collaborators were the first to demonstrate in normal liver that centrizonal hepatocytes take up bile acids equally well but they excrete them at a much lower rate than periportal hepatocytes do[4]. We have confirmed these findings in our control animals, extraction being equal during ante- and retrograde perfusion but excretion rate (α_1) being markedly slower during retrograde perfusion.

Oral bile acid feeding has been used by several investigators to expand the bile acid pool[5-8]. Although we did not measure the bile acid pool in the present experiments, the increase in bile flow and basal bile salt secretion was comparable to that in another study using the same feeding schedule[8]. It can be assumed, therefore, that we induced an 8-fold expansion of the bile acid pool similar to that described by previous investigators[8]. Expansion of the bile acid pool has been shown to increase the maximal rate of taurocholate secretion in vivo[5-8]; this has been ascribed to recruitment of hepatocytes[5,6] and to an induction of bile acid receptors[8]. Our results suggest two different phenomena induced by bile acid feeding. In the absence of exogenous taurocholate the excretion rate constant is even slower in bile acid-fed than in control rats. This could be due to induction of bile acid-binding proteins in the hepatocyte[17]. It is not known whether these binders have a preferential acinar localization but the delay in excretion observed during retrograde perfusion is compatible with such a hypothesis. When excretion is studied during a period of moderate taurocholate load, by contrast, a marked acceleration in taurocholate secretion is observed in pool-expanded, but not in control rats. We submit that this phenomenon is due to saturation of the bile acid binders, and excretion thus becomes the rate-limiting step. Under these experimental conditions it becomes obvious that the secretory characteristics of centrizonal hepatocytes have been dramatically changed; they now have the secretory characteristics of periportal hepatocytes. These findings suggest that the previously-described increase in cholic acid receptors induced by bile acid feeding[8] could be occurring mostly in centrizonal hepatocytes.

Two serendipitous observations deserve comment. Livers of cholate-fed animals exhibited significantly higher oxygen consumption than those of control rats (table 1). This is probably due to the induction of Na,K-ATPase as described by Wannagat et al. after cholate feeding[6]. The other one relates to the increase in bile flow observed during retrograde perfusion (fig.). Mention of this phenomenon has also been made by Groothuis et al.[4]. The reason for it is unknown; its occurrence in the absence of exogenous bile acids suggests that it could be related to asymmetric distribution of ionic pumps; improved oxygenation of centrizonal hepatocytes is thought to be mainly responsible for formation of bile salt-independent canalicular bile flow[3].

In conclusion, our study has shown that expansion of the bile acid pool by cholate feeding alters the secretory characteristics of centrizonal hepatocytes, making them behave more like periportal hepatocytes, when challenged with a moderate taurocholate load. In the absence of exogenous bile acids, however, they secrete bile acids more sluggishly, perhaps secondarily to induction of cytosolic bile acid-binding proteins. Taken together, these two mechanisms could protect centrizonal hepatocytes from potentially toxic levels of bile acids.

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Immunohistochemical localization of glutamine synthetase in human liver

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Summary. Glutamine synthetase (GS) of human liver was recognized with a polyclonal antibody to pig brain GS, but failed to stain with an antibody against rat liver GS. Using the latter antibody GS of human liver was shown to be localized within small rings of 1 to 3 hepatocytes surrounding the terminal hepatic venules. This pattern was analogous to that seen in rat and mouse liver.
Key words. Ammonia detoxification; enzyme distribution; glutamine synthetase; human liver; immunohistochemistry.

The strikingly heterogeneous localization of glutamine synthetase (GS) within liver parenchyma of mammals, characterized by the exclusive expression of the enzyme protein within 7 to 8% of the hepatocytes arranged in small rings of 1 to 3 cells surrounding the terminal hepatic venules, was first shown for the rat [1-4] and later on also for the mouse [5]. Recently, a similar distribution of this enzyme was described for some other mammalian species, while the distribution of GS in uricotelic animals such as birds was found to be homogeneous [6]. These findings suggest that GS in ureotelic species, e.g. mammals, is restricted to a small population of hepatocytes which may act as scavenger cells for ammonia [1, 7]. Preliminary results indicated that this concept may hold for human liver [2]. In the present study we investigated in detail the distribution of GS in human liver using antibodies against different GS species. As demonstrated herein there seems to be a strong immunological difference between GS molecules from different sources.

Materials and methods. Small fragments of human liver from patients between 24 and 68 years of age (total of 17 specimens) were obtained during autopsy 8–20 h post-mortem or from surgical biopsies in association with liver transplantations. The histological appearance of all liver specimens was classified as normal, except three which showed some fatty infiltration. The fragments were either snap-frozen in liquid nitrogen or isopentane-cooled by dry ice or fixed by injection of 3.5% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4) into open vessels, whenever possible, as well as by immersion in the same solution for 1 to 3 h. They were then rinsed with PBS and embedded in paraffin. Further processing for immunofluorescence followed the procedure of Gebhardt and Mecke [1] and that for immunohistochemistry using the unlabeled peroxidase-antiperoxidase (PAP) technique was performed as described recently [8, 9]. Two pri-

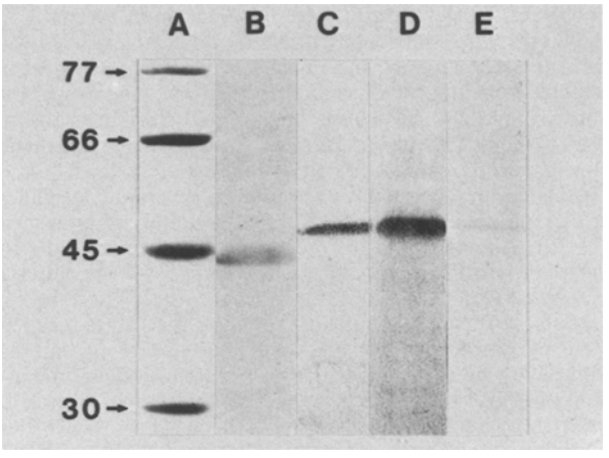

Figure 1. Immunoblotting of GS in homogenates from different sources with an antiserum against GS from pig brain. Lane A: marker proteins (staining with amido black); lane B: rat liver; lane C: rat kidney; lane D: rat brain; lane E: human liver.

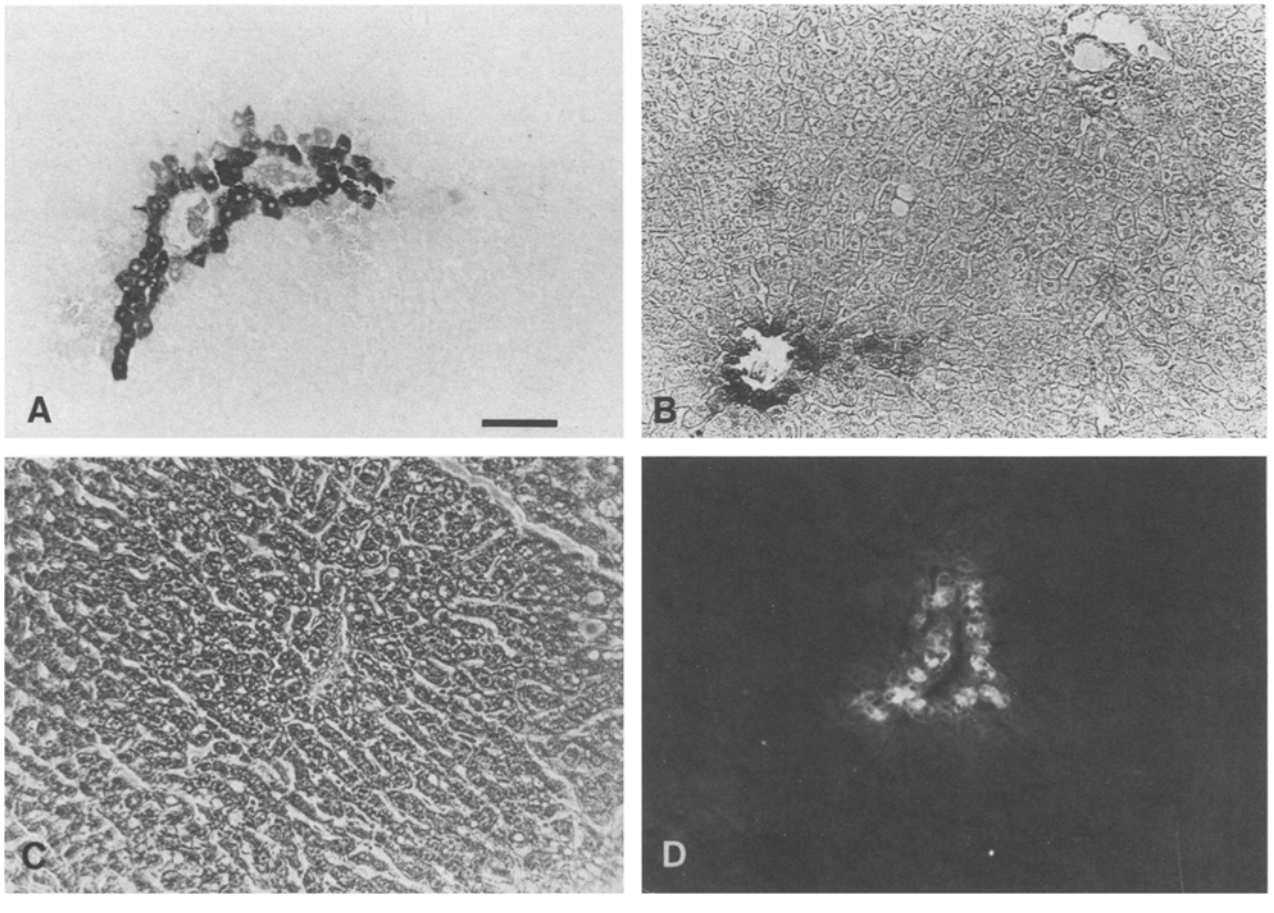

Figure 2. Immunohistochemical localization of GS in rat and human liver using an antiserum against GS from pig brain *A* PAP staining of rat liver (bright field). *B* PAP staining of a paraffin section of human liver, 33-year-old male (phase contrast). *C* Phase contrast micrograph of a cryotome section of human liver, 68-year-old male. *D* Fluorescence micrograph corresponding to (*C*). Scale bar = 50 µm.

Different recognition of GS from various sources by different antisera.

Species	Organ	Anti-GS-antiserum	
		Rat liver	Pig brain
Rat	Liver	+	+
	Kidney	−	+
	Brain	−	+
Mouse	Liver	+	+
	Kidney	−	+
Rabbit	Liver	+	+
Human	Liver	−	+

mary antibodies against GS were used. The first was raised against pig brain GS[1] and the second against rat liver GS prepared as described recently[9].

For Western blot analysis homogenates of unfixed human liver or of various tissues of rat, mouse, and rabbit were prepared in sample buffer, and aliquots of 50 to 70 µg protein were applied to discontinuous SDS-polyacrylamide gel electrophoresis according to Laemmli[10]. Electrophoretic transfer on nitrocellulose, saturation of unspecific binding sites and development with antibodies and peroxidase staining was performed according to Gebhardt and Schäfer-Degenhart[11].

Results and discussion. Two polyclonal antisera prepared against GS from either rat liver or pig brain showed a strikingly different organ and species specificity. The antiserum against the rat liver enzyme recognized GS only in the liver of rat, mouse and rabbit, but not of man, whereas the antiserum against the pig brain enzyme reacted with GS of different organs and all species studied including man (table). The latter antiserum also recognized GS from malignant human gliomas[12]. Western blot analysis (fig. 1) showed that the subunits of GS of rat liver had a M_r of 42,000 and those of GS of rat kidney and brain had M_r of 48,000 and 49,000, respectively. The subunits of GS of human liver displayed a M_r of 49,000. These results indicate that some molecular differences exist between the GS of rodent and human liver, resulting in a markedly different immunological response. Despite these differences the GS enzymes of mammalian liver and brain must share at least some immunological determinants responsible for their cross-reactivity with the proper antiserum. The results of the immunoblotting analysis exclude that this cross-reactivity is due to the presence of different subunits in the native enzyme octamer.

Immunohistochemical localization of GS in cryotome and paraffin sections of human liver using the cross-reacting antiserum against pig brain GS revealed the exclusive presence of GS in some few perivenous hepatocytes arranged in continuous rings, 1 to 2 cells thick, around the terminal hepatic venules (fig. 2). Although the intensity of the staining decreased to some extent if the liver material was taken after long post-mortem intervals, there was no change in the distribution of the enzyme. Furthermore, in livers from healthy patients the distribution was independent on the age, whereas in patients with various kinds of (even mild) liver diseases (e.g. alcoholic fatty liver, viral hepatitis, sarcoidosis) a tremendous loss of GS around the terminal hepatic venules was apparent[13].

The described localization of human liver GS is equivalent to that found for ureotelic mammals[1,6]. It is, therefore, highly conceivable that recently developed concepts of ammonia detoxification[4,7,14] and pH regulation[14,15] apply not only to some experimental animals such as the rat, but also to man. This assumption may bear important implications for certain kinds of liver diseases characterized by a disturbed localization of GS, e.g. liver cirrhosis[13], zonal liver damage[9] or hepatocarcinogenesis[16].

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