Heterogeneity of rat liver parenchyma in cholesterol 7 α -hydroxylase and bile acid synthesis

Bernhard UGELE,*† Herman J. M. KEMPEN,† Rolf GEBHARDT,* Piet MEIJER,† Hans-Jörg BURGER* and Hans M. G. PRINCEN†

*Physiologisch-Chemisches Institut, Universität Tübingen, D-7400 Tübingen, Federal Republic of Germany, and †Gaubius Institut TNO, 2300 AP Leiden, The Netherlands

Periportal and perivenous hepatocytes were isolated from rat liver by digitonin/collagenase perfusion for investigating the acinar distribution of bile acid synthesis. The specific activity of cholesterol 7 α -hydroxylase (EC 1.14.13.17) was 7.9-fold higher in perivenous cells than in periportal hepatocytes. Mass production of bile acids differed 4.4-fold between cultured perivenous and periportal hepatocytes. In contrast, the levels of free cholesterol in homogenates and microsomes derived from both subfractions were similar. Feeding of rats with the bile-acid-sequestering anion-exchange resin colestid resulted in a pronounced stimulation of cholesterol 7 α -hydroxylase activity and bile acid mass production, but decreased the perivenous/periportal ratio of both parameters. These results demonstrate that bile acid synthesis predominates in the perivenous hepatocytes, possibly owing to feedback suppression by bile acids from the enterohepatic circulation. Furthermore, the opposite acinar localization of cholesterol and bile acid biosynthesis provides an interesting alternative to current views of the regulation of their metabolic pathways.

INTRODUCTION

Hepatocytes of the microvascular unit of hepatic parenchyma, the liver acinus, are heterogeneous with respect to subcellular structure, enzyme content and metabolic functions (for recent reviews see [1,2]). One plausible reason for this heterogeneity seems to be the execution of different, i.e. opposing, metabolic pathways in different zones of the liver. However, different types of zonation may exist. For instance, whereas in carbohydrate metabolism zonation seems rather dynamic and functional, that of glutamine and urea synthesis seems to be very static and structural [3].

Concerning cholesterol metabolism, much less is known about the possible zonation of biosynthetic and biotransformation reactions. It is a well-established fact that the regulation of cholesterol synthesis is dominated by the enzyme activity of HMG-CoA reductase. Recently, in intact liver of control rats HMG-CoA reductase was found by immunofluorescence techniques to be localized within about 20% of the hepatocytes which are scattered randomly about the portal triad [4,5]. However, under drastic conditions (i.e. simultaneous administration of the HMG-CoA reductase inhibitor mevinolin and of the bile acid sequestrant cholestyramine) HMG-CoA reductase could be induced in almost all hepatocytes [4,5].

Excretion of cholesterol into bile, either as free cholesterol or following its conversion into bile acids, is the predominant pathway through which cholesterol is eliminated from the mammalian body [6]. Thus the rate of bile acid formation is an important regulator of cholesterol homeostasis. The rate-limiting step in bile acid synthesis is the 7 α -hydroxylation of cholesterol catalysed by microsomal cytochrome P-450-dependent cholesterol 7 α -hydroxylase (EC 1.14.13.17) [7,8]. In general, the bulk of various isoforms of cytochrome P-450 [2,9,10], as well as the related NADPH-cytochrome P-450 reductase [11,12] and the NADPH-generating enzymes [13–15], are predominant

in the perivenous area. However, for cholesterol 7 α -hydroxylase nothing is known about such a heterogeneous distribution within the acinus. In general, the regulation of this enzyme is still a matter of debate [8]. There is substantial evidence, however, suggesting that the regulation is brought about by the amount and/or nature of bile acids entering the liver via the enterohepatic circulation [16–21] as well as by hormones [22]. In the present study we have investigated the acinar distribution of cholesterol 7 α -hydroxylase activity, using periportal and perivenous subfractions of hepatocytes isolated by the digitonin/collagenase perfusion technique of Quistorff [23] and Lindros & Penttilä [24]. The reliability of this approach was recently corroborated in our laboratory, using glutamine synthetase as a marker enzyme for perivenous hepatocytes [25–28]. Furthermore, this cell-culture approach allowed the quantitative assessment of functional parameters such as the rate of bile acid synthesis and cholesterol content.

MATERIALS AND METHODS

Materials

Materials used for isolation and culture of rat hepatocytes, determination of cholesterol 7 α -hydroxylase activity, mass determination of bile acids and cholesterol have been described previously [26,29–31].

Animals

Male Sprague–Dawley rats (200–280 g) were kept in a strictly controlled 12 h-light/12 h-dark cycle (lights on 06:00–18:00 h) on a standardized diet (Alma H 1003, containing 22.4% protein, 6.5% fat and 58.6% carbohydrate; Botzenhardt, Kempten, Germany) and tap water *ad libitum*. For preparation of hepatocytes, animals were killed between 09:00 and 10:00 h. In selected cases the diet was supplemented with 5% (w/w) colestid (Upjohn,

Abbreviations used: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; PP-cells, PV-cells, hepatocyte preparations enriched in either periportal or perivenous cells respectively.

† To whom correspondence should be sent. Present address: I. Frauenklinik der Universität München, Maistrasse 11, D-8000 München 2, Federal Republic of Germany.

Puurs, Belgium), containing the bile acid-sequestering anion-exchange resin colestipol, 1 week before cell preparation [33].

Isolation and cultivation of hepatocytes

Normal liver parenchymal cells were isolated by the two-step collagenase perfusion technique of Seglen [34] modified as described previously [29].

Perivenous and periportal subfractions of hepatocytes were isolated by the digitonin/collagenase perfusion technique of Quistorff [23] and Lindros & Penttilä [24], with modifications described elsewhere [26]. The volumes of digitonin necessary for destruction of periportal and perivenous zones were similar in normal and colestid-treated livers. For both control and colestid-treated rats, the yield of PP-cells was slightly higher compared with PV-cells, but no significant differences between the two groups of rats were apparent [PP-cells 163.6 ± 46.7 ($n = 14$) and 216 ± 31.8 ($n = 3$), PV-cells 143.5 ± 45.8 ($n = 12$) and 141 ± 73.6 ($n = 3$) in millions, for control and colestid-treated rats respectively]. Viability, assessed by Trypan Blue exclusion, was $81.7 \pm 3.9\%$ ($n = 5$), $81.6 \pm 5.5\%$ ($n = 14$) and $83.4 \pm 4.0\%$ ($n = 12$) for normal hepatocytes, PP-cells and PV-cells from untreated rats and $83.8 \pm 3.7\%$ ($n = 3$) and $82 \pm 5.7\%$ ($n = 3$) for PP- and PV-cells from colestid-treated rats respectively. Cell suspensions from digitonin/collagenase perfusions with a viability index of less than 70% were discarded.

The efficiency of enrichment of periportal and perivenous hepatocytes was monitored by measurements of glutamine synthetase activity. The mean of the perivenous/periportal activity ratio was 56 for cell preparations from normal rats and 87 from colestid-treated rats. Additionally estimated perivenous/periportal ratios of alanine aminotransferase (0.64) and pyruvate kinase (1.11) activity were in good agreement with published values for normal rats [24,26,27].

The cells were seeded on collagen-coated [29] plastic tissue-culture dishes (60 mm diameter; Greiner, Nürtingen, Germany) at a density of 1.25×10^5 cells/cm² and were maintained in Williams medium E supplemented with 10% (v/v) newborn-calf serum, 2 mM-glutamine, penicillin (50 units/ml)/streptomycin (50 µg/ml) at 37 °C in an atmosphere of CO₂/humidified air (1:19). During a 2 h inoculation period, viable hepatocytes had attached strongly to the collagen matrix. Dead cells were removed by washing the monolayer three times with cold Hanks Balanced Salt Solution (4 °C) before harvesting for the determination of cholesterol 7 α -hydroxylase activity. For quantification of bile acid mass production, cells were washed twice with Williams medium E (37 °C) and incubated with serum-free medium for 8 h as stated above.

Preparation of microsomes

This was done as described earlier [31].

Enzyme assays

The activity of glutamine synthetase was determined by the γ -glutamyltransferase assay with the modification reported previously [35]. Alanine aminotransferase and pyruvate kinase were determined as described by Bergmeyer & Bernt [36]. Cholesterol 7 α -hydroxylase activity was measured in homogenates and microsomes as described in detail [31,32].

Protein and cholesterol determinations

Protein and cholesterol were assayed as described by Lowry *et al.* [37] and Gamble *et al.* [38] respectively.

Quantification of mass production of bile acids

Bile acids in cells and medium were quantified by g.l.c., essentially as described by Davis *et al.* [39], with modifications

[22,30]. Bile acid synthesis was calculated as the amount in medium and cells after the 8 h incubation period minus the amount in cells at the beginning of the incubation. Total bile acid production was the sum of synthesis of cholic, chenodeoxycholic and α - and β -muricholic acids.

RESULTS

Heterogeneous distribution of cholesterol 7 α -hydroxylase

The specific activity of cholesterol 7 α -hydroxylase in PV-cells was 7.9-fold higher than in PP-cells after 2 h in short-term culture (Table 1). Similar differences in cholesterol 7 α -hydroxylase activities were found in homogenates or microsomes prepared from freshly isolated PP- and PV-cells, indicating that the observed differences did not arise during the 2 h culture period. The mean activities of the corresponding liver homogenates, which were prepared from a small liver lobule ligated before digitonin infusion during each preparation, as well as the values for hepatocytes prepared from the whole liver acinus by conventional collagenase perfusion technique (Table 1), were well in between those of the two subfractions after 2 h in culture, indicating that the digitonin treatment had no effect on the cholesterol 7 α -hydroxylase activity in the isolated viable cells.

Concerning the kinetics of the enzyme reaction, no difference in saturation of the enzyme with substrate between both populations was detectable (saturated at 120–130 µM). Therefore, since the enzyme assays were performed under saturating substrate conditions, in which free cholesterol from cells comprised maximally 35% of total unesterified cholesterol in the assay [32], the differences in cholesterol 7 α -hydroxylase activity cannot be attributed to differences in substrate availability for the enzyme.

Effect of colestid feeding on heterogeneity of cholesterol 7 α -hydroxylase activity

In rats treated with 5% colestid for 1 week before cell preparation, cholesterol 7 α -hydroxylase activity in the whole liver was enhanced about 5-fold (Table 1). Colestid contains the agent colestipol, which shows a similar bile-acid-sequestering mechanism in the gut to cholestyramine. The enzymic data on the PP- and PV-cell fractions revealed an 8.2-fold induction in the PP-cells, compared with a slight increase of 1.9-fold only in the PV-cells. Thus colestid treatment decreased the perivenous/periportal ratio of cholesterol 7 α -hydroxylase activity from 7.9 to 1.8.

Bile acid mass production

The above enzymic data suggest that bile acid synthesis predominates in the PP-cells. To confirm these findings, we measured the mass production of bile acids in the cultured PP- and PV-cells during 2–10 h after plating. Similarly to cholesterol 7 α -hydroxylase activity, bile acid synthesis in PV-cells was clearly higher than in PP-cells (Table 2). In this case a perivenous/periportal ratio of 4.4 was obtained. Again, the mean value of the total preparations was well in between those of the subfractions. As expected, there was also a drastic stimulation of bile acid production in both PP- and PV-cells after pretreatment of donor animals with colestipol before cell isolation. Similarly to cholesterol 7 α -hydroxylase activity, bile acid synthesis increased 4.6-fold in PP-cells and 2.0-fold in PV-cells, resulting in a decrease in the periportal/perivenous ratio from 4.4 in the control livers to 1.9 in the stimulated organ.

Furthermore, the heterogeneity is evident also at the level of individual bile acids. As shown in Table 2, the percentage of cholic acid synthesized by PP-cells was more than twice that

Table 1. Specific activity of cholesterol 7 α -hydroxylase in hepatocytes prepared from the whole liver acinus, in periportal and perivenous hepatocytes of normal livers and livers of colestipol-treated rats and in the corresponding liver homogenates

After an inoculation period of 2 h in Williams medium E supplemented with 10 % newborn-calf serum, cells were washed and harvested for the determination of cholesterol 7 α -hydroxylase as described. The corresponding liver homogenates were prepared from a small liver lobule which was ligated before digitonin infusion during preparation of PP- and PV-cells or before collagenase digestion during preparation of cells from whole acinus respectively. Values shown are means $\pm$ S.D. for n different hepatocyte isolations and are expressed in pmol/h per mg of protein; n.d., not determined.

	Control	PV/PP ratio	Colestid-treated rats	PV/PP ratio
PP-cells	170 $\pm$ 81 (n = 8)	7.9	1386 $\pm$ 504 (n = 3)	1.8
PV-cells	1345 $\pm$ 345 (n = 8)		2506 $\pm$ 649 (n = 3)	
Cells from whole acinus	645 $\pm$ 136 (n = 5)		n.d.	
Corresponding homogenates	453 $\pm$ 112 (n = 10)		2231 $\pm$ 342 (n = 6)	

Table 2. Bile acid mass production of hepatocytes from the whole liver acinus and from periportal and perivenous hepatocytes of normal livers and livers of colestipol-treated rats

After an inoculation period of 2 h in Williams medium E supplemented with 10 % newborn-calf serum, cells were washed and incubated for 8 h in serum-free medium. Afterwards, bile acids in the medium and cells were extracted and quantified by g.l.c. The values were corrected for the bile acid content in the cells before the incubation. All values shown are means $\pm$ S.D. of n different hepatocyte isolations. 'Total bile acid synthesis' values shown are expressed in μ g of bile acids/mg of cell protein synthesized during 8 h. For individual bile acids, values shown are percentages of total mass production.

	Control livers			Colestid-treated rats	
	Periportal (n = 4)	Perivenous (n = 5)	Whole acinus (n = 5)	Periportal (n = 3)	Perivenous (n = 3)
Total bile acid synthesis	0.12 $\pm$ 0.08	0.50 $\pm$ 0.20	0.33 $\pm$ 0.15	0.53 $\pm$ 0.09	1.02 $\pm$ 0.40
Cholic acid (%)	52 $\pm$ 9	19 $\pm$ 6	30 $\pm$ 12	45 $\pm$ 3	43 $\pm$ 6
β -Muricholic acid (%)	36 $\pm$ 17	77 $\pm$ 8	67 $\pm$ 14	52 $\pm$ 6	54 $\pm$ 8
Other bile acids* (%)	12 $\pm$ 15	4 $\pm$ 4	3 $\pm$ 4	4 $\pm$ 2	3 $\pm$ 3

* Minor bile acids formed by the cultured rat hepatocytes were chenodeoxycholic and in some experiments α -muricholic acid, which both may be converted into β -muricholic acid.

synthesized by PV-cells. Interestingly, these differences disappeared after colestipol treatment.

Cholesterol content

In order to examine the hypothesis advanced by others, that the availability of the substrate and/or the cholesterol concentration of the microsomal membrane may act as an effector of cholesterol 7 α -hydroxylase activity or bile acid biosynthesis, we also examined the free cholesterol content in homogenates and microsomes prepared from freshly isolated cells of both cell fractions. In the cell homogenates [whole acinus 28.5 $\pm$ 3.3 (n = 6), PP-cells 28.3 $\pm$ 2.6 (n = 8), PV-cells 28.1 $\pm$ 4.1 (n = 8) nmol of cholesterol/mg of total cell protein] as well as in the microsomes [whole acinus 79.4 $\pm$ 10.4 (n = 4), PP-cells 84.3 $\pm$ 8.7 (n = 3), PV-cells 78.3 $\pm$ 7.5 (n = 3) nmol of cholesterol/mg of microsomal protein], no significant differences between the periportal and perivenous fractions were detected.

DISCUSSION

The results reported here demonstrate that the dynamic zonation of liver parenchyma plays an important role in regulation of bile acid synthesis in the rat. Under normal feeding

conditions cholesterol 7 α -hydroxylase as well as bile salt production predominate in the perivenous area. The very high perivenous/periportal ratios of about 8 for cholesterol 7 α -hydroxylase activity and more than 4 for bile acid synthesis represent a very strong heterogeneity of liver parenchyma, reported so far for very few functions only (see [40]). The observed difference between the periportal/perivenous ratio of cholesterol 7 α -hydroxylase (at a given time) and that of bile acid synthesis (during a certain period) may be due to an inconstant enzyme activity. However, it cannot be excluded that this fact indicates the involvement of additional regulatory mechanisms superimposed on the activity of the key enzyme. Nevertheless, since microsomes and homogenates of both cell fractions show similar cholesterol concentrations, a regulation of bile acid synthesis by the availability of endogenous substrate seems rather unlikely.

Regulation of bile acid synthesis by feedback inhibition

The present data strongly support the current concept that bile acids regulate their own synthesis via feedback inhibition. Since periportal and perivenous hepatocytes are equally able to take up bile acids [27,41], the microanatomy of the liver lobule gives rise to an acinar concentration gradient of bile acids during blood

Fig. 1. Hypothetical scheme of the relationship between the acinar bile acid concentration gradient and the cholesterol 7 α -hydroxylase activity (shading) in periportal (PP) and perivenous (PV) hepatocytes in normal and colestid-treated rats

The decrease in the bile acid concentration gradient in the acinus is reflected by the stimulation of the cholesterol 7 α -hydroxylase activity, particularly in periportal cells.

flow through the liver. Direct evidence for such a gradient was obtained by different groups with various approaches [41–43]. The high bile salt concentration in the periportal area of the acinus may suppress cholesterol 7 α -hydroxylase activity to a larger extent than with the low concentration seen by the perivenous hepatocytes, thus restricting bile acid synthesis to the perivenous zone (Fig. 1). Such a relationship between plasma bile salt concentration and cholesterol 7 α -hydroxylase activity had already been assumed years ago [44,45]. This hypothesis is further supported by feeding of cholestyramine analogues, which strongly decreases bile acid concentration in the portal blood [46]. The resulting shorter gradient decreases the range of down-regulation within the acinus (Fig. 1).

This interpretation is supported by the demonstration of feedback inhibition of bile acid synthesis and cholesterol 7 α -hydroxylase activity in pig hepatocytes [19,21]. In these pig hepatocytes the decay of the transport capacity for taurocholate is moderate [19,47], whereas in rat hepatocytes a dramatic decline of Na⁺-dependent bile acid uptake is found ([47,48]; B. Ugele & R. Gebhardt, unpublished work). This difference may be responsible for the fact that in some reports no direct inhibition of bile acid synthesis by bile acid added to rat hepatocytes in culture [49,50] or to rabbit hepatocytes [51] was found.

Relationship between cholesterol synthesis and bile acid synthesis

In the past, various papers have dealt with the possible tight coupling between the biosynthesis of cholesterol and its transformation into bile acids, or between the respective rate-limiting enzyme activities, leading to rather contradictory conclusions. Furthermore, there is still some confusion about the contribution of different functional pools of cholesterol (preformed versus newly synthesized) as the precursor not only of the total mass production of bile acids but also of the formation of individual bile acids [45,52–57] (for review see [8]).

Singer *et al.* [4] have described a strikingly heterogeneous distribution of HMG-CoA reductase, by immunofluorescence microscopy. This enzyme was found exclusively localized in periportal hepatocytes in normal control rats. The opposite acinar localization of the rate-limiting enzymes of the cholesterol synthesis and bile acid synthesis described here would restrict a

direct linkage between the two pathways to a small amount of periportal hepatocytes, at least in control livers. This metabolic compartmentation could explain how different cholesterol pools may contribute to the synthesis of bile acids to a different extent under various experimental conditions. Furthermore, the observed differences in relative contribution of cholic acid to total bile acid synthesis between PP- and PV-cells may provide an explanation for the suggested different substrate pools for the individual bile acids. Stimulation of bile acid synthesis and/or induction of cholesterol synthesis leads to an expansion of both pathways within the acinus, thus enlarging the overlapping area. Consequently, in control livers preformed cholesterol taken up by the hepatocytes from the plasma should be the preferred substrate of bile acid synthesis, whereas in stimulated livers the utilization of newly synthesized cholesterol should increase. This hypothesis is supported by the findings by Long *et al.* [58] that in acute bile-fistula rats the bulk of bile acid is derived from preformed cholesterol, whereas with more prolonged fistula times an increasing fraction of the bile acids originated from the newly synthesized cholesterol.

In conclusion, the findings presented demonstrate that the concept of 'metabolic zonation' of the liver parenchyma has to be extended to cholesterol and bile acid metabolism.

This work was supported in part by a NATO grant for international collaboration in research (research grant no. 35/81).

REFERENCES

1. Gumucio, J. J. (1989) *Hepatology* **9**, 154–160
2. Jungermann, K. & Katz, N. (1989) *Physiol. Rev.* **69**, 708–751
3. Gebhardt, R., Burger, H.-J., Heini, H. G., Schreiber, K.-L. & Mecke, D. (1988) *Hepatology* **8**, 822–830
4. Singer, I. I., Kawaka, D. W., Kazakis, D. W., Alberts, A. W., Chen, J. S., Huff, J. W. & Ness, G. C. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5556–5560
5. Li, A. C., Tanaka, R. D., Callaway, K., Fogelman, A. M. & Edwards, P. A. (1988) *J. Lipid Res.* **29**, 781–796
6. Danielsson, H. & Sjövall, J. (1975) *Annu. Rev. Biochem.* **44**, 233–253
7. Myant, N. B. & Mitropoulos, K. A. (1977) *J. Lipid Res.* **18**, 135–153
8. Björkhem, I. (1985) *New Compr. Biochem.* **12**, 231–278
9. Ji, S., Lemaster, J. J. & Thurmann, R. G. (1981) *Mol. Pharmacol.* **19**, 513–516
10. Bengtsson, G., Julkunen, A., Penttilä, K. E. & Lindros, K. O. (1986) *J. Pharmacol. Exp. Ther.* **240**, 663–667
11. McManus, M. E., Hall, P. de la M., Stupans, I., Brennan, J., Burgess, W., Robson, R. & Birket, D. J. (1987) *Mol. Pharmacol.* **32**, 189–194
12. Taira, Y., Greenspan, P., Kapke, G. F., Redick, J. A. & Baron, J. (1980) *Mol. Pharmacol.* **18**, 304–312
13. Hildebrand, R. (1980) *Adv. Anat. Embryol. Cell Biol.* **60**, 1–54
14. Morrison, G. R., Broch, F. E., Karl, I. E. & Shank, R. (1965) *Arch. Biochem. Biophys.* **111**, 448–464
15. Deutsch, H. F. & Rieder, R. (1979) *Histochemistry* **60**, 43–52
16. Shefer, S., Hauser, S., Berkensky, I. & Mosbach, E. H. (1969) *J. Lipid Res.* **10**, 646–655
17. Spady, D. K. & Dietschy, J. M. (1987) in *Bile Acids and the Liver* (Paumgartner, G., Stiehl, A. & Gerok, W., eds.), pp. 15–24. MTP Press, Lancaster
18. Hall, R., Kok, E. & Javitt, N. B. (1988) *FASEB J.* **2**, 152–156
19. Kwekkeboom, J., van Voorthuizen, E. M., Princen, H. M. G. & Kempen, H. J. M. (1988) *Biochem. Biophys. Res. Commun.* **155**, 850–856
20. Stange, E. F., Scheibner, J. & Ditschuneit, H. (1989) *J. Clin. Invest.* **84**, 173–180
21. Kwekkeboom, J., Princen, H. M. G., van Voorthuizen, E. M. & Kempen, H. J. M. (1990) *Hepatology* **12**, 1209–1215
22. Princen, H. M. G., Meijer, P. & Hofstee, B. (1989) *Biochem. J.* **262**, 341–348

23. Quistorff, B. (1985) *Biochem. J.* **229**, 221–226
24. Lindros, K. O. & Penttilä, K. E. (1985) *Biochem. J.* **228**, 757–760
25. Gebhardt, R. & Mecke, D. (1983) *EMBO J.* **2**, 567–570
26. Burger, H.-J., Gebhardt, R., Mayer, C. & Mecke, D. (1989) *Hepatology* **9**, 22–28
27. Ugele, B., Locher, M., Burger, H.-J. & Gebhardt, R. (1987) in *Bile Acids and the Liver* (Paumgartner, G., Stiehl, A. & Gerok, W., eds.), pp. 153–160, MTP Press, Lancaster
28. Ugele, B., Kempen, H. J. M., Gebhardt, R., Meijer, P., Burger, H.-J. & Princen, H. M. G. (1989) in *Trends in Bile Acid Research* (Paumgartner, G., Stiehl, A. & Gerok, W., eds.), pp. 53–61, Kluwer Academic Publishers, Dordrecht, Boston and London
29. Gebhardt, R. & Jung, W. (1982) *J. Cell Sci.* **56**, 233–244
30. Kwekkeboom, J., Princen, H. M. G., van Voorthuizen, E. M. & Kempen, H. J. M. (1990) *Biochim. Biophys. Acta* **1042**, 386–394
31. Princen, H. M. G., Huijsmans, C. M. G., Kuipers, F., Vonk, R. J. & Kempen, H. J. M. (1986) *J. Clin. Invest.* **78**, 1064–1071
32. Princen, H. M. G., Meijer, P., Kwekkeboom, J. & Kempen, H. J. M. (1988) *Anal. Biochem.* **171**, 158–165
33. Kempen, H. J. M., Vos-van Holstein, M. P. M. & de Lange, J. (1982) *J. Lipid Res.* **23**, 823–830
34. Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
35. Gebhardt, R. & Williams, G. M. (1986) *Cell Biol. Toxicol.* **2**, 9–20
36. Bergmeyer, H. U. & Bernt, E. (1974) *Methoden der enzymatischen Analyse*, pp. 785–791 and 800–804, Verlag Chemie, Weinheim
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
38. Gamble, W., Vaughan, M., Kruth, H. S. & Avigan, J. (1978) *J. Lipid Res.* **19**, 1068–1070
39. Davis, R. A., Hyde, P. M., Kuan, J.-C. W., Malone-McNeal, M. & Archambault-Schexnayder, J. (1983) *J. Biol. Chem.* **258**, 3661–3667
40. Jungermann, K. (1986) *Enzyme* **35**, 161–180
41. Buscher, H.-P., Fricker, G., Gerok, W., Kurz, G., Müller, M., Schneider, S., Schramm, U. & Schreyer, A. (1987) in *Bile Acids and the Liver* (Paumgartner, G., Stiehl, A. & Gerok, W., eds.), pp. 95–100, MTP Press, Lancaster
42. Groothuis, G. M. M., Hardonck, M. J., Keulemans, K. P. T., Nieuwenhuis, P. & Meijer, D. K. F. (1982) *Am. J. Physiol.* **243**, G455–G462
43. Jones, A. L., Hradek, G. T., Renston, R. H., Wong, K. Y., Karlaganis, G. & Paumgartner, G. (1980) *Am. J. Physiol.* **238**, G233–G237
44. Hofmann, A. F. (1980) in *Communication of Liver Cells* (Popper, H., Bianchi, L., Gudat, F. & Reutter, W., eds.), pp. 43–49, MTP Press, Lancaster
45. Cronholm, T., Crustedt, T. & Sjövall, J. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 331–359, Academic Press, New York
46. Botham, K. M., Lawson, M. E., Beckett, G. J., Percy-Robb, I. W. & Boyd, G. S. (1981) *Biochim. Biophys. Acta* **665**, 81–87
47. Kwekkeboom, J., van Voorthuizen, E. M., Princen, H. M. G. & Kempen, H. J. M. (1989) *Biochem. Biophys. Res. Commun.* **162**, 619–625
48. Föllmann, W., Petzinger, E. & Kinne, K.-H. R. (1990) *Am. J. Physiol.* **258**, C700–C712
49. Davis, R. A., Highsmith, W. E., Malone-McNeal, M., Archambault-Schexnayder, J. & Kuan, C. W. (1983) *J. Biol. Chem.* **258**, 4079–4082
50. Kubaska, W. M., Gurley, E. C., Hylemon, P. B., Guzelian, P. S., Vlahcevic, Z. R. (1985) *J. Biol. Chem.* **260**, 13459–13463
51. Whitting, M. J., Wishart, R. A., Growing, M. R., McManus, M. E. & Mackinnon, A. M. (1989) *Biochim. Biophys. Acta* **1001**, 176–184
52. Ayaki, Y., Tsuma-Date, T., Endo, S. & Ogura, M. (1981) *Steroids* **38**, 495–509
53. Björkhem, I. (1986) *Biochim. Biophys. Acta* **877**, 43–49
54. Björkhem, I. & Akerlund, J.-E. (1986) *J. Lipid Res.* **29**, 136–143
55. Kempen, H. J. M., Vos-van Holstein, M. P. M. & de Lange, J. (1983) *J. Lipid Res.* **24**, 316–323
56. Mitropoulos, K. A., Myant, N. B., Gibbons, G. F., Balasubramaniam, S. & Reeves, B. E. A. (1974) *J. Biol. Chem.* **246**, 6052–6056
57. Mitropoulos, K. A., Venkatesan, S., Balasubramaniam, S. & Peters, T. J. (1978) *Eur. J. Biochem.* **82**, 419–429
58. Long, T. T., Jakoi, L., Steven, R. & Quarfordt, S. (1978) *J. Lipid Res.* **19**, 872–878

Received 14 August 1990/7 December 1990; accepted 7 January 1991