Absorption of cholesterol by the gallbladder

Dewey H. Neiderhiser, Clifford K. Harmon, and Harold P. Roth

Medical Service, Veterans Administration Hospital and the Departments of Medicine and Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Abstract To study whether cholesterol is absorbed by the gallbladder, we instilled from 1 to 300 nmoles of [4-^{14}C]cholesterol dissolved in 1 ml of guinea pig bile into the in situ guinea pig gallbladder. The bile used in these experiments contained 7 μmole/ml bile acid, 27 nmole/ml lecithin, and 8 nmole/ml cholesterol. To this bile, we added the radioactive cholesterol, from 0 to 1 μmole/ml egg lecithin, and 100 μg/ml of bromosulfophthalein, a nonabsorbable marker. After 1 nmole [4-^{14}C]cholesterol was instilled in bile without added lecithin, 77 ± 8% of the radioactivity was demonstrated to be in the gallbladder wall 6 hours later. The proportion of cholesterol absorbed by the gallbladder decreased as the concentration of added lecithin or bile salts was increased. Radioautography showed radioactivity in the mucosa, and subcellular fractionation of homogenized cells by centrifugation showed cholesterol in the mitochondrial (23 ± 4%) and microsomal (18 ± 4%) fractions. Studies on specific activity suggested that there was net absorption of cholesterol and not merely an exchange of cholesterol in the contents for cholesterol in the wall. This study presents evidence that significant quantities of cholesterol but not cholesterol ester can be absorbed by the guinea pig gallbladder. We also found that the absorbed cholesterol can be converted to cholesterol ester and the relevance of these findings to cholesterosis in man are discussed.

Supplementary key words gallstones • micelles • lipid absorption • radioautography • cholesterol metabolism

The gallbladder epithelium has the ability to absorb various substances (1). Water and ion transport have been extensively studied (2, 3). Relatively little work has been done on the transport of lipids by this epithelium, yet lipid absorption could have major significance in gallstone formation. Cholesterol, the principal component of most human gallstones, is solubilized in bile together with lecithin and bile salts (4, 5) in the form of mixed micelles. In previous studies (6, 7) we have shown that significant quantities of lipid can be absorbed by the gallbladder. Unesterified oleic acid is readily absorbed and is both metabolized by the gallbladder and transported from the gallbladder and metabolized by the liver (6). More recently, we demonstrated that lecithin, one of the components of bile necessary for the micellar solubilization of cholesterol is only slightly absorbed by the gallbladder (7). This study is concerned with the absorption of cholesterol. In man, bile is often fully saturated with cholesterol and at times supersaturated (8). Therefore, absorption of cholesterol could be a protective mechanism which prevents its precipitation. Further, absorption might be relevant to cholesterosis since, in this disorder, large quantities of cholesterol ester accumulate in the gallbladder wall (9). In the present study, we find that significant quantities of cholesterol are absorbed by the in situ gallbladder. Studies on the distribution of absorbed cholesterol within the gallbladder epithelium are also presented.

MATERIALS AND METHODS

Materials

[4-^{14}C]Cholesterol (52 mCi/mmole) and cholesteryl [1-^{14}C]-oleate (53 mCi/mmole) were purchased from New England Nuclear, Boston, Mass., and [1,2-^{3}H]cholesterol (39 Ci/mmole) was purchased from Mallinckrodt, St. Louis, Mo. Carrier cholesterol was purchased from Calbiochem, La Jolla, Calif., and recrystallized twice from aqueous ethanol. The recrystallized cholesterol had a mp of 149–50°C (lit. 149°C) (10). The radioactive cholesterol and carrier cholesterol were found to be free of contaminants by TLC on silica gel G glass plates (Analtech Inc., Newark, Del.) with the solvent system containing petroleum ether–diethyl ether–acetic acid 80:20:1 (v/v) (11). Cholesterol and cholesteryl oleate had R_f values of 0.25 and 0.78, respectively.

[4-^{14}C]Cholesteryl acetate was prepared by acylation of [4-^{14}C]cholesterol and carrier cholesterol (0.1 μmole/μCi) in acetic anhydride–pyridine 20:1, 10 volumes per gram of cholesterol (12). After recrystallization two times from aqueous ethanol, the [4-^{14}C]cholesteryl acetate had a mp of 115–16°C (lit. 116°C) (10) and was found to be free of contaminants by TLC with the solvent system described above.

Abbreviations: TLC, thin-layer chromatography; BSP, bromosulfophthalein; SD, standard deviation; SEM, standard error of mean.

[1] Presented in part at the meeting of the Ninth International Congress of Gastroenterology, Paris, France, July, 1972. A preliminary report has been published in abstract form (*Gastroenterology*. 1974, **66**: 876).

[2] Supported by funds from the Veterans Administration, Project No. 6003-01.

Cholesteryl acetate had a R_f value of 0.65. Both radioactive cholesteryl oleate and cholesteryl acetate were found to be free of digitonin-precipitable cholesterol.

Sodium glycochenodeoxycholate (Calbiochem, lot 800458) contained less than 1% free bile acid by TLC (13) and after alkaline hydrolysis (14) yielded only one spot on TLC corresponding to chenodeoxycholic acid.

Cholesterol determination

To determine the concentration of cholesterol in a pool or gallbladder wash, the aqueous solution (usually 4 ml) was extracted two times with 2 ml of chloroform–methanol 4:1, then two times with 2 ml of chloroform–methanol 2:1, and finally two times with 2 ml of diethyl ether. The extracts were combined and the organic solvent was removed at 40°C under nitrogen. The lipid extract was then made to a volume of 2 ml in chloroform–methanol 1:1 and 1.5 ml was used for cholesterol determination. The organic solvent was removed and the residue was dissolved in 0.5 ml of acetone–alcohol 1:1. After addition of 1 drop of 1% acetic acid, 0.25 ml of 0.5% digitonin in 50% alcohol was added. The cholesterol digitonide was sedimented by centrifugation, and washed with acetone–ether 1:2 and then with diethyl ether. The digitonide was dissolved in 0.2 ml acetic acid and assayed by a micro modification of the Sperry-Webb procedure (14).

Gallbladder absorption

Male guinea pigs (Beaumanor Farms, Aurora, Ohio) weighing 250–300 g were used in all experiments. Guinea pig hepatic bile and gallbladder bile were obtained as previously described (15) and stored separately at −10°C. Prior to use, the bile samples were thawed, centrifuged at 500 g for 10 min, and the total bile acid content was measured by the enzymatic method of Admirand and Small (8). The bile used for this study was a mixture of hepatic and gallbladder bile which contained 7.2 ± 0.2 μmole/ml total bile acid. The mean lecithin concentration of this bile was 27 nmole/ml (7) and the concentration of cholesterol was 8.2 ± 1.1 nmole/ml. We dissolved various amounts of [4-^{14}C]cholesterol (1–300 nmole), [4-^{14}C]cholesteryl acetate or cholesteryl [1-^{14}C]oleate (1 nmole), 100 μg BSP, a nonabsorbable marker (6, 15) and egg lecithin (0 to 1 μmole/ml) in 1 ml of this guinea pig bile. These solutions are referred to as the pool bile. The pool bile was rendered aseptic by passage through a 0.45 μ Millipore filter. The in situ guinea pig gallbladders were prepared as previously described (6, 15). The cystic duct was separated from the blood vessels and cannulated. We then instilled 1 ml of the appropriate pool bile into the gallbladder and ties off the cystic duct. The guinea pig was placed in a metabolic chamber similar to that described by Shipley et al. (16), and allowed to recover. Expired CO_2 was collected at hourly intervals in 2 N NaOH. At the scheduled time, the guinea pig was killed and its gallbladder was isolated. The mucosa was exhaustively washed four times with 0.5 ml of 0.1% albumin in Krebbs-Ringer phosphate buffer, pH 7.2 (6, 15) and the contents and washes were combined and made to a volume of 5 ml. The combined contents and washes were then assayed for BSP, radioactivity, and, in some instances, cholesterol. The gallbladders were then extracted with boiling chloroform–methanol 2:1 and the resulting extract was made to a volume of 4 ml. An aliquot (0.5 ml) of the expired CO_2 in 2 N NaOH was assayed for radioactivity. The liver was homogenized in 30 ml of water and made to a volume of 50 ml with water. An aliquot (0.5 ml) was assayed for radioactivity. In some experiments, 20 ml of the liver homogenate was extracted with chloroform–methanol 4:1 and an aliquot of the resulting extract was assayed for radioactivity. The carcass was homogenized in 500 ml of water and 10 g of this was aliquoted and dissolved in 10 ml of 50% NaOH. This solution was made to a volume of 50 ml with water and an aliquot (0.5 ml) was assayed for radioactivity. In some experiments, feces were collected and dried in a vacuum oven overnight. One g of feces was hydrolyzed in 10 ml of 2 N NaOH in an autoclave at 120°C for 3 hr, acidified to pH 2.0 with 6 N HCl, and the bile acids and sterols were extracted with diethyl ether. The sterols in an aliquot of this extract were separated from the bile acids by precipitation with digitonin. An aliquot of the fecal extract and the separated sterols and bile acids were then radioassayed. In certain experiments, the gallbladder mucosa was separated from the wall and radioactivity in mucosa and wall were measured separately. To determine whether the absorbed [^{14}C]cholesterol was present in the gallbladder wall or liver as free sterol or ester, an aliquot of the lipid extract, usually 0.1 ml, was separated by TLC with the solvent system petroleum ether–diethyl ether–acetic acid 80:20:1. The spots corresponding to standard cholesterol and cholesteryl oleate were scraped into scintillation vials and assayed for radioactivity.

Radioautography

For histologic examination, samples of guinea pig gallbladders, after exhaustive washing with 0.1% albumin solution, were fixed in buffered formalin and sections were prepared, stained and examined by an independent investigator (Pathology Service, Veterans Administration Hospital). For radioautography, frozen sections were prepared from gallbladders into which 1 ml of bile containing 0.6 Ci [1,2-^{3}H]-cholesterol (10 nmole) had been instilled for 6 hr. The unstained frozen sections were coated with Nuclear Track Emulsion NTB-2 (Eastman Kodak Co., Rochester, N. Y.), exposed for 22 days and the radioautograph was prepared according to a standard procedure (17). The exposed sections were stained with hematoxylin and eosin to indicate the location of the gallbladder epithelium.

Subcellular fractionation (Fig. 1)

Guinea pig gallbladders were excised 4 hr after instillation of 1 ml of bile to which 10 nmole of [4-^{14}C]cholesterol (1 μCi) had been added. The gallbladders were exhaustively washed as described above. The gallbladder epithelium was exposed and the mucosa cells were scraped free from the muscular and connective tissue with the edge of a glass slide. The mucosa cells were transferred to a Potter-Elvehjem type homogenizer with a Teflon pestle and homogenized in cold (4°C) 0.25 M

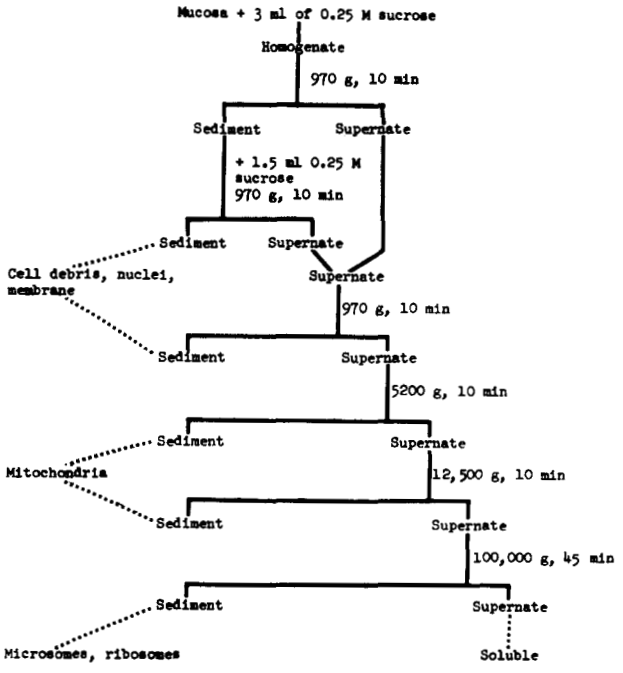

Fig. 1. Procedure for fractionation of gallbladder mucosa cell components by centrifugation.

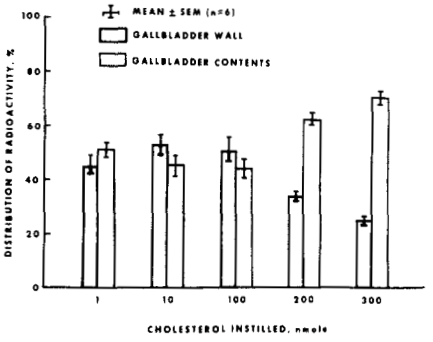

Fig. 2. Effect of cholesterol concentration on gallbladder absorption. [4-^{14}C]Cholesterol (1–300 nmole, 200,000–250,000 dpm) and 1 μmole of egg lecithin were dissolved in 1 ml of bile which contained 7 μmole/ml bile salts, 27 nmole/ml lecithin and 8 nmole/ml cholesterol. BSP (100 μg/ml) was used as the nonabsorbable marker (6, 15). One ml of this solution was instilled into the in situ guinea pig gallbladder for 6 hr. The height of each bar represents the mean % ± SEM; six animals in each group.

sucrose (3 ml per gallbladder mucosa) with 15 strokes of the pestle. The homogenizer was washed with 0.5 ml of 0.25 M sucrose and an aliquot (0.1 ml) was taken from the combined homogenate and wash to determine the percentage of [4-^{14}C]-cholesterol in the mucosa cells. The homogenate was centrifuged at 970 *g* for 10 min (2800 rpm in a Sorvall RC-2 centrifuge, SS-34 rotor, Ivan Sorvall, Inc., Norwalk, Conn.) and the resulting sediment was rehomogenized in 1.5 ml of 0.25 M sucrose with 5 strokes of the homogenizer and centrifuged at 970 *g* for 10 min. The supernatant fraction was combined with the original supernate and recentrifuged at 970 *g*. The sediment obtained from centrifugation at 970 *g* contained nuclei, cells and debris (**Fig. 1**). The mitochondrial fraction was obtained by centrifuging the 970 *g* supernate at 5200 *g* (6700 rpm) for 10 min and then at 12,500 *g* (10,000 rpm) for 10 min. The microsomal fraction was obtained by centrifuging the 12,500 *g* supernate at 100,000 *g* for 45 min (Spinco ultracentrifuge, Beckman Instruments, Spinco Div., Palo Alto, Calif.). Aliquots (0.1 ml) of the supernates and resuspended sediments were removed for radioassay at each step of the centrifugation procedure. For electron microscopy, representative pellets of the mitochondrial and microsomal fractions were fixed in hypertonic glutaraldehyde fixative and examined by an independent investigator (Pathology Service, Veterans Administration Hospital).

Experimental criteria

To be accepted as a satisfactory experiment, each study had to meet the criteria previously established (6, 15), i.e., greater than 90% of the instilled BSP marker was recovered at the end of the experiment. For studies in which the instilled solution remained in the gallbladder for 6 or 24 hr, the contents were concentrated from 1 ml to less than 0.4 ml. When the solution remained in the gallbladder for 4 hr, the contents were concentrated from 1 ml to less than 0.6 ml. With these as criteria, greater than 80% of the experiments were satisfactory.

Collateral experiments

Millipore filtration. To test for solubilization of added [4-^{14}C]cholesterol in our test bile solutions, the specific activity of cholesterol was determined before and after passage of the bile plus [4-^{14}C]cholesterol through a 0.22 μ Millipore filter. This procedure has been used by us (14, 18) and by other investigators (19, 20) to separate micellar solutions of cholesterol from precipitated cholesterol. We studied three solutions to which 100, 200, and 300 nmole of [4-^{14}C]cholesterol (200,000 to 250,000 dpm) had been added per ml of bile. The specific activity of these solutions before passage through the Millipore filter was 2281, 1217, and 610 dpm per nmole, respectively. Greater than 95% of the radioactivity and cholesterol passed through the Millipore filter and the specific activity of cholesterol in these solutions was 2195, 1196, and 595 dpm per nmole, respectively.

Gel filtration. We studied the behavior of bile to which 300 nmole/ml of [4-^{14}C]cholesterol (200,000 to 250,000 dpm) had been added. Two ml of bile which contained 410,000 dpm and 556 nmole of cholesterol was chromatographed on a Sephadex G-75 column (35 × 200 mm) and eluted with 0.05 M borate buffer, pH 7.2 as previously described (5). Ninety-four percent of the radioactivity was eluted in the high molecular weight fraction along with 97% of the cholesterol. The specific activity of cholesterol in the solution added to the column was 738 dpm/nmole; the specific activity of the high molecular weight fraction was 727 dpm/nmole of cholesterol.

TABLE 1. Absorption of cholesterol by the in situ guinea pig gallbladder[a]

	Cholesterol	Radioactivity	Specific Activity
	nmole	*dpm*	*dpm/nmole*
Solution instilled (pool bile)	315 ± 16	248,200 ± 14,200	776 ± 73
Gallbladder contents after 6 hr	230 ± 17 (72 ± 7%)[b]	177,500 ± 20,200 (71 ± 7%)[b]	776 ± 114

[a] The bile used in these experiments contained 7 μmole/ml bile salts, 27 nmole/ml lecithin, and 8 nmole/ml cholesterol. To this bile was added 100 μg/ml of BSP, a nonabsorbable marker. [4-^{14}C]Cholesterol (300 nmole, 248,200 ± 14,200 dpm) and 1 μmole egg lecithin were dissolved per ml of bile. One ml of this solution was instilled into the in situ guinea pig gallbladder for 6 hr. Values represent mean ± SD; six animals in each group.

[b] Values in parentheses represent mean % ± SD of solution instilled.

Histology. Guinea pig gallbladders used for histologic examination were immediately fixed after washing with 0.1% albumin solution in buffered formalin and were not extracted with chloroform–methanol. Gallbladder sections were prepared and stained with hematoxylin–eosin and examined by a pathologist. An acute inflammatory reaction on the serosal surface consisting of edema, extravasated erythrocytes, neutrophils and fibrin were attributed to the operative procedure (7). Attention was given to the mucosa, lamina propria and inner portion of the muscularis layer. After instillation of pool bile containing added cholesterol (300 nmole/ml) and lecithin (1 μmole/ml) for 6, 24, and 144 hr, the mucosa remained intact. No ulceration or focal erosion was observed, which is in contrast to our results in previous experiments (7) when lysolecithin was instilled instead of lecithin. The mucosa was assessed as normal by the pathologists. At 144 hr there was a slight increase in the number of plasma cells and lymphocytes in the lamina propria and fibrous connective tissue was found in the lamina propria.

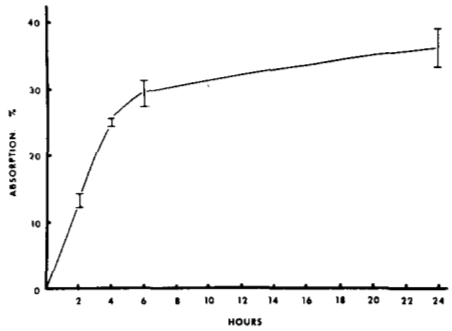

Fig. 3. Time course of cholesterol absorption by the guinea pig gallbladder. [4-^{14}C]Cholesterol (300 nmole, 200,000–250,000 dpm) and 1 μmole of egg lecithin were dissolved per ml of bile as described in Table 1. One ml of this solution was instilled for the indicated time. A line connects the mean ± SD of each set of observations; three animals in each set.

RESULTS

Cholesterol absorption by the gallbladder

Cholesterol absorption by the guinea pig gallbladder in situ was studied after instillation of from 1 nmole to 300 nmole of added [4-^{14}C]cholesterol (**Fig. 2**). This radioactive cholesterol was added to guinea pig bile which contained 8.2 ± 1.1 nmole/ml of cholesterol. This bile contained sufficient bile salts (7.2 μmole/ml) and added egg lecithin (1.0 μmole/ml) to solubilize these concentrations of cholesterol. Six hours after instillation of 1 nmole of added [4-^{14}C]-cholesterol, 45 ± 4% of the instilled radioactivity was in the gallbladder wall and 51 ± 3% remained in the contents. Comparable distribution was observed after instillation of 10 and 100 nmole of added [4-^{14}C]cholesterol. However, when the amount of radioactive cholesterol was increased to 200 nmole and 300 nmole, the gallbladder wall absorbed only 34 ± 2% and 25 ± 2% respectively of the instilled cholesterol.

There was net absorption of cholesterol rather than exchange since the specific activity of the cholesterol in the gallbladder contents (**Table 1**) 6 hr after instillation of 300 nmole of added [4-^{14}C]cholesterol was the same as that of the solution instilled. Chemical analysis of the contents showed that 230 ± 17 nmole or 72 ± 7% of the amount instilled (315 ± 16 nmole) remained in the gallbladder at the end of the six hour experimental period (Table 1). The radioactivity in the contents represented a comparable proportion (71 ± 7%) (Table 1).

Time course of cholesterol absorption

The time course of cholesterol absorption by the guinea pig gallbladder is given in **Fig. 3.** [4-^{14}C]Cholesterol (300 nmole) was added to 1 ml of guinea pig bile which contained 8 nmole of cholesterol, 7 μmole of bile salts, and 1 μmole of added egg lecithin. Cholesterol absorption increased with time; 14 ± 1% was absorbed after 2 hr of instillation, 25 ± 0.3% was absorbed after 4 hr, 29 ± 2% after 6 hr, and 36 ± 3% 24 hr later. The distribution of this absorbed [4-^{14}C]cholesterol 24 hr after instillation is given in **Table 2.** For six experi-

TABLE 2. Distribution of radioactivity 24 hr after instillation of [4-^{14}C]cholesterol[a]

	% of Radioactivity Instilled
Gallbladder contents	62.8 ± 7.5
Gallbladder wall	35.4 ± 3.6
Carcass	3.3 ± 1.8
Liver	0.9 ± 0.3
Feces	0.3 ± 0.2

[a] [4-^{14}C]Cholesterol (300 nmole, 1,500,000–1,700,000 dpm) and 1 μmole egg lecithin were dissolved per ml of guinea pig bile as described in Table 1. One ml of this solution was instilled into the in situ guinea pig gallbladder for 24 hr. Values represent mean % recovery ± SD for six animals.

TABLE 3. Distribution of radioactivity between cholesterol and cholesterol ester in pool bile, gallbladder contents and gallbladder extract[a]

Time	Number[b]		Cholesterol	Cholesterol Ester
hr			%	
0	6	Pool bile	94 ± 2	0
6	6	Gallbladder contents	94 ± 2	0
6	6	Gallbladder extract	94 ± 2	3 ± 2
24	6	Gallbladder contents	94 ± 2	0
24	6	Gallbladder extract	90 ± 3	5 ± 2
144	3	Gallbladder contents	94 ± 2	1
144	3	Gallbladder extract	76 ± 11	18 ± 8

[a] This table presents the TLC analysis of radioactivity in pool bile, gallbladder contents, and gallbladder extract after instillation of 300 nmole [4-^{14}C]cholesterol as described in Table 1. The gallbladder contents and gallbladder wall contained 71 ± 7% and 25 ± 4% 6 hr after instillation of the pool bile; after 24 hr the values were 63 ± 8% and 35 ± 4% and after 144 hr the values were 44 ± 3% and 30 ± 5%, respectively. Values represent mean ± SD.

[b] For gallbladder contents and gallbladder extracts, number represents number of animals.

mental animals, 62.8 ± 7.5% of the instilled radioactivity was recovered in the gallbladder contents and washes, and the gallbladder wall accounted for 35.4 ± 3.6%. Small but detectable quantities of radioactivity were recovered in liver (0.9 ± 0.3%), carcass (3.3 ± 1.8%) and feces (0.3 ± 0.2%). No detectable radioactivity was found in urine or expired CO_2. Of the radioactivity excreted in the feces, 8 ± 3% was present as digitonin precipitable sterols; the remainder was in non-digitonin precipitable bile acids.

Cholesterol ester formation by the gallbladder

The radioactivity in aliquots of the gallbladder contents and extracts from the gallbladder wall at 6, 24, and 144 hr after instillation of [4-^{14}C]cholesterol (300 nmole/ml) were separated by TLC and the proportions in cholesterol and in cholesterol ester were determined (**Table 3**). In addition, we analyzed the pool bile instilled for cholesterol and cholesterol ester. No radioactivity was demonstrated in cholesterol ester in the bile instilled or in the gallbladder contents 6 and 24 hr after instillation; at 144 hr, the gallbladder contents contained a small (< 1%) amount of cholesterol ester. However, the gallbladder extract 6 hr after instillation contained 3 ± 2% of the radioactivity as cholesterol ester. After 24 hr, cholesterol ester contained 5 ± 2% of the radioactivity and after 144 hr, the proportion of radioactivity present as cholesterol ester was 18 ± 8%.

TABLE 4. Effect of added bile salts and lecithin on the incorporation of [4-^{14}C]cholesterol into the in situ guinea pig gallbladder[a]

Bile Salts Added	Egg Lecithin Added	Distribution of Radioactivity	
		Wall	Contents
μmole/ml	*μmole/ml*	*% of instilled*	
0	0	77 ± 8	20 ± 9
0	0.1	81 ± 6	16 ± 4
0	0.5	65 ± 9	31 ± 5
0	1.0	45 ± 9	51 ± 7
10	0	38 ± 6	58 ± 7

[a] Bile salt (glycochenodeoxycholate) or egg lecithin were added to guinea pig bile as described in Table 1. [4-^{14}C]Cholesterol (1 nmole, 200,000–250,000 dpm) was dissolved per ml of bile. One ml of this solution was instilled into the in situ guinea pig gallbladder for 6 hr. Values represent mean ± SD; six animals in each group.

TABLE 5. Absorption of cholesterol and cholesterol ester by the in situ guinea pig gallbladder[a]

Compound Instilled	Distribution of Radioactivity	
	Wall	Contents
	% of instilled	
Cholesterol	80 ± 4	19 ± 6
Cholesteryl acetate	8 ± 1	92 ± 4

[a] [4-^{14}C]Cholesterol or [4-^{14}C]cholesteryl acetate (1 nmole, 200,000–250,000 dpm) and 0.1 μmole egg lecithin were dissolved per ml of bile as described in Table 1. One ml of this solution was instilled into the gallbladder for 6 hr. Values represent mean ± SD; six animals in each group.

Effect of added lecithin and bile salts on cholesterol absorption

The amount of cholesterol absorbed by the gallbladder epithelium after 6 hr instillation varied with the concentration of lecithin and bile salts present in the bile (**Table 4**). The bile used in these experiments contained 7 μmole/ml bile salts, 27 nmole/ml lecithin and 8 nmole/ml cholesterol. When 1 nmole of [4-^{14}C]cholesterol was dissolved in 1 ml of this bile and instilled into the gallbladder for 6 hr, 77 ± 8% of the instilled radioactivity was absorbed by the gallbladder wall. Addition of 0.1 μmole/ml of egg lecithin did not significantly alter the quantity of radioactive cholesterol absorbed, but addition of 0.5 and 1.0 μmole/ml egg lecithin diminished the quantity to 65 ± 9% and 45 ± 9%, respectively. When 10 μmole/ml of chenodeoxycholate was added without any

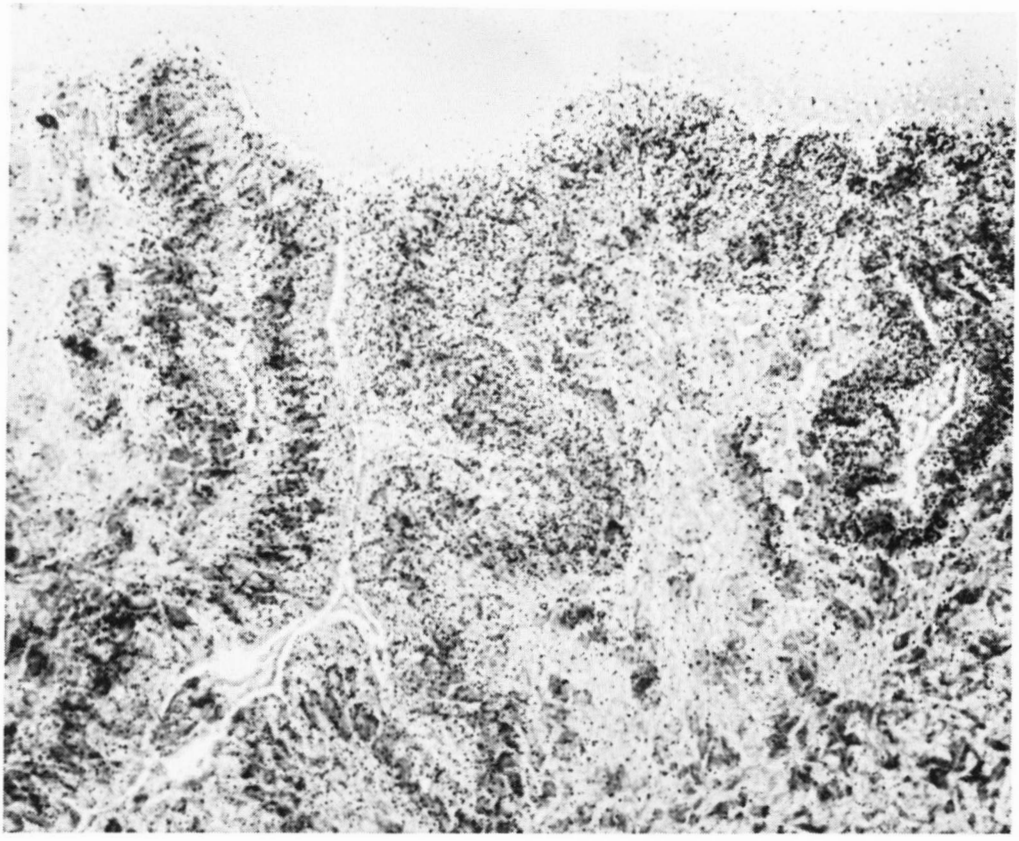

Fig. 4. Radioautograph of guinea pig gallbladder epithelium. [1,2-^{3}H]Cholesterol (0.6 Ci, 10 nmole) and 0.1 μmole of egg lecithin were dissolved per ml of bile as described in Table 1. One ml of this solution was instilled into the in situ guinea pig gallbladder for 6 hr. Frozen sections were prepared and coated with Nuclear Track Emulsion NTB-2. Exposure time was 22 days. Hema- toxylin–eosin. Phase contrast microscopy. × 100.

additional lecithin, the amount of instilled radioactivity absorbed decreased to 38 ± 6%.

Comparison of cholesterol and cholesterol ester absorption

[4-^{14}C]Cholesterol (1 nmole) or [4-^{14}C]cholesteryl acetate (1 nmole) and 0.1 μmole of egg lecithin were dissolved in 1 ml of bile containing 7 μmole/ml bile salts, 27 nmole lecithin, and 8 nmole of cholesterol. The absorption of radioactivity by the in situ guinea pig gallbladder was compared. Cholesteryl acetate was used as the test cholesterol ester because it is soluble in the bile (18). Cholesteryl [1-^{14}C]oleate is not soluble in this micellar solution (18) and when tested it precipitated from the solution. The gallbladder wall absorbed 80 ± 4% of the [4-^{14}C]cholesterol but only 8 ± 1% of the [4-^{14}C]cholesteryl acetate (**Table 5**). There did not appear to be any hydrolysis of the cholesteryl acetate since, at the end of the 6 hr experimental period, TLC demonstrated all of the radioactivity in the contents to be present as cholesteryl acetate.

TABLE 6. Subcellular distribution of [4-^{14}C]cholesterol incorporated into the in situ guinea pig gallbladder epithelium[a]

Fraction	Distribution of Radioactivity	Identification
	%	
Homogenate	100	
970 *g* Sediment	36 ± 6	cell debris, nuclei, membrane
12,500 *g* Sediment	23 ± 4	mitochondria[b]
100,000 *g* Sediment	18 ± 4	microsomes, ribosomes[b]
100,000 *g* Supernate	4 ± 2	soluble

[a] [4-^{14}C]Cholesterol (10 nmole, 1,900,000–2,200,000 dpm) and 0.1 μmole egg lecithin were dissolved per ml of bile as described in Table 1. One ml of this solution was instilled into the in situ guinea pig gallbladder for 4 hr. Of the instilled radioactivity, 78 ± 9% was recovered in the mucosa cells and 21 ± 6% was in the gallbladder contents. Values represent mean ± SD for six animals.

[b] Demonstrated by electron microscopy.

Distribution of cholesterol within the gallbladder mucosa

When [4-^{14}C]cholesterol (10 nmole) and 0.1 μmole of egg lecithin were dissolved in 1 ml of guinea pig bile containing 7 μmole/ml of bile salts, 27 nmole/ml of lecithin and 8 nmole/ml of cholesterol and then instilled into the gallbladder for 4 hr, 78% of the radioactivity was recovered in the gallbladder wall and 21% in the contents. Separation of mucosa from the remainder of the gallbladder wall showed that the major proportion of the radioactivity was present in the mucosa; less than 3% of the instilled radioactivity was recovered in the other layers of the gallbladder wall. As a further means of identifying where the absorbed cholesterol was accumulating, radioautography was performed on the gallbladder sections after instillation of [1,2-^{3}H]cholesterol. The localization of the developed grains in the photographic film suggests that the cholesterol accumulated in the mucosa layer (**Fig. 4**).

To determine the subcellular fraction of the epithelium in which the cholesterol was localized, isolated mucosa cells were homogenized 4 hr after instillation of [4-^{14}C]cholesterol (Fig. 1). When the homogenate was fractionated by centrifugation, 23 ± 4% of the radioactivity was recovered in the mitochondrial fraction and 18 ± 4% was recovered in the microsomal fraction (**Table 6**). The nature of the materials present in the mitochondrial and microsomal fractions was confirmed by electron microscopy. Bile acid analysis showed that all of the bile salts instilled in the gallbladder were recovered in the contents and wash at the end of the 4 hr experimental period; no bile acid was detected in the subcellular fractions. Ostrow (21) also reported that bile salts are not absorbed by the gallbladder.

DISCUSSION

This study demonstrates a significant net absorption of cholesterol from the gallbladder contents by the gallbladder wall. Cholesterol has been shown to bind to the mucous on the surface of the intestinal epithelium and this has been proposed as a step in the absorption of cholesterol by the intestine (22, 23). In our studies, we instilled micellar solutions of radioactive cholesterol dissolved in bile into the guinea pig gallbladder for various times. Absorption of cholesterol increased as the time of instillation increased and the absorbed cholesterol accumulated in the epithelial cells of the gallbladder. In the present study, some radioactive cholesterol was found on the surface of the gallbladder epithelium but radioautography suggested that the major proportion of absorbed cholesterol entered the mucosa cells. Further evidence of entry into the mucosa was obtained by cell fractionation of the gallbladder epithelium after exposure to radioactive cholesterol. Of the radioactivity that disappeared from the contents, 81% was demonstrated to be in the various cell fractions and over half of this was in the mitochondrial and microsomal fractions.

Although the major proportion of cholesterol absorbed from the gallbladder lumen was recovered in the gallbladder wall, we did demonstrate that approximately 5% of the instilled radioactivity was in feces, carcass, and liver 24 hr after instillation of radioactive cholesterol. The major proportion of the radioactivity in the feces was present as bile acids, the major excretory end product of cholesterol metabolism. Further evidence for absorption and metabolism of cholesterol by the gallbladder was obtained from our studies on cholesterol ester formation. We found that 18% of the radioactivity present in the gallbladder wall 144 hr after instillation of cholesterol was present in the cholesterol ester fraction.

In our studies, the proportion of cholesterol absorbed by the gallbladder diminished as the concentration of cholesterol increased. However, this represents an increase in the absolute amount of cholesterol absorbed. In man, the concentration of cholesterol in bile is considerably greater than in the guinea pig and the bile is often saturated or supersaturated (8). Absorption of quantities of cholesterol no larger than those observed in the guinea pig could provide a measure of protection against supersaturation and precipitation of cholesterol.

Absorption of cholesterol by the gallbladder also diminished as there was an increase in the concentration of lecithin or bile salts, the agents that solubilize cholesterol in bile. Rampone (24) reported a comparable finding with respect to absorption of cholesterol by the intestine; absorption of cholesterol decreased as interluminal concentration of lecithin was raised. Smith and Treadwell (22) also reported an inhibition of intestinal absorption of cholesterol by bile salts. Increased concentrations of lecithin and bile salts enhance the ability of the bile to solubilize cholesterol and cholesterol absorption would seem less necessary as a protective mechanism for such bile. However, the effect of these high concentrations on absorption of cholesterol from a saturated bile such as is seen in man remains to be demonstrated.

In cholesterosis, large quantities of cholesterol ester accumulate in the gallbladder wall, predominately in the submucosal layer (9). On the basis of our study, cholesterosis does not appear to be due to the absorption by the gallbladder wall of cholesterol ester from the gallbladder lumen. However, our data suggest that in the normal gallbladder, ester can be derived from the unesterified cholesterol normally present in the gallbladder lumen. Thus, cholesterosis could represent an abnormality of transport of cholesterol ester out of the gallbladder with an accumulation in the submucosa.

We thank Dr. W. A. Morningstar, Pathology Service, Veterans Administration Hospital, for histologic examinations of the gallbladders.

Manuscript received 7 October 1974 and in revised form 17 June 1975; accepted 6 October 1975.

REFERENCES

1. Diamond, J. M. 1968. Transport mechanisms in the gallbladder. *In* Handbook of Physiology, Section 6, Alimentary Canal, Vol. V, Bile; Digestion; Ruminal physiology. C. F. Code, editor. American Physiological Society, Washington, D. C. 2451–2482.

2. Wheeler, H. O. 1963. Transport of electrolytes and water across the wall of rabbit gallbladder. *Amer. J. Physiol.* **205**: 427–438.
3. Dietschy, J. M. 1964. Water and solute movement across the wall of the everted rabbit gallbladder. *Gastroenterology.* **47**: 395–408.
4. Small, D. M. 1967. Physicochemical studies on cholesterol gallstone formation. *Gastroenterology.* **52**: 607–610.
5. Neiderhiser, D. H., H. P. Roth, and L. T. Webster, Jr. 1966. Studies on the importance of lecithin for cholesterol solubilization in bile. *J. Lab. Clin. Med.* **68**: 90–102.
6. Neiderhiser, D. H., F. M. Pineda, L. J. Hejduk, and H. P. Roth. 1971. Absorption of oleic acid by the guinea pig gallbladder. *J. Lab. Clin. Med.* **77**: 985–992.
7. Neiderhiser, D. H., W. A. Morningstar, and H. P. Roth. 1973. Absorption of lecithin and lysolecithin by the gallbladder. *J. Lab. Clin. Med.* **82**: 891–897.
8. Admirand, W., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47**: 1043–1052.
9. Rains, A. J. H. 1964. Gallstones, Causes and Treatment. C. C. Thomas, Springfield, Ill. 65.
10. Fieser, L. F., and M. Fieser. 1959. Steroids. Reinhold, New York, N. Y. 28.
11. Mangold, H. K., R. Kammereck, and D. C. Malins. 1962. Thin-layer chromatography as an analytical and preparative tool in lipid radiochemistry. *Microchem. J.* **2**: 697–714.
12. Blatt, A. H. 1943. Organic Synthesis. Wiley, New York, N. Y. 193.
13. Kritchevsky, D., D. S. Martak, and C. H. Rothblat. 1963. Detection of bile acids by thin-layer chromatography. *Anal. Biochem.* **5**: 388–392.
14. Neiderhiser, D. H., and H. P. Roth. 1968. Cholesterol solubilization by solutions of bile salts and bile salts plus lecithin. *Proc. Soc. Exp. Biol. Med.* **128**: 221–225.
15. Ostrow, J. D. 1967. Absorption of bile pigments by the gallbladder. *J. Clin. Invest.* **46**: 2035–2052.
16. Shipley, R. A., E. B. Chudzik, A. P. Gibbons, K. Jongedyk, and D. O. Brummond. 1967. Rate of glucose transformation in the rat by whole body analysis after glucose-^{14}C. *Amer. J. Physiol.* **213**: 1149–1158.
17. Kaye, C. I., R. M. Maenza, and N. Lane. 1966. Cell replication in rabbit gallbladder. An autoradiographic study of epithelial and associated fibroblast renewal in vivo and in vitro. *Gastroenterology.* **51**: 670–680.
18. Neiderhiser, D. H., and H. P. Roth. 1972. The effect of modifications of lecithin and cholesterol on the micellar solubilization of cholesterol. *Biochim. Biophys. Acta.* **270**: 407–413.
19. Hegardt, F. G., and H. Dam. 1971. The solubility of cholesterol in aqueous solutions of bile salts and lecithin. *Z. Ernaehrungswiss.* **10**: 223–233.
20. Holzbach, R. T., M. Marsh, M. Olszewski, and K. Holan. 1973. Cholesterol solubility in bile: evidence that supersaturated bile is frequent in healthy man. *J. Clin. Invest.* **52**: 1467–1479.
21. Ostrow, J. D. 1969. Absorption by the gallbladder of bile salts, sulfobromophthalein and iodipamide. *J. Lab. Clin. Med.* **74**: 482–494.
22. Smith, A. L., and C. R. Treadwell. 1958. Effect of bile acids and other factors on cholesterol uptake by inverted intestinal sacs. *Amer. J. Physiol.* **195**: 773–778.
23. Swell, L., E. C. Trout, Jr., R. Hopper, H. Field, Jr., and C. R. Treadwell. 1959. The mechanism of cholesterol absorption. *Ann. N. Y. Acad. Sci.* **72**: 813–825.
24. Rampone, A. J. 1972. Bile salt and non-bile salt components in bile affecting micellar cholesterol uptake by rat intestine in vitro. *J. Physiol.* **227**: 889–898.