

Developmental Appearance of Ammonia-Metabolizing Enzymes in Prenatal Murine Liver

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ABSTRACT To resolve an apparent discrepancy in the developmental appearance of glutamine synthetase (GS) protein in rat [Gaasbeek Janzen et al. (1987) *J. Histochem. Cytochem.*, 35:49–54] and mouse [Bennett et al. (1987) *J. Cell Biol.*, 105:1073–1085] liver, we have investigated its expression during liver development in the mouse and compared it with that of carbamoylphosphate synthetase I (CPS). The expression of glutamate dehydrogenase was used as a marker to identify all hepatocytes in these strongly hematopoietic livers. GS protein accumulation starts in mouse hepatocytes at embryonic day (ED) 15. The first hepatocytes in which the enzyme accumulates were found around the major hepatic veins. CPS protein was found to accumulate in mouse hepatocytes from ED 13 onward: first, at the center of the median and lateral lobes, but temporarily not at the periphery of these lobes and not at the caudate lobe. The initial phase of accumulation of GS and CPS protein was characterized by a heterogeneity in enzyme content between hepatocytes. By ED 17, both enzymes were detectable in all hepatocytes at the center of the median and lateral lobes. This event marked the onset of the development of the complementary distribution of the enzymes typical of zonal heterogeneity in the adult mammalian liver. However, during the perinatal period, the pericentral hepatocytes temporarily accumulated CPS protein. We also observed heterochrony between species in the appearance of CPS protein in the small intestine. *Microsc. Res. Tech.* 39:413–423, 1997. © 1997 Wiley-Liss, Inc.

INTRODUCTION

Ammonia fixation and detoxification is one of the characteristic functions of the mammalian liver. We have recently reviewed the regulatory mechanisms that underlie the developmental appearance of ammonia-metabolizing enzymes within the liver (Dingemans and Lamers, 1995). In the present survey, we focus on the spatial and temporal expression patterns of these enzymes in prenatal murine liver. Such an analysis would provide insight into (1) the sequential aspects of hepatocyte maturation and (2) the relationship between the development of a mature liver architecture and the appearance of metabolic zonation.

Ammonia metabolism is closely linked to amino acid metabolism. The finding that the three major ammonia-metabolizing enzymes in liver, glutamate dehydrogenase (GDH) [EC 1.4.1.3], carbamoylphosphate synthetase I (CPS) [EC 6.3.4.16], and glutamine synthetase (GS) [EC 6.3.1.2], have a distinct timing for their appearance in prenatal hepatocytes suggests that their functional roles may not be closely linked. GDH is expressed in rat hepatocytes as soon as they differentiate from the embryonic foregut (Gaasbeek Janzen et al., 1988; Moorman et al., 1990; Westenend et al., 1986). Because this expression coincides with the development of oxidative metabolism (Shepard et al., 1970), it is likely that GDH activity is required for deamination. In contrast, the expression of ornithine cycle enzymes in hepatocytes, including CPS, starts relatively late during rat and mouse liver development but may vary between mammalian species. In rat and mouse hepato-

cytes, CPS expression is not observed until the beginning of the fetal period (Dingemans et al., 1996; Gaasbeek Janzen et al., 1988; Moorman et al., 1990; Morris et al., 1989). However, in hepatocytes of human embryos, the expression of this enzyme begins shortly after the liver has differentiated from the embryonic foregut (Dingemans and Lamers, 1994; van Roon et al., 1990). These interspecies differences in the developmental appearance of ornithine cycle enzymes in the liver are probably related to the intrauterine growth rate of the embryo (Dingemans and Lamers, 1994; Meijer et al., 1990), which occurs earlier in development in species with a slow growth rate (human) than in species with a relatively rapid growth rate (rat and mouse). Species with a rapid prenatal growth rate apparently use the available amino acids almost exclusively for protein accretion, whereas species with a slower prenatal growth rate might oxidize amino acids to a greater extent for the generation of energy. Observations in several species, including the human, indicate that the developmental appearance of hepatic GS protein is a birth-associated event (Dingemans and Lamers, 1994; Gaasbeek Janzen et al., 1987; Lamers et al., 1987; Moorman et al., 1989b; Shiojiri et al., 1995).

This conclusion, however, contradicts the results of Bennett et al. (1987) that GS can be demonstrated

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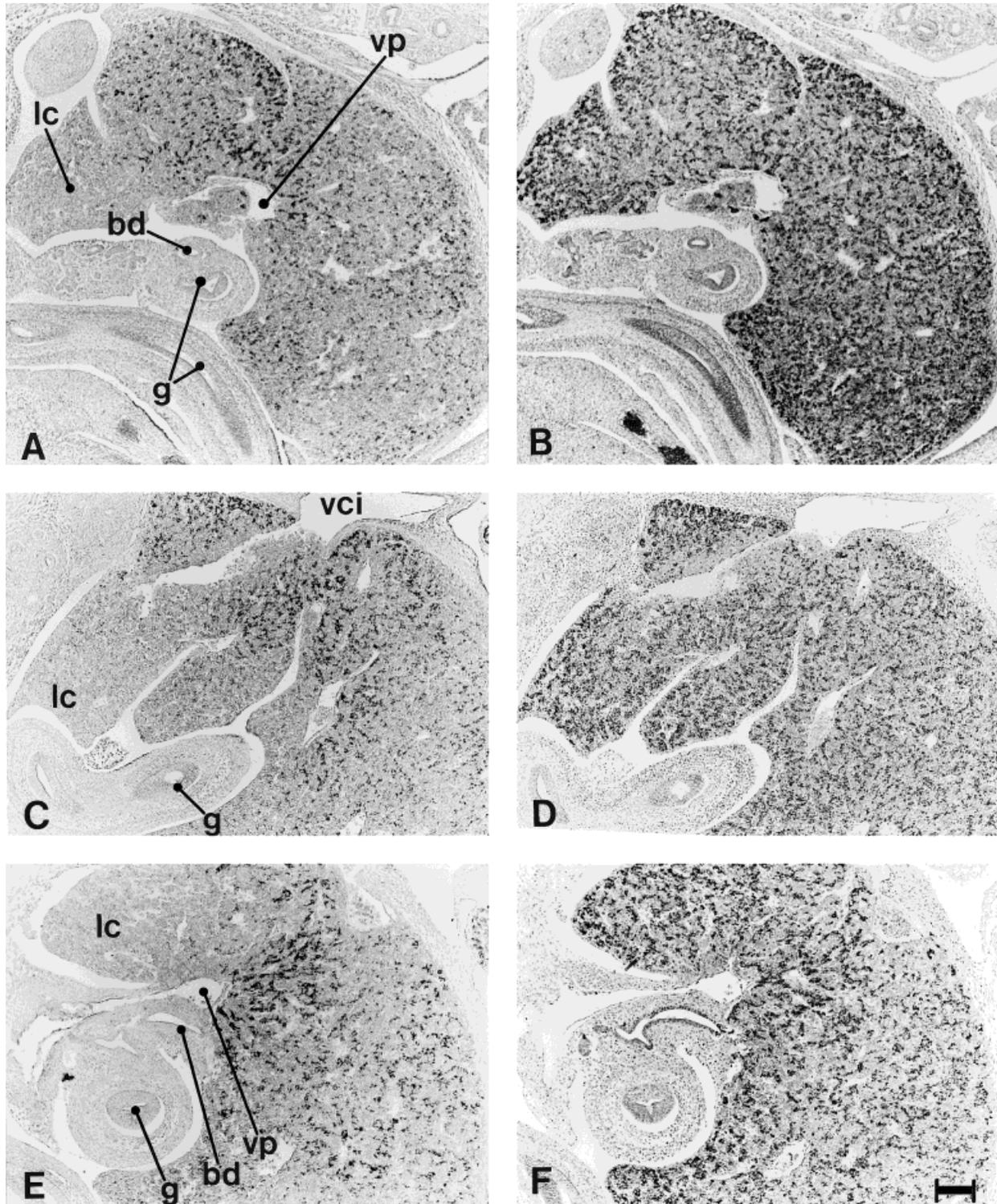


Fig. 1. Regional distribution of CPS (A,C,E) and GDH (B,D,F) protein in sagittal sections of the liver of two ED 13 Swiss mouse embryos. A-B, C-D, and E-F are adjacent serial sections. Dorsal is toward the top and anterior towards the right. The top panels are paramedian sections, the sections in the middle panels pass through

the inferior caval vein (vci), and the sections in the lower panels pass through the portal vein (vp). Note the sparse, median distribution of CPS-positive hepatocytes and the virtual absence of staining in the caudate lobe (lc); all hepatocytes are positive for GDH. Also note that the gut (g) is negative for CPS. bd, bile duct. Bar, 200 μ m.

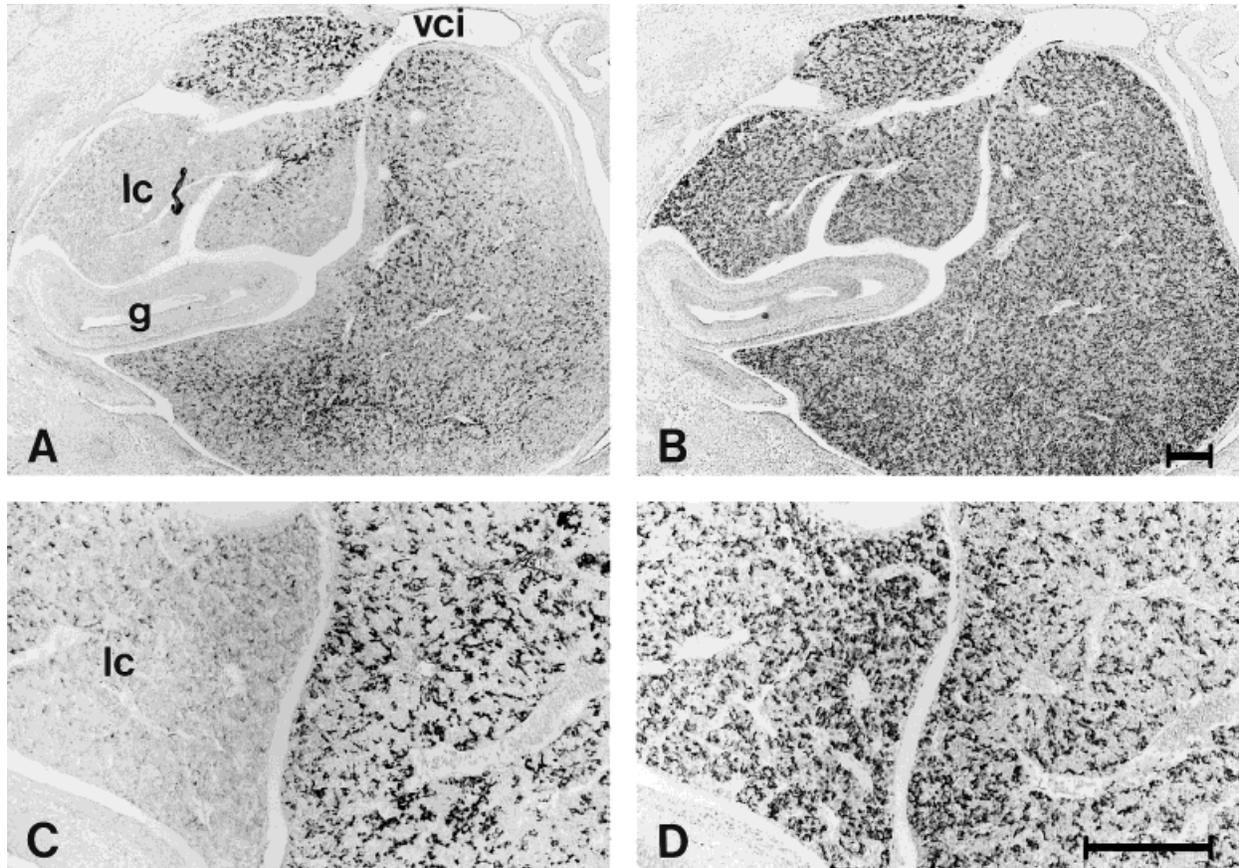


Fig. 2. Regional distribution of CPS (A,C) and GDH (B,D) proteins in sagittal sections of the liver of two ED 14 Swiss mouse embryos. A–B and C–D are adjacent serial sections. Dorsal is toward the top and anterior toward the right. The top panels are sections that pass through the inferior caval vein (vci). The bottom panels show a

magnification of the caudate and median lobe. Note the slight increase in the number of CPS-positive hepatocytes, except for the caudate lobe (lc). All hepatocytes are positive for GDH. Also note that the gut (g) is still CPS negative (A). Bars, 200 μ m.

immunohistochemically in mouse hepatocytes as early as embryonic day (ED) 15. The observation that GS mRNA can already be detected in substantial quantities in the liver of early mouse (Kuo et al., 1988) and rat embryos (Moorman et al., 1990) raised additional questions concerning the generality of the conclusion that GS protein appears late in prenatal development or concerning the antisera and/or histological methods used to demonstrate the intrahepatic presence of GS. Therefore, we obtained the antiserum used by Bennett et al. from Dr. R.E. Miller (Miller et al., 1978) and compared its staining properties in mouse liver with that of the antiserum raised against pig-brain GS (Gebhardt and Mecke, 1983) that we previously used to study the developmental appearance of GS in rat liver (Gaasbeek Janzen et al., 1987).

HETEROGENEOUS DISTRIBUTION OF AMMONIA-METABOLIZING ENZYMES IN NORMAL MOUSE AND RAT LIVER

The heterogeneous distribution pattern of GS and CPS in adult mouse liver (see also Bennett et al., 1987; Kuo et al., 1988; Shiojiri et al., 1995; Smith and Campbell, 1988) is comparable to that in rat liver (Gaasbeek Janzen et al., 1984; Gebhardt and Mecke,

1983; Moorman et al., 1988), in which GS occupies a 1- to 2-hepatocyte-thick layer around the central (efferent) veins and CPS a complementary 9- to 12-hepatocyte-thick layer around the portal (afferent) veins. Similar to the rat (Lamers et al., 1988), the cellular concentration of GDH is highest in hepatocytes of the pericentral zone and gradually decreases toward the portal vein.

DISTRIBUTION OF AMMONIA-METABOLIZING ENZYMES IN THE DEVELOPING MOUSE LIVER AND GUT

Glutamate Dehydrogenase

As observed previously in the rat (Gaasbeek Janzen et al., 1988), GDH protein is expressed in all hepatocytes of the embryonic mouse liver (Figs. 1–4). This mitochondrial enzyme is, therefore, an excellent marker to visualize the topographic distribution of hepatocytes in prenatal livers when hepatocytes are intermingled with numerous hematopoietic foci. At ED 19, that is, just before birth, expression of GDH protein becomes restricted to the pericentral hepatocytes (not shown).

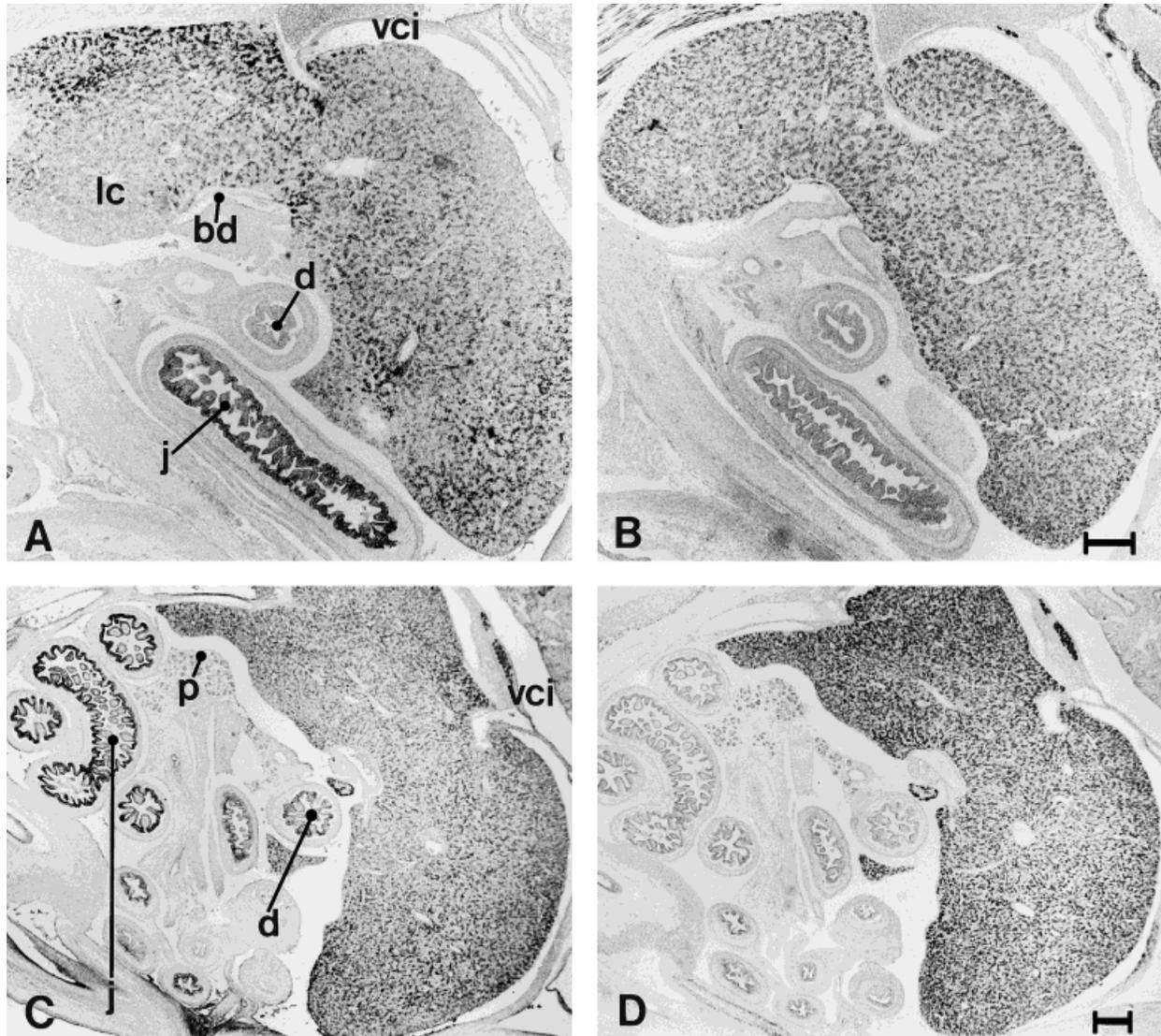


Fig. 3. Regional distribution of CPS (A,C) and GDH (B,D) proteins in sagittal sections of the liver of an ED 15 (A,B) and an ED 16 (C,D) Swiss mouse embryo. A–B and C–D are adjacent serial sections. Dorsal is toward the top and anterior toward the right. The top panels are sections that pass through the inferior caval vein (vci), and the sections in the lower panels are slightly more medial and oblique. Note the increase in the number of CPS-positive hepatocytes in the entire

liver (A,C), although the caudate lobe (lc) is still less intensely stained (A). All hepatocytes are positive for GDH. Also note that the gut has now become positive for CPS. The strongest staining is observed at ED 15 (A) in the jejunum (j) and notably less in the duodenum (d); at ED 16 (C), this difference in staining has disappeared. The staining for GDH in the gut is less intense than that for CPS. bd, bile duct; p, pancreas. Bars, 200 μ m.

Glutamine Synthetase

Similar staining patterns were obtained with the Miller and the Gebhardt antisera. A comparison of the staining properties of the Miller and Gebhardt antisera is shown in Figure 5. However, we found a notably higher degree of background staining with the Miller antiserum than with the Gebhardt antiserum. As development progressed, this background staining gradually decreased in intensity and virtually disappeared at birth. This result suggests that the Miller antiserum recognizes additional proteins or isoenzymes in prenatal mouse liver that may or may not be related to GS. Definitive proof for this would require Western blot analysis.

Regional (interlobular) heterogeneity. A marked regional heterogeneity could be observed in the distribution of hepatocytes in which GS protein first accumulates at ED 15. In general, these GS-positive cells appear to be associated topographically with the distribution of the major branches of the hepatic veins. In particular, the hepatocytes near the intrahepatic part of the inferior caval vein were distinctive due to their local accumulation and intense staining (Fig. 6). These pronounced regional differences in GS protein accumulation had disappeared by ED 16 (not shown). The timing of the appearance of GS protein in the caudate lobe was not different from that elsewhere in the liver.

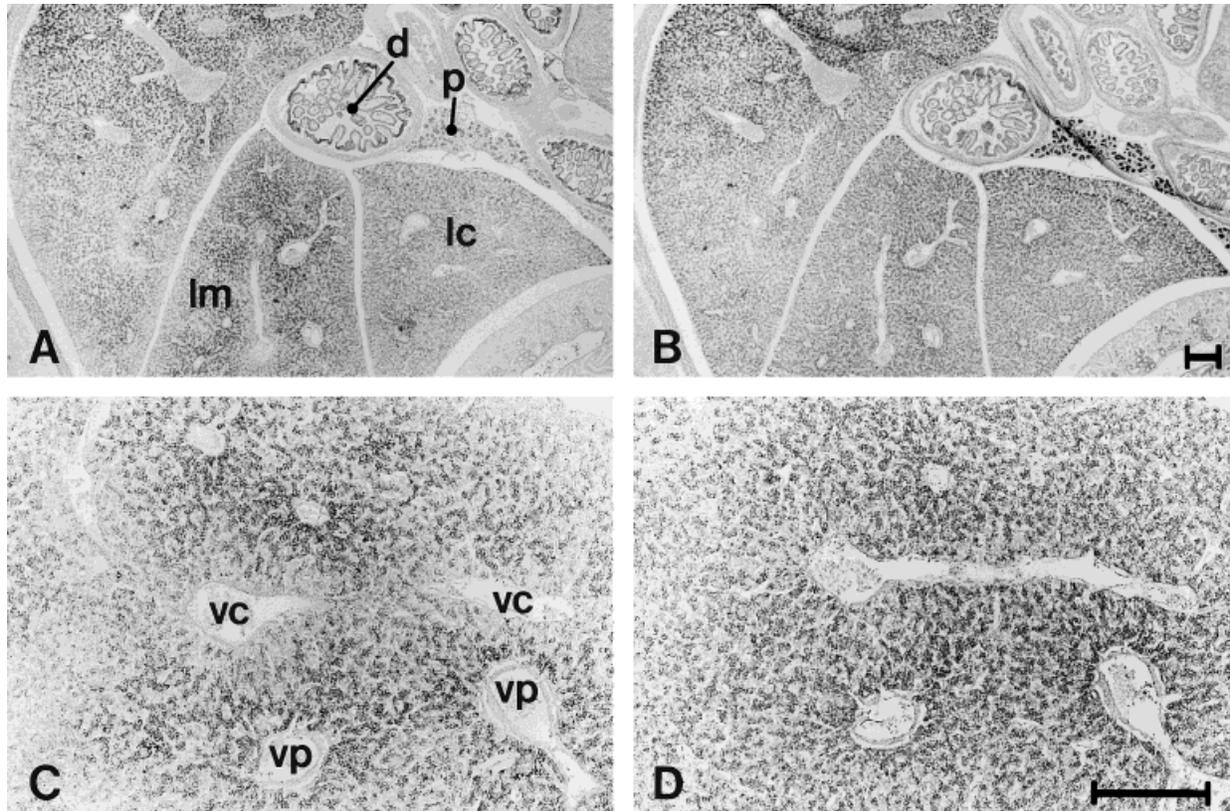


Fig. 4. Regional and intralobular distribution of CPS (A,C) and GDH (B,D) proteins in sagittal sections of the liver of an ED 17 Swiss mouse fetus. A–C are adjacent serial sections, and D is a higher magnification of B. The top panels are sections that pass through the median and caudate lobes and through the duodenum (d). Dorsal is toward the bottom and anterior toward the left. The bottom panels

show a magnification of the median lobe (lm). Note the first appearance of zonal interhepatocyte heterogeneity in the staining intensity for CPS (A,C), which is least pronounced in the caudate lobe (lc) (A). All hepatocytes are still positive for GDH. p, pancreas; vc, central vein; vp, portal vein. Bars, 200 μ m.

Intercellular (intralobular) heterogeneity. GS protein is first detected in hepatocytes at ED 15 (Fig. 6). The number of hepatocytes accumulating this enzyme then further increases with development (Figs. 5, 7). By ED 18, most hepatocytes are stained by the antisera (Fig. 7). At this stage, a lobular heterogeneity in enzyme content, which is complementary to that of CPS, becomes discernable. This heterogeneity becomes gradually more pronounced so that by the end of the first postnatal week GS staining is confined to a layer of two to three hepatocytes surrounding the central veins (Fig. 7). The establishment of the adult distribution pattern of GS is, therefore, not interrupted perinatally as with CPS (see next section).

Gut. The small intestine, the bile, and the pancreatic ducts are negative for GS during the entire prenatal period.

Carbamoylphosphate Synthetase I

Regional (interlobular) heterogeneity. Similar to our previous observations in the rat (Gaasbeek Janzen et al., 1988), the first accumulation of CPS protein is observed mainly in hepatocytes at the center of the liver (Fig. 1). The accumulation of the enzyme then gradually spreads toward the peripheral parts of the median and the right and left lateral lobes. By ED 17, these peripheral areas have also become positive. In contrast

to the developmental appearance of CPS protein in the median and lateral lobes, the enzyme is virtually absent from the subcapsular zones of the liver, including the entire caudate lobe, until ED 16 (Figs. 1–3). In addition, the development of the typical intralobular heterogeneity is delayed a few days in this lobe (Fig. 4).

Intercellular (intralobular) heterogeneity. CPS protein is first detected in hepatocytes at ED 13, albeit in a small number of hepatocytes that are located mainly at the center of the liver (Fig. 1). As with GS (see previous section), the number of hepatocytes accumulating this enzyme gradually increases during further development (Figs. 2, 3; compare the staining pattern of CPS with that of GDH; see also Gaasbeek Janzen et al., 1988). By ED 16, most hepatocytes of the median and lateral lobes have become CPS positive (Fig. 3). This maturation of the hepatocytes in a large part of the liver is marked by a decline in the cellular concentration of CPS protein in the hepatocytes surrounding the central veins (Fig. 4), that is, by the appearance of the adult distribution pattern of the enzyme. Toward birth and during the first 2 postnatal weeks, this intercellular heterogeneity in enzyme content temporarily disappears (Figs. 7, 8) but is firmly reestablished at weaning, which is comparable to the situation in the rat (Gaasbeek Janzen et al., 1985).

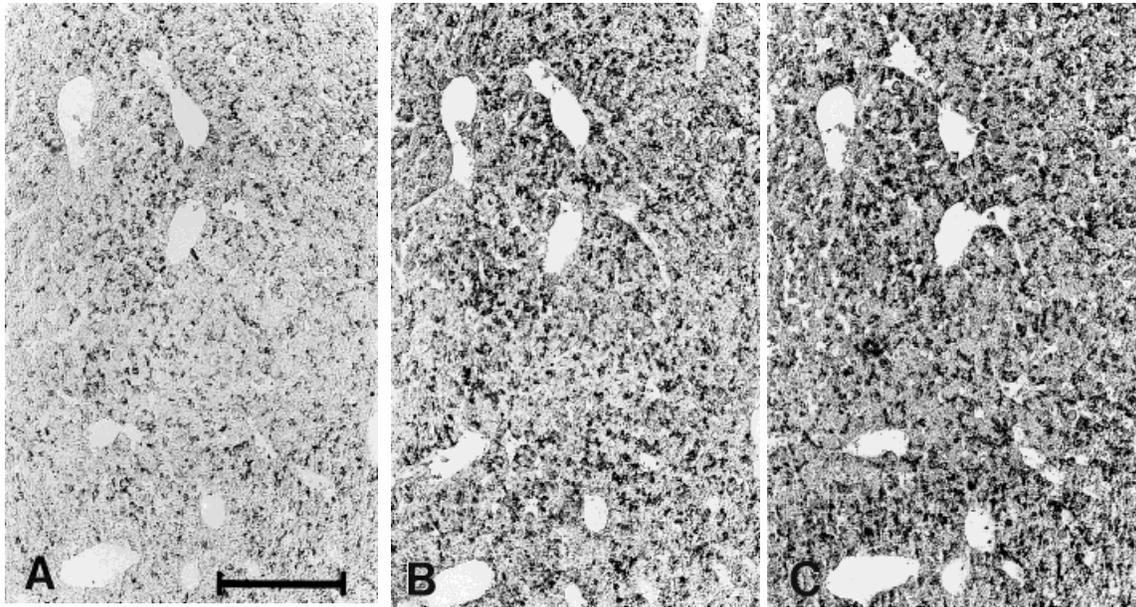


Fig. 5. Distribution of GS (A,B) and CPS (C) proteins in serial sections of the liver of an ED 16 Swiss mouse embryo. A: Gebhardt antiserum. B: Miller antiserum. Bar, 200 μ m.

Gut. The small intestine, particularly the jejunum, is positive for CPS from ED 15 onward (Figs. 3, 4). During the next 2 days, the duodenum and the ileum also become positive. Nevertheless, a proximal-to-distal gradient in cellular enzyme concentration remains present in the ileum. The bile and the pancreatic ducts are always negative (Fig. 3); thus, the junction with the duodenum becomes well demarcated.

DISTRIBUTION OF AMMONIA-METABOLIZING ENZYMES IN THE DEVELOPING RAT LIVER AND GUT

We have previously described the overall picture of the appearance of CPS and GS mRNA and protein in rat liver and gut (Gaasbeek Janzen et al., 1986, 1987, 1988; Moorman et al., 1990). However, in these studies, the presence of a regional heterogeneity in the appearance of ammonia-metabolizing enzymes was not clearly delineated. Figure 9 shows that in rat embryonic liver, as in the mouse (see previous section), the subcapsular zones and the caudate lobe are also distinct in that the accumulation of CPS mRNA is markedly retarded. At this stage (i.e., ED 20), GS mRNA begins to accumulate in the pericentral hepatocytes.

PRENATAL REGULATION OF GLUTAMINE SYNTHETASE EXPRESSION

This survey has focused on the developmental appearance of ammonia-metabolizing enzymes in mouse liver. The major reason for this analysis was to determine whether mouse and rat differ in their developmental timing of the expression of hepatic GS protein. Previous *in situ* hybridization studies (Kuo et al., 1988; Moorman et al., 1990) have revealed a strong and homogeneous expression of GS mRNA in the liver of early mouse and rat embryos. Moorman et al. (1990) showed that GS mRNA is expressed in rat hepatocytes from ED 13 (comparable to \sim ED 11.5 in the mouse; see Butler

and Juurlink, 1987 for comparative time tables of embryonic development) onward. However, using immunohistochemistry, we could not demonstrate GS protein in rat hepatocytes until ED 20 (Gaasbeek Janzen et al., 1987), whereas Bennett et al., (1987) reported that mouse hepatocytes already contain immunohistochemically detectable amounts of GS at ED 15 (comparable to \sim ED 16.5 in the rat). It should be noted that Kuo et al. (1988) did not study mouse embryos before this stage. Our analysis showed that GS protein starts to accumulate in mouse hepatocytes at ED 15. The same results were obtained regardless of whether the Miller or Gebhardt antiserum was used, although the Gebhardt antiserum produced a lower background in prenatal mouse liver. Our data are in agreement with those of Darnell and colleagues (Bennett et al., 1987), but in contrast to a more recent study (Shiojiri et al., 1995) which reports that GS immunoreactivity is not observed in mouse liver until 2–3 days after birth. This discrepancy apparently depends upon the effective antigen concentration of GS and its sensitivity for the fixation protocol. Preliminary experiments (Notenboom et al., unpublished data) indeed show that addition of glacial acetic acid to the fixation medium dramatically diminishes immunohistochemical staining for GS in prenatal hepatocytes. These experiments also revealed that GS protein can be detected in rat hepatocytes at ED 17–18, that is, 2–3 days earlier than previously recognized (see Gaasbeek Janzen et al., 1987), provided that acetic acid is omitted during tissue fixation. Notwithstanding, the accumulation of GS protein in fetal hepatocytes following the onset of transcription appears to be delayed in both species. Since our technique reveals steady-state levels of mRNAs and proteins, the reason for this delay can be either a poor translational efficiency or instability of the protein in the early embryonic liver (cf. our discussion in de Groot et al., 1986, 1987).

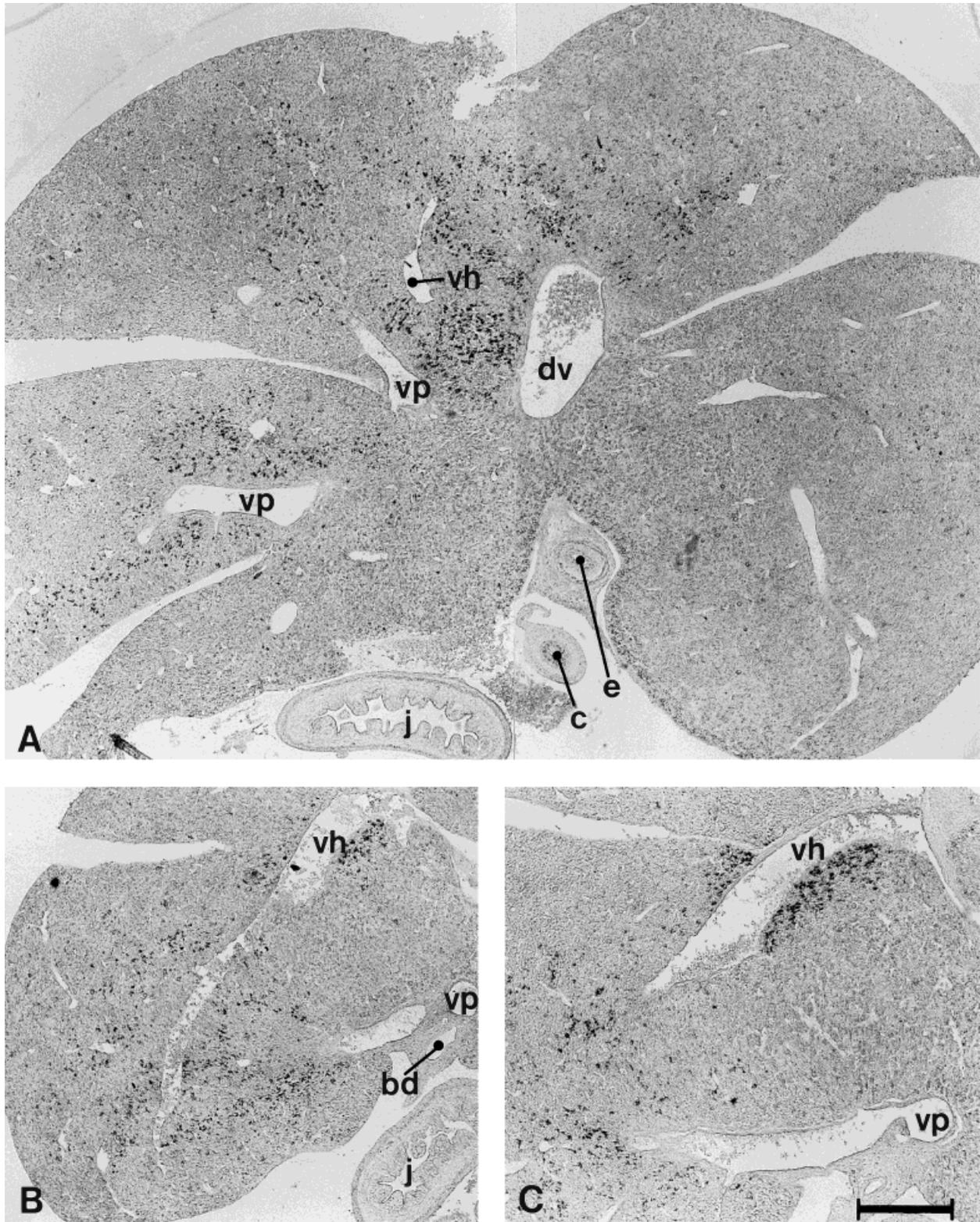


Fig. 6. Regional distribution of GS protein in sagittal sections of the liver of two ED 15 Swiss mouse embryos stained with the Gebhardt antiserum. **A** shows a section through the middle of the liver, and **B** shows a section through the hepatic vein (vh) just before it

drains into the inferior caval vein. **C** shows a higher magnification of a section near that of **B**. bd, bile duct; c, colon; dv, ductus venosus; e, esophagus; j, jejunum; vp, portal vein. Bar, 200 μ m.

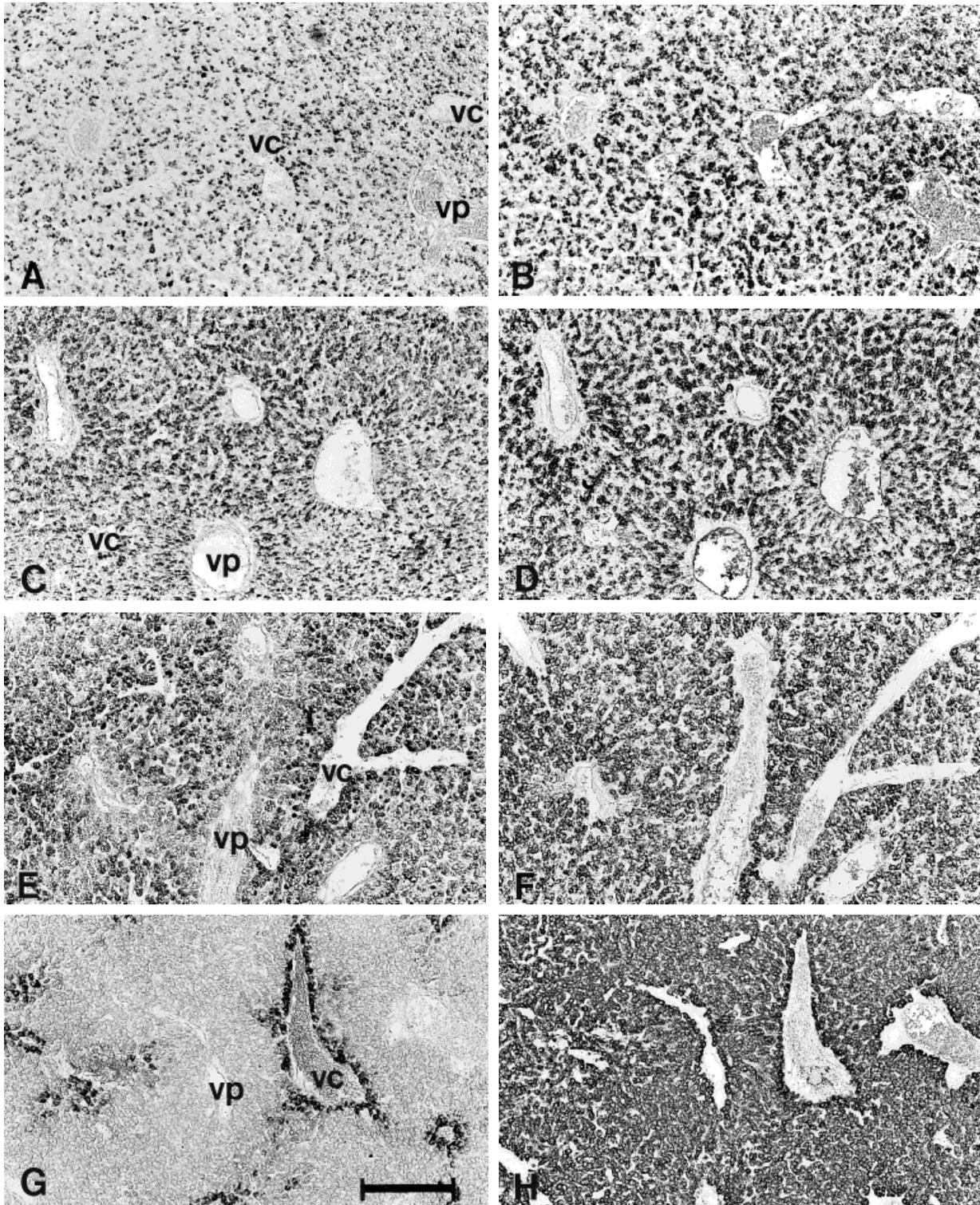


Fig. 7. Intralobular distribution of GS (A,C,E,G) and CPS (B,D,F,H) proteins in serial sections of the liver of an ED 17 (A,B), an ED 18 (C,D), an ED 19 (i.e., perinate) (E,F) Swiss mouse fetus, and a 8-day-old postnatal mouse (i.e., neonate) (G,H). Panels A,C,E,G: Miller antiserum. vc, central vein; vp, portal vein. Bar, 200 μ m.

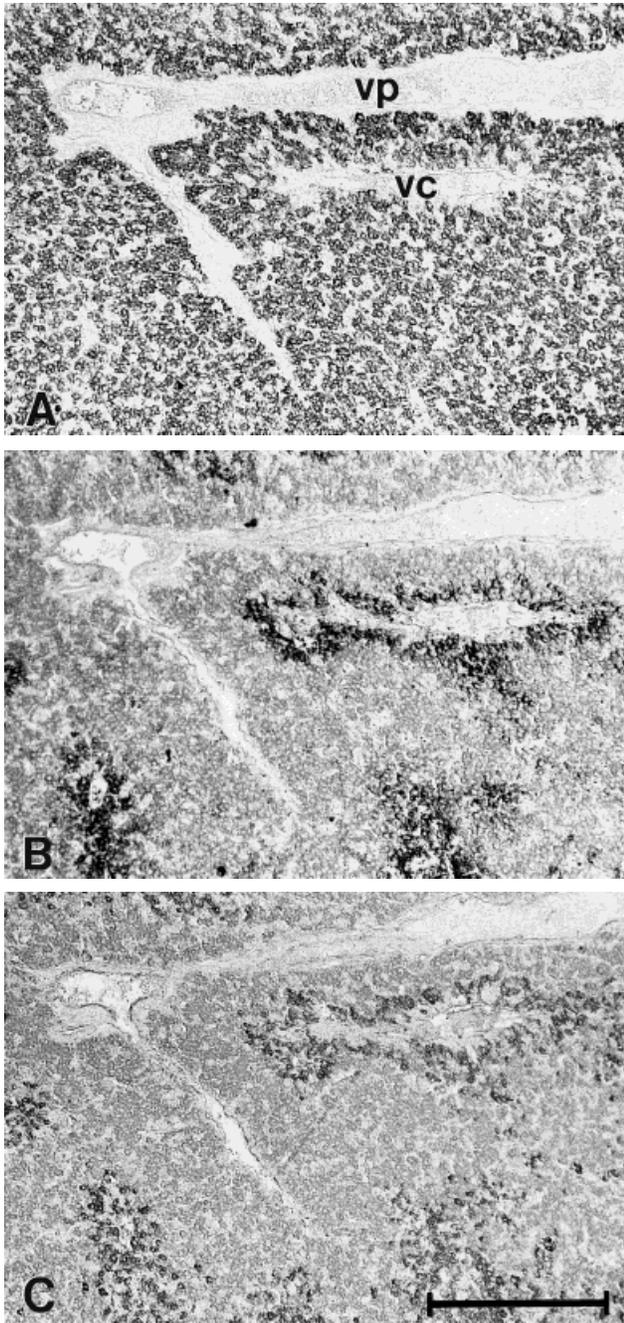


Fig. 8. Intralobular distribution of CPS (A), GS (B) and GDH (C) proteins in serial sections of the liver of a 1-day-old postnatal (i.e., perinate) Swiss mouse. Note the near-homogeneous staining of hepatocytes for CPS and the pronounced heterogeneity for GS and for GDH. vc, central vein; vp, portal vein. Bar, 200 μ m.

REGIONAL DIFFERENCES IN THE ONSET OF EXPRESSION OF CARBAMOYLPHOSPHATE SYNTHETASE I AND GLUTAMINE SYNTHETASE

The asynchronous regional accumulation of CPS and GS protein within the liver is striking. Because in mouse and rat (Gaasbeek Janzen et al., 1988; Moorman

et al., 1990) embryonic liver the distribution pattern of CPS mRNA and protein is the same, this heterogeneity is regulated at the pretranslational and, hence, probably at the transcriptional level. The distribution pattern of GS mRNA, however, is homogeneous in mouse and rat embryonic liver (Kuo et al., 1988; Moorman et al., 1990) suggesting that the differential accumulation of GS protein depends on a posttranscriptional level of regulation (see also de Groot et al., 1987).

The developmental appearance of CPS protein in mouse and rat liver appears to be strictly topographical: central versus peripheral portions of the lobes and medial plus lateral lobes versus caudate lobe. In contrast, GS protein accumulation in mouse liver is initially limited to the hepatocytes surrounding the larger hepatic veins. We also observed such a distribution pattern during the prenatal accumulation of GS protein in spiny mouse liver, even though the development of the architecture of these livers is more advanced (Lamers et al., 1987). These regional differences in the onset of enzyme accumulation can be due to a lack of inducing factors or the presence of repressing factors. The inability to stimulate CPS expression in hepatocytes of cultured intact rat embryos by hormones and the instantaneous expression of this enzyme after hormonal stimulation of the same hepatocytes with explantation into primary culture points to the presence of repressing factor(s) rather than to a lack of inducing factor(s) in such embryos (Westenend et al., 1986). As stated before, a posttranscriptional level of regulation must be responsible for the accumulation of GS protein. No candidates for these presumably novel regulatory factors have been identified so far.

We have evaluated the possibility that local differences in cell-cycle kinetics are responsible for differences in gene expression or protein accumulation. However, the initial accumulation of CPS protein in embryonic hepatocytes was found to proceed independently of the position of the cell in the cell cycle (van Roon et al., 1989a). Thus, this finding argues against a role for the cell cycle. Differences in the timing of the developmental appearance of enzymes, possibly resulting from differences in the source of the lobar perfusate [i.e., umbilical vs. portal (systemic) blood], have been described for the left and right lobes of the fetal liver (Chianale et al., 1988). However, the caudate lobe differs from the other liver lobes in that it is supplied with blood by both these vessels (Couinaud, 1957). Thus, the source of the blood supply also seems to be a less likely explanation for the observed regional heterogeneity in gene expression.

DEVELOPMENT OF A COMPLEMENTARY DISTRIBUTION OF CARBAMOYLPHOSPHATE SYNTHETASE I AND GLUTAMINE SYNTHETASE

In the present survey, we have found that the typical distribution of CPS and GS around the afferent and efferent vessels, respectively, develops as soon as all hepatocytes within a lobule have started to accumulate both proteins. Our *in vitro* studies have shown that, with hormonal stimulation the initial accumulation of hepatocyte-specific proteins occurs stochastically (van Roon et al., 1989b). This accumulation seems to be caused by an initially discontinuous transcription of the

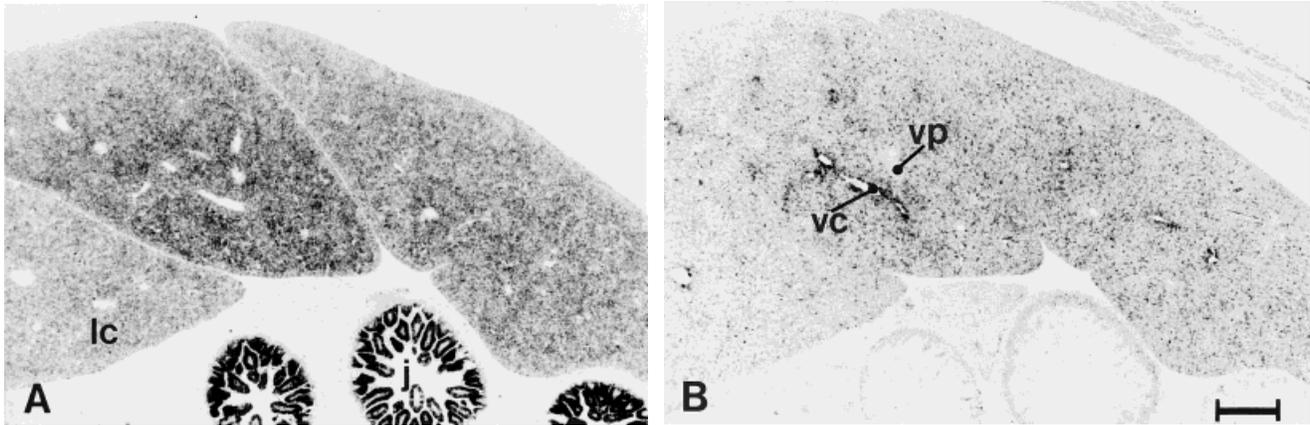


Fig. 9. Regional and intralobular distribution of CPS (A) and GS (B) mRNAs in serial sections of an ED 20 Wistar rat fetus. Note the marked difference in signal intensity for the CPS cDNA probe of the subcapsular zones and the caudate lobe (lc) vs. the rest of the liver, and

the extremely intense hybridization signal in the enterocytes of the jejunum (j). The accumulation of GS mRNA transcripts in the pericentral hepatocytes of rat liver can first be demonstrated at this stage. vc, central vein; vp, portal vein. Bar, 200 μ m.

responsible genes, which is no longer observed in mature hepatocytes (Dingemans et al., 1994). This condition may also exist during the initial accumulation of GS protein. Thus, the acquisition of the capacity to coordinately and simultaneously accumulate cell-specific proteins by hepatocytes indeed appears to be a distinct landmark on the pathway to maturation. Our observations suggest that the presence of such a degree of maturation is a prerequisite for the establishment of the zonal heterogeneity in enzymic phenotype, which is so characteristic of all adult mammalian livers. Transplantation studies have shown that this developmental phenomenon coincides with that of the formation of lobules, the architectural units of the liver (Notenboom et al., 1996). Regardless, the finding of a complementary distribution of enzymes in prenatal liver, like that observed in the adult, is indicative of a prenatal initiation of hepatic involvement in ammonia metabolism.

The findings in the mouse support our previous conclusion that the development of zonal heterogeneity proceeds independently of the process of birth (Lamers et al., 1987). This conclusion was based on a comparison of hepatic development in altricial (rat) and precocial (spiny mouse) rodents. It remains to be established as to why zonal heterogeneity of CPS is more pronounced in prenatal mouse liver than in prenatal rat liver (Gaasbeek Janzen et al., 1988). The question of why accumulation of CPS protein resumes perinatally in the pericentral hepatocytes of altricial species (mouse and rat) (present review; Gaasbeek Janzen et al., 1985, 1988) but not in those of a precocial species (the spiny mouse) (Lamers et al., 1987) also needs clarification. Furthermore, it is unclear as to why the periportal hepatocytes of the mammalian liver *in situ* never accumulate GS. We have previously ascribed the loss of the capacity of hepatocytes to express GS or CPS in response to stimulating signals to the establishment of compartments of gene expression (Lamers et al., 1989; Moorman et al., 1989a). In this respect, the observations in the mouse support our previous conclusion that the pericentral compartment becomes established earlier than the periportal compartment (Lamers et al., 1987).

CARBAMOYLPHOSPHATE SYNTHETASE I AND GLUTAMINE SYNTHETASE IN THE SMALL INTESTINE

The finding that CPS protein begins to accumulate in the jejunum at ED 15 of mouse development differentiates this species from the rat, in which this enzyme is first detected at ED 14 (comparable to ~ED 12.5 in the mouse), and from the human, in which the enzyme is first observed at approximately 8 weeks of gestation (comparable to ~ED 16 in the mouse). The functional consequences of this heterochrony (i.e., a different timing of the same developmental process; Gould, 1977) between species in intestinal enzyme accumulation are presently unclear. This paucity in our understanding is further stressed by the finding that CPS and GS become complementarily expressed in the fetal gut of human and rodent embryos (Moorman et al., unpublished data). CPS is confined to the small intestine, whereas GS is found in the distal stomach with a junction between both at the intestinal boundary of the pylorus. Because of the high concentration of CPS in the prenatal enterocyte, the enzyme probably exerts an important function in the embryo, possibly in arginine metabolism.

CONCLUSION

To summarize, the present survey highlights the successive steps in the process of liver maturation, which are necessary to establish the zonal pattern of gene expression characteristic of the adult mammalian liver. After hepatocytes have acquired the capacity to express hepatocyte-specific genes during their differentiation from the embryonic foregut, the number of hepatocytes that actually accumulate these proteins gradually increases. Once all hepatocytes participate equally in enzyme accumulation, regional differences in gene expression emerge that are related to the vascular architecture of the organ and that lead to the establishment of the periportal and pericentral zones. This zonation in turn emphasizes that the hepatic lobule is a determinant rather than a reflection of the pattern of gene expression within the liver.

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REFERENCES

- Bennett, A.L., Paulson, K.E., Miller, R.E., and Darnell, J.E., Jr. (1987) Acquisition of antigens characteristic of adult pericentral hepatocytes by differentiating fetal hepatoblasts in vitro. *J. Cell Biol.*, 105:1073-1085.
- Butler, H., and Juurlink, B.H.J. (1987) *An Atlas for Staging Mammalian and Chick Embryos*. CRC Press, Boca Raton, Florida.
- Chianale, J., Dvorak, C., Farmer, D.L., Michaels, L., and Gumucio, J.J. (1988) Cytochrome P-450 gene expression in the functional units of the fetal liver. *Hepatology*, 8:318-326.
- Couinaud, C. (1957) *Le foie. Etudes anatomiques et chirurgicales*. Masson, Paris.
- de Groot, C.J., Zonneveld, D., de Laaf, R.T.M., Dingemans, M.A., Mooren, P.G., Moorman, A.F.M., Lamers, W.H., and Charles, R. (1986) Developmental and hormonal regulation of carbamoyl-phosphate synthase gene expression in rat liver: Evidence for control mechanisms at different levels in the perinatal period. *Biochim. Biophys. Acta*, 866:61-67.
- de Groot, C.J., ten Voorde, G.H.J., van Aniel, R.E., te Kortschot, A., Gaasbeek Janzen, J.W., Wilson, R.H., Moorman, A.F.M., Charles, R., and Lamers, W.H. (1987) Reciprocal regulation of glutamine synthetase and carbamoylphosphate synthetase levels in rat liver. *Biochim. Biophys. Acta*, 908:231-240.
- Dingemans, M.A., and Lamers, W.H. (1994) Expression patterns of ammonia-metabolizing enzymes in the liver, mesonephros, and gut of human embryos and their possible implications. *Anat. Rec.*, 238:480-490.
- Dingemans, M.A., de Boer, P.A.J., Moorman, A.F.M., Charles, R., and Lamers, W.H. (1994) The expression of liver-specific genes within rat embryonic hepatocytes is a discontinuous process. *Differentiation*, 56:153-162.
- Dingemans, M.A., and Lamers, W.H. (1995) Gene expression and development of hepatic nitrogen metabolic pathways. In: *Nitrogen Metabolism and Excretion*. P.J. Walsh and P.A. Wright, eds. CRC Press, Boca Raton, Florida, pp. 229-242.
- Dingemans, M.A., de Jonge, W.J., de Boer, P.A.J., Mori, M., Lamers, W.H., and Moorman, A.F.M. (1996) Development of the ornithine cycle in rat liver: Zonation of a metabolic pathway. *Hepatology*, 24:407-411.
- Gaasbeek Janzen, J.W., Lamers, W.H., Moorman, A.F.M., de Graaf, A., Los, J.A., and Charles, R. (1984) Immunohistochemical localization of carbamoyl-phosphate synthetase (ammonia) in adult rat liver; evidence for a heterogeneous distribution. *J. Histochem. Cytochem.*, 32:557-564.
- Gaasbeek Janzen, J.W., Moorman, A.F.M., Lamers, W.H., and Charles, R. (1985) Development of the heterogeneous distribution of carbamoyl-phosphate synthetase (ammonia) in rat-liver parenchyma during postnatal development. *J. Histochem. Cytochem.*, 33:1205-1211.
- Gaasbeek Janzen, J.W., Moorman, A.F.M., Lamers, W.H., and Charles, R. (1986) Plasticity of the phenotype of cells derived from the embryonic foregut. *Acta Histochem. (Suppl.)*, 32:115-119.
- Gaasbeek Janzen, J.W., Gebhardt, R., ten Voorde, G.H.J., Lamers, W.H., Charles, R., and Moorman, A.F.M. (1987) Heterogeneous distribution of glutamine synthetase during rat liver development. *J. Histochem. Cytochem.*, 35:49-54.
- Gaasbeek Janzen, J.W., Westenend, P.J., Charles, R., Lamers, W.H., and Moorman, A.F.M. (1988) Gene expression in derivatives of embryonic foregut during prenatal development of the rat. *J. Histochem. Cytochem.*, 36:1223-1230.
- Gebhardt, R., and Mecke, D. (1983) Heterogeneous distribution of glutamine synthetase among rat liver parenchymal cells in situ and in primary culture. *EMBO J.*, 2:567-570.
- Gould, S.J. (1977) *Ontogeny and Phylogeny*. Harvard University Press, Cambridge.
- Kuo, C.F., Paulson, K.E., and Darnell, J.E. Jr. (1988) Positional and developmental regulation of glutamine synthetase expression in mouse liver. *Mol. Cell Biol.*, 8:4966-4971.
- Lamers, W.H., Gaasbeek Janzen, J.W., te Kortschot, A., Charles, R., and Moorman, A.F.M. (1987) Development of enzymic zonation in liver parenchyma is related to development of acinar architecture. *Differentiation*, 35:228-235.
- Lamers, W.H., Gaasbeek Janzen, J.W., Moorman, A.F.M., Charles, R., Knecht, E., Martínez-Ramón, A., Hernández-Yago, J., and Grisolia, S. (1988) Immunohistochemical localization of glutamate dehydrogenase in rat liver: Plasticity of distribution during development and with hormone treatment. *J. Histochem. Cytochem.*, 36:41-47.
- Lamers, W.H., Moorman, A.F.M., and Charles, R. (1989) The metabolic lobulus, a key to the architecture of the liver. *Cell Biol. Rev.*, 19:5-26.
- Meijer, A.J., Lamers, W.H., and Chamuleau, R.A.F.M. (1990) Nitrogen metabolism and ornithine cycle function. *Physiol. Rev.*, 70:701-748.
- Miller, R.E., Hackenberg, R., and Gershman, H. (1978) Regulation of glutamine synthetase in cultured 3T3-L1 cells by insulin, hydrocortisone, and dibutyryl cyclic AMP. *Proc. Natl. Acad. Sci. USA*, 75:1418-1422.
- Moorman, A.F.M., de Boer, P.A.J., Geerts, W.J.C., van de Zande, L., Lamers, W.H., and Charles, R. (1988) Complementary distribution of carbamoylphosphate synthetase (ammonia) and glutamine synthetase in rat liver acinus is regulated at a pretranslational level. *J. Histochem. Cytochem.*, 36:751-755.
- Moorman, A.F.M., Charles, R., and Lamers, W.H. (1989a) Development of hepatocyte heterogeneity. *Cell Biol. Rev.*, 19:27-41.
- Moorman, A.F.M., Vermeulen, J.L.M., Charles, R., and Lamers, W.H. (1989b) Localization of ammonia-metabolizing enzymes in human liver: Ontogenesis of heterogeneity. *Hepatology*, 9:367-372.
- Moorman, A.F.M., de Boer, P.A.J., Das, A.T., Labruyère, W.T., Charles, R., and Lamers, W.H. (1990) Expression patterns of mRNAs for ammonia-metabolizing enzymes in the developing rat: The ontogenesis of hepatocyte heterogeneity. *Histochem. J.*, 22:457-468.
- Morris, S.M. Jr., Kepka, D.M., Sweeney, W.E. Jr., and Avner, E.D. (1989) Abundance of mRNAs encoding urea cycle enzymes in fetal and neonatal mouse liver. *Arch. Biochem. Biophys.*, 269:175-180.
- Notenboom, R.G.E., de Boer, P.A.J., Moorman, A.F.M., and Lamers, W.H. (1996) The establishment of the hepatic architecture is a prerequisite for the development of a lobular pattern of gene expression. *Development*, 122:321-332.
- Shepard, T.H., Tanimura, T., and Robkin, M.A. (1970) Energy metabolism in early mammalian embryos. *Dev. Biol. (Suppl.)*, 4:42-58.
- Shiojiri, N., Wada, J.-I., Tanaka, T., Noguchi, M., Ito, M., and Gebhardt, R. (1995) Heterogeneous hepatocellular expression of glutamine synthetase in developing mouse liver and in testicular transplants of fetal liver. *Lab. Invest.*, 72:740-747.
- Smith, D.D., Jr., and Campbell, J.W. (1988) Distribution of glutamine synthetase and carbamoyl-phosphate synthetase I in vertebrate liver. *Proc. Natl. Acad. Sci. USA*, 85:160-164.
- van Roon, M.A., Eier, W., Charles, R., and Lamers, W.H. (1989a) The initial accumulation of carbamoylphosphate synthetase in embryonic rat hepatocytes, and the cell cycle. *Differentiation*, 41:139-147.
- van Roon, M.A., Aten, J.A., van Oven, C.H., Charles, R., and Lamers, W.H. (1989b) The initiation of hepatocyte-specific gene expression within embryonic hepatocytes is a stochastic event. *Dev. Biol.*, 136:508-516.
- van Roon, M.A., Zonneveld, D., de Boer, P.A.J., Moorman, A.F.M., Charles, R., and Lamers, W.H. (1990) Regulation of hepatocyte-specific gene expression in cultures of human embryonic hepatocytes. *Biol. Neonate*, 58:152-159.
- Westenend, P.J., Dahmen, R., Charles, R., and Lamers, W.H. (1986) Hormonal inducibility of liver-specific enzymes in cultured rat embryos. *Acta Morphol. Neerl.-Scand.*, 24:165-180.