, and 2) expression of functional enzyme activity in Xenopus oocytes injected with rat tissue RNA. On Northern blots, the cDNA probe for the pancreatic enzyme hybridized to a message in rat liver and intestinal RNA (Figures 1 and 4) that is identical in size to that detected in rat pancreas. Injection of rat tissue mRNA into Xenopus oocytes resulted in the expression of functional CEH activity with properties identical to those of the tissue enzyme activity ( "Results" section; see also Reference 12). These two methods were consistent: the only samples of tissue RNA that led to expression of the functional enzyme in oocytes were those with detectable CEH mRNA on blotting analysis.

Our results demonstrate that similar to the expression of enzyme activity in rat liver and intestine, there was also highly variable expression of CEH mRNA in these tissues. However, there was no correlation between levels of mRNA and enzyme activity (Tables 2 and 3). Particularly striking in both rat tissues was the presence of significant enzyme activity in the absence of detectable message. A likely explanation is that CEH activity can be derived largely from sources exogenous to these tissues.

We and others have previously provided data to support this possibility. Diversion of pancreatic secretions in rats results in a rapid and significant decline in CEH activity in the intestine. Using immunological and ultrastructural methods, respectively, have shown that at least part of the intracellular CEH pool in rat enterocytes was derived from uptake of pancreatic enzyme from the intestinal lumen. The intracellular enzyme is catalytically active and can be exported from the cells into the mesenteric lymph (References 5 and 29 and R. Zolfaghari and E. Fisher, unpublished observations). Because this lymph enters the circulation via the thoracic duct, it must contribute to the CEH activity in rat plasma that we have previously reported. Finally, because CEH binds to the surface of hepatic cells, the liver-associated activity may represent enzyme cleared from the plasma. In many ways, this model is similar to the metabolic pathway of another lipase, namely LPL. LPL is synthesized in adipocytes and striated muscle, transported across endothelial cells, and released into the circulation. The LPL in plasma binds to the liver cell membrane and is internalized. Although the presence of tissue CEH enzyme activity in the absence of CEH mRNA may be explained by having an exogenous source, we have no explanation for the variable presence of the mRNA itself.

Another potential explanation for the absence of CEH mRNA in rat tissues despite enzymatic activity is that the half-life of CEH mRNA is significantly shorter than that of the protein. However, the finding of abundant CEH mRNA in the absence of enzyme activity in the liver of rat 2 (Figure 1, Table 2) is not consistent with this model. Nonetheless, although we do not consider it likely, we have not formally excluded the possibility that rat liver contains two pools of CEH mRNA, with an actively translated pool having a significantly shorter half-life than an inactive pool.

Other striking aspects of our results are the differences between rats and rabbits, namely, that rabbit liver contains neither CEH mRNA nor enzyme activity and that the majority of rabbit intestinal samples had easily detectable CEH mRNA. The functional consequences of these differences are not known. However, it is interesting to note that rabbit (compared with rat) bile is poor in free cholesterol and that rabbit liver can be easily induced to accumulate cholesteryl esters by dietary manipulation. Both phenomena may be associated with the reduced ability to hydrolyze cholesteryl esters in rabbit liver.

If, as we hypothesized for the rat, hepatic CEH activity can originate from the pancreas, we were intrigued by the lack of hepatic CEH activity in the rabbit. There may be fundamental differences between rats and rabbits in the intestinal uptake and intracellular metab-
olism of CEH. For example, the majority of rabbit intestinal samples had the capacity for intracellular synthesis, consistent with a relative inability of the rabbit enterocyte to import luminal CEH. The ability of the rabbit enterocyte to synthesize CEH may explain why the intestinal homogenate activity in the rat was clearly independent of CEH mRNA levels (Table 3), whereas in the rabbit, tissue activity tended to follow mRNA relative abundance (Table 4). Finally, the lack of rabbit plasma CEH activity may indicate that export of CEH from the rabbit enterocyte is also limited. The basis for these phenomena may involve variations in enterocyte processing of CEH or cell-surface components that bind CEH, such as proteins and the glycocalyx. There may also be species differences in the structure of the CEH protein, thus altering its properties and the glycolcayx.

In summary, using a variety of approaches including specific DNA and antibody probes as well as in vitro translation, we have demonstrated that in rat liver and intestine, CEH mRNA levels and tissue enzyme activity are not correlated. There are also major differences in the distribution of CEH mRNA and tissue activity in rats and rabbits. We propose that factors related to the intestinal or intravascular transport of CEH underlie these findings. The elucidation of these factors is likely to contribute to our understanding of species-specific differences in the regulation of cholesterol and cholesteryl ester metabolism.

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References


Tissue and species differences in bile salt-dependent neutral cholesteryl ester hydrolase activity and gene expression.

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