Ann. Rev. Pharmacol. Toxicol. 1978. 18:17–35

DEVELOPMENTAL ASPECTS OF DRUG CONJUGATION, WITH SPECIAL REFERENCE TO GLUCURONIDATION

❖6703

G. J. Dutton
Department of Biochemistry, Medical Sciences Institute, The University, Dundee DD1 4HN, Scotland

INTRODUCTION

Drug metabolism has been divided into two successive phases (1). Phase 1 involves hydroxylation, dealkylation, hydrolysis, or similar modifications of the administered molecule. Phase 2 consists of one or more biosynthetic reactions whereby endogenous polar molecules are conjugated with the products of Phase 1 or with the administered drug itself if it possesses a conjugable grouping. These conjugates are generally readily excreted, although certain of them are biologically active and may contribute towards further metabolism of the administered drug or its derivative (2).

This review concerns developmental aspects of Phase 2 reactions, principally glucuronidation. Glucuronidation is the most important Phase 2 reaction in vertebrates because of the variety of species and tissues performing it, the range of molecular structures glucuronidated, and the net amount of conjugate formed (3). We concentrate on significant advances in the field and whenever possible on the natural mechanisms regulating perinatal development of Phase 2 reactions. References cannot be comprehensive; for general aspects, reviews are preferentially listed, including the excellent surveys in this series (4–7), which discuss many studies of conjugation not duplicated here. Development in Phase 1 has been recently well covered (8).

Literature was examined up to January 1977; a few outstanding later contributions are included, and unpublished information kindly made available subsequently is added to the reference list.

The conjugation reactions treated are (*a*) glucuronidation, (*b*) other glycosylations, (*c*) sulfation, (*d*) acetylation, (*e*) conjugation with amino acids, (*f*) conjugation with glutathione, and (*g*) methylation. These reactions are virtually all deficient

0362-1642/78/0415-0017$01.00

in the fetus and develop perinatally. Despite its obvious practical importance, the pattern of their development—and still less the mechanism responsible—has not in most cases been rigorously or extensively studied; this review is a plea for work in that area. All conjugations expend ATP to synthesize or activate the endogenous conjugating group or, more rarely, to "activate" the xenobiotic. In all of them, xenobiotics will compete with endogenous compounds routinely conjugated (e.g. *bilirubin* and steroids); competition will be for the conjugating agent and in some cases also for conjugating enzymes. Expenditure of time and energy on Phase 2 reactions for xenobiotics may thus understandably embarrass the fetus or neonate, already biochemically committed to a profound metabolic readjustment. Conjugation of endogenous compounds therefore cannot be entirely ignored in this review.

GLUCURONIDATION

All known biosynthetically formed xenobiotic glucuronides are glycosides of β-D-glucopyranosiduronate structure. Groups conjugated with glucuronic acid comprise hydroxyl, carboxyl, amino, imino, and sulfhydryl groups of aromatic, aliphatic, or heterocyclic nature (3); aglycon linkage can be through *O*-, *N*-, *S*-, and also (9) directly *C*-atoms. Reviews are extensive (10–13). We must mention excretion of glucuronides briefly before treating recent studies on the development of the enzyme mechanism itself.

Perinatal Excretion of Glucuronides

PRENATAL EXCRETION Glucuronidation occurs to a limited extent in the intact fetus but probably not in the placenta (13). Except for steroids its documentation is scanty (13). Glucuronides may cross the placenta slowly (14) and can accumulate in fetal tissues or amniotic fluid, as long known for steroid glucuronides. Many such "glucuronides" have not been satisfactorily characterized. These steroid glucuronides are assumed to be formed in the fetal liver and/or gut before excretion into meconium, amniotic fluid, and fetal gall bladder bile. Only small amounts are found. O'Donoghue (15) has reported drug conjugates (glucuronides?) of fetal origin in amniotic fluid.

POSTNATAL EXCRETION Excretion of glucuronides by infants and neonates has been well documented in this series (5–7). Generally, urinary glucuronide excretion is low at birth, though often quite detectable. Occasional high values may result from induction following maternal medication (e. g. 16).

Genetic differences in glucuronidation are becoming evident. Wilson (5) reviewed racial variations, and there are suggestions of high and low glucuronidating strains in man (see Discussion in Reference 54); identical twins metabolized the predominantly glucuronidated *p*-hydroxyphenylhydantoin at identical rates (17).

INCREASED EXCRETION AFTER INDUCTION Increased glucuronide excretion following phenobarbital treatment has been detailed previously (5). Data on phenobarbital half-lives for use in hyperbilirubinemia therapy are given by Boréus et al (18), who note conjugation of the drug metabolites (presumably as glucuronides)

within 4 hr of birth. Accelerated plasma bilirubin clearance in infants of drug-addicted mothers is now regularly noted (5, 6).

The value of D-glucarate excretion as an index of neonatal glucuronidation remains unproved; Levy et al (19) found no correlation with acetaminophen clearance and point out the partial compensation of low glucuronidation by sulfation. They suggest that an observation of high serum bilirubin and *high* glucarate excretion could result from UDP-glucuronic acid forming glucarate rather than forming glucuronides. Such complexities reduce the usefulness of the glucarate excretion test.

Perinatal Development of Glucuronidating Enzymes

GLUCURONIDATING ENZYMES The mechanism of glucuronidation (10, 13) and its feeder pathways (11) have been reviewed extensively. The aglycone (here R.OH) is glucuronylated by glucuronyl transfer from UDP-glucuronic acid (UDPGlcUA):

$$R.OH + UDPGlcUA \rightarrow R.O.glucuronide + UDP.$$

The microsomal enzyme UDP-glucuronosyltransferase (EC 2.4.1.17) acts reversibly for a few substrates, but in vivo appears predominantly to synthesize glucuronides. UDPGlcUA is formed from UDP-glucose by cytoplasmic UDP-glucose dehydrogenase (EC 1.1.1.22):

$$UDP\text{-}glucose + NAD^+ \rightarrow UDPGlcUA + NAD.H.$$

Synthesis of UDP-glucose itself requires a carbohydrate source and ATP.

UDP-GLUCURONYLTRANSFERASE

General development Much has recently been learned about this enzyme, modifying earlier (10) views. Its marked latency, heterogeneity, and regulation by the membrane environment are discussed in reference (13).

Low levels of the transferase in fetal tissues, including those of man, were noted many years ago following the original observations of poor glucuronidation in sliced neonatal mouse liver by Levvy's group (see 10). The subsequent vast literature has been reviewed (10–13), some of it recently in this series (5, 6). Here we need only outline the developmental pattern of the enzyme(s).

In all early fetal tissues (i.e. up to first half of gestation) the enzyme activity is either undetectable or below 20% of adult levels, for the wide range of substrates examined. Subsequently it rises, at a rate dependent on substrate and species (12). Just before birth in the rat, for example, liver transferase activity towards *p*-nitrophenol is above adult value, whereas towards bilirubin it is below 10% adult value. Activities towards two groups of substrates seem to develop (20) as if they belonged respectively to the late-fetal and the neonatal clusters of Greengard (21). Moreover, components of the cluster can vary with the species (12, 13). In man, prenatal values appear low for all substrates. Rane et al (22) for example, confirming earlier work, detected no transferase activity towards several xenobiotics in microsomes from human fetal liver at 13–22 weeks; Burchell (23) detected activity towards estriol in

10-week fetal liver and kidney, but at 17 weeks, when it had increased some tenfold, it was still only 16% adult values. Unlike that of some fetal animals (24), human fetal gut is even less active than fetal liver (23, 24). Liver and kidney so far appear quantitatively to be the main human fetal sites of glucuronidation. Their low activity contributes to the fetal accumulation of steroid glucuronides noted above, for the placenta does not form glucuronides (10, 13, 23, 25). Other fetal sites (e.g. adrenal) should be investigated for possible glucuronidation of certain xenobiotics.

Few studies continue beyond weaning, yet UDP-glucuronyltransferase activity towards pregnanediol (26) does not significantly develop in female rat until 33 days after birth and surges again dramatically (fourfold) at 60-65 days, when puberty is normally attained. This valuable study was carried on until 90 days, and revealed that activity towards testosterone rose until day 33; activities to estrone and bilirubin plateaued earlier, around weaning. However, age-dependent activation of the enzyme was not allowed for.

Age-dependent activation—that is, variation with age of the conditions required to activate the enzyme optimally in vitro—complicates assay of the transferase in perinatal tissues. Much early work needs reassessment, although serious misinterpretation is unlikely to have occurred. First noted by Winsnes (27), it could give rise to an artifactual "development" of the enzyme (28, 29). Perinatal activities are best measured over a range of activations from zero to above optimal, alongside adult preparations similarly treated (28–30). Another reason for cautious interpretation is the age-dependent sedimentation characteristics of the microsomal fraction [see references in (6)]; homogenates are therefore preferable for screening.

Although the fetal enzyme is the more readily destroyed by activation procedures, its low activities do not seem an artifact of homogenate preparation; gentler homogenization does not increase activity and glucuronidation remains low in slices of fetal liver even when, as with chick (31), UDPGlcUA is not limiting.

Likewise, low fetal transferase activity is not due to inhibition of an existing enzyme. No inhibitor from fetal tissues has been demonstrated. Absence of a possible rate-limiting activator also appears unlikely. Although the endogenous activator UDP-*N*-acetylglucosamine is high prenatally (32), when added to early human fetal liver microsomes (± ATP) (22), or over a range of concentrations to rat fetal liver preparations, it does not result in the appearance of transferase activity (G. J. Wishart, unpublished observations). Once the enzyme has appeared, UDP-*N*-acetylglucosamine activates the transferase.

Most probably, the deficient amount of transferase is responsible for the low fetal activity; increased transferase synthesis or decreased breakdown, or both, will accompany the natural development of activity.

Are fetal and adult transferases different? Dissimilarities occur between infant and adult mouse liver enzyme preparation in apparent $K_{bilirubin}$ and in response to deoxycholate (33). The latter, of course, reflects the age-dependent activation and presumably a progressive change in the enzyme's membrane environment. Here we confront the vexed question of how far the catalytic specificity of the transferase depends on its membrane environment. It must be stressed that various kinetic or

physical differences reported between perinatal and adult microsomal-bound or "solubilized" transferases cannot prove the existence of different enzyme proteins. This tempting conclusion must await purification of the catalytic entity.

Mechanism of the natural development of UDP-glucuronyltransferase activity Precocious development of UDP-glucuronyltransferase has been more intensively studied than that of other Phase 2 enzymes (*a*) to assist, therapeutically, detoxication in the premature or neonate and (*b*) to elucidate the mechanism of the natural development. Studies have utilized xenobiotic and endogenous substrates and both xenobiotic and endogenous inducers. They have employed embryo chick and fetal mammal. As *a* has been well covered previously in this series (5, 6), we concentrate on *b* and deal separately with avian embryo and mammalian fetus.

1. *Chick embryo.* Advantages of using the independent readily manipulated avian embryo for preliminary studies have been described (34, 35). Early references are given in these reviews. Transferase activity is detectable in chick-embryo liver from days 4-8, remains negligible until hatching on day 21, and surges to adult levels within 1-3 days (34–37). If embryo liver or kidney is cultured, activity spontaneously and precociously reaches or surpasses adult levels. This rise involves some morphological maturation, is independent of culture method, tissue organization or cell division, but requires nutrients and directly depends on protein synthesis. The induced enzyme (like adult, but unlike embryonic, enzyme) can be activated (29).

Transferase does not increase, but is merely maintained, when embryo liver is cultured on the chorioallantoic membrane (CAM); subsequent culture in vitro is required to increase the enzyme. Induction in vivo thus appears to be initiated by removal from a repressive embryonic environment in ovo. Despite earlier hints (34) no in ovo repressor has been noted, and the embryonic hypophysis is not responsible (38). Possibly, inducing factors accumulate in the isolated cells. A similar spontaneous induction, equally unexplained, has recently been seen in the synthesis of adult plasma protein by cultured embryo liver (39).

Phenobarbital directly induced transferase synthesis in the liver explants or cultured cells (40), and when injected into the egg overcame the repression dramatically; activity could rise to over 30 times adult value, whether the latent or activated enzyme was measured (41). Notably, competence was not age limited, response occurring [like that for spontaneous induction (37)] as early as 96 hr. However, certain effects of phenobarbital on the cultured tissue (40) suggested that the natural regulation of transferase changed in some way between days 5 and 11 in ovo. Injected phenobarbital increased the transferase in both embryonic liver and kidney (41), and in both the microsomes and nuclear envelope (42) of the embryonic liver.

Would endogenous inducers such as hormones also overcome the in ovo repression? And might they increase in vivo about the hatching period? Unlike xenobiotics, hormones are subject to evolved controls, and operate within fine limits of administration. Indeed, injected hormones gave erratic results (36). To ensure physiological release, and to detect possibly unknown inducers, adult tissues were grafted on the CAM, and transferase subsequently measured in host-embryo liver. Activity appeared precociously and at adult levels when the cephalic area of chicken hypoph-

ysis was grafted (38). Host liver only responded to the secreted factor after 13 days of age, contrasting with its age-independent response to phenobarbital. Pituitaries from earlier embryos were ineffective but those from embryos about to hatch were more effective than those from adults (38). This suggested that a peak of "factor" in vivo accounted for the post-hatching surge of transferase.

Using simple infusion in ovo, Leakey & Dutton (43) showed that the factor was corticotropin, with thyrotropin acting synergistically. Corticosteroids possessing the 4-pregnene structure and a 11-hydroxy or oxo group also evoked precocious transferase activity, with the same 13-day competence limit, when infused to a level consistent with that occurring in plasma over hatching (36). Other steroids and hormones tried were ineffective. Stimulation was also observed in culture (36), so that competence to respond to glucocorticoid is a function of the embryo liver cell itself. Summing up, the surge of UDP-glucuronyltransferase, and of overall glucuronidation (36), during hatching in chick would seem to be due to corticosteroid, whose production is stimulated by corticotropin and whose action is assisted by thyroxine via thyrotropin.

2. *Mammalian fetus.* With the mammalian fetus, certain differences are evident and their relevance to the maternal dependence of the fetus is not yet clear. One example is the response to phenobarbital. Administration of phenobarbital to the mother prenatally or to the neonate accelerates liver transferase development in early life (5, 6), but unlike chick embryo, the fetus, in mice at least, appears not able to respond in this way until just before birth, even though the drug is similarly concentrated in fetal and maternal liver and the transferase has been stimulated in the latter from early in gestation (44); this occurs with transferase activities which, to different substrates, exhibit at birth either adult values or virtually zero values. Another difference lies in the absence (30), or low degree (23, 28), of spontaneous induction in fetal liver cultured in vitro.

Similarities were found in the developmental mechanism, however. Mammalian pituitary gland grafted on the CAM stimulated transferase in host-embryo liver, and explants of fetal liver responded on culture in vitro with glucocorticoids even more readily than embryo-liver explants, because they exhibited no concurrent background of spontaneous induction. Dexamethasone, corticosterone, and other corticosteroids (all 2 μM) with the 4-pregnene structure and a hydroxy or oxo group at C-11 precociously raised transferase in 14-day rat fetal liver explants from negligible levels to adult male values within 4 days' culture (30); over this period in utero the enzyme had reached only some 15% adult values. By cycloheximide pulsing, the rise was shown to depend on protein synthesis. Several lines of evidence, including rate of amino acid incorporation and delayed exposure to hormone, indicated that explants cultured without glucocorticoids were viable. Confirmation of a specific effect came from a similarly precocious rise in utero, following injection of glucocorticoids into the mother on days 14, 15, and 16 of gestation. By day 17 fetal liver transferase had reached adult male levels. In control fetuses, injected with carrier only, levels remained negligible on day 17. This precocious surge was already evident 24 hr after the first injection (45, 46). In utero the more persistent dexamethasone was more effective than the natural glucocorticoids. This was the first

evidence in fetal mammalian tissue of precocious UDP-glucuronyltransferase activity brought about by known compounds of an endogenous nature. Their involvement perinatally is consistent with the surge in corticosterone levels observed in fetal rat plasma from day 18 (47). Significantly, the natural rise in fetal liver transferase is not observed in decapitated fetuses from adrenalectomized mothers (46). It is thus reasonable to suppose that (via corticotropin) glucocorticoids act at the mammalian liver cell, as at that of chick embryo, and cause the perinatal surge of liver transferase activity to certain substrates.

Not all the various transferase activities are so induced. Important evidence for the functional heterogeneity of the enzyme came from the observation in rat liver (45) that whereas activities towards *o*-aminophenol, *p*-nitrophenol, 1-naphthol, and serotonin were induced in culture or in utero by glucocorticoids over days 14–17, those towards morphine, bilirubin, testosterone, and chloramphenicol were not. Significantly, the former group develop, in Wistar rat, to adult levels prenatally, while the latter reach them postnatally (20); they would represent the late-fetal and neonatal clusters of Greengard (21) respectively. Other late-fetal enzymes, such as glycogen synthetase, are induced precociously in rat by glucocorticoids (48) and it remains to be seen whether transferase activities of the second group are stimulated by inducers of the neonatal group or whether they require different, or additional, factors including that of time.

Transferase activities of the first group are also stimulated precociously in lung, upper alimentary tract, and kidney of fetal rats following dexamethasone injection of the mother (46). An enzyme need not be maintained through adulthood by the hormone which triggered its onset (21); it is therefore not inconsistent that transferase activities remained stable in male rats adrenalectomized 10 days previously (G. J. Wishart and M. T. Campbell, unpublished observations).

There is no evidence yet of other hormones inducing or repressing perinatal transferase development (30). Progesterone, a suggested competitor with endogenous inducers for binding sites, was by itself and in absence of serum ineffective as inducer or repressor in culture (30); its slight stimulation following maternal injection (46) was probably from metabolism to glucocorticoids. Growth hormone, repressing development of Phase 1 metabolism (5), had no effect on transferase in rat fetal tissue (G. J. Wishart, unpublished observations) or in infant mice (49).

Bilirubin, biliverdin, and heme have so far failed to accelerate development of transferase activity to bilirubin, or to xenobiotics, in culture or after injection (M.T. Campbell and G. J. Wishart, unpublished observations) despite the many indirect suggestions that they do influence the process (13).

Reports of the effect on the transferase and glucuronidation of starvation or protein deprivation are contradictory (13,50). This is understandable, because although restricted supply of carbohydrate for UDPGlcUA or of amino acids for enzyme protein can be expected to delay glucuronidation, concomitant changes in the membrane environment (13) can affect the enzyme's activation or inactivation during assay and by this means at times suggest an accelerated transferase development. References to the still controversial inhibitory factor in breast-milk are discussed elsewhere (13).

DEVELOPMENT OF OVERALL GLUCURONIDATION By overall glucuronidation we mean the formation of glucuronides by intact cells or tissues. Generally, this follows the pattern seen in disrupted cells or microsomes. Two factors are involved.

Membrane conformation Homogenization must change membrane conformation. Membrane conformation may well (51) contribute to low perinatal glucuronidation, but is difficult to study and clearly is not wholly responsible.

UDP-glucuronic acid availability Compared with those in adult, liver UDPGlcUA levels are low in fetal mammal but high in embryo chick (13, 31). This could account for the relatively more rapid development of glucuronidation in liver slices from hatched chick than from neonatal mammals (31). However, levels of UDPGlcUA found in a liver extract need not reflect its *availability* to the membrane-bound transferase in vivo. Apart from compartmentational factors, turnover of UDPGlcUA must be considered.

Levels of UDP-glucose dehydrogenase in liver are even less reliable as an indication of availability of UDPGlcUA to the liver transferase; UDPGlcUA formed by the abundant extrahepatic dehydrogenase could be transferred to the liver. Liver dehydrogenase is low in fetal mammals (31, 52) and high in embryo chick (31); in mice it surges late-fetally and at weaning, rising further at puberty in female animals (31). The dehydrogenase is stimulated by phenobarbital (31) in fresh and cultured chick embryo liver, and by glucocorticoids in cultured rat fetal liver (C. Petrou and G. J. Wishart, unpublished observations). Its stimulation may therefore contribute to the increased overall glucuronidation observed in fetal mammalian liver after exposure to glucocorticoids in culture or in utero (30, 46).

Increased destruction of UDPGlcUA during assay is not responsible for the low transferase levels in fetal broken-cell preparations (13).

Knowledge of the mechanism regulating perinatal onset of the glucuronidation enzymes, incomplete as it is, is greater than that concerning the other Phase 2 enzymes, which are discussed below.

OTHER GLYCOSYLATIONS

β-D-Glucosides, β-D-xylosides, and β-D-*N*-acetylaminoglycosides are now recognized as minor conjugates in mammalian tissues, being biosynthesized analogously to the β-D-glucuronides, by means of the respective UDP-sugars and UDP-glycosyltransferases; the enzymes appear specific for their nucleotides. Groups conjugated resemble those glucuronidated. Work up to 1974 has been summarized (13).

Although glucosides of xenobiotics substitute largely for glucuronides in invertebrates (53) and in adult mammals may increase relatively as UDPGlcUA falls (13), fetal glucosidation remains proportionately as low as glucuronidation and develops at a roughly similar rate (13, 30). No UDP-glucosyltransferase for estriol could be found in human fetal liver (30). Heirwegh's group observed that the three UDP-glycosyltransferases responsible for bilirubin conjugation in rat developed similarly

over birth, although at first the glucosyltransferase appeared less rapidly than did the glucuronyl- and xylosyltransferases (54). Others (55), using a different strain, found that one-day-old rats conjugated 50% of the bilirubin as xyloside and glucoside, and that these glycosylations developed more rapidly than glucuronidation both ontologically and on induction by phenobarbital.

SULFATION

Sulfation in the pharmacological sense gives rise biosynthetically to sulfate esters of the structure $R.O.SO_3^-$, ionized half-esters of sulfuric acid. Such sulfations are predominantly of phenolic hydroxyl groups, alcoholic hydroxyl or primary amino groups being sulfated to a lesser extent. Sulfation, as a conjugation reaction, is relatively expensive in ATP and sulfur; it is, more so than glucuronidation, required for transport and metabolic interconversion as well as for simple excretion.

These points are discussed in recent extensive reviews (56–58). Developmentally, little information is available (58).

Sulfation Enzymes

Sulfate is activated in two stages before transfer to an acceptor. First, ATP-sulfate adenylyl transferase (EC 2.7.7.4) (ATP-sulfurylase) catalyzes the reaction:

$$ATP + SO_4^{-2} \rightleftharpoons \text{adenosine-5'-phosphosulfate (APS)} + \text{pyrophosphate.}$$

Second, ATP-adenylylsulfate 3-phosphotransferase (EC 2.7.1.25) (APS-kinase) catalyzes

$$ATP + APS \rightarrow \text{3-phosphoadenosine 5'-phosphosulfate (PAPS)} + ADP.$$

Then a sulfotransferase (e.g. EC 2.8.2.1) catalyzes the conjugation:

$$PAPS + R.OH \rightarrow PAP + R.O.SO_3^-.$$

The first two enzymes are in mammalian cytosol; sulfotransferases for drugs and small endogenous molecules (e.g. steroids) are also cytoplasmic.

Perinatal Sulfation

Early developmental studies could not distinguish between the various stages in sulfation, and so require cautious interpretation. Because PAPS, like UDPGlcUA, is required for synthesis of large molecules such as glycans, ATP-sulfurylase and APS-kinase must be present in the fetus. The sulfurylase, like UDPGlucose dehydrogenase, may be low in liver—10–20% adult at birth (59, 60); it is high in fetal adrenal and other sites [for reference see (58)]. Reports on mammalian APS-kinase development have not been found. As with glucuronidation, it is difficult to assess the limitation imposed on conjugate excretion by low liver levels of the enzymes forming the activated donor molecule. From the scanty literature, excretion of sulfates of drugs or endogenous compounds appears almost as high in the neonate

as in the adult. Sulfotransferase levels required for this sulfoconjugation are attained during fetal life, very early in the case of steroid conjugation (14).

Heterogeneity of sulfotransferase is certain (56–58), the enzymes being soluble and readily separable. Two broad groups briefly concern us here.

SULFATION OF STEROIDS Fetal or placental sulfation of steroids has been thought necessary (14, 56) for protection against circulating maternal steroid, and sulfation of steroids is required for certain of their metabolic transformations (61). Estrogen sulfotransferase approaches adult level in late human-fetal liver, and in human-fetal adrenal gland is much higher (59). The human fetus is therefore acknowledged to possess high sulfotransferase activities towards steroids, and the fetoplacental sulfation of these compounds is exhaustively discussed by Pasqualini (14); he quotes evidence of fetal hypothalamo-pituitary factors controlling fetal steroid sulfate production, there being a deficiency of these sulfates, together with adrenal atrophy, in anencephalic monsters. No detailed mechanism appears to be known.

SULFATION OF DRUGS As noted above, the neonate tends to excrete drugs preferentially as sulfate, which suggests that this system is more developed than glucuronidation. Fetal phenol sulfotransferases appear to develop less rapidly than steroid transferases (59, 60, 62), but at birth may approach adult levels for some substrates. Sulfotransferases evolved to deal with endogenous steroids may possess overlapping specificity towards xenobiotics, and the intrusion of a cycle of sulfated and desulfated drug into the fetal steroid sulfate economy could prove deleterious. With the limited information available, it is premature to discuss this matter. Two points may profitably be made. Yaffe et al (62) noted differential inhibition of liver phenol sulfotransferases in 1- and 28-day-old mice by salt concentration and suggested a difference in protein structure; recent work (63) suggests that certain observed differences in phenol sulfotransferases could arise from conformational changes dependent on their redox state. The soluble sulfotransferases are more easily studied than are their insoluble UDP-glucuronyltransferase analogues, and the mechanism of their development should be investigated.

Dodgson notes (58) a nondialyzable inhibitor of phenolsulfotransferase found in gut homogenate supernatants over birth, which accounted for a spurious fall in the otherwise rapidly developing enzyme. It could be a PAPS-destroying protein analogous to the UDPGlcUA-pyrophosphatase notorious in glucuronidation studies but, according to Dr. G. Powell (personal communication), some PAPS may still be present after incubation.

ACETYLATION

Acetylation occurs at aromatic amino or hydrazino groups (64). Endogenous acceptors may include 5(OH)-tryptamine and histamine (65). Comprehensive reviews are available (64, 65). Acetylation requires acetyl coenzyme A and the soluble enzyme

acetyl coenzyme A:arylamine *N*-acetyltransferase (EC 2.3.1.5), found in many mammalian tissues:

$$CH_3CO.S.CoA + R.NH_2 \rightarrow R.NH.CO.CH_3 + CoA.SH.$$

Two other acetylation mechanisms for xenobiotic arylamines are known in mammals; a carboxyl esterase (EC 3.1.1) and an *N*-acetyltransferase employing hydroxamic acids as acetyl donors (77). Only *N*-acetyltransferases of the group EC 2.3.1.5 have been studied developmentally.

Perinatal Acetylation

In this field little has yet been done (65, 66). Fichter & Curtis (66) found that premature infants acetylated sulfonamides less readily than did newborns, and both were below adult capabilities. Vest's group (67, 68) found that although acetylation was low in newborns it was proportionately higher than conjugation with glycine; in older children the glycinated derivatives increased. Placental tissue is capable of limited acetylation, e.g. of *p*-aminobenzoate, but not of isoniazid (69).

Weber's group (70) showed that, for isonicotinic acid hydrazide, partially purified liver *N*-acetyltransferase (from rapid-acetylating rabbits) reaches 100% adult levels only at 3–4 weeks; it rises from low levels at 6 days to some 15–20% at 2 weeks, thereafter remaining constant until a surge at 3 weeks. The authors suggest that as well as different forms of transferase existing in different tissues there may be different forms of the enzyme specific for various stages of development: in infant rabbits, the limiting K_m for the acceptor substrate changes to adult values between 2–3 weeks of age, the period when the specific enzyme activity remains constant. Enzyme from infant animals was also less heat-stable than from adults.

CONJUGATION WITH AMINO ACIDS

Acylation reactions of pharmacological interest also include conjugation of aromatic carboxyl groups with the α-amino group of certain amino acids. Conjugation with amino acids is expensive in ATP and amino acid. It appears to be used when glucuronidation capacity is low, either permanently, as in members of the superfamily Feleidae (71) or temporarily, as at birth in man. A recent review is by Weber (65).

Acylation Enzymes

Here the xenobiotic acid is activated, rather than the endogenous donor; e.g. with benzoic acid and glycine:

(*a*) benzoate + ATP → benzoyl AMP + pyrophosphate;
(*b*) benzoyl AMP + CoASH → benzoyl.S.CoA + AMP;
(*c*) benzoyl.S.CoA + L-glycine → benzoylglycine + CoASH.

The first two reactions are mitochondrial and apparently identical with those activating endogenous fatty acids. The third reaction uses one of several acyltransferases (EC 2.3.1.73) found both in mitochondria and cytoplasm (65).

Perinatal Acylation

Levy (72) noted that human neonates excreted relatively more glycinated salicylate than did adults. The early work of Vest's group (67) indicated that *p*-aminobenzoate and benzoate were less readily glycinated by neonates than by infants or older children and that prematures were still less efficient; glycination increased over acetylation as the babies grew older. In rat liver (73) a glycine *N*-acyltransferase, with benzoyl CoA as substrate, occurred only as traces in fetus and neonate; it increased slowly to a maximum at 30 days; activity of this enzyme paralleled excretion of conjugate.

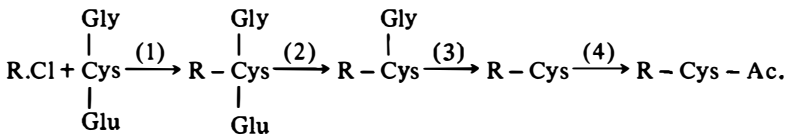
CONJUGATION WITH GLUTATHIONE: THE SYNTHESIS OF MERCAPTURIC ACIDS

Very full reviews are available (74–76); the later reviews (post 1975) deal more satisfactorily with the substrate specificities of the enzymes.

This form of conjugation recently has become intensively studied. Glutathione (GSH) protects the body against the toxic effects of the many xenobiotic electrophiles in several ways, well summed up by Grover (76): the key enzymes, the glutathione transferases, (*a*) may conjugate a very wide range of aromatic hydrocarbons, arylamines, organic halides, phenols, esters, chlorides, etc, which are then either excreted as biliary GSH-conjugates or further metabolized to a biliary or urinary mercapturic acid; (*b*) they may covalently bind with toxic electrophile metabolites of, for example, carcinogenic azo dyes or aromatic hydrocarbons, thus biologically inactivating both the toxic compound and the enzyme; and (*c*) they may transport compounds that are less electrophilic, such as bilirubin, to sites suitable for excretion. We deal only with the first, the true enzymic conjugation.

Conjugation with Glutathione, and Mercapturic Acid Formation

Mercapturic acid formation occurs in four steps. The first is conjugation of the xenobiotic with the tripeptide glutathione, spontaneously or, usually, by the cytoplasmic glutathione-*S*-transferases (EC 2.5.1.18):



A second step (2) removes the glutamine by γ-glutamyltransferase; a third (3) removes the glycine by cysteinylglycinase; and the fourth (4) acetylates the cysteine remaining, by an *N*-acetyltransferase.

Development of Liver Glutathione Levels

Although the GSH-GSSG ratio is identical, GSH levels are lower in fetal than in adult rat liver; they rise from 12% to 33% over the last 6 days before birth, reaching adult values at 10 days of age (77). Acetaminophen administration does not deplete GSH significantly until about 10 days, and depletion is at adult proportionality by 15 days of age (77).

Development of Glutathione S-Transferases

Several of these enzymes have now been isolated as homogeneous proteins (78). Though all are specific for GSH, they display overlapping specificities for a wide range of second substrates. Earlier literature employed falsely specific terms such as *epoxide* or *aryl transferase.* Little is yet known of the developmental mechanism of these separate enzymes.

Early studies (see 74, 75, 79) noted a slow increase of bromsulfothalein excretion with GSH over several postnatal weeks in animals. With aryl epoxide as substrate (80), 20% of adult activity in 18-day fetal rat liver and lung rose to 100% at 20 days of infancy; this pattern was also seen in guinea pig (81), with minor differences in substrate and tissue.

In man, maturation may be somewhat more rapid. Chasseaud (82) found GSH-*S*-transferase activity in human fetal liver at (admittedly postmortem) adult levels, and with naphthalene-1,2-oxide as substrate many human fetal and placental tissues were above those in adult rat liver (83).

GSH-*S*-transferase *B* is now known to be ligandin. In several animals and man, hepatic ligandin is absent fetally, appears perinatally, and reaches adult levels within the first week of life (84). Hales & Neims (85), using immunological and catalytic assays for ligandin in rat, found it to rise from 20% adult at 1 day to 100% in 4–5 weeks; they noted that slight differences occurred between the various transferases in rates of development. Importantly, they investigated its perinatal inducibility. Phenobarbital induced it in rat postnatally as early as 5 days of age, and the percentage increase of induced over uninduced enzyme was constant at any given age. Throxine or cortisol injected for 3 successive days before sacrifice at 6 or 18 days of age did not precociously induce the transferase towards the chlorinated nitrobenzene substrates; these cortisol injections had successfully induced tyrosine aminotransferase.

Ligandin has been reported to develop precociously in vivo. 'Y' protein (ligandin), assayed as a ligand (86), accumulated more rapidly in livers of fetal Gunn rats (congenitally hyperbilirubinemic) than in those of normal Wistar fetuses, suggesting bilirubin or a precursor as an endogenous inducer. Gatmaitan et al, (87) using mutant mice that lack many endoplasmic reticulum enzyme activities, found that homozygotes possessed catalytic ligandin activity double that of heterozygous littermates or newborn controls, and equal to that of control adult mice. The authors suggest that here, also, induction occurs by an accumulation of substrate(s) normally metabolized by the endoplasmic reticulum.

METHYLATION

Methylation is a relatively minor pathway in drug conjugation. Recent reviews cover endogenous and xenobiotic methylation (88, 89). Microsomal or cytoplasmic methyltransferases catalyze methyl transfer from *S*-adenosylmethionine to phenols, thiols, and aliphatic or heterocyclic amino groups.

Catechol-*O*-methyltransferase (90) appears low in fetal and neonatal liver of both rat and man. In rat it increases over 9–35 weeks and in man increases tenfold by maturity (91). In chick liver it peaked on day 7 of embryo life, fell to a minimum at day 16, and then slowly rose for 10 days after hatching (92); the pattern in chick heart was broadly similar.

Phenylethanolamine *N*-methyltransferase methylates many drugs (93) and may require glucocorticoids for its development (94), being absent in decapitated newborns unless the mother had been treated with glucocorticoids or corticotropin.

DECONJUGATION

Although enzymes hydrolyzing conjugates coexist with the biosynthetic transferases in developing tissues, there is no evidence that they contribute significantly to the low conjugations in immature mammals. β-Glucuronidase of the endoplasmic reticulum, as distinct from that of the lysomes, may participate in a deconjugation-reconjugation cycle (95), as probably do the sulfatases (56, 59); *total* cellular β-glucuronidase is usually increased in younger tissue (10). Circulating conjugates could be hydrolyzed by any fetally or perinatally increased hydrolyases in the blood; however, blood esterases, for example, are low in the human fetus and neonate (96).

CONCLUSIONS

Phase 2 reactions are virtually all low in the early fetus and although certain activities approach high levels perinatally, the neonate is clearly at risk when subject to demands on its conjugative capacity. Endogenous and xenobiotic compounds must compete for energy, material, and often for catalytic sites. Relative to other species investigated, man appears less well-equipped perinatally to deal with Phase 2 reactions than with those of Phase 1.

Heterogeneity within the various Phase 2 transferases is in some cases proved and in all is likely. Overlapping specificities may not be confined to the glutathione-*S*-transferases, for obviously Phase 2 enzymes have not been evolved to deal separately with the products of the modern organic chemist. When the transferases are membrane-bound, as in glucuronidation, specificities are further obscured by substrate behavior at the membrane and their unresolved heterogeneity detracts from the published developmental patterns. It would seem essential to solubilize the catalytic entity, purify the protein, and determine the role—for assay in vitro and for activity in vivo—of the developing membrane environment. Kinetic studies on insoluble, largely impure or imperfectly reconstituted enzymes should at present be designed to that end. We may then be able to decide whether a given fetal

or perinatal transferase is a different protein from its adult analogue, represents a different mixture of isozymes, or derives different properties through operating in a different cellular environment. The cytoplasmic enzymes, however, appear suited for immediate investigation.

The little already known of developmental patterns of Phase 2 enzymes, and of their inducibility by drugs, has been used to prevent much distress; if available sooner, it might have prevented more. Our present major task is to discover what triggers the natural onset of both fetal and neonatal Phase 2 transferases. We may then be able to treat more rationally any delayed development of these important enzymes, extrapolating results from animals to man in the manner suggested by Greengard (97).

ACKNOWLEDGMENTS

Whatever value this review possesses lies in its indication of how little is known of the perinatal pharmacology of Phase 2 reactions, and how readily fundamental information can be obtained. Nevertheless, the author may have omitted significant advances. He accepts full responsibility for such ignorance or misjudgement, while gratefully acknowledging information received from colleagues, particularly from Drs. L. F. Chasseaud, A. K. Done, G. Powell, A. B. Roy, F. Sjöqvist, W. W. Weber, J. T. Wilson, and S. J. Yaffe; and helpful discussion with Drs. B. Burchell, M. T. Campbell, and G. J. Wishart.

Quoted work from the author's laboratory has been supported by the Medical Research Council, Science Research Council, Scottish Hospitals Endowment Research Trust, and the Wellcome Trust.

Literature Cited

1. Williams, R. T. 1959. *Detoxication Mechanisms.* London: Chapman & Hall. 2nd ed.
2. Mulder, G. J., Hinson, J. A., Gillette, J. R. 1977. Generation of reactive metabolites of *N*-hydroxy-phenacetin by glucuronidation and sulfation. *Biochem. Pharmacol.* 26:189–96
3. Smith, R. L., Williams, R. T. 1966. Implication of the conjugation of drugs and other exogenous compounds. In *Glucuronic Acid.* ed. G. J. Dutton, pp. 457–91. New York: Academic
4. Mirkin, B. L. 1970. Developmental pharmacology. *Ann. Rev. Pharmacol.* 10:255–72
5. Wilson, J. T. 1972. Developmental pharmacology: A review of its application to clinical and basic science. *Ann. Rev. Pharmacol.* 12:423–50
6. Yaffe, S. J., Juchau, M. R. 1974. Perinatal pharmacology. *Ann. Rev. Pharmacol.* 14:219–38
7. Done, A. K., Cohen, S. N., Strebel, L. 1977. Pediatric clinical pharmacology and the "therapeutic orphan". *Ann. Rev. Pharmacol. Toxicol.* 17:561–73
8. Neims, A. H., Warner, M., Loughnan, P. M., Aranda, J. V. 1976. Developmental aspects of the hepatic cytochrome P450 monooxygenase system. *Ann. Rev. Pharmacol. Toxicol.* 16: 427–45
9. Richter, W. J., Alt, K. O., Dieterle, W., Faigle, J. W., Kriemler, H.-P., Mory, H., Winkler, T. 1975. C-Glucuronides, a novel type of drug metabolites. *Helv. Chim. Acta* 58:2512–17
10. Dutton, G. J. 1966. The biosynthesis of glucuronides. See Ref. 3, pp. 185–299
11. Miettinen, T. A., Leskinen, E. 1970. Glucuronic acid pathway. In *Metabolic Conjugation and Metabolic Hydrolysis,* ed. W. H. Fishman, 1:157–237. New York: Academic
12. Dutton, G. J. 1971. Glucuronide-form-

ing enzymes. *Handb. Exp. Pharmacol.* 28(2):378–400
13. Dutton, G. J., Burchell, B. 1976. Newer aspects of glucuronidation. In *Progress in Drug Metabolism,* ed. J. W. Bridges, L. F. Chasseaud, 2:1–70. London: Wiley
14. Pasqualini, J. R. 1970. Metabolic conjugation and hydrolysis of steroid hormones in the fetoplacental unit. See Ref. 11, 2:153–259
15. O'Donoghue, S. E. F. 1971. Distribution of pethidine and chlorpromazine in maternal, foetal and neonatal biological fluids. *Nature* 229:124–25
16. Rane, A. 1974. Urinary excretion of diphenylhydantoin metabolites in newborn infants. *J. Pediatr.* 85:543–45
17. Wilson, J. T., Hojer, B., Rane, A. 1976. Loading and conventional dose therapy with phenytoin in children: Kinetic properties of parent drug and main metabolite in plasma. *Clin. Pharmacol. Ther.* 20:48–58
18. Boréus, L. O., Jalling, B., Kallberg, N. 1975. Clinical pharmacology of phenobarbital in the neonatal period. In *Basic and Therapeutic Aspects of Perinatal Pharmacology,* ed. P. L. Morselli, S. Garattini, F. Sereni, pp. 331–40. New York: Raven
19. Levy, G., Khanna, N. N., Soda, D. M., Tsuzuki, O., Stern, L. 1975. Pharmacokinetics of acetaminophen in the human neonate: Formation of aceteminophen glucuronide and sulfate in relation to plasma concentration and D-glucaric acid excretion. *Pediatrics* 55: 818–25
20. Wishart, G. J., Mossman, S., Donald, A. M., Dutton, G. J. 1977. Differential stimulation of foetal rat liver UDP-glucuronosyltransferase activity towards certain substrates after glucocorticoid treatment in culture and *in utero,* and during development. *Biochem. Soc. Trans.* 5:723–25
21. Greengard, O. 1971. Enzymic differentiation in mammalian tissues. *Essays Biochem.* 7:159–205
22. Rane, A., Sjöqvist, F., Orrenius, S. 1973. Drugs and fetal metabolism. *Clin. Pharm. Ther.* 14:666–72
23. Burchell, B. 1974. UDP-Glucuronyltransferase activity towards oestriol in fresh and cultured foetal tissues from man and other species. *J. Steroid Biochem.* 5:261–67
24. Stevenson, I. H., Dutton, G. J. 1962. Glucuronide synthesis in kidney and gastrointestinal tract. *Biochem. J.* 82: 330–40
25. Aitio, A. 1974.UDPglucuronyltransferase activity in various rat tissues. *Int. J. Biochem.* 5:325–30
26. Fuchs, M., Rao, G. S., Rao, M. L., Breuer, H. 1977. Studies on the properties of an enzyme forming the glucuronides of pregnanediol and the pattern of development of steroid glucuronyltransferases in rat liver. *J. Steroid Biochem.* 8:235–41
27. Winsnes, A. 1971. Age and sex dependent variability of the activation characteristics of UDP-glucuronyltransferase *in vitro. Biochem. Pharmacol.* 20: 1249–58
28. Leakey, J. E. A., Donald, A. M. 1976. Activation *versus* induction of foetal enzymes during culture: A problem illustrated by increased uridine diphosphate glucuronic acid-5-hydroxytryptamine glucuronosyltransferase activity through organ culture on rafts and paper strips. *Biochem. Soc. Trans.* 4:1071–72
29. Dutton, G. J., Wishart, G. J., Leakey, J. E. A., Goheer, M. A. 1977. Conjugation with glucuronic acid and other sugars. In *Drug Metabolism from Microbe to Man,* ed. D. V. Parke, R. L. Smith, pp. 71–90. London: Taylor & Francis
30. Wishart, G. J., Goheer, M. A., Leakey, J. E. A., Dutton, G. J. 1977. Precocious development of UDP-glucuronosyltransferase activity during organ culture of foetal rat liver in the presence of glucocorticoids. *Biochem. J.* 166: 249–53
31. Fyffe, J., Dutton, G. J. 1975. Induction of UDPglucose dehydrogenase during development, organ culture, and exposure to phenobarbital. Its relation to levels of UDPglucuronic acid and overall glucuronidation in chicken and mouse. *Biochim. Biophys. Acta* 411: 41–49
32. Zhivkov, V., Tosheva, R., Zhivkova, Y. 1975. Concentration of uridine diphosphate sugars in various types of vertebrates. *Comp. Biochem. Physiol.* 51B: 421–24
33. Krasner, J., Juchau, M. R., Yaffe, S. J. 1973. Postnatal developmental changes in hepatic bilirubin UDP-glucuronyltransferase. Studies on the solubilised enzyme. *Biol. Neonate* 23:381–90
34. Dutton, G. J. 1973. Developmental studies on some enzymes associated with 'detoxication'. *Enzyme* 15:304–17

35. Dutton, G. J. 1975. Control of UDP-glucuronyltransferase activity. *Biochem. Pharmacol.* 24:1835–41
36. Leakey, J. E. A., Wishart, G. J., Dutton, G. J. 1976. Precocious development of UDP-glucuronyltransferase activity in chick embryo liver following administration of adrenocorticotrophic hormone and of certain 11β-hydroxycorticosteroids. *Biochem. J.* 158: 419–26
37. Scherer, G. K. 1975. Tissue interaction in chick liver development: A re-evaluation. II. Parenchymal differentiation: Mesenchymal influence and morphogenetic independence. *Dev. Biol.* 46: 296–08
38. Wishart, G. J., Dutton, G. J. 1975. Precocious development of glucuronidating and hydroxylating enzymes in chick embryos treated with pituitary grafts. *Biochem. J.* 152:325–31
39. Grieninger, G., Granick, S. 1975. Synthesis and differentiation of plasma proteins in cultured embryonic chicken liver cells: A system for study of regulation of protein synthesis. *Proc. Natl. Acad. Sci. USA* 72:5007–11
40. Burchell, B., Dutton, G. J., Nemeth, A. M. 1972. Development of phenobarbital-sensitive control mechanisms for uridine diphosphate glucuronyltransferase activity in chick embryo liver. *J. Cell Biol.* 55:448–56
41. Wishart, G. J., Dutton, G. J. 1975. Release by phenobarbital of the repression of UDP-glucuronyltransferase *in ovo*. *Biochem. Pharmacol.* 24:451–54
42. Fry, D. J., Wishart, G. J. 1976. Apparent induction by phenobarbital of uridine diphosphate glucuronyltransferase activity in nuclear envelopes of embryonic chick liver. *Biochem. Soc. Trans.* 4:265–66
43. Leakey, J. E. A., Dutton, G. J. 1975. Precocious development *in vivo* of UDP-glucuronyltransferase and aniline hydroxylase by corticosteroids and ACTH, using a simple new continuous-flow technique. *Biochem. Biophys. Res. Commun.* 66:250–54
44. Burchell, B., Dutton, G. J. 1975. Delayed induction by phenobarbital of UDPglucuronyltransferase activity towards bilirubin in fetal liver. *Biol. Neonate* 26:122–28
45. Wishart, G. J., Dutton, G. J. 1977. Precocious development *in utero* of certain UDP-glucuronyltransferase activities in rat fetuses exposed to glucocorticoids. *Biochem. Biophys. Res. Commun.* 75:125–29
46. Wishart, G. J., Dutton, G. J. 1977. Regulation of onset of development of UDP-glucuronyltransferase activity towards *o*-aminophenol by glucocorticoids in late foetal rat liver *in utero*. *Biochem. J.* 168:507–11
47. Kamoun, A. 1970. Activité cortico-surrenale au cours de la lactation et du developpement pré- et postnatal chez le rat. I. Concentration et cinétique de disparition de la corticosterone. *J. Physiol. Paris* 62:5–32
48. Jacquot, R. L., Plas, C., Nagel, J. 1973. Two examples of physiological maturation in rat fetal liver. *Enzyme* 15:296–303
49. Simons, J. A. 1972. The effect of growth hormone, thyroxine and testosterone on the ontogeny of bilirubin UDP-glucuronyltransferase in mouse liver. *Biochem. Med.* 6:53–58
50. Catz, C. S., Winick, M., Yaffe, S. J. 1971. The effect of early malnutrition on drug metabolism. *12th Int. Congr. Pediatr. Wien, Osterreich* 29:17–19
51. Schröter, W. 1972. Intracellulärer Transport und Membran des endoplasmatischen Reticulums der Leberzelle: Neue Aspekte in der Genese der transitorischen Neugeborenenbilirubinämie. *Monatsschr. Kinderheilkd.* 120: 119–22
52. Brown, A. K., Zuelzer, W. W. 1958. Studies on the neonatal development of the glucuronide conjugating system. *J. Clin. Invest.* 37:332–40
53. Smith, J. N. 1977. Comparative detoxication of invertebrates. See Ref. 29, pp. 219–32
54. Heirwegh, K. P. M., Blanckaert, N., Fevery, J. 1976. Methods of determination and nature of diazo-positive bile pigments present in body fluids in neonatal jaundice and related conditions. *Birth Defects. Orig. Artic. Ser.* 12:293–306
55. Vaisman, S. L., Lee, K. S., Gartner, L. M. 1976. Xylose, glucose, and glucuronic acid conjugation of bilirubin in the newborn rat. *Pediatr. Res.* 10: 967–71
56. Roy, A. B. 1971. Sulphate-conjugation enzymes. *Handb. Exp. Pharmacol.* 28(2):536–63
57. Dodgson, K., Rose, F. A. 1970. Sulfoconjugation and sulfohydrolysis. See Ref. 11, pp. 239–325
58. Dodgson, K. 1976. Conjugation with sulphate. See Ref. 29, pp. 91–104

59. Wengle, B. 1966. Distribution of some steroid sulphokinases in foetal human tissues. *Acta Endocrinol. Copenhagen* 52:607–18
60. Spencer, B., Raftery, J. 1966. Sulphate activation and sulphotransferase in foetal guinea pigs and in developing hen's eggs. *Biochem. J.* 99:35P
61. Lebeau, M.-C., Baulieu, E.-E. 1973. On the significance of the metabolism of steroid hormone conjugates. See Ref. 11, 3:151–87
62. Yaffe, S. J., Krasner, J., Catz, C. S. 1968. Variations in detoxication enzymes during development. *Ann. NY Acad. Sci.* 151:887–99
63. Cresswell, D. 1975. *Catalytic properties of sulphotransferases.* PhD thesis, Univ. Wales, Cardiff. 79 pp.
64. Weber, W. W. 1973. Acetylation of drugs. See Ref. 11, pp. 249–96
65. Weber, W. W. 1971. Acetylating, deacetylating and amino acid conjugating enzymes. *Handb. Exp. Pharmacol.* 28(2):564–83
66. Fichter, E. G., Curtis, J. A. 1955. Sulfonamide administration in newborn and premature infants. *Am. J. Dis. Child.* 90:596–97
67. Vest, M. F., Rossier, R. 1963. Detoxification in the newborn: The ability of the newborn infant to form conjugates with glucuronic acid, glycine, acetate and glutathione. *Ann. NY Acad. Sci.* 111:183–98
68. Vest, M. F., Salzberg, R. 1965. Conjugation reactions in the newborn infant: The metabolism of *p*-aminobenzoic acid. *Arch. Dis. Child.* 40:97–105
69. Juchau, M. R., Niswander, K. R., Yaffe, S. J. 1968. Drug metabolising systems in homogenates of human immature placentas. *Am. J. Obstet. Gynecol.* 100:348–56
70. Cohen, S. N., Baumgartner, R., Steinberg, M. S., Weber, W. W. 1973. Changes in the physicochemical characteristics of rabbit liver *N*-acetyltransferase during postnatal development. *Biochim. Biophys. Acta* 304:473–81
71. Hirom, P. C., Idle, J. R., Millburn, P. 1977. Comparative aspects of the biosynthesis of excretion of xenobiotic conjugates by non-primate mammals. See Ref. 29, pp. 299–329
72. Levy, G. 1975. Salicylate pharmacokinetics in the human neonate. See Ref. 18, pp. 319–30
73. Brandt, I. K. 1966. Glycine acyltransferase activity in developing rat liver. *Biochem. Pharmacol.* 15:994–95
74. Boyland, E. 1971. Mercapturic acid conjugation. *Handb. Exp. Pharmacol.* 28(2):584–608
75. Chasseaud, L. F. 1976. Conjugation with glutathione and mercapturic acid excretion. In *Glutathione: Metabolism and Function,* ed. I. M. Arias, W. B. Jakoby, pp. 77–114. New York: Raven
76. Grover, P. L. 1977. Conjugations with glutathione. See Ref. 29, pp. 105–22
77. Lambert, G. H., Thorgeirsson, S. S. 1976. Glutathione in the developing mouse liver. I. Developmental curve and depletion after acetaminophen treatment. *Biochem. Pharmacol.* 25: 1777–81
78. Jakoby, W. B., Habig, W. H., Keen, J. H., Ketley, J. N., Pabst, M. J. 1976. Glutathione *S*-transferases: Catalytic aspects. See Ref. 75, pp. 189–211
79. Klaassen, C. D. 1975. Biliary excretion of drugs: Role of ligandin in newborn immaturity and in the action of microsomal enzyme inducers. *J. Pharmacol. Exp. Ther.* 195:311–19
80. Mukhtar, H., Bresnick, E. 1976. Glutathione-S-epoxide transferase during development and the effect of partial hepatectomy. *Cancer Res.* 36:937–40
81. James, M. O., Bend, J. R., Foureman, F. C., Law, F. C., Bend, J. 1977. The perinatal development of epoxide-metabolising activities in liver and extrahepatic organs of guinea pig and rabbit. *Drug Metab. Dispos.* 5:19–28
82. Chasseaud, L. F. 1973. Distribution of enzymes that catalyse reactions of glutathione with α, β-unsaturated compounds. *Biochem. J.* 131:765–69
83. Juchau, M. R., Namkung, M. J. 1974. Studies on the biotransformation of naphthalene-1,2-oxide in fetal and placental tissues of humans and monkeys. *Drug Metab. Dispos.* 2:380–85
84. Arias, I. M., Fleischner, G., Kirsch, R., Mishkin, S., Gatmaitan, Z. 1976. On the structure, regulation and function of ligandin. See Ref. 75, pp. 175–88
85. Hales, B. F., Neims, A. H. 1976. Developmental aspects of glutathione-S-transferase B (ligandin) in rat liver. *Biochem. J.* 160:231–36
86. Foliot, A., Housset, E., Ploussard, J. P., Petite, J. P., Infante, R. 1973. Study of Y and Z, two cytoplasmic-bilirubin binding proteins. Their development in the liver of foetal and newborn Wistar and Gunn rats. *Biomedicine* 19:488–91
87. Gatmaitan, Z., Lewis, S., Turchin, H., Arias, I. M. 1977. Premature development of ligandin (GSH transferase B) in

mice with an inherited defect in endoplasmic reticulum-Golgi structure and function. *Biochem. Biophys. Res. Commun.* 75:337–41
88. Axelrod, J. 1971. Methyltransferase enzymes in the metabolism of physiologically active compounds and drugs. *Handb. Expt. Pharmacol.* 28(2):609–19
89. Mudd, S. H. 1973. Biochemical mechanisms in methyl group transfer. See Ref. 61, pp. 297–350
90. Guldbert, H. C., Marsden, C. A. 1975. Catechol-*O-methyltransferase*: pharmacological aspects and physiological role. *Pharmacol. Rev.* 27:135–206
91. Agathopoulous, A., Nicolopolous, D., Matsaniotis, N., Papadatos, C. 1971. Biochemical changes of catechol-O-methyltransferase during development of human liver. *Pediatrics* 47:125–28
92. Ignarro, L. J., Shideman, F. E. 1968. Catechol-*O*-methyltransferase and monoamine oxidase activities in the heart and liver of the embryonic and developing chick. *J. Pharmacol. Exp. Ther.* 159:29–37
93. Fuller, R. W., Hunt, J. M. 1965. Substrate specificity of phenylethanolamine *N*-methyl transferase. *Biochem. Pharmacol.* 4:1896–97
94. Margolis, F. L., Roffi, J., Jost, A. 1966. Norepinephrine methylation in fetal rat adrenals. *Science* 154:275–76
95. Schöllhammer, I., Poll, D. S., Bickel, M. H. 1975. Liver microsomal beta-glucuronidase and UDP-glucuronyltransferase. *Enzyme* 20:269–76
96. Ecobichon, D. J., Stephens, D. S. 1973. Perinatal development of human blood esterases. *Clin. Pharmacol. Ther.* 14: 41–47
97. Greengard, O. 1977. Enzymic differentiation of human liver: Comparison with the rat model. *Pediatr. Res.* 11: 669–76