Pharmacology & Therapeutics 118 (2008) 250–267

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

The ontogeny of drug metabolism enzymes and implications for adverse drug events

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ARTICLE INFO

Keywords:
Gene expression regulation
Developmental
Cytochrome P450
Flavin-containing monooxygenase
Alcohol dehydrogenase
Aldehyde oxidase
Paroxonase
Epoxide hydrolase
Glutathione transferase
Sulfotransferases
Glucuronosyl transferase
Ontogeny
Drug metabolism

Abbreviations:
bZIP, basic/leucine zipper
C/EBP, CCAAT/enhancer binding protein
DBP, D-element binding protein
DHEA, dehydroepiandrosterone
DM, dextromethorphan
DME, drug metabolizing enzyme
DX, dextrorphan
GFR, glomerular filtration rate
HNF1, hepatic nuclear factor 1
LAP, liver activator protein
LIP, liver inhibitory protein
PAR, proline and acidic acid rich
RT-PCR, reverse transcriptase-coupled polymerase chain reaction.

ABSTRACT

Profound changes in drug metabolizing enzyme (DME) expression occurs during development that impacts the risk of adverse drug events in the fetus and child. A review of our current knowledge suggests individual hepatic DME ontogeny can be categorized into one of three groups. Some enzymes, *e.g.*, CYP3A7, are expressed at their highest level during the first trimester and either remain at high concentrations or decrease during gestation, but are silenced or expressed at low levels within one to two years after birth. SULT1A1 is an example of the second group of DME. These enzymes are expressed at relatively constant levels throughout gestation and minimal changes are observed postnatally. ADH1C is typical of the third DME group that are not expressed or are expressed at low levels in the fetus, usually during the second or third trimester. Substantial increases in enzyme levels are observed within the first one to two years after birth. Combined with our knowledge of other physiological factors during early life stages, knowledge regarding DME ontogeny has permitted the development of robust physiological based pharmacokinetic models and an improved capability to predict drug disposition in pediatric patients. This review will provide an overview of DME developmental expression patterns and discuss some implications of the data with regards to drug therapy. Common themes emerging from our current knowledge also will be discussed. Finally, the review will highlight gaps in knowledge that will be important to advance this field.

Contents

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doi:10.1016/j.pharmthera.2008.02.005

1. Introduction

1.1. Historical perspective

There is ample historical evidence from therapeutic misadventures that drug disposition and response are substantially different in children versus adults. Often cited is the administration of chloramphenicol to neonates at doses that were extrapolated from those found effective and safe in adult patients. These children exhibited symptoms referred to as grey baby syndrome consisting of emesis, abdominal distension, abnormal respiration, cyanosis, cardiovascular collapse and death. Studies subsequently demonstrated that an immature UDP glucuronosyl transferase system, resulting in impaired metabolism and clearance, was primarily responsible (Weiss et al., 1960). However, increased drug sensitivity is not universal in children versus adults. Thus, children exhibit increased resistance to acetaminophen toxicity relative to adults, apparently because of an increased capacity for sulfate conjugation early in life (Alam et al., 1977). Nevertheless, the example of chloramphenicol-induced grey baby syndrome, as well as other age-specific adverse drug events, were major impetuses for legislative changes to encourage pediatric clinical trials both in the United States (1997 FDA Modernization Act; 2002 Best Pharmaceuticals for Children Act; and the 2007 FDA Revitalization Act) and in Europe (Regulation EC No. 1901/2006 on Medicinal Products for Paediatric Use). There also has been a concerted effort to better understand life-stage-dependent changes in drug metabolism and disposition.

1.2. The challenge

Changes in pharmacokinetic parameters during development (Alcorn & McNamara, 2003) contribute substantially to the differences in therapeutic efficacy and adverse drug reactions observed in children (Kearns et al., 2003a). Of these parameters, changes in drug metabolizing enzyme (DME) expression, as exemplified by the example of grey baby syndrome described above, are recognized as making a major contribution to the overall pharmacokinetic differences between adults and children (Hines & McCarver, 2002; McCarver & Hines, 2002). However, the knowledge needed to better understand and more importantly, predict therapeutic dosing and avoidance of adverse reactions during maturation remains incomplete. This gap in knowledge is despite the increasing prescription of off-label medications for pediatric diseases based on adult efficacy data, particularly in the neonatal and pediatric intensive care settings (Cuzzolin et al., 2006). Advances in human developmental pharmacology that would address this knowledge gap have faced several challenges. Of major importance have been ethical and logistical problems in obtaining suitable tissue samples for *in vitro* studies. Increasing the significance of these problems was the realization that substantial species differences exist in both DME primary structure and regulatory mechanisms, causing concern regarding the ability to readily extrapolate data from animal model systems to humans. Furthermore, dynamic changes in gene expression occur during different stages of ontogeny. Thus, the common study design involving a small number of tissue samples representing a narrow time window, or the pooling of samples across large windows of time, has lead to data from which definitive conclusions are difficult to make. The science also has been hampered by the promiscuous nature of many of the DME making it difficult to identify specific probe substrates or develop highly specific antibodies. Questions regarding the cross-reactivity of antibodies raised against animal model antigens also have been raised. In addition, the lack of appreciation of the complexity of some of the loci encoding human DMEs has lead to the utilization of non-specific probes, and the mis-belief that transcript levels would correlate well with protein and activity levels [see Rich and Boobis (1997) for a discussion of many of these latter points].

The objective of this review is to summarize our current knowledge regarding the ontogeny of key human hepatic enzymes that potentially impact xenobiotic pharmacokinetics and indirectly, pharmacodynamics. The review also will try to put this knowledge into the context of other developmental changes that have a significant impact on pharmacokinetics.

2. Physiological factors impacting drug disposition during development

Several physiological parameters undergo changes during development that can impact drug disposition [see Kearns et al. (2003a) for a recent review]. For example, intragastric pH is elevated in the neonate relative to later life stages resulting in lower bioavailability of weakly acidic drugs. Maturation of intestinal motor activity takes place during early infancy and also impacts drug absorption. Similar to what has been observed in the liver, intestinal enzymes and transporters that influence drug absorption are likely to undergo developmental changes and alter bioavailability, although this is an understudied area. There also are age-dependent changes in body composition that will impact the volume of drug distribution and thus, overall disposition. Furthermore, changes in the major drug binding plasma proteins occur with age (McNamara & Alcorn, 2002). However, anatomical and functional changes in the liver and kidney appear to have a quantitatively more important influence on pharmacokinetics,

including changes in the enzymes responsible for xenobiotic biotransformation.

2.1. Liver development: relative size and microsomal content

Although the enzymes involved in drug disposition are located in many tissues, they are most abundant in the liver. Hepatic organogenesis begins from the fetal mesoderm and endoderm during the fourth week of gestation and progresses rapidly with the fundamental components of the liver being formed by the end of the first trimester. By 12 weeks, the smooth endoplasmic reticulum is developing and is present in large amounts by mid-gestation [see review by Ring et al. (1999)]. The presence of several enzymes involved in drug metabolism have been detected as early as 8 to 10 weeks gestation [*e.g.*, FMO1 (Koukouritaki et al., 2002) and CYP3A5 and 3A7 (Stevens et al., 2003)]. However, adding to the complexity of this dynamic process, the ratio of liver to body mass is not constant and in fact, is considerably greater in infants and young children. This difference accounts for some, but not all of the developmental differences observed in drug metabolism between adults and children (Murry et al., 1995; Noda et al., 1997). Scaling by normalizing to a 70-kg individual using the 3/4 power allometric rule [$\text{Activity}_{70\text{-kg}} = \text{Activity}/(\text{Weight}/70\text{-kg})^{0.75}$] is sometimes used to adjust for the differences in liver size relative to body mass in pediatric versus adult individuals [*e.g.*, Zaya et al. (2006)].

Recent studies have determined that microsomal protein content also changes with age. Although there is considerable interindividual variability, the average microsomal protein content of a 30 year old was observed to be 40 mg/g liver with a decline to 31 mg/g liver for the average 60 year old (Barter et al., 2007). Extending these studies using a set of pediatric samples suggested an increase in microsomal protein content from birth to the maximum observed at approximately 30 years of age. Thus, the average neonate exhibits a microsomal protein content of only 26 mg/g liver (A. Rostami-Hodjegean, Clinical Pharmacology, University of Sheffield, UK, personal communication, and SimCYP ADME Simulator Software, Simcyp, Ltd., Sheffield, UK). Data from this laboratory is consistent with this observation and furthermore, would suggest significantly less microsomal content in the fetal liver (Fig. 1). For drugs undergoing microsomal enzyme-dependent metabolism, the age-dependent changes in microsomal content between pediatric patients and adult patients would effect drug disposition in a direction opposite to that of the changes in liver size relative to body mass.

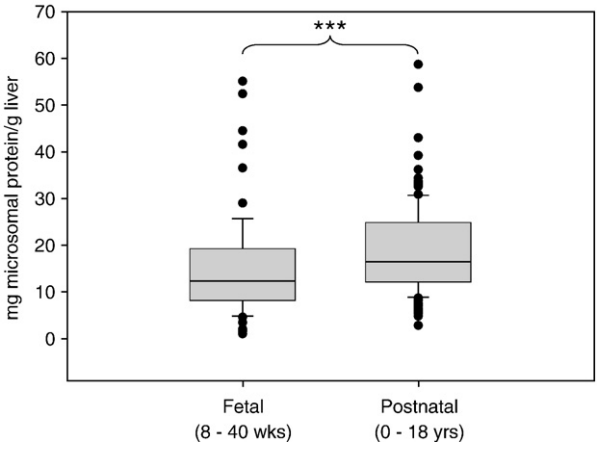

Fig. 1. Changes in microsomal content during early life stages. Microsomal suspensions were prepared from 234 human liver samples from donors ranging in age from 8 weeks gestation to 18 years after birth. Details regarding the demographics of the donors and the experimental procedure for preparing the microsomal suspensions were described by Koukouritaki et al. (2002). Microsomal content was determined based on the starting weight of the material used for the preparations and corrected for yield based on the average values reported by Barter et al. (2007). Data are plotted as medians (bars), interquartile values (boxes) and 10th to 90th percentiles (whiskers). Outliers were defined as 1.5 times the interquartile values. Fetal and postnatal data were compared using a Mann Whitney Rank Sums Test (SigmaStat Version 3.1, Systat Software, Chicago, IL) (*** = $P < 0.001$).

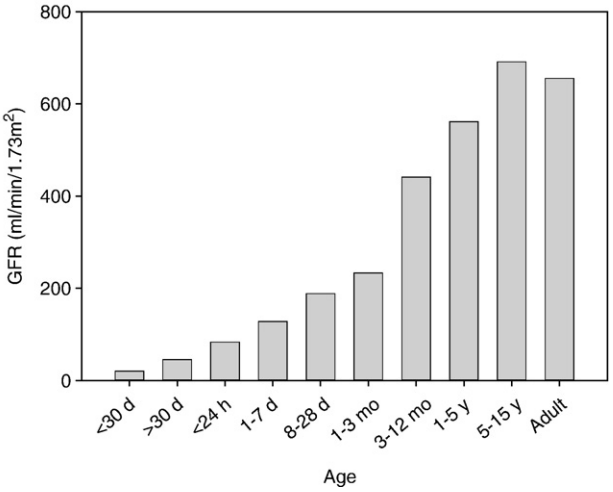

Fig. 2. Changes in renal GFR as a function of age during development. Mean GFR within different age brackets in the fetus, child and adult. Data from Alcorn and McNamara (2003) and references therein.

2.2. Renal structure and function

The maturation of kidney structure and function has a profound impact on those drugs that depend on renal clearance for elimination and/or termination of pharmacological action, a topic that was recently reviewed by Alcorn and McNamara (2003). Nephrogenesis begins as early as 9 weeks and is complete by 36 weeks gestation. However, vasoconstriction and reduced renal blood flow result in a substantially diminished glomerular filtration rate (GFR) in the term infant versus the adult. With parturition and the resulting decrease in vascular resistance and increase in cardiac output and renal blood flow, GFR increases rapidly and approaches adult levels by the first year of life (Fig. 2). Despite these parturition associated events, GFR is more tightly correlated with post-conceptional age than postnatal age, clearly suggesting that maturation of renal structure continues to influence GFR in the postnatal period. Tubular secretion and reabsorption also play an important role in overall renal drug clearance. At birth, the renal tubules are not yet mature, either structurally or functionally, leading to activity that is only 20 to 30% of adult values. Increases to adult levels of tubular secretion are attained by seven to eight months. Information on the ontogeny of specific renal transport enzymes in the human remains deficient and would greatly aid in our understanding of early life stage differences in drug response and risk for adverse events.

3. Metabolic factors impacting drug disposition during development

Several groups demonstrated low-level expression of one or more cytochromes P450 early in fetal liver development using either probe substrates (Cresteil et al., 1982; Pasanen et al., 1987; Lee et al., 1991), fractionation and purification (Cresteil et al., 1982), western blotting (Kitada et al., 1991), and/or by reverse transcriptase-coupled polymerase chain reaction (RT-PCR) DNA amplification (Hakkola et al., 1994). However, all of these approaches were limited by their specificity and many were severely limited by their sample-size and range of ages covered. With the development of highly specific antibody probes, more sensitive detection techniques, and a greater understanding of gene complexity as

a benefit of the human genome project, a more complete knowledge of developmental expression patterns has been achieved.

3.1. Oxidative enzymes

3.1.1. Alcohol dehydrogenase (ADH)

The ADH (EC 1.1.1.1) family of enzymes are encoded by seven genes (*ADH1A, 1B, 1C, 4, 5, 6* and *7*) clustered on human chromosome 4q21–q25. The enzymes function as dimers and catalyze the oxidation and reduction of a wide variety of alcohols and aldehydes, respectively (Edenberg & Bosron, 1997). Little is known about the *ADH4*, *5*, *6* and *7* developmental expression patterns. However, because of their role in ethanol oxidation and the suspected role this pathway has on risk for alcohol-related birth defects, considerable attention has been given to the ontogeny of the *ADH* class 1 genes, whose encoded proteins can form homodimers or heterodimers among the three subfamilies. Smith et al. (1971) provided definitive evidence for the progressive expression of ADH1A, ADH1B and ADH1C, during development. The expression of ADH in liver, lung, kidney, and intestine from 222 individuals ranging in age from 9 weeks gestation to greater than 20 years postnatal age was examined using starch gel electrophoresis. In 48 fetal liver samples with a mean gestational age of 13.5 weeks (based on crown–rump length), only the ADH1A enzyme was detectable. However, by 16 weeks, both ADH1A and ADH1B were measurable, although ADH1A predominated and appeared to be expressed at higher levels than observed at 13.5 weeks. By 19 weeks, products from all three loci were observed, with ADH1A being somewhat greater than ADH1B and ADH1C being present at low levels in some samples. In 30 week premature infants ($n=10$), ADH1A and ADH1B levels were equivalent, but still greater than ADH1C. By 36 weeks, ADH1B expression in premature infants dominated over the other two class 1 alleles. In the adult, hepatic ADH1A expression was low to non-detectable, whereas expression from the *ADH1B* and *ADH1C* loci was equivalent. The results from these studies would suggest that birth, but not gestational age, determines the activation of *ADH1C* expression. In addition to the progressive change in ADH1 subfamily expression, there was an overall increase such that enzyme levels in adults were 10-fold higher than those observed in first trimester fetal samples (Smith et al., 1972).

Similar studies in other tissues demonstrated that the progressive change in ADH1 expression was tissue-specific (Smith et al., 1971). In lung, no differences between the fetal and adult samples were observed and only ADH1C was detectable. ADH expression in the intestine and kidney was low, was dominated by expression from the *ADH1C* locus, and did not change appreciably with age. The conclusions regarding the ontogeny of hepatic ADH enzymes are largely in agreement with a study in which steady-state concentrations of the different ADH class 1 transcripts were determined by northern blot analysis (Estonius et al., 1996). In two different fetal liver samples of unspecified age, ADH1A, ADH1B and ADH1C transcripts were observed in one, whereas ADH1B and ADH1C transcripts dominated in the second. However, differing from the report by Smith et al. (1971), ADH1A transcripts dominated in the lung, whereas in the fetal kidney, only transcripts for both ADH1B and ADH1C were present. ADH class 1 transcripts also were present in most other adult tissues with the exception of brain, kidney and placenta. ADH4 and ADH7 transcripts were observed only in fetal liver at concentrations similar to that seen in the adult. Faint signals also were observed in the adult small intestine and pancreas. Similar to the class I transcripts, ADH5 transcripts were widely distributed, being detectable at approximately the same concentration in all fetal and adult tissues examined with the possible exception of the brain. In this tissue, the ADH5 transcripts appeared higher in the fetus than in adult. Although tempting to draw conclusions from these data, the unknown correlation between ADH protein and transcript levels in the various tissues and as a function of age make it difficult to do so with any confidence.

3.1.2. Aldehyde oxidase (AOX)

There are two human AOX (EC 1.2.3.1) family members, AOX1 and AOX2, encoded on chromosome 2q33. Aldehyde oxidase is important for the metabolism of a wide variety of aldehyde-containing and *N*-heterocyclic drugs and xenobiotics, including 5-fluoropyrimidine, quinine, methotrexate, 6-mercaptopurine, and zonisamide, although the relative specificity of AOX1 versus AOX2 for these compounds is poorly defined. Tayama et al. (2007) characterized AOX ontogeny in a cohort of Japanese children by measuring the urinary AOX-dependent oxidation products of N1-methylnicotinamide, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide. The study cohort consisted of 101 children of both sexes ranging in age from shortly after birth to 10 years of age. Pediatric values were compared to the same urinary products in 26 adults, ages 20 to 60 years. AOX activity in neonates was 10 to 15% of that observed in adults. However, activity increased in a nearly linear fashion to adult levels at one year of age. Considerable interindividual variation was observed with a four-fold range in activity in individuals greater than one year of age. No difference was observed between males and females at any age. No data were available on fetal liver AOX activity, but assuming activity no greater than that observed in neonates, fetal enzyme levels would not be expected to be greater than 10% of those observed in adults. Finally, it is unclear whether the measured N1-methylnicotinamide oxidation activity represents AOX1, or both AOX1 and AOX2.

3.1.3. Cytochromes P450

The human cytochrome P450 superfamily of enzymes (EC 1.14.14.1) is encoded by 59 functional genes (full-length transcripts produced) that have been divided into 18 families and 42 subfamilies based on divergent evolution. However, the vast majority of drug and toxicant metabolism is carried out by the 23 enzymes belonging to families one through three, which are the focus of this review. The remaining cytochromes P450 are more specialized enzymes involved in the synthesis and degradation of important endogenous signaling molecules.

3.1.3.1. Cytochrome P4501 family (CYP1)

CYP1A1 (EC 1.14.14.1) is the principal enzyme responsible for the metabolic activation of polycyclic aromatic hydrocarbons to toxic metabolites in the lung (Roberts-Thomson et al., 1993; Shou et al., 1994). In unexposed individuals, CYP1A1 expression is low or absent. However, the gene is highly induced by exposure to polycyclic aromatic hydrocarbons derived from cigarette smoking and other combustion processes (McLemore et al., 1990), a phenomenon regulated by the aryl hydrocarbon receptor. CYP1A1 does not appear to be inducible or expressed constitutively in the adult human liver (McManus et al., 1990; Edwards et al., 1998). However, the presence or absence of CYP1A family members in human fetal liver is controversial. Early studies using both probe substrates and polyclonal antibodies suggested that members of the CYP1A family (CYP1A1 and CYP1A2) were either not expressed, or were expressed at very low levels in the human fetal second or third trimester liver (Cresteil et al., 1982; Cresteil et al., 1985). Hakkola et al. (1994) also were not able to detect either CYP1A1 or CYP1A2 mRNA in any of 16 fetal liver samples that ranged in age from 11 to 24 weeks gestation using RT-PCR, results that were recently corroborated in a set of 63 fetal liver samples representing ages from 22 to 44 weeks gestation (Bieche et al., 2007). Consistent with these data, as well as the earlier reports, Lee et al. (1991) demonstrated significant cytochrome P450-dependent metabolic ability in liver tissue ($n=3$–6) from 7 to 8.5 week fetuses using phenoxazone ethers as probe substrates. However, the observed activity was not inhibited with rat anti-CYP1A antibody or by the addition of 7,8-benzoflavone, consistent with catalysis by enzymes other than those belonging to the CYP1A family.

In contrast to the above negative reports, Omiecinski et al. (1990) utilized RT-PCR to detect highly variable amounts of CYP1A1 mRNA

in three of four fetal liver samples that ranged in age from 6.5 to 12 weeks gestation. Further supporting expression in fetal liver, several studies also were able to detect CYP1A1 protein in fetal liver that again, was highly variable (Kitada et al., 1992; Murray et al., 1992). In a follow-up of their previous negative study, Yang et al. (1995) observed CYP1A1-dependent catalytic activity in liver microsomes isolated from 7 to 8.5 week gestation fetuses that was inhibited by an anti-rat CYP1A1 antibody and 7,8-benzoflavone. They also were able to detect CYP1A1 mRNA in these same tissues. Furthermore, using a combined cytosolic and microsomal tissue fraction, they showed that their earlier negative results (Lee et al., 1991) were due to an inhibitory factor present in the soluble fraction. None of these studies were able to detect CYP1A2 expression in fetal tissue.

The reason for the conflicting results regarding fetal liver CYP1A1 expression is unknown, although the lack of expression in the absence of an exposure to aryl hydrocarbon receptor ligands may offer a partial explanation. However, the negative studies all reported using second and third trimester tissues while those reporting detectable expression were restricted to the first trimester. Thus, the weight of evidence would suggest that CYP1A1 is constitutively expressed at low levels in fetal liver, at least during organogenesis, but that expression then declines to non-detectable levels during the remaining gestational period and beyond. Of considerable interest, this conclusion would be consistent with Shao et al. (2007) who demonstrated low levels of CYP1A1 transcripts in fetal hematopoietic stem cells, suggesting that the decline in these cells with maturation of the liver may coincide and explain the loss of fetal CYP1A1 expression and its absence in the adult liver (see Section 4).

Using caffeine as a metabolic probe, Cazeneuve et al. (1994) observed a change in metabolic pattern consistent with very low to no CYP1A2 expression in fetal (n=10, 19 to 35 weeks gestation) and neonatal (n=10, 0.5 h to 4 weeks postnatal age) liver and onset of expression in infants (n=9, 1.5 to 10 months postnatal age). However, even in this age group, mean expression levels were still 10-fold less than those observed in adults. These results were largely consistent with earlier *in vivo* results (Carrier et al., 1988) that measured urinary caffeine metabolites. A delayed CYP1A2 ontogenesis was further confirmed by Sonnier and Cresteil (1998) using methoxyresorufin as a probe substrate and an anti-rat polyclonal CYP1A1 antibody that cross-reacted with both human CYP1A1 and 1A2. However, these investigators were able to separate both CYP1A isoforms by electrophoresis and demonstrated the absence of any CYP1A1 in all of their tissue samples. CYP1A2 was not detectable in 10 fetal liver samples (ages 14 to 40 weeks). A progressive increase in CYP1A2 catalytic activity and protein levels was observed in postnatal samples with neonates (n=38) at 4–5% of adult levels, samples from one to three month old infants (n=23) at 10 to 15% of adult levels, samples from three to 12 month old children at 20 to 25% of adult levels, and samples from children from one to nine years of age (n=6) at 50 to 55% of adult levels. Interestingly, dietary differences can alter the rate of CYP1A2 maturation with formula fed infants acquiring activity faster than breast-fed infants (Le Guennec & Billon., 1987; Blake et al., 2006).

CYP1B1 would appear to be largely an extrahepatic cytochrome P450. Although Hakkola et al. (1997) reported variable levels of CYP1B1 mRNA in three of six fetal livers studied using RT-PCR, a more recent study by Bieche et al. (2007) failed to reproduce these results. Also using RT-PCR, this group was unable to detect CYP1B1 mRNA in 63 fetal liver samples (ages 22 to 44 weeks gestation) or in 12 adult liver biopsy samples. Thus, it would appear this member of the CYP1 family will contribute little if any to hepatic drug metabolism at any age, although it represents a major enzyme in many extrahepatic tissues for which possible ontogenic changes in expression are unknown.

3.1.3.2. Cytochrome P4502A subfamily (CYP2A)

The human *CYP2A* gene family consists of three members, CYP2A6, 2A7 and 2A13. However, the dominant CYP2A7 transcript is alternatively spliced resulting in a truncated, inactive protein (Ding et al., 1995). Furthermore, even the full-length protein appears to be devoid of any detectable activity (Yamano et al., 1990). While CYP2A6 is expressed at relatively high levels in the adult liver (Shimada et al., 1994), CYP2A13 is the dominant form in the nasal mucosa (Su et al., 2000). Coumarin 7-hydroxylation is a probe metabolic activity for hepatic CYP2A6 (Yamano et al., 1990), but the enzyme also is active toward the oxidative metabolism of nicotine (Messina et al., 1997), as well as several other protoxicants (Liu et al., 1996). CYP2A13 exhibits high activity towards the tobacco-specific procarcinogen, 4-(methylnitrosoamino)-1,-(3-pyridyl)-1-butanone (Su et al., 2000). Early studies suggested the lack of CYP2A expression in human fetal liver between 13 and 23 weeks gestation (n=20), although protein expression (likely CYP2A6) was readily detectable in adult liver (Raunio et al., 1990) and as early as 17 weeks after birth (n=1) (Crespi et al., 1990). These findings were largely confirmed by Gu et al. (2000) who reported finding immunoreactive hepatic CYP2A protein in only one of 6 fetal liver samples that ranged in age from 14 to 18 weeks gestation. Thus, it would appear that hepatic CYP2A6 expression is a postnatal event, although the exact pattern of expression remains to be elucidated.

In contrast to what was observed in the liver, CYP2A protein was readily detectable in microsomes prepared from fetal olfactory mucosa between 13 and 18 weeks gestation (n=8) (Gu et al., 2000). By pre-concentrating the olfactory microsomes prior to immunoblot analysis, this same group was able to better quantify protein and demonstrated fetal CYP2A levels of 0.8 to 5.3 pmol/mg microsomal protein in five samples, 14 to 26 weeks gestation. Furthermore, there was a trend toward increased expression as a function of gestational age. Interestingly, CYP2A expression in the fetal olfactory mucosa was higher than observed in the adult tissue, which ranged from 0.1 to 1.1 pmol/mg microsomal protein (n=7) (Chen et al., 2003). Additional studies will be needed to better define the CYP2A olfactory mucosa developmental expression pattern and determine at what age maximal expression is observed.

3.1.3.3. Cytochrome P4502C subfamily (CYP2C)

The human *CYP2C* subfamily consists of four genes, *CYP2C8*, *2C9*, *2C18* and *2C19*, clustered within an approximate 500 kbp locus on chromosome 10q24. The encoded enzymes account for about 18% of the total cytochrome P450 in adult liver (Shimada et al., 1994) and are responsible for the oxidative metabolism of ~29% of clinically relevant drugs (Williams et al., 2004). CYP2C9 is the major hepatic CYP2C enzyme, followed by CYP2C19 and CYP2C8 (Edwards et al., 1998). CYP2C9 substrates include warfarin, phenytoin, diclofenac, ibuprofen, tolbutamide, losartan (Miners & Birkett, 1998), and indomethacin (Nakajima et al., 1998), while CYP2C19 substrates include the anticonvulsant, mephenytoin (Goldstein et al., 1994) and the antidepressasnt, *S*-citalopram (Von Moltke et al., 2001). Most proton pump inhibitors also are CYP2C19 substrates, *e.g.*, omeprazole (Andersson et al., 1993) and lansoprazole (Sohn et al., 1997), several of which are commonly used in children (Litalien et al., 2005). Very little is known about the ontogeny of CYP2C8 and 2C18 and nothing is known about the ontogeny of any of the CYP2C enzymes in extrahepatic tissues. However, because of the common use of several CYP2C9 and 2C19 substrates in pediatric patients (Loughnan et al., 1977; Van et al., 2001; Shaffer et al., 2002), the activity of CYP2C9 in the conversion of arachidonic acid to known signaling molecules (Daikh et al., 1994; Rifkind et al., 1995), and an association between elevated CYP2C9 expression and sudden infant death syndrome (Treluyer et al., 2000), there has been considerable interest in the ontogeny of both enzymes.

Two or three specific proteins that were immunoreactive to rabbit anti-mouse Cyp2c7 polyclonal antibody were detected in 11/17 fetal liver samples that ranged in age from 13 to 23 weeks gestation (Maenpaa et al., 1993). Although these three proteins most likely represented CYP2C9, 2C19 and 2C8, no attempt was made to further define which

specific enzymes were reacting with this antibody. Current knowledge of the electrophoretic mobility of these enzymes in denaturing gels is consistent with the most consistently detectable protein being CYP2C9. These results are in contrast to a report by Treluyer et al. (1997) in which CYP2C protein immunoreactive against a rabbit anti-human CYP2C19 antibody was not detected in any of 55 fetal liver samples ranging between 16 and 40 weeks gestation. Levels of CYP2C protein that were 4 to 5% of those observed in adults were detectable in 11 liver samples from newborns less than 1 day old. In neonatal samples greater than 1 day old and up to one year of age, CYP2C protein levels ranged between 22 and 30% of adult values. In general, the measured protein levels correlated well with tolbutamide hydroxylase activity in these same liver samples.

Koukouritaki et al. (2004) characterized CYP2C9 and 2C19 ontogeny in a bank of 237 human liver samples from donors ranging in age from 8 weeks gestation to 18 years after birth. Western blotting with rabbit anti-human CYP2C9 and anti-human CYP2C19 antibodies were used along with diclofenac 4-hydroxylase and (*S*)-mephenytoin 4′-hydroxylase activity assays. CYP2C9 was detectable in 46/55 tissue samples from donors that were between 8 and 24 weeks gestation at 1% (mean ± SD = 0.3 ± 0.2 pmol/mg) of reported adult values (Wrighton et al., 1990) (Shimada et al., 1994). CYP2C9 levels increased significantly in tissue samples between 25 and 40 weeks (n = 16) to levels that were approximately 10% (5.0 ± 4.0 pmol/mg) of adult values. A further increase in CYP2C9 was observed in postnatal samples from donors that were 0 to 5 months of age (n = 92) to protein levels that were approximately 25% of those seen in adults (11.8 ± 7.0 pmol/mg). However, samples from this age bracket also exhibited the greatest interindividual differences such that approximately 50% of the samples exhibited CYP2C9 levels that ranged in values no different than those observed in the third trimester samples while many samples exhibited values as high as those observed in the post-puberty samples. This observation is consistent with earlier *in vivo* pharmacokinetic data in which an age-dependent decrease in phenytoin half-life was observed over the first two weeks of life (Loughnan et al., 1977) as was a delayed acquisition of saturable phenytoin metabolism in neonates (Bourgeois & Dodson, 1983). In tissue samples from donors between 5 months and 18 years of age, mean CYP2C9 levels were approximately 50% of adult values (18.0 ± 6.1 pmol/mg). Good correlation was observed between measured CYP2C9 protein levels and diclofenac 4-hydroxylase activity in each age bracket. The failure to observe adult CYP2C9 values, even in the post-puberty samples (n = 20), was consistent with the study by Treluyer et al. (1997) and suggests a late CYP2C9 maturation. Yet, these conclusions do not appear to be consistent with *in vivo* pharmacokinetic studies in pediatric patients wherein metabolic ability in children over one year of age is no different than adults [*e.g.*, Leff et al. (1986) and Andrew et al. (1994)]. These apparent discrepancies are likely due to developmental changes in the liver size to body weight ratio and other factors (see above), as first suggested by Chiba et al. (1980) and later demonstrated by Takahashi et al. (2000) and illustrate the rather large effect this physiological change can have on drug disposition.

The study by Koukouritaki et al. (2004) is the only one that has attempted to differentiate the ontogeny of CYP2C19 from 2C9. Unlike the latter enzyme, CYP2C19 was detectable in all fetal liver tissue samples examined (ages 12 weeks 40 weeks) at levels that were 10 to 20% of those observed in adults (3.4 ± 2.2 pmol/mg microsomal protein, n = 71). No significant change in CYP2C19 content was observed during gestation. Again, unlike CYP2C9, there was no apparent change in CYP2C19 levels at birth. Rather, there was a trend for a near linear increase to enzyme levels 50 to 75% of those observed in adults over the first five months after birth (11.7 ± 7.9 pmol/mg microsomal protein, n = 54). Enzyme levels indistinguishable from adult levels were observed in post-puberty samples (14.9 ± 4.1 pmol/mg microsomal protein). These data were consistent with the (*S*)-mephenytoin 4′-hydroxylase activities observed in these same samples. A comparison of relative CYP2C9 and 2C19 levels in individual samples revealed that CYP2C19 is the dominant prenatal CYP2C enzyme with a transition to CYP2C9 as the dominant postnatal enzyme at or around birth.

3.1.3.4. Cytochrome P4502D6 (CYP2D6)

Although accounting for less than 2% of total adult hepatic cytochrome P450 (Shimada et al., 1994), CYP2D6 is important for the oxidative metabolism of approximately 12% of clinically relevant drugs (Williams et al., 2004). Furthermore, *CYP2D6* is highly polymorphic with over 80 different alleles identified to date, including several complete loss-of-function, reduced function, and multiple copy number alleles that are relatively common. A wide range of metabolic capacities result from the inheritance of different allele combinations. CYP2D6 substrates include propafenone (Lee et al., 1990), paroxetine (Ereshefsky et al., 1995), risperidone (Mannens et al., 1993) and atomoxetine (Ring et al., 2002), the latter of particular relevance to pediatrics. In addition to these important reactions, CYP2D6 also catalyzes the *O*-demethylation of codeine to the active moiety, morphine (Dayer et al., 1988), and dextromethorphan to dextrorphan (Perault et al., 1991). These latter two reactions have been used as both *in vitro* and *in vivo* CYP2D6 phenotypic probes, dextromethorphan *O*-demethylation being particularly useful in pediatric populations.

Measuring both codeine and dextromethorphan *O*-demethylation, Ladona et al. (1991) were unable to detect any activity in 10 Caucasian or 12 Asian liver samples from fetuses 14 to 24 weeks of age. The lack of activity was consistent with the inability to detect CYP2D6 protein in the same samples by western blot analysis. In a much more extensive study, Treluyer et al. (1991) similarly measured CYP2D6 protein and dextromethorphan *O*-demethylase activity, but also CYP2D6 mRNA levels in a group of fetal liver samples <30 weeks of age (n = 60), >30 weeks to 40 weeks (n = 15), newborns <24 h (n = 11; mean gestational age at birth = 29.3 weeks ± 3.3 weeks), newborns 1 to 7 days of age (n = 13), newborns 8 to 28 days (n = 8), infants ages 4 weeks to 5 years (n = 12), and adults (n = 8). CYP2D6 protein was undetectable in 47% of the fetal <30 wk age samples whereas 30% of the samples exhibited values that were at least 5% of those observed in the adult samples. In fetal samples from donors >30 weeks gestational age, the number of negative samples was reduced to 20% whereas the number of samples exhibiting values at least 5% of those observed in adults rose to 47%. In the 1–7 day old newborn age bracket, all 13 samples had detectable CYP2D6 protein, but at a mean level that still was only 5% of that observed in adults. CYP2D6 protein content in this newborn group also was independent of gestational age, suggesting an important contribution from a birth-dependent control process. Thereafter, there was a steady, age-dependent increase in CYP2D6 protein levels such that newborns 7–28 days of age exhibited mean levels that were 30% of adults and infants 4 weeks to 5 years, 70% of adults. Dextromethorphan *O*-demethylase activity closely followed this same developmental pattern. Given the polymorphic nature of the *CYP2D6* locus, the maturation of positive phenotype as a percentage of the total sample in each age bracket was of interest, consistent with the notion that during early life stages, there is a maturation process to a phenotype that would be predicted from genotype. Interestingly, the ontogeny of CYP2D6 mRNA exhibits a very different pattern than that observed for the protein or catalytic activity. CYP2D6 transcript levels were detectable in fetal samples and increased as a function of age, exceeding adult values in newborn and infant samples by two- to three-fold. There was a close correlation between CYP2D6 protein and mRNA levels in adult samples (r^2 = 0.95, P < 0.005), but not in fetal, newborn or infant samples, suggestive of a translational control mechanism overlying the clearly evident transcriptional regulation. However, more recent studies have revealed highly variable, but essentially equivalent hepatic transcript levels from both the *CYP2D6* and *CYP2D7P* loci (median = 3.20 transcript copies/pg total mRNA, range = 0.32 to 14.81; and median = 3.38 transcript copies/pg total mRNA, range = 0.46 to 14.25, respectively). Furthermore, many of the transcripts represented splice variants incapable of translation to functional protein (Endrizzi

et al., 2002; Gaedigk et al., 2005). Thus, the high levels of CYP2D mRNA reported by Treluyer et al. (1991) most likely represented a composite of transcripts from both the *CYP2D6* and *2D7P* loci. Furthermore, the presence of these variant transcripts appears to be more abundant during early life stages versus the adult (Gaedigk et al., 2005).

Dextromethorphan *O*-demethylase activity, as measured by the ratio of dextromethorphan to dextrorphan (DM/DX) in an overnight urine sample, also was used to assess CYP2D6 phenotype during the first year of life in 193 infants (Blake et al., 2007). A total of 892 measurements were taken during regularly scheduled well-child examinations at 0.5, 1, 2, 4, 6 and 12 months after birth. There was a clear correlation between the DM/DX ratio and an assigned activity score based on genotype that did not appear to change during the study period, suggesting concordance between phenotype and genotype by 0.5 months of age. Furthermore and in contrast to what one would have predicted from the previous *in vitro* study (Treluyer et al., 1991), there was no change in the DM/DX ratio as a function of age. There was considerable interindividual variation, as the range in DM/DX ratio at each time-point varied nearly 1000-fold (10th to 90th percentile).

Johnson et al. (in press) have suggested that the discrepancy between the *in vitro* and *in vivo* data on CYP2D6 ontogeny may be largely explained by the added complexity of renal functional maturation that was not considered in the *in vivo* study by Blake et al. (2007) (see Section 2.2 above). However, a recently completed *in vitro* study by the author and colleagues (Stevens et al., manuscript submitted) suggests a CYP2D6 developmental expression pattern more consistent with the *in vivo* data reported by Blake et al. (2007). Perhaps more importantly, the latter study only reported urinary metabolic ratios from which it is difficult if not impossible to assess changes in the absolute amount of parent drug and metabolite that would be more informative with respect to changes in biological parameters directly impacting pharmacokinetics.

3.1.3.5. Cytochrome P4502E1 (CYP2E1)

CYP2E1 accounts for approximately 7% of total hepatic cytochrome P450 in the adult liver (Shimada et al., 1994), but is involved in the oxidative metabolism of only about 2.5% of clinically relevant drugs (Williams et al., 2004). The enzyme is important for the oxidative metabolism of several therapeutics, including acetaminophen, halothane and chlorzoxazone. The enzyme also has an important role in the bioactivation of many small molecular weight toxicants, including ethanol, benzene, toluene, *N*-nitrosodimethylamine, and halogenated alkanes (Tanaka et al., 2000). CYP2E1 expression is regulated at multiple levels that remain poorly defined (Novak & Woodcroft, 2000).

The first attempt to determine CYP2E1 ontogeny was reported by Vieira et al. (1996), who examined enzyme content and catalysis by western blotting and chlorzoxazone hydroxylase activity, respectively, in liver microsomal samples and CYP2E1 transcripts by slot blot analysis with normalization to 28S rRNA. In addition, genomic DNA was isolated from liver samples to probe for possible changes in *CYP2E1* methylation. Liver samples were grouped according to age similar to what had been done for CYP2D6 (above): fetal samples < 30 weeks of age (n = 66), > 30 weeks to 40 weeks (n = 16), newborns < 24 h (n = 13; mean gestational age at birth = 30.3 weeks ± 3.5 weeks), newborns 1 to 7 days of age (n = 20), newborns 8 to 28 days (n = 21) and infants ages 1 to 3 months (n = 26), infants ages 3 to 12 months (n = 18), children 1 to 10 years (n = 7) and adults (n = 16). CYP2E1 protein was undetectable in any of the fetal samples. However, the enzyme was readily detectable in the newborn samples at levels approximately 10% of adult values. There was a steady increase in CYP2E1 levels, with infants 3 to 12 months of age exhibiting mean values about 30% of adults and children between 1 and 10 years of age exhibiting mean values no different than adults. There was a close association between CYP2E1 protein levels and catalytic activity as a function of age (r = 0.778, P < 0.001). In contrast, CYP2E1 transcript levels showed only modest differences between fetal samples > 30 weeks and 28 days after birth, consistent with the possible involvement of translational or post-translational control mechanisms during this time period. A significant increase in transcript levels was observed between 1 and 3 months and in samples representing the 3 to 12 month age bracket, transcript levels were 50% of those observed in adults. Differential digestion of genomic DNA with the methylation insensitive *Msp*I and methylation sensitive *Hpa*II enzymes followed by Southern blot analysis revealed that demethylation of a CG bp near the transcription start site correlated with the onset of *CYP2E1* expression, as did several other sites in exon 1 or intron 1. These data are consistent with demethylation playing an important role in controlling CYP2E1 ontogeny. Interestingly, these same sites remained hypermethylated in lung and kidney tissue wherein CYP2E1 expression remained low or absent (Vieira et al., 1998).

The absence of fetal hepatic CYP2E1 reported by Vieira et al. (1996) is consistent with the more limited studies by some groups (Wrighton et al., 1988; Komori et al., 1990; Jones et al., 1992; Hakkola et al., 1994), but contrasts with others (Carpenter et al., 1996; Boutelet-Bochan et al., 1997; Khalighi et al., 1999). In an attempt to resolve this controversy, Johnsrud et al. (2003) quantified CYP2E1 protein content in 238 liver samples ranging in age from 8 weeks gestation to 18 years by western blot analysis and linear regression, using recombinant CYP2E1 protein as a standard. The enzyme was undetectable in 10 first trimester fetal liver samples but was present in 18 of 49 second trimester samples (median = 0.35 pmol/mg microsomal protein) and 12 of 15 third trimester samples (median = 6.7 pmol/mg microsomal protein). These data were consistent with nearly all of the previous reports, as those that reported an absence of fetal liver CYP2E1 protein content utilized samples from the first or second trimester while those reporting the presence of fetal liver CYP2E1 utilized samples from the late second or third trimester period. Postnatal hepatic CYP2E1 increased slowly after birth, attaining a median level of 8.8 pmol/mg microsomal protein in the neonatal period (n = 42; undetectable in 8 samples, range = 0.7 to 70.1), 23.8 pmol/mg microsomal protein between 31 and 90 days after birth (n = 29; range = 10.0 to 43.3) and values equivalent to those reported in adults, 41.4 pmol/mg microsomal protein (n = 94; range = 17.9 to 94.7) in samples between 91 days and 18 years after birth. Based on multiple linear regression analyses and MANOVA, increasing postnatal age, age plus the presence of at least one copy of the *CYP2E1*1D* allele [a promoter polymorphism linked to increased gene expression in obese individuals and individuals consuming alcohol (McCarver et al., 1998)], and ethnicity were all associated with greater CYP2E1 expression. Gender and post-mortem interval failed to enter into the models. These data also were used to model interindividual pharmacokinetic differences in toluene internal dose (Nong et al., 2006). Based on exposures of 1 ppm toluene over 24 h, predicted neonatal exposures (area under venous blood concentration versus time) ranged from 0.16 to 1.01 μg/mL × h depending on CYP2E1 content. The model in older children predicted levels similar to those observed in adults, *i.e.*, 0.16 to 0.43 μg/mL × h. Thus, at an equivalent exposure, the low CYP2E1 levels in neonatal liver are predicted to result in higher internal doses than those observed in older children and adults. Furthermore, it is likely this model would extrapolate to other solvents for which CYP2E1 is intimately involved in determining clearance.

Because of CYP2E1's ability to activate several neurotoxicants known for their teratogenic activity (*e.g.*, ethanol and toluene), there also has been considerable interest in the ontogeny of this enzyme in the brain. CYP2E1 transcripts, protein and catalytic activity were present in the human fetal brain as early as 7.7 weeks gestation. Transcript and protein levels, which were higher than those measured in hepatic tissue, remained relatively constant through 16 weeks, although activity levels appeared to double between 7.7 and 11.5 weeks (Boutelet-Bochan et al., 1997). Thus, it would appear CYP2E1 present in the fetal brain may well play a role in the neurotoxicity caused by *in utero* exposure to agents such as ethanol, toluene and other solvents.

3.1.3.6. Cytochrome P4503A subfamily (CYP3A)

The *CYP3A* subfamily consists of four genes, *CYP3A4*, *3A5*, *3A7* and *3A43*, encoded at an approximate 270 kbp locus on human chromosome 7q21.1. CYP3A43 has minimal if any xenobiotic metabolizing activity and is expressed at low levels where detectable. In contrast, CYP3A4, 3A5, and 3A7 are collectively the most abundant hepatic members of the cytochromes P450 and account for nearly 46% of the oxidative metabolism of clinically relevant drugs (Williams et al., 2004). Although these three enzymes share at least 85% sequence identity, a property which has proven an obstacle to independent measurement, they exhibit considerable differences in substrate specificity and expression. In adult liver, CYP3A4 comprises 10 to 50% of the total cytochrome P450 (Shimada et al., 1994) and also exhibits high levels of expression in the intestine (Paine et al., 1997). CYP3A5 levels in both liver and intestine can exceed CYP3A4, subject to the presence of the *CYP3A5*1* allele (Kuehl et al., 2001; Lin et al., 2002). CYP3A7 is the dominant enzyme in fetal liver, but can be expressed at substantial levels (10 to 40% of total CYP3A levels) in the adult liver and intestine, dependent on the presence of the *CYP3A7*1C* allele (Sim et al., 2005).

The first strong evidence for CYP3A age-dependent expression was reported by Komori et al. (1990) using oligonucleotide probes specific for CYP3A7 (cytochrome P450 HFL) and CYP3A4 (cytochrome P450 NF). A strong hybridization signal was observed with the CYP3A7 probe against total RNA isolated from six fetal livers ranging in age from 13 to 23 weeks, but not against total RNA isolated from seven adult livers ranging in age from 33 to 70 years. The opposite pattern was observed with the CYP3A4 probe. At the protein level, CYP3A5 was shown to be expressed in a limited number of fetal (1/5; ages not specified) and postnatal (8/20) liver samples with no apparent correlation with age (Wrighton et al., 1990). The first evidence of CYP3A-dependent metabolic activity in fetal liver (14 to 27 weeks gestation) was obtained using the *N*-demethylation of codeine and dextromethorphan as metabolic probes (Ladona et al., 1991). Codeine *N*-demethylase activity ranging from 125 to 1470 pmol/min/mg microsomal protein was observed in 15 tissue samples while dextromethorphan *N*-demethylase activity ranged from 0 to 163 pmol/mg microsomal protein/min in 11 tissue samples. However, the interpretation of these data is complicated by the overlapping substrate specificities of the CYP3A isoforms. Thus, CYP3A4 has the greatest activity towards the *N*-demethylation of dextromethorphan, but below a substrate concentration of 1 mM, both CYP3A7 and 3A5 exhibit activities that are 33% and 17%, respectively, of that exhibited by CYP3A4 (Drs. Robin E. Pearce and J. Steven Leeder, Children's Mercy Hospital, Kansas City, MO, personal communication). Yang et al. (1994) employed multiple substrates and western blotting with a CYP3A antibody to demonstrate the presence of CYP3A-dependent activity and protein in early fetal tissue (7 to 8.5 weeks). However, the same antibody was unable to detect any immunoreactive protein in heart, lung, brain, or kidney. Amplification of CYP3A4 transcripts and sequence analysis provided strong evidence for CYP3A7 and to a lesser extent, CYP3A5, but not CYP3A4. Subsequent studies by this same group also demonstrated CYP3A7-dependent *N*-demethylation of imipramine (Chen et al., 1999) and 4-hydroxylation of retinoic acid (Chen et al., 2000) in fetal liver tissue. Similar results have been reported by at least two other groups. Thus, Schuetz et al. (1994) reported CYP3A7 and CYP3A5, but not CYP3A4 transcripts in six fetal liver samples (6 to 12 weeks gestational age). In adult tissue, CYP3A4 transcripts that varied in concentration by about 10-fold were observed in 13/13 samples. Of interest, CYP3A7 transcripts also were detected in 7/13 adult tissue samples, which was the first report of the limited expression of this gene in adults. Hakkola et al. (2001) reported CYP3A7 and 3A5, but not 3A4 transcripts in 12 fetal liver samples (9 to 12 weeks gestation). CYP3A7 transcript levels varied 77-fold while the 3A5 transcript levels were relatively constant. CYP3A7 protein also was detected in each of the samples, but CYP3A5 protein was only detected in a single sample. Thus, these early studies clearly demonstrated the largely fetal-specific expression of CYP3A7 and postnatal expression of CYP3A4 while CYP3A5 appeared to be expressed in a limited number of samples during both life stages. However, the dynamics of the transition between CYP3A7 and 3A4 had not been determined.

LaCroix et al. (1997) attempted to characterize the ontogeny of CYP3A7 and 3A4 using the same tissue samples that this group previously used to characterize CYP2E1 developmental expression (see Section 3.1.3.5) (Vieira et al., 1996). Using a non-specific anti-CYP3A antibody, equal levels of total CYP3A protein expression were observed in all age brackets. However, using a CYP3A4-specific hybridization probe, steady-state fetal CYP3A4 transcripts were determined to be only 10% of adult levels in samples <30 weeks gestational age ($n=32$), increasing to 20% of adult levels in fetal samples >30 weeks gestational age, and then achieving 30 to 60% of adult values within 1 week after birth. Using recombinant enzyme, the 16α-hydroxylation of dehydroepiandrosterone (DHEA) was shown to be catalyzed by CYP3A7 two- to three-times more efficiently than by CYP3A4. In contrast, the 6β-hydroxylation of testosterone was catalyzed three-times more efficiently by CYP3A4 than by CYP3A7. Thus, these two activities were used as probes to determine the ontogeny of these two enzymes. DHEA 16α-hydroxylase activity (CYP3A7) ranged from 0.48 to 1.31 nmol/min/mg microsomal protein in fetal samples ($n=34$) through neonates 1–7 days after birth ($n=12$), but then declined steadily to 0.56 pmol/min/mg microsomal protein in neonates 8 to 28 days after birth ($n=8$), 0.34 nmol/min/mg microsomal protein in infants 1 to 3 months of age, 0.17 nmol/min/mg in children from 3 to 12 months of age and finally to 0.07 nmol/min/mg microsomal protein in adult tissue samples. In contrast, testosterone 6β hydroxylase activity was only 0.004 to 0.008 nmol/min/mg microsomal protein in fetal samples ($n=16$). Activity rose steadily after birth to 0.012 to 0.015 nmol/min/mg microsomal protein in neonates up to seven days of age ($n=12$), 0.035 to 0.052 nmol/min/mg microsomal protein in neonates and infants from 8 days to 1 year of age, and reached adult levels of 0.120 to 0.130 nmol/min/mg microsomal protein in children greater than one year of age. However, this study did not consider the possible contribution of CYP3A5 to any of these activities. A somewhat similar approach was taken by Stevens et al. (2003), but using differential metabolism of DHEA. Measuring both DHEA 7β-and 16α-hydroxylase activity, the V_{max}/K_M quotients for CYP3A4, 3A5 and 3A7 were 0.423, 0.0134 and 0.0125 and 0.433, 0.002, and 1.32, respectively. Thus, DHEA 7β-hydroxylation was considered a preferential activity for CYP3A4 while DHEA 16α-hydroxylation was considered a preferential activity for CYP3A7. A multiple linear regression model was developed using different ratios of recombinant CYP3A4 and CYP3A7 enzymes and used to determine relative levels of both enzymes in a bank of liver samples from donors ranging in age from 8 weeks gestation to 18 years after birth. In addition, CYP3A5 levels were determined immunologically. Consistent with earlier studies, CYP3A5 protein was observed in 119/228 samples, but there was no apparent change in enzyme levels as a function of age (mean = 6.0 ± 5.2 pmol/mg microsomal protein for those samples with detectable protein). CYP3A7 was expressed at high but variable levels, 289.5 ± 103.7 pmol/mg microsomal protein, in all fetal samples examined ($n=11$, 13 to 40 weeks) while CYP3A4 was detectable in 7/11 samples, but at levels that were nearly 100-fold less than CYP3A7 (3.4 ± 2.6 pmol/mg microsomal protein). After birth, there was an approximate 50% decline in neonatal (0 to 30 days after birth) CYP3A7 enzyme levels (142.2 ± 100.5 mg microsomal protein, $n=14$) while little or no change was observed in CYP3A4 levels (6.5 ± 3.7 pmol/mg microsomal protein, $n=14$). In infants between 30 days and 1 year of age, CYP3A7 levels again decreased by nearly five-fold (27.3 ± 27.9 pmol/mg microsomal protein, $n=27$) while CYP3A4 increased modestly (8.4 ± 7.3 pmol/mg microsomal protein, $n=27$). In contrast to the earlier report by LaCroix et al. (1997), CYP3A4 levels in children between 1 and 10 years of age remained well below adult levels (13.1 ± 17.5 pmol/mg microsomal protein, $n=22$

versus 128.9±99.9 pmol/mg microsomal protein, $n=11$), although the data on CYP3A7 were consistent with an observed decrease to 3.3±4.8 pmol/mg microsomal protein ($n=22$). Thus, these studies would suggest that in many individuals, CYP3A7 remains the dominant CYP3A enzyme in children up to one year after birth and that CYP3A4 expression increases steadily, but does not reach adult levels until well after one year of age. These conclusions regarding CYP3A4 postnatal ontogeny are consistent with an *in vivo*, longitudinal study of dextromethorphan *N*-demethylase activity (Blake et al., 2007; Johnson et al., in press), as well as a metabolic study of amprenavir in fetal and postnatal liver samples (Treluyer et al., 2003). Furthermore, the variable, but high levels of CYP3A7 expression in fetal liver is consistent with a report by Leeder et al. (2005) in which mean CYP3A7 mRNA and protein levels were determined to be 14,200±15,000 transcripts/ng total RNA and 234.8±123.1 pmol/mg microsomal protein, respectively, in 54 fetal liver samples (11 to 32 weeks gestational age). Protein levels correlated well with testosterone 2α-and DHEA 16α-hydroxylation activities. This same group also demonstrated significant correlations between NR1I2 (pregnane X receptor) and NR1I3 (constitutive androstane receptor) expression and both CYP3A7 fetal liver and CYP3A4 postnatal liver expression (Vyhlidal et al., 2006), consistent with the known role each of these nuclear receptors have in regulating the expression of these genes. Yet the degree of association also clearly indicated the involvement of other factors.

The potential clinical implications of the CYP3A7/3A4 transition is illustrated by studies on the pharmacokinetics and pharmacodynamics of cisapride, a gastroprokinetic agent that was commonly used in pediatric patients presenting with feeding intolerance, apnea, and bradycardia due to gastroesophageal reflux. Due to a higher than acceptable incidence of cisapride-related QT prolongation in adult patients associated with excessive dose or high plasma levels of parent drug, cisapride was removed from the market and used only through a limited access program for specific diseases, including feeding intolerance in neonates. However, a prospective study in this same patient population demonstrated a significant increase in QT prolongation not associated with adult risk factors (Bernardini et al., 1997). Given the evidence that cisapride is metabolized primarily by CYP3A4, but not CYP3A5 or CYP3A7 (Pearce et al., 2001), it was hypothesized that neonates would show deficient metabolic ability (Treluyer et al., 2001). Consistent with this hypothesis, cisapride metabolism was only detectable in 4/7 microsomal preparations from fetal or neonatal liver aged less than 7 days and was low in those preparations that did show activity. The hypothesis was further confirmed by the *in vivo* study reported by Kearns et al. (2003b) in which the cisapride terminal elimination rate constant was shown to increase from approximately 0.02 h^{-1} in 30 week post-conceptional age patients to 0.20 h^{-1} in 52 week post-conceptional age patients. Other kinetic parameters also were consistent with compromised cisapride metabolic clearance in premature and term neonates. Thus, the differential substrate specificity of CYP3A4 and CYP3A7 combined with the developmental transition between these two enzymes can have a significant impact on the risk for adverse drug reactions in neonatal patients, particularly those born prematurely.

3.1.4. Flavin-containing monooxygenases (FMO)

The FMO family of enzymes (EC 1.14.13.8) are important for the oxidative metabolism of a wide variety of therapeutics, toxicants and endogenous compounds containing nucleophilic nitrogen-, sulfur-, selenium-, or phosphorous-heteroatoms. Common therapeutic substrates include tamoxifen, itopride, benzydamine, olopatidine, and xanomeline (Krueger & Williams, 2005). Eleven human *FMO* genes have been identified. *FMO1*, *2*, *3*, *4* and *6P* are contained within a cluster at chromosome 1q24.3. *FMO5* lies outside the cluster and in the opposite orientation at 1q21.1. A second cluster is found at 1q24.2, but consists entirely of pseudogenes, *FMO7P-11P* (Hernandez et al., 2004). The FMO enzymes active in xenobiotic metabolism include FMO1, 2 and 3 (Krueger & Williams, 2005).

Evidence for a developmental transition between human hepatic FMO1 and FMO3 was first reported by Dolphin et al. (1996). FMO1, FMO3 and FMO4 mRNA levels were quantified in individual fetal liver, kidney, lung and brain and adult liver, kidney and lung tissue samples from donors of unspecified ages. Although considerable interindividual variability was observed, FMO1 transcripts were the most abundant in fetal liver (1.7 to 3.1 nmol/cell, $n=2$) and kidney (2.7 to 4.8 nmol/cell, $n=2$), but were only present at low levels in lung and brain (0.1 to 0.3 nmol/cell, $n=1$ each). In the adult, FMO1 transcripts only were observed in the kidney (0.3 to 2.7 nmol/cell, $n=2$). FMO3 transcripts were present at low levels in the fetal liver (0.2 nmol/cell, $n=2$) and lung (0.2 nmol/cell, $n=1$), but not kidney. In contrast, FMO3 was the most abundant in the adult liver (1.7 to 3.3 nmol/cell, $n=4$) and also was expressed at low levels in the kidney and lung (0.2 to 0.9 nmol/cell, $n=2$ and $n=1$, respectively). Finally, FMO4 was expressed at low levels in all fetal tissues tested (0.1 to 0.3 nmol/cell), except brain, and did not change as a function of life stage. The restriction of FMO1 expression to the fetal liver was confirmed by Yeung et al. (2000) using immunological approaches to measure FMO1 protein levels in fetal and adult liver, kidney and small intestine. FMO1 levels of 14.4±3.5 pmol/mg microsomal protein (mean±SD, $n=5$) were observed in fetal liver, but expression was not detected in four adult livers. Consistent with the earlier studies measuring transcript levels (Dolphin et al., 1996), FMO1 protein levels were highest in the adult kidney, 47±9 pmol/mg microsomal protein ($n=4$). However, this enzyme also was observed in the adult small intestine at 2.9±1.9 pmol/mg microsomal protein ($n=7$). High levels of FMO1 expression in the adult kidney also was reported by Krause et al. (2003).

Although the studies by Dolphin et al. (1996) and Yeung et al. (2000) supported the concept of a developmental transition between human hepatic FMO1 and FMO3, several questions remained unanswered, including the time-frame of the transition, whether or not coordinate regulation of both genes was involved, whether there were changes in expression at either locus within the developmental stages examined, and whether there were interindividual differences in the timing of the FMO1/FMO3 transition. To address some of these questions, Koukouritaki et al. (2002) quantified FMO1 and FMO3 protein levels in 240 human liver samples representing ages from 8 weeks gestation to 18 years after birth. This study revealed that hepatic FMO1 expression was largely limited to the fetus and that the highest level of expression (mean±SD=7.8±5.3 mol/mg microsomal protein, $n=25$) was observed at gestational ages less than 15 weeks. FMO1 expression was detectable in 96% of the samples examined during this time-frame. FMO1 expression subsequently declined by approximately 50% in each subsequent trimester and was essentially silenced within 3 days after parturition in a birth-, rather than gestational age-dependent process. Furthermore, the percentage of samples with detectable FMO1 decreased to 90% ($n=33$) in the second trimester, 70% ($n=34$) in the third trimester, and during the first three days after birth, to only 16% ($n=147$). Low levels (0.8±1.0 mol/mg microsomal protein, $n=13$) of FMO3 expression were detectable in about 15% of first trimester fetal liver samples, but expression was non-detectable throughout the remaining gestational period. Low FMO3 expression levels (1.1±3.3 mol/mg microsomal protein, $n=37$) also were observed in approximately 25% of the neonatal liver samples. However, it was only after 1 month and before 10 months of age that detectable FMO3 was observed in most samples with mean levels of 4.7±5.9 mol/mg microsomal protein ($n=67$). These data suggested birth is necessary, but not sufficient for the onset of FMO3 expression. Mean FMO3 protein levels of 12.7±8.0 mol/mg microsomal protein ($n=43$), about 50% of adult values, were observed in tissue samples from individuals between 10 months and 11 years of age. In post-puberty tissue samples up to 18 years, a gender-independent, near linear increase in FMO3 expression was observed to levels approaching adult values (26.9±8.6 pmol/mg microsomal protein, $n=19$). The above studies suggest that not only is the ontogeny of hepatic FMO1 and FMO3

regulated independently, but that the ontogeny of FMO1 itself is regulated differently in different tissues. Support for this supposition has been reported by several laboratories. Zhang and Cashman (Zhang & Cashman, 2006) described FMO transcript levels in human brain as a function of age and demonstrated a decline in FMO1 mRNA levels from 18 to 21 weeks gestation through 15 to 40 years. In contrast, FMO2, 3, 4 and 5 expression was unchanged. FMO1 transcripts also were present at relatively high levels in both fetal and adult nasal mucosa (Zhang et al., 2005). Finally, Shephard et al. (2007) have shown that FMO1 expression in kidney and fetal liver is regulated by the use of different promoters combined with alternative splicing.

3.1.5. Paroxonase (PON1)

Plasma PON1 (EC 3.1.1.2) appears to have an endogenous role in the metabolism and clearance of oxidized lipids, thus protecting against vascular disease (Aviram et al., 1998; Durrington et al., 2001). However, this enzyme also is actively involved in the metabolism of a number of organophosphorous compounds, including soman, sarin, diazoxon, and chlorpyrifos oxon (Costa et al., 1999). By measuring plasma arylesterase activity longitudinally, Cole et al. (2003) determined PON1 expression levels in nine individuals between birth and 25 months of age. PON1 expression was shown to increase two- to seven-fold before reaching plateau levels at 10 to 25 months. Most interesting, different individuals reached plateau levels at different times; some as early as four months and in some, levels were still rising at the end of the 25 month study period. Given that PON1 levels and activity are important in determining risk for organophosphorous compound-induced toxicity, these data would be consistent with young children having increased sensitivity.

3.2. Conjugation enzymes

3.2.1. Epoxide hydrolase (EPHX)

Although several mammalian EPHX (EC 3.3.2.3) exist, two are known for their important role in detoxifying often highly reactive xenobiotic epoxides by the addition of water to form dihydrodiols, microsomal epoxide hydrolase (EPHX1) and soluble epoxide hydrolase (EPHX2). More recently, EPHX2 also has been recognized for its critical role in the inactivation of epoxyeicosatrienoic acid signaling molecules that exhibit potent vasodilatory, antiinflammatory, and fibrinolytic effects. As such, EPHX2 has received attention as a potential therapeutic target (Morisseau & Hammock, 2005). EPHX1 and EPHX2 are encoded by distinct genes on chromosome 1q42.1 and 8p21–p12, respectively.

Pacifici and Rane (1982) and Pacifici et al. (1983a) were the first to demonstrate fetal EPHX1 activity in various tissues between 14 and 25 weeks gestation. Styrene EPHX1 activity (mean ± SD, nmol/min/mg microsomal protein, $n=7$) was reported to be 5.9 ± 1.6 in fetal liver, 3.6 ± 1.0 in adrenals, 0.6 ± 0.1 in lung, 0.5 ± 0.4 in kidney and 0.3 ± 0.1 in intestine. In contrast, EPHX1 activity in adult liver ($n=6$, ages 29 to 69) was approximately two-fold higher, 13.8 ± 2.4 nmol/min/mg microsomal protein. Although clear differences were noted between fetal and adult liver, there was no apparent change with gestational age. By increasing the sample-size to 20 and extending the age range to 10 to 25 weeks gestation, a weak correlation between gestational age and microsomal EPHX1 activity became apparent ($r=0.6$), but this relationship was driven heavily by one or two samples (Pacifici & Rane, 1983b). The differences between fetal and adult liver EPHX1 were corroborated by Cresteil et al. (1985) who measured both EPHX1 protein levels and benzo[*a*]pyrene 4,5-oxide hydrolase activity in microsome preparations from 14 fetal liver samples, ages 17 to 32 weeks, and nine adult liver samples. EPHX1 activities ranged from 1.0 to 3.3 nmol/min/mg protein in the fetal samples, 6.0 to 9.7 nmol/min/mg protein in the adult samples, and there was an excellent correlation between activity and protein concentration ($r=0.939$). None of the above studies were able to demonstrate a change in EPHX1 activity or protein levels as a function of gestational or postnatal age, although the substantial difference between fetal and adult liver samples was highly reproducible. Age-dependent changes were demonstrated by Omiecinski et al. (1994) who determined EPHX1 catalytic activity, protein and mRNA levels in 18 fetal liver samples, ages 7.6 to 21.9 weeks using benzo[*a*]pyrene 4,5-oxide as a substrate, a monospecific peptide antibody against human EPHX1, and oligonucleotide probes, respectively. Activity ranged from 41 pmol/min/mg protein in the 7.6 week samples to 306 pmol/min/mg protein in the older fetal samples. A high degree of correlation was observed between age and activity ($r=0.82$), as well as between protein levels and activity ($r=0.93$). Mean (±SD) adult hepatic EPHX1 activity was reported as 424 ± 236 pmol/min/mg protein ($n=15$). No correlation was observed between activity and mRNA content in either the fetal or adult samples.

Mean fetal liver EPHX2-dependent styrine oxide hydrolase activity was determined to be 0.23 ± 0.02 nmol/min/mg protein ($n=10$) while adult values were 0.83 ± 0.05 nmol/min/mg protein (Pacifici et al., 1983a). Similar results were obtained with stilbene oxide as an EPHX2 substrate with mean (±SD) activity in the fetal liver being 55.2 ± 89.6 pmol/min/mg protein (ages 14 to 27 weeks, $n \cong 40$) and five-fold higher levels in the adult liver, 303.2 ± 73.2 pmol/min/mg protein (ages 30 to 72 years, $n \cong 40$) (Pacifici et al., 1988). Similar to the earlier studies on EPHX1, there was no apparent change in EPHX2 activity as a function of gestational or postnatal age, although a correlation might be apparent with tissue samples from a wider range of gestational ages.

3.2.2. Glutathione S-transferase (GST)

The GST enzymes (EC 2.5.1.18) catalyze the nucleophilic attack of reduced glutathione against hydrophobic xenobiotics containing electrophilic carbon, nitrogen or sulfur atoms, thereby detoxifying these compounds. Substrates include a variety of halogenonitrobenzenes, arene oxides, quinones, and unsaturated carbonyls. Although there are three families of GST enzymes, microsomal, cytosolic and mitochondrial, this review will focus on the cytosolic enzymes, as these are considered most important for drug and toxicant metabolism (Hayes et al., 2005).

The cytosolic GST enzyme family consists of 16 genes within six subfamilies: *GSTA* (alpha), *GSTM* (mu), *GSTO* (omega), *GSTP* (pi), *GSTT* (theta), and *GSTZ* (zeta) (Nebert & Vasiliou, 2004). The encoded enzymes function as dimers with members of the GSTA and GSTM families being able to form heterodimers with each other. The enzymes exhibit overlapping substrate specificities, which has made it difficult to characterize the expression of individual genes based on catalytic activity (Hayes et al., 2005).

Pacifici and Rane (1982) and Pacifici et al. (1983a) demonstrated GST activity toward styrene oxide in fetal liver, adrenals, lungs, kidney, and intestine (ages 14 to 25 weeks gestation, $n=10$) that varied little among the tissues examined and ranged from 2.6 to 5.6 nmol/min/mg protein. Activity against this same substrate increased to a level of 9.95 ± 1.75 nmol/min/mg protein in adult liver ($n=14$, ages 29 to 69 years). However, members of the GSTM, GSTT and GSTP families also exhibit activity towards this substrate making it difficult to conclude anything regarding the expression of specific proteins from these studies. The first attempt to differentiate between GST enzymes was accomplished using starch gel electrophoresis and chromatofocusing (Strange et al., 1985) that permitted an examination of GSTM (GST1), GSTA (GST2) and GSTP (GST3) ontogeny. Tissue samples were divided into five age groups: group 1, 10–20 weeks gestation ($n=15$); group 2, 21–30 weeks gestation ($n=33$); group 3, 31–42 weeks gestation ($n=14$); group 4, term infants who died between 2 and 67 weeks postnatal age ($n=38$); and adults ($n=6$). A progressive increase in GSTM expression was observed as a function of age. Low expression of GSTM was observed in a small number of the group 1 and group 2 samples accounting for 2.5 to 16.5% of the total GST activity. However, readily detectable protein was present in 7/14 of the group 3 tissue samples accounting for 5.0 to 24.5% of total activity. The postnatal samples (group 4) exhibited GSTM activities that were no different than that observed in adults, accounting for 14.9 to

55.3% of total GST activity. In contrast, GSTA was expressed at relatively high levels within each of the age groups, accounting for 45 to 90% of the total GST activity, with no apparent change with age. The developmental GSTP expression pattern was the opposite of GSTM, exhibiting the highest activity in the group 1 samples (30 to 50% of total GST activity), declining to 10 to 20% of total GST activity in the group 2 samples, and becoming undetectable in any of the postnatal and adult liver tissue. A subsequent study by this same group (Strange et al., 1989) further refined the enzymes being examined by distinguishing between GSTA1 and A2, as well as expanding the number of study samples (Fig. 3). GSTA1 and A2 were expressed in fetal liver and there was a significant 1.5- to 2.0-fold increase at birth, but no change between neonatal and adult samples. GSTA2 was present at levels approximately 10% of GSTA1 levels. GSTM also was expressed in fetal liver and similar to the GSTA enzymes, increased four- to five-fold at birth, approaching adult levels. Finally, GSTP was expressed at the highest levels in early gestation (<20 weeks) and declined progressively with age to nearly non-detectable levels in adults. GSTA1 and A2 also were detected in fetal lung, but at levels 0.5 to 1% of that observed in the liver. Unlike what was observed in liver, no increase in expression was seen at birth. GSTM and GSTP also were detectable in fetal lung at similar levels as those observed in liver and decreased by approximately 50% and 30% at birth, respectively (Beckett et al., 1990). GSTA1 and GSTA2 were both expressed in fetal kidney at similar levels (0.042 ± 0.05 and 0.03 ± 0.05 μg/mg cytosolic protein, respectively, $n=8$). Levels of both enzymes increased 5- to 10-fold at birth. GSTM and GSTP were present in both fetal and postnatal kidney samples at similar levels as those observed in liver (Beckett et al., 1990). Immunohistochemistry demonstrated GSTA and GSTP in both collecting tubules and developing nephrons in fetal kidney, but after 35 weeks gestation, GSTA was restricted to the proximal tubule while GSTP was restricted to the distal and collecting tubules and the loop of Henley (Hiley et al., 1989). A similar approach demonstrated widespread expression of these same enzymes, as well as GSTO in many fetal tissues (van Lieshout et al., 1998; Yin et al., 2001). However, the demonstration that the GST enzymes are expressed in specific cell-types within lung and kidney also suggests that the earlier measured enzyme levels from total tissue homogenates likely underestimated cellular expression levels.

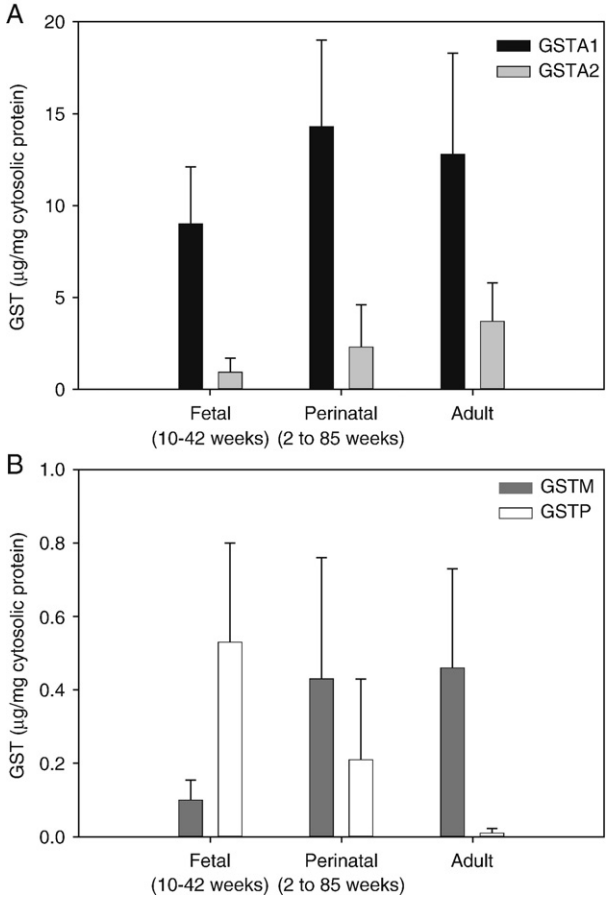

Fig. 3. GSTA1, A2, M and P ontogeny. GSTA1 and A2 (**A**) and GSTM and P (**B**) protein levels were determined by chromatofocusing using starch gel electrophoresis in cytosolic preparations from liver tissues from donors that had been divided into fetal, perinatal, and adult age brackets. Data from Strange et al. (1989).

The above studies clearly demonstrate gene-specific developmental expression patterns for selected members of the GST enzyme family. Furthermore, the relatively high levels and ubiquitous expression would suggest important roles for these enzymes during development. Yet, our knowledge currently is limited to a select few of the 16 genes that constitute the *GST* family (Nebert & Vasiliou, 2004). Thus, GST ontogeny remains an important, but understudied area.

3.2.3. Sulfotransferase (SULT)

The family of SULT enzymes (EC 2.8.2.1) catalyze the conjugation of a sulfonate moiety donated by 3′-phosphoadenosine-5′-phosphosulfate to a wide variety of electrophilic substrates, generally resulting in inactivation and facilitated elimination from the body. The human SULT enzymes are encoded by three gene families based on divergent evolution and function, the phenol SULTs (*SULT1*), the hydroxysteroid SULTs (*SULT2*) and a more recently discovered brain-specific SULT (*SULT4*). The SULT1 family is subdivided into four subfamilies encoding seven genes (*SULT1A1-1A3/4*; *SULT1B1*, *SULT1C2* and *1C4*; and *SULT1E1*) and the SULT2 family into two subfamilies encoding two genes (*SULT2A1* and *2B1*). A single gene encodes the only known brain-specific SULT, *SULT4A1* (Blanchard et al., 2004). No information is available regarding the ontogeny of SULT1A2, 1B1, 1C4, 2B1 or 4A1.

Early studies utilized probe substrates to begin characterizing SULT ontogeny with the inherent problem, perhaps not appreciated at the time, of overlapping substrate specificity. Mean (±SD) 2-naphthol sulfotransferase activity, most likely catalyzed by one or more members of the SULT1A family, was reported to be 0.18 ± 0.12 nmol/min/mg protein in fetal liver (ages from 14 to 27 weeks, $n=30$) increasing approximately three-fold to 0.63 ± 0.22 nmol/min/mg protein in adults (number and ages unspecified) (Pacifici et al., 1990). In contrast, Duanmu et al. (2006) described the ontogeny of SULT1A1 by determining protein levels by western blotting; quantifying by linear regression using recombinant protein. Substantial expression was observed in fetal liver that was not significantly different from liver tissue donated from individuals 0 to 12 months of age or >12 months to 18 years (Fig. 4A). These data are consistent with earlier studies by Richard et al. (2001) and Stanley et al. (2005) using both *p*-nitrophenol and thyroxine as substrates, as well as the more recent study by Adjei et al. (2008). Stanley et al. (2005) also demonstrated high levels of SULT1A1 in the choroid plexus using *p*-nitrophenol as a probe substrate (45 pmol/min/mg), but much less so in other regions of the brain (<5 pmol/min/mg protein). A very different expression pattern was observed in the developing lung where there appeared to be a progressive decrease in SULT1A1 protein levels as a function of both prenatal and postnatal age and a significant two-fold decrease in expression when comparing mean prenatal to postnatal SULT1A1 protein levels (Hume et al., 1996). However, all of these studies need to be reconsidered in the context of the findings recently reported by Hebbring et al. (2007) in which a SULT1A1 copy number polymorphism was described that occurs with different frequencies in different population groups.

SULT1A3 ontogeny has been characterized using dopamine as a probe substrate. The enzyme was widely expressed in fetal tissue (ages 18 to 25 weeks, $n=6$) with the highest level in fetal gut (mean ± SD = 200 ± 133 pmol/min/mg) followed by fetal lung, liver (122 ± 52 pmol/min/mg and 96 ± 37 pmol/min/mg, respectively) and kidney (37 ± 30 pmol/min/mg). Dopamine sulfotransferase activity was

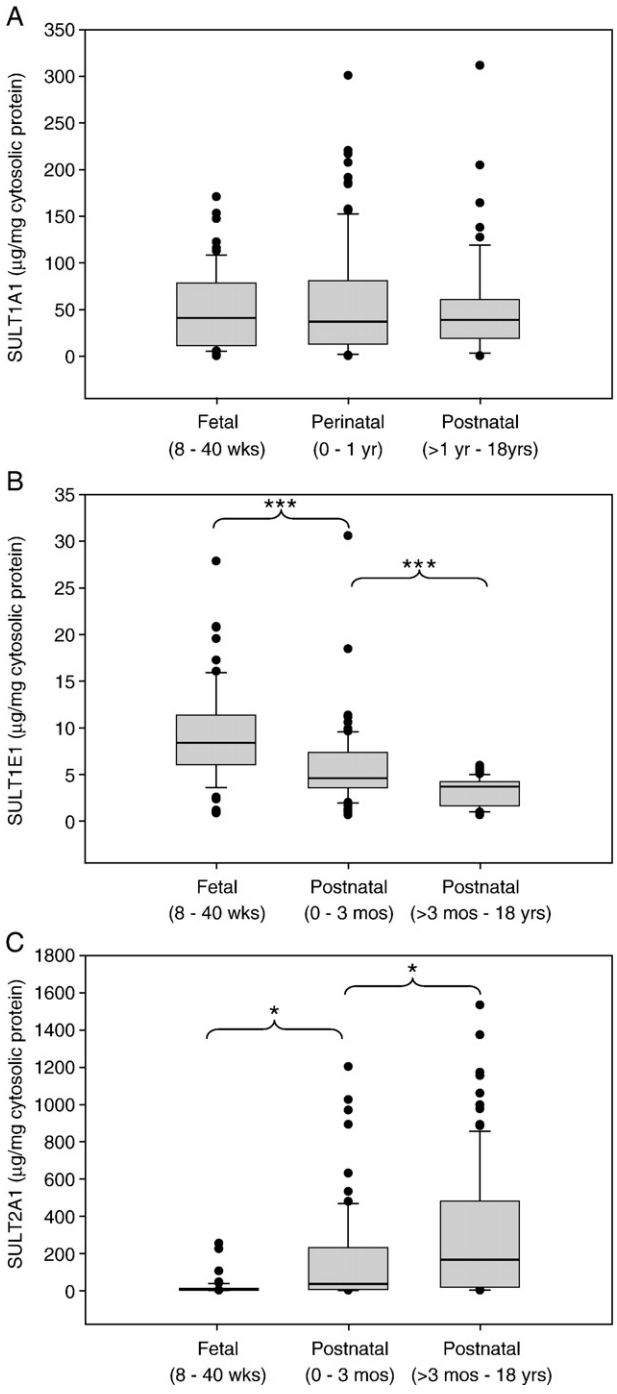

Fig. 4. SULT1A1, 1E1 and 2A1 ontogeny. SULT1A1 (**A**), SULT1E1 (**B**) and SULT2A1 (**C**) protein levels were determined by quantitative western blotting in cytosolic preparations from fetal (n=68), postnatal <1 year (n=105), and postnatal >1 year (n=59) liver samples. Data are plotted as medians (bars), interquartile values (boxes) and 10th to 90th percentiles (whiskers). Outliers were defined as 1.5 times the interquartile values. Data in different age brackets were compared using Kuskal–Wallis one way ANOVA on ranks (SigmaStat Version 3.1, Systat Software, Chicago, IL) (*=P<0.05; ***=P<0.001). Data from Duanmu et al. (2006).

essentially unchanged in adult gut (188±33 pmol/min/mg), but was decreased in lung (78±33 pmol/min/mg), liver (30±15 pmol/min/mg) and kidney (11±7 pmol/min/mg) (Cappiello et al., 1991). Substantially greater changes were reported by Richard et al. (2001) and Stanley et al. (2005) with a 10-fold decrease in hepatic SULT1A3 levels between fetal (mean±SD=75.6±52.3 pmol/min/mg, ages 10 to 22 weeks, n=31) and postnatal liver (6.7±7.6 pmol/min/mg, n=13) and a six-fold decrease between fetal (39.7±18.8 pmol/min/mg, n=5) and postnatal lung (6.6±10.0 pmol/min/mg, n=15). Low SULT1A3 activity also was detected in various regions of the fetal brain (<5 pmol/min/mg protein). However, the complexity of interpreting data related to *SULT1A3* expression has increased with the finding that the gene has been duplicated in most if not all individuals, resulting in a locus referred to as *SULT1A3/4*. Both gene copies are transcriptionally active and result in an identical protein (Hildebrandt et al., 2004).

Estrone and/or ethinyl estradiol have been used commonly as probe substrates for SULT1E1. Miki et al. (2002) detected widespread expression of this enzyme in a variety of fetal and adult tissues, but were unable to determine any trends in expression as a function of age. Expression in most fetal tissues, including relatively high levels in the fetal choroid plexus, was confirmed by Stanley et al. (2005). This latter group also provided evidence using western blotting for a decrease in SULT1E1 expression between fetal and adult liver. An inverse correlation between hepatic SULT1E1 protein levels and early age was confirmed by Duanmu et al. (2006) who demonstrated a progressive decrease in expression in fetal liver from the first to third trimester, a further decrease between fetal and perinatal (0 to 3 months after birth) and a final decrease between the perinatal and tissue samples from older children (Fig. 4B). Higher expression levels also were demonstrated in male versus female fetal liver tissue, which the author's speculated might protect the male fetus from excessive estrogen levels.

Although the ontogeny of SULT1C2 has not been documented, transcripts were detected in fetal kidney and liver with the greater concentration in the former tissue (Her et al., 1997). In a subsequent study, the highest level of SULT1C2 protein was observed in fetal small intestine, followed by fetal kidney and liver. Low protein levels were observed in a variety of other fetal tissues. Furthermore, protein levels appeared less in adult liver. No SULT1C1 was detectable in fetal brain (Stanley et al., 2005).

DHEA sulfation can be used to probe for SULT2A1 enzyme levels. Barker et al. (1994) used this reaction to demonstrate relatively low levels of SULT2A1 activity in fetal liver, but substantially increased levels in postnatal samples and adults. Furthermore, there seemed to be a relatively good correlation between activity and protein levels as determined by immunodot blot analysis. A somewhat similar trend was observed in fetal and postnatal lung, although lung SULT2A1 levels appeared to match those seen in postnatal samples by the third trimester (Hume et al., 1996). A subsequent study by this same group documented SULT2A1 enzyme activity in fetal liver (~84 pmol/min/mg protein), adrenal (~422 pmol/min/mg protein), kidney (~49 pmol/min/mg protein), small bowel (~56 pmol/min/mg protein) and lung (~7 pmol/min/mg protein), but no activity in brain. A substantial increase was observed in adult liver (~323 pmol/min/mg protein) (Stanley et al., 2005). Duanmu et al. (2006) confirmed this SULT2A1 developmental expression pattern. SULT2A1 was readily detectable in fetal liver (ages 8 to 40 weeks, n=68), but increased significantly in the perinatal period (0 to 3 months, n=71), achieving adult levels in samples older than 3 months of age (n=95) (Fig. 4C).

3.2.4. UDP glucuronosyltransferase (UGT)

Using uridine 5'-diphosphoglucuronic acid as a donor, the UGT enzymes (EC 2.1.4.17) catalyze the conjugation of hydrophobic compounds containing functional groups, often added during oxidative drug metabolism, to form β-D-glucopyranosiduronic acids. These products generally are inactive and are targets for facilitated renal and biliary elimination from the body. UGT-dependent glucuronidation represents a major route of drug clearance, accounting for nearly 15% of this activity in the adult human (Williams et al., 2004). Two UGT gene families exist in the human that encode 16 genes named based on the divergent evolution of their amino acid sequence. Nine functional genes exist in the *UGT1* family, *UGT1A1*, *1A3*, *1A4*, *1A5*, *1A6*,

1A7, *1A8*, *1A9* and *1A10*, on chromosome 2q37 and seven within the *UGT2* family, *UGT2A1*, *UGT2B4*, *2B7*, *2B10*, *2B11*, *2B15* and *2B17* on chromosome 4q13 to q13.2 (Tukey & Strassburg, 2000). UGT ontogeny was reviewed by de Wildt et al. (1999). Summarizing their assessment of the literature at that time, UGT1A1, the major enzyme responsible for bilirubin glucuronidation, was essentially undetectable in fetal liver. Enzyme expression was observed to increase immediately after parturition in a process independent of gestational age. Adult levels of the enzyme were attained by three to six months of age. Using estrone as a probe substrate, UGT1A3 was shown to be present in fetal and neonatal liver at levels approximately 30% of those in adult tissue, but the pattern of ontogeny was unknown. UGT1A6 was present in fetal liver at 1 to 10% of adult levels. Beginning at birth and independent of gestational age, expression increased slowly, achieving 50% of adult values by six months of age. However, UGT1A6 maturation was not complete until puberty. Because of its specificity for morphine glucuronidation, several studies have been performed on the ontogeny of UGT2B7. In fetal liver samples from donors 15 to 27 weeks gestational age, UGT2B7 was detectable at levels that were 10 to 20% of adult values. Progressive increases in expression were observed with age such that adult expression levels were attained by two to three months after birth. Finally, UGT2B17 was shown to be expressed in the fetal liver at levels <10% of adults. Expression increased to 10% of adult values in the neonate, but the pattern of subsequent maturation is unknown.

Strassburg et al. (2002) conducted an exhaustive analysis of hepatic UGT ontogeny in 16 infant liver samples, ages 6 to 24 months, comparing these data to that obtained from 12 adult patients, ages 12 to 75 years. In addition, RNA from two fetal samples at 20 weeks gestation was available. UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10 and 2B15 expression levels were examined using quantitative RT-PCR to measure transcript levels, immunological detection of protein, and activity assays with a variety of substrates. No UGT transcripts were detected in the two 20 week gestation fetal samples. Although these data conflict with earlier reports wherein low levels of UGT activity and/or protein level were detected, all of the available data are consistent with the onset of UGT activity during the latter half of the second trimester or the third trimester. Transcript levels in the infant samples were binned into three age brackets, 6 to 12 months, 13 to 18 months, and 19 to 24 months, and compared among themselves and to adult levels. No difference was observed between any of these age brackets and adult expression for UGT1A1 or UGT2B7 transcripts. In contrast, there was a progressive increase in UGT1A9 transcript levels as a function of age. For UGT2B4, there were no differences among the different pediatric age groups which all exhibited protein levels 30 to 40% of those in adults. The catalytic activities within all of the pediatric age groups were lower than that observed in the adult samples. These data are consistent with a report by Zaya et al. (2006) in which the maturation of epirubicin glucuronidation, catalyzed by UGT2B7, was evaluated *in vitro*. This study demonstrated a progressive increased in UGT2B7 activity across five age groups, <1 year of age, 1 to 5 years of age, 6 to 11 years of age, 12 to 17 years of age, and adult. However, allometric scaling using the 3/4 power rule (Anderson et al., 1997) predicted that UGT2B7-dependent clearance activity would be attained by 2 to 3 months of age, consistent with *in vivo* studies.

4. Cell-specific expression and ontogeny

In the human fetus, the liver is the major site of multilineage hematopoiesis with initial activity detectable by five weeks gestation, maximal activity by 15 weeks gestation, and then a decline and eventual disappearance of activity at or around birth. During the peak of this activity, hematopoietic stem cells and precursors account for nearly 50% of the total cells in the developing liver. This is in contrast to the adult and even perinatal liver where parenchymal hepatocytes dominate (Morrison et al., 1995). Given this major change in function from hematopoiesis to anabolism/catabolism associated with gluconeogenesis, xenobiotic biotransformation, and other metabolic pathways, one must question what role cell-specific expression of the DME might have during ontogeny in the fetal liver and how this might effect risk for adverse drug reactions. Although this remains an area of much needed study, Richard et al. (2001) demonstrated much higher SULT1A1 expression in hepatic hematopoietic stem cells at 16 weeks gestation than in neighboring hepatocytes. In the adult, moderate SULT1A1 expression was observed in hepatocytes. Overall, however, hepatic SULT1A1 expression has been reported to remain relatively constant during development (Richard et al., 2001; Stanley et al., 2005; Duanmu et al., 2006; Adjei et al., 2008). Thus, taken together, these observations would suggest a decline in hematopoietic cell-specific SULT1A1 expression during the prenatal period and a corresponding increase in hepatocyte-specific SULT1A1 expression. More recently, Shao et al. (2007) addressed the question of cell-specific expression more directly, measuring the expression of several cytochromes P450 and GST enzymes in both hematopoietic stem cells and total fetal liver. Neither CYP1A2 nor CYP3A7 were detectable in hematopoietic stem cells. Thus, the high levels of CYP3A7 expression reported in fetal liver would appear to be restricted to fetal hepatocytes. Low but indistinguishable levels of CYP1A1 mRNA were detected in both hematopoietic stem cells and hepatocytes. In contrast, although low levels of both CYP3A4 and 3A5 transcripts, protein and activity were observed in hematopoietic stem cells, much higher levels were observed in total fetal liver, suggesting the expression of these enzymes also was primarily in fetal hepatocytes. Among the GST enzymes studies, GSTM1, M2, M4 and P1 were expressed at substantially higher levels in hematopoietic stem cells versus total fetal liver whereas GSTM3 and T1 were expressed at equivalent levels. GSTA1, A2, A3 and A4 were all expressed at much higher levels in total fetal liver than in hematopoietic stem cells. Thus, the hematopoietic stem cells of the fetal liver would appear to have a high capacity for GSTM-and P1-dependent conjugation, but much less capacity for cytochrome P450-mediated oxidation. However, the results also suggest future studies should consider cell-specific expression in the fetal liver when characterizing DME ontogeny.

5. Regulation of drug metabolizing enzyme ontogeny

Knowledge regarding specific mechanisms regulating the developmental expression of the DME genes is extremely limited. Factors regulating the ontogeny of the class 1 *ADH* genes (*ADH1A*, *ADH1B*, and *ADH1C*) have been reviewed by Edenberg (2000). All of the class 1 *ADH* contain TATA boxes within their basal promoters. All three of these genes also contain a CCAAT/enhancer binding protein (C/EBP) element between the TATA box and the transcription start site that is capable of binding both C/EBPα and C/EBPβ, although there is some evidence that C/EBPα may preferentially bind this proximal element. The C/EBP proteins belong to the larger basic/leucine zipper (bZIP) family of transcription factors (Schrem et al., 2004). A second C/EBP responsive element is located immediately upstream of the TATA box in *ADH1B* and *ADH1C*, but not *ADH1A*. This second element also is able to bind members of the proline and acidic acid rich (PAR) b/ZIP family of factors (Schrem et al., 2004). Finally, all three class 1 *ADH* genes contain a more distal hepatocyte nuclear factor 1 (HNF1) site at approximately position-200 that primarily binds HNF1α in liver tissue. Co-transfection studies with an HNF1α expression vector stimulated *ADH1A*, but not *ADH1B* or *ADH1C* promoter activity in the human hepatoma cell line, HepG2. Mutation of the HNF1 site did reduce constitutive *ADH1B* promoter activity.

The developmental and tissue-specific expression pattern of the HNF1α, C/EBPα, C/EBPβ, and PAR transcription factors is consistent with a role in *ADH* ontogeny. Thus, HNF1 is expressed in the kidney, liver, intestine and pancreas of adult animals, but also is detected in the liver during the earliest stages of organogenesis (Cereghini, 1996). HNF1α likely contributes to the onset of hepatic *ADH1A* expression during the first trimester through the distal site and in

combination with other factors, the later onset of *ADH1B* and *1C* expression.

C/EBPα expression is observed in differentiated hepatocytes. In rodents, hepatic expression increases late in gestation, peaks in the perinatal period, and is then maintained at moderate levels in adult animals (Birkenmeier et al., 1989). Thus, C/EBPα may well contribute to the overall increase in *ADH1* expression as a function of age, but also the onset of *ADH1B* later in gestation. A pattern similar to C/EBPα also has been observed for C/EBPβ. Multiple studies in rodents reported low levels of C/EBPβ mRNA and protein in fetal liver and greatly increased levels of C/EBPβ mRNA and protein in adult liver (*e.g.*, Descombes and Schibler (1991)). However, multiple forms of C/EBPβ are known to exist due to translation from alternative ATG start codons and/or through proteolytic cleavage, the former process apparently regulated by the CUG binding protein 1 (Schrem et al., 2004). The dominant form in fetal liver is C/EBPβ liver inhibitory protein (LIP) in which a downstream ATG site is selected, eliminating the transactivation domain but retaining the full bZIP DNA binding domain. Thus, C/EBPβ LIP serves as a competitive repressor. The dominant C/EBPβ form in adult liver is liver activating protein (LAP), a near full-length protein that retains all of the functional domains. In rodents, the hepatic C/EBPβ LAP:LIP ratio increases significantly during development from about 3:1 before birth to about 15:1 in adults with a transient perinatal peak of LAP expression (Descombes & Schibler, 1991). Thus, assuming a similar pattern in human, the developmental change in C/EBPβ LAP:LIP also likely contributes to both the increase in *ADH1* expression as a function of age, but also the postnatal onset of *ADH1C* expression.

The PAR transcription factor family members exhibit a temporal-specific expression pattern that is consistent with a role in regulating the postnatal transition observed with ADH and other DME (Cereghini, 1996). Three members of the PAR family, D-element binding protein (DBP), thyrotroph embryonic factor (TEF) and hepatic leukemia factor (HLF), act as transcriptional activators and share a C-terminal motif important for enhancer function. The fourth member of the PAR family, E4 binding protein 4 (E4BP4) (or nuclear factor, interleukin 3 regulated, NFIL3), shares the unique bZIP domain and recognizes the same consensus DNA sequence, but does not contain a PAR motif. Instead, E4BP4 functions as an active repressor, having a unique C-terminal motif that interacts with the TATA box binding protein, DR1 (Cowell & Hurst, 1994; Cowell & Hurst, 1996). Of the positively acting PAR family members, DBP and TEF hepatic expression is only observed postnatally (Mueller et al., 1990; Drolet et al., 1991; Nagy et al., 1994). The active repressor, E4BP4, is expressed in fetal liver (Hulme et al., 2000) and at low levels in adult liver (Lai & Ting, 1999). Thus, PAR transcription factor family ontogeny is consistent with a role in regulating the postnatal onset of *ADH1C* expression.

What about the other DME? HNF1α has been shown to be a critical factor for the expression of *FMO1* and as such, likely contributes to the onset of expression of this gene during the first trimester (Luo & Hines, 2001). The C/EBPβ LAP:LIP ratio regulates *CYP3A4* expression (Martinez-Jimenez et al., 2005), as does the PAR transcription factor family member, DBP (Ourlin et al., 1997). Both of these factors may contribute to the postnatal regulation of this gene. These factors likely will be implicated in the expression of other DME as more is learned about regulatory mechanisms. However, other mechanisms will be involved, including a possible role for changes in DNA methylation, a process that has been implicated in the birth-associated increase in *CYP2E1* expression (Vieira et al., 1996). Finally, changes in chromatin structure through histone modifications, a well recognized transcriptional regulatory mechanism during development (Kiefer, 2007), also is likely to be involved.

6. Summary and conclusions

Although oversimplified, the ontogeny of individual DME can be categorized into one of three groups. As typified by CYP3A7, FMO1, SULT1A3/4, SULT1E1, and perhaps ADH1A, some enzymes are expressed at their highest level during the first trimester and either remain at high concentrations, or decrease during gestation, but are silenced or expressed at low levels within one to two years after birth. An obvious question is whether or not these enzymes have an important endogenous function during hepatic development. CYP3A5, 2C19, and SULT1A1 are examples of enzymes that can be categorized in a second group. These enzymes are expressed at relatively constant levels throughout gestation. Moderate postnatal increases in CYP2C19 are observed within the first year, but not CYP3A5 or SULT1A1. ADH1C, ADH1B, CYP1A2, 2C9, 2D6, 2E1, 3A4, FMO3, and SULT2A1 are more typical of a third group of enzymes that are not expressed or are expressed at low levels in the fetus. For many, the onset of expression can be seen in either the second or third trimester. However, substantial increases in expression are observed within the first one to two years after birth. This third category of ontogeny also appears to include the largest number of enzymes, although this might change as our knowledge regarding DME ontogeny expands.

For those DME that belong to group three, *i.e.*, those that undergo a perinatal onset or significant increase in perinatal expression, most if not all exhibit greater interindividual variability during this time-frame compared to other age brackets. This observation is most apparent in scatter plots of the developmental expression data presented in some of the original reports on FMO3 (Koukouritaki et al., 2002), CYP2C9 (Koukouritaki et al., 2004), CYP2E1 (Johnsrud et al., 2003), and CYP3A4 (Stevens et al., 2003). As an example, both CYP2C9 and 2E1 exhibited an approximate 100-fold range of expression in the perinatal period, which was approximately two-times greater than that observed within any other age bracket. This is largely explained by what appears to be variability in the postnatal increase in expression for both enzymes. Thus, during the neonatal period, nearly 50% of the samples exhibited CYP2C9 and 2E1 expression levels that were no different than those observed in the fetal third trimester samples while the remaining samples exhibited CYP2C9 and 2E1 expression levels that were similar or approached the maximum observed over the entire sample-set. Somewhat similarly, the largest variation in CYP3A7 expression (>100-fold) was observed in the infant samples, likely explained by variation in the silencing or suppression of this gene during this period of time. In the case of FMO3, interindividual differences in the onset of expression during the first years of life are likely a major cause for the case reports of transient trimethylaminuria in children (Mayatepek & Kohlmüeller, 1998). Thus, there would appear to be windows of hypervariability during the ontogeny of many of the DME that would have a significant impact on risk for adverse drug reactions and/or failed efficacy and would not be predicted based on pharmacogenetic studies in adults.

For multiple enzyme families with members belonging to both group one and group three categories, the term “developmental switch” is often used to describe the transition between the predominant fetal enzyme form to the predominant adult enzyme form. Such a “developmental switch” within the CYP3A family, wherein hepatic CYP3A7 expression dominates in the fetus while hepatic CYP3A4 expression dominates in the adult, has long been recognized (LaCroix et al., 1997). A similar phenomenon occurs within the FMO family between FMO1 and FMO3. However, less obvious and well described are developmental switches in other enzyme systems. Thus, when individual CYP2C9 and 2C19 expression levels were compared, CYP2C19 was the dominant enzyme in most individuals during fetal development and it was only after birth that CYP2C9 expression increases and becomes the dominant CYP2C enzyme. However, in none of these systems is there any evidence for coordinated inverse regulation between these “fetal” and “adult” enzymes, suggesting that the term “developmental switch” is a misnomer and that developmental transition is a more appropriate description of this process. Consideration of these data also suggests that for enzyme systems such as CYP2C9 and 2C19 that appear to share some regulatory mechanisms in the adult, mechanisms regulating ontogeny are strikingly different.

This review also illustrates several knowledge gaps in this field. Although improving, there remains a need for additional studies to define the true ontogeny of many of the enzyme systems, particularly in extra-hepatic tissues. Too much of our current knowledge is based on data from tissue samples or *in vivo* studies that utilized samples or recruited patients, respectively, representing narrow windows of time or omitting what would appear to be critical time windows. Conclusions drawn from such studies can be contradictory and misleading, as was observed for fetal hepatic CYP2E1 expression (see Section 3.1.3.5 above). Finally, the mechanisms regulating DME ontogeny remain poorly understood. Associations between specific transcription factor ontogeny and DME ontogeny have been made, but such associations hardly offer definitive proof for control mechanisms. Furthermore, it is highly likely that epigenetic mechanisms are playing an important role, as has been shown for CYP2E1 (Vieira et al., 1996). There also is a need to elucidate the mechanism or mechanisms whereby the increase or onset of expression of many of the category three enzymes are linked to the birth process, independent of gestational age, and the underlying cause for the inter-individual variation in this process. Despite these knowledge gaps, the field has progressed to a point to support the development of robust physiologically based pharmacokinetic models that provide a much improved means of predicting age-specific xenobiotic disposition (Clewell et al., 2004; Ginsberg et al., 2004; Johnson et al., 2006; Nong et al., 2006). Further advances in the field will only improve these valuable tools.

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