Bile acids and lipids in isolated rat hepatocytes: content, synthesis, and release, as affected by cholestyramine treatment of the donor rats

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Abstract Contents of bile acids and lipids, as well as rates of triglyceride synthesis, were determined in isolated hepatocytes from control or cholestyramine-fed rats (denoted below as "control" or "treated" hepatocytes, respectively). During a 3-hr incubation period, total bile acid production was markedly higher in "treated" cells than in "control" cells. With both kinds of cells a marked fall in production rate occurred after the first hour of incubation. Newly produced bile acids appeared in the conjugated form with both kinds of hepatocytes. "Control" cells produced only taurine-conjugated, while "treated" cells made both taurine-conjugated and glycine-conjugated bile acids. However, with exogenous taurine (0.5 mM), the latter cells also produced taurine-conjugated bile acids only. With both kinds of cells, cholic and β-muricholic acids, but not dihydroxylated bile acids, appeared as newly formed species during the incubation. Addition of dialyzed rat serum to the incubation did not result in a stimulation of bile acid production, with either kind of hepatocytes. "Treated" cells had a slightly higher content of free cholesterol than control cells; contents of other lipids were not different. Fractional release of bile acids and lipids into the medium did not differ between the two kinds of cells. Triglyceride synthesis from added [^{14}C]palmitate (0.5 mM) was 1.8-fold higher in "treated" than in "control" hepatocytes.—**Kempen, H. J. M., M. P. M. Vos-Van Holstein, and J. de Lange.** Bile acids and lipids in isolated rat hepatocytes: content, synthesis, and release, as affected by cholestyramine treatment of the donor rats. *J. Lipid Res.* 1982. **23:** 823–830.

Supplementary key words enzymatic-luminometric bile acid quantitation • β-muricholic acid • bile acid conjugation • triglyceride formation from exogenous palmitate

The liver is the major organ responsible for the catabolism of cholesterol. The conversion of this sterol into bile acids is accomplished by a number of oxidative reactions modifying both nucleus and side chain (1), and is metabolically irreversible.

Enhancement of bile acid formation may potentially cause a depletion of cholesterol from blood plasma and from nonhepatic tissues. Cholestyramine has frequently been used for that purpose since 1959 (2), acting by sequestration of bile acids in the gut lumen, and increasing their fecal excretion and that of neutral sterols (3). The consequent decrease in back-flow of bile acids to the liver is a signal for this organ to elevate its bile acid production, which is achieved by induction of cholesterol-7α-hydroxylase (4), the first and rate-limiting enzyme in this catabolic pathway (5).

Cholestyramine, by increasing the conversion of cholesterol into bile acids, as well as by reducing the transport of cholesterol from the gut to the liver, would create a want of cholesterol in this organ, which can be met in two different ways. The first of these is the well-known stimulation of de novo cholesterol biosynthesis, observed after giving cholestyramine to experimental animals (6, 7) or man (8). The second manner is the one that would indeed lead to depletion of cholesterol from plasma and extrahepatic tissues, namely an increased uptake of lipoprotein-cholesterol by the liver from the blood. Only recently could this effect of cholestyramine be confirmed experimentally in rabbits (9). It might well be that the inefficacy of cholestyramine to lower plasma cholesterol levels, seen in the rat (6) and in some human patients, is due to a failure of this second response to occur. In these instances, the heightened de novo cholesterol synthesis is probably (more than) sufficient to fulfil the need.

Another flaw of bile acid-sequestrants as hypocholesterolemic agents has recently been discovered in clinical studies. Treatment of hypercholesterolemic patients with colestipol (10) or cholestyramine (11) appeared to increase the plasma level and hepatic synthesis of triacylglycerol, indicating an enhanced VLDL production. Since VLDL particles are catabolized to LDL in the human circulation, this effect could counteract the lowering of the LDL-cholesterol level in the plasma, the goal of resin-therapy.

In an attempt to obtain more insight into the diverse actions of cholestyramine on hepatic bile acid and lipid metabolism, we have investigated contents and production rates of various bile acids and lipids in isolated he-

Abbreviations and trivial names: cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid; dihydroxycholanoic acids, sum of 3α,7α-dihydroxy- and 3α,12α-dihydroxy-5β-cholanoic acids; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HSD, 3-hydroxysteroid dehydrogenase.

patocytes obtained from control or cholestyramine-treated rats. For our purpose, we have employed a sensitive enzymatic–luminometric method for bile acid determination, to be described in the present study. It will be shown in this report that rat hepatocytes have a relatively high content of β-muricholic acid, which is a normal but minor component in rat bile (12, 13) and normal rat liver tissue (14). As the result of cholestyramine treatment we find a strong increase in the rates of bile acid synthesis, as well as an enhanced triglyceride synthesis from added palmitate.

MATERIALS AND METHODS

Materials

3-Hydroxysteroid dehydrogenase Grade II (HSD) was obtained from Sigma, and was purified in order to avoid high blank values in the luminometric procedure described below. This was done by application of 30 mg of crude enzyme, dissolved in 2 ml of 10 mM NH_4HCO_3 buffer, on a column (40 × 2.5 cm) of BioGel P-10 (BioRad), which was then eluted with the same buffer. The material in the void volume fraction was lyophilized, weighed, and stored at −10°C. For daily use, a fresh solution of 1 mg of dry material per ml was prepared in 30 mM Tris–HCl, 1 mM Na_2 EDTA, pH 7.2. Reagents for the luminometric assay of NADH were obtained as a kit ('Lumase'®, cat. no. 4780) from Lumac. Cholylglycinehydrolase was from Schwarz-Mann; cholestyramine ('Questran'®) was from Mead-Johnson; β-muricholic acid was from Steraloids; other reference bile acids were from Supelco; thin-layer silica plates (0.25 thickness) were from Merck (art. nr. 5721); cartridges of C-18-silica were from Waters (part no. 51910); radiolabeled compounds were from The Radiochemical Center.

Animals

Male Wistar rats (6 weeks old) were obtained from the Centraal Proefdierenbedrijf TNO (Zeist, The Netherlands), and housed in a temperature-(20°C) and light-(6:00 on–18:00 off) controlled room for at least 1 week before starting any treatment. During that period they had free access to pelleted chow (RMH-B, Hope Farms, Woerden, The Netherlands) and to bottled tap water. For incorporation of cholestyramine in the diet, the food pellets were ground in a Waring blendor, and the resulting flour was mixed with an equal weight of a warm solution of 2% (w/v) agar in distilled water in which 2% (w/v) cholestyramine was suspended. The slurry was left to solidify, and then cut into cubes to be fed to the rats. Control animals received the same cubes, made without cholestyramine. This food was given ad libitum for 5–7 days before the rats were killed. Food intake and weight gain were similar for control and cholestyramine-fed rats.

Rat serum

Rats fed the control diet were bled under ether anaesthesia by puncture of the aorta at the abdominal bifurcation. About 10 ml of blood was collected in a syringe containing 1 ml of 25 mM EDTA in saline (pH 7.4). After centrifugation for 10 min at 3,000 rpm, the supernatant was collected and recentrifuged for 15 min at 5,000 rpm. The resulting supernatant was dialyzed for 18 hr at 4°C against 500 ml of Krebs-Henseleit medium, containing 1.3 mM $CaCl_2$ and buffered with bicarbonate and HEPES at pH 7.4. The fibrin formed during the dialysis was removed by centrifugation. Portions of this serum were used directly for incubations with hepatocytes.

Hepatocytes: isolation, incubation, viability

Rats were killed for hepatocyte isolation between 9 and 10 AM, as described previously (15). Hepatocytes were incubated in a final volume of 2.5 ml in Krebs-Henseleit medium, containing 1.3 mM $CaCl_2$, 2% bovine serum albumin (fatty acid-poor), and buffered with 25 mM $NaHCO_3$ and 20 mM HEPES at pH 7.4. Further additions to the incubation medium are specified under Results. Incubations were done as described (15), and were terminated after 0, 60, or 180 min by addition of 9 ml of chloroform–methanol 1:2 to the whole incubate, or by first separating cells and medium (15) before solvent addition.

Cells of both control and cholestyramine-fed rats were viable as judged by trypan blue exclusion (more than 90% of the cells directly after isolation, and more than 75% after 3 hr incubation). Furthermore, the intracellular ATP level was maintained at a high value (between 7 and 10 nmol/mg dry weight) during the entire 3-hr incubation period. Both kinds of cells contained 800–1200 nmol glycogen/mg dry weight directly after their isolation; this was lost mainly by conversion to glucose and lactate, at a rate of 100–200 nmol/hr per mg dry weight during the incubation.

Extraction and separation of lipids and bile acids

The tubes were supplemented with a trace amount of [^{14}C]taurocholic acid, in order to be able to correct for losses in the subsequent purification steps. Chloroform and water (3.0 ml each) were then added to induce phase separation (16), which was accelerated by centrifugation. The lower layer containing the lipids was aspirated and dried under a stream of N_2 at 60°C. Part (1/4) of the residue was spotted as a streak on a silica chromatoplate

in order to separate and recover the various lipid classes as previously described (15).

The upper layer was collected for bile acid determination. In separate tests this upper layer was found to contain more than 90% of the conjugated and unconjugated bile acids. The upper layer was freed from most of the methanol by blowing with N_2 at 60°C, and then brought to dryness by evaporation in a Speed-Vac® apparatus (Savant). The dry residues were dissolved in 1 ml of distilled water and applied on a cartridge of C18-silica, which was prewetted by rinsing with methanol and then with water. Bile acids[1] were absorbed (more than 90%) from water onto this material. The cartridge was washed twice with 5 ml of distilled water, after which the bile acids were eluted quantitatively with 2 ml of methanol. The methanol eluates were lyophilized in the Speed-Vac apparatus. The residues were either applied on a thin layer of silica for chromatographic separation of taurine-conjugated, glycine-conjugated, or free bile acids (see below), or were treated with cholylglycinehydrolase. Incubation with the latter enzyme was carried out in 1 ml of buffer containing 20 mM sodium acetate, 20 mM EDTA, 0.3% mercaptoethanol, and 0.8 unit of the deconjugating enzyme, pH 5.6. The mixtures were left overnight at room temperature and then acidified to pH 1. Free bile acids were isolated by extraction (16) and collection of the lower layer. After removal of the chloroform by blowing with N_2 at 60°C, the residues were applied on a thin layer of silica for separation of the various bile acid species.

For thin-layer chromatography the samples were spotted as streaks (four on each plate), using methanol–water 4:1 as the transfer solvent. Appropriate reference bile acids (20 nmol each) were spotted at the left and right sides of the plates. For separation of the conjugated bile acids, the plates were developed in chloroform–methanol–glacial acetic acid–water 15:5:1:1 (by volume) as described by O'Moore and Percy-Robb (17). The deconjugated bile acids were separated on plates developed in benzene–dioxane–glacial acetic acid 20:10:2 (mixture S2 of Eneroth (18)). In the latter separation, chenodeoxycholic and deoxycholic acids had the same R_f value; bile acid material with this R_f was therefore designated as dihydroxycholanoic acids. After drying, the plates were sprayed at the vertical edges with water to visualize and mark the reference bile acids. The areas containing the bile acids from the hepatocytes were scraped off and the scrapings were extracted twice with 2 ml of ethanol. The ethanol was removed again by blowing with N_2 at 60°C.

[1] The following bile acids were tested: cholic, chenodeoxycholic (both as the taurine and glycine conjugates and in the unconjugated form) and β-muricholic (unconjugated).

Determination of bile acids and lipids

The quantity of the various bile acids recovered from the chromatoplates was determined by an enzymatic–luminometric method. For that purpose the dry residues were dissolved and incubated for 45 min at 30°C in 600 μl of a mixture containing 83 mM Tris-HCl, 0.7 mM NAD^+, and 67 μg of purified HSD, pH 9.0. The NADH generated in this incubation was quantitated by luminometry. For that purpose 10 μl (1/60) of the incubate was injected into tubes containing 50 μl of the 'Lumase'® mixture and placed in a Packard luminometer ('Picolite'®), and light production was measured for 60 sec. Duplicate measurements were done for each HSD incubation. In separate tests, the slope of the curve, relating the light response to 1/60 of the molar amount of reference bile acid given to the HSD incubation, was found to be identical for all reference bile acids used in this work. Moreover, the same slope was found for the curve relating light response to the molar amount of pure, freshly dissolved NADH, injected directly into the Lumase®. The latter observation demonstrates that the various bile acids were quantitatively converted into 3-keto bile acids during HSD incubation under the above conditions.

In this procedure, amounts of NADH injected into the Lumase® were kept between 10 and 200 pmol, thus enabling quantitation between 0.6 and 12 nmol of bile acid. This range could be extended to lower values by decreasing the volume of the HSD incubation and to higher values by dilution of the HSD incubation with Tris-HCl, pH 9. The light response was calibrated every hour during each measuring session and was found to remain reasonably stable in the period between 3 and 8 hr after the preparation of a fresh Lumase® mixture.

The remaining parts of the HSD incubates of the (tauro)cholate-containing material were mixed with 10 ml of Picofluor® (Packard) and counted for ^{14}C radioactivity. The results, obtained by luminometric assay of the various bile acids in each hepatocyte extract, were corrected for the ^{14}C loss found for that particular extract. Quantities of the various lipid classes, obtained after chromatographic separation, were determined as previously described (15).

Triglyceride synthesis from added palmitate was determined in dilute hepatocyte suspensions (containing 1–2 mg dry weight per incubation of 2.5 ml) in the same medium as described previously (15), supplemented with 0.5 mM [1-^{14}C]palmitate (0.1 μCi per incubation). Radioactivity in triglycerides was determined after extraction and thin-layer chromatography of the lipids as described (15), and was found to increase linearly between 10 and 50 min after the start of the incubation. Free fatty acids were also recovered from the chromatoplates

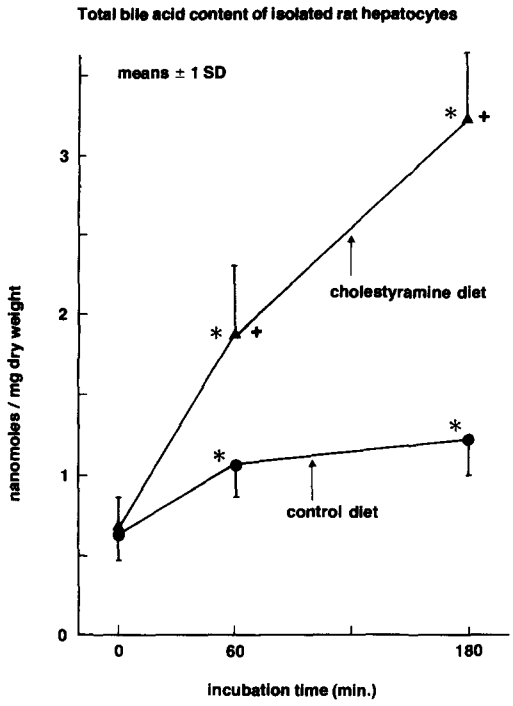

Fig. 1. Effect of incubation time on total bile acid content of isolated hepatocytes plus medium, obtained from control (● —— ●) or cholestyramine-fed (▲ —— ▲) rats. Values indicated with an asterisk are significantly higher ($P < 0.001$) than the corresponding values at t = 0. Values indicated with a cross are significantly higher ($P < 0.001$) than the corresponding control value.

by scraping and elution with chloroform, and were assayed for mass (19) and radioactivity. Consumption of FFA in that period did not exceed 20% of the initial amount. Triglyceride synthesis was calculated as:

$$\frac{\text{cpm accumulated in triglycerides between 10 min and 50 min}}{\text{cpm/nmol of fatty acids at 10 min}} \times 1.5 \times 1/3,$$

giving nmol triglycerides formed per hour in the incubation.

Data were expressed per mg dry cell weight and given as mean ± 1 SD. Statistical significance of differences was evaluated using Student's *t*-test for paired observations or for populations.

RESULTS

Content and production of bile acids in isolated hepatocytes and release in the incubation medium

As shown in **Fig. 1,** the total content of bile acid (considered to be the sum of all separately determined bile acids) was similar in freshly isolated hepatocytes of both control and cholestyramine-fed rats. However, during in vitro incubation, hepatocytes of cholestyramine-fed rats produced much more bile acid than their control counterparts. It is also clear from Fig. 1 that the rate of bile acid production in both types of hepatocytes was higher during the first hour than during the next 2 hours of incubation.

In both types of hepatocytes, more than 90% of the bile acids were in the conjugated form, either before or during incubation (**Fig. 2**). In freshly isolated hepatocytes from control rats, the bile acids were conjugated predominantly with taurine, and during incubation they produced only taurine-conjugated bile acids. In hepatocytes of resin-treated rats, the bile acids were conjugated about equally to both taurine and glycine, and both taurine- and glycine-conjugated bile acids were produced during incubation.

The high degree of conjugation to glycine, found for the bile acids produced by hepatocytes of cholestyramine-fed rats, was surprising in view of the many reports that bile acids excreted by the rat in vivo are conjugated mainly with taurine.[2] A possible reason for this abnormal conjugation pattern in vitro could be a relative lack of taurine in the isolated hepatocytes. This was checked by addition of exogenous taurine (0.5 mM). As shown in **Table 1,** this resulted in a shift of the conjugation pattern, such that newly produced bile acids were conjugated solely with taurine and not with glycine. The rate of total bile acid production was not affected by the taurine addition (Table 1).

In the experiments shown in Fig. 2, bile acids were measured separately in cells and medium. After 3 hr of incubation the following values were found for the percent of bile acid occurring outside the cells: taurine conjugates, 28 ± 10 and 38 ± 14; glycine conjugates, 36 ± 16 and 39 ± 10; and unconjugated acids, 65 ± 5 and 58 ± 5; for cells of control (n = 5) and cholestyramine-fed rats (n = 7), respectively.

In both types of hepatocytes, the trihydroxylated species cholic and β-muricholic acids comprised the majority of the total bile acids (**Fig. 3**). The contents of both of these species were increased significantly during the incubation while that of dihydroxycholanoic acid was not. In hepatocytes of cholestyramine-treated rats, production rates of both cholic and β-muricholic acids were higher and more sustained than those in control cells.

Finally, the effect of addition of dialyzed rat serum to the incubation medium was studied. As shown in **Table 2,** production of the various bile acid species tended to be inhibited by this addition, using hepatocytes of both control and cholestyramine-fed rats. This inhibition

[2] This was confirmed in our cholestyramine-fed rats. In bile samples obtained directly after a fistula of the common duct was established, 85% of the bile acids were conjugated with taurine. Kempen, H. J. M., and M. P. M. Vos-Van Holstein. Unpublished observation.

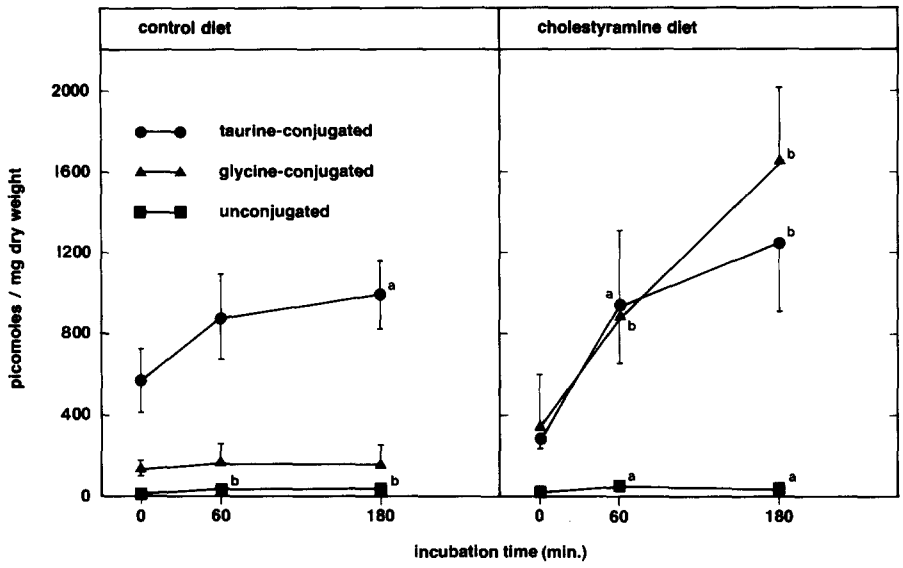

Fig. 2. Effect of incubation time on the contents of taurine-conjugated (● —— ●), glycine-conjugated (▲ —— ▲), or unconjugated (■ —— ■) bile acids in isolated hepatocytes plus medium, obtained from control (n = 5; left panel) or cholestyramine-fed (n = 7; right panel) rats. Values indicated with a letter symbol are significantly higher than the corresponding value at t = 0 (a, $P < 0.05$; b, $P < 0.001$). For % in medium, see text.

reached statistical significance for total bile acid production in hepatocytes of control rats.

Content and release of lipids; triglyceride synthesis from added palmitate

Directly after their isolation, hepatocytes of control and cholestyramine-fed rats had similar contents of phospholipids, triglycerides, and esterified cholesterol; however, the latter cells had significantly more free cholesterol (**Table 3,** first row). After 3 hr incubation, the phospholipid and esterified cholesterol contents were decreased and that of triglycerides was increased, while no change was detected in the free cholesterol in both types of hepatocytes (Table 3, second row). The fractions of each of the various lipids present in the medium after 3 hr incubation were not different for the two types of hepatocytes, although clearly different from each other (Table 3, third row). Hepatocytes of cholestyramine-fed rats displayed a significantly higher rate of triglyceride synthesis from added palmitate (0.5 mM) than hepatocytes of control rats (**Fig. 4**).

DISCUSSION

Several groups have reported on bile acid production by isolated rat hepatocytes (20–24). Our results for cholic acid production agree quantitatively with those of Whiting and Edwards (22) and of Botham et al. (23), who used gas–liquid chromatography and radioimmunoassay, respectively. Earlier, much higher values were reported (20, 21) by authors who used an enzymatic method for bile acid quantitation. Whiting and Edwards (22) have shown, however, that the enzymatic method can give rise to spuriously high values owing to errors that apparently are avoided by the extraction, purification, and separation of bile acids employed in the present work. We have also confirmed the findings of the above-named authors

TABLE 1. Effect of addition of taurine to incubations of isolated hepatocytes, obtained from cholestyramine-fed rats, on the conjugation pattern of bile acids produced during a 3-hr incubation period

	Taurine-Conjugated	Glycine-Conjugated	Unconjugated	Sum
		nmol/mg dry weight per 3 hr		
Control	1.27 ± 0.55	1.19 ± 0.41	0.02 ± 0.02	2.46 ± 0.73
+ Taurine (0.5 mM)	2.48 ± 0.52[a]	0.02 ± 0.17[a]	0.05 ± 0.05	2.51 ± 0.69

Data are means ± 1 SD of four separate experiments.
[a] Significantly different ($P < 0.05$) from control value (*t*-test for paired observations).

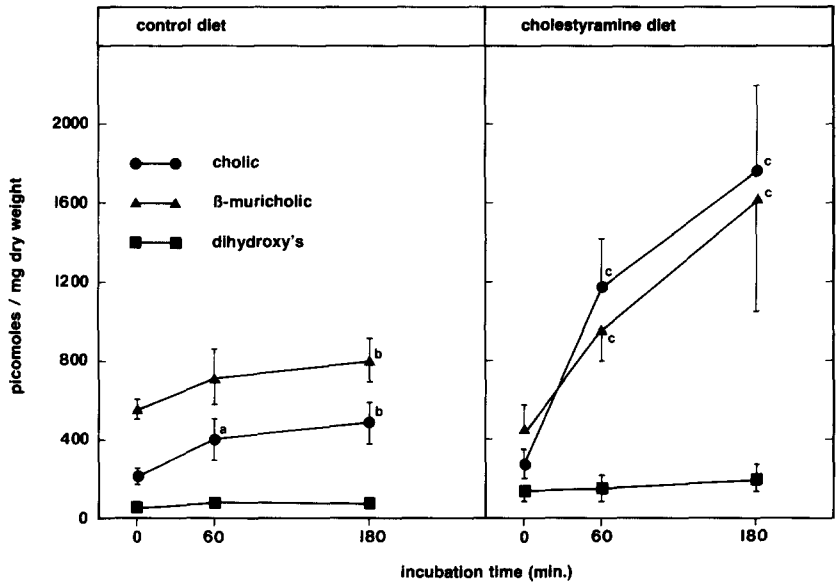

Fig. 3. Effect of incubation time on contents of cholic (● —— ●), β-muricholic (▲ —— ▲), or dihydroxycholanoic (■ —— ■) acids in isolated hepatocytes plus medium, obtained from control (n = 7; left panel) or cholestyramine-fed (n = 9; right panel) rats. Dihydroxy's represent the sum of chenodeoxycholic and deoxycholic acids. Values indicated with a letter symbol are significantly higher than the corresponding values at t = 0 (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$).

(22, 23) that the rate of bile acid production by hepatocytes of cholestyramine-fed rats is much higher than by hepatocytes of control rats, but that with both types of hepatocytes production decreases markedly after the first hour of incubation. The production rate in the first hour of incubation of control hepatocytes was similar to that in the intact rat in vivo (cf. Table 2 in ref. 22). In contrast, the lower rate in the next hours was similar to the rate of bile acid excretion reported for the isolated rat liver after the first hour of perfusion (25), and for isolated rat hepatocytes during the first 24 hr of a suspension culture (24).

The marked fall in bile acid production after the first hour of incubation does not seem to result from a general loss of cell viability, since trypan blue exclusion and ATP content remained high during 3 hr of incubation. Instead, it may result from exhaustion of a cholesterol pool specially destined for conversion into bile acids. This pool might be one that is filled in vivo by influx of cholesterol from the blood. However, this explanation seems unlikely, because we did not find stimulation of bile acid production by addition of rat serum, and because others have also observed low production rates during more prolonged in vitro experiments in the presence of serum (24) or whole blood (25).

While hepatocytes from control rats were found to produce only taurine-conjugated bile acids during the incubation, those of cholestyramine-fed rats conjugated their newly-made bile acids about equally with taurine and glycine. However, after addition of taurine to the

TABLE 2. Effect of addition of dialyzed rat serum (20% by volume) on bile acid production by isolated hepatocytes, obtained from control or cholestyramine-fed rats, during a 3-hr incubation period

Diet	Serum Added	Cholate	β-Muricholate	Dihydroxy Acids	Sum
		nmol/mg dry weight per 3 hr[a]			
Control	−	0.35 ± 0.15	0.30 ± 0.11	−0.01 ± 0.03	0.64 ± 0.26
	+	0.22 ± 0.10	0.28 ± 0.17	−0.06 ± 0.05	0.43 ± 0.28[b]
Cholestyramine	−	1.11 ± 0.27	1.08 ± 0.34	0.08 ± 0.06	2.22 ± 0.46
	+	0.81 ± 0.16	0.76 ± 0.21	−0.01 ± 0.06[b]	1.55 ± 0.06

[a] Data represent means ± 1 SD of four separate experiments with either type of hepatocytes. For each experiment a different serum preparation was used. The bile acid production found in the incubations with added serum is corrected for the (variable) contents of bile acids in the serum.

[b] Significantly less ($P < 0.05$) than production in the absence of serum (*t*-test for paired observations).

TABLE 3. Contents of lipids in hepatocytes of control or cholestyramine-fed rats before and after 180-min incubation, and release of these lipids into the incubation medium

	Control (n = 6)				Cholestyramine-fed (n = 6)			
			Cholesterol				Cholesterol	
	Phospholipids	Triglycerides	Free	Esterified	Phospholipids	Triglycerides	Free	Esterified
Initial content[a] at t = 0	102 ± 11	21.0 ± 2.3	13.2 ± 0.7	1.25 ± 0.36	112 ± 15	20.6 ± 5.0	18.2 ± 2.7[d]	1.72 ± 0.36
Percent[b] of initial content[a] at t = 180 min	86.6 ± 12.4	126.2 ± 16.5	105.3 ± 7.8	70.8 ± 12.7	80.5 ± 10.7	110.2 ± 3.2	100.7 ± 4.2	91.4 ± 22.7
Percent release[c] into the medium at t = 180 min	8.5 ± 0.7	16.9 ± 6.0	7.0 ± 1.2	9.4 ± 2.0	8.8 ± 2.4	17.4 ± 1.3	7.3 ± 2.4	13.7 ± 1.0

[a] Expressed as nmol/mg dry cell weight.
[b] Equation = (content at t = 180 min of cells and medium together/content at t = 0) × 100.
[c] Equation = (amount in medium/amount in cells plus medium) × 100.
[d] Significantly different from control value in control hepatocytes ($P < 0.001$).

incubation medium, the newly-formed acids were conjugated exclusively to taurine also in the latter type of cells. It may be concluded that, without a supply of exogenous taurine, the availability of this amino acid is insufficient relative to the high rate of bile acid production in these cells, and that glycine then is used as second-best substrate for conjugation.

A notable observation in our studies was the high content and production of β-muricholic acid. This bile acid occurs in relatively low amounts in normal rat liver (14) and is formed as a metabolite from chenodeoxycholic acid (12, 13). It has not been detected before in isolated rat hepatocytes, but its formation and excretion by the isolated perfused rat liver has been described (26).

During 3 hr incubation, bile acid production by hepatocytes of control rats amounted to about 0.6 nmol/mg dry weight, while cells of cholestyramine-fed rats made about 2.5 nmol/mg dry weight. Nevertheless, we have been unable to detect a change in the mass content of free cholesterol after the incubation of either cell type (Table 3). In our procedure, a mass change of 1.5 nmol/mg dry weight (about 10% of the initial mass) should have been detected. The constant mass of free cholesterol during incubation of hepatocytes of cholestyramine-fed rats, therefore, indicates that in these cells cholesterol synthesis de novo must have occurred at about the same rate as formation of bile acids from cholesterol. Furthermore, this implies that de novo synthesis of cholesterol was much higher in cells of cholestyramine-fed rats than in those of control rats. An increased rate of cholesterol synthesis, as monitored by incorporation of ^3H from tritiated water into cholesterol, indeed has been reported for livers (7) or hepatocytes (27) isolated from cholestyramine-fed rats.

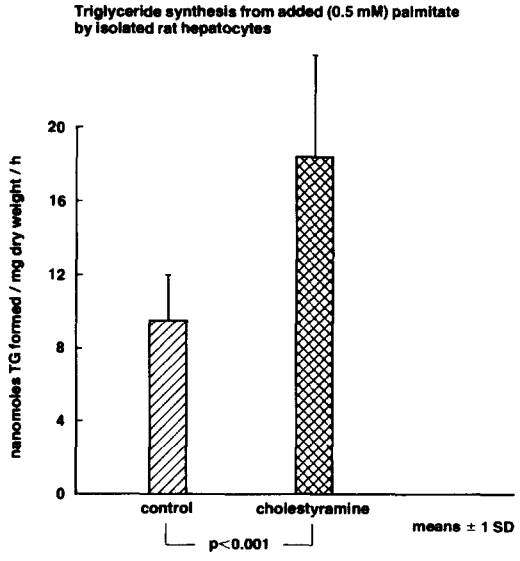

Fig. 4. Triglyceride synthesis from added 0.5 mM [1-^{14}C]palmitate by hepatocytes, obtained from control (n = 8) or cholestyramine-treated (n = 14) rats.

In hepatocytes of cholestyramine-fed rats we find a significantly higher cholesterol content than in cells of control rats. This suggests that the cholestyramine treatment in vivo has resulted in a slightly larger increase in cholesterol synthesis and/or uptake from the blood than in bile acid formation.

Finally, we have observed an increased rate of triglyceride formation from added palmitate after cholestyramine-feeding of the donor rats. This effect may be mediated by an enhanced activity of the enzyme phosphatidate phosphatase, shown to occur after cholestyramine treatment (28). If this mechanism applies also for the human situation, it would explain the increase in triglyceride and VLDL production observed in some cholestyramine-treated patients (10, 11).

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REFERENCES

1. Danielsson, H., and K. Einarsson. 1969. Formation and metabolism of bile acids. *In* The Biological Basis of Medicine. Vol. 5. E. E. Bittar and N. Bittar, editors. Academic Press, New York. 279–315.
2. Bergen, S. S., Jr., T. B. van Itallie, D. M. Tennent, and W. H. Sebrell. 1959. Effect of an anion exchange resin on serum cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **102:** 676–678.
3. Tennent, D. M., H. Siegel, M. E. Zanetti, G. W. Kuron, W. H. Ott, and F. J. Wolf. 1960. Plasma cholesterol lowering action of bile acid binding polymers in experimental animals. *J. Lipid Res.* **1:** 469–473.
4. Shefer, S., S. Hauser, and E. H. Mosbach. 1968. 7α-Hydroxylation of cholestanol by rat liver microsomes. *J. Lipid Res.* **9:** 328–333.
5. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1970. Biochemical site of regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **11:** 404–411.
6. Huff, J. W., J. L. Gilfillan, and V. M. Hunt. 1963. Effect of cholestyramine, a bile acid binding polymer, on plasma cholesterol and fecal bile acid excretion in the rat. *Proc. Soc. Exp. Biol. Med.* **114:** 352–355.
7. Liersch, M. E. A., C. A. Barth, H. J. Hackenschmidt, H. L. Ullman, and K. F. Decker. 1973. Influence of bile salts on cholesterol synthesis in the isolated perfused rat liver. *Eur. J. Biochem.* **32:** 365–371.
8. Nazir, D., L. Horlick, B. J. Kudchodkar, and H. S. Sodhi. 1972. Mechanisms of action of cholestyramine in the treatment of hypercholesterolemia. *Circulation.* **46:** 95–102.
9. Slater, H. R., C. J. Packard, S. Bicker, and J. Shepherd. 1980. Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit. *J. Biol. Chem.* **255:** 10210–10213.
10. Witztum, J. L., G. Schonfeld, and S. W. Weidman. 1976. The effects of colestipol on the metabolism of very low density lipoproteins in man. *J. Lab. Clin. Med.* **88:** 1008–1018.
11. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. *J. Lipid Res.* **19:** 1017–1024.
12. Matschiner, J. T., T. A. Mahowald, W. H. Elliott, E. A. Doisy, Jr., S. L. Hsia, and E. A. Doisy. 1957. Bile acids. I. Two new bile acids from rat bile. *J. Biol. Chem.* **225:** 771–779.
13. Samuelsson, B. 1959. On the metabolism of chenodeoxycholic acid in the rat. Bile acids and steroids 85. *Acta Chem. Scand.* **13:** 976–983.
14. Greim, H., D. Trülzsch, J. Roboz, K. Dressler, P. Czygan, F. Hutterer, and H. Popper. 1972. Mechanisms of cholestasis. 5. Bile acids in normal rat livers and in those after bile duct ligation. *Gastroenterology.* **63:** 837–845.
15. Kempen, H. J. M. 1980. Lipoprotein secretion by isolated rat hepatocytes: characterization of the lipid-carrying particles and modulation of their release. *J. Lipid Res.* **21:** 671–680.
16. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Biophys.* **37:** 911–917.
17. O'Moore, R. R. L., and I. W. Percy-Robb. 1973. Analysis of bile acids and their conjugates in jejunal juice by thin-layer chromatography and direct densitometry. *Clin. Chim. Acta.* **43:** 39–47.
18. Eneroth, P. 1963. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4:** 11–16.
19. Regouw, B. J. M., P. J. H. C. Cornelissen, R. A. P. Helder, J. B. F. Spijkers, and Y. M. M. Weeber. 1971. Specific determination of free fatty acids in plasma. *Clin. Chim. Acta.* **31:** 187–195.
20. Anwer, M. S., R. Kroker, and D. Hegner. 1975. Bile acid secretion and synthesis by isolated rat hepatocytes. *Biochem. Biophys. Res. Commun.* **64:** 603–609.
21. Gardner, B., and M. S. Chenouda. 1978. Studies of bile acid secretion by isolated rat hepatocytes. *J. Lipid Res.* **19:** 985–991.
22. Whiting, M. J., and A. M. Edwards. 1979. Measurement of cholic acid synthesis and secretion by isolated rat hepatocytes. *J. Lipid Res.* **20:** 914–918.
23. Botham, K. M., G. J. Beckett, I. W. Percy-Robb, and G. S. Boyd. 1980. Bile acid synthesis in isolated rat liver cells. The effect of 7α-hydroxycholesterol. *Eur. J. Biochem.* **103:** 299–305.
24. Yousef, I. M., J. Ho, and K. N. Jeejeebhoy. 1978. Bile acid synthesis in isolated rat hepatocytes. *Can. J. Biochem.* **56:** 780–783.
25. Percy-Robb, I. W., and G. S. Boyd. 1970. The synthesis of bile acids in perfused rat liver subjected to chronic biliary drainage. *Biochem. J.* **118:** 519–530.
26. Ogura, M., M. Goto, and Y. Ayaki. 1978. Formation of bile acids in haemoglobin-free perfused rat livers. *J. Biochem. (Tokyo).* **83:** 527–535.
27. Fears, R. B., and B. Morgan. 1976. Studies on the response of cholesterol biogenesis to feeding in rats. Evidence against the existence of diurnal rhythms. *Biochem. J.* **158:** 53–60.
28. Angelin, B., I. Björkhem, and K. Einarsson. 1981. Influence of bile acids on the soluble phosphatidic acid phosphatase in rat liver. *Biochem. Biophys. Res. Commun.* **100:** 606–612.