Modulation of low density lipoprotein receptor activity by bile acids: differential effects of chenodeoxycholic and ursodeoxycholic acids in the hamster[1]

Mauro Malavolti, Hans Fromm,[2] Susan Ceryak, and Ingram M. Roberts

Division of Gastroenterology, Department of Medicine, George Washington University Medical Center, Washington, DC 20037, and Gastroenterology Unit, Department of Medicine, Montefiore Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Abstract Hamsters were fed chenodeoxycholic acid (CDC), ursodeoxycholic acid, (UDC), or no bile acid. [^{14}C]Sucrose-labeled hamster low density lipoprotein (LDL) and methylated human LDL were infused intravenously to study LDL receptor-dependent and LDL receptor-independent organ uptake, respectively, of LDL. Biliary CDC increased during both CDC and UDC treatment. The UDC enrichment of bile after UDC feeding was relatively small. Bile acid synthesis was suppressed after both bile acid treatments. Under the condition of an acute bile fistula, the hamster LDL uptake increased in the liver, heart, and adrenals in the CDC-treated animals. During an intact enterohepatic circulation, the hepatic uptake of hamster LDL, which accounted for a major portion of the total uptake, was increased after UDC treatment. The hamster LDL uptake in the colon, which represented only a small fraction of the total uptake, increased after CDC treatment. When hamster LDL was infused at increasing concentrations, its uptake was significantly higher in the UDC-treated than in the control and CDC-treated animals. The methylated human LDL uptake showed no significant changes in the different treatment groups under either experimental condition. The study shows significantly different effects of CDC and UDC on LDL receptor activity. Since these differences are expressed in spite of a similar suppression of bile acid synthesis, UDC may directly influence LDL receptor activity.—**Malavolti, M., H. Fromm, S. Ceryak, and I. M. Roberts.** Modulation of low density lipoprotein receptor activity by bile acids: differential effects of chenodeoxycholic and ursodeoxycholic acids in the hamster. *J. Lipid Res.* 1987. **28:** 1281–1295.

Supplementary key words LDL uptake • bile acid synthesis • biliary bile acid composition

Low density lipoproteins (LDL) are catabolized by receptor-dependent (1,2) and receptor-independent mechanisms (3, 4). The activity of the LDL receptor provides an important mechanism for the maintenance of cholesterol homeostasis in the body (1–5). Since bile acids represent a major product of cholesterol metabolism, changes in the synthesis of bile acids and, consequently, of cholesterol, result in appropriate modifications of the activity of the LDL (apoB, E) receptor (6–9).

An acceleration of bile acid synthesis due, for example, to an enhanced bile acid loss leads to an increased demand for the precursor, cholesterol, and a consequent activation of the LDL receptor (6, 8–10). The therapeutic use of bile acids, such as chenodeoxycholic acid (CDC) and its 7β-epimer, ursodeoxycholic acid (UDC), has raised the question as to their influence on LDL catabolism (11–14). Long-term treatment of gallstone patients with CDC has been shown to be associated with minor, but statistically significant increases in serum LDL cholesterol (11, 12). In contrast to CDC, UDC does not seem to influence serum levels of LDL cholesterol (15). The differential response of serum cholesterol to the two bile acid epimers could be an expression of opposing effects they may exert on the activity of the LDL receptor. One mechanism, which could be responsible for these divergent effects on LDL receptor function, relates to the difference between CDC and UDC which has been shown to exist

Abbreviations: LDL, low density lipoproteins; CDC, chenodeoxycholic acid; UDC, ursodeoxycholic acid; GLC, gas–liquid chromatography. The chemical names of the bile acids referred to are: 3α,7α-dihydroxy-5β-cholan-24-oic acid, chenodeoxycholic acid; 3α,7β-dihydroxy-5β-cholan-24-oic acid, ursodeoxycholic acid; 3α-hydroxy-5β-cholan-24-oic acid, lithocholic acid; 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, cholic acid; 3α,12α-dihydroxy-5β-cholan-24-oic acid, deoxycholic acid.

[1]This paper was presented in part at the Annual Meeting of the American Gastroenterological Association, New York, NY, May 1985, and at the Plenary Session of the Annual Meeting of the American Society for the Study of Liver Diseases, Chicago, IL, November 1986.

[2]Address reprint requests to: Hans Fromm, M.D., Division of Gastroenterology, Department of Medicine, George Washington University Medical Center, The Burns Memorial Building, 2150 Pennsylvania Avenue NW, #606, Washington, DC 20037.

in man as far as the modulation of bile acid synthesis is concerned. While CDC suppresses bile acid synthesis, UDC appears to have either no (16–20), or a stimulating (21) influence. Whether mechanisms unrelated to bile acid synthesis, such as, for example, direct physicochemical interactions of certain bile acids with either the uptake function of the LDL receptor or its recycling between cell surface and intracellular compartments, could also be involved in the modulation of its activity, is unknown. The present study, therefore, aims at investigating, in a hamster model, *1*) the effect of long-term feeding of CDC and UDC on LDL receptor-dependent and LDL receptor-independent uptake of LDL in the major organs; and *2*) the relation between LDL uptake and bile acid synthesis in the two bile acid treatment groups.

METHODS

Animals

A total of 116 male Golden Syrian hamsters (Harlan Sprague-Dawley, Indianapolis, IN) was studied. The numbers of animals in the different study groups are listed in the figures and tables. Three groups of hamsters, each animal weighing 100–130 g, were fed a 0.027% cholesterol rodent chow diet (Ralston Purina Co., St. Louis, MO) for 4 weeks. One group received the diet alone (control), the second received, in addition, 0.1% CDC, and the third the same dose of UDC. There were no differences among the treatment groups as far as food intake and weight gain were concerned. Both CDC and UDC were well tolerated by the animals. They had no diarrhea, and the livers appeared macroscopically normal. Light-microscopic examination of the liver, after 4 weeks of CDC (n = 6), UDC (n = 4), and control (n = 6) feeding, respectively, in 16 randomly chosen hamsters, showed normal findings in 14 of them, and mild to moderate abnormalities in the remaining two. The abnormalities consisted of bile duct proliferation in one CDC-fed hamster, and of fatty changes in one control animal. CDC was supplied by Dr. Falk GmbH & Co. (Freiburg, West Germany) and UDC by Tokyo Tanabe Co., Ltd. (Tokyo, Japan). CDC was >98% pure and UDC was >99% pure as judged by gas–liquid chromatography (GLC) (22–24). The animals were kept on alternating 12-h light and 12-h dark cycling. All LDL infusion experiments were started during the light cycle between 9 AM and 12 noon. Each study on a given day was carried out in three animals, one of each group. The sequence in which the animals were studied was randomized.

Bile acid analysis of gallbladder bile

Bile acid composition of gallbladder bile was determined by GLC methods previously established in this laboratory (22–24).

Measurement of fecal bile acids and of bile acid synthesis

During the fifth week of treatment, animals were housed individually in metabolic cages. Complete 24-h fecal collections were obtained for 8 days from each hamster. The dietary intake was measured. In each animal, three 24-hr fecal samples from the second half of the collection period were used for bile acid analysis. Fecal bile acids were analyzed qualitatively and quantitatively by GLC as previously described (23).

Bile acid synthesis was estimated by subtracting the dietary intake of bile acids from the total fecal bile acid excretion (19, 25).

LDL and albumin preparation

Hamster LDL was prepared from untreated animals that had been fed a 0.027% cholesterol rodent chow diet. The animals were decapitated and bled between 9 AM and 12 noon. The blood was collected in sterile glass tubes containing EDTA (final concentration 4 mM, pH 7.4). The plasma was separated by centrifugation at 300 *g* for 15 min at 4°C. Plasma lipoproteins were separated by density gradient ultracentrifugation (26). In brief, samples of plasma were adjusted to d 1.21 g/ml with solid potassium bromide, and 4.0-ml aliquots were pipetted into 13.2-ml polyallomer centrifuge tubes (Beckman Instruments, Palo Alto, CA). A discontinuous gradient was prepared as described by Redgrave, Roberts, and West (26). All salt solutions contained EDTA, 0.1 mg/ml, and were prepared from potassium bromide and sodium chloride according to Havel, Eder and Bragdon (27). Ultracentrifugation was carried out at 10°C for 24 hr at 286,000 *g* and 41,000 RPM using the SW41 Beckman rotor. The layers corresponding to the densities from 1.020 to 1.060 g/ml were removed from the tubes by needle aspiration. The aspirated layers were transferred to another tube which was filled with salt solution at a density of 1.060 g/ml (27).

The ultracentrifugation was repeated at the above described conditions for 24 hr. The lipoprotein fraction with a density of 1.060 g/ml, which concentrated in the top layer of the ultracentrifuged tube, was removed by needle aspiration and dialyzed against 0.9% sodium chloride and 0.01% EDTA at a pH range from 7.2 to 7.4. Parallel to the isolation of homologous hamster LDL, human LDL was prepared to study the LDL receptor-independent LDL uptake. The procedures for the preparation of LDL from human plasma were identical to those for the isolation of LDL from hamster plasma. The apoprotein content of both the hamster and human LDL preparations migrated in one band in the position of apolipoprotein B in the vertical slab gel electrophoresis, which was performed according to the method of Laemmli (28). Hamster and human LDL as well as hamster albumin (Research Plus, Inc., Bayonne, NJ) were labeled

with [^{14}C]sucrose as described by Pittman et al. (29). The specific activities (dpm/μg of protein) were 1188 ± 718.6 (mean ± SEM) for the hamster LDL, 2112 ± 566.2 for human LDL, and 3885 ± 244.9 for hamster albumin. The labeled LDL and albumin in the exclusion volume were dialyzed at pH 7.4 (29).

The human LDL was reductively methylated as described by Weisgraber, Innerarity, and Mahley (30) in order to study the LDL receptor-independent uptake of LDL. Methylated LDL has been shown to be taken up by LDL receptor-independent mechanisms (30, 31). In the majority of the preparations, the labeled LDL was chromatographed on silica gel G thin-layer plates in n-propanol–ethyl acetate–water 70:20:10 (vol/vol) to determine the presence of unbound sucrose. No radioactivity was detected in the position of free sucrose.

Assessment of purity and integrity of LDL preparations

For most preparations, labeled hamster LDL and methylated human LDL were also subjected to SDS polyacrylamide gel electrophoresis (28). After silver staining (32), the vertical lanes containing the LDL preparations were cut from the gel and sliced into 7-mm bands. The bands were incubated at 60°C for 24 hr with 1 ml of 30% H_2O_2 until dissolved, and the radioactivity was determined in a liquid scintillation counter. All of the radioactivity on the gel was in the band corresponding to the apolipoprotein B standard.

The LDL preparations (^{14}C-labeled hamster LDL, unlabeled human LDL, ^{14}C-labeled human LDL, and ^{14}C-labeled methylated human LDL) were also characterized by scanning electron microscopy. The size and shape of the LDL particles were comparable to those previously reported by other authors (5).

The LDL preparations were also analyzed for protein, cholesterol, triglyceride, and phospholipid content. The total protein concentration was measured using the method of Bradford (33). Enzymatic assays were used to measure cholesterol and triglyceride (Bio-Dynamics, Boehringer Mannheim, Indianapolis, IN) as well as phospholipid (Nippon Shoji Kaisha, Ltd., Higashi-Ko, Osaka, Japan) concentrations. The concentrations of these components in the different LDL preparations were virtually identical to those described in the literature (27).

The LDL and albumin preparations were refrigerated at 4°C and used within 48 hr.

Measurement of LDL tissue uptake

During the infusion experiments, the animals were kept under diethyl ether anesthesia. The animals were weighed and the respective labeled albumin or LDL preparations were administered via a jugular vein catheter (Silastic®, ID, 0.012 in.; OD, 0.025 in., Dow Corning Corporation, Midland, MI) in a bolus, containing 20 μg of protein, followed by a constant infusion for 1 hr at a rate of 1 μg of protein in 70 μl per min. This infusion rate resulted in constant plasma levels of ^{14}C radioactivity which was measured at 15, 30, and 60 min in all animals. For the measurement of plasma radioactivity, 50-μl blood samples were withdrawn from the vena cava of the hamsters. The dose and rate at which LDL was infused were chosen according to data both from other investigators and from experiments in this study (9). A bolus of 20 μg of LDL protein, followed by a constant infusion at a rate of 1 μg/min, results in hepatic tissue uptake values which approach a plateau, i.e., levels that are close to maximal receptor mediated uptake in the liver (see Fig. 6). It should be emphasized, however, that the dose infused in the present study differed considerably from the trace amount used by other investigators (9). The use of higher doses may allow a better distinction in the LDL uptake between the different treatment groups. In half of the LDL-infused animals studied, a bile fistula was constructed 10 min after the beginning of the injection of the bolus of albumin and LDL, respectively. The cystic duct was ligated and a cannula (Silastic, ID, 0.012 in.; OD, 0.025 in., Dow Corning Corporation, Midland, MI) was placed into the common bile duct. After completing the LDL infusion at 60 min, a sample of at least 100 μl of urine was withdrawn from the urinary bladder by needle aspiration. The animals were killed at 60 min, and the organs were rapidly removed.

The organs were rinsed with 0.9% NaCl, blotted on filter paper, and weighed. With the exception of the liver, which was cut into several sections, the organs were combusted in toto in a Packard Oxidizer (Packard Instrument Corporation, Downers Grove, IL) (34). The ^{14}C radioactivity in the combusted organs as well as in triplicate blood, bile, and urine samples was determined by scintillation counting. The LDL tissue uptake was calculated by subtracting the [^{14}C]albumin tissue space from the [^{14}C]-LDL tissue space. The tissue spaces were expressed by relating the tissue radioactivity to the volume of plasma which contains the same amount of radioactivity (35, 36). The respective tissue space was calculated by the following formula:

$$\frac{\text{dpm}}{\text{tissue weight, g}} \times \frac{1}{\text{dpm/plasma volume, } \mu\text{l}} = \frac{\mu\text{l}}{\text{g}}.$$

The uptake in the different organs was calculated by multiplying the tissue uptake by the respective organ weight, which was normalized for an animal weighing 100 g.

Infusions of hamster LDL at different concentratrions and for prolonged periods of time

Since LDL infusions at low concentrations may not represent a sufficient challenge to the LDL receptor-mediated uptake process, differences among the treatment groups may not be detectable. The results of one

TABLE 1. Biliary bile acid composition

Treatment	CDC	UDC	LC	C	DC	Other
			% of total			
Control (n = 9)	29.2 ± 1.89	1.0 ± 0.97	1.6 ± 1.52	43.0 ± 4.84	20.0 ± 2.61	5.3 ± 3.65
CDC (n = 9)	71.2 ± 3.61[a]	0.7 ± 0.41	4.5 ± 1.05	11.8 ± 1.92[b]	5.0 ± 1.34[b]	6.7 ± 3.79
UDC (n = 9)	55.4 ± 4.45[a]	17.4 ± 4.48[a]	5.9 ± 1.05[c]	10.1 ± 1.74[b]	8.9 ± 1.68[b]	2.0 ± 1.10

Results are expressed as mean ± SEM. CDC, chenodeoxycholic acid; UDC, ursodeoxycholic acid; LC, lithocholic acid; C, cholic acid; DC, deoxycholic acid; Other, unidentified gas-liquid chromatographic peaks.

Respective bile acid in comparison to control fed animals: [a], $P < 0.01$; [b], $P < 0.02$; [c], $P < 0.05$.

recent study by other authors, which failed to show an effect of UDC on LDL uptake, may relate to this possibility (37). Therefore, experiments were carried out in which hepatic LDL uptake was measured after intravenous infusion of three different concentrations of hamster LDL. Each concentration was studied simultaneously in one animal of each treatment group. The injection of a bolus of 5, 20, and 40 μg, respectively, of LDL protein was followed by a 15-min constant infusion of [^{14}C]sucrose-labeled hamster LDL at a rate of 0.25, 1.0, and 2.0 μg per min, respectively.

In addition to these studies of the LDL receptor-dependent uptake, increasing concentrations of [^{14}C]sucrose-labeled methylated human LDL were infused in control animals. The LDL concentrations infused were similar to those used in the hamster LDL studies. A bolus of 10, 20, and 100 μg was followed by a 15-min constant infusion of 0.5, 1.0, and 5.0 μg per min, respectively. The hepatic LDL uptake increased linearly under these conditions, underlining the LDL receptor-independent nature of the uptake of the methylated human LDL (3, 4).

The steady state conditions of the hamster LDL infusions were evaluated by the performance of longer periods of hamster LDL infusions. Two hamsters, one control and one CDC-treated, received a bolus of 20 μg of [^{14}C]sucrose-labeled hamster LDL intravenously, which was followed by a 3-hr constant infusion at a rate of 1 μg/min. The plasma radioactivity remained constant throughout the entire infusion study between 15 min and 3 hr.

Analysis of total, HDL and LDL cholesterol in serum

Serum was analyzed after the animals had been treated for 6 weeks. HDL cholesterol was measured enzymatically after dextran sulfate-$MgCl_2$ precipitation of apoB-containing lipoproteins in fresh serum samples (38). The completeness of the precipitation was confirmed by the absence of apoB-containing lipoproteins in the supernatant, as assessed by agarose electrophoresis (39). LDL cholesterol was estimated from the measurement of total cholesterol, total triglycerides, and HDL cholesterol, using the empirical relationship of Friedewald (40).

Statistical analyses

The data were expressed as mean ± standard error of the mean (SEM). The statistical significance of the differences among the study groups was calculated by the two-way analysis of variance and the Student-Neuman-Keuls test (41).

RESULTS

Body and organ weights

The body weights of the control, CDC- and UDC-treated animals at the time of study, which were 129 ± 3.8, 125 ± 4.3, and 121 ± 3.6 g, respectively, did not differ significantly. There were also no statistically significant differences in the organ weights among the treatment groups.

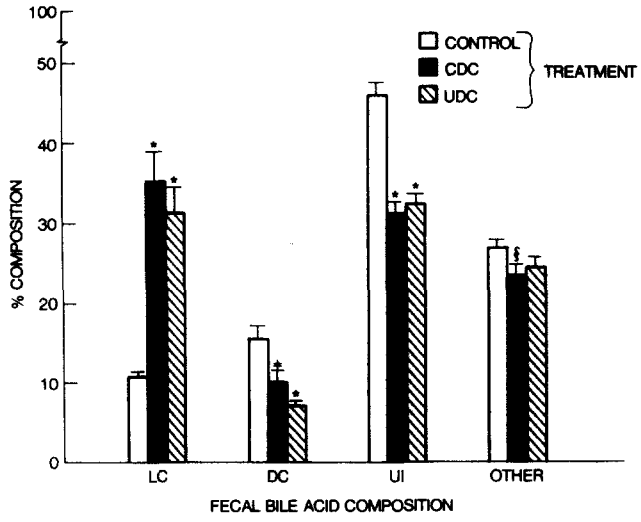

Fig. 1. Fecal bile acid composition after control, chenodeoxycholic acid (CDC), and ursodeoxycholic acid (UDC) treatment. Abbreviations: LC, lithocholic acid; DC, deoxycholic acid; UI, one major unidentified GLC peak; other: combined area under the curve of six other unidentified peaks in the gas chromatogram (See also text of Result section). *, $P < 0.01$ versus control; ‡, $P < 0.02$ versus control; §, $P < 0.05$ versus control.

TABLE 2. Rate of bile acid synthesis

Treatment group[a]	Number of Animals	Total Fecal Bile Acid Excretion	Rate of Bile Acid Synthesis (Total fecal bile acid excretion minus dietary bile acid intake)
		mg/24 hr	
Control	13	5.01 ± 0.505	5.01 ± 0.505
Chenodeoxycholic acid	13	7.13 ± 0.910	0.87 ± 0.708[b]
Ursodeoxycholic acid	13	6.01 ± 0.818	−0.57 ± 0.768[b]

[a] For description of treatment regimens see legend of Fig. 1 as well as text of Methods section.
[b] Denotes statistical significance ($P < 0.01$) of difference between respective treatment and control groups.

Biliary bile acid composition

The biliary bile acid composition in the three treatment groups is shown in **Table 1**. The CDC content in bile increased significantly during both CDC and UDC treatment. The increase in biliary UDC during UDC feeding was statistically significant. However, the UDC treatment-induced enrichment of bile in UDC in the hamsters was considerably smaller than that seen in man (about 17% vs. approximately 60%) (13). Both the CDC and UDC treatment groups were also characterized by a significant decrease in the biliary content of cholic and deoxycholic acids.

Fecal bile acids and rate of bile acid synthesis

The fecal bile acid composition in the different treatment groups is shown in **Fig. 1**. Lithocholic acid, deoxycholic acid, and one unidentified bile acid, which represented a major peak, constituted about 75% of the total fecal bile acids. About 20 of the remaining 25% were distributed over six other gas chromatographic peaks. The structures of the bile acids represented by these peaks were not identified. Although differences in the sizes of these peaks among the treatment groups were statistically significant on several occasions, the magnitude of the differences was relatively small, not exceeding 3% in any instance. In both the CDC- and UDC-fed hamsters, lithocholic acid increased significantly, whereas deoxycholic acid and the major unidentified bile acid peak decreased significantly.

The rates of bile acid synthesis in the three treatment groups are listed in **Table 2**. Both the CDC and UDC feedings effected a significant depression of bile acid synthesis to less than one-fifth of the control value.

Studies in bile fistula animals

Tissue uptake. The total hamster LDL uptake in all organs combined was significantly ($P < 0.05$) higher in the CDC-treated animals than in the control and UDC-treated animals **(Table 3)**. The percent uptake of LDL in the different organs is shown in **Table 4**. In all three treatment groups, a major portion of the LDL receptor-dependent uptake of LDL took place in the liver. It accounted for approximately one-third of the total. There were no significant differences between the treatment groups as far as the percent LDL uptake in the different organs was concerned. The LDL uptake in the different organs expressed as both μl/g and μl/organ is shown in **Fig. 2** and **Fig. 3**. The receptor-dependent uptake of LDL in the liver and heart was significantly higher in the CDC group than in the control and UDC-treated hamsters (Fig. 2). In addition to the liver and heart, the adrenals showed significantly higher LDL uptake in the CDC group when it was calculated in terms of organ uptake (μl/organ). The receptor-independent uptake of LDL in the different organs showed no significant differences among the treatment groups.

Excretion of radioactivity into bile and urine. As expected (8, 34), only small amounts of the radioactivity administered as [^{14}C]sucrose-labeled hamster LDL and reductively methylated human LDL, respectively, were excreted into bile and urine. About 0.1% of the administered radioactivity was excreted in bile during the 50-min collection.

TABLE 3. Total organ uptake of LDL in the different treatment groups

Group	^{14}C-Labeled Hamster LDL			^{14}C-Labeled Methylated Human LDL		
	Control	CDC	UDC	Control	CDC	UDC
			μl/organ, mean ± SEM			
Acute bile fistula	1327.4 ± 407.58	3376.5 ± 1173.28[a,b]	1287.0 ± 498.72	520.6 ± 188.60	1042.6 ± 365.65	1246.7 ± 502.00
Intact enterohepatic circulation	1313.6 ± 392.30	1096.5 ± 257.80	1476.2 ± 224.00	439.9 ± 70.40	700.1 ± 341.67	905.1 ± 212.52

[a] Bile acid versus respective control-fed animals: $P < 0.05$.
[b] CDC versus respective UDC-fed animals: $P < 0.05$.

TABLE 4. Percent uptake of LDL in the different organs in the acute bile fistula animals

Organ	^{14}C-Labeled Hamster LDL			^{14}C-Labeled Methylated Human LDL		
	Control	CDC	UDC	Control	CDC	UDC
Liver	22.4 ± 6.48	26.9 ± 14.86	27.1 ± 3.18	26.8 ± 6.32	24.0 ± 8.69	29.2 ± 11.20
Adrenal	0.3 ± 0.20	0.2 ± 0.07	0.2 ± 0.08	0.1 ± 0.10	0.1 ± 0.01	0.1 ± 0.03
Aorta	0.1 ± 0.02	0.04 ± 0.02	0.1 ± 0.03	0.2 ± 0.17	0.04 ± 0.01	0.1 ± 0.04
Lung	5.3 ± 1.68	6.3 ± 3.41	7.8 ± 1.13	30.2 ± 9.29	13.3 ± 1.6	12.7 ± 4.38
Heart	0.1 ± 0.15	0.5 ± 0.22	0	0.1 ± 1.0	0.04 ± 0.37	0.8 ± 0.74
Brain	0.9 ± 0.17	0.8 ± 0.40	1.3 ± 0.44	0.2 ± 0.17	0.03 ± 0.21	0.6 ± 0.33
Kidney	0	0.3 ± 1.18	0	5.3 ± 5.35	2.0 ± 2.34	0
Spleen	0	0.1 ± 0.11	0	0.1 ± 0.58	0	0
Stomach	1.0 ± 0.44	0.8 ± 0.52	1.27 ± 0.8	0.5 ± 1.03	0.8 ± 0.29	0.9 ± 0.20
Small intestine	0.9 ± 0.38	0.2 ± 0.68	1.61 ± 0.70	0.8 ± 0.59	1.4 ± 0.44	1.0 ± 0.41
Colon	0.7 ± 0.27	0.6 ± 0.13	0	0	0.3 ± 0.11	0
Cecum	0.9 ± 0.59	1.1 ± 0.31	0.8 ± 0.83	0	1.4 ± 1.07	0.4 ± 0.18
Testes	0	0.2 ± 0.17	0.2 ± 0.36	0	0.4 ± 0.18	0.3 ± 0.08
Epididymis	0.3 ± 0.05	0.1 ± 0.05	0.1 ± 0.03	0	0.5 ± 0.38	0.1 ± 0.04
Seminal vesicles	0.6 ± 0.26	0.2 ± 0.06	0.3 ± 0.11	0.1 ± 0.59	0.4 ± 0.15	0.2 ± 0.13
Prostate	0.1 ± 0.06	0.04 ± 0.01	0.1 ± 0.03	0.03 ± 0.02	0.1 ± 0.04	0.1 ± 0.06
Bladder	0	0	0	0.2 ± 0.20	0	0
Pancreas	0.02 ± 0.10	0.3 ± 0.11	0.2 ± 0.12	1.1 ± 0.7	0.1 ± 0.09	0.3 ± 0.08
Thyroid	0.6 ± 0.11	0.6 ± 0.22	0.3 ± 0.23	0.1 ± 0.18	0.1 ± 0.22	0.3 ± 0.15
Thymus	0.03 ± 0.03	ND	0.02 ± 0.02	ND	ND	ND
Fat	9.2 ± 5.38	8.8 ± 2.16	21.8 ± 8.12	6.9 ± 4.02	2.8 ± 2.77	8.7 ± 4.09
Skin	15.3 ± 5.49	23.6 ± 9.90	6.5 ± 3.75	6.3 ± 4.17	10.8 ± 8.55	14.9 ± 5.37
Muscle	42.6 ± 0.47	29.1 ± 10.91	31.6 ± 10.78	24.6 ± 14.25	41.9 ± 8.94	31.9 ± 12.45
Gallbladder	0.1 ± 0.10	0	0	0	0	0

Values are given as mean ± SEM; ND, no data.

The radioactivity in the 100-μl urine samples ranged from 0 to 0.07% of the administered amount. No statistically significant differences were found in either the biliary or the urinary radioactivity between the different treatment and infusion groups.

Studies in animals with an intact enterohepatic circulation

Tissue uptake. No significant differences were found in the total as well as in the percent LDL organ uptake in the different treatment groups (Table 3 and **Table 5**). However, the tissue uptake of the homologous hamster LDL, expressed as both μl/g and μl/organ, was significantly increased in the liver after UDC treatment in comparison to that after both control and CDC treatment **(Fig. 4** and **Fig. 5**). The hamster LDL uptake in the colon was significantly higher in the CDC-fed than in the control animals (Figs. 4 and 5). The changes in the tissue uptake of methylated human LDL in the different treatment groups were statistically not significant (Figs. 4 and 5).

The results of the studies in which increasing concentrations of homologous hamster LDL were infused simultaneously in three groups of hamsters, three animals per group, are shown in **Fig. 6**. Although the levels of receptor-dependent hepatic LDL uptake at 15 min represent single measurements, the mean ± SEM values of the 60-min uptake after a 20-μg bolus–1 μg/min infusion dose are shown in the graph for comparison. The uptake after 60 min was, as expected, higher than that after 15 min in both the UDC and CDC treatment groups. The 15-min level of the hepatic LDL uptake in the control group was comparable to the upper range of the respective 60-min measurements. The hepatic receptor-dependent 15-min LDL uptake reached different plateau levels in the three treatment groups. The LDL uptake levels at the different infusion concentrations were significantly higher in the UDC-treated than in the control ($P < 0.01$) and CDC-treated ($P < 0.01$) animals. The LDL uptake values of the control group were situated between those of the UDC and those of the CDC treatment groups. However, the difference between the control and CDC treatment groups was not statistically significant.

Excretion of radioactivity in urine. The urinary excretion of the radioactivity in the animals with an intact enterohepatic circulation was virtually indistinguishable from that in the bile fistula hamsters, i.e., it ranged from 0 to 0.02% of the administered dose in the 100-μl urine sample.

Total, HDL and LDL cholesterol in serum. The total as well as HDL and LDL cholesterol values in serum, after a 6-week control, CDC- and UDC-treatment, respectively, are listed in **Table 6**. In comparison to the animals treated with UDC, those that were fed CDC showed a slightly higher total serum cholesterol ($P = 0.072$), but a lower fraction of cholesterol in HDL ($P = 0.020$). The HDL fraction in the CDC treatment group was also

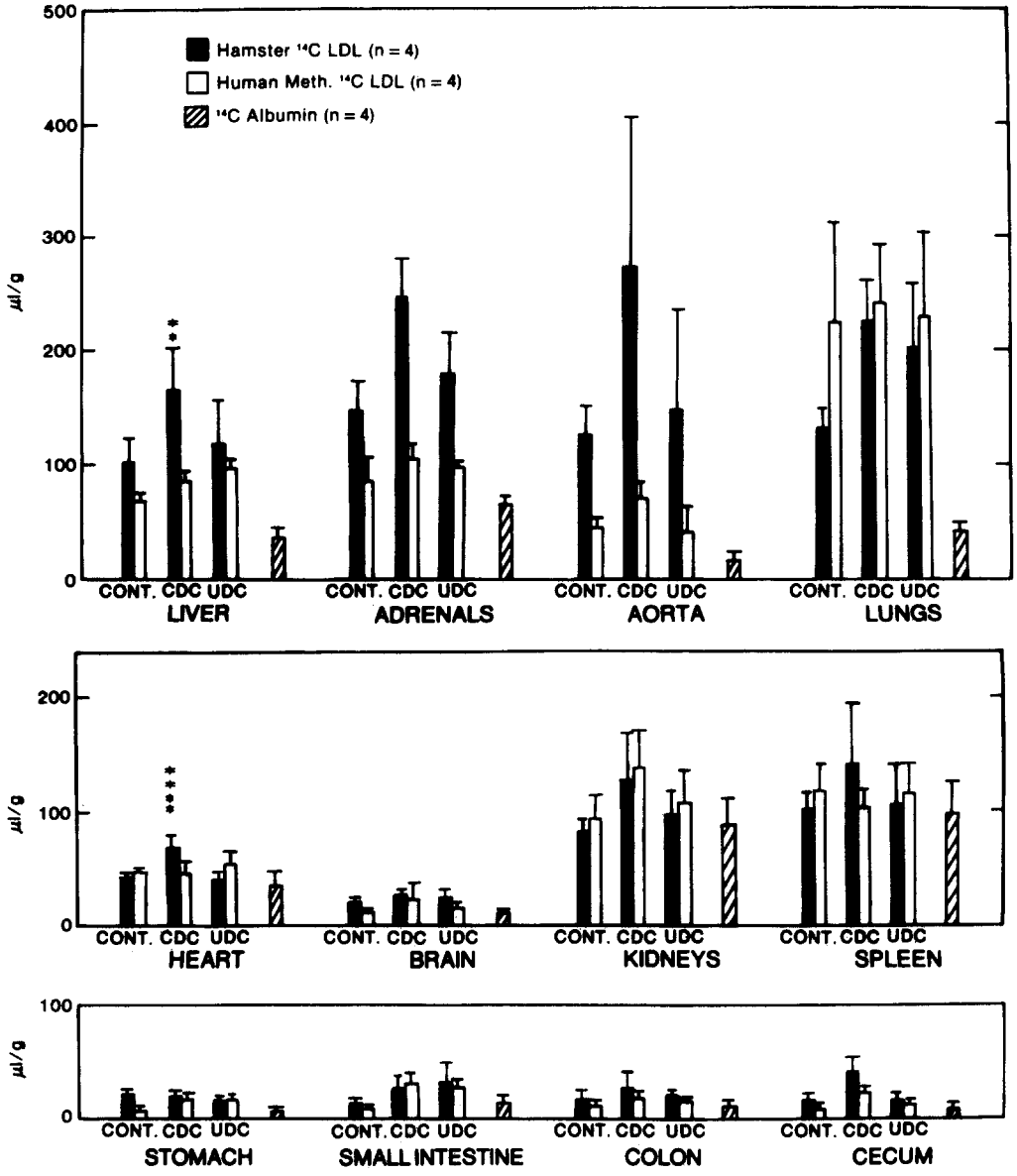

Fig. 2. Tissue spaces of low density lipoprotein (LDL) and albumin in different organs of the control (CONT), chenodeoxycholic acid-treated (CDC), and ursodeoxycholic acid treated-(UDC) bile fistula hamsters, after 1 hr of intravenous infusion of [^{14}C]sucrose-labeled hamster LDL, methylated human LDL, and albumin, respectively (mean ± SEM). Tissue uptake of LDL is calculated by subtracting the albumin spaces in the different organs from the respective LDL spaces. For calculation of tissue space, see text of Method section. *, $P < 0.05$; **, $P < 0.01$ versus control treatment; ‡, $P < 0.05$, ‡‡, $P < 0.01$ versus treatment with the other bile acid.

slightly lower than that in the controls ($P = 0.055$). The differences in the LDL levels among the treatment groups were statistically not significant.

DISCUSSION

The choice of a hamster model for the present studies relates to important similarities that exist between the hamster and humans as far as the metabolism of cholesterol and lipoproteins is concerned. The similarities pertain to the use of LDL as a cholesterol carrier (9, 42) as well as to the ability of the hamster to either form cholesterol gallstones or to dissolve them during CDC and UDC treatment (43–45). The response of the hamster to bile acid therapy in this study, as far as the enrichment of bile with the administered bile acid is concerned, was similar to that reported by other investigators (37).

When the CDC- and UDC-induced changes in bile acid metabolism in the hamsters are compared with those

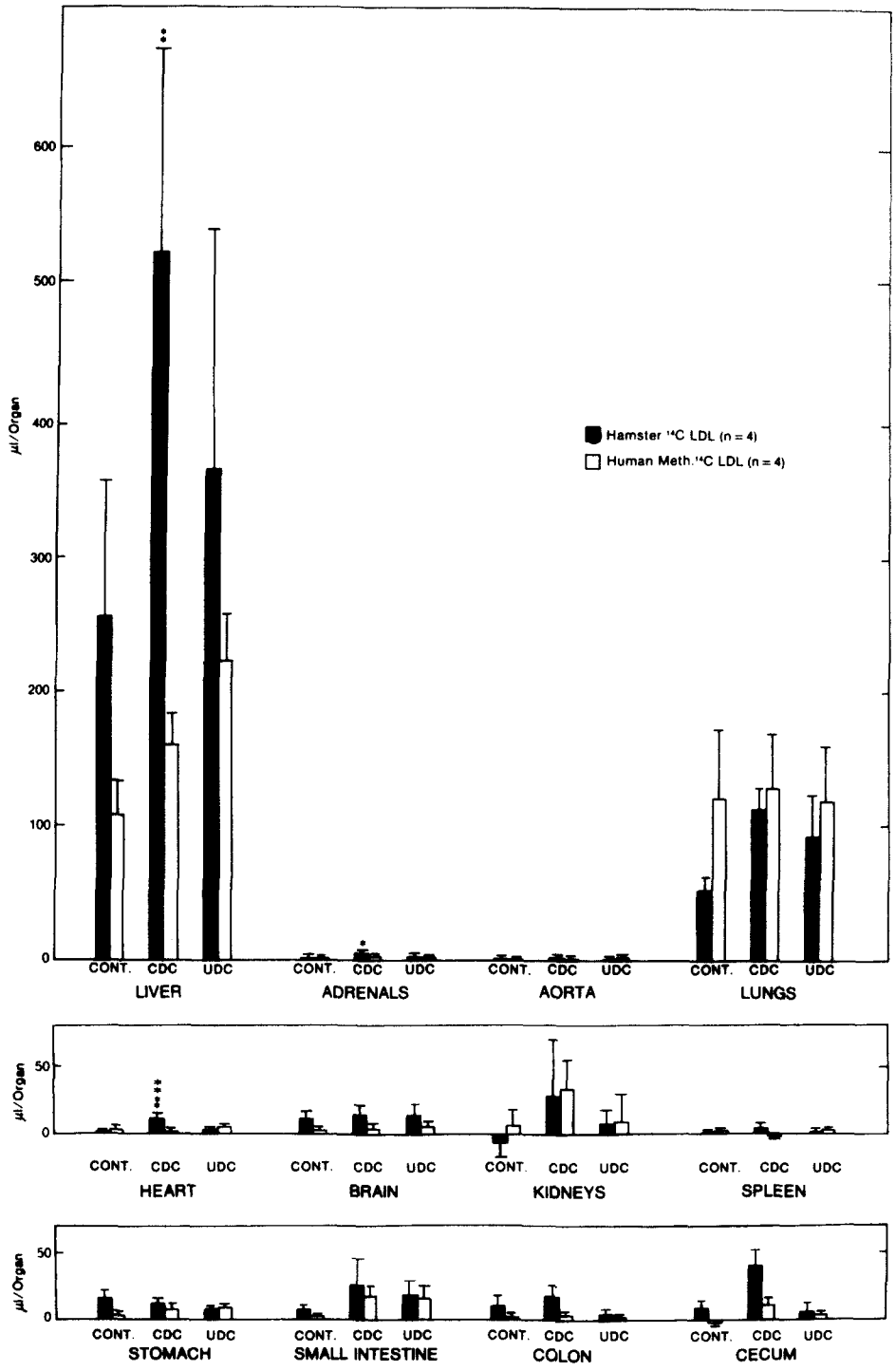

Fig. 3. Organ uptake of LDL, normalized for a 100-g animal, in the different treatment groups of bile fistula hamsters, after 1 hr of intravenous infusion of [^{14}C]sucrose-labeled hamster LDL and methylated human LDL, respectively (mean ± SEM). For corresponding tissue uptake, and for explanation of symbols and abbreviations, see Fig. 2.

previously observed in humans, both similarities and distinct differences can be noted. Similarities were found during CDC treatment and concerned the enrichment of bile with CDC, the biliary decrease in both cholic and deoxycholic acids, as well as a marked suppression of bile acid synthesis (13, 14, 16). However, distinct dissimilarities between the findings in the hamsters and those known to occur in humans were apparent during UDC

TABLE 5. Percent uptake of LDL in the different organs in the animals with an intact enterohepatic circulation

Organ	^{14}C-Labeled Hamster LDL			^{14}C-Labeled Methylated Human LDL		
	Control	CDC	UDC	Control	CDC	UDC
Liver	39.1 ± 15.35	42.6 ± 12.47	45.2 ± 8.87	51.5 ± 9.64	62.3 ± 33.86	31.3 ± 8.31
Adrenal	0.2 ± 0.09	0.3 ± 0.17	0.1 ± 0.03	0.2 ± 0.05	0.02 ± 0.28	0.2 ± 0.03
Aorta	0.03 ± 0.01	0.1 ± 0.09	0.1 ± 0.05	0.1 ± 0.02	0.1 ± 0.06	0.1 ± 0.04
Lung	6.4 ± 0.03	5.9 ± 1.15	4.0 ± 0.89	9.5 ± 2.97	16.7 ± 9.16	14.9 ± 7.41
Heart	0	0	0.1 ± 0.13	0.14 ± 0.37	0	0.3 ± 0.19
Brain	0.2 ± 0.06	0.2 ± 0.16	0.2 ± 0.14	0.4 ± 0.13	0	0.4 ± 0.15
Kidney	0	0	0	1.0 ± 0.84	0	0
Spleen	0.5 ± 0.12	0.6 ± 0.2	0.2 ± 0.16	0.8 ± 0.54	0	0.2 ± 0.63
Stomach	1.1 ± 0.79	1.2 ± 0.45	0.6 ± 0.36	0.6 ± 0.69	0	0.6 ± 0.38
Small intestine	0.4 ± 0.30	1.6 ± 0.39	0.4 ± 0.32	0	0	2.9 ± 1.58
Colon	0	1.1 ± 0.73	0.1 ± 0.11	0.1 ± 0.08	0	0.4 ± 0.31
Cecum	0.01 ± 0.30	1.1 ± 0.80	0.3 ± 0.27	0.1 ± 0.36	0	1.1 ± 0.20
Testes	0	1.1 ± 0.55	0.4 ± 0.28	0	0.2 ± 0.36	0.9 ± 0.43
Epididymis	0.3 ± 0.23	0.5 ± 0.15	0.2 ± 0.10	0.5 ± 0.32	0.1 ± 0.24	0.4 ± 0.23
Seminal vesicles	0	0.4 ± 0.47	0.2 ± 0.18	1.0 ± 0.89	0.5 ± 0.65	0
Prostate	0.2 ± 0.06	0.1 ± 0.08	0.1 ± 0.02	0.2 ± 0.07	0.1 ± 0.04	0.1 ± 0.03
Bladder	0	0	0.01 ± 0.15	0	0	0
Pancreas	0.2 ± 0.03	0.1 ± 0.12	0.2 ± 0.06	0.6 ± 0.27	0.3 ± 0.17	0.1 ± 0.10
Thyroid	0.5 ± 0.22	0.8 ± 0.38	0.4 ± 0.21	0.1 ± 0.25	0	0.2 ± 0.21
Thymus	0.03 ± 0.04	0.02 ± 0.02	0.04 ± 0.03	0.2 ± 0.09	0.03 ± 0.03	0.04 ± 0.01
Fat	10.4 ± 3.23	15.8 ± 1.75[a]	2.6 ± 0.92	19.0 ± 9.83	14.6 ± 10.14	13.2 ± 6.67
Skin	5.1 ± 3.42	13.0 ± 8.09	13.0 ± 5.66	8.0 ± 7.88	6.1 ± 5.53	3.0 ± 2.65
Muscle	39.2 ± 13.72	14.7 ± 8.72	32.1 ± 8.94	7.8 ± 7.86	22.0 ± 16.93	30.1 ± 7.07
Gallbladder	0.2 ± 0.13	0.1 ± 0.2	0	0.04 ± 0.03	0.1 ± 0.06	0

Values are given as mean ± SEM.
[a]CDC versus respective UDC-fed animals, $P < 0.05$.

feeding. In contrast to humans, the hamsters showed *a*) only a small increase in biliary UDC (17% vs. approximately 60% in humans (13)); *b*) a significant increase in biliary CDC; and *c*) a marked suppression in bile acid synthesis. The striking difference between hamsters and humans, as far as the UDC-induced changes of biliary bile acid composition are concerned, is probably the result of a rapid intestinal and hepatic biotransformation of UDC to CDC (22, 24, 46). Since CDC, in contrast to UDC, suppresses bile acid synthesis, the biliary enrichment in CDC during UDC treatment leads to the observed decrease in bile acid synthesis.

The serum levels of total and LDL cholesterol in the three treatment groups were very comparable to those previously described in the literature (37). Similar to the report by the other authors, both measurements were slightly higher in the animals that received CDC than those that were fed UDC or a control diet.

The distribution of the uptake of hamster LDL among the various organs observed in the present study was, with the exception of fat, muscle, and skin tissues, comparable to that reported by other investigators (Table 5) (9). The uptake in fat, skeletal muscle, and skin was somewhat higher than that reported in the literature. However, in agreement with other authors, a major portion of the receptor-mediated LDL uptake took place in the liver (19).

The results of the present study show that CDC and UDC exert different effects on LDL receptor activity. These differences between the two bile acid epimers were expressed as significant changes of both serum cholesterol and hepatic uptake of homologous LDL. In comparison to both CDC and control treatment, UDC feeding led to a significant increase in hepatic uptake of LDL. In the CDC-fed animals, the uptake was slightly lower than that in the controls. Although this difference was consistently present at all LDL infusion levels studied (Fig. 6), it did not reach statistical significance. It is of note that the approximately 20% decrease in hepatic hamster LDL uptake, which was observed in the present study in the CDC-fed versus the control animals, after the infusion of the 20 μg dose, is identical to the percentage figure given by other investigators (37). However, the UDC-induced increase in hepatic hamster LDL uptake in the present study is in disagreement with data by the other group of investigators who found the LDL uptake to be unchanged after UDC treatment (37). These differences between the results of the present study and those of the other group of authors may relate to differences in both the dosage of LDL infused and the experimental design. As has already been emphasized (see Methods section), higher doses of LDL were infused in the present study. It was felt that the higher doses, in contrast to trace amounts, may permit a better detection of an enhanced uptake capacity. As far as

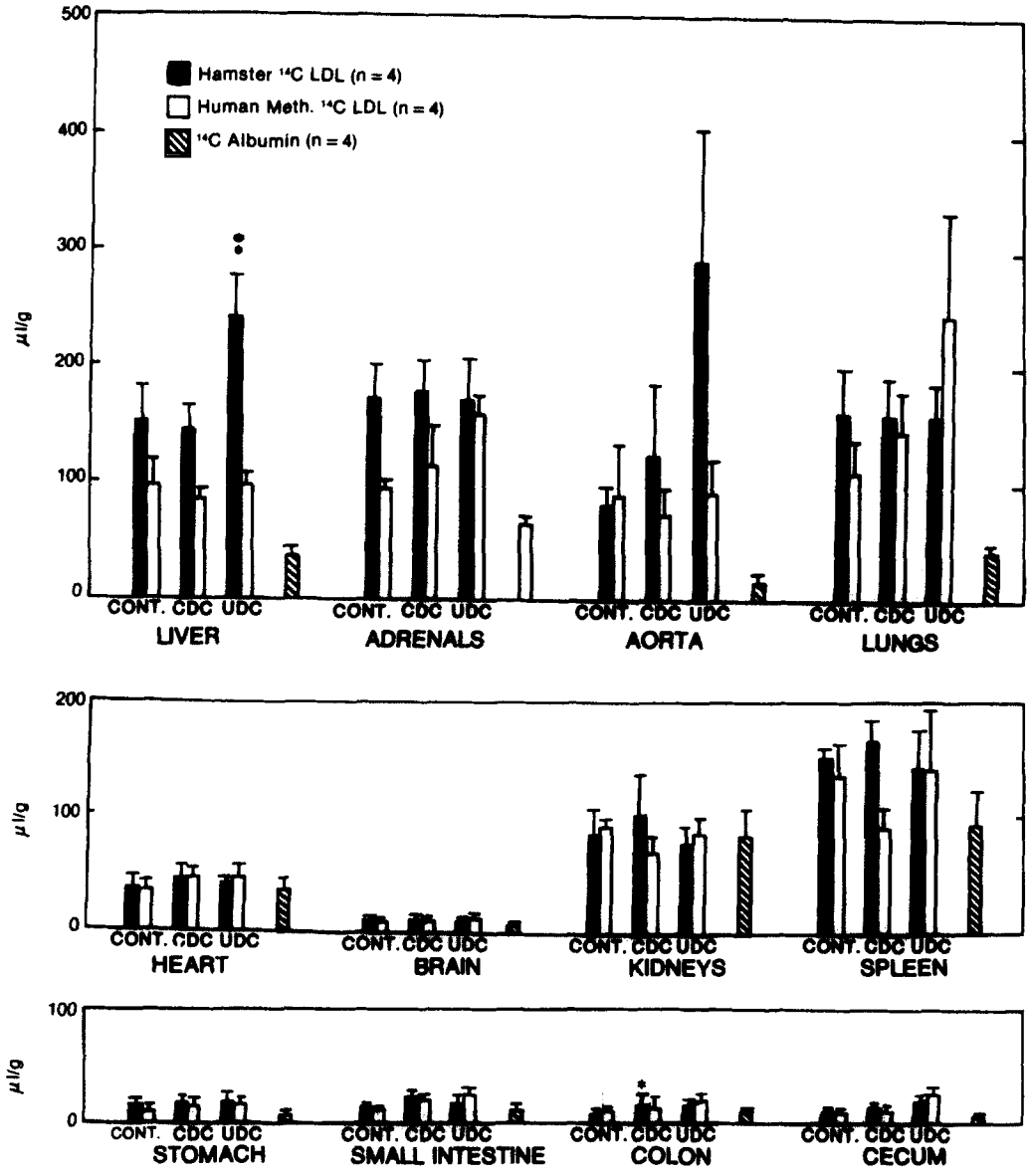

Fig. 4. Tissue spaces of LDL and albumin in different organs of the different treatment groups of hamsters with an intact enterohepatic circulation, after 1 hr of intravenous infusion of [^{14}C]sucrose-labeled hamster LDL, methylated human LDL, and albumin, respectively (mean ± SEM). For calculation of tissue uptake and for explanation of symbols and abbreviations, see Fig. 2.

the experimental design is concerned, three animals, one of each treatment group, were always studied simultaneously to control for any possible diurnal, seasonal, or other variables. In the study by the other authors, the LDL uptake in the CDC- and UDC-fed hamsters was apparently evaluated on the basis of previously obtained control data (37). The decrease in LDL receptor activity in the CDC-fed animals was probably related to the observed depression of bile acid synthesis. Although cholesterol synthesis was not measured in this study, other investigators found, in the hamster, that CDC, in contrast to UDC feeding, induces a significant reduction in hepatic cholesterol synthesis (37). The rapid rise in hepatic LDL receptor activity, which occurred in the liver, heart, and adrenals, in the present study, in the CDC-fed animals after construction of the bile fistula, could be an expression of an acute cellular demand for cholesterol. Since both bile acid and cholesterol synthesis are suppressed by CDC, a bile fistula-induced acute depletion of the cells of bile acids and cholesterol could

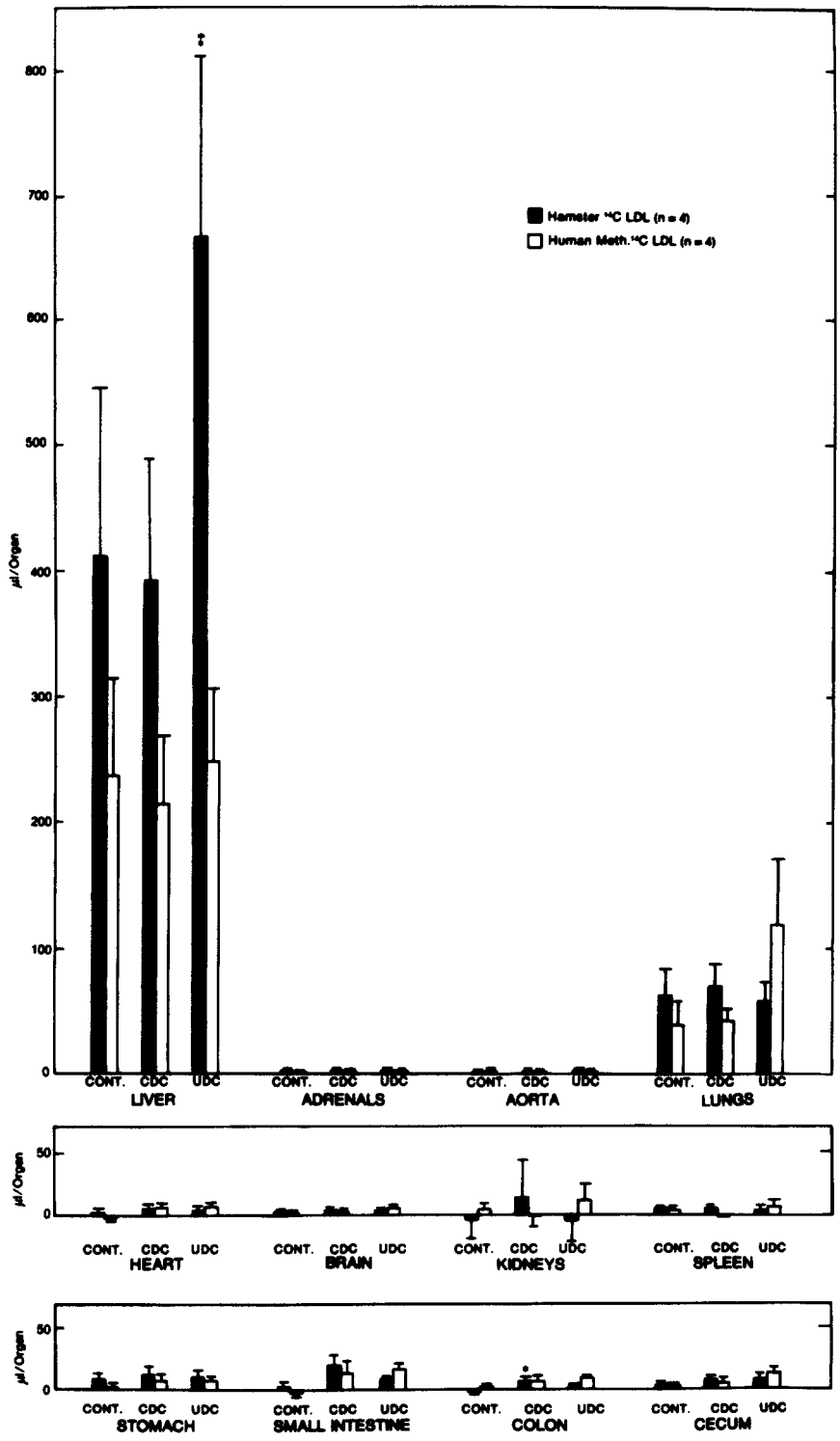

Fig. 5. Organ uptake of LDL, normalized for a 100-g animal, in the different treatment groups of hamsters with an intact enterohepatic circulation, after 1 hr of intravenous infusion of [^{14}C]sucrose-labeled hamster LDL and methylated human LDL, respectively (mean ± SEM). For corresponding tissue uptake, see Fig. 4, and for explanation of symbols and abbreviations, see Fig. 2.

provide a particularly strong stimulus for an activation of the LDL receptor. The findings are, therefore, consistent with observations by other investigators, which indicate that the activity of the apoB, E receptor is subject to rapid regulation by appropriate stimuli, such as the rate of bile acid synthesis (8, 47).

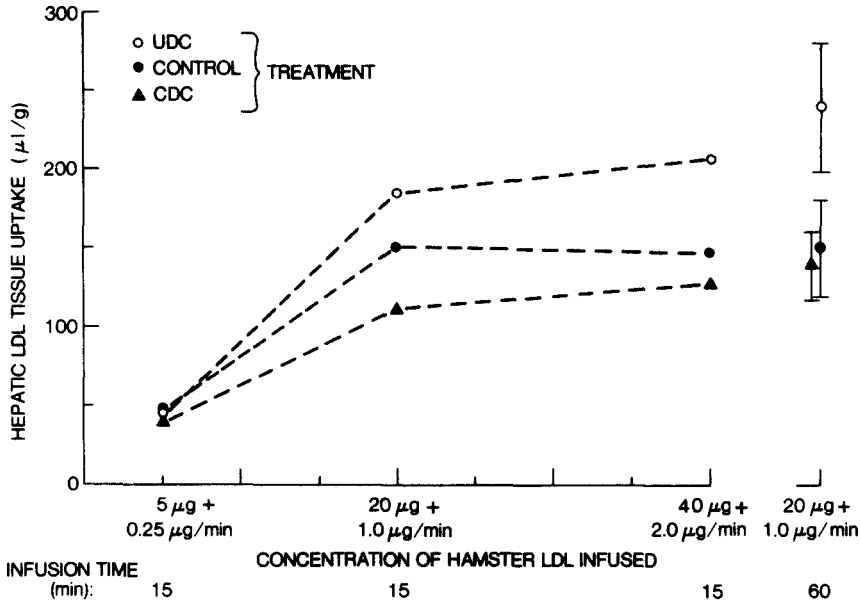

Fig. 6. Hepatic uptake of LDL after intravenous infusion of different concentrations of homologous hamster LDL. The injection of a bolus of 5, 20, or 40 μg, respectively, of LDL protein was followed by a 15-min constant infusion of [^{14}C]sucrose-labeled hamster LDL at a rate of 0.25, 1.0, or 2.0μg/min, respectively. A total of nine hamsters was studied, three in each treatment group. For comparison, to the right of the curves, the hepatic LDL uptake values are shown of the animals with an intact enterohepatic circulation that received a bolus of 20 μg of hamster LDL intravenously, followed by a constant 1-hr infusion at a rate of 1 μg/min. When all hamster LDL infusion studies were considered, the hepatic LDL uptake after treatment with UDC was significantly higher than that after both the control ($P < 0.01$) and CDC ($P < 0.01$) treatment. When the 15-min infusion studies were considered alone, the increase in hepatic LDL uptake was significant ($P < 0.05$) only when the animals treated with UDC were compared with those fed CDC.

However, in spite of a marked depression of bile acid synthesis, UDC feeding resulted in a stimulation of receptor-dependent LDL uptake in the liver. Since bile acid synthesis was similarly depressed after both CDC and UDC treatment, mechanisms other than the rate of bile acid production must be responsible for the divergent effects of the two epimers on the receptor activity. It appears possible that UDC has a direct influence on LDL receptor function.

TABLE 6. Total, HDL, and LDL cholesterol in serum

Treatment Group	Serum Cholesterol		
	Total	HDL	LDL
	mg/dl	%	%
Control	127.7 ± 7.68	50.9 ± 1.80	21.1 ± 2.56
CDC	136.5 ± 6.04[a]	45.1 ± 2.01[a,b]	24.5 ± 3.07
UDC	123.8 ± 2.21	51.1 ± 0.91	17.4 ± 2.67

Values are given as mean ± SEM.
[a]CDC in comparison to UDC-treated animals, $P = 0.072$ (total cholesterol), $P = 0.020$ (HDL).
[b]CDC in comparison to controls, $P = 0.055$.

The possibility for the existence of such a direct effect is supported by other examples, in which it has been shown that bile acids may be able to directly affect lipoprotein metabolism in certain cells. Davidson, Kollmer, and Glickman (48) showed, in the rat, that bile diversion led to a substantial decrease in the production of apoB in the jejunal enterocyte. Sodium taurocholate infusion into the jejunum reversed apoB synthesis to normal levels. It appears, therefore, possible that, under certain conditions, similar interactions may exist between bile acids and LDL receptor.

Angelin et al. (8) observed rapid down-regulation of apoB, E receptor activity in the dog after intravenous infusion of taurocholate. Although bile acid synthesis was not measured in that study, the authors considered a taurocholate-induced depression of bile acid synthesis as the most likely mechanism of the decrease in the receptor-mediated uptake of LDL. Angelin et al. (8) also discussed the possibility of a detergent effect of taurocholate on the cell surface receptor. However, such a mode of action was dismissed as less probable, since the expression of the apoE receptor, in contrast to that of the apoB, E receptor, was not significantly altered by the taurocholate infusion (8).

It is now well appreciated that CDC and UDC are characterized by fundamental differences as far as their physicochemical, biochemical, and physiological properties are concerned (14). The differences between the two bile acid epimers are particularly striking in regard to their effect on membrane structure, cytotoxicity, intestinal fluid, and electrolyte secretion, as well as hepatobiliary cholesterol transport, (14, 19–21, 49–51). The main explanation for the different biological behavior of CDC and UDC probably lies in their different physicochemical actions. While lipid solubilization by CDC is micellar in nature, that by UDC is marked, in addition, by a liquid crystalline mechanism (52, 53).

The finding in this study, that hepatic hamster LDL uptake does not change in the UDC-treated animals after construction of an acute bile fistula, could be related to the possibility that the acute cellular demand for cholesterol synthesis is sufficiently met by cholesterol synthesis (which is, in contrast to the CDC-fed animals, not depressed (37)).

The uptake in the control hamsters of methylated human LDL, which is representative for the receptor-independent transport of LDL into the cell, was similar to that described by other investigators (3, 4). It is of interest that neither CDC nor UDC treatment influenced the receptor-independent LDL uptake. This finding appears to indicate that the effect of the bile acids on the apoB, E receptor is relatively specific, an observation which is consistent with findings by Angelin et al. (8).

In summary, the present study shows significant differences between the two physiologically and clinically important bile acids, CDC and UDC, in their effects on LDL receptor activity. The differences are apparent under conditions of both the intact enterohepatic circulation and an acute bile fistula model. An additional major finding is that the differences in LDL receptor function are expressed in spite of a similar suppression of bile acid synthesis. The results of the study are, therefore, consistent with the possibility that the presence, in the enterohepatic circulation, of certain bile acids, such as UDC, directly induces changes in hepatic LDL receptor activity.

This study was supported by Grant ROl AM 35695 from the National Institutes of Health. Dr. Malavolti is the recipient of a NATO Science Fellowship Award. Dr. Fromm was the recipient of Research Career Development Award AM-00290 from the National Institutes of Health. The authors thank Dr. Lucien Nochomovitz, Department of Pathology, George Washington University Medical Center, Washington, DC for the light microscopic examination of the hamster livers.

Manuscript received 29 September 1986, in revised form 16 March 1987, and in re-revised form 1 June 1987.

REFERENCES

1. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein receptors in the liver: control signals for plasma cholesterol traffic. *J. Clin. Invest.* **72:** 743–747.
2. Mahley, R. W., D. Y. Hui, and T. L. Innerarity. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man: apo-B,E and apo-E receptors. *J. Clin. Invest.* **68:** 1197–1206.
3. Pittman, R. C., T. E. Carew, A. D. Attie, J. L. Witztum, Y. Watanabe, and D. Steinberg. 1982. Receptor-dependent and receptor-independent degradation of low density lipoprotein in normal rabbits. *J. Biol. Chem.* **257:** 7994–8000.
4. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1986. Receptor-independent low density lipoprotein transport in the rat in vivo. *J. Clin. Invest.* **76:** 1113–1122.
5. Glickman, R. M., and S. M. Sabesin. 1982. Lipoprotein metabolism. *In* The Liver: Biology and Pathophysiology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 123–142.
6. Kesaniemi, T. A., and S. M. Grundy. 1982. Significance of low density lipoprotein production in the regulation of plasma cholesterol level in man. *J. Clin. Invest.* **70:** 13–22.
7. Scallen, T. H., and A. Sanghvi. 1983. Regulation of three key enzymes in cholesterol metabolism by phosphorylation–dephosphorylation. *Proc. Natl. Acad. Sci. USA.* **80:** 2477–2480.
8. Angelin, B., C. A. Raviola, T. L. Innerarity, and R. W. Mahley. 1983. Regulation of hepatic lipoprotein receptors in the dog. Rapid regulation of apolipoprotein B,E receptors, but not of apolipoprotein E receptors, by intestinal lipoproteins and bile acids. *J. Clin. Invest.* **71:** 816–831.
9. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
10. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
11. Schoenfield, L. J., and J. M. Lachin. 1981. The Steering Committee and The National Cooperative Gallstone Study Group. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: the National Cooperative Gallstone Study. *Ann. Intern. Med.* **95:** 257–282.
12. Albers, J. J., S. M. Grundy, P. A. Cleary, D. M. Small, J. M. Lachin, L. J. Schoenfield, and the National Cooperative Gallstone Study Group. 1982.: the effect of chenodeoxycholic acid on lipoproteins and apolipoproteins. *Gastroenterology.* **82:** 638–646.
13. Fromm, H., J. W. Roat, V. Gonzalez, R. P. Sarva, and S. Farivar. 1983. Comparative efficacy and side effects of ursodeoxycholic and chenodeoxycholic acids in dissolving gallstones: a double-blind controlled study. *Gastroenterology.* **85:** 1257–1264.
14. Fromm, H. 1984. Gallstone dissolution and the cholesterol–bile acid–lipoprotein axis. Propitious effects of ursodeoxycholic acid. *Gastroenterology.* **87:** 229–233.
15. Fromm, H. 1985. Serum lipid and lipoprotein analysis in patients treated with chenodeoxycholic and ursodeoxycholic acids. *In* Recent Advances in Bile Acid Research. L. Barbara, R. H. Dowling, A. F. Hofmann, and E. Roda, editors. Raven Press Books, Ltd., New York. 311–312.
16. Danzinger, R. G., A. F. Hofmann, J. L. Thistle, and L. J. Schoenfield. 1983. Effect of oral chenodeoxycholic acid on bile acid kinetics and biliary lipid composition in women with cholelithiasis. *J. Clin. Invest.* **52:** 2809–2821.

17. Salen, G., A. Colalillo, D. Verga, E. Bagan, G. S. Tint, and S. Shefer. 1980. Effect of high and low doses of ursodeoxycholic acid on gallstone dissolution in humans. *Gastroenterology.* **78:** 1412–1418.
18. Thistle, J. L., N. R. LaRusso, A. F. Hofmann, J. Turcotte, G. L. Carlson, and B. J. Ott. 1982. Differing effects of ursodeoxycholic or chenodeoxycholic acid on biliary cholesterol saturation and bile acid metabolism in man. A dose response study. *Dig. Dis. Sci.* **27:** 161–168.
19. Hardison, W. G. M., and S. M. Grundy. 1984. The effect of ursodeoxycholate and its taurine conjugate on bile acid synthesis and cholesterol absorption. *Gastroenterology.* **87:** 130–135.
20. von Bergmann, K., M. Epple-Gutsfeld, and O. Leiss. 1984. Differences in the effects of chenodeoxycholic and ursodeoxycholic acids on biliary lipid secretion and bile acid synthesis in patients with gallstones. *Gastroenterology.* **87:** 136–143.
21. Nilsell, K., B. Angelin, B. Leijd, and K. Einarsson. 1983. Comparative effects of ursodeoxycholic acid and chenodeoxycholic acid on bile acid kinetics and biliary lipid secretion in man. Evidence for different modes of action on bile acid synthesis. *Gastroenterology.* **85:** 1248–1256.
22. Fromm, H., G. L. Carlson, A. F. Hofmann, S. Farivar, and P. Amin. 1980. Metabolism in man of 7-ketolithocholic acid: precursor of cheno- and ursodeoxycholic acids. *Am. J. Physiol.* **239:** G161–166.
23. McJunkin, B., H. Fromm, R. P. Sarva, and P. Amin. 1982. Factors in the mechanism of diarrhea in bile acid malabsorption: fecal pH —a key determinant. *Gastroenterology.* **80:** 1454–1464.
24. Fromm, H., R. P. Sarva, F. Bazzoli, S. Ceryak, and L. Mendelow. 1983. Formation of ursodeoxycholic acid from chenodeoxycholic acid in the human colon: studies of the role of 7-ketolithocholic acid as an intermediate. *J. Lipid Res.* **24:** 841–853.
25. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
26. Redgrave, T. G., D. C. K. Roberts, and C. E. West. 1975. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal. Biochem.* **65:** 42–49.
27. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
28. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
29. Pittman, R. C., S. R. Green, A. D. Attie, and D. Steinberg. 1979. Radiolabeled sucrose covalently linked to protein: a device for quantifying degradation of plasma proteins catabolized by lysosomal mechanisms. *J. Biol. Chem.* **254:** 6876–6879.
30. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of the lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053–9062.
31. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
32. Switzer, R. C., C. R. Merril, and S. Shifrin. 1979. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.* **98:** 231–237.
33. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
34. Fromm, H., P. J. Thomas, and A. F. Hofmann. 1973. Sensitivity and specificity in tests of distal ileal function: prospective comparison of bile acid and vitamin B12 absorption in ileal resection patients. *Gastroenterology.* **64:** 1077–1090.
35. Koelz, H. R., B. C. Sherrill, S. D. Turley, and J. M. Dietchy. 1982. Correlation of low and high density lipoprotein binding in vivo with rates of lipoprotein degradation in the rat. *J. Biol. Chem.* **257:** 8061–8072.
36. Munford, R. S., J. M. Andersen, and J. M. Dietschy. 1981. Sites of tissue binding and uptake in vivo of bacterial lipopolysaccharide-high density lipoprotein complexes: studies in the rat and squirrel monkey. *J. Clin. Invest.* **68:** 1503–1513.
37. Spady, D. K., E. F. Stange, L. E. Bilhartz, and J. M. Dietschy. 1986. Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver. *Proc. Natl. Acad. Sci. USA.* **83:** 1916–1920.
38. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate–Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
39. Elevitch, F. R., S. B. Aronson, T. V. Aronson, T. V. Feichtmeir, and M. L. Enterline. 1966. Thin gel electrophoresis in agarose. *Am. J. Clin. Pathol.* **46:** 692–697.
40. Friedewald W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
42. Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ. 1–457.
41. Pittman, R. C., A. D. Attie, T. E. Carew, and D. Steinberg. 1979. Tissue sites of degradation of low density lipoprotein: application of a method for determining the fate of plasma proteins. *Proc. Natl. Acad. Sci. USA.* **76:** 5345–5349.
43. Bergman F., and W. Van der Linden. 1967. Diet-induced cholesterol gallstones in hamsters: prevention and dissolution by cholestyramine. *Gastroenterology.* **53:** 53: 418–421.
44. Sue, S. O., G. G. Bonorris, J. W. Marks, S. Vimadalal, and L. J. Schoenfield. 1982. Dissolution of cholesterol gallstones by bile acids in hamsters. *Am. J. Med. Sci.* **284:** 18–23.
45. Singhal, A. K., J. Finver-Sadowsky, C. K. McSherry, and E. H. Mosbach. 1983. Effect of cholesterol and bile acids on the regulation of cholesterol metabolism in hamster. *Biochim. Biophys. Acta.* **752:** 214–222.
46. Fedorowsky T., G. Salen, G. S. Tint, and E. H. Mosbach. 1979. Transformation of chenodeoxycholic acid and ursodeoxycholic acid by human intestinal bacteria. *Gastroenterology.* **77:** 1068–1073.
47. Stahl, P., and A. L. Schwartz. 1986. Receptor-mediated endocytosis. *J. Clin. Invest.* **77:** 657–662.
48. Davidson, N. O., M. E. Kollmer, and R. M. Glickman. 1986. Apolipoprotein B synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **27:** 30–39.
49. Igimi, H., and M. C. Carey. 1980. pH-Solubility relations of chenodeoxycholic and ursodeoxycholic acids: physical-chemical basis for dissimilar solution and membrane phenomena. *J. Lipid Res.* **21:** 72–90.
50. Kimura T., M. Shimamura, A. Yamaguchi, T. Katayama, T. Kurita, and A. Tanaka. 1981. Solubilization of cultured

cell membrane by bile acids. *Acta Hepatol. Jpn.* **22:** 1–7.
51. Caspary, W. F., and K. Meyne. 1980. Effects of chenodeoxy- and ursodeoxycholic acid on absorption, secretion and permeability in rat colon and small intestine. *Digestion.* **20:** 168–174.
52. Corrigan, O. I., C. C. Su, W. I. Higuchi, and A. F. Hofmann. 1980. Mesophase formation during cholesterol dissolution in ursodeoxycholate-lecithin solutions: new mechanism for gallstone dissolution in humans. *J. Pharm. Sci.* **69:** 869–870.
53. Salvioli G., H. Igimi, and M. C. Carey. 1983. Cholesterol gallstone dissolution in bile. Dissolution kinetics of crystalline cholesterol monohydrate by conjugated chenodeoxycholate-lecithin and conjugated ursodeoxycholate-lecithin mixtures: dissimilar phase equilibria and dissolution mechanisms. *J. Lipid Res.* **24:** 701–720.