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Localization of Glutamine Synthetase in Adult and Fetal Liver of the Tree Shrew (*Tupaia belangeri*)

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ABSTRACT: The adult and prenatal tree shrew's liver consists of organized plates of hepatocytes, arranged as single cell layers separated by sinusoidal capillaries. Each hepatic lobule appears irregular in shape rather than hexagonal. Portal tracts, including those in prenatal animals, contain a distinct portal vein. Immunohistochemical analysis of hepatic tissues revealed a similar distribution of glutamine synthetase (GS) protein in both fetuses and adults. GS was absent from the periportal hepatocytes. The gene was strongly expressed in only a single, continuous layer of hepatocytes surrounding the central vein, expressed as a discontinuous, patchy pattern around the smaller sublobular veins, and was absent from the hepatocytes surrounding the thick-walled sublobar and hepatic veins. The changing expression pattern of GS around the sublobar veins suggests that the sinusoids near the wall of the larger efferent veins no longer drain to these vessels directly.

KEYWORDS: Tupaia, glutamine synthetase, liver, hepatocyte.

INTRODUCTION

Important functions of the mammalian liver are ammonia detoxification and glutamine homeostasis[1]. The bulk of ammonia is detoxified by the urea cycle, which is strategically localized in the upstream, periportal hepatocytes. The urea cycle has a high capacity for ammonia detoxification, but due to the low affinity for ammonia (~1-2 mM) of the first enzyme of the cycle, carbamoylphosphate synthetase, further clearance of the blood is necessary. The pericentral expression of the high-affinity enzyme glutamine synthetase (GS) (K_M ammonia ~ 0.01 mM) is therefore thought to be of crucial importance for effective elimination of ammonia. The distribution of GS is restricted to a few hepatocytes around the efferent venules[2, 3]. Ever since the acinar concept of liver structure was proposed more than 30 years ago[4], evidence has accumulated that the hepatocytes in different regions of the acinus may perform complementary (as in the case of ammonia detoxification) or even antagonistic functions (glycolysis as opposed to gluconeogenesis, lipid synthesis as opposed to lipid oxidation, etc.)[5, 6]

Immunohistochemistry has localized GS in one to three layers of pericentral hepatocytes in adults rats and man[6]. In the rat, GS appears in hepatocytes of the pericentral zone only shortly before birth[7], but in the spiny mouse, a precocial rodent, expression is detectable much longer before birth. In humans, on the other hand, GS could be demonstrated only after birth[8]. To investigate whether the human condition is an exception to the rule that a long gestational period is associated with early expression of GS, we studied its expression in the liver of both adult and fetal tree shrews as representatives of a primate relative. We found that GS is expressed relatively early in tree shrew development, suggesting that the late, perinatal expression of GS in human liver is exceptional.

MATERIALS AND METHODS

Common tree shrews, weighing 110-120 g, were purchased from Ayuttaya province in Thailand. Tree shrews carry 2-4 fetuses. The dam was anesthetized with diethyl ether. The body was perfused with 0.9% NaCl via a cannula that was inserted into the aorta. The right atrium was cut to allow the effluent to flow out. When the effluent was clear, 4% phosphate-buffered formaldehyde was perfused through the same cannula. The liver was dissected out and immersed in the same fixative overnight. Fetuses were dissected and fixed in 4% phosphate-buffered formaldehyde overnight. Livers were dehydrated in a graded series of ethanol and embedded in paraffin. Sections of 5 μm thickness were collected on poly-L-lysine-coated glass slides. The sections were boiled for 10 minutes in sodium citrate (10 mM, pH 6.0) to retrieve epitopes and to

inactivate endogenous phosphatase. After blocking nonspecific sites with 10% goat serum diluted in Teng-T (10 mM TrisHCl, pH 7.4, 5 mM EDTA, 150 mM NaCl, 0.25% gelatin, 0.05% Tween-20), GS monoclonal antibody #G45050 (0.1% in goat serum/Teng-T; Transduction Laboratories, Lexington, KY) was applied overnight at 20°C. After rinsing 3 times with 0.5 M Na-acetate, the sections were incubated for 1 hour at 20°C with goat anti-mouse IgG, conjugated with alkaline phosphatase (1% in goat serum/Teng-T). After rinsing 3 times with 0.5 M Na-acetate, the sections were incubated with 0.4 mg/ml NitroBlueTetrazolium chloride, 0.19 mg/ml 5-Bromo-4-Chloro-3-Indolyl Phosphate (NBT/BCIP) and 50 mM $MgSO_4$, 100 mM TrisHCl (pH 9.5) (Roche, Mannheim, Germany). Sections in which the primary antibody was omitted, served as negative controls. After dehydration in a graded series of ethanol, a coverslip was mounted using Permount.

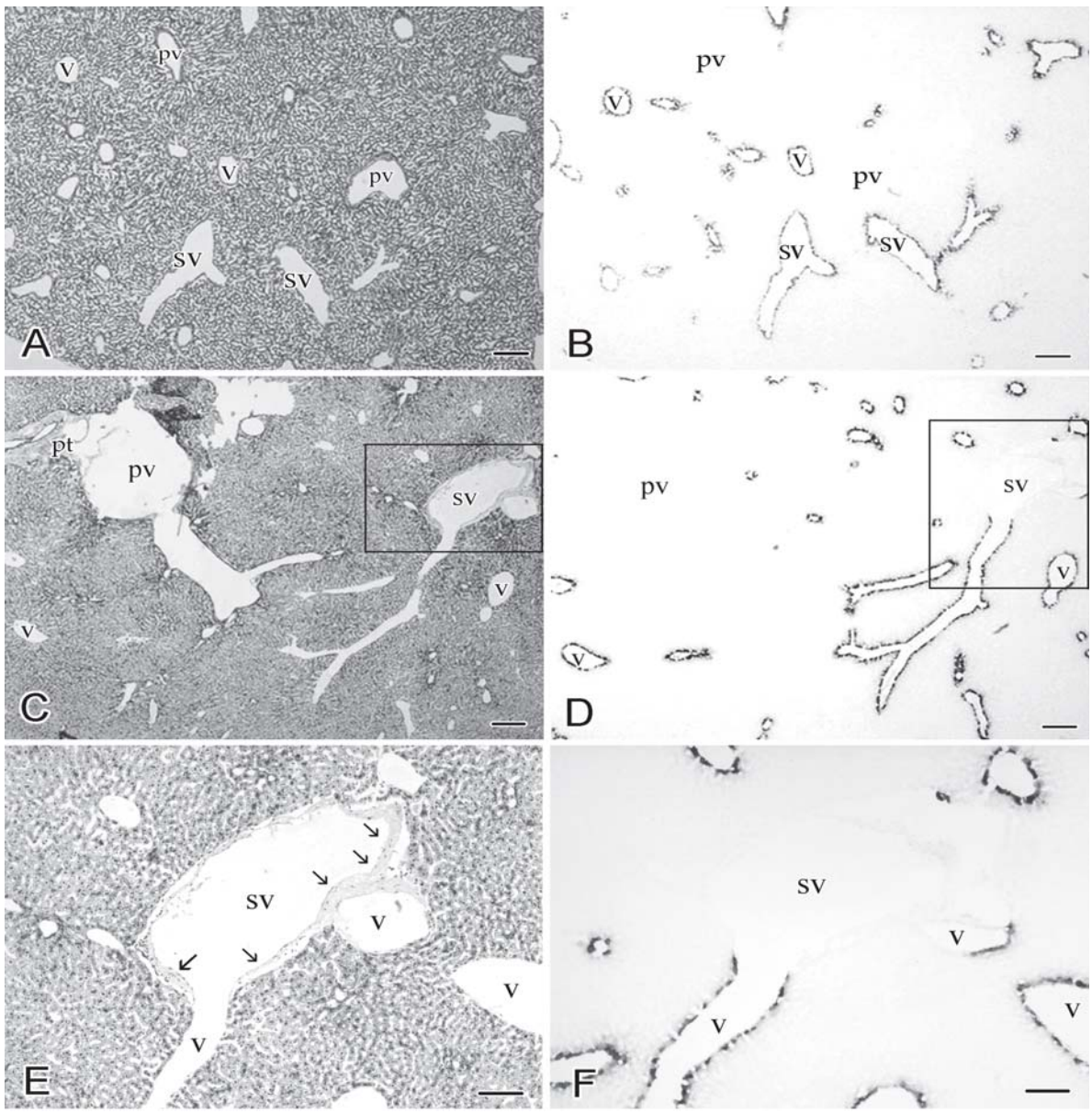

Fig 1. Glutamine synthetase expression in adult liver of Tupaia. Panels A,C and E were stained with H&E, whereas panels B, D and F are serial sections stained for the presence of glutamine synthetase. Panels E and F are a magnification of panels C and D (boxed area). Hepatocytes surrounding central (v) and small sublobular veins (sv) stain positive for GS proteins whereas hepatocytes surrounding the portal veins (pv) never express GS (panels B, D). The GS-positive hepatocytes are not present around the larger sublobar hepatic veins (sv) with a relatively thick wall (arrows) (panels C-F). Abbreviation: portal tract. Scale bars: 200 mm (A-D) and 100 mm (E-F).

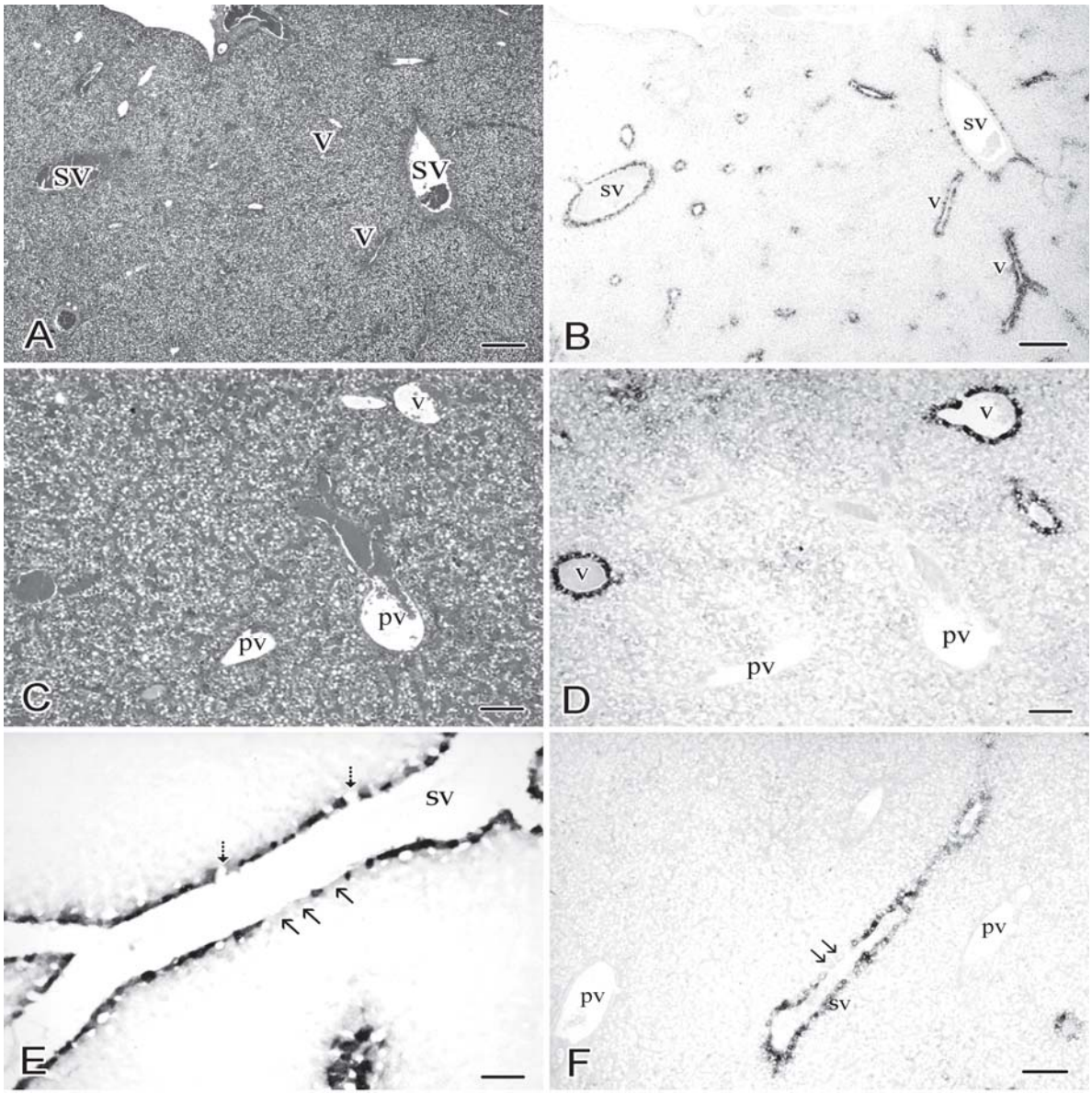

Fig 2. Glutamine synthetase expression in fetal liver of Tupaia. Panels A and C are stained with H&E, whereas panels B and D are serial sections stained for the presence of glutamine synthetase. Panel E and F are higher magnifications of GS-stained sections. The GS-positive hepatocytes surround the central vein (v) and small sublobular veins (sv), but some hepatocytes bordering these veins do not express GS yet (panels E and F, arrows; broken arrows identify sinusoids emptying into the vein). GS expression is not found around the portal veins (pv). Scale bars: 200 mm (A-B), 100 mm (C-D,F) and 50 mm (E).

Result

Adult Liver

The adult tree shrew's liver resembles the liver of other mammalian species in that it consists of parallel plates of hepatocytes that are separated by sinusoidal capillaries. Each plate is one-cell thick, however, the shape of liver lobule cannot be identified (Fig. 1A). The conducting portal vein is surrounded by a layer of smooth muscle. Each vein is bundled with the bile duct and hepatic artery to form the portal tract, which itself is largely formed by connective tissue (Fig. 1C). All portal blood is delivered to the lobule through the inlet venules that open into the sinusoids. The sinusoids, in turn, drained blood into a central vein, from which the blood was conducted into the sublobular vein and finally into the hepatic vein (Fig. 1C).

We found that GS was localized in a single layer of hepatocytes surrounding the central veins (Fig. 1B), but was absent from in the hepatocytes that surround

the larger sublobular hepatic veins (Fig. 1D). Veins that were surrounded by GS-containing hepatocytes were 30-50 μm wide central veins and collecting veins that were not more than 200 μm in diameter (Fig. 1D). Strikingly, only a single layer of hepatocytes was clearly positive (Fig. 1B), the staining in the adjacent second layer of hepatocytes being far less intensive. GS was not found in hepatocytes surrounding the sublobular veins with relatively thick walls (Fig. 1 E, F). Figure 1E, F shows a clear example of the drainage of a collecting vein that is surrounded by GS-positive hepatocytes into a large sublobular vein that is no longer surrounded by GS-positive hepatocytes.

Fetal Liver

In general, the fetal liver had a similar architecture as that of the adult tree shrew. Hepatocytes were also arranged in plates, each consisting of a single cell layer. As already noted for the adult liver, the shape of fetal liver lobule can not be identified either (Fig. 2A). The portal tract exhibited a distinct portal vein, which could be identified more easily than the hepatic artery and bile duct because the connective tissue around the portal tract was much less developed than that in adult liver (Fig.2C). The central vein and sublobular vein were filled with blood.

GS was identified in the layer of hepatocytes that directly borders the central veins (Fig. 2B). Interestingly, the levels of expression of GS in the positive cells varied, with nearly negative cells neighboring clearly positive ones (Fig. 2E, F).

Discussion

We have demonstrated in this study that GS was detectable in the hepatocytes around the efferent hepatic veins as a continuous single cell layer in both adult and fetal tree shrews. The expression pattern of this enzyme is, therefore, similar to that found in the livers of rat, mouse, pig, monkey and man[9]. In contrast to the narrow pericentral distribution in the mammalian liver, GS expression in avian and reptile livers shows no zonation but is present in all hepatocytes[2]. Our observation that GS-immunoreactive hepatocytes were prominent around the central and small sublobular veins, but absent from the larger branches of the sublobular hepatic veins with thicker walls (Fig. 1F) fits with similar findings in pigs and rats [3,9,10]. The result suggests that the hepatocytes that border the larger efferent hepatic vessels no longer represent the downstream part of their lobule, but drain, instead, into nearby central veins. The finding that scattered hepatocytes lining the central and sublobular veins in the fetal liver (Fig. 2E,F) are still in the process of accumulating GS shows that, in this respect, Tupaia liver also resembles rat and pig liver[7,9]. Apparently, GS expression requires a very pronounced downstream, pericentral environment. Both direct interaction between the hepatic venous endothelium and hepatocytes [11,12,13,14] and factors produced by upstream, periportal hepatocytes and secreted into the sinusoids that regulate liver cell heterogeneity [5,15,16] have been postulated to represent this downstream environment.

The abundant expression of GS in prenatal tree shrew liver differs markedly from the lack of GS expression in prenatal human liver[8], even though the two species are a primate relative and a primate, respectively. In this respect, tree shrews resemble mammals such as mice, rats and spiny mice that, even though their gestational period differs markedly, all express GS as soon as the hepatic veins can be identified as draining agglomerates of hepatocytes[17]. In fact, the first GS-expressing hepatocytes were identified around vessels that drain directly into the caval vein, that is, subsequently grow to form the largest branches of the hepatic vein[17]. These findings suggest that, as the veins increase in size when the liver grows and additional lobules are added[18], they are transformed from vessels with a sinusoidal function into vessels with a conducting function. Such larger vessels require stronger and, hence, thicker walls, that no longer allow for a direct interaction with the adjacent hepatocytes. Tree-shrews, which have a gestational period of 45-55 days[19], therefore seem to be an ideal species to compare liver development with that in the developing human, because of their relatively close phylogenetic relationship. They also appear to be very useful to compare liver development with rodents to establish the conditions that mediate discontinuation of GS expression as the central veins increase in size to become sublobular and lobar vein, because this feature is first seen prenatally in the tree shrew and only postnatally in rodents.

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References

1. Haussinger D, Lamers WH, Moorman AF (1992) Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* **46**: 72-93.
2. Smith DD and Campbell JW (1988) Distribution of glutamine synthetase and carbamoylphosphate synthetase I in vertebrate liver. *Proc Natl Acad Sci USA.* **85**: 160-4.
3. Lamers WH, Vermeulen JL, Hakvoort TB, Moorman AF (1999) Expression pattern of glutamine synthetase marks transition from collecting into conducting hepatic veins. *J Histochem Cytochem.* **47**:1507-12.

4. Rappaport AM, Borowy ZJ, Lougheed W, Lotto W (1954) Subdivision of hexagonal liver lobules into a structural and functional unit: Role in hepatic physiology and pathology. *Anat Rec* **119**:11-3.
5. Jungermann K and Katz N (1989) Functional specialization of different hepatocyte populations. *Physiol Rev* **69**: 708-64.
6. Lamers WH, Hilberts A, Furt E, Smith J, Jonges GN, Noorden CJF, Janzen JWG, et al., (1989) Hepatic enzymic zonation: a reevaluation of the concept of the liver acinus. *Hepatology* **10**: 72-6.
7. Lamers WH, Gaasbeek Janzen JW, Kortschot te A, Charles R and Moorman AFM (1987) The development of enzymic zonation in liver parenchyma is related to the development of the acinar architecture. *Differentiation* **35**: 228-35.
8. Moorman AFM, Vermeulen JLM, Charles R and Lamers WH (1989)
Localization of ammonia metabolizing enzymes in human liver: ontogenesis of heterogeneity. *Hepatology* **9**: 367-72.
9. Wagenaar GTM, Moorman AFM, Chamuleau RAFM, Deutz NEP, Gier CD, Boer PA, Verbeek FJ, et al., (1994) Vascular branching pattern and zonation of gene expression in the mammalian liver. A comparative study in rat, mouse, cynomolgus monkey, and pig. *Anat Rec* **239**:441-52.
10. Gaasbeek Janzen JW, Gebhardt R, ten Voorde GH, Lamers WH, Charles R, Moorman AFM (1987) Heterogenous distribution of glutamine synthetase during rat liver development. *J Histochem Cytochem* **35**:49-54.
11. Schols L, Mecke D, and Gebhardt R (1990) Reestablishment of the heterogeneous distribution of hepatic glutamine synthetase during regeneration after CCl_4-intoxication. *Histochemistry* **94**: 49-54.
12. Kuo FC and Darnell JE (1991) Evidence that interaction of hepatocytes with the collecting (hepatic) veins triggers position-specific transcription of the glutamine synthetase and ornithine aminotransferase genes in the mouse liver. *Mol Cell Biol* **11**:6050-8.
13. Gebhardt R, Burger H-J, Heini H, Schreiber K-L, Mecke D (1988) Alterations of hepatic enzymes levels and of the acinar distribution of glutamine synthetase in response to experimental injury in the rat. *Hepatology* **8**:822-30.
14. Gebhardt R (1990) Altered acinar distribution of glutamine synthetase and different growth response of cultured enzyme-position and enzyme-negative hepatocytes after partial hepatectomy. *Cancer Res* **50**:4407-10.
15. Gebhardt R, Gaunitz F, Mecke D (1994) Heterogeneous (positional) expression of glutamine synthetase: features, regulation and implications for hepatocarcinogenesis. *Adv Enzyme Regul* **36**:27-56.
16. Wagenaar GTM, Chamuleau RA, Haan JG de, Maas MA, Boer PA, Marx F, Moorman AFM, et al., (1993) Experimental evidence that the physiological position of the liver within the circulation is not a major determinant of zonation of gene expression. *Hepatology* **18**: 1144-53.
17. Notenboom RG, Moorman AFM, Lamers WH (1997) Developmental appearance of ammonia-metabolizing enzymes in prenatal murine liver. *Microsc Res Tech* **39**: 413-23.
18. Ekataksin W, Wake K (1991) Liver units in three dimensions. I. Organization of argyrophylic connective tissue skeleton in porcine liver with particular reference to the compound hepatic lobule. *Am J Anat* **191**:113-53
19. Martin RD (1968) Reproduction and ontogeny in tree-shrews (*Tupaia belangeri*), with reference to their general behavior and taxonomic relationships. *Z Tierpsychol* **25(5)**:505-32.