Regulation of cholesterol metabolism in the ethionine-induced premalignant rat liver

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Abstract The early premalignant liver provides a model in which to study metabolic alterations that may be permissive for the development of full malignancy. Although there are biochemical changes in this model, there are no detectable morphological ones when compared with a normal, fully differentiated liver. The maintenance of cholesterol homeostasis, essential for proper functioning of mammalian cells, is known to be altered in malignancy. We used the ethionine-induced premalignant liver model to study the effects of the premalignant state on cellular parameters involved in the maintenance of hepatic cholesterol homeostasis. Cholesterol synthesis was elevated about twofold in the livers of rats treated with ethionine as was the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, its rate limiting enzyme. There was no change in reductase activation state. Acyl coenzyme A:cholesterol acyltransferase (ACAT) was decreased about 30%, and cholesterol 7α-hydroxylase, about 50%. There was no significant change in neutral cholesteryl ester hydrolase activity, but acid hydrolase activity was decreased. There was little change in low density lipoprotein receptor protein as determined by immunoblotting. Biliary lipid secretion was in the normal range when expressed per gram liver; however, bile flow was doubled. The ethionine-fed animals were mildly hypocholesterolemic and had an altered serum lipoprotein pattern. Cholesterol synthesis and HMG-CoA reductase activity exhibited decreased sensitivities to inhibition by dietary cholesterol when compared to control livers. However, sensitivity to intragastrically administered mevalonolactone was not altered. Although ACAT activity was increased by mevalonolactone administration to levels similar to those in untreated animals, it was not increased in the ethionine-fed animals by feeding cholesterol. The ethionine-induced premalignant liver responded to ethinyl estradiol treatment in a manner similar to that of the control, i.e., profound hypolipidemia, increased low density lipoprotein receptors, decreased reductase activity, and increased cholesterol esterification. Thus, these livers retained their estrogen responsiveness. Taken together, the data demonstrate that the major elements involved in maintaining hepatic cholesterol homeostasis are present in the premalignant liver, although in some cases at levels that are different from the control. However, the susceptibility to regulation was altered in these livers to suggest markedly decreased availability of cholesterol of exogenous origin to the regulatory compartment(s). Further, coupling of the different elements involved in maintenance of hepatic cholesterol homeostasis appeared to have been changed. —**Erickson, S. K., S. R. Lear, M. E. Barker, and T. A. Musliner.** Regulation of cholesterol metabolism in the ethionine-induced premalignant rat liver. *J. Lipid Res.* 1990. **31:** 933–945.

Supplementary key words cholesterol homeostasis • liver function • LDL • HMG-CoA reductase • acyl CoA:cholesterol acyltransferase • dietary cholesterol • ethinyl estradiol

The maintenance of cholesterol homeostasis is basic to mammalian cells. It is required for normal cell growth and function; thus, alterations or defects in the regulation of cholesterol metabolism can have profound effects on cells.

The regulation of cholesterol metabolism becomes aberrant during development of malignancy (1, 2). However, the nature of the defect(s) has not been elucidated. A common feature of hepatomalignancy is loss of sensitivity to regulation of cholesterol synthesis by dietary cholesterol (1). This has been observed both in transplantable hepatomas (3, 4) and in primary hepatomas from several species including humans (1, 5, 6). It is an early event in the development of chemical carcinogen-induced malignancy and is observed regardless of the inducing carcinogen employed (1). The defect is evident after exposure to carcinogens but before malignancy is histologically identifiable (7). Evidence for loss of regulation in presumptive human hepatopremalignancy also has been reported (8).

Ethionine, a methionine analogue, can induce hepatic malignancy in rats (for review see Farber (9)). After several months on an ethionine-containing diet, rats develop a high incidence of hepatomas within the following 12–18 months (9). When rats were fed a diet containing 0.25% ethionine for 3–5 weeks, hepatic cholesterol syn-

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; MVA, mevalonolactone; CE, cholesteryl ester.

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thesis was increased, and in selected animals, was not inhibited by including 5% cholesterol in the diet (7). We observed previously (10) that the livers of such ethionine-fed animals had decreased numbers of cell membrane receptors recognizing chylomicron remnants, the lipoproteins that deliver cholesterol of intestinal origin to the liver. Membrane receptors recognizing chylomicron remnants also have been reported to be decreased in hepatomas (11–15).

However, we also found that despite the decrease in lipoprotein receptors, the livers of the ethionine-fed rats did accumulate cholesterol when cholesterol was added to the diet. These observations suggested that feeding ethionine induced changes in cellular cholesterol metabolism and its regulation in addition to the decrease in binding sites for chylomicron remnants. To examine this possibility we studied the responses of key elements of hepatic cholesterol metabolism in the ethionine-induced premalignant liver: *a)* hepatic cholesterol synthesis measured in vivo using [^{3}H]OH; *b)* the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme for sterol synthesis; *c)* the activity of acyl coenzyme A:cholesterol acyltransferase (ACAT), responsible for intracellular cholesterol esterification; *d)* the activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis; *e)* the activities of the neutral and acidic cholesterol ester hydrolases; and *f)* some aspects of biliary and lipoprotein metabolism.

MATERIALS AND METHODS

Experimental procedures

Male Sprague-Dawley rats, 180–200 g (Simonsen, Gilroy, CA or Bantin and Kingman, Newark, CA) were housed under either reverse (lights on 3 PM; lights off 3 AM) or normal (lights on 6AM; lights off 6 PM) illumination. They were allowed free access to food and water at all times.

For induction of the early premalignant liver, rats were fed ground rat chow (Purina) containing 0.25% D,L-ethionine (Sigma, St. Louis, MO) and 0.45% corn oil. Control animals received ground rat chow containing 0.45% corn oil only. Unless otherwise stated, the diets were fed for 4 weeks.

In some experiments, 0.3% cholate, 5% lard, and 2% cholesterol were added to each diet for the last 48-h period. In other experiments, 5% or 0.1% cholesterol was added to the diet for the last 48-h period. In still other experiments mevalonolactone was administered intragastrically at 1 mg/g body weight 2 h before the animals were killed as described previously (16). The control animals received the same volume of vehicle. In yet other experiments ethinyl estradiol (5 mg/kg body weight) was administered (17) for the last 3 days of the experiments. The control animals received the vehicle alone.

For the studies in which the reversibility of the effects of ethionine were examined, the animals were fed the ethionine-containing diet for 4 weeks and then both they and their controls received the standard Purina rat chow diet for 1 for 2 weeks.

All protocols were approved by the Animal Studies Subcommittee of the Research Development Committee at the VA Medical Center, San Francisco.

Rates of cholesterol and fatty acid synthesis were determined in vivo using [^{3}H]OH based on the method of Jeske and Dietschy (18) as described previously (19). Briefly, rats were injected with 50 mCi [^{3}H]OH and killed 1 h later. Samples of serum, liver, and the entire small intestine were removed and saponified in alcoholic KOH. The nonsaponifiable lipids were extracted with petroleum ether and separated by thin-layer chromatography on silica gel G plates developed in benzene–ethyl acetate 5:1 (v:v) (20). The bands corresponding to cholesterol were scraped into scintillation vials and counted in a liquid scintillation counter. After extraction of the nonsaponifiable lipids, the residue was acidified with HCl, extracted with petroleum ether, the extract was taken to dryness under N_2, resuspended, and aliquots were counted for incorporation of [^{3}H]OH into fatty acids. The results are expressed as micromoles [^{3}H]OH incorporated into product in 1 h per g wet weight of tissue or per ml serum and were corrected for the specific activity of [^{3}H]OH in the plasma.

Rat liver microsomes were prepared and HMG-CoA reductase was assayed as described previously (21) except that mevalonolactone was separated by ion exchange chromatography (22). Microsomal ACAT was assayed as described previously (23) except that a 2-min (initial rate) or 15-min (to estimate microsomal cholesterol substrate pool size) assay time was used. In some cases, ACAT activity was also measured using a [^{3}H]cholesterol:egg phosphatidylcholine liposome assay as described previously (24). Cholesterol 7α-hydroxylase activity was determined by a modification of the method of Nicolau et al. (25) as described previously (24).

The acidic and neutral cholesteryl ester hydrolase activities were assayed in liver homogenates by a method based on that described by Brecher et al. (26) using cholesteryl [^{14}C]oleate:egg phosphatidylcholine liposomes (1:66 mole ratio) prepared by sonication. The lipid mixture was sonicated under argon in a sonication bath for four 10-min bursts. Aliquots of homogenate were assayed at pH 4.5 (acidic hydrolase) or pH 8.0 (neutral hydrolase) using the assay conditions described by Brecher et al. (26). The assays were linear with the protein and substrate concentrations chosen over the 60-min time period. The [^{14}C]oleate released was differentially extracted (27) and radioactivity was determined. The results are ex-

pressed as dpm [^{14}C]oleate released per h per mg homogenate protein.

Rat liver membrane-associated LDL receptor levels were estimated using a preparation enriched in sinusoidal plasma membranes prepared based on the method of Touster et al. (28). Briefly, portions of livers were homogenized in 5 volumes of 0.12 M sucrose, 30 mM EDTA, 0.15 M KCl, 0.1 M K phosphate, pH 7.4, in a glass-Teflon Potter Elvehjem homogenizer by 3 strokes at low speed. The homogenate was centrifuged for 15 min at 12,000 *g* to sediment nuclei, mitochondria, lysosomes, and cell debris. The supernatant was centrifuged 1 h at 105,000 *g*. The pellet was resuspended by homogenizing 10 strokes in a Dounce homogenizer and the membranes were collected by centrifugation for 1 h at 105,000 *g*. The membranes were resuspended in homogenizing buffer at approximately 10 mg protein/ml and frozen at −70°C until further use.

Potential changes in recovery of sinusoidal plasma membrane that might have been induced by feeding ethionine were assessed by the ^{125}I-labeled wheat germ agglutinin method of Chang, Bennett, and Cuatrecasas (29) as described previously (30). There were no significant differences between control and ethionine-treated (7.7 ± 1.5 vs 7.7 ± 1.9% ^{125}I recovered). The specific activities also were similar, 1.08 ± 0.23 versus 1.10 ± 0.18 cpm/mg protein (normalized to cpm recovered in whole homogenate; average of three animals in each group). Thus, ethionine feeding did not appear to drastically alter sinusoidal plasma membrane distribution or recovery.

Relative quantitation of the LDL receptor content of these membranes was done by SDS gel electrophoresis on 7.5% acrylamide gels followed by transfer to nitrocellulose and immunoblotting (31) using an LDL receptor-specific antiserum kindly provided by Dr. Janet Boyles of the Gladstone Foundation, UCSF. This antiserum was raised in rabbits against the bovine adrenal LDL receptor purified by SDS gel electrophoresis. It recognizes the rat hepatic LDL receptor. The immunoblotted receptors were visualized with donkey anti-rabbit IgG labeled with ^{125}I (Amersham) followed by autoradiography. Also run on each gel were: *a)* molecular weight standards, *b)* a reference standard sinusoidal rat liver membrane-enriched preparation from rats treated with ethinyl estradiol, and *c)* membranes of *Giardia muris*, which do not appear to contain mammalian-like LDL receptors and thus, served as a measure of nonspecific binding. After autoradiography, the bands were quantitated by densitometry and the peak areas for each band were normalized to that of the reference standard. Each sample was run in triplicate. In preliminary studies, standard curves were constructed of membrane protein concentration versus LDL receptor peak area for membranes from control and ethinyl estradiol-treated rats in order to determine the linearity range for LDL receptor estimation. Subsequent determinations were all made using membrane protein concentrations that fell within the linear range of these curves. A representative autoradiogram of immunoblots is shown in **Fig. 1**.

Liver morphology was examined by microscopy. Pieces of liver lobes were fixed by immersion or, alternatively, the entire liver was perfused with 2.5% glutaraldehyde, 0.8% paraformaldehyde in 0.13 M $NaHCO_3$, pH 7.4. Tissue was cut into slices and embedded in JB-4 plastic for light microscopy, or into 1 mm^3 pieces and embedded in Epon for electron microscopy. Dehydration was carried out with graded ethanols; tissue was embedded, sectioned, and examined with a Zeiss light microscope or a Zeiss 10 CA electron microscope.

Serum lipoproteins were separated by ultracentrifuga-

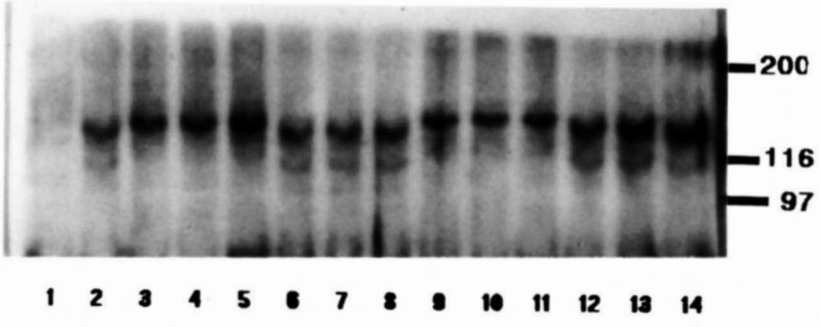

Fig. 1. Immunoblot of rat hepatic LDL receptors. Animals were fed a control diet or one containing 0.25% ethionine for 4 weeks. Ethinyl estradiol (1 mg/kg body weight) was administered for 3 days prior to killing. Liver sinusoidal plasma membrane fractions were prepared as described in Methods. Aliquots were analyzed by SDS gel electrophoresis followed by immunoblotting with a rabbit antiserum specific for LDL receptors as described in Methods. The second, lower molecular weight band observed in the liver membranes from ethinyl estradiol-treated animals is thought to be a specific breakdown product of the LDL receptor (for discussion see Friedman et al. (15)). Lane 1, *Giardia* membranes; lane 2, reference standard rat liver membranes; lanes 3–5, control membranes, 50 μg protein; lanes 6–8, control + ethinyl estradiol membranes, 10 μg protein; lanes 9–11, ethionine membranes, 50 μg protein; lanes 12–14, ethionine + ethinyl estradiol membranes, 10 μg protein.

tion at d < 1.063 g/ml (apoB-containing lipoproteins: very low density, (VLDL), low density (LDL) lipoproteins, VLDL and chylomicron remnants) and at d 1.063–1.21 g/ml (high density lipoproteins, HDL), or at d < 1.21 g/ml for total lipoproteins, according to Havel, Eder, and Bragdon (32). Serum lipoprotein patterns were analyzed by nondenaturing gradient gel electrophoresis (33) using either whole serum or the d < 1.21 g/ml fractions from pooled sera. Gradient gels were stained for lipid, calibrated, and scanned by densitometry as described by Nichols, Krauss, and Musliner (33).

Bile was collected over a 4-h period after cannulation of the common bile duct. The volume of bile was determined and cholesterol, phospholipid, and bile acid contents were estimated as described previously (19). Biliary ions were determined as described previously by Van Dyke, Stephens, and Scharschmidt (34).

Total cholesterol was determined enzymatically (35) after chloroform–methanol extraction of samples of serum, liver homogenate, liver microsomes, and serum lipoprotein fractions.

Serum albumin, bilirubin, alkaline phosphatase, and alanine aminotransferase were assayed using Sigma kits #631, #550, #245, #505, respectively. Serum glucose was determined using the YSI 23A glucose analyzer. Serum glutathione was determined according to Tietze (36).

Liver DNA synthesis was assessed by measuring incorporation of [^{3}H]thymidine into DNA (50 μCi [^{3}H]thymidine injected intraperitoneally/200 g body weight 1 h before killing) as described previously (37). Liver protein synthesis was estimated by measuring incorporation of [^{3}H]leucine into tricholoroacetic acid-precipitable protein in liver and in serum samples (50 μCi [^{3}H]leucine injected intraperitoneally/200 g body weight 1 h before killing).

Microsomal cytochrome P450 content was estimated spectrophotometrically as described previously (21). Protein was estimated by the biuret method (38) or according to Lowry et al. (39) using bovine serum albumin as reference standard.

Statistical methods

Two-tailed Student's *t*-test was used to assess the statistical significance of changes in any parameters measured.

RESULTS

The effects of ethionine feeding on general liver integrity and function were determined by microscopy and by biochemical techniques. Examination at both the light microscope and electron microscope levels showed no consistent morphological changes in the livers of the ethionine-fed animals compared with those from controls (data not shown). A number of serum parameters indicative of general liver function were examined (**Table 1**). Although these parameters measured in the ethionine-treated rats fell within published ranges for normal rats (40–42), relative to the control animals in this study, small (10–20%) but statistically significant changes were observed in all the serum parameters measured.

Potential effects of ethionine feeding on hepatic DNA and protein synthesis were assessed. Rates of hepatic

TABLE 1. General biochemical characteristics of the ethionine-induced premalignant liver model

	A. Serum Parameters					
Treatment	Albumin	Bilirubin	Alkaline Phosphatase	Alanine Aminotransferase	Glucose	Glutathione
	g/dl	*mg/dl*	*Sigma units /ml*	*SF units/ml*	*mg/dl*	μg/ml
Control	4.75 ± 0.17 (22)	0.195 ± 0.018 (28)	8.47 ± 0.44 (15)	28.3 ± 1.5 (12)	146 ± 3 (12)	27.2 ± 2.4 (12)
0.25% Ethionine-fed	5.61 ± 0.17 (20)	0.344 ± 0.041 (27)	17.23 ± 1.23 (12)	38.5 ± 3.7 (11)	123 ± 5 (12)	48.6 ± 3.4 (12)
	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.02$	$P < 0.001$	$P < 0.001$
	(lit. normal range = 2.71–5.03)	(lit. normal range = 0.12–0.40)	(lit. normal range = 22 ± 10 (± SD))	(lit. normal value = 27 units)	(lit. normal range = 90–168)	

	B. Liver Parameters			
		[^{3}H]Leucine Incorporation into Protein		
	[^{3}H] Thymidine Incorporation into DNA	Liver	Serum	Liver Microsomal Cytochrom P450
	dpm/μg DNA	*dpm × 10^{-6}/mg protein*		*μmol/mg protein*
Control	10.1 ± 2.3 (15)	2602 ± 247 (11)	5530 ± 515 (11)	0.97 ± 0.06 (24)
0.25% Ethionine-fed	11.4 ± 1.4 (12)	3309 ± 355 (11)	4990 ± 152 (11)	0.71 ± 0.04 (23)
	NS	NS	NS	$P < 0.001$

Animals were fed a control diet or one containing 0.25% ethionine for 4 weeks. Serum and hepatic parameters indicative of liver function were assayed as described in Methods. The results are expressed as mean ± SE. The number of animals is in parentheses. NS, difference not statistically significant.

DNA synthesis were in the normal range (Table 1). Thus, this treatment did not lead to liver regeneration where 20- to 50-fold changes in incorporation of [^{3}H]thymidine into DNA are observed (cf. Bucher, Patel, and Cohen (43)). General hepatic protein synthesis as determined by incorporation of [^{3}H]leucine into TCA-precipitable protein also was unaffected by ethionine feeding (Table 1), and there were no statistically significant changes in accumulation of radiolabeled protein in serum (Table 1). Liver microsomal cytochrome P450 content was decreased 27% in the ethionine-fed animals as compared with controls (Table 1).

Taken together, these data indicate that ethionine feeding at 0.25% for 4 weeks did not cause gross nonspecific pathological changes either morphologically or biochemically in the livers of the treated animals. However, consistent with its hepatospecific effects, feeding ethionine did cause modest changes in some parameters commonly measured to assess liver function.

Ethionine feeding altered serum and serum lipoprotein cholesterol contents but had little effect on liver cholesterol content. Serum total cholesterol was about 30% lower in the rats fed 0.25% ethionine in the diet for 4 weeks ($P < 0.05$, **Table 2**). This mild hypocholesterolemia might be the result of changes in cholesterol absorption or lipoprotein metabolism. Relatively more cholesterol was recovered in the d 1.006–1.063 g/ml (VLDL + IDL + LDL + chylomicron remnants) fraction of serum from the ethionine-fed animals than in the d 1.063–1.21 g/ml (HDL) fraction when compared to the distribution of cholesterol in these fractions from control sera. The ratio for cholesterol recovered in the d 1.006–1.063 g/ml fraction to the d 1.063–1.21 g/ml fraction was 1.84 ± 0.09 (four determinations; sera pooled from three ethionine-fed animals for each determination) versus 0.99 ± 0.26 for control (four determinations; sera pooled from three control animals for each determination; $P < 0.05$). Thus, a relative increase in content of cholesterol in the d 1.006–1.063 g/ml (VLDL + LDL + IDL + chylomicron remnant) fraction was found that could reflect accumulation of cholesterol-enriched remnants of intestinal or hepatic origin or hepatic secretion of cholesterol-enriched lipoprotein particles.

The mild hypocholesterolemia induced by feeding ethionine and the changes in relative distribution of cholesterol between the apoB-containing lipoproteins and HDL led us to examine serum lipoprotein patterns by agarose gel electrophoresis. There were no marked differences between the control and ethionine-fed animals. (S. K. Erickson and J. P. Kane, unpublished observations) Feeding the animals a cholesterol-cholate-lard diet also resulted in no consistent differences amongst sera from control and ethionine-fed rats when analyzed by this technique. However, analyses by nondenaturing gradient gel electrophoresis (31) of individual unspun sera as well as pooled d < 1.21 g/ml fractions revealed distinct differences in LDL and HDL subspecies distribution (**Fig. 2**). The major LDL species from the ethionine-fed animals had a larger peak particle diameter than the LDL subspecies present in control sera. In addition to this large LDL species, particles intermediate in size between LDL and the major VLDL band were present in increased amounts in the sera from ethionine-fed rats, again suggestive of remnant accumulation. The HDL species from the treated animals were shifted to a smaller particle size distribution compared with controls. In other work (10), we found that serum apoE concentration was increased; the increase was observed in all the lipoprotein classes, but was most marked in the LDL density region.

No significant changes in liver homogenate or liver microsomal cholesterol contents were induced by ethionine feeding (Table 2). Feeding a diet containing cholesterol, cholate, and lard for 48 h before killing induced significant increases in the cholesterol content of liver homogenates that were comparable in both control and ethionine-fed rats; however, there were no statistically significant changes in microsomal cholesterol content in either group (Table 2). The cholesterol-cholate-lard diet caused a 50% increase (Table 2) in serum total cholesterol in the ethionine-fed animals which, however, did not

TABLE 2. Effect of ethionine feeding on serum and hepatic cholesterol contents

Treatment	Serum Cholesterol	Liver Cholesterol	Liver Microsomal Cholesterol
	mg/dl	*μg/mg homogenate protein*	*μg/mg microsomal protein*
Control	64.8 ± 6.4 (26)	15.0 ± 0.6 (26)	34.1 ± 1.6 (26)
+ Cholesterol, cholate, lard diet, 48 h	118.1 ± 31.3 (9)	31.1 ± 3.1 (12)	41.0 ± 4.1 (12)
	$P < 0.05$	$P < 0.001$	NS
Ethionine-fed	46.6 ± 4.0 (26)[a]	14.4 ± 0.7 (26)	34.8 ± 1.8 (26)
+ Cholesterol, cholate, lard diet, 48 h	70.3 ± 21.2 (7)	25.8 ± 2.0 (12)	37.6 ± 3.0 (12)
	NS	$P < 0.001$	NS

Serum was collected and liver homogenates and microsomes were prepared and analyzed as described in Methods. Values are the mean ± SE; the number of animals is in parentheses; NS, difference not statistically significant.

[a]Different from control at $P < 0.05$.

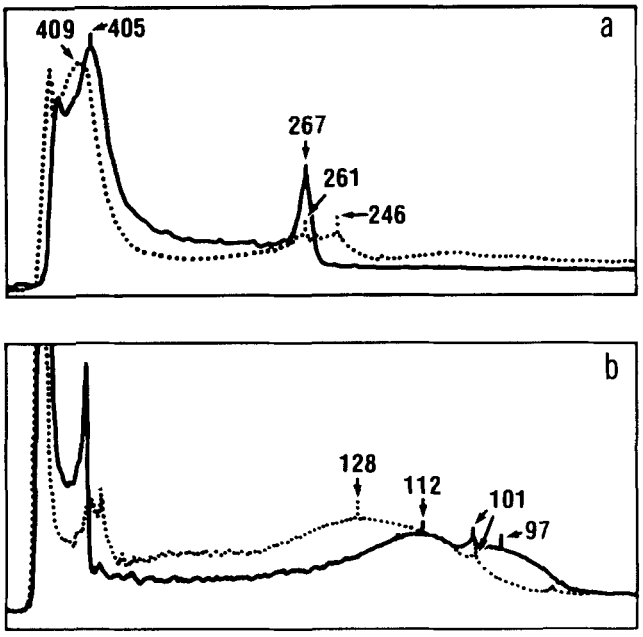

Fig. 2. Effect of ethionine feeding on serum lipoprotein patterns as analyzed by nondenaturing gradient gel electrophoresis. Animals were fed a control diet or one containing 0.25% ethionine for 4 weeks. Blood was collected, red cells were sedimented, and the d < 1.21 g/ml fraction was collected as described in Methods. The samples were dialyzed and analyzed by nondenaturing gradient gel electrophoresis (31). The numbers refer to the particle size in Ångstroms. Similar patterns were observed on analysis of unspun sera from both groups. Panel a: 2–16% gel (for separation of VLDL and LDL species); panel b: 4–30% gel (for separation of HDL species); control, (---); ethionine-fed, (—).

reach statistical significance. The increase in controls fed this diet was greater (80%; Table 2). Feeding a 5% or 0.1% cholesterol diet for 48 h did not result in statistically significant changes in serum, liver homogenate, or microsomal cholesterol contents in either group (data not shown).

The small elevations in serum bilirubin observed in the ethionine-fed animals suggested that hepatic uptake and/or excretion of bilirubin had decreased and that biliary metabolism in general, including that of cholesterol, might have been altered by ethionine feeding. Bile flow in the animals fed ethionine increased almost twofold (**Table 3**); therefore, these livers were not cholestatic. A similar result was reported earlier (44) for animals administered oral ethionine, albeit using a somewhat different protocol that resulted in morphological changes in the liver at the time points examined. Millimolar concentrations of Na^+, K^+, and Cl^- in bile from the ethionine-fed animals were similar to those in bile from control animals, but HCO_3^- was slightly increased, 22% (Table 3). Since bile flow was increased in the ethionine-fed animals, the biliary output of these ions expressed per g liver was increased. When biliary output of cations (Na^+, K^+) and anions (Cl^-, HCO_3^-, and bile salts) was compared, there was a small increase in total anions in ethionine-fed animals, suggesting that a slight increase in unmeasured biliary cations may have occurred. These might have included ethionine metabolites.

Despite the increased bile flow and biliary ion output, the biliary mass output of bile acids, cholesterol, and phospholipid expressed per g liver per h was not different from control values (Table 3). Thus, the biliary concentrations of bile salts, cholesterol, and phospholipid were decreased significantly in the ethionine-fed animals. The decrease in bile salt concentration was in part compensated for by the increase in HCO_3^-. Since bile salt output per g liver was similar in both sets of animals, bile salt-independent bile flow specifically may have been affected by feeding ethionine.

Previous work by Horton and Sabine (7) showed that ethionine feeding increased the rate of hepatic choles-

TABLE 3. Effects of ethionine feeding on biliary content and bile flow

Treatment	Bile Flow[a]	Biliary Lipid		
		Bile Acids	Cholesterol	Phospholipids
	ml/h/g liver		*μmol/h/g liver*	
Control (7)	0.079 ± 0.008	2.37 ± 0.18	0.055 ± 0.004	0.26 ± 0.02
Ethionine-fed (8)	0.163 ± 0.023	2.41 ± 0.43	0.058 ± 0.010	0.26 ± 0.04
	$P < 0.01$	NS	NS	NS

	Biliary Ions				
	Na^+	K^+	Cl^-	HCO_3^-	Bile Acids
			mM		
Control (7)	141 ± 2.9	5.2 ± 0.2	87.4 ± 1.3	25.6 ± 1.1	32.0 ± 4.0
Ethionine-fed (8)	139 ± 2.0	4.8 ± 0.2	92.5 ± 1.8	31.3 ± 1.4	15.5 ± 3.0
	NS	NS	NS	$P < 0.01$	$P < 0.01$

Rats were fed either the control or 0.25% ethionine diet for 4 weeks. The common bile duct was cannulated under light anesthesia and the animals were placed in restraining cages. Bile was collected over a 4-h period. The bile volume was measured as well as the body and liver weights of the animals. Aliquots of the bile samples were assayed for bile acids, cholesterol, phospholipids, and ions as described in Methods. Values are expressed as mean ± SE; the number of animals is in parentheses; NS, difference not statistically significant.

[a]These values are equivalent to about 0.3 ml/100 g body weight/h for controls versus about 0.6 ml/100 g body weight/h for ethionine-fed animals.

TABLE 4. Effect of ethionine feeding on hepatic and intestinal cholesterol and fatty acid syntheses and the accumulation of newly synthesized cholesterol in the serum

Treatment	Cholesterol			Fatty Acids	
	Liver	Intestine	Serum Accumulation	Liver	Intestine
	μmol [³H]OH incorporated into sterol/g tissue/h		*μmol [³H]OH incorporated into sterol/ml/h*	*μmol [³H]OH incorporated into fatty acids/g tissue/h*	
Control	1.64 ± 0.19 (17)	0.70 ± 0.05 (18)	0.17 ± 0.02 (9)	2.30 ± 0.17 (17)	2.45 ± 0.19 (18)
Ethionine	3.71 ± 0.47 (19)	0.67 ± 0.05 (19)	0.42 ± 0.06 (10)	3.60 ± 0.39 (19)	2.44 ± 0.13 (19)
	$P < 0.001$	NS	$P < 0.01$	$P < 0.005$	NS

Incorporation of [³H]OH into hepatic and intestinal cholesterol and fatty acids and the accumulation of radiolabel in serum cholesterol over a 1-h time period were measured in vivo as described in Methods. Values are the mean ± SE; the number of animals is in parentheses; NS, not statistically significant.

Note: Similar differences between control and ethionine-fed animals are observed when values are expressed per organ: for control liver, 22.78 ± 2.21 μmol [³H]OH incorporated into sterol/organ; for liver from ethionine-fed animals, 49.94 ± 4.23 μmol/organ; different at $P < 0.001$. For intestine, control is 7.58 ± 0.54 μmol [³H]OH incorporated into sterol/intestine and for ethionine-fed, 7.77 ± 0.54 μmol/intestine; difference not statistically significant.

terol synthesis when studied in vivo using the drug AY9944 and measuring production of 7-dehydrocholesterol. We confirmed and extended these studies using [³H]OH incorporation in vivo to study the rates of synthesis of cholesterol and fatty acids in both the liver and intestine. Incorporation of [³H]OH into hepatic cholesterol was increased 2.2-fold in the ethionine-fed animals (**Table 4**). Incorporation of [³H]OH into liver fatty acids also was increased, 1.6-fold. There was no effect of ethionine feeding on incorporation of [³H]OH into either fatty acids or cholesterol in the intestine (Table 4). Accumulation of radiolabeled cholesterol in the serum was higher, 2.4-fold (Table 4), in the animals fed ethionine. This increase was likely due, at least in part, to the increased synthesis in the liver inasmuch as no change was observed in intestinal synthetic rate.

It had been reported previously that feeding ethionine in the diet abolished feedback inhibition of hepatic cholesterol synthesis by dietary cholesterol in selected animals (7). We examined sensitivity to feedback inhibition by feeding a spectrum of cholesterol-containing diets for 48 h, a time period that is sufficient for inhibition of cholesterol synthesis to occur in control animals. Hepatic cholesterol synthesis was inhibited to a lesser degree in ethionine-fed animals with all cholesterol-containing diets compared to control animals (**Fig. 3A and B**). In ethionine-fed rats no inhibition by the 0.1% cholesterol diet was observed, while in controls, this diet resulted in 57% inhibition. Feeding the 5% cholesterol diet resulted in 43% inhibition in the ethionine-fed group compared with 85–95% in the control group; while feeding the cholesterol-cholate-lard diet led to about 75% inhibition in the ethionine-fed animals (compared with 95% or more in controls; Fig. 3B). The latter diet was fed to minimize any potential differences in absorption between the control and ethionine-fed animals. Intestinal cholesterol synthesis in both control and ethionine-fed animals responded similarly to these diets (data not shown).

Next, we examined the effects of ethionine feeding on key intracellular enzymes of cholesterol metabolism. Ethionine feeding for 4 weeks increased the activity of HMG-CoA reductase approximately twofold whether measured at the peak or nadir of its diurnal rhythm (**Table 5**). The magnitude of its diurnal increase was similar to that of controls. Hepatic ACAT activity was lower in the ethionine-fed animals (Table 5) both at its peak and nadir of activity in control animals, and its diurnal rhythm was no longer apparent. Cholesterol 7α-hydroxylase activity, when measured at the diurnal peak, was suppressed by feeding ethionine (Table 5). Liver homogenate neutral cholesteryl ester hydrolase activity was unchanged under the assay conditions used, while acidic cholesteryl ester hydrolase activity was decreased (Table 5). Since the cholesterol content of the liver homo-

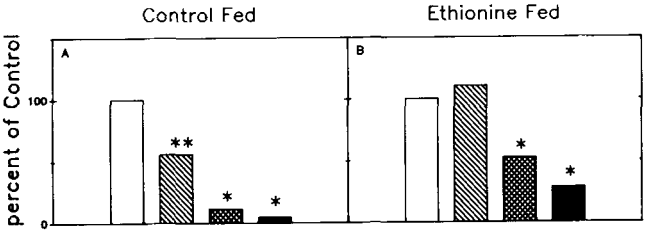

Fig. 3. Effect of ethionine feeding on the regulation of hepatic cholesterol synthesis by dietary cholesterol. Animals were fed a diet containing 0.25% ethionine or a control diet for 4 weeks as described in Methods. During the last 48 h of feeding, cholesterol was added to the diets of half the ethionine-fed and half the control-fed animals. Cholesterol synthesis was assayed by incorporation of [³H]OH into sterols in vivo as described in Methods. Noncholesterol-fed values in the two groups were: control-fed = 1.88 ± 0.16 μmol [³H]OH incorporated into sterol/g liver/h (n = 17); ethionine-fed = 4.58 ± 0.33 μmol [³H]OH incorporated into sterol/g liver/h (n = 9), different at $P<0.001$. Statistical significance was determined by comparison of experimental values with those from animals fed diet without addition of cholesterol. Panel A:control; panel B:ethionine-fed; □, diet without addition of cholesterol; ▨, 0.1% cholesterol diet; ▩, 5% cholesterol diet; ■, 2% cholesterol, 0.3% cholate, 5% lard diet; *, statistically significant at $P<0.05$; **, statistically significant at $P<0.02$.

TABLE 5. Effect of ethionine feeding on the activities of hepatic HMG-CoA reductase, ACAT, cholesterol 7α-hydroxylase, and the acidic and neutral cholesteryl ester hydrolases

Treatment	HMG-CoA Reductase	ACAT	Cholesterol 7α-Hydroxylase	Cholesteryl Ester Hydrolase	
				pH 4.5	pH 8.0
	nmol MVA/min/mg protein	*nmol CE/min/mg protein*	*nmol 7α-hydroxycholesterol/min/mg protein*	*oleate dpm released/h/mg protein*	
Control diet					
Peak (6)	0.706 ± 0.074	0.260 ± 0.026	0.11 ± 0.02	352 ± 51	320 ± 18
Trough (6)	0.333 ± 0.024	0.158 ± 0.024			
	$P < 0.001$	$P < 0.05$			
Ethionine diet					
Peak (6)	1.71 ± 0.144**	0.169 ± 0.030*	0.04 ± 0.01	209 ± 14	364 ± 26
Trough (7)	0.631 ± 0.104***	0.130 ± 0.026			
	$P < 0.02$	NS	$P < 0.01$	$P < 0.05$	NS

Animals were treated as described in Table 1 except that animals were taken at the peak or trough of the diurnal rhythm of cholesterol synthesis. Liver microsomes were prepared and assayed as described in Methods. Values are the mean ± SE; the number of animals is in parentheses; NS, not statistically significantly different.

Different from control at: *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.02$

genates was similar in the control and ethionine-fed rats, it is possible that the decreased activity of the acidic hydrolase reflects a decrease in the content of this lysosomal enzyme itself.

After 1 week of ethionine feeding, HMG-CoA reductase had begun to increase, reaching a maximum by 4 weeks (**Fig. 4**). Although HMG-CoA reductase was still susceptible to inhibition by dietary cholesterol at 1 week, after 2 and 4 weeks of ethionine feeding, this sensitivity was markedly decreased (data not shown). ACAT activity was decreased maximally after 1 week of feeding (Fig. 4) and did not respond to dietary cholesterol at 1 week or thereafter (data not shown).

We next examined the reversibility of the changes in HMG-CoA reductase and ACAT activities induced by ethionine. Animals were fed 0.25% ethionine in the diet for 4 weeks and then placed on a control diet for 1 or 2 weeks. After either 1 or 2 weeks on the control diet, the serum bilirubin levels were indistinguishable from control values (data not shown). However, after 1 week on the control diet, both ACAT and HMG-CoA reductase in the livers of animals fed the ethionine diet were still abnormal (Fig. 4); by 2 weeks, both activities approached control levels (Fig. 4). Thus, the expression of these two enzyme activities averaged over the whole liver apparently had not been irreversibly altered by feeding the ethionine-containing diet for 4 weeks.

The activation state of HMG-CoA reductase has been reported to be increased in transplantable hepatomas (45). Phosphorylation of HMG-CoA reductase correlates with a decrease in enzyme activity (46) while it has been shown that ACAT activity can be increased when rat liver microsomes are treated with Mg^{2+}-ATP and a kinase in vitro, (47). Thus, a general decrease in hepato-cellular phosphorylation state could result in enhanced reductase activity with a decrease in ACAT activity as observed in the ethionine-fed animals.

The amounts of HMG-CoA reductase activity that were in the active form (as defined by the ratio between activities in microsomes prepared in the presence of 50 mM NaF to block phosphatase action or its absence) were similar in both groups (**Table 6**). However, both the amounts of activity insensitive to NaF and total activity in liver microsomes from ethionine-fed animals were about twice as high as in controls. Both increases correlated with the approximate twofold elevation in the incorporation of [^{3}H]OH into cholesterol in vivo in the

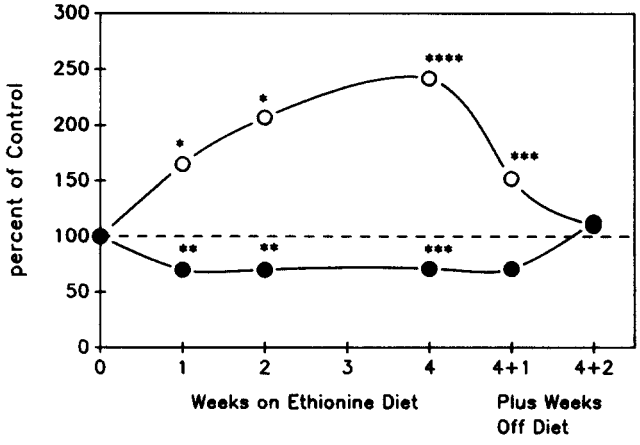

Fig. 4. Time course of changes in HMG-CoA reductase and ACAT induced by ethionine feeding and the reversibility of these effects. Animals were fed either a 0.25% ethionine-containing diet or a control diet for the times indicated. For the reversibility studies, after 4 weeks of feeding, both sets of animals received the control diet for 1 or 2 weeks. Liver microsomes were prepared and assayed for HMG-CoA reductase and ACAT activity as described in Methods. Each point represents the average of determinations from at least three pairs of animals. The recovery experiments represent two separate experiments with three pairs of animals in each set; (○), HMG-CoA reductase; (●), ACAT; *, statistically significant at $P<0.05$; **, statistically significant at $P<0.02$; ***, statistically significant at $P<0.01$; ****, statistically significant at $P<0.001$.

TABLE 6. Effect of ethionine feeding on the activation state of HMG-CoA reductase and ACAT

Treatment	HMG-CoA Reductase	Ratio	ACAT	Ratio
	nmol MVA/min/mg protein		*nmol CE/min/mg protein*	
Control (15)				
− NaF	1.141 ± 0.097		0.282 ± 0.027	
+ NaF	0.219 ± 0.022	0.19	0.359 ± 0.052	1.27
	$P < 0.001$		NS	
Ethionine (15)				
− NaF	2.361 ± 0.217***		0.188 ± 0.012**	
+ NaF	0.403 ± 0.059*	0.17	0.207 ± 0.053*	1.10
	$P < 0.001$		NS	

Animals were treated and liver microsomes were prepared as described in Table 5 except that only diurnal peak animals were used. The livers were divided into two portions and microsomes were prepared in the presence or absence of 50 mM NaF. Values are mean ± SE; the number of animals is in parentheses; NS, not statistically significantly different.

Different from control at: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ethionine-fed animals. Since both the total amount and the amount of NaF-insensitive reductase activity were increased in the ethionine-fed animals, the amount of enzyme protein likely had increased. Inclusion of NaF in the buffer for preparation of liver microsomes had little effect on ACAT activity from either control or ethionine-fed animals (Table 6).

Administration of mevalonolactone intragastrically results in inhibition of hepatic HMG-CoA reductase (16) and increased hepatic ACAT activity (23) within 2 h. Ethionine feeding did not block the mevalonolactone-induced changes in these enzymes (**Table 7**). Although the magnitude of the decrease in HMG-CoA reductase (ca. 75%) was similar in the control and ethionine-fed animals, the specific activities in the ethionine-fed animals remained two- to threefold higher than in controls after mevalonolactone treatment. Thus, although the sensitivity was similar to control animals, the end result was retention of higher hepatic HMG-CoA reductase activity in the ethionine-treated animals.

ACAT activity was induced by mevalonolactone to similar levels in both types of animal (Table 7). This effect appeared to be due in part to an increase in cholesterol availability. The microsomal cholesterol substrate pool was expanded two- to threefold by mevalonolactone treatment in both types of animal (3.70 ± 0.34 nmol/mg for control + MVA vs 1.80 ± 0.12 for control + vehicle, $P<0.001$; and 4.76 ± 0.76 nmol/mg for ethionine-fed + MVA vs 1.25 ± 0.10 for ethionine-fed + vehicle; $P<0.001$). To estimate whether alterations in ACAT activity were due to changes in amount of enzyme activity, the enzyme was assayed using [^{3}H]cholesterol:phospholipid liposomes. The decrease in hepatic ACAT activity in the ethionine-fed animals was maintained in this assay (23,536 ± 1,674 dpm CE/min/mg for ethionine-fed vs 40,748 ± 2,434 dpm CE/min/mg protein in control, $P<0.001$). Intragastric administration of mevalonolactone resulted in little change in ACAT activity was measured by this assay. In the control, ACAT activity was 40,748 ± 2,434 dpm CE/min per mg protein versus

TABLE 7. Effects of ethionine feeding on the response of HMG-CoA reductase and ACAT to intragastric administration of mevalonolactone

Treatment	HMG CoA Reductase	% Decrease	ACAT	% Increase
	nmol MVA/min/mg protein		*nmol CE/min/mg protein*	
Control (12)	0.834 ± 0.145		0.261 ± 0.032	
+ MVA (12)	0.215 ± 0.078	74	0.366 ± 0.027	40
	$P < 0.005$		$P < 0.01$	
Ethionine (11)	2.004 ± 0.295**		0.166 ± 0.031*	
+ MVA (12)	0.466 ± 0.113*	77	0.450 ± 0.034	71
	$P < 0.001$		$P < 0.001$	

Animals were treated as in Table 5 except that the animals were administered 200 mg of mevalonolactone intragastrically 2 h before killing. Controls received the vehicle alone. Liver microsomes were prepared and assayed as in Methods. Values are the mean ± SE; the number of animals is in parentheses.

Different from control at: *, $P < 0.05$; **, $P < 0.01$.

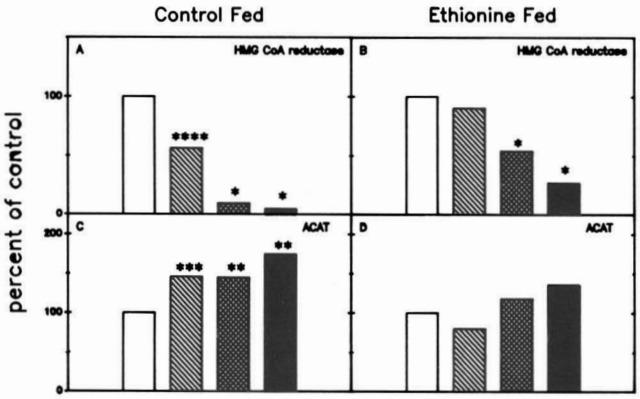

Fig. 5. Effect of ethionine feeding on the regulation of hepatic HMG-CoA reductase and ACAT activities by dietary cholesterol. Animals were treated as in the legend to Fig. 3. Liver microsomes were prepared and HMG-CoA reductase and ACAT activities were assayed as described in Methods. Noncholesterol-fed values in the two groups were: for HMG-CoA reductase: control-fed = 1.01 ± 0.18 nmol MVA/min/mg protein (n = 24); ethionine-fed = 1.80 ± 0.29 nmol MVA/min/mg protein (n = 24), different at $P<0.05$; for ACAT: control-fed = 0.24 ± 0.02 nmol CE/min/mg protein (n = 24); ethionine-fed = 0.17 ± 0.01 nmol CE/min/mg protein (n = 24), different at $P<0.01$. Statistical significance was determined by comparison of experimental values with those for animals fed diet without addition of cholesterol; panels A and C, controls; panels B and D, ethionine-fed; □, diet without addition of cholesterol; ▨, 0.1% cholesterol diet; ▩, 5% cholesterol diet; ■, 2% cholesterol, 0.3% cholate, 5% lard diet; *, statistically significant at $P<0.05$; **, statistically significant at $P<0.02$; ***, statistically significant at $P<0.01$; ****, statistically significant at $P<0.001$.

42,990 ± 1,879 for the MVA-treated control animals, while in the ethionine-treated animals the activity was 23,536 ± 1,674 dpm/min per mg versus 31,066 ± 5,345 after MVA treatment.

Sensitivity to regulation by dietary cholesterol was decreased for both HMG-CoA reductase and ACAT activities in the livers of the ethionine-fed animals. HMG-CoA reductase activity was not inhibited in ethionine-fed animals who received the 0.1% cholesterol diet for 48 h (**Fig. 5B**), whereas in control animals receiving this diet, it was inhibited 46% (Fig. 5A). In ethionine-fed animals receiving 5% cholesterol or those fed the cholesterol-cholate-lard diet, reductase activity was decreased by 30–40% (Fig. 5B). This was in contrast to the control animals where HMG-CoA reductase activity was inhibited 90% or more by these two treatments (Fig. 5A). Hepatic ACAT activity in the ethionine-fed animals was not increased significantly after feeding any of the cholesterol-containing diets (Fig. 5D) unlike what was observed in the controls (Fig. 5C). Feeding the cholesterol-cholate-lard diet for 48 h had no statistically significant effect on LDL receptor protein levels in sinusoidal liver plasma membrane-containing preparations from either the control or ethionine-fed animals nor was there any significant difference in LDL receptors induced by ethionine feeding. Expression of these receptors as assessed by immunoblotting was: for control, 7.42 ± 2.40 units versus 8.39 ± 2.19 units for controls fed the cholesterol-cholate-lard diet (average of values for liver membrane preparations from four animals in each group); for ethionine-fed, 5.35 ± 1.66 units for ethionine-fed versus 8.80 ± 1.50 units for ethionine-fed + cholesterol-cholate-lard diet (average of four animals in each group).

Estrogens can serve as cancer promotors (48). The liver is a target organ for estrogen action, and one of the effects of estrogen administration is to alter hepatic cholesterol metabolism. Inasmuch as hepatomas have often lost the ability to respond to estrogens, we studied the responses of the ethionine-induced premalignant liver to ethinyl estradiol. The changes induced in rat hepatic cholesterol metabolism by ethinyl estradiol in normal animals (49–51) were also expressed in the ethionine-fed animals (**Table 8**). Hypocholesterolemia was induced in both sets of animals. HMG-CoA reductase activity was suppressed to similar extents in the ethionine-fed animals and in control animals. However, the increase in ACAT activity induced by ethinyl estradiol was lower in the ethionine-fed rats. There was little effect of ethionine feeding on membrane-associated hepatic LDL receptor protein compared with control (Table 8), and ethinyl estradiol administra-

TABLE 8. Effects of ethionine feeding on sensitivity of hepatic cholesterol metabolism to ethinyl estradiol

Treatment	Serum Cholesterol	ACAT	HMG-CoA Reductase	LDL Receptor Content
	mg/dl	*nmol CE/min/mg protein*	*nmol MVA/min/mg protein*	*units/μg* membrane protein
Control	57.4 ± 2.1	0.297 ± 0.031	0.408 ± 0.060	19.1 ± 1.6
+ Ethinyl estradiol	8.0 ± 3.2	0.623 ± 0.046	0.108 ± 0.020	99.6 ± 4.1
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Ethionine	58.2 ± 4.7	0.316 ± 0.027	1.233 ± 0.134	16.2 ± 3.5
+ Ethinyl estradiol	3.9 ± 1.2	0.458 ± 0.030**	0.321 ± 0.113*	113.0 ± 7.2
	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$

Rats were treated as described in Table 1. In the last 3 days of treatment, half of the control and half of the ethionine-fed rats received ethinyl estradiol (1 mg/200 g body weight) (17). The other half received the vehicle alone. Serum cholesterol and hepatic ACAT, HMG-CoA reductase, and LDL receptors were measured as described in Methods. Values are the mean ± SE from two separate experiments with three pairs of animals in each.
Different from control + ethinyl estradiol at: *, $P < 0.05$; **, $P < 0.02$.

tion induced these receptors in the ethionine-fed rat liver to levels similar to those in controls (Table 8). Thus, ethionine-induced premalignancy did not appear to result in massive changes in the sensitivity of hepatic cholesterol metabolism to estrogen.

DISCUSSION

Early premalignancy, in which cells show biochemical but no morphologically detectable changes when compared with normal, fully differentiated cells, provides a model in which to study alterations in metabolism that may be permissive for development of the fully malignant state. Since the maintenance of cholesterol homeostasis is essential for proper functioning of mammalian cells and has been shown to be altered in malignancy, we chose to study the effects of the hepatocarcinogen ethionine on cellular parameters involved in the maintenance of hepatic cholesterol homeostasis in the fully mature liver. We reasoned that studying the responses of hepatic cholesterol metabolism to induction of early premalignancy would provide insight as to whether changes occurred in a single parameter only, or whether a constellation of changes in the key elements regulating hepatic cholesterol metabolism was involved.

The regulation of cholesterol metabolism in the early premalignant liver is reminiscent of that reported for primary and transplantable hepatomas (1). When cholesterol absorption was maximized by feeding a diet containing 2% cholesterol together with 0.3% cholate and 5% lard, both cholesterol synthesis and HMG-CoA reductase were inhibited in the premalignant liver, although not to the extent observed in controls. Moreover, when 5% or 0.1% cholesterol were fed, the inhibitory effects on cholesterol synthesis and HMG-CoA reductase were further decreased in the ethionine-fed animals. This is in marked contrast to the effects in control animals. This difference in sensitivity might be explained in part by the decreased numbers of hepatic cell membrane lipoprotein receptors for chylomicron remnants in ethionine-fed animals (10). We found that levels of membrane-associated hepatic LDL receptors, which also recognize chylomicron remnants (52), were unchanged in the ethionine-fed animals. This suggests that in these livers, LDL receptors may not play an important role in uptake of dietary cholesterol and mediation of the dietary feedback phenomena.

Other factors also may be important in the decreased sensitivity of the premalignant liver to dietary cholesterol. In contrast to cholesterol synthesis and HMG-CoA reductase activity, hepatic ACAT activity in the ethionine-fed animals was insensitive to regulation by dietary cholesterol. This suggested either a specific abberation in sensitivity to regulation at the level of the enzyme itself or a difference in cholesterol compartmentation or delivery within the cell. Since the acidic, probably lysosomal, cholesteryl ester hydrolase activity in liver homogenate was decreased in these animals, this suggested that an alteration in intracellular availability of lipoprotein-derived cholesterol within the cells at the lysosomal level might have occurred. ACAT activity responded to intragastrically administered mevalonolactone in a manner similar to that in control livers, suggesting that the access pathway for endogenous cholesterol to the enzyme was intact although access of exogenous cholesterol to ACAT (or a regulator activated by lipoprotein uptake) might have been changed by induction of premalignancy.

The observation that hepatic sinusoidal membrane LDL receptor protein content in both the control and ethionine-fed rats was not affected by increased dietary cholesterol despite increased serum and hepatic cholesterol contents accompanied by suppression of cholesterol synthesis, at least over the time span of these experiments, supports the findings of Spady, Turley, and Dietschy (53) that in the normal rat, cholesterol synthesis and LDL uptake can be regulated independently.

Since we observed no detectable changes by microscopy in vasculature or sinusoidal architecture in these livers, changes in accessibility of lipoproteins to the cell are an unlikely explanation for the decreased sensitivity of hepatic cholesterol metabolism to dietary cholesterol in ethionine-fed rats. Further, since neither cholesterol nor fatty acid synthesis in the intestine was affected by ethionine feeding, it seems unlikely that a gross defect in intestinal lipoprotein secretion explained the decreased effects of dietary cholesterol in these animals. This is in agreement with the work of Horton and Sabine (7) and our previous work (10) which reported that the appearance of intragastrically administered radiolabeled cholesterol in the plasma followed the same time course in control and ethionine-fed animals, suggesting that cholesterol absorbance was not grossly altered. Results from this study suggested that the major effect of feeding ethionine was to induce intrahepatic changes that contributed to the decreased sensitivity of the liver to dietary cholesterol.

Thus, the induction of premalignancy appears to have its effect on cholesterol metabolism and its regulation not by deletion of elements known to be involved in these processes in the mature, fully differentiated liver, but rather by altering expression of the levels of these elements and their sensitivity to regulatory stimuli.

We would like to thank Dr. Rebecca Van Dyke of the Gastroenterology Unit, Department of Medicine, UCSF for determination of Na^+, K^+, Cl^-, and HCO_3^- in samples of bile; Dr. Janet Boyles of the Gladstone Foundation, UCSF, for antiserum to the LDL receptor; Dr. Claire CaJacob of the Department of Pharmaceutical Chemistry, UCSF, for assistance with the cytochrome P450 spectral analyses; Dr. Keelong Hong of the Cancer Research Institute, UCSF, for assistance with preparation of cholesteryl ester-phospholipid liposomes; and Dr. Martin

Heyworth of the V. A. Medical Center, San Francisco for the gift of *Giardia muris* membranes. We are especially grateful to Dr. Marvin D. Siperstein for invaluable discussion during the course of this work. We are indebted to Ms. Maggie Joe and Ms. Pamela Herranz for typing the manuscript. This work was supported by grants CA15979, DK38553, AM26743 (Liver Center, UCSF) and HLB Program Project Grant 18574 (Lawrence Berkeley Laboratory, UCB) from the National Institutes of Health, by a grant from the Academic Senate Committee on Research, University of California, San Francisco, and by the Veterans Administration.

Manuscript received 19 October 1989 and in revised form 19 December 1989.

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