Biliary cholesterol secretion and bile acid formation in the hamster: the role of newly synthesized cholesterol[1]

Jürgen Scheibner, Michael Fuchs, Erwin Hörmann, Gisela Tauber, and Eduard F. Stange[2]

Department of Internal Medicine, Medical University of Ulm, Ulm, and Department of Internal Medicine, Medical University of Lübeck, Lübeck, Germany

Abstract In order to define the source of cholesterol for bile acid synthesis and biliary cholesterol, hamsters with an extracorporeal bile duct received an intraperitoneal bolus of [^{3}H]water labeling newly synthesized cholesterol. Thereafter the enterohepatic circulation was interrupted and a nutrient solution was infused during the experimental period of 78 h. In a separate group, pravastatin was administered (54–78 h) to allow discrimination of ^{3}H-labeled cholesterol recycling from plasma and newly synthesized hepatic cholesterol late during the experiment. In controls, newly synthesized biliary cholesterol and primary bile acids derived from cholesterol newly synthesized during the experiment amounted to 5% and 12% immediately after depletion of the bile acid pool (6–9 h), respectively. After longterm bile diversion these proportions increased to 56–63%, whereas 71% of plasma cholesterol was labeled. Pravastatin inhibited the secretion of biliary cholesterol, cholate, and chenodeoxycholate by 30, 50, and 44%, respectively. In contrast, the preinfusion tritium label was suppressed by a maximum of 16%, 14%, and 26%, respectively, reflecting the contribution of cholesterol newly synthesized in the hepatocyte as opposed to labeled cholesterol recycling from the plasma. It is concluded that in the hamster newly synthesized cholesterol is of minor importance as substrate for bile acid synthesis as well as biliary cholesterol, both under near physiologic conditions and after long-term bile diversion. Moreover, the hepatic cholesterol pools subserving the synthesis of the primary bile acids are identical but appear to be different from that of biliary cholesterol directly after the depletion of the enterohepatic bile acids.—**Scheibner, J., M. Fuchs, E. Hörmann, G. Tauber, and E. F. Stange.** Biliary cholesterol secretion and bile acid formation in the hamster: the role of newly synthesized cholesterol. *J. Lipid Res.* 1994. **35:** 690–697.

Supplementary key words bile acid synthesis • bile fistula • biliary cholesterol • pravastatin • HMG-CoA reductase inhibitor

Ample evidence suggests a close regulatory linkage between hepatic cholesterol and bile acid synthesis (1–9). In addition, several in vivo studies have investigated the role of newly synthesized and preformed cholesterol for biliary cholesterol and bile acids in rats more directly. Using tritiated water (10–13), [$^{18}O_2$]inhalation (14), or inhibitors of cholesterol synthesis (15–20) this proportion was estimated to be 5–28% for biliary cholesterol and 25–80% for bile acids. It is still under debate whether cholesterol and bile acids derive from the same (21, 22) or from separate cholesterol pools in the rat (10, 23–27).

Although the rat has been used extensively for the investigation of cholesterol and bile acid metabolism, the hamster seems to be a more adequate model. As a consequence of the relatively low capacity of hepatic cholesterol synthesis, variations in cholesterol balance are regulated by the modulation of LDL transport in hamsters (28–32) much like in humans. Moreover, excess dietary cholesterol intake results in an increase of biliary cholesterol output (33). Finally, the hamster has a gallbladder and displays a bile acid pattern (34) similar to humans. Therefore hamsters may be used as a model for the pathogenesis of cholesterol gallstones (35–38).

However, there is no information on the quantitative importance of newly formed cholesterol as a substrate for bile acid synthesis in the hamster and only few data are available concerning the origin of cholesterol for biliary secretion in this species. In an elegant study, Turley, Spady, and Dietschy (39) showed that the bile acid-binding resin cholestyramine increased the proportion of newly synthesized cholesterol in bile from 5% in controls to 21% but the total secretion rate remained unchanged.

We have recently developed a new model in the free-moving hamster with an extracorporeal bile duct allowing experiments in recovered animals and avoiding the postoperative stress period. The biliary lipid secretion in these animals was several-fold higher than reported previ-

Abbreviations: HPLC, high performance liquid chromatography; LDL, low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

[1]This paper is dedicated to Professor G. Paumgartner on the occasion of his 60th birthday.

[2]To whom correspondence should be addressed at: Department of Internal Medicine, Division of Gastroenterology, Medical University of Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Germany.

ously in animals with an acute bile fistula and probably reflects the physiologic rates (40). Using this model it could be shown that newly synthesized cholesterol plays a minor role as a source of both biliary cholesterol and bile acid synthesis, not only directly after the depletion of the bile acid pool but also after derepression of bile acid synthesis.

MATERIALS AND METHODS

Materials

[24-^{14}C]taurocholic acid (57.7 mCi/mmol), [^{3}H]water (100 mCi/ml), and [4-^{14}C]cholesterol (59 mCi/mmol) were obtained from Amersham Corp. (Braunschweig, Germany). Pravastatin sodium was a gift from Bristol-Myers Squibb GmbH (Regensburg, Germany). All chemicals used were of the highest grade available commercially.

Animals and diet

Female Golden Syrian hamsters (*Mesocriteus aureatus*) in the weight range of 110 to 130 g were purchased from the Zentralinstitut für Versuchstierzucht, Hannover, Germany. The animals were kept under light-cycling (light: 6:00 AM–6:00 PM) conditions and fed a pelleted standard diet (Altromin 1314, Altromin, Lage, Germany) for an adjustment period of at least 1 week prior to surgery. The experimental protocol had been approved by an official veterinary commission and complied with the national guidelines for the care and use of laboratory animals.

Surgical procedures and experimental setup

Under fluothane anesthesia (ICI Pharma, Plankstadt, Germany) an extracorporeal bile duct was established as described in detail previously (40). After a postoperative recovery period of 1 week, the loop was interrupted and both ends were connected to polyethylene tubing. The infusate was administered intraduodenally and hepatic bile was collected in 3-h intervals using a fraction collector (Isco-Retriever II, Colora, Lorch, Germany).

After an intraperitoneal injection of 100 mCi [^{3}H]water, an intraduodenal infusion was given to a control group (n = 11) for 78 h at a rate of 1 ml per h (Precision pump Unita Ib; B. Braun, Melsungen, Germany). It contained nutrients and minerals as described previously (41) and 0.09 μCi [24-^{14}C]taurocholic acid per 100 g per h as well as tritiated water at the specific activity of body water. Another group of animals (n = 8) was supplied with the same solution, but received in addition 1 mg pravastatin sodium per 100 g per h during the final 24 h of the experiment. At the end of the infusion the animals were bled by puncture of the aorta to obtain EDTA-plasma for measurement of plasma cholesterol. Glutamate dehydrogenase, alanine aminotransferase, and alkaline phosphatase activity were estimated using commercial kits (Boehringer, Mannheim, Germany).

Analytical procedures

Bile flow was measured gravimetrically. To estimate the specific activity of body water, 50 μl of every bile fraction was diluted and counted by liquid scintillation (Rackbeta 1217, LKB, Freiburg, Germany).

Four hundred fifty μl of bile was evaporated under vacuum to remove the tritiated water from the hydrate shell of biliary cholesterol and bile acids, as described previously (10). Thereafter 14 nCi of [4-^{14}C]cholesterol was added to 100 μl of resuspended bile to calculate the recovery of biliary cholesterol. Using the method of Tietz et al. (42), bile acids and biliary cholesterol were extracted by isopropanol. The evaporated sample was extracted with methanol–petroleum ether to separate the bile acids from biliary cholesterol.

The incorporated ^{3}H from tritiated water and the absorbed ^{14}C from taurocholate were measured in the unconjugated bile acids by HPLC with online liquid scintillation spectrometry (Ramona-6-LS, RSM, Straubenhardt, Germany) after the preparation of the methanol extract, as described previously (10). The ^{14}C activity was estimated in 25 μl of resuspended bile by liquid scintillation.

The amount of incorporated ^{3}H and added ^{14}C of biliary cholesterol was quantitated by the preparation of the petroleum ether extract followed by HPLC separation as described previously (40). The plasma cholesterol was analyzed as described (10). The conjugated bile acids were measured in 100 μl of the resuspended bile by HPLC (43).

Calculations and definitions

The total synthesis rates of bile acids were calculated from the molar mass of conjugated bile acids. After the depletion of the bile acid pool, the synthesis rates of bile acids and biliary cholesterol corresponded to their secretion rates.

The amount of newly synthesized ("de novo") biliary and plasma cholesterol as well as of bile acids derived from newly synthesized cholesterol was calculated from the tritium label as detailed recently (10, 11, 13, 44). The calculation was based upon the actual specific activity of tritiated water in each bile fraction. The newly synthesized cholesterol and bile acids formed from newly synthesized cholesterol during the experiment may have included labeled cholesterol recycling from the plasma as well as from direct hepatic synthesis. The infusion experiment with pravastatin was performed to allow discrimination between these two pathways.

The synthesis from unlabeled cholesterol was calculated from the difference of total and newly synthesized rates and was designated as synthesis from preformed cholesterol.

The results are given in mean values ± 1 SEM. The two-tailed Student's *t* test for unpaired or paired samples, as applicable, was used to assess statistical significance.

RESULTS

Food intake and body weight were similar in both treatment groups and did not change significantly during the experiments (data not shown).

Control experiment

As shown in **Fig. 1A,** the specific activity of bile water rose rapidly after intraperitoneal injection of [^{3}H]water. The bile flow ranged from 218 to 281 μl per h (Fig. 1B) and showed a minimal diurnal rhythm similar to that of bile acid secretion.

The total synthesis of cholate (**Fig. 2A**) derepressed approximately twofold from a basal rate of 818 nmol per 100 g per h with a clearcut diurnal rhythm. The synthesis of cholate from newly synthesized cholesterol increased continuously with a parallel diurnal rhythm. In contrast, the cholate synthesis from preformed cholesterol (Fig. 2B) decreased gradually, also with a flat diurnal rhythm. Following pool depletion after 9 h, the proportion of cholate from newly formed cholesterol (Fig. 2C) amounted to 12% and increased continuously during derepression to approximately 63% after 75–78 h.

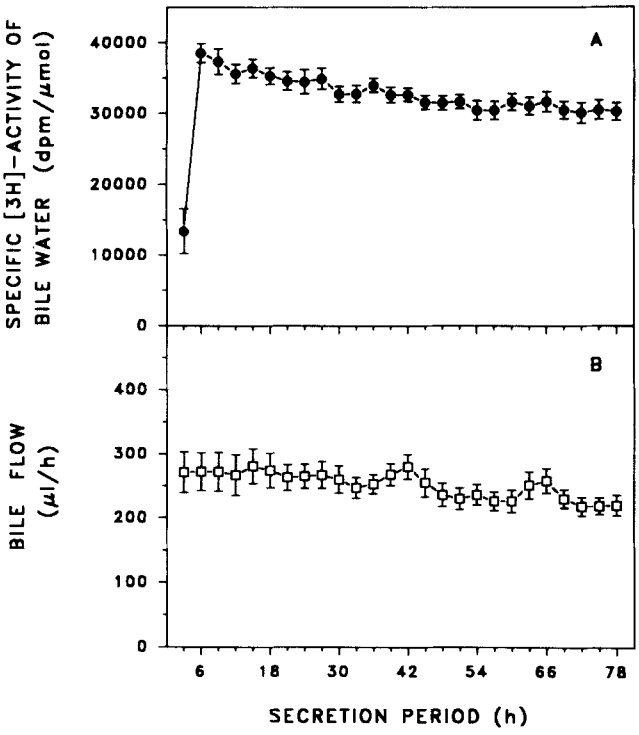

Fig. 1. Specific ^{3}H activity of bile water (panel A) and bile flow (panel B) of female hamsters. Hamsters recovered for 7 days from the operation and were given an intraperitoneal bolus of 100 mCi [^{3}H]water. Thereafter, the enterohepatic circulation was interrupted and an intraduodenal infusion of 0.09 μCi [^{14}C]taurocholate per 100 g per h and tritiated water at the calculated specific activity of body water was administered for 78 h. The secreted bile was collected during 3-h intervals. Values represent the means ± 1 SEM of 11 animals.

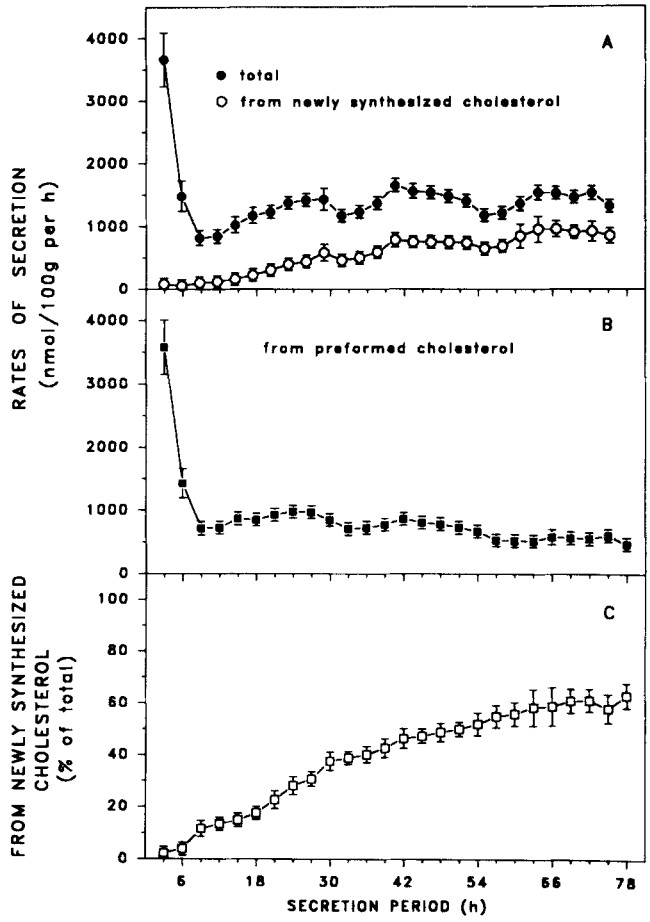

Fig. 2. Cholate secretion of hamsters after interruption of enterohepatic circulation. Panel A: From total (preformed plus newly synthesized) cholesterol and from newly synthesized cholesterol. Panel B: From preformed cholesterol. Panel C: Relative proportions from newly synthesized cholesterol. Experimental setup was as described in the legend of Fig. 1. Values represent the means ± 1 SEM of 11 animals.

The total synthesis of chenodeoxycholate (**Fig. 3A**) increased from 185 to a maximum of 274 nmol per 100 g per h after 66 h. Similar to cholate, chenodeoxycholate synthesis from newly synthesized cholesterol (Fig. 3A) increased continuously with a flat diurnal rhythm. After the depletion of the bile acid pool, the chenodeoxycholate synthesis from preformed cholesterol (Fig. 3B) decreased slightly, again with a minimal diurnal variation. The proportion of chenodeoxycholate from newly synthesized cholesterol (Fig. 3C) amounted to 12% at 9 h reaching a plateau of 56% after 54 h.

In contrast to cholate and chenodeoxycholate, biliary cholesterol (**Fig. 4A**) was secreted with a pronounced diurnal variation during the derepression of bile acid synthesis, increasing maximally 3.5-fold. The secretion rate of "de novo" cholesterol exhibited an early plateau up to 30 h and increased with a diurnal rhythm thereafter. The amount of preformed cholesterol remained unchanged (Fig. 4B) with diurnal variation during the full ex-

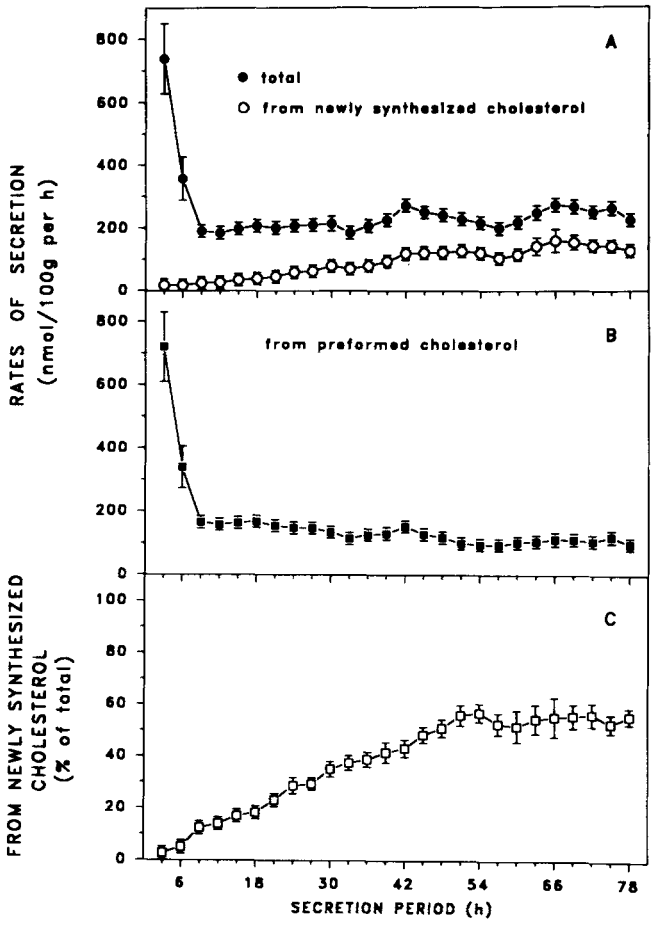

Fig. 3. Chenodeoxycholate secretion of hamsters after interruption of enterohepatic circulation. Panel A: From total (preformed plus newly synthesized) cholesterol and from newly synthesized cholesterol. Panel B: From preformed cholesterol. Panel C: Relative proportions from newly synthesized cholesterol. Experimental setup was as described in the legend of Fig. 1. Values represent the means ± 1 SEM of 11 animals.

perimental period. The proportion of "de novo" cholesterol (Fig. 4C) varied from 5% (6–9 h) to a plateau reaching approximately 60% after 60 h.

Taken together, biliary cholesterol was derived from "de novo" cholesterol to a significantly lower extent compared to cholate and chenodeoxycholate ($P < 0.05$ and $P < 0.01$, respectively) only during an early phase of the experiment. At the end of the experiment 71 ± 6% of plasma cholesterol was newly synthesized, which was only slightly different from the tritium label of cholate, chenodeoxycholate, and biliary cholesterol after 75–78 h. The total plasma cholesterol level amounted to 3.2 ± 0.2 μmol per ml at this time point.

Pravastatin experiment

The bile flow during the pravastatin infusion (54–78 h) was not significantly different from that of controls (data not shown). Compared to the preinfusion period (51–54 h), pravastatin suppressed the total cholate synthesis as well as formation from "de novo" cholesterol by 50% ($P < 0.01$) and 54% ($P < 0.01$) after 63 h (**Fig. 5A**), respectively. Thus, the rate of cholate synthesis from preformed cholesterol also decreased similarly (from 896 to 477 nmol per 100 g per h, $P < 0.05$). Before starting the pravastatin infusion, 51% of cholate was synthesized from "de novo" cholesterol; after 63–66 h this proportion was maximally reduced by 14% ($P > 0.05$).

Compared to the reference value at 54 h, the chenodeoxycholate synthesis rates from total and "de novo" cholesterol were reduced by 44% ($P < 0.01$) and 54% ($P < 0.01$) after 63 h, respectively (Fig. 5B). Accordingly, the synthesis from preformed cholesterol was similarly suppressed (167 to 92 nmol per 100 g per h) after 63 h ($P < 0.01$). Immediately prior to the pravastatin infusion (51–54 h), the relative synthesis from "de novo" cholesterol amounted to 35%. With the onset of the pravastatin infusion, the proportion from "de novo" cholesterol decreased maximally by 26% ($P < 0.01$) after 66 h.

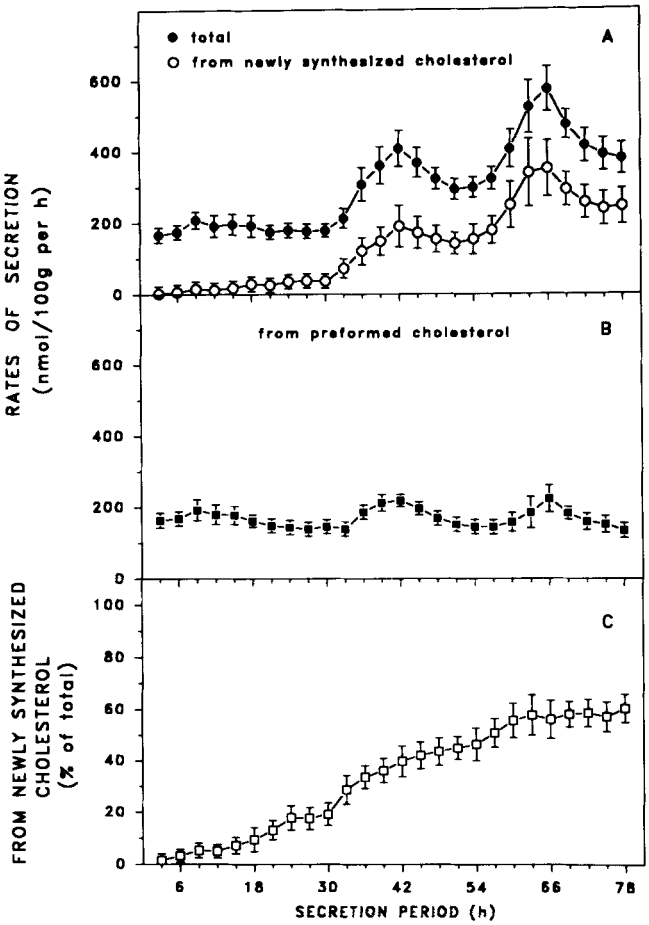

Fig. 4. Biliary cholesterol secretion of hamsters after interruption of enterohepatic circulation. Panel A: Total (preformed plus newly synthesized) cholesterol and newly synthesized cholesterol. Panel B: Preformed cholesterol. Panel C: Relative proportions of newly synthesized cholesterol. Experimental setup was as described in the legend of Fig. 1. Values represent the means ± 1 SEM of 11 animals.

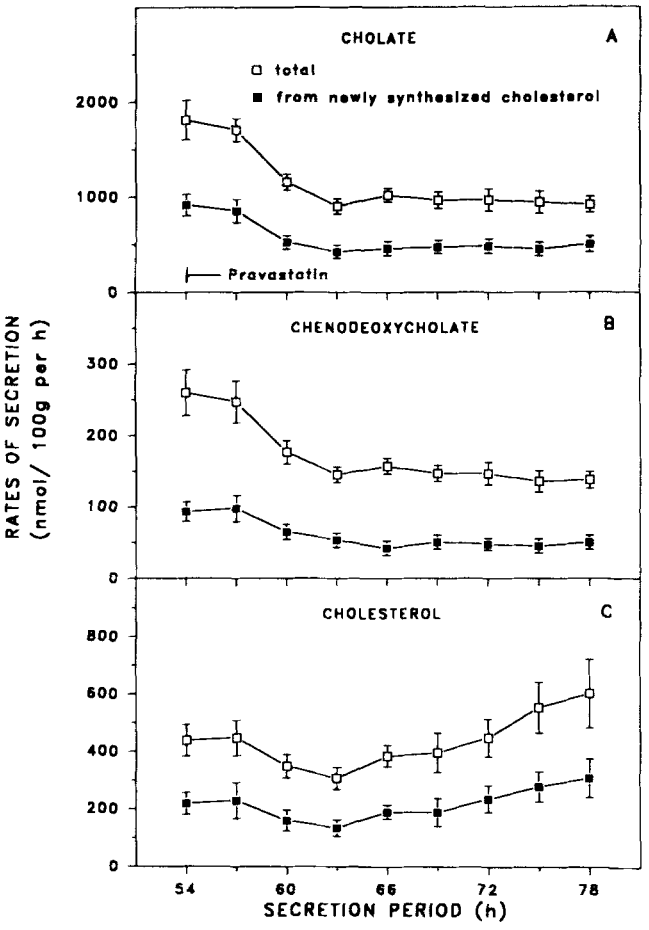

Fig. 5. Secretion of cholate (A), chenodeoxycholate (B) and biliary cholesterol (C) of hamsters before and during pravastatin infusion. Experimental setup was as described in the legend of Fig. 1. In addition, the animals received 1 mg of pravastatin/100 g per h during the last 24 h. Values represent the means ± 1 SEM of 8 animals.

The biliary secretion of total and "de novo" cholesterol decreased by 30% ($P < 0.05$) and 39% ($P < 0.05$) after 63 h, respectively, compared to the 54-h bile fraction of the pravastatin group (Fig. 5C). In contrast to bile acids, pravastatin reduced the secretion of preformed cholesterol only slightly (218 to 173 nmol per 100 g per h) ($P < 0.01$) at 60–63 h. In the pravastatin experiment the relative amount of "de novo" cholesterol secreted decreased from 49% (51–54 h) by 16% to a nadir at 60–63 h ($P > 0.05$).

The infusion of pravastatin reduced the proportion of "de novo" plasma cholesterol to 50 ± 2% ($P < 0.01$ compared to controls), corresponding to the ^{3}H label of cholate and biliary cholesterol at the end of the experiment. The total cholesterol concentration was reduced to 2.6 ± 0.2 μmol per ml plasma.

The liver enzymes glutamate dehydrogenase, alanine aminotransferase, and alkaline phosphatase were in the range of unoperated animals in both groups (data not shown). The control and pravastatin-treated hamsters secreted 103% and 110% of the intraduodenally infused [^{14}C]taurocholate in bile (54–78 h), respectively, suggesting that pravastatin had no effect on intestinal absorption and hepatic secretion of the bile acids.

DISCUSSION

In the past hamsters were used as a suitable model for the investigation of the pathogenesis of pigment and cholesterol gallstones (35–38). In this context, some relevant data are available about the role of hepatic cholesterogenesis in the secretion of cholesterol in hamster bile (39). In contrast, nothing is known in the hamster about the relative importance of newly synthesized versus preformed cholesterol for bile acid synthesis under physiologic conditions or after stimulation of the biosynthesis of bile acids by long term bile depletion. To clarify this point, female hamsters were fitted with an extracorporeal bile duct (40) and bile was diverted for 78 h. The proportions from newly synthesized and preformed cholesterol were defined for biliary cholesterol, cholate, and chenodeoxycholate by labeling "de novo" cholesterol with tritiated water (10, 13, 45–47). The HMG-CoA reductase inhibitor pravastatin was infused after derepression of bile acid synthesis to allow differentiation of the labeled proportion derived directly from hepatocytic cholesterogenesis versus recycling lipoprotein [^{3}H]cholesterol.

In the present study the bile acid pool was secreted within 9 h in controls, and immediately thereafter an approximation of the physiologic rate of bile acid synthesis may be obtained (40, 41, 48). At this time point only 12% of cholate and chenodeoxycholate derived from "de novo" cholesterol. After a comparable short-term depletion of bile, male (10) and female rats (13) synthesized similar proportions of these bile acids from tritium-labeled "de novo" cholesterol (4–19%). This low contribution of cholesterol synthesis may appear to contrast with the coordinated regulation of cholesterol and bile acid synthesis observed under various conditions in the rat (1–9). On the other hand, during cholesterol feeding, both synthesis rates clearly dissociate (49) and their usual link does not necessarily imply a quantitative role of "de novo" cholesterol in bile acid formation.

In contrast to both primary bile acids, only 5% of cholesterol secreted into bile was newly synthesized in the present study. This is in good agreement with data published by Turley et al. (39), also using female hamsters and a similar tritiated water method. In female rats, 18% of biliary cholesterol was labeled with [^{3}H]water (11), whereas in male animals only 8–9% of biliary cholesterol was newly synthesized (10, 39). A somewhat higher proportion (28%) of newly synthesized cholesterol was found in male rats by Long et al. (20) by inhibiting the terminal cholesterogenesis with triparanol. Thus, much

like in the rat (10), in the hamster de novo cholesterol appears to play only a minor role as a substrate for bile acid synthesis and biliary cholesterol secretion directly after depletion of the bile acid pool, i.e., under rather physiologic conditions. Furthermore, it is concluded that cholate and chenodeoxycholate are synthesized from a common or similar cholesterol precursor pool in the hamster, whereas the biliary secretion of "de novo" cholesterol probably derives from a different cholesterol pool. This is supported by the retarded secretion of the ^{3}H-label of cholesterol until 30 h, compared to the bile acids. A common precursor pool was also reported for the primary bile acids of humans (50) and the rat (21, 22), whereas we and others found separate pools in the rat (10, 23–27).

After bile diversion for 78 h, bile acids and biliary cholesterol were labeled to approximately 60% in control hamsters. In comparison, 64% of cholate was labeled in the rat, but 84–88% of chenodeoxycholate, muricholate, and biliary cholesterol (10). Interestingly, in hamsters the flux of tritium-labeled cholesterol decreased in cholate and biliary cholesterol maximally by 15% and in chenodeoxycholate by 26% only during a short period of 6–9 h after the start of the pravastatin infusion. This rapid effect may be assumed to reflect the limited amount formed directly from newly synthesized cholesterol in the liver because under these conditions plasma labeling remains high and the small intestine in the absence of luminal fat does not secrete newly formed cholesterol, at least in the rat (51). Thus, after long-term exposure to tritiated water, labeled cholesterol and bile acids are mostly derived from labeled plasma lipoproteins and, to a limited extent, from hepatic synthesis.

The hamster is known to compensate for its low cholesterol synthesis (52) by the up-regulation of the hepatic LDL uptake (30). As a consequence, the slight increase of the ^{3}H-label at the end of the pravastatin administration may be explained by the enhanced uptake of tritium-labeled LDL-cholesterol, whereas the metabolism of intrahepatic preformed, unlabeled cholesterol probably remained unchanged. Alternatively, hepatic HMG-CoA reductase may have escaped the inhibitory action of pravastatin by an increase in enzyme synthesis (53). The dose of pravastatin used prevented the normal further increase in the labeling of biliary lipids between 54 and 78 h and may therefore be assumed to have blocked cholesterol synthesis efficiently. This is also supported by the observed reduction of plasma cholesterol and by a 71% inhibition of hepatic sterol synthesis by a lower single oral dose of 1.33 mg pravastatin per kg body weight of rats (54).

As shown by others in the rat (15, 17, 18), the application of a HMG-CoA reductase inhibitor resulted in a suppression of bile acid synthesis and biliary cholesterol secretion. The inhibition of 44–50% seen for bile acids in the hamster is comparable to data of Muraca et al. (16) in humans. At this point it is remarkable that not only substrate but also the enzyme mass (55) and mRNA levels (56, 57) of cholesterol 7α-hydroxylase were reduced by statins in the rat. As a consequence, it is not feasible to derive a dominant role of newly synthesized cholesterol for bile acid synthesis merely from the suppressive effect of an HMG-CoA reductase inhibitor (17) because a part of its action is due to direct inhibition of 7α-hydroxylase rather than HMG-CoA reductase.

It may be concluded that bile acids and biliary cholesterol derive preferentially from preformed cholesterol pools after short-term bile depletion which may be assumed to be close to the physiological condition. This contribution does not dramatically increase after long-term interruption of the enterohepatic circulation probably because, in the hamster, bile acid synthesis does not derepress as much as in the rat. Furthermore, a common cholesterol precursor pool for the primary bile acids that is distinct from that of biliary cholesterol is suggested.

The careful technical assistance of Mrs. C. Engstler, Mrs. E. Preiss, and Mrs. R. Barnikel is gratefully acknowledged. This study was supported by the Deutsche Forschungsgemeinschaft (Sta 172/3-5).

Manuscript received 16 June 1993 and in revised form 11 November 1993.

REFERENCES

1. Danielsson, H. 1972. Relationship between diurnal variations in biosynthesis of cholesterol and bile acids. *Steroids.* **20:** 63–72.
2. Myant, N. B., and H. A. Eder. 1961. The effect of biliary drainage upon the synthesis of cholesterol in the liver. *J. Lipid Res.* **2:** 363–368.
3. Boyd, G. S., N. A. Scholan, and J. R. Mitton. 1989. Factors influencing cholesterol 7α-hydroxylase activity in the rat liver. *Adv. Exp. Med. Biol.* **4:** 443–456.
4. Myant, N. B., and K. A. Mitropoulos. 1977. Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18:** 135–153.
5. Björkhem, I. 1986. Effects of mevinolin in rat liver: evidence for a lack of coupling between synthesis of hydroxymethylglutaryl-CoA reductase and cholesterol 7α-hydroxylase activity. *Biochim. Biophys. Acta.* **877:** 43–49.
6. Shefer, S., F. G. Zaki, and G. Salen. 1983. Early morphologic and enzymatic changes in livers of rats treated with chenodeoxycholic and ursodeoxycholic acids. *Hepatology.* **3:** 201–208.
7. Hamprecht, B., R. Roscher, G. Waltinger, and C. Nüssler. 1971. Influence of bile acids on the activity of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Eur. J. Biochem.* **18:** 15–19.
8. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1973. Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol-7α-hydroxylase in the rat. *J. Lipid Res.* **14:** 573–580.
9. Danielsson, H., and G. Johansson. 1974. Effects of long-term feeding of chenodeoxycholic acid on biosynthesis and metabolism of bile acids in the rat. *Gastroenterology.* **67:** 126–134.

10. Scheibner, J., M. Fuchs, M. Schiemann, G. Tauber, E. Hörmann, and E. F. Stange. 1993. Bile acid synthesis from newly synthesized vs. preformed cholesterol precursor pools in the rat. *Hepatology.* **17:** 1095–1102.
11. Turley, S. D., and J. M. Dietschy. 1981. The contribution of newly synthesized cholesterol to biliary cholesterol in the rat. *J. Biol. Chem.* **256:** 2438–2446.
12. Robins, S. J., and H. Brunengraber. 1982. Origin of biliary cholesterol and lecithin in the rat: contribution of new synthesis and preformed hepatic stores. *J. Lipid Res.* **23:** 604–608.
13. Stange, E. F., D. K. Spady, and J. M. Dietschy. 1985. Characterization of the cholesterol precursor pools used for bile acid synthesis in the rat. *In* Enterohepatic Circulation of Bile Acids and Sterol Metabolism. Falk Symposium 42. G. Paumgartner, A. Stiehl and W. Gerok, editors. MTP Press Ltd., Lancaster, UK. 29–36.
14. Björkhem, I., and A. Lewenhaupt. 1979. Preferential utilization of newly synthesized cholesterol as substrate for bile acid biosynthesis. *J. Biol. Chem.* **254:** 5252–5256.
15. Pandak, W. M., D. M. Heuman, P. B. Hylemon, and Z. R. Vlahcevic. 1990. Regulation of bile acid synthesis. IV. Interrelationship between cholesterol and bile acid biosynthesis pathways. *J. Lipid Res.* **31:** 79–90.
16. Muraca, M., G. Baggio, L. Miconi, M. T. Vilei, S. Martini, C. Gabelli, C. Belluco, M. Lise, and G. Crepaldi. 1991. Acute effects of HMG-CoA reductase inhibitors on biliary lipids in patients with interrupted enterohepatic circulation. *Eur. J. Clin. Invest.* **21:** 204–208.
17. Kempen, H. J. M., J. De Lange, M. P. M. Vos-Van Holstein, P. van Wachem, R. Havinga, and R. J. Vonk. 1984. Effect of ML-236B (Compactin) on biliary excretion of bile salts and lipids, and on bile flow, in the rat. *Biochim. Biophys. Acta.* **794:** 435–443.
18. Davis, R. A., C. A. Musso, M. Malone-McNeal, G. R. Lattier, P. M. Hyde, J. Archambault-Schexnayder, and M. Straka. 1988. Examination of bile acid negative feedback regulation in rats. *J. Lipid Res.* **29:** 202–211.
19. Pandak, W. M., Z. R. Vlahcevic, D. M. Heuman, and P. B. Hylemon. 1990. Regulation of bile acid synthesis. V. Inhibition of conversion of 7-dehydrocholesterol to cholesterol is associated with down-regulation of cholesterol 7α-hydroxylase activity and inhibition of bile acid synthesis. *J. Lipid Res.* **31:** 2149–2158.
20. Long, T. T., L. Jakoi, R. Stevens, and S. Quarfordt. 1978. The sources of rat biliary cholesterol and bile acid. *J. Lipid Res.* **19:** 872–878.
21. Ogura, M., J. Shiga, and K. Yamasaki. 1971. Studies on the cholesterol pool as the precursor of bile acids in the rat. *J. Biochem.* **70:** 967–972.
22. Vlahcevic, Z. R., T. Cronholm, T. Curstedt, and J. Sjövall. 1980. Biosynthesis of 5α- and 5β-cholanoic acid derivatives during metabolism of [1,1-^{2}H]- and [2,2,2-^{2}H]ethanol in the rat. *Biochim. Biophys. Acta.* **618:** 369–377.
23. Mitropoulos, K. A., N. B. Myant, G. F. Gibbons, S. Balasubramaniam, and B. E. A. Reeves. 1974. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. *J. Biol. Chem.* **249:** 6052–6056.
24. Staple, E., and S. Gurin. 1954. The incorporation of radioactive acetate into biliary cholesterol and cholic acid. *Biochim. Biophys. Acta.* **15:** 372–376.
25. Normann, T., and K. R. Norum. 1976. Newly synthesized hepatic cholesterol as precursor for cholesterol and bile acids in rat bile. *Scand. J. Gastroenterol.* **11:** 427–432.
26. Ayaki, Y., Y. Ogura, S. Kitayama, S. Endo, and M. Ogura. 1983. Difference between cholic acid and chenodeoxycholic acid in dependence upon cholesterol of hepatic and plasmatic sources as the precursor in rats. *Steroids.* **41:** 509–521.
27. Ayaki, Y., T. Tsuma-Date, S. Endo, and M. Ogura. 1981. Role of endogenous and exogenous cholesterol in liver as the precursor for bile acids in rats. *Steroids.* **38:** 495–509.
28. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
29. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
30. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
31. Spady, D. K., E. F. Stange, L. E. Bilhartz, and J. M. Dietschy. 1986. Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver. *Proc. Natl. Acad. Sci. USA.* **83:** 1–5
32. Malavolti, M., H. Fromm, S. Ceryak, and I. M. Roberts. 1987. Modulation of low density lipoprotein receptor activity by bile acids: differential effects of chenodeoxycholic and ursodeoxycholic acids in the hamster. *J. Lipid Res.* **28:** 1281–1295.
33. Kajiyama, G., S. Kubota, H. Sasaki, T. Kawamoto, and A. Miyoshi. 1981. Lipid metabolism in the development of cholesterol gallstones in hamsters. The effect of dietary cholesterol on its biliary concentration and lithogenicity. *Hiroshima J. Med. Sci.* **30:** 141–143.
34. Kuroki, S., S. Muramoto, T. Kuramoto, and T. Hoshita. 1983. Sex differences in gallbladder bile acid composition and hepatic steroid 12α-hydroxylase activity in hamsters. *J. Lipid Res.* **24:** 1543–1549.
35. Cohen, B. I., T. Setoguchi, E. H. Mosbach, C. K. McSherry, R. J. Stenger, S. Kuroki, and R. D. Soloway. 1987. An animal model of pigment cholelithiasis. *Am. J. Surg.* **153:** 130–138.
36. Pearlman, B. J., G. G. Bonorris, M. J. Phillips, A. Chung, S. Vimadalal, J. M. Marks, and L. J. Schoenfield. 1979. Cholesterol gallstone formation and prevention by chenodeoxycholic and ursodeoxycholic acids. *Gastroenterology.* **77:** 634–641.
37. Dam, H., and F. Christensen. 1952. Alimentary production of gallstones in hamsters. *Acta Pathol. Microbiol. Scand.* **30:** 236–242.
38. Handelsman, B., G. Bonorris, J. W. Marks, and L. J. Schoenfield. 1982. Enrichment of bile with tauroursodeoxycholic acid and biliary cholesterol saturation in hamsters. *Am. J. Physiol.* **243:** G424–G427.
39. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1983. Alteration of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed and newly synthesized cholesterol. *Gastroenterology.* **84:** 253–264.
40. Fuchs, M., J. Scheibner, E. Hörmann, G. Tauber, and E. F. Stange. 1992. Enterohepatic circulation in hamsters with an extracorporeal bile duct. *J. Lipid Res.* **33:** 1383–1392.
41. Stange, E. F., J. Scheibner, C. Lutz, and H. Ditschuneit. 1988. Feedback regulation of bile acid synthesis in the rat by dietary vs. intravenous cholate or taurocholate. *Hepatology.* **8:** 879–886.
42. Tietz, P. S., J. L. Thistle, L. J. Miller, and N. F. LaRusso. 1984. Development and validation of a method for measur-

ing the glycine and taurine conjugates of bile acids in bile by high-performance liquid chromatography. *J. Chromatogr.* **336:** 249–257.
43. Swobodnik, W., U. Klüppelberg, J. G. Wechsler, M. Volz, G. Normandin, and H. Ditschuneit. 1985. Rapid and accurate reversed-phase high-performance liquid chromatographic determination of conjugated bile acids in human bile for routine clinical applications. *J. Chromatogr.* **339:** 263–271.
44. Andersen, J. M., and J. M. Dietschy. 1979. Absolute rates of cholesterol synthesis in extrahepatic tissues measured with ^{3}H-labeled water and ^{14}C-labeled substrates. *J. Lipid Res.* **20:** 740–752.
45. Kempen, H. J., M. Vos-van Holstein, and J. de Lange. 1983. Bile acids and lipids in isolated rat hepatocytes. II. Source of cholesterol used for bile acid formation, estimated by incorporation of tritium from tritiated water, and by the effect of ML-236B. *J. Lipid Res.* **24:** 316–323.
46. Lakshmanan, M. R., and R. L. Veech. 1977. Measurement of rate of rat liver sterol synthesis in vivo using tritiated water. *J. Biol. Chem.* **252:** 4667–4673.
47. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
48. Eriksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. *Proc. Soc. Exp. Biol. Med.* **94:** 578–582.
49. Shefer, S., L. B. Nguyen, G. Salen, G. C. Ness, I. R. Chowdhary, S. Lerner, A. K. Batta, and G. S. Tint. 1992. Differing effects of cholesterol and taurocholate on steady state hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels in the rat. *J. Lipid Res.* **33:** 1193–1200.
50. Schwartz, C. C., Z. R. Vlahcevic, L. G. Halloran, R. Nisman, and L. Swell. 1977. Evidence for a common hepatic cholesterol precursor site for cholic and chenodeoxycholic acid synthesis in man. *Proc. Soc. Exp. Biol. Med.* **156:** 261–264.
51. Stange, E. F., and J. M. Dietschy. 1985. The origin of cholesterol in the mesenteric lymph of the rat. *J. Lipid Res.* **26:** 175–184.
52. Turley, S. D., and J. M. Dietschy. 1988. The metabolism and excretion of cholesterol by the liver. *In* The Liver: Biology and Pathobiology. I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter and D. A. Shafritz, editors. Raven Press Ltd., New York. 617–641.
53. Ma, P. T. S., G. Gil, T. C. Südhof, D. W. Bilheimer, J. L. Goldstein, and M. S. Brown. 1986. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. *Proc. Natl. Acad. Sci. USA.* **83:** 8370–8374.
54. Bocan, T. M. A., E. Ferguson, W. McNally, P. D. Uhlendorf, S. B. Mueller, P. Dehart, D. R. Sliskovic, B. D. Roth, B. R. Krause, and R. S. Newton. 1992. Hepatic and nonhepatic sterol synthesis and tissue distribution following administration of a liver selective HMG-CoA reductase inhibitor, CI-981: comparison with selected HMG-CoA reductase inhibitors. *Biochim. Biophys. Acta.* **1123:** 133–144.
55. Nguyen, L. B., S. Shefer, G. Salen, G. Ness, R. D. Tanaka, V. Packin, P. Thomas, V. Shore, and A. Batta. 1990. Purification of cholesterol 7α-hydroxylase from human and rat liver and production of inhibiting polyclonal antibodies. *J. Biol. Chem.* **265:** 4541–4546.
56. Jones, M. P., W. M. Pandak, D. M. Heuman, J. Y. L. Chiang, P. B. Hylemon, and Z. R. Vlahcevic. 1993. Cholesterol 7α-hydroxylase: evidence for transcriptional regulation by cholesterol or metabolic products of cholesterol in the rat. *J. Lipid Res.* **34:** 885–892.
57. Pandak, W. M., Z. R. Vlahcevic, J. Y. L. Chiang, D. M. Heuman, and P. B. Hylemon. 1992. Bile acid synthesis. VI. Regulation of cholesterol 7α-hydroxylase by taurocholate and mevalonate. *J. Lipid Res.* **33:** 659–668.