Studies on the regulation of cholesterol 7α-hydroxylase and HMG-CoA reductase in rat liver: effects of lymphatic drainage and ligation of the lymph duct

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Abstract Lymphatic drainage leads to a significant stimulation of both the cholesterol 7α-hydroxylase and HMG-CoA reductase activity in rats (Björkhem et al. 1978. *Biochem. Biophys. Res. Commun.* **85:** (532–540). This finding was confirmed here and it was also shown that ligation of the lymph duct leads to a similar but less pronounced effect. Ligation of the lymph duct or lymph fistulation of bile duct-ligated or cholestyramine-treated rats did not further increase 7α-hydroxylase or the HMG-CoA reductase activity. However, treatment of lymph fistula rats with cholestyramine led to a significant further stimulation of both 7α-hydroxylase and HMG-CoA reductase activity. Intravenous infusion of lymph into bile fistula rats led to a significant inhibition of both cholesterol 7α-hydroxylase activity and HMG-CoA reductase activity. A corresponding infusion of cholesterol-enriched Intralipid led to inhibition of HMG-CoA reductase without effect on cholesterol 7α-hydroxylase activity. The results show that cholesterol 7α-hydroxylase is feedback-regulated by bile acids in a situation where the flux of cholesterol to the liver is interrupted also. The possibility is discussed that there is a factor in the lymph that down-regulates cholesterol 7α-hydroxylase. If such a factor exists, it requires an intact enterohepatic circulation for its effect. The stimulatory effect of cholestyramine on HMG-CoA reductase also in lymph fistula rats shows that the previously demonstrated suppressive effect of bile acids on HMG-CoA reductase is not only due to the effect of bile acids on intestinal absorption of cholesterol. The bile acid may suppress the HMG-CoA reductase either directly or indirectly by affecting cholesterol 7α-hydroxylase. —**Åkerlund, J-E., and I. Björkhem.** Studies on the regulation of cholesterol 7α-hydroxylase and HMG-CoA reductase in rat liver: effects of lymphatic drainage and ligation of the lymph duct. *J. Lipid Res.* 1990. **31:** 2159–2166.

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In previous work from this laboratory (1), it was shown that lymphatic drainage of rats on a cholesterol-free diet containing various triglyceride mixtures increased the activity of the rate-limiting enzyme in bile acid biosynthesis, cholesterol 7α-hydroxylase. This finding was later confirmed in rats on an ordinary cholesterol-free pellet diet (2, 3). The degree of stimulation was not related to the volume of the lymph flow, the magnitude of loss of triglycerides, cholesterol, and albumin through the lymph, or the nutritional status as judged from serum cholesterol, serum triglycerides, and serum albumin. The possibility was discussed that chylomicrons in the lymph may have a direct inhibitory effect on the 7α-hydroxylation of cholesterol.

That cholesterol-containing chylomicrons in the lymph are of regulatory importance for the activity of the hepatic HMG-CoA reductase is well documented, and a stimulatory effect of lymphatic drainage would thus be expected. Since cholesterol feeding leads to a stimulatory effect on cholesterol 7α-hydroxylase, most probably by induction of enzyme synthesis (4, 5), lymphatic drainage would rather be expected to decrease the activity of cholesterol 7α-hydroxylase.

In order to further study the possibility of a regulatory factor in the lymph, we have repeated the previous experiments with lymph fistula rats and also studied the effect of ligation of the lymph duct and infusion of lymph into bile fistula rats. In addition, we have combined lymph duct fistulation or lymph ligation with other treatments known to stimulate cholesterol 7α-hydroxylase and HMG-CoA reductase activity. It was believed that such studies would give useful information concerning the relative importance of bile acids and cholesterol for regulation of the above key enzymes in cholesterol synthesis and degradation.

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MATERIAL AND METHODS

[4-^{14}C]Cholesterol and [3-^{14}C]HMG-CoA with specific radioactivities of 55–60 mCi/mmol were obtained from NEN Research Products, Dreieich, FRG. The labeled cholesterol was purified by aluminum oxide chromatography immediately before use (6). 7α-[$^{2}H_3$]Hydroxycholesterol was prepared as described previously (6). Intralipid 10% was obtained from Kabi Vitrum (Stockolm, Sweden). To this Intralipid, additional 10% soybean oil was added, containing 50 mg of cholesterol per ml. The final concentration of cholesterol in the Intralipid was about 5 mg/ml (13 μmol/ml). The mixture was mixed with an Ultraturax and sonicated to produce a stable emulsion. It should be pointed out that Intralipid is an emulsion with particles less than 1 μm. The cholesterol added to the Intralipid should dissolve in these particles. All cofactors were obtained from Sigma Chemical (St. Louis, MO). All solvents used were of highest analytical grade.

Animals and animal treatments

Male rats (200–250 g) of Sprague-Dawley strain were used. With some exceptions (see Results) they were given free access to a commercial pellet diet containing less than 0.2% sterols (1). In some other experiments the rats were fed a corresponding diet as a powder containing 5% cholestyramine (Questran^{R}, Bristol-Myers) for 6 days prior to being killed. In some experiments the bile duct was cannulated when the rats were under ether anesthesia. The animals were then kept in restraining cages for 48 h prior to being killed. In other experiments the thoracic lymph duct was cannulated just proximal to the cisterna magnum through an abdominal approach (7, 8). The proximal part of the lymph duct was ligated. The animals were kept in restraining cages for 72 h while the lymph was drained. The corresponding control animals in each set of experiments were sham-operated and then treated in the same way. The rats with a biliary fistula or lymph fistula were given the ordinary pellet diet, but were given 0.6% (w/v) NaCl instead of water. In some experiments a central venous catheter was placed in the vena cava via the vena jugularis while the rats were under ether anesthesia. These animals were infused with Intralipid enriched with cholesterol (see above) or with saline (0.9% w/v) or with lymph for 48 h. The rate of infusion was 1 ml/h. In some experiments lymph fistula rats were fed cholestyramine in a powder diet for 3 days prior to cannulation of the lymph duct. The control group was then fed a diet without cholestyramine whereas the treatment group was fed cholestyramine, prior to the operation, for additional 4 days.

The animal operations were approved by the Local Ethical Committee for animal experiments.

Preparations of subcellular fractions

Homogenates of rat liver were prepared in 50 mM Tris-Cl^{-1} buffer, pH 7.4, containing 0.3 M sucrose, 50 mM NaCl, 10 mM EDTA, and 10 mM DTT (10% homogenate, w/v). In some specific experiments (cf Results) NaF was used instead of NaCl in order to inhibit phosphatase activity. A microsomal fraction was prepared by centrifugation at 20,000 *g* for 15 min and recentrifugation of the supernatant at 100,000 *g* for 1 h. Half of the microsomal fraction was resuspended in the homogenizing medium and recentrifuged at 100,000 *g* for 1 h. This fraction was used for assay of HMG-CoA reductase. Half of the original microsomal fraction was recentrifuged at 100,000 *g* in a homogenizing medium lacking DTT. The resulting fraction was used for assay of cholesterol 7α-hydroxylase activity.

Assay of cholesterol 7α-hydroxylase activity

After preparation of a microsomal fraction as above, incubations with 10 μg of [4-^{14}C]cholesterol dissolved in 1 mg of Tween 80 were performed as described previously in a total volume of 3 ml of 0.1 M potassium phosphate buffer, pH 7.4 (6). In some experiments the labeled cholesterol was replaced with various amounts of unlabeled cholesterol (0–300 μg). 7α-[$^{2}H_2$]Hydroxycholesterol was added to the incubation mixture after the incubation, before the extraction steps. The conversion of exogenous [4-^{14}C]cholesterol into 7α-hydroxycholesterol was determined by radioscanning after thin-layer chromatography (6) and corresponding conversion of endogenous cholesterol was determined by combined gas–liquid chromatography–mass spectrometry as described previously (6). In all experiments, the total conversion of both the endogenous microsomal cholesterol and the exogenous [4-^{14}C]cholesterol was calculated.

Assay of HMG-CoA reductase activity

After preparation of the microsomal fraction as outlined above, incubations with [3-^{14}C]HMG-CoA and subsequent analysis of incubation mixtures were performed essentially as described by Brown, Goldstein, and Dietschy (9). In this assay, the microsomal fraction, 40 μl, is preincubated for 15 min at 30°C in a total volume of 200 μl containing 0.1 M phosphate buffer, pH 7.4, 10 mM imidazole buffer, pH 7.4, 5 mM dithiothreitol, 10 mM EDTA, 3 mM NADP, 12 mM glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase. The reaction is then initiated with the addition of 90 nmol (0.5 μCi) of [3-^{14}C]HMG-CoA, dissolved in 25 μl of distilled water, giving a total substrate concentration of 400 μM. The incubation continues for 15 min and is stopped by the addition of 25 μl of 6 M HCl. Tritium-labeled mevalonic acid (0.01 μCi) together with 3 mg of unlabeled mevalonic acid

lactone is added to the incubation mixture, which is then further lactonized, subjected to thin-layer chromatography, and analyzed for radioactivity.

Assay of microsomal cholesterol, microsomal protein, lymph cholesterol, lymph triglycerides

Microsomal free cholesterol was determined by isotope dilution-mass spectrometry after extraction as described previously (10) with modifications (described in ref. 11). The protein concentration was determined according to the method of Lowry et al. (12). Lymph cholesterol and lymph triglycerides were determined by enzymatic methods (13, 14). The liver microsomal content of free cholesterol in the different groups of animals was the following: untreated 29 ± 4 μg/mg protein, cholestyramine-treated 27 ± 2 μg/mg, bile duct-ligated 38 ± 3 μg/mg, lymph duct-ligated 25 ± 3 μg/mg, lymph duct-fistulated 30 ± 2 μg/mg (means ± SD).

RESULTS

Lymphatic drainage leads to a pronounced loss of fluid. When the animals were given 0.6% NaCl in drinking water this loss of fluid was, in general, 40–80 mg/24 h. Under such conditions, there was no significant weight loss during the time of the experiment. When only water was given as drinking fluid, the flow of lymph was reduced to about 10–30 ml, and the weight of the animals was significantly reduced. In spite of that similar effects were obtained on the two hepatic enzymes studied. In all experiments presented here, 0.6% NaCl in drinking water was used.

The loss of albumin, cholesterol, and triglycerides in lymph (means ± SD, n = 8) was found to be 0.15 ± 0.05 g; 0.04 ± 0.02 mmol; 0.4 ± 0.2 μmol/24 h, respectively. These results are similar to those obtained in our previous study (1). In that study we showed that the lymph fistulation led to a significant reduction of serum albumin (about 20%), whereas serum cholesterol and serum triglyceride levels were unaffected. In **Table 1**, the results of two typical experiments with lymph fistula rats are shown. In accordance with the previous study (1) cholesterol 7α-hydroxylase was stimulated about twofold and the HMG-CoA reductase activity about threefold as compared to sham-operated rats. In several similar experiments the degree of stimulation of cholesterol 7α-hydroxylase varied between 50% and 130%. It should be noted that cholesterol 7α-hydroxylase activity in the sham-operated rats (fixed in cages in the same way as lymph fistula rats) was about 70% higher than the corresponding activity in untreated rats. This may be due to a stress effect, and it is well known that catecholamines and glucocorticoids may stimulate the activity to some extent (4). In the present experiments the lymph was drained for 72 h instead of 48 h used in the previous study. The same degree of stimulation was obtained after 48 as after 72 h, provided the lymph flow was high immediately after the operation in the former case. There was a tendency, however, to a reduced flow of lymph in some animals during the first 24 h. Thus, treatment for 72 h was regarded to give more reproducible results with respect to degree of stimulation.

It is noteworthy that the degree of stimulation of 7α-hydroxylation of endogenous microsomal cholesterol and total cholesterol (endogenous + exogenous) was similar. We have previously shown that cholesterol 7α-hydroxylase is almost completely saturated (80–90%) with the endogenous microsomal cholesterol under most experimental conditions. (5). this means that the total formation of 7α-hydroxycholesterol in an assay where a small amount of exogenous labeled cholesterol is added should reflect the maximal capacity of the enzyme. In order to measure the degree of saturation of cholesterol 7α-hydroxylase also in

TABLE 1. Effects of lymphatic drainage and ligation of the lymph duct

Treatment		7α-Hydroxylation of Cholesterol		HMG-CoA Reductase Activity
		Endogenous Substrate	Total Substrate	
		pmol/min/mg		*pmol/min/mg*
Exp. 1	Untreated (n = 6)	22 ± 2	27 ± 2	380 ± 50
Exp. 2	Sham-operated (n = 4)	38 ± 14	44 ± 13	267 ± 56
	Lymph fistula (n = 4)	88 ± 5** (+130%)	101 ± 8** (+130%)	699 ± 69** (+162%)
Exp. 3	Sham-operated (n = 4)	36 ± 2	40 ± 3	340 ± 62
	Lymph fistula (n = 5)	73 ± 38* (+103%)	76 ± 17* (+90%)	1136 ± 147** (+234%)
Exp. 4	Sham-opeated (n = 4)	40 ± 6	45 ± 7	300 ± 51
	Lymph-ligated (n = 4)	64 ± 7* (+60%)	74 ± 9* (+64%)	824 ± 139* (+175%)

Values are given as means ± SEM.
*$P < 0.05$, Student's t-test.
**$P < 0.01$, Student's t-test.

the liver of lymph fistula rats, incubations were performed with liver microsomes from three such animals together with increasing amounts of added unlabeled cholesterol (5). The degree of saturation of cholesterol 7α-hydroxylase was found to be 69 ± 3% as compared to 83 ± 5% in the corresponding controls. This means that the degree of saturation of the enzyme is slightly lower in the lymph-fistula animals than in the controls. As a consequence the assay used here may lead to a slight underestimation of the degree of stimulation of the enzyme activity obtained after lymphatic drainage.

In the evaluation of the stimulatory effect of lymphatic drainage on HMG-CoA reductase activity, the extent of activation of the enzyme must be taken into account. Thus rat liver HMG-CoA reductase is known to be activated by protein phosphatases during the preparation of microsomes, and this activation can be prevented by inclusion of sodium fluoride in the medium during the preparation (15). Inclusion of sodium fluoride in the homogenizing medium reduced HMG-CoA reductase activity to 21 ± 7% (mean ± SD) in liver microsomes from four control rats and to 17 ± 3% (mean ± SD) in liver microsomes from four lymph fistula rats. The difference was not significant from the statistical point of view, and it can be concluded that differences in the degree of activation cannot explain the threefold stimulation of HMG-CoA reductase activity by lymphatic drainage.

Fistulation is a relatively traumatic model, and attempts were therefore made to achieve the same effects by ligation of the lymph duct. Since a ligation may lead to transfer of lymph from small lymph vessels not normally used to the circulation, such treatment would be expected to lead to less pronounced effects than lymphatic drainage. Several series of experiments were performed, and in most of them there was at least a small stimulatory effect on both cholesterol 7α-hydroxylase activity and HMG-CoA reductase activity. A typical result is shown in Table 1. In all the experiments where an effect on cholesterol 7α-hydroxylase was noted, a similar or more marked effect was seen on HMG-CoA reductase. Although the results of the ligation experiments are less convincing, they confirm the results of the lymph fistulation experiments.

Cholestyramine treatment leads to a stimulatory effect on both cholesterol 7α-hydroxylase and HMG-CoA reductase and the possibility was tested that ligation of the lymph duct could further increase the two activities. As shown in **Table 2**, however, no such additional stimulation was seen. Ligation of the bile duct also leads to a stimulatory effect on both HMG-CoA reductase and cholesterol 7α-hydroxylase (16). Also in this case, lymph duct ligation did not further stimulate the two activities (Table 2). Lymphatic drainage also did not increase HMG-CoA reductase or cholesterol 7α-hydroxylase activity in bile duct-ligated rats. In fact, cholesterol 7α-hydroxylase activity was slightly depressed by lymphatic drainage.

The results obtained above with untreated lymph fistula or lymph-ligated rats may be explained if it is assumed that there is a factor in the lymph that inhibits cholesterol 7α-hydroxylase. If this hypothetical factor is dependent upon bile acids for its absorption from the intestine, the lack of stimulatory effect of lymph fistulation in cholestyramine-treated and bile duct-ligated rats can also be explained. The effects of lymph fistulation on HMG-CoA reductase activity are all consistent with the well-documented inhibitory effect of cholesterol-containing chylomicrons on the enzyme activity. The possibility must therefore also be considered that the effects on cholesterol 7α-hydroxylase activity are secondary to those on HMG-CoA reductase activity.

In order to further substantiate the possible presence of a specific inhibitor of cholesterol 7α-hydroxylase in the lymph, attempts were made to infuse lymph from rats on a cholesterol-free diet into rats with a biliary fistula. The reason for using bile fistula rats with an up-regulated bile acid biosynthesis was that such rats lose a volume of bile similar to that possible to infuse. Effects were obtained first when the infusion rate of lymph was 1 ml/h or more,

TABLE 2. Combined treatment with lymph ligation or lymph drainage with cholestyramine or bile duct ligation

Treatment		7α-Hydroxylation of Cholesterol		HMG-CoA Reductase Activity
		Endogenous Substrate	Total Substrate	
		pmol/min/mg		*pmol/min/mg*
Exp. 5	Bile duct ligated (n = 4)	110 ± 10	129 ± 11	867 ± 122
	Bile and lymph ducts ligated (n = 5)	96 ± 7[a] (−13%)	113 ± 8[a] (−12%)	832 ± 156[a] (−4%)
Exp. 6	Bile duct ligated (n = 5)	127 ± 16	150 ± 17	855 ± 37
	Bile duct ligated and lymph fistulation (n = 5)	87 ± 5* (−31%)	101 ± 6* (−33%)	819 ± 56[a] (−4%)
Exp. 7	Cholestyramine treatment and sham operation (n = 5)	64 ± 10	81 ± 11	2739 ± 181
	Cholestyramine treatment and lymph duct ligation (n = 5)	63 ± 11[a] (−2%)	83 ± 10[a] (+2%)	2841 ± 286[a] (+4%)

[a]Not statistically significant.
*$P < 0.05$, Student's t-test.

TABLE 3. Effect of infusion of lymph and cholesterol in bile fistula rats

Treatment		7α-Hydroxylation of Cholesterol		HMG-CoA Reductase Activity
		Endogenous Substrate	Total Substrate	
		pmol/min/mg		*pmol/min/mg*
Exp. 8	Bile fistula infused with NaCl (n = 4)	56 ± 4	80 ± 7	2883 ± 260
	Bile fistula infused with lymph (n = 4)[a]	40 ± 4* (−29%)	52 ± 8* (−35%)	1432 ± 371* (−50%)
Exp. 9	Bile fistula infused with NaCl (n = 4)	64 ± 4	86 ± 5	2780 ± 230
	Bile fistula infused with cholesterol (n = 4)[b]	54 ± 2[c] (−16%)	79 ± 3[c] (−8%)	570 ± 81** (−79%)

[a]Cholesterol load was about 20 μmol/24 h.
[b]Cholesterol load was about 310 μmol/24 h.
[c]Not statistically significant.
*$P < 0.05$, Student's *t*-test.
**$P < 0.01$, Student's *t*-test.

corresponding to about half of the volume of lymph produced by a lymph fistula rat during the same period of time. In all experiments where an inhibitory effect was seen on cholesterol 7α-hydroxylase, a similar effect was also seen on HMG-CoA reductase. One set of experiments in which a significant effect was seen on both enzyme activities is shown in **Table 3**. In this case the degree of inhibition of cholesterol 7α-hydroxylase activity and HMG-CoA reductase activity was 35% and 50%, respectively. The amount of lymph infused (1 ml/h) in the experiment shown in Table 1 corresponded to a load of about 0.08 g of albumin, 20 μmol of cholesterol, and 0.2 μmol of triglyceride/24 h.

In view of the technical difficulties (clotting of the lymph) and the relatively small effects obtained, no further attempts were made to characterize the postulated inhibitory factor in the lymph by infusion experiments.

For reasons of comparison, cholesterol-containing Intralipid was also infused in bile-fistula rats (cf ref. 5). In this case there was a marked depression of HMG-CoA reductase (80%) with little or no effect on cholesterol 7α-hydroxylase (Table 3). It should be pointed out that the cholesterol load in the Intralipid-treated animals (about 310 μmol/24 h) was more than 10-fold higher than that in the lymph-treated animals.

A notable finding was that the level of HMG-CoA reductase activity was always considerably and consistently higher (two- to fivefold) in the cholestyramine-treated rats and the bile fistula rats than in the lymph fistula rats and the lymph duct-ligated rats (Tables 1–4). If the stimulatory effect of interruption of the enterohepatic circulation of bile acids on HMG-CoA reductase activity is due only to malabsorption of cholesterol from the intestine, similar degree of stimulation would have been expected. In order to evaluate the relative importance of the flux of cholesterol and the flux of bile acids on the activity of HMG-CoA reductase, the effect of cholestyramine treatment on the enzyme activity was studied in lymph fistula rats. As shown in **Table 4**, cholestyramine treatment led to a significant further stimulation in the lymph fistula rats. Also, cholesterol 7α-hydroxylase activity was stimulated about twofold.

It may be concluded that if there is a specific lymphatic factor of importance for the activity of cholesterol 7α-hydroxylase, this factor can only be responsible for part of the regulation.

DISCUSSION

Relation between HMG-CoA reductase activity and cholesterol 7α-hydroxylase activity

Under most experimental conditions, HMG-CoA reductase and cholesterol 7α-hydroxylase activity covariate (for a review, see ref. 4). The only known condition where the two enzyme activities change in opposite

TABLE 4. Effects of combined treatment with cholestyramine and lymph ligation

Treatment		7α-Hydroxylation of Cholesterol		HMG-CoA Reductase Activity
		Endogenous Substrate	Total Substrate	
		pmol/min/mg		*pmol/min/mg*
Exp. 10	Lymph fistulated rats on control diet (n = 5)	73 ± 15	76 ± 17	1136 ± 147
	Lymph fistulated rats on cholestyramine (n = 5)	183 ± 24** (+150%)	200 ± 20** (+163%)	2588 ± 163** (+128%)

**$P < 0.01$, Student's *t*-test.

direction is treatment with dietary cholesterol. Even a relatively low content of cholesterol in the diet effectively depresses HMG-CoA reductase activity. With a high load of cholesterol (at least 0.5% or more in diet) for several days, cholesterol 7α-hydroxylase activity increases. We have previously shown that the latter stimulation is not due to a simple saturation of a substrate-unsaturated enzyme (5). It was recently shown that the cholesterol-induced stimulation of cholesterol 7α-hydroxylase is due to induction of enzyme synthesis (17).

If cholesterol-containing chylomicrons in the lymph have an inhibitory effect on HMG-CoA reductase and a stimulatory effect on cholesterol 7α-hydroxylase, lymphatic drainage would be expected to increase HMG-CoA reductase and to inhibit cholesterol 7α-hydroxylase. The latter effect would, however, only be expected if part of the activity of cholesterol 7α-hydroxylase is normally dependent upon induction by cholesterol. In contrast to the above expectation, however, lymphatic drainage was found to stimulate cholesterol 7α-hydroxylase. Due to the possible traumatic effect of a lymph fistula with loss of considerable fluid, attempts were made to find a less traumatic animal model. It would, for example, be difficult to study the combined effect of lymphatic and biliary drainage due to the even greater loss of fluid under such conditions. The effects of ligation of the lymph duct were therefore also studied. Due to a possible dilatation and utilization of lymph vessels not normally used, such treatment could be expected to lead to less pronounced effects than lymphatic drainage. In most sets of experiments, however, a significant stimulatory effect of lymph ligation was seen on both cholesterol 7α-hydroxylase and HMG-CoA reductase activity.

Regulation of cholesterol 7α-hydroxylase

In principle there are two possible explanations for the effects of lymph fistulation or lymph duct ligation on cholesterol 7α-hydroxylase. First, there may be a factor in the lymph that down-regulates cholesterol 7α-hydroxylase. The finding that lymphatic drainage or ligation of the lymph duct of rats with interrupted enterohepatic circulation did not further stimulate cholesterol 7α-hydroxylase activity can be explained if it is assumed that the factor is derived from the intestine and requires bile acids for its absorption. Second, a primary increase in HMG-CoA reductase activity may lead to a secondary increase in cholesterol 7α-hydroxylase activity by some unknown mechanism. The primary cause of the effect of lymphatic drainage would then be the loss of cholesterol through the lymph, which leads to increased HMG-CoA reductase activity.

Support for the first hypothesis was obtained by infusing lymph in a bile fistula rat. This infusion reduced both HMG-CoA reductase and cholesterol 7α-hydroxylase to a similar degree. In contrast, infusion of cholesterol reduced HMG-CoA reductase only, without affecting cholesterol 7α-hydroxylase. Due to technical difficulties and due to the relatively small effects obtained, it was not possible to further characterize the postulated lymphatic factor. For practical reasons, it was only possible to infuse about half the volume of lymph produced by a rat during the time of infusion. If there is a linear relationship between the flux of the inhibitory factor into the liver and the degree of suppression of cholesterol 7α-hydroxylase, the inhibitory effect of the postulated lymphatic factor is substantial. Since treatment of lymph fistula animals with cholestyramine led to a more than twofold stimulation, however, it is evident that only part of the regulatory effect of bile acids on cholesterol 7α-hydroxylase activity can be mediated by the lymphatic factor.

The second hypothesis may be consistent with most of the present experimental findings, although the results of the lymph infusion experiments are better explained by the first hypothesis. An argument against a direct coupling between cholesterol 7α-hydroxylase activity and HMG-CoA reductase activity is that we have previously shown that HMG-CoA reductase inhibitors have little or no effect on cholesterol 7α-hydroxylase activity under normal conditions (18, 19). It should be borne in mind, however, that treatment with mevinolin causes a compensatory increase in the amount of HMG-CoA reductase protein, and the decrease in enzymatic activity in vivo is probably relatively small. In acute experiments in vivo with bile fistula rats, an HMG-CoA reductase inhibitor seems to have an inhibitory effect on bile acid biosynthesis (20). Our present finding that infusion of cholesterol depresses HMG-CoA reductase but not cholesterol 7α-hydroxylase in a bile fistula rat does not exclude the possibility that a primary increase in HMG-CoA reductase activity may cause a secondary increase in cholesterol 7α-hydroxylase activity.

At the present state of knowledge, the first hypothesis appears to be the most attractive one. Identification of the postulated inhibitory factor and a demonstration of its specificity is required, however, before the second hypothesis can be ruled out.

Regardless of whether or not there is a regulatory factor in the lymph, the results with the combined treatment with cholestyramine and lymphatic drainage show that the flux of bile acids is most important for regulation of cholesterol 7α-hydroxylase. This is different from the regulatory model suggested by Davis et al. (21, 22) on the basis of experiments with isolated hepatocytes. Apparently, synthesis and availability of cholesterol seem to be the most important factors for bile acid formation in such cells. Thus it was shown that the HMG-CoA reductase inhibitor mevinolin caused a markedly reduced synthesis of both cholesterol and bile acids. The reason for the different results obtained with isolated hepatocytes and with in vivo models could be that the cells may be cholesterol-

depleted during isolation and incubation. Under such conditions, the normally substrate-saturated cholesterol 7α-hydroxylase (5) may be unsaturated, with the result that the formation of bile acids would be dependent upon the activity of HMG-CoA reductase. A similar situation may occur under drastic in vivo conditions, e.g., by combining biliary drainage with treatment with a potent cholesterol synthesis inhibitor.

Regulation of HMG-CoA reductase

The mechanism of regulation of HMG-CoA reductase by bile acids is controversial. Weis and Dietschy (23) did not observe any influence of taurocholate on cholesterol synthesis in bile fistula rats and suggested that the inhibitory effect of bile acids on cholesterol synthesis may be related to their stimulatory effect on absorption of cholesterol in the intestine. However, Hamprecht et al. (24) were able to demonstrate a reduction of HMG-CoA reductase activity in lymph fistula rats infused with cholate, suggesting the bile acids have an effect on the enzyme that is not mediated by effects on absorption of cholesterol. The present finding that cholestyramine treatment stimulates HMG-CoA reductase activity also in lymph fistula animals is in accord with the results by Hamprecht et al. (24) and clearly shows that only a part of the regulatory effect of bile acids on hepatic HMG-CoA reductase can be mediated by effects on intestinal absorption of cholesterol. The bile acids may either suppress HMG-CoA reductase directly, or their effect on this enzyme may be mediated by effects on cholesterol 7α-hydroxylase. It can be hypothesized that the activity of the latter enzyme may be of importance for a small pool of cholesterol that may be the direct regulator of HMG-CoA reductase.

It should be emphasized that the present study was performed with rats on a diet practically free of cholesterol. The relative importance of the flux of bile acids for the regulation of hepatic HMG-CoA reductase should be less with a dietary cholesterol load. In accordance with this, it was shown here that it is possible to markedly depress HMG-CoA reductase by intravenous infusion of cholesterol also in rats with an up-regulated HMG-CoA reductase activity due to biliary drainage.

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