DEVELOPMENTAL ASPECTS OF THE HEPATIC CYTOCHROME P450 MONOOXYGENASE SYSTEM

❖6658

Allen H. Neims, Margaret Warner, Peter M. Loughnan, and Jacob V. Aranda

Roche Developmental Pharmacology Unit, Departments of Pharmacology and Therapeutics, and Pediatrics, McGill University, and the Montreal Children's Hospital Research Institute, Montreal, Quebec

In addition to their intrinsic value as probes in the study of differentiation, the interactions between xenobiotics and the developing organism are of immediate therapeutic and toxicologic consequence (1). The magnitude of inadvertent exposure of the fetus and newborn to drugs is well documented. Surveys have revealed that the gravid woman is exposed to an average of three to ten drugs per pregnancy (2–5). In addition to transplacental acquisition of drugs and metabolites (6, 7), exposure of the perinate continues postnatally through suckling (8, 9). Recent advances in perinatal care continue to redefine the dimensions of exposure. The regionalization of high-risk obstetrical and neonatal care has been associated with evolution of an attitude of aggressive diagnostic and therapeutic intervention. Attention must now be devoted to the influence of drugs on the cardiorespiratory measurements involved in diagnostic fetal monitoring (10, 11). Indeed, the practice of fetal therapeutics has materialized with approaches designed to modify the maturational process (12), including glucocorticoids to prevent respiratory distress syndrome (13, 14) and phenobarbital to lessen the degree of hyperbilirubinemia (15, 16). Recent survey in a neonatal intensive care unit has revealed an average exposure of 3.4 drugs per infant excluding nursery routines (17); exposure was inversely related to birth weight, and 71 different drugs were utilized in the 320 consecutive infants surveyed.

The adverse actions of drugs upon the human fetus and newborn have been tabulated recently (18–20). Delayed effects, such as vaginal adenocarcinoma after in utero exposure to diethylstilbesterol (21) and hyperkinetic behavioral anomalies in rats after neonatal exposure to lead (22), dramatize the scope of the problem (23). Nonetheless, the need for further information has been tempered by the moral dilemmas inherent to fetal and neonatal research (24). The recent appearance of

numerous books, clinics and symposia (25–32), and comprehensive reviews (33, 34) has allowed us to restrict the subject of this review to fetal and neonatal aspects of the mammalian hepatic cytochrome P450 monooxygenase complex in relation to developmental biology (35–37).

DEVELOPMENT OF HEPATIC MONOOXYGENASE ACTIVITY IN NONPRIMATE MAMMALS

Developmental aspects of the hepatic cytochrome P450 monooxygenase system in nonprimate mammals have been studied for almost two decades (38, 39), and comprehensive reviews are available (34, 40). The capacity of hepatic tissue to catalyze the xenobiotic monooxygenase reaction is presented schematically as a function of the age of the animal from which liver was obtained in Figure 1. The various developmental sequences depicted in Figure 1 apply to different species, including rat (41–48), mouse (49, 50), rabbit (39, 51–53), hamster (54), guinea pig (38, 55, 56), opossum (57), swine (34, 58–60), and ferret (61). The figure was compiled to emphasize the similarities, rather than the differences, in separate studies.

The specific developmental pattern(s) exhibited by each animal may vary with substrate (e.g. 47, 61), strain (62), sex (e.g. 41, 46), and details of tissue preparation (homogenate, postmitochondrial supernatant, or microsomal pellet) including the techniques of cell disruption and differential centrifugation (63). An important consideration involves the standard selected for expression of results since the liver undergoes appreciable anatomical and biochemical modification perinatally (64,

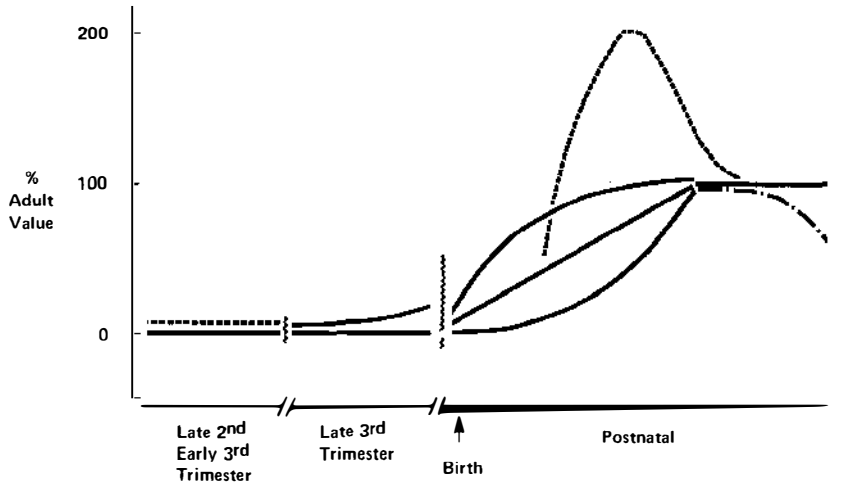

Figure 1 Patterns of development of hepatic monooxygenase activity for different nonprimate mammalian species. See text.

65). The standard employed (body weight or surface area; liver weight, DNA, or protein; microsomal protein or phospholipid; etc) has remained a function of the purpose of each investigation.

Aspects of each phase of development of hepatic monooxygenase activity merit discussion. In contrast to the substantial xenobiotic monooxygenase activity exhibited by midgestational human fetal liver preparations, studies of nonprimate fetal liver have not revealed comparable activity (34, 53, 55, 66), even in animals that achieve considerable maturity in utero (55) or experience a long gestational period (34). Although miniscule, catalytic activity and/or components of the monooxygenase system are measurable in liver from second or third trimester fetal swine (34, 58–60), guinea pig (55), rat (67), and mouse (50).

Many (e.g. 55, 67), but not all, investigations of nonprimate mammals have revealed a definite, albeit small, increase in monooxygenase activity near term. After birth, all animals studied to date have exhibited marked increases in hepatic monooxygenase activity, the time course of which can be measured in days, weeks, or even months. It seems likely that the rapidity with which this sequence occurs is determined at least in part by the relative state of maturity of each animal at birth. During postnatal maturation it has not been unusual to observe levels of hepatic monooxygenase activity that exceed subsequent adult values by two to three fold (45, 47, 55). After puberty, monooxygenase activity toward a few substrates decreases (43, 44, 48).

The influence of fetal and neonatal events upon expression of the sex differences has received recent attention. "Imprinting" by androgens of certain behavioral characteristics and the release of gonadotropins have been recognized for some time in rodents and primates (cf 68). De Moor & Denef (69, 70) observed that the masculine pattern of adult rat hepatic cortisol metabolism could be altered by neonatal castration. A male pattern was expressed after puberty in such animals only in association with the administration of testosterone in the neonatal period; similar androgen exposure in female pups elicited postpubertal masculine hydroxylation patterns. Gonadectomy and/or treatment with androgen after weaning were without relevant effect. The imprinting of hepatic steroid metabolism has been studied in detail by Einarsson and colleagues (71, 72). Chung et al (73) have demonstrated applicability of imprinting to the hepatic xenobiotic monooxygenase reaction, and some evidence has been presented for involvement of pituitary factor(s) (74). Levin et al (75) have evaluated the effects of imprinting on the relative proportions of fast and slow turning-over forms of cytochrome P450 in rat liver microsomal preparations. The significance of these findings deserves emphasis.

CORRELATES OF MONOOXYGENASE ACTIVITY

Various components and catalytic activities that have been associated with the hepatic monooxygenase complex (cytochrome P450, NADPH-cytochrome P450 reductase, NADPH-cytochrome *c* reductase, NADPH-oxidase, cytochrome b_5, and cytochrome b_5 reductase) have been studied singly or in combination in livers of developing rat (41, 42, 47, 48), rabbit (52, 53), swine (34, 60), guinea pig (55),

and ferret (61). In general, the developmental sequences for cytochrome P450 and NADPH-cytochrome P450 reductase resemble closely the developmental patterns for monooxygenase activity depicted in Figure 1. In rabbit (53) and swine (34), the postnatal increments in the content of cytochrome P450 and the activity of NADPH-cytochrome P450 reductase occur synchronously. In rat (41, 76) and ferret (61), however, maturation of NADPH-cytochrome P450 reductase activity lags relative to the increase in cytochrome P450 content despite the presence of substantial NADPH-cytochrome *c* reductase activity at, or shortly after, birth (41, 61, 76, 77). Certain phenomena reviewed below could be involved in retarding the reducibility of cytochrome P450; these include developmental alterations in (*a*) the species of cytochrome P450, (*b*) endogenous ligands affixed to cytochrome P450, and (*c*) membrane composition and structure (see also reference 76).

In adult rats, the activity of NADPH-cytochrome P450 reductase is considered to be rate limiting with respect to microsomal hydroxylations (78). Although much effort has been devoted to detailed correlations of component data with monooxygenase activity for the purpose of identifying the rate-limiting component during development, we agree with the analysis by Short et al (34) who state, "It seems most likely that after birth the monooxygenase system develops largely as a unit, with some species and substrate variation in the postnatal increase in reducibility of cytochrome P450 by NADPH-cytochrome c reductase." In different developmental studies, the component (or catalytic activity) that correlates best with the development of overall monooxygenase activity has been found to vary with substrate (e.g. 51), species, and sex (41). Other possible rate determinants, such as a deficiency in NADPH or NADH, an age-dependent variation in the ratio of low/high spin iron in cytochrome P450, or an age-dependent change in the stimulatory effects of type I substrates, are not thought to be of quantitative significance (79). An important, unresolved problem relates to possible age-related variation in the relative proportions of different forms (80) of cytochrome P450 (including cytochrome P448), or even the existence of a distinct fetal or perinatal cytochrome (81).

Substrates that generate type I difference spectra with adult hepatic microsomes, elicit reverse type I (modified type II) spectra with preparations from neonatal animals (e.g. 34) and human fetuses (82–84). Although interpretation of the reverse type I difference spectrum is incompletely resolved (85), its occurrence in perinatal hepatic preparations raises the possibility that inhibitory, endogenous ligands are affixed to the monooxygenase complex. Ligand candidates include phospholipids present in immature endoplasmic reticulum (81) and a variety of steroids, the composition and proportions of which are known to fluctuate considerably during maturation (e.g. 86). More specifically, certain metabolites of progesterone inhibit the monooxygenase reaction (87–89). The potential affinity of steroidal ligands for the monooxygenase complex is emphasized by the findings of Juchau and coworkers (90) that relate to human placental preparations and the ability of androstenedione to prevent binding of carbon monoxide to cytochrome P450.

It is generally held that the monooxygenase complex is associated with the smooth endoplasmic reticulum (SER) (91). Electron microscopic investigations (34, 55, 76) of nonprimate mammalian liver have revealed little or no SER before birth;

its appearance postnatally mimics the development of monooxygenase activity (cf 34, 55, 76). The proliferation of SER requires net synthesis of membrane lipid as well as protein. Certain enzymes involved in phospholipid biosynthesis exhibit sharp increases in activity perinatally (92).

Studies with solubilized components of the monooxygenase complex have revealed an absolute requirement for phospholipids (93). The chain length and degree of unsaturation of the fatty acid moieties of phosphatidylcholine determine the extent of monooxygenase activity exhibited by the reconstituted system. In vivo, changes in the fatty acid composition of microsomal phosphatidylcholine have been observed during both postnatal development (94) and xenobiotic induction (95). The linoleic acid content of microsomal phosphatidylcholine parallels monooxygenase activity during phenobarbital induction and withdrawal; changes in oleic acid content correlate best with monooxygenase activity after 3-methylcholanthrene induction. Interestingly, one of the developmental changes in microsomal lipid composition of rabbit liver is an increase in the linoleic acid content of phosphatidylcholine after the tenth postnatal day (94). Finally, current concepts of the mechanism of action of membrane-bound multienzyme complexes have been summarized eloquently by Strittmatter and colleagues (96) for the case of cytochrome b_5 and its reductase; the potential influence of composition and physical state of the membrane upon monooxygenase activity during maturation clearly merits further investigation.

The inverse relationship between monooxygenase activity and microsomal lipid peroxidation is a subject of current investigative interest (97, 98), but quantitative comparisons are complex in part because of the deficiencies inherent in the use of malonic dialdehyde production as a measure of lipid peroxidation. The rate of microsomal lipid peroxidation has been found to increase after the neonatal period in rats and swine (99, 100). A chemically unidentified hepatic cytosol factor of ca 10,000 daltons by gel filtration is capable of inhibiting lipid peroxidation and enhancing monooxygenase activity (101, cf 102); this factor is present at birth and does not seem to fluctuate significantly during subsequent maturation (103).

DEVELOPMENT OF THE MONOOXYGENASE SYSTEM IN PRIMATE LIVER

Certain aspects of the development of hepatic monooxygenase activity in man differ significantly from the sequence depicted in Figure 1. Although little is known about perinatal events, the presence of substantial monooxygenase activity in the hepatic microsomes from the midgestational human fetus has been established. Reference is made to the excellent reviews on this subject in the past few years by Yaffe & Juchau (33), Pelkonen & Kärki (104), Rane et al (105), Short et al (34), and Netter (106). In 1969 and 1970, in vitro demonstration of hepatic xenobiotic monooxygenase activity in livers from late first and second trimester human fetuses was provided independently by various research groups. Pelkonen et al (107) found that the human fetal liver metabolized chlorpromazine, *p*-nitrobenzoic acid, and hexobarbital, and Arvela et al (108) observed 3,4-benzpyrene hydroxylase activity (cf 49).

Yaffe et al (109) demonstrated that hepatic microsomes obtained from 14 to 25 week abortuses catalyzed the hydroxylation of endogenous substrates such as laurate and testosterone, and significantly, that cytochrome P450, as well as the electron transport enzymes, were measurable. Gustafsson & Lisboa (110) had earlier demonstrated catalysis of the oxidation of testosterone by microsomal preparations from human fetal liver. Similarly, Juchau observed the reduction of *p*-nitrobenzoic acid (111).

Both hepatic monooxygenase activity (111–114) and SER (115, 116) are demonstrable as early as the sixth week of gestation. Current data suggest that an increase in monooxygenase activity occurs during the first trimester of gestation with subsequent plateau during the second trimester. Pelkonen (112, 113) observed a positive correlation between fetal weight and levels of hepatic monooxygenase activity for fetuses from 8 to 13, but not 13 to 21, weeks of gestation. Yaffe et al (109) also found no significant age-related increase in cytochrome P450, NADPH-cytochrome *c* reductase, or laurate hydroxylase during midgestation.

A bibliographic summary of data pertaining to monooxygenase activity and components in hepatic preparations from the midgestational human fetus is presented in Table 1. Many of the parameters that influence monooxygenase activity and its variability in animal studies discussed above apply also to study of human fetal tissue. Specifically, characteristics of cell disruption and differential centrifugation are different in fetal and adult liver (119, 124). Although striking interindividual variability exists, hepatic monooxygenase activity toward most substrates is lower than comparative human adult values. Levels of fetal hepatic monooxygenase activity relative to adult tissue vary as a function of substrate, ranging from a few percent [oxidation of 3,4-benzpyrene expressed per gram of liver (117)] to more than 100%

Table 1 Cytochrome P450 monooxygenase activity in early and midgestational human fetal liver

Substrates	References	Component/Activity	References
Aminopyrine	(28%: 117)[a] (84, 113) (109, 120)[b]	Cytochrome P450	(28%: 117)[a] (84, 109, 118–121)
4,16-Androstadien-3-one	(145%: 122)[a]	Cytochrome P450 reductase	(109)
Aniline	(32%: 117)[a] (84, 113, 120, 123, 124)	NADPH-cytochrome *c* reductase	(49%: 117)[a] (84, 109, 119)
3,4-Benzpyrene	(1–3%: 113, 117)[a] (84, 112, 120, 121, 126–128) (109)[b]	Cytochrome b_5	(109)
		NADPH oxidase	(111)
Chlorpromazine	(112, 114, 128)		
Desmethylimipramine	(129, 130)		
Diazepam	(10%: 131)[a] (132)		
Ethylmorphine	(123)		
Hexobarbital	(37%: 117)[a]		
Laurate	(109)		
N-Methylaniline	(112, 126, 128)		
N,N-Dimethylaniline	(133)		
Neoprontosil	(120)		
p-Nitroanisole	(49)[b]		
p-Nitrobenzoate	(111, 112, 114, 120, 128)		
Testosterone	(109, 110)		

[a] Percentage of adult value; only studies in which comparative adult values are reported have been included.
[b] Inconsistent or negative activity.

[formation of 16 β-hydroxyepitestosterone from 4,16-androstadien-3-one expressed per mg microsomal protein (122) or aniline hydroxylation expressed per gram of liver (123)]. For most substrates, midgestational fetal liver preparations have exhibited 20–40% of adult activity. Components of the monooxygenase complex are present in comparable concentrations, with cytochrome P450 approaching one third of adult levels and NADPH cytochrome *c* reductase approaching one half of adult activity (117).

Consideration of changes in fractional liver weight has led Pelkonen & Kärki (104) to comment that "fetal and adult capacities to metabolize foreign compounds in vitro are at a surprisingly similar level." The question of in vivo activity of the monooxygenase complex in midgestation remains unresolved. Demonstration of metabolites of diazepam (e.g. 134) and chlorpromazine (135) in the midgestational fetus are suggestive of activity in vivo, but details of the placental transfer of many metabolites remain unexplored.

The potential significance of the presence of substantial monooxygenase activity early in human gestation with respect to teratology, carcinogenesis, and other aspects of toxicity has been emphasized (66, 67). Indeed, Rane & Gustafsson (122) have provided indirect evidence for the production of epoxides in fetal liver by documenting the formation of a 16,17-transglycolic metabolite from 4,16-androstadien-3-one. Similar preparations also catalyze the generation of potentially toxic N-oxides from N,N-dimethylaniline through a process presumably independent of cytochrome P450 (133).

Studies in the third trimester of gestation, and in the neonatal period, have been limited mainly by the difficulties inherent in sample collection. Thus far, two postmortem studies demonstrate that livers from premature and full-term newborn infants contain hepatic monooxygenase activity or possible components thereof. Soyka (136) has demonstrated the presence of cytochrome b_5 and NADPH-cytochrome *c* reductase in livers from two infants of 33 and 40 weeks gestation. Studies of Aranda et al with specimens from seven premature and full-term infants from 28 to 41 weeks gestation revealed the presence of cytochrome P450, NADPH-oxidase and cytochrome *c* reductase, aminopyrine N-demethylase, aniline *p*-hydroxylase (137), and microsomal lipid peroxidation (99). Considerable overlap between the highest perinatal and the lowest adult values was observed. In contrast to the midgestational plateau, a positive correlation was demonstrated between postconceptional age (gestational age + postnatal age) and the activities of aniline *p*-hydroxylase, NADPH-oxidase, NADPH-cytochrome *c* reductase, as well as cytochrome P450 content. Since the number of infants studied was limited, and the more premature infants tended to die at a younger age, it is impossible to distinguish which parameter correlates best with the increase in monooxygenase activity: gestational age, postnatal age, or postconceptional age. It is therefore difficult at this time to conclude with confidence from in vitro hepatic data whether or not there is a birth-related, significant increase in monooxygenase activity to or beyond adult values in the postnatal period. It is of considerable interest that the stumptail monkey, which, like man, exhibits appreciable midgestational hepatic monooxygenase activity (138; cf 120), experiences substantial increments in activity perinatally;

the hepatic microsomal content of cytochrome P450 increases about four-fold from 10 days before to 10 days after birth. Studies of the contents of diazepam and N-desmethyldiazepam in umbilical artery and vein have provided some evidence for in vivo function of the monooxygenase complex in term fetuses (139).

Although extrahepatic monooxygenase reactions are not reviewed herein, substantial effort has been devoted to their study in certain fetal tissues. The placental monooxygenase systems have been reviewed by Juchau (140) and Netter & Bergheim (141). It seems that human placental cytochrome P450 exhibits high selectivity in ligand binding, and that few xenobiotics are likely to be substrates for the monooxygenase complex (140). Human placental aryl hydrocarbon hydroxylase activity is well documented, especially in conjunction with maternal cigarette smoking (121, 125, 142–145). Other fetal tissues, including adrenal, kidney, gastrointestinal tract, and lung do exhibit monooxygenase activity (114, 120, 121, 126, 127, 145). Fetal adrenal, compared to liver, is reported to contain higher concentrations of cytochrome P450 (111, 120, 121) and to exhibit substantial monooxygenase activity (120, 121, 126). The perinatal development of monooxygenase activity, as well as detoxification systems, in extrahepatic tissues of animals has been studied intensively by Fouts and colleagues (e.g. 146).

DRUG OXIDATION IN THE HUMAN NEONATE

The appreciable midgestational monooxygenase activity of primate liver raises the possibility that man, unlike laboratory animals, may not experience a substantial postnatal increase in xenobiotic oxidative capacity. Since in vitro data pertaining to hepatic monooxygenase activity in perinatal man are sparse, the subsequent discussion attempts to utilize pharmacokinetic information to probe the question. Although conclusions reached about in vitro systems from pharmacokinetic data must be considered tentative, the few direct developmental comparisons that have been conducted in animals are supportive (44), as are the results of animal toxicology (147). Nonetheless, developmental aspects of distribution, hepatic uptake, storage, conjugation, and biliary and renal elimination, as well as extrahepatic oxidative metabolism (see above), are likely to contribute to the overall pharmacokinetic profile.

The disposition of diphenylhydantoin (DPH) by full-term infants has been studied in considerable detail. Metabolite patterns support applicability of such data to questions regarding oxidative metabolic capacity. Horning et al (7) have demonstrated by gas chromatographic-mass spectrometric techniques the presence of 5-phenyl-5-(4-hydroxyphenyl)-hydantoin (HPPH) and its glucuronide, as well as 5-phenyl-5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-hydantoin, in the urine of infants who had been exposed to DPH transplacentally or postnatally. Identical metabolites have been found in adult urine. Indeed, the relative proportions of DPH, HPPH, and conjugated HPPH in urine from neonates who had acquired DPH (and perhaps, metabolites) transplacentally resemble closely those seen in urine from adult man (148, 149). Since HPPH may retard the elimination of DPH, accumulation of the unconjugated metabolite postnatally could complicate interpretation of

pharmacokinetic data; accumulation did not occur, at least in the plasma of the one neonate studied (150). The binding of DPH to newborn plasma protein is decreased relative to adult values (151), and the deficiency is accentuated by hyperbilirubinemia (152) and increased plasma concentrations of fatty acids (153). Adult-like binding is attained by the age of 3 months (154).

Pharmacokinetic investigations of DPH in infants have been conducted by Mirkin [7 newborns, transplacental acquisition (155, 156)], Rane et al [7 newborns, transplacental acquisition (157)], Baughman & Randinitis [1 newborn, transplacental acquisition (158)], Jalling et al [steady state plasma levels in 6 infants, 5 of whom were more than 3 weeks old (159)], and Loughnan et al [15 infants from birth to 24 months, single-dose decay curves and steady state concentrations (160)]. The interpretation of events in each study and the consequent method of data analysis have influenced conclusions. Mirkin (155), although noting that elimination was "very slow on postpartum days 1 and 2, and increased markedly on postpartum day 3," elected to pool data and derived an average plasma half-life of about 60 hr. Comparative plasma half-lives in adults are much shorter: 11 to 31 hr. Rane et al (157) suggested that possibly the early slow phase of elimination reflected saturation kinetics. Analysis of the subsequent first-order decay curves omitted the events of the first few postnatal days and yielded plasma half-lives (6.6–34 hr) similar to adult values. Jalling et al (159) observed surprisingly low steady state plasma DPH concentrations after multiple intramuscular or oral doses in older infants. Loughnan et al (160) confirmed these observations, and found in single intravenous dose studies that the low steady state plasma concentrations reflected primarily a substantial diminution in half-life (5 to 7 hr) relative to the adult.

Although the question as to why apparent rates of elimination of DPH increase during the neonatal period is unresolved, we believe that the data reflect primarily a maturation of metabolic oxidative capacity. This attitude has been influenced significantly by observation (160) of a number of infants with seizures, who, after an intravenous loading dose of DPH (12 mg/kg) on the first or second postnatal day, received a maintenance (oral or intravenous) dose of 8 mg/kg per day; the plasma DPH concentrations of some infants increased to peak values (> 20 μg/ml) within a few days, only to decline to values of less than 5 μg/ml a few weeks later without alteration in dose/kg. Notably, the elimination rates of other compounds that undergo metabolic oxidation, but do not exhibit saturation kinetics at therapeutic concentrations in adults, can change similarly in the neonatal period. These agents include phenobarbital (see below), tolbutamide (161), and aminopyrine (162). Other drugs have displayed slow rates of elimination in the early postnatal period, but the time at which rates increase is less clearly defined; these include acetanilid (163), diazepam (139, 164–166), amobarbital (167), nortriptylene (168), and mepivacaine (169).

In order to probe postnatal maturation of oxidative capacity, the DPH plasma concentration versus time relationships of individual patients (155–160) have been analyzed to calculate plasma half-lives for three arbitrary age groupings (Figure 2). When half-life changed with age in an individual patient, separate estimations of half-life were made using at least 3 plasma concentration values. In some instances

DPH half-lives were estimated by calculations based on steady state plasma concentrations with assumption of an apparent volume of distribution applicable to that age (160). Such procedures have limitations, and the values generated can be regarded only as approximations. For these full-term infants, mean DPH plasma half-lives of about 80, 15, and 6 hr were derived for the age groupings 0–2, 3–14, and 14–150 days, respectively. Large interindividual variability was seen during the first few postnatal days with half-lives ranging from fewer than 10 to greater than 100 hr; the variability diminished with age. It is difficult to assess the effect of xenobiotic induction upon this maturational sequence. Many of the infants were exposed to phenobarbital along with DPH, and the barbiturate (or DPH) could accelerate maturation of oxidative (170, 171) as well as conjugative (e.g. 15, 16) processes. No dependence of DPH elimination rate on coincident exposure to phenobarbital was apparent.

Speculatively, the data presented in Figure 2 may indicate that the full-term infant is born with about 30% of the relative adult capacity to eliminate DPH. This conclusion compares favorably with in vitro measures of hepatic monooxygenase activity in second and third trimester human fetuses and the perinatal stumptail monkey (see above). The apparent rapid increase in metabolic capacity to levels about two to three fold greater than those of the adult is not without in vitro precedent in animal experiments (see above).

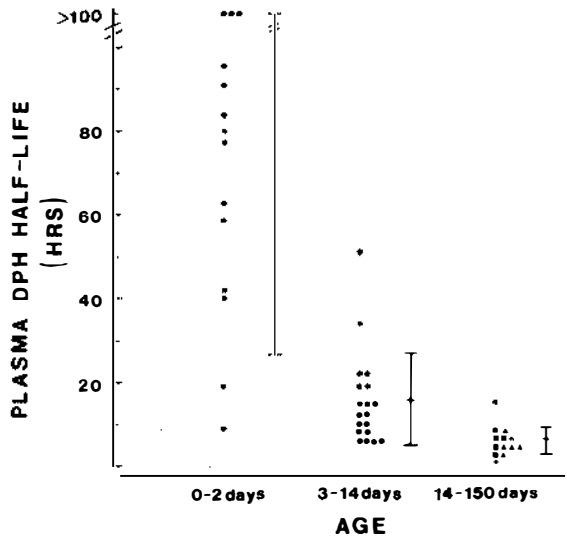

Figure 2 Plasma DPH half-lives measured in infants grouped according to postnatal age. Each infant received the drug either transplacentally (●) or as an initial therapeutic dose (■). Those half-lives calculated from steady state plasma DPH concentrations are indicated (▲). Means and standard deviations are shown. Data were obtained from several published studies (see text).

Even with the assumption that such in vivo data reflect maturation of the xenobiotic monooxygenase complex, generalization to other drugs is not possible. Four of five infants who had acquired carbamazepine transplacentally eliminated the drug in the immediate postnatal period as rapidly as adults (172). Whether or not subsequent rates of elimination would have exceeded adult values is unknown.

The elegant studies of Jalling, Boréus and colleagues (170, 171, 173–175), as well as the earlier investigations of Melchior et al (176) and Heinze & Kampffmeyer (177), permit a similar analysis of the disposition of phenobarbital by neonates and infants. In these investigations, patients acquired the barbiturate transplacentally or were treated postnatally. As seen in Figure 3, the neonates and infants exhibited mean phenobarbital plasma half-lives of greater than 200 hr, about 100 hr, and about 50 hr for the age intervals, 0–5, 5–15, and 30–900 days postnatal, respectively. Many aspects of analysis resemble closely the situation observed with DPH. The large interindividual variability seen in newborns diminished with age. The mean plasma half-life of the drug during the first five postnatal days was twice the adult value, but decreased to about half of the adult value by the age of one month.

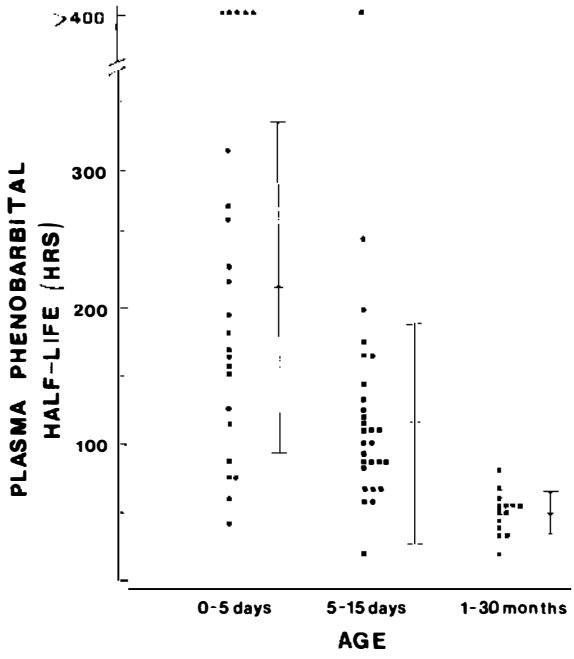

Figure 3 Plasma phenobarbital half-lives in infants grouped according to postnatal age. Some infants received the drug transplacentally (●); in others, half-lives were measured following termination of phenobarbital therapy (■). Means and standard deviations are shown. Data were obtained from several published sources (see text).

With current neonatal intensive care practices, the survival of premature infants is not negligible even when birth occurs in the late second or early third trimester of pregnancy, a circumstance not applicable to the usual animal models. An understanding of the postnatal maturation of xenobiotic oxidative capacity in prematures is of obvious clinical and biological consequence. With respect to modes of drug elimination other than oxidation, the newborn premature is deficient relative to the full-term infant although many functions improve within a few weeks of birth (178). Unfortunately, data that bear upon oxidation are sparse, and it is difficult to separate the effects of illness and nutritional status. Diazepam is eliminated more slowly by premature than full-term newborns (164, 166) but subsequent events are not clear. Unpublished studies in this laboratory indicate that two methylxanthines, caffeine and theophylline, display long plasma half-lives in premature infants (birth weight $<$1500 g) for at least the first 2 to 4 weeks postpartum. The plasma half-life for caffeine in such infants was greater than 2 days (compared to about 4 hr in adults) and 16 to $>$60 hr for theophylline [compared to 3.5 hr in children aged 1 to 4 years (179)]. Unfortunately, comparative data in full-term infants are unavailable, but Horning et al (7) have noted a decreased content of the demethylated metabolites of caffeine in urine from full-term infants.

REGULATORY MECHANISMS

The physiological factors responsible for the various developmental sequences displayed by the hepatic monooxygenase complex are not well defined, and may be multiple and/or species-specific. We have considered it appropriate in this review to view the monooxygenase complex as a functional unit that increases in capacity at certain critical periods in development. This conception is not meant to detract from the important differences in the developmental character of individual components or catalytic activities of the complex, but rather to emphasize a relationship to the problems of developmental biology. Similar control mechanisms may act also upon the development of certain other enzymes involved in xenobiotic disposition, such as UDP-glucuronyl transferase (180) and epoxide hydrase (e.g. 181). The recent finding that inducibilities of both UDP-glucuronyl transferase and monooxygenase, but not epoxide hydrase (182), are under genetic control of the "Ah locus" in mice is provocative in this recard.

It is difficult to classify the monooxygenase developmental pattern confidently within the clusters described by Greengard (36). In some species and with certain substrates, activity has increased in the late fetal period; nevertheless, the prominent increment is usually observed postnatally. In guinea pig with chlorcyclizine or benzo(a)pyrene as substrate (55), the pattern might be classified as "neonatal," whereas in the ferret with ethylmorphine as substrate (61), little increase in activity is noted until late in the suckling period. It is generally held that the event of birth per se in some way triggers the development of the monooxygenase complex, but to our knowledge direct evidence is lacking because of the difficulties inherent in the acquisition of significantly premature (or postmature) laboratory animals.

Discussions of potential physiologic control mechanisms must distinguish between direct inhibition of the monooxygenase complex, on the one hand, and agents that act at the level of transcription or translation, on the other. Applicability of one does not exclude modulation by the other. With respect to direct inhibition, the observation of reverse type I difference spectra in human fetal and neonatal animal hepatic microsomal preparations can be interpreted to indicate the presence of such potentially inhibitory endogenous ligands. Interest has focused primarily on the effects of certain steroids and phospholipids (see above). Particularly, metabolites of progesterone that are present in the plasma of pregnant women and fetuses (86) are potent inhibitors of conjugative (183) and oxidative (87–89) enzymes. Endogenous ligands have been implicated to some extent also in explanations of the perinate's decreased plasma protein binding capacity (184, 185). The action of such inhibitors of monooxygenase activity could extend into the suckling period with transmission through milk. Precocious development of coumarin 3-hydroxylase activity has been observed in rats that were weaned early, and the increase could be prevented by administration of 5 β-pregnane-5βol-20-one (89; cf 62). Other studies, however, have failed to demonstrate dependence of monooxygenase activity on the time of weaning (34, 46). It seems that most data reviewed herein indicate that the primary deficit in fetal hepatic monooxygenase activity relates more to decreased contents of the appropriate components and membrane structure than to direct enzyme inhibition.

Substantial effort has been devoted to study of fetal and perinatal induction of monooxygenase activity by xenobiotics. Most studies with nonprimate mammals have revealed that inducibility of monooxygenase activity in fetal liver by barbiturates or polycyclic hydrocarbons is negligible until near or after term (34, 45, 51, 55, 186). Experiments designed to evaluate inducibility of the hepatic monooxygenase system in midgestational human fetal liver by phenobarbital, other drugs, and cigarette smoking have yielded equivocal (128) or negative (145) results. Certain investigations in rodents have revealed some inducibility of hepatic 3, 4-benzpyrene hydroxylation as early as the end of the second trimester of pregnancy (66). After birth, inducibility by xenobiotics increases in striking fashion and has been found to exceed relative adult values later in maturation; significantly, monooxygenase activity returns to the expected basal level for age after withdrawal of the inducer (187). Oesch and colleagues (67) have explored in detail inducer specificity with respect to aryl hydrocarbon monooxygenase and epoxide hydrase activities in rat liver 1 to 3 days antepartum. The minimal responsiveness of midgestational fetal liver (including primates, above) has been hypothesized to reflect any one or a number of maturational parameters, including insufficient exposure in utero (120, 188) and absence of appropriate receptor macromolecules (188). The sequential development in responsiveness of rat liver tyrosine aminotransferase to cAMP, glucagon, hydrocortisone, dietary protein, and pyridoxine (36) offers interesting comparison. The requisite priming effect of hydrocortisone for precocious responsiveness of tryptophan oxygenase and glucokinase to inducibility by substrates may serve also as an informative analogy (36).

Investigations that involve potential physiological inducers are sparse. In liver cultures, hydrocortisone is required for glycogen storage (36), development of bile canaliculi (189), and development of tyrosine transaminase activity (36), but has no effect on aryl hydrocarbon hydroxylase activity (189a, b). The effects of thyroxine on the mixed function oxidase activity in neonatal rats have been studied (190). Conclusions are difficult to summarize because of the fluctuating effects of thyroxine with age. Finally, the possible effects of diet and arterial oxygen tension require clarification.

The presence of fetal repressors has been suggested by certain experiments. In chick embryo cultures, the precocious development of conjugative (35, 191) and oxidative enzymes (192) occurs upon removal of the embryo from the egg. The development of UDP glucuronyltransferase activity in these chick embryo cultures required protein synthesis, but not the presence of adrenal steroids; it was independent of the rate of cell proliferation (35). The potential in ovo repressor(s) has not been chemically identified. Interestingly, livers removed at an early embryonic age achieve higher levels of transferase activity on culture than those extirpated later (35). The injection of phenobarbital in ovo does provoke precocious development of the transferase in situ (35, 193).

Growth hormone has been suggested as a repressor of hepatic monooxygenase activity in the rat (42). The plasma level of growth hormone in the rat at birth is about 200 ng/ml and decreases to adult levels of about 40 ng/ml during the first 21 postnatal days. Adult rats treated with growth hormone have displayed a diminution in hepatic microsomal drug metabolism (194), and the postnatal muturation of oxidative capacity of young rats was slowed, but not prevented, by a single injection of growth hormone (195). Henderson & Kersten (46, 196) have commented that the increase in hepatic monooxygenase activity, both postnatally and during liver regeneration, in rats correlates well with the cessation of active liver growth. The asynchrony of parenchymal cell perinatal maturation with respect to content of glucose-6-phosphatase is of interest in this regard (197, 198). Provocative evidence for a repressor in rat fetal liver has been presented by Klinger et al (199) who demonstrated that the administration of supernatant fractions from fetal liver to weanling rats prevented subsequent induction of monooxygenase activity by phenobarbital, but exerted less effect on basal levels. It was noted that the effects of such manipulations were inconsistent.

Whether the low fetal hepatic monooxygenase activity relates to the lack of effective induction or the existence of repression, or a combination thereof, it should reflect decreased rates of component synthesis or increased degradation. Little data bear directly upon this question, but Short et al (34) have noted an *increase* in the rate of degradation of cytochrome P450 between the age of 5 days and 8 weeks in the pig. Relationships of perinatal maturation of oxidative capacity to membrane structure and the incorporation of heme into the apoprotein of cytochrome P450 (121) require clarification. Although δ-aminolevulinic acid synthetase is thought not to be rate limiting with respect to heme synthesis in fetal rat liver (200), Siekevitz (201) has reported evidence suggestive of the presence of the apoprotein of cytochrome P450 in hepatic microsomes from near-term fetal animals.

CONCLUSION

An attempt has been made to examine developmental aspects of the hepatic cytochrome P450 monooxygenase system as a functional unit in fetal and neonatal mammals including man. In man, monooxygenase activity is detected in liver preparations as early as the sixth week of gestation and increases to a plateau (ca 20–40% of comparative adult values) in midgestation. Aside from other primates, laboratory mammals exhibit miniscule levels of hepatic monooxygenase activity midgestationally, but experience a substantial increase more or less rapidly after birth. Pharmacokinetic and sparse in vitro studies suggest tentatively that primates, like other mammals, experience sharp increases in activity perinatally, but the temporal relationship to birth and the situation in prematures is unclear. Substantial variability is seen in both midgestational in vitro data and early postnatal pharmacokinetic data. The physiological factors involved in differentiation of the monooxygenase unit are unresolved. Indeed, the relative contributions of direct inhibition (e.g. by phospholipids or steroids) as compared to inhibition at a transcriptional or translational level are not yet defined. Certain areas wherein insufficient information is available have been emphasized; they include perinatal aspects of (*a*) membrane structure and composition, (*b*) physiological inducers and/or repressors, (*c*) relationship to cell division, (*d*) endogenous inhibitors, and (*e*) incorporation of heme into cytochrome P450. Continued progress in this area promises to bear significantly on the related clinical problem of drug therapy of the premature and full-term infant, as well as teratology and perinatal toxicology.

Literature Cited

1. Stern, L. 1975. *Basic and Therapeutic Aspects of Perinatal Pharmacology,* ed. P. L. Morselli, S. Garattini, F. Sereni, pp. 7–12. New York: Raven
2. Peckham, C. H., King, R. W. 1963. *Am. J. Obstet. Gynecol.* 87:609–20
3. Bleyer, W. A., Au, W. Y. W., Lange, W. A., Raisz, L. G. 1970. *J. Am. Med. Assoc.* 213:2046–48
4. Hill, R. M. 1973. *Clin. Pharmacol. Ther.* 14:654–59
5. Forfar, J. O., Nelson, M. M. 1973. *Clin. Pharmacol. Ther.* 14:632–42
6. Meschia, G., Battaglia, F. C., Bruns, P. D. 1967. *J. Appl. Physiol.* 22:1171–78
7. Horning, M. G., Butler, C. M., Nowlin, J., Hill, R. M. 1975. *Life Sci.* 16:651–71
8. Ayd, F. J., ed. 1973. *Int. Drug Ther. Newslett.* 8:33–39
9. Vorherr, H. 1974. *Postgrad. Med.* 56:97–104
10. Yeh, S. Y., Paul, R. H., Cordero, L., Hon, E. H. 1974. *Obstet. Gynecol.* 43:363–73
11. Van Petten, G. R. 1975. *Br. Med. Bull.* 31:75–79
12. Reynolds, J. W. 1974. *Clin. Obstet. Gynecol.* 17:95–114
13. Liggins, G. C., Howie, R. N. 1972. *Pediatrics* 50:515–25
14. Avery, M. E. 1975. *Br. Med. Bull.* 31:13–17
15. Wilson, J. T. 1972. *Ann. Rev. Pharmacol.* 12:423–50
16. Maurer, H. M. et al 1968. Lancet II:122–24
17. Aranda, J. V., Cohen, S., Neims, A. H. 1975. *Abstr. Can. Pediatr. Soc.,* Toronto, Canada
18. Pomerance, J. J., Yaffe, S. J. 1973. *Curr. Probl. Pediatr.* 4:1–60
19. Silverman, H. M. 1974. *Drug Intell. Clin. Pharm.* 8:690–93
20. Shepard, T. H. June 1974. *Dis. Mon.,* pp. 1–32
21. Herbst, A. L., Ulfelder, H., Poskanzer, D. C. 1971. *N. Engl. J. Med.* 284: 878–81
22. Silbergeld, E. K., Goldberg, A. M. 1974. *Exp. Neurol.* 42:146–57
23. Neims, A. H., Chung, L. W. K., Weimar, W. R., Hales, B. 1975. In *Clinical Pharmacology of Psychoactive Drugs,*

ed. E. M. Sellers, pp. 15–33. Toronto: Addict. Res. Found. Ontario
24. Mirkin, B. L. 1975. *Clin. Res.* 23: 106–13
25. Boreus, L. O., ed. 1973. *Fetal Pharmacology.* New York: Raven
26. Dancis, J., Hwang, J. C. 1974. *Perinatal Pharmacology: Problems and Priorities.* New York: Raven
27. Morselli, P. L., Garattini, S., Sereni, F., eds. 1975. *Basic and Therapeutic Aspects of Perinatal Pharmacology.* New York: Raven
28. Ankermann, H. 1973. *Entwicklungs-Pharmakologie.* Berlin: VEB Verlag Volk Gesundheit
29. *Pediatr. Clin. North Am.* 1972. Vol. 19
30. *Clin. Pharmacol. Ther.* 1973. Vol. 14
31. *Pediatrics.* Part II. 1974. Vol. 54
32. *Clin. Perinatol.* 1975. Vol. 2
33. Yaffe, S. J., Juchau, M. R. 1974. *Ann. Rev. Pharmacol.* 14:219–38
34. Short, C. R., Kinden, D. A., Stith, R. 1975. *Drug Metab. Rev.* In press
35. Nemeth, A. M. 1973. *Enzyme* 15:286–95
36. Greengard, O. 1971. *Essays Biochem.* 7:159–205
37. Rutter, W. J., Pictet, R. L., Morris, P. W. 1973. *Ann. Rev. Biochem.* 42: 601–46
38. Jondorf, W. R., Maickel, R. P., Brodie, B. B. 1958. *Biochem. Pharmacol.* 1: 352–54
39. Fouts, J. R., Adamson, R. H. 1959. *Science* 129:897–98
40. Gillette, J. R., Stripp, B. 1975. *Fed. Proc.* 34:172–78
41. MacLeod, S. M., Renton, K. W., Eade, N. R. 1972. *J. Pharmacol. Exp. Ther.* 183:489–98
42. Wilson, J. T., Frohman, L. A. 1974. *J. Pharmacol. Exp. Ther.* 189:255–70
43. Sims, P., Grover, P. L. 1967. *Nature London* 216:77–78
44. Kato, R., Vassanelli, P., Frontino, G., Chiesara, E. 1964. *Biochem. Pharmacol.* 13:1037–51
45. Feuer, G., Liscio, A. 1970. *Int. J. Clin. Pharmacol.* 3:30–33
46. Henderson, P. Th. 1971. *Biochem. Pharmacol.* 20:1225–32
47. Gram, T. E., Guarino, A. M., Schroeder, D. H., Gillette, J. R. 1969. *Biochem. J.* 113:681–85
48. Basu, T. K., Dickerson, J. W. T., Parke, D. V. W. 1971. *Biochem. J.* 124:19–24
49. Pomp, H. B., Schnoor, M., Netter, K. J. 1969. *Dtsch. Med. Wochenschr.* 94: 1232–40
50. Nebert, D. W., Goujon, F. M., Gielen, J. E. 1972. *Nature London New Biol.* 236:107–10
51. Pantuck, E., Conney, A. H., Kuntzman, R. 1968. *Biochem. Pharmacol.* 17: 1441–47
52. Fouts, J. R., Devereux, T. R. 1972. *J. Pharmacol. Exp. Ther.* 183:458–68
53. Rane, A., Berggren, M., Yaffe, S., Ericsson, J. L. F. 1973. *Xenobiotica* 3:37–48
54. Nebert, D. W., Gelboin, H. V. 1969. *Arch. Biochem. Biophys.* 134:76–89
55. Kuenzig, W., Kamm, J. J., Boublik, M., Burns, J. J. 1975. See Ref. 27, pp. 289–300
56. Kuenzig, W. et al 1974. *J. Pharmacol. Exp. Ther.* 191:32–44
57. Wilson, J. T. 1970. *Pediatric Pharmacology. FDA Symp.*, pp. 34–37
58. Short, C. R., Davis, L. E. 1970. *J. Pharmacol. Exp. Ther.* 174:185–96
59. Short, C. R., Maines, M. D., Westfall, B. A. 1972. *Biol. Neonate* 21:54–68
60. Short, C. R., Stith, R. D. 1973. *Biochem. Pharmacol.* 22:1309–19
61. Ioannides, C., Parke, D. V. 1975. See Ref. 27, pp. 245–53
62. Yaffe, S. 1968. *Report 58th Ross Conf. Pediatr. Res.*, pp. 58–63
63. Chatterjee, I. B., Price, Z. H., McKee, R. W. 1965. *Nature London* 207: 1168–70
64. Greengard, O., Federman, M., Knox, W. E. 1972. *J. Cell Biol.* 52:261–72
65. Herzfeld, A., Federman, M., Greengard, O. 1973. *J. Cell Biol.* 57:475–83
66. Robinson, J. R., Felton, J. S., Thorgeirsson, S. S., Nebert, D. W. 1975. See Ref. 27, pp. 155–69
67. Oesch, F. 1975. See Ref. 27, pp. 53–64
68. Resko, J. A. 1975. *Fed. Proc.* 34: 1650–55
69. De Moor, P., Denef, C. 1968. *Endocrinology* 82:480–92
70. Denef, C. 1974. *Endocrinology* 94: 1577–82
71. Einarsson, K., Gustafsson, J. A., Goldman, A. S. 1972. *Eur. J. Biochem.* 31:345–54
72. Einarsson, K., Gustaffsson, J. A., Stenberg, A. 1973. *J. Biol. Chem.* 248: 4987–97
73. Chung, L. W. K., Raymond, G., Fox, S. 1975. *J. Pharmacol. Exp. Ther.* 193:621–30
74. Chung, L. W. K., Raymond, G. 1975. *Pharmacologist* 17:209
75. Levin, W., Ryan, D., Kuntzman, R., Conney, A. H. 1975. *Mol. Pharmacol.* 11:190–200

76. Dallner, G., Siekevitz, P., Palade, G. E. 1966. *J. Cell Biol.* 30:97–117
77. Uehleke, H., Reiner, O., Hellmer, K. H. 1971. *Res. Commun. Chem. Pathol. Pharmacol.* 2:793–805
78. Davies, D. S., Gigon, P. L., Gillette, J. R. 1969. *Life Sci.* 8:85–91
79. Klinger, W. et al See Ref. 27, pp. 255–64
80. Gunsalus, I. C., Pederson, T. C., Sligar, S. G. 1975. *Ann. Rev. Biochem* 44:377–407
81. Iba, M., Soyka, L. F., Schulman, M. P. 1975. *Fed. Proc.* 34:784
82. Rifkind, A., Lauersen, N., Bennett, S., New, M. 1974. *Pediatric Res.* 8:366
83. Rane, A., von Bahr, C., Orrenius, S., Sjöqvist, F. See Ref. 25, pp. 278–301
84. Pelkonen, O. 1973. *Biochem. Pharmacol.* 22:2357–64
85. Schenkman, J. B., Cinti, D. L., Orrenius, S. 1973. *Drug Metab. Dispos.* 1:111–19
86. Deshpande, G. N., Turner, A. K., Sommerville, L. F. 1960. *J. Obstet. Gynaecol. Br. Commonw.* 67:954–61
87. Soyka, L. F., Long, R. J., 1972. *J. Pharmacol. Exp. Ther.* 182:320–27
88. Soyka, L. F., Deckert, F. W. 1974. *Biochem. Pharmacol.* 23:1629–39
89. Kardish, R., Feuer, G. 1972. *Biol. Neonate* 20:58–67
90. Juchau, M. R., Lee, Q. H., Zachariah, K. 1975. *Pharmacologist* 17:208
91. Holtzman, J. L., Gram, T. E., Gigon, P. L., Gillette, J. R. 1968. *Biochem. J.* 110:407–12
92. Getz, G. S., Wentworth, J., Laiken, S. 1970. *Adv. Lipid Res.* 8:175–223
93. Lu, A. Y. H., Levin, W. 1974. *Biochim. Biophys. Acta* 344:205–40
94. Baldwin, J., Cornatzer, W. E. 1968. *Lipids* 3:361–67
95. Davison, S. C., Wills, E. D. 1974. *Biochem. J.* 140:461–68
96. Rogers, M. J., Strittmatter, P. 1974. *J. Biol. Chem.* 249:895–900
97. Kamataki, T., Kitagawa, H. 1973. *Biochem. Pharmacol.* 22:3199–3207
98. Wills, E. D. 1968. *Biochem. J.* 113:325–32
99. Renton, K. W. 1975. *Hepatic microsomal lipid peroxidation and its effects on drug metabolism.* PhD thesis. McGill Univ., Montreal, Canada. 192 pp.
100. Short, C. R. 1975. *Pharmacologist* 17: 208
101. Kotake, A. N., Peloria, L. B., Abbott, V. S., Mannering, G. J. 1975. *Biochem. Biophys, Res. Commun.* 63:209–16
102. Kamataki, T., Ozawa, N., Kitada, M., Kitagawa, H. 1974. *Biochem. Pharmacol.* 23:2485–90
103. Warner, M. 1975. *Studies on the ligand-binding protein and other components of Z-fraction.* PhD thesis. McGill Univ., Montreal, Canada. 146 pp.
104. Pelkonen, O., Kärki, N. T. 1973. *Life Sci.* 13:1163–80
105. Rane, A., Sjöqvist, F., Orrenius, S. 1973. *Clin. Pharmacol. Ther.* 14: 666–72
106. Netter, K. J. 1971. *Arch. Gynaekol.* 211:112–33
107. Pelkonen, O., Vorne, M., Kärki, N. T. 1969. *Acta Physiol. Scand. Suppl.* 330:69–74
108. Arvela, P., Vorne, M., Järvinen, P., Kärki, N. 1970. *Abstract, Reg. Congr. Int. Union Physiol. Sci. Brasov, Romania*
109. Yaffe, S. J., Rane, A., Sjöqvist, F., Boréus, L.-O., Orrenius, S. 1970. *Life Sci.* 9:1189–1200
110. Gustafsson, J. A., Lisboa, B. P. 1968. *Steroids* 11:555–63
111. Juchau, M. R. 1971. *Arch Int. Pharmacodyn. Ther.* 194:346–58
112. Pelkonen, O. 1973. *Arch Int. Pharmacodyn. Ther.* 202:281–87
113. Pelkonen, O., Kärki, N. T. 1973. *Biochem. Pharmacol.* 22:1538–40
114. Pelkonen, O., Vorne, M., Jouppila, P., Karki, N. T. 1971. *Acta Pharmacol. Toxicol.* 29:284–94
115. Koga, A. 1971. *Analyt. Entwickl. Gesch.* 135:156–61
116. Zamboni, L. 1965. *J. Ultrastruct. Res.* 12:509–24
117. Pelkonen, O., Kaltiala, E. H., Larmi, T. K. I., Kärki, N. T. 1973. *Clin. Pharmacol. Ther.* 14:840–46
118. Pelkonen, O., Kärki, N. T. 1971. *Acta Pharmacol. Toxicol.* 30:158–60
119. Ackermann, E., Rane, A., Ericsson, J. L. E. 1972. *Clin. Pharmacol. Ther.* 13:652–62
120. Juchau, M. R., Pedersen, M. G. 1973. *Life Sci.* 12:193–204
121. Rifkind, A. B., Bennett, S., Forster, E. S., New, M. I. 1975. *Biochem. Pharmacol.* 24:839–46
122. Rane, A., Gustafsson, J. A. 1973. *Clin. Pharmacol. Ther.* 14:833–39
123. Rane, A., Ackermann, E. 1972. *Clin. Pharmacol. Ther.* 13:663–70
124. Ackermann, E., Rane, A. 1971. *Chem. Biol. Interact.* 3:233–34
125. Juchau, M. R. 1971. *Toxicol. Appl. Pharmacol.* 18:665–75

126. Pelkonen, O., Arvela, P., Kärki, N. T. 1971. *Acta Pharmacol. Toxicol.* 30: 385–95
127. Juchau, M. R., Pedersen, M. G., Symms, K. G. 1972. *Biochem. Pharmacol.* 21:2269–72
128. Pelkonen, O., Jouppila, P., Kärki, N. T. 1973. *Arch. Int. Pharmacodyn. Ther.* 202:288–97
129. Rane, A., Ackermann, E. 1971. *Acta Pharmacol. Toxicol.* 29:Suppl. 4, 84
130. Rane, A., Sjöqvist, F., Orrenius, S. 1971. *Chem. Biol. Interact.* 3:305–6
131. Ackermann, E., Richter, K. See Ref. 27, pp. 311–18
132. Idänpään-Heikkila, J. E., Jouppila, P. I., Puslakka, J. O., Vorne, M. S. 1971. *Am. J. Obstet. Gynecol.* 109:1101–6
133. Rane, A. 1974. *Clin. Pharmacol. Ther.* 15:32–38
134. Erkkola, R., Kanto, J., Sellman, R. 1974. *Acta Obstet. Gynecol. Scand.* 53:135–38
135. Pelkonen, O., Jouppila, P., Järvinen, P., Kärki, N. T. 1973. In *Perinatal Medicine,* ed. H. Brossart, J. M. Cruz, A. Huber, L. S. Prod'hom, J. Sistek, pp. 306–7. Bern: Hans Huber
136. Soyka, L. F. 1970. *Biochem. Pharmacol.* 19:945–51
137. Aranda, J. V., MacLeod, S. M., Renton, K. W., Eade, N. R. 1974. *J. Pediatr.* 85:534–42
138. Dvorchik, B. H., Stenger, V. G., Quattropani, S. L. 1974. *Drug Metab. Dispos.* 2:539–44
139. Mandelli, M. et al 1975. *Clin. Pharmacol. Ther.* 17:564–72
140. Juchau, M. R. 1975. See Ref. 27, pp. 29–38
141. Netter, K. J., Bergheim, P. 1975. See Ref. 27, pp. 39–52
142. Nebert, D. W., Winker, J., Gelboin, H. V. 1969. *Cancer Res.* 29:1763–69
143. Welch, R. M. et al 1969. *Clin. Pharmacol. Ther.* 10:100–109
144. Juchau, M. R. et al 1974. *Drug Metab. Dispos.* 2:79
145. Pelkonen, O., Jouppila, P., Kärki, N. T. 1972. *Toxicol. Appl. Pharmacol.* 23: 399–407
146. Bend, J. R., James, M. O., Devereux, T. R., Fouts, J. R. 1975. See Ref. 27, pp. 229–43
147. Goldenthal, E. I. 1971. *Toxicol. Appl. Pharmacol.* 18:185–207
148. Rane, A. 1974. *J. Pediatr.* 85:543–45
149. Reynolds, J. W., Mirkin, B. L. 1973. *Clin. Pharmacol. Ther.* 14:891–97
150. Hoppel, C., Rane, A., Sjöqvist, F. 1975. See Ref. 27, pp. 341–45
151. Ehrnebo, M., Agurell, S., Jalling, B., Boréus, L. O. 1971. *Eur. J. Clin. Pharmacol.* 3:189–93
152. Rane, A., Lunde, P. K. M., Jalling, B., Yaffe, S. J., Sjöqvist, F. 1971. *J. Pediatr.* 78:877–82
153. Fredholm, B. B., Rane, A., Persson, B. 1975. *Pediatr. Res.* 9:26–30
154. Loughnan, P. M., Greenwald, A., Neims, A. H. 1974. *Clin. Res.* 22:726A
155. Mirkin, B. L. 1971. *J. Pediatr.* 78: 329–37
156. Mirkin, B. L. 1971. *Am. J. Obstet. Gynecol.* 109:930–33
157. Rane, A., Garle, M., Borga, O., Sjöqvist, F. 1974. *Clin. Pharmacol. Ther.* 15:39–45
158. Baughman, F. A., Randinitis, E. J. 1970. *J. Am. Med. Assoc.* 213:466
159. Jalling, B., Boréus, L. O., Rane, A., Sjöqvist, F. 1970. *Pharmacol. Clin.* 2:200–202
160. Loughnan, P. M., Watters, G., Neims, A. H. 1975. *Ped. Res.* 9:285
161. Nitowsky, H. M., Matz, L., Berzofsky, J. A. 1966. *J. Pediatr.* 69:1139–49
162. Reinicke, C., Rogner, G., Frenzel, Y., Maak, B., Klinger, W. 1970. *Pharmacol. Clin.* 2:167–72
163. Vest, M. F., Streiff, R. R. 1949. *Am. J. Dis. Child.* 98:688–93
164. Morselli, P. L. et al 1973. *J. Perinat. Med.* 1:133–41
165. Cree, J. E., Meyer, J., Hailey, D. M. 1973. *Br. Med. J.* 4:251–55
166. Morselli, P. L. et al 1974. In *Drug Interactions,* ed. P. L. Morselli, S. Garattini, S. N. Cohen, pp. 259–70. New York: Raven
167. Krauer, B. et al 1973. *Clin. Pharmacol. Ther.* 14:442–46
168. Sjöqvist, F., Bergfors, P. G., Borga, O., Lind, M., Ygge, H. 1972. *J. Pediatr.* 80:496–500
169. Meffin, P., Long, G. J., Thomas, J. 1973. *Clin. Pharmacol. Ther.* 14: 218–25
170. Boréus, L. O., Jalling, B., Kallberg, N. 1975. See Ref. 27, pp. 331–40
171. Jalling, B., Boreus, L. O., Kallberg, N., Agurell, S. 1973. *Eur. J. Clin. Pharmacol.* 6:234–38
172. Rane, A., Bertilsson, L., Palmer, L. 1975. *Eur. J. Clin. Pharmacol.* 8: 283–84
173. Jalling, B. 1976. *Acta Paediatr. Scand.* In press
174. Walling, A., Jalling, B., Boréus, L. O. 1974. *J. Pediatr.* 85:392–98

175. Jalling, B. 1974. *Dev. Med. Child. Neurol.* 16:781–93
176. Melchior, J. C., Svensmark, P., Trolle, D. 1967. *Lancet* 2:860–61
177. Heinze, E., Kampffmeyer, H. G. 1971. *Klin. Wochenschr.* 49:1146–47
178. Gladtke, E., Heimann, G. See Ref. 27, pp. 393–403
179. Loughnan, P. M. et al 1976. *J. Pediatr.* In press
180. Dutton, G. J. 1966. *Biochem. Pharmacol.* 15:947–51
181. Oesch, F., Morris, N., Daly, J. W., Gielen, J. E., Nebert, D. W. 1973. *Mol. Pharmacol.* 9:692–96
182. Owens, I. S., Nebert, D. W. 1975. *Pharmacologist* 17:217
183. Hsia, D. Y. Y., Riabov, S., Dowben, R. M. 1963. *Arch. Biochem. Biophys.* 103:181–85
184. Chignell, C. F., Vesell, E. S., Starkweather, D. K., Berlin, C. M. 1971. *Clin. Pharmacol. Ther.* 12:897–901
185. Hadjian, A. J., Chedin, M., Cochet, C., Chambaz, E. M. 1975. *Pediatr. Res.* 9:40–45
186. Welch, R. M., Gommi, B., Alvares, A. P., Conney, A. H. 1972. *Cancer Res.* 32:973–78
187. Müller, D., Forster, D., Dietze, H., Langenberg, R., Klinger, W. 1973. *Biochem. Pharmacol.* 22:905–10
188. Fouts, J. R. 1973. See Ref. 25, pp. 305–20
189. Lambiotte, M., Verbrœdt, A., Benedetti, E. L. 1972. *C.R. Acad. Sci. Ser. D.* 275:2539–42
189a. Whitlock, J. P., Miller, H., Gelboin, H. V. 1974. *J. Cell Biol.* 63:136–45
189b. Nebert, D. W., Gelboin, H. V. 1968. *J. Biol. Chem.* 243:6250–61
190. Aranda, J. V. 1975. *Thyroxin and development of hepatic microsomal drug oxidation and electron transport in man and rats.* PhD thesis. McGill Univ., Montreal. 233 pp.
191. Dutton, G. J. 1973. *Enzyme* 15:304–17
192. Poland, A., Kappas, A. 1971. *Mol. Pharmacol.* 7:697–705
193. Burchell, B., Dutton, G. J., Nemeth, A. M. 1972. *J. Cell Biol.* 55:448–56
194. Wilson, J. T. 1969. *Biochem. Pharmacol.* 18:2029–31
195. Wilson, J. T. 1970. *Nature London* 225:861–63
196. Henderson, P. Th., Kersten, K. J. 1970. *Biochem. Pharmacol.* 19:2343–51
197. Leskes, A., Siekevitz, P., Palade, G. E. 1971. *J. Cell Biol.* 49:264–87
198. Leskes, A., Siekevitz, P., Palade, G. E. 1971. *J. Cell Biol.* 49:288–302
199. Klinger, W. 1973. See Ref. 28, pp. 51–126
200. Woods, J. S., Dixon, R. L. 1972. *Biochem. Pharmacol.* 21:1735–44
201. Siekevitz, P. 1973. *J. Supramol. Struct.* 1:471–89