PERINATAL PHARMACOLOGY[1] ❖6590

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The perinatal period has been recognized as an identifiable area of pharmacological endeavor by reviews in Volumes 8, 10, and 12 of this series (1–3) and in Volumes 17 and 24 of the *Annual Review of Medicine* (4, 5). These reviews initially identified adverse effects in the fetus and newborn infant; however, as data accumulated, the importance of the influence of the developmental stage of the host upon pharmacologic responses was emphasized.

The purpose of this paper is to discuss drug disposition in the perinatal organism with major emphasis on the human fetus and newborn infant. The specific areas that have been singled out for discussion include placental transfer, distribution of drugs within the feto-placental unit, fetal drug metabolism and absorption, protein binding, metabolism, and excretion in the neonate. Awareness of the differences that exist in the prenatal host in these important parameters may minimize the adverse effects previously seen at this age.

DRUG DISPOSITION IN THE FETUS

Until recently, drugs were rarely administered for the treatment of fetal disorders. However, rapid progress in the diagnosis of such disorders has led an increasing number of physicians to consider the various possibilities for intrauterine treatment with medicinal chemicals (6). Nevertheless, the present lack of knowledge concerning the pharmacologic-toxicologic effects of drugs on the human fetus and newborn infant would greatly hamper the physician in his attempts to evaluate intelligently the risk-benefit ratios of various drug regimens. Of even greater concern is the

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exposure of unborn and newborn children to drugs administered for treatment of the maternal organism rather than the fetus or neonate. In addition, a large number of foreign chemicals of no therapeutic value either to the mother or her child enter into the maternal circulation, and subsequently into the fetal or neonatal circulation (via placental transfer or the maternal milk respectively). Such chemicals may enter unintentionally as a result of contact with an ever-increasing chemical contamination of the environment, including contaminants in food and water, or as a result of intentional drug abuse.

One of the greatest challenges to modern pharmacologists is to increase understanding of the pharmacology of human development to the extent that rational guidelines for risk-benefit ratios during pregnancy might be developed for a wide range of specific chemical agents, particularly therapeutic agents and other foreign chemicals to which pregnant and lactating women are, or may be, frequently exposed. Observations of the effects of drugs on the fetus or offspring represents a necessary empirical approach toward the resolution of these problems; however, research into basic mechanistic aspects will provide a much more satisfactory long-range approach. In particular, studies of the mechanisms regulating the rates of access to and egress from fetal and neonatal pharmacologic-toxicologic receptors seem to be of prominent importance.

Transplacental Drug Movement

Recent studies on the transplacental passage of drugs have begun to question earlier concepts that suggested a direct relationship between the physicochemical properties of drug molecules and their rates of transfer between maternal and fetal circulations (7). Experimental evidence has been provided for the participation of a number of previously neglected factors that may regulate the rate and extent of placental drug transfer. These include the following:

1. Binding of drugs to placental macromolecules. Experiments performed by Tjalve et al (8) tended to indicate that such an effect could occur with nicotine. Even though such drug binding could markedly retard transplacental drug movement, systematic studies of this phenomenon appear to be nonexistent.

2. Active transport of drug molecules from the fetal to maternal circulation. McNay & Dayton (9) administered triamterene, a substituted pteridine diuretic agent, intravenously into the circulation of fetal lambs as well as into the maternal systemic circulation during the last month of pregnancy. Although an active transport mechanism was not verified, the rate of transfer from the fetal to maternal circulation was calculated to be 150 times the rate of transfer from the maternal to fetal circulation. A recent report (10) has provided evidence for the presence of a Mg^{2+}-dependent $Na^+ + K^+$-activated ATPase (ouabain sensitive) as well as a Ca^{2+}-ATPase (ouabain insensitive) in human term placentas. Ouabain, dinitrophenol, and Na^+-free media also reduced the uptake of α-aminoisobutyric acid into placental tissue slices of rabbits and humans (11). These studies are highly interesting from the viewpoint of transplacental drug movement because of the apparent involvement of ATPase enzymes in the active transport of organic ions.

3. Differences in affinity for adult vs fetal blood proteins. Evidence is now plentiful that drugs can bind to fetal or neonatal plasma proteins to different extents and

with different affinity constants as compared with their binding to analogous maternal plasma proteins (12–15). The implications of these phenomena with respect to maternal-fetal drug distribution are obvious.

4. Biotransformation of drugs during transplacental passage. Morgan et al (16) have provided experimental evidence that both placental monoamine oxidase and catechol-O-methyltransferase could influence the rate of transfer of norepinephrine and isoproterenol between the fetal and maternal circulations. It is increasingly recognized that the placenta contains enzymes capable of catalyzing the biotransformation of drug substrates and that such reactions could play a role in the determination of rates of the transplacental movement of certain drugs. This subject has been reviewed recently by Juchau (17).

5. Biotransformation of drugs in fetal tissues. This topic has been reviewed recently by Rane et al (18). Fetal tissues, particularly in primates, contain enzyme systems that can catalyze the biotransformations of a variety of drug substrates. As one example, various studies indicated that newborn infants (and therefore presumably the human fetus at term) could metabolize lidocaine quite rapidly and that, as might be expected, the maternal fetal lidocaine plasma concentration ratio was well above unity, even following repeated administrations of the drug (19, 20).

6. The ability of various vasoactive drugs to exert effects on the utero-placental-umbilical vascular systems that in turn may result in changes in placental perfusion. This topic also has been the subject of a recent review (21). Since rates of placental drug exchange are strongly related to rates of placental perfusion, pronounced effects on drug transfer rates could be expected with vasoactive drugs. Thus, by virtue of the drug's own pharmacologic effect, its rate of transfer from maternal to fetal circulations may be disproportional to its physicochemical properties. Although many drugs are known to exhibit profound effects on this vascular system, no specific examples of such an effect on drug transfer have been reported. Mirkin, however, has alluded to such a phenomenon in a recent review (22).

In addition to the above considerations, Oh & Mirkin (23) found that, whereas certain drugs that are nearly 100% ionized at pH 7.4 (eg. salicylates) would pass into brain tissues with considerable difficulty, their rates of transplacental passage were quite rapid. Even bis-quaternary ammonium compounds are capable of entering the fetal circulation (24), albeit at extremely slow rates. Thus it seems imperative that the question once commonly posed as to whether a given drug would enter the fetal circulation must now be replaced by the questions, "At what rate? What is the extent and time course of its accumulation on the fetal side? What is the relative exposure and distribution of the drug in the fetus vs mother?" It also seems apparent that the many factors governing the kinetics of drug disposition in utero now require a systematic evaluation.

Drug Distribution In Utero

Rapid advances in the study of fetal drug distribution have been made with the aid of autoradiographic techniques. Studies with whole body autoradiography have led Ullberg (25) to the conclusion that the more "unphysiological" a compound is, the more is its fetal-maternal distribution determined by purely physicochemical properties, whereas the distribution of "physiological" substances such as vitamins,

hormones, amino acids, minerals, etc are strongly influenced by specific mechanisms including active transport, facilitated diffusion, specific binding to endogenous macromolecules, etc. Ullberg also pointed out that even though many drugs cross the placental barrier more readily than they cross the blood-brain barrier, some drugs, such as certain tertiary amines, accumulate much more readily in the brain than in fetal tissues. Ullberg also presented additional evidence to indicate that various amino acid analogs may be actively transported by the placenta from the maternal to the fetal circulation.

The intrauterine distribution of a large number of specific chemical agents have been studied in experimental animals (for recent reviews see 22, 25) with whole body autoradiographic techniques that allow comparisons of the distribution of drugs in fetal vs maternal tissues. These studies have indicated that the relative distribution of most drugs in fetal organs tend to parallel that observed in the corresponding maternal organs except that the maternal liver appears to be much more capable than the fetal liver of sequestering drugs. As expected, organs with high lipid content, such as the liver, lungs, intestines, and adrenal glands, tend to concentrate fat-soluble drugs. It has frequently been found that drugs will accumulate selectively and strongly in a single type of fetal tissue. Tetracycline accumulation in the fetal skeleton, thiouracil in the fetal thyroid, chloroquine and chlorpromazine in the pigment of the fetal eye, and diphenylhydantoin and progesterone in the fetal adrenal gland (22, 25) represent examples of the phenomenon. It was found that Vitamin B_{12} accumulated more than a hundredfold in the fetus if a low dose was administered.

A recent study of the maternal-fetal distribution of trans-Δ^9-tetrahydrocannabinol (THC) indicated that even though THC distributed to the fetus in only very low concentrations, the drug exhibited a definite affinity for the fetal central nervous system (26). Although it is now apparent that certain drugs distribute in fetal tissues with fairly uniform pattern and others distribute with a more selective affinity for given organs or tissues, sufficient data are not yet available to allow broad generalizations or predictions of fetal distribution from a knowledge of the chemical structure of the drug molecule.

Studies of the passage of drugs from the maternal circulation to the uterine fluid and subsequently into the preimplantation blastocyst also have been reviewed recently (27, 28). A large number of drugs and chemicals now are known to penetrate the blastocyst and alter or arrest the normal developmental process. Particularly interesting were some of the studies by Fabro (28) which indicated that the pregnant state could modify the degree to which drugs pass from the maternal circulation into the uterine fluid. In nonpregnant rabbits the uterine fluid/plasma radioactivity ratio ranged between 0.78 and 1.75 following the administration of ^{3}H-nicotine. In 6 day pregnant does, however, there was 5–10 times more radioactivity in the uterine fluid than in the plasma. This increase in accumulation also could be effected by treatment of nonpregnant does either with human chorionic gonadotrophin or progesterone. Cotinine, a more polar metabolite of nicotine, did not appear to pass into the uterine fluid as readily as unchanged nicotine. Thus, it would seem possible that active transport of certain drugs from the maternal circulation into the uterine fluid

can occur and that such processes could be influenced markedly by the hormonal status of the host. Concentrations of drugs in human Fallopian tubular fluid have not been measured. They are urgently required because of their clinical importance.

Fetal (and Neonatal) Drug Biotransformation

Chemical alterations of drugs and other foreign compounds in tissues of the fetus and placenta have received increasing attention during the past few years. A significant stimulus for this research was the recent observation of Yaffe et al (29) that human fetal liver microsomes contained necessary electron transport components (NADPH-specific cytochrome *c* reductase and cytochrome P-450) for drug hydroxylation reactions. These investigators were able to demonstrate significant rates of mixed-function oxidation of endogenous substrates such as testosterone or laurate, but variable results were obtained with drug substrates. Positive results were later obtained for the oxidative N-demethylation of desmethyl imipramine and ethyl morphine and *p*-hydroxylation of aniline in human fetal liver preparations in vitro (18, 30, 31).

These findings were somewhat surprising in view of the inability of a large number of investigators to detect significant quantities of cytochrome P-450 or P-450-dependent drug hydroxylation reactions in liver microsomes from fetuses of a wide variety of animal species, even at comparatively late stages of gestation (32–39). The possibility remains, however, that such phenomena do not represent a genetically determined species difference but rather a difference in the extent of exposure of humans vs experimental animals to chemicals that can induce or stimulate these systems in the fetus. The bulk of the currently available evidence, nevertheless, does not support this idea.

Prior to the report of Yaffe et al (29) other investigators had reported the oxidative biotransformation of several drug substrates in post mitochrondrial supernatant fractions of human fetal liver homogenates (40) but were unable to detect cytochrome P-450 in the microsomal fractions (41). This possibly was due to the fact that the human fetal liver homogenates were centrifuged at 12,000 X g for 20 min prior to the sedimentation of the microsomal fraction at 100,000 X g. From the experiments of Ackermann et al (42) and Chatterjee et al (43) it would be expected that recoveries of endoplasmic reticulum elements in those microsomal pellets would be very low and that detection of cytochrome P-450 therefore would be quite difficult. Electron microscopic studies of various human fetal hepatic homogenate subfractions revealed (42) that the endoplasmic reticulum appeared to be converted partly to long, slender cisternae and partly to uniform microsomes during homogenization. This led to a substantial loss of microsomal marker enzyme activity into the low speed subfractions. Later, Pelkonen & Karki (44) confirmed the presence of significant quantities of cytochrome P-450 in human fetal liver microsomal fractions.

It should be pointed out that various investigators also have reported negative results with respect to the mixed-function oxidation of several xenobiotic substrates in human fetal liver preparations: N-demethylation of N-monomethyl *p*-nitroaniline (45), O-demethylation of *p*-nitroanisole (45), N-demethylation of aminopyrine

(46), and hydroxylation of 3,4-benzpyrene (29, 31). Several subsequent investigations, however, indicated that hydroxylation of 3,4-benzpyrene would be catalyzed in human fetal hepatic tissues as well as a large number of other human fetal tissues at several stages of gestation (46, 49). Specific activities were one to two orders of magnitude lower as compared with analogous preparations of rat liver microsomes under similar reaction conditions. [Transplacental induction of the fetal benzpyrene hydroxylating system did not appear to occur readily, as judged both from animal experiments (50, 51) as well as from studies in humans (46–49, 52)]. Thus far, positive results have been reported for the N-demethylation of aminopyrine (29), hydroxylation of 3,4-benzpyrene (46, 49), N-demethylations of ethylmorphine (18, 31), and desmethylimipramine (53) and *p*-hydroxylation of aniline (31, 46). Again, enzyme induction in certain cases could account for some of the apparently conflicting data. Another explanation may be found in methodological differences when using indirect measurements of activity.

The human fetal adrenal gland also has attracted attention as a site of drug biotransformation. Following the observation that this organ appeared to be much more active than other human fetal tissues with respect to aromatic nitro group reduction (54), Juchau & Pedersen (46) showed that microsomal fractions of fetal adrenal homogenates contained exceptionally high concentrations of cytochrome P-450 and were active with respect to catalysis of hydroxylations of 3,4-benzpyrene and aniline as well as the reductions of aromatic nitro groups and azo linkages. Specific concentrations of cytochrome P-450 as well as specific activities of the drug metabolic reactions were considerably higher than those observed in the fetal liver under the reaction conditions used. A very similar pattern of fetal drug biotransformation was observed in a subhuman primate species. In spite of these observations, however, significant catalysis of the N-demethylation of aminopyrine or the $\omega(\omega-1)$ oxidation of laurate could not be detected in the human fetal adrenal gland (55). Such studies are of considerable interest because of the comparatively very large size of the human fetal adrenal gland and the extremely important role it plays in steroid biosynthesis and biotransformation during fetal development. The similarities between drug and steroid hydroxylation reactions suggest that drugs could act as alternative substrates for at least some of the many steroid hydroxylases present in that organ.

The first fetal tissue to be encountered by drugs circulating in the maternal plasma of humans is the syncitial trophoblast of the placenta. It is possibly for that reason that several investigators have studied drug biotransformation reactions in various preparations of human placental tissues in recent years. Evidence has accumulated that the placenta is capable of catalyzing the biotransformation of various types of drug substrates (55). The types of reactions catalyzed indicate that this tissue exhibits a much higher degree of substrate specificity than the extremely versatile hepatic tissues. Most of the reactions also appear to occur at considerably slower rates, although hydroxylation of 3,4-benzpyrene can occur at rates approaching those observed in analogous rat liver preparations (56). This appears to occur in genetically susceptible individuals who have been exposed to relatively large quantities of polycyclic aromatic hydrocarbons present in the environment, particularly

in tobacco smoke (52, 57–59). Pretreatment of experimental animals with such hydrocarbons markedly increases rates of placental hydroxylation of 3,4-benzpyrene and a limited number of other xenobiotic substrates, presumably via induction of the components of the hydroxylase systems in a large number of tissues. Studies on the placental hydroxylase have indicated that induction of the enzyme system in this organ occurs much more readily near term than during the early stages of gestation. This appears to apply to humans (52, 59) as well as to experimental animals (60, 61). The higher growth rate of the placenta during early gestation may be responsible for such observations, for many studies have shown that drug hydroxylation reactions tend to occur at very slow rates in such rapidly growing tissues as regenerating liver, fetal and neonatal liver, and liver tumors. However, hepatic drug hydroxylating enzymes appear to be more responsive to enzyme induction in younger animals (38).

As compared with drug oxidation-reduction reactions, the study of conjugation reactions in fetal tissues have received only slight attention. Earlier reports (62, 63) indicated that the human fetal kidney was more active than the fetal liver with respect to the catalysis of glucuronide formation using O-aminophenol or 4-methylumbelliferone as substrates. The activities observed were, however, very low as compared with human adult liver. Recent studies, however, have reported negative results with respect to the capacity of the human fetal liver to exhibit significant glucuronyl transferase activity during early gestation (18, 64). 4-Methylumbelliferone, α-naphthol, and p-nitrophenol were tested as aglycone acceptors.

It has been demonstrated that pre-exposure to certain drugs, notably phenobarbital, can enhance glucuronyl transferase activity in fetal livers near term (65–67) and by this mechanism contribute to an observed decrease in jaundice by increasing the rate of bilirubin glucuronidation. Other effects of phenobarbital such as an increase in bile flow and increase in anion acceptor protein also play a role. Recently, evidence that heroin addiction may exert a similar effect in humans has appeared in the literature (68).

The fetal liver and adrenal glands are extremely active with respect to the catalysis of sulfate transfer and, although no specific examples have been given, sulfurylation of foreign phenolic compounds (e.g. morphine, diethylstilbesterol, etc) could be an important factor in fetal drug distribution as well as in transplacental drug movement and modification of drug actions on the fetus. The highly active sulfatase enzymes present in the placenta also may be significant in this regard.

Some preliminary investigations have indicated that human fetal tissues may catalyze various other drug conjugation reactions including acetylation and glycine conjugation (69). Uher (70) was able to demonstrate significant rates of acetylation of sulfamethoxypyrimidine in cultures of human trophoblast.

Investigators also have continued to search for an explanation for the low rates of mixed-function oxidation and glucuronidation observed with xenobiotic substrates in fetal or neonatal hepatic tissues. Fouts (71) noted that most studies have indicated that hepatic parenchymal cells are the principal sites of most drug hydroxylation reactions and that the proportion of parenchymal cells to reticuloendothelial cells is quite low in fetal livers. In addition, the studies of Leskes et al (72,

73) indicated that the smooth-surfaced endoplasmic reticulum (SER) was essentially absent before birth and developed asynchronously in hepatocytes after birth. Koga, however, was able to show the presence of SER as early as 6 weeks of fetal age in human hepatocytes (74), an observation that coincides with the reported capacity of human fetal livers to catalyze mixed-function oxidation of drug substrates as measurable rates during early gestation.

Feuer & Liscio (75) postulated that the low levels of drug hydroxylating activity observed in newborn animals was due to the presence of relatively high levels of maternal inhibitory factors (female gonadal hormones) in the fetal and neonatal circulation. This postulate was based on the following observations: 1. Pregnant female rats exhibited longer hexobarbital sleeping times than nonpregnant females. 2. Early weaning accelerated the development of drug metabolic activities in rats. 3. There was a rapid increase in drug metabolic activity postpartum. Although such studies suggest that female sex hormones inhibit drug metabolic activity (several previous studies, in vitro and in vivo have shown this to be the case) they do not indicate to what extent the gonadal hormones contribute to the low activities observed. However, certain metabolites of progesterone were shown to be extremely potent inhibitors of drug biotransformation in vitro (76). The fact that inducing agents can markedly increase rates of drug hydroxylation reactions in tissue culture systems but appear to be much less effective in intact pregnant animals (or humans) also leads one to suspect that maternal factors may be responsible.

Because the hepatic content of cytochrome P-450 and the capacity of fetal and neonatal livers to catalyze drug hydroxylation seem to increase in parallel during development, Woods & Dixon (77) investigated aspects of the hepatic capacity of prenatal rats, rabbits, and guinea pigs to synthesize hemoproteins. They found that the activity of the enzyme that catalyzes the rate limiting step of heme biosynthesis (δ-aminolevulinic acid synthetase) was four to eight times higher in the livers of prenatal animals than in those of corresponding adult animals. The fetal enzyme, however, appeared to be much less susceptible to induction or repression, and it was postulated that this resistance to regulatory control might in some way account for the decreased cytochrome P-450 concentrations and drug hydroxylating activity observed in immature hepatic cells.

A number of other factors have been proposed to explain the low rates of mixed-function oxidation, reduction, and glucuronidation of xenobiotic substrates in the fetus and neonate. These include the following: 1. Inability of inducing agents to reach fetal hepatic tissues in quantities sufficient to yield adequate induction due to clearance of the inducer by other maternal, placental, or fetal tissues (46). 2. Inability of inducing agents to bind to critical proteins in fetal hepatic tissues (71). 3. Presence of repressors (or possibly other inhibitors) in the fetal livers (78). 4. The influence of growth hormone (STH) on rapidly growing tissues (79). Various aspects of these possibilities have been discussed in recent reviews (71, 80). It would seem likely that none of the above items can be viewed as the sole explanation but that each may act as a single contributing factor in decreased microsomal drug biotransformation in the prenatal and perinatal periods.

DRUG DISPOSITION IN THE NEONATE

During intrauterine existence the fetus has at its disposal the metabolic and excretory capabilities of the maternal organism. Once the umbilical cord is severed the neonate must use his own mechanisms to distribute and then eliminate xenobiotic substances. In contrast to the fetus, drug concentration at receptors is governed by the traditional process of absorption, distribution, metabolism, and excretion. These will be discussed in the following sections, although it must not be forgotten that drugs may also be present in the neonate as a consequence of medications administered to the mother prior to or during delivery.

Absorption

The gastrointestinal tract of the neonate undergoes a marked change in function shortly after birth, e.g. transit time is prolonged, gastric pH decreased, and permeability increased. In addition, the gastrointestinal tract represents a relatively larger portion of the body in the newborn than in later life. Therefore, it is not surprising that the bioavailability of drugs given orally differs from that seen in the adult organism. One of the first investigations of drug absorption in the newborn infant demonstrated that triple sulfa-suspension (mixture of equal weights of sulfadizaine, sulfamerazine, and sulfamethazine) was absorbed less well in the low birthweight (premature) infant than in the full term infant (81). The hypoglycemic effect of orally administered insulin in newborn infants was observed only during the first few minutes after birth. Insulin is absorbed intact at this time. After several hours, gastric pH decreases and hydrolyzes the insulin in the gastrointestinal lumen (82). The absorption of riboflavin is found to be much lower in the neonate than in the older infant (83). The same percentage of dose was absorbed. The absorptive process lasted 16 hr in the newborn in contrast to only 3–4 hr in the older organism. The authors hypothesize that the specialized intestinal transport process for riboflavin is much less active in the newborn and that the slow absorption is accounted for by passive diffusion over a much longer segment of the gastrointestinal tract. Prolonged transit time mentioned above would also support this view. Another possibility is that because riboflavin was administered as a phosphate salt, the phosphatase-mediated conversion to riboflavin in the intestine was rate limiting in the neonate and delayed the absorption.

No systematic studies of the absorption of pharmacologic agents in newborn infant have been conducted. The available data deals mainly with the absorption of antibiotics because these are used so frequently in the newborn infant. When the intramuscular route has been compared with the oral route, higher serum concentrations as anticipated have been achieved following parenteral administration. O'Connor et al (84) compared serum levels of sodium nafcillin achieved after oral administration in newborn infants and adults. A significantly higher concentration was reached in the newborn. The area under the serum concentration curve was five times greater in the newborn than in adults given equivalent doses on the basis of body weight. While the more prolonged serum concentrations are undoubtedly due

to decreased renal excretion, there is no question that the absorptive process from the GI tract is functioning at a greater rate. Silverio & Poole (85) have recently compared ampicillin absorption in newborn infants and adults. The results are in agreement with those found with nafcillin with the area under the curve approximately three times greater in the newborn infant. They also contrasted two dosage forms, anhydrous and trihydrate, and found that the anhydrous was absorbed to a greater extent than the trihydrate in a ratio similar to that seen in adults. Thus, the ionized form of the penicillin, monobasic nafcillin, or amphoteric ampicillin, does not appear to affect its absorption in the neonate. Jusko (86) has analyzed, kinetically, data available in the literature and has contrasted the availability of ampicillin in the newborn infant when administered by mouth or by intramuscular injection. About two thirds of the oral dose was absorbed in the newborn, and this can be compared with about 30% availability of ampicillin from capsules in adults. A new antimycotic agent, chlortrimazole, has been found to be well absorbed in neonates including premature infants with serum concentrations achieved sufficient to effect systemic Candida infections (87).

Digoxin is another drug frequently used in the newborn. When absorption by oral and intramuscular routes was compared after the administration of labeled digoxin (88) to infants with severe congenital cardiac malformations who required digitalization, absorption was a rapid and in the same range as that seen in adults. Digoxin could be detected in blood 5 min after oral administration, reaching a peak concentration in 1–3 hr. The intramuscular route delivered the drug into the circulation 1 min after injection, and peak levels were achieved 15–30 min later.

The parenteral route is also used frequently to administer drugs in the sick neonate. In general, drugs injected into skeletal muscles or subcutaneous tissues are absorbed rapidly. Comparative studies between these two sites in newborn infants are not available and are needed because, under hypoxic conditions, newborns may selectively curtail circulation in muscles and skin, hindering absorption of drugs given by these two routes. Kupferberg & Way (89) found no difference in the rate of disappearance of morphine from its injection site after subcutaneous administration of the alkaloid in a dosage of 50 mg/kg to 16 and 32 day old rats. These investigators directly analyzed the amount of free morphine remaining at the injection site (right hind limb) at various time intervals following administration. Mention should be made of absorption via the skin. While no studies have been carried out, the toxicity of hexachlorphene when used in the routine bathing of newborn infants has recently attracted considerable attention in the United States at large. Consideration of the histology of the infants' skin in contrast to the adults' clearly indicates less impediment to absorption, particularly of lipophilic compounds. The tragic results with hexachlorophene are therefore not surprising.

Recently Kandall et al (90) measured the activities of the enzymes β-glucuronidase and UDP-glucuronal transferase in the gastrointestinal tract during the perinatal period. Both of these enzymes are concerned with the deconjugation of bilirubin. Activity of β-glucuronidase before birth was relatively high while transferase activity was barely detectable. During the first 4 days after birth, β-glucuronidase reached a level nearly seven times that of adult activity, whereas glucuronyl trans-

ferase activity approximated adult activity. β-Glucuronidase activity in the perinatal period was predominantly located in lysosomes in contrast to a microsomal location later in development. The authors speculate that the excess capacity of the intestine for deconjugation of bilirubin, over that of conjugation, favors the active reabsorption of bilirubin and therefore contributes significantly to the hyperbilirubinemia seen in the neonate. While this relationship remains to be more clearly demonstrated by kinetic studies, the data do serve to exemplify the changing biochemistry of the developing gastrointestinal tract and the potential role that this may have in drug absorption.

Distribution and Drug Plasma Protein Binding

Differences in the distribution of pharmacologic agents in the neonate as compared with the adult may be attributable to variations in membrane permeability or in protein binding. Changes in body composition in the young subject may also result in quantitative alterations in drug distribution. Body water content is higher in the newborn than in the adult and in the newborn infant varies from 85% of body weight in small prematures to 70% in the full-term infant. Fat content is likewise altered with a marked decrease being found in the infant born prematurely. These variations in body composition were associated with increases in the volume of distribution of sulfonamides and bromsulphalein (91). Several studies have shown a differential permeability from blood to brain as a function of the age of the host. This is of particular importance for psychopharmacologic agents whose action in the newborn may also be modified because of differences in brain receptors at this age. The distributional aspects have been reviewed previously (4). Preliminary experiments in our laboratories have shown that the malnourished preweanling rodent is more susceptible to the action of hexobarbital, presumably because of variations in distribution and binding to brain receptors.

The plasma protein binding of diphenylhydantoin has been studied in mixed cord plasma by means of an ultrafiltration technique using carbon-14 labeled diphenylhydantoin (92) at concentrations of 16 mμg/ml (mean therapeutic concentration). The unbound fraction of diphenylhydantoin in 13 normal infants was 10.6 ± 1.4%. The corresponding value in adult plasma was 7.4 ± 0.7%. In addition to the lower degree of binding noted in cord plasma, the range of values in individual samples was much greater than in adult plasma. Binding of diphenylhydantoin was also investigated in 20 hyperbilirubinemic infants in whom the concentration of total bilirubin (mainly unconjugated) varied from 4.5–24.5 mg/ml of serum. There was a definite correlation between the size of the unbound fraction of diphenylhydantoin and the total concentration of bilirubin. At concentrations of bilirubin greater than 20 mg/100 ml, the unbound fraction of diphenylhydantoin was twice as high as in plasma from nonhyperbilirubinemic infants. Correlation became even greater when the bilirubin/albumin ratio was plotted against the percentage of unbound diphenylhydantoin. This strengthens the hypothesis that diphenylhydantion and bilirubin may compete for the same binding site on the albumin molecule. This difference in binding may account for the greater adverse effects often seen in the newborns when

diphenylhydantoin is administered, even though the dose is reduced to take the size of the patient into consideration.

The binding of salicylate to albumin fractionated from pooled neonatal cord serum was found to be quite different from that seen with adult serum (93). The apparent association constant of $1.7 \times 10^5 M^{-1}$ in the infant was one third of the adult value of $4.0 \times 10^5 M^{-1}$. Nafcillin binding to pooled cord serum was also studied by the same investigators using the technique of equilibrium dialysis. The percentage of the drug bound increased from 16% at a low antibiotic concentration (5 μg/ml) up to 28% and 200 μg/ml. This represents a considerable difference from adult values reported in the literature (94) of 86% of the antibiotic bound. Furthermore, examination of the Scatchard plots of nafcillin binding in newborn cord serum suggested that binding occurred to some protein fraction other than albumin. Electrophoresis on acetate membranes of cord serum incubated with nafcillin and subsequent biological assay of each band, showed that α-1-globulin had a greater affinity for nafcillin than albumin. This contrasts with the reported binding to adult serum for penicillins (94).

Many drugs have been suspected of displacing bilirubin from its binding to albumin. This has serious clinical consequences (neurotoxicity) in the newborn infant whose capacity to metabolize bilirubin is limited and whose blood-brain barrier is more permeable than in the adult. The physicochemical properties of bilirubin have hampered examination of its binding to albumin in aqueous systems. Krasner (95) was able to circumvent this problem by using dimethylsulfoxide as the solvent. Association constants for the interaction of bilirubin with albumin were obtained at varying dimethylsulfoxide concentrations, and these were plotted as a function of the concentration of the solvent. This resultant curve was then extrapolated from 10% dimethylsulfoxide to 0% to obtain the apparent association constant for the binding of bilirubin to albumin. Albumin purified from cord serum appeared to bind bilirubin with a greater affinity than adult albumin with apparent k values of $5.2 \times 10^7 M^{-1}$ and $2.4 \times 10^7 M^{-1}$ respectively. Because large quantities of serum were required in the equilibrium dialysis method, the same author developed a fluorometric assay that measured the direct interaction of bilirubin with albumin in microliter quantities (96). He was able to measure bilirubin binding capacity and to determine whether or not other pharmacologic agents could displace bilirubin from its binding to albumin. Sodium salicylate displaced bilirubin from its binding to albumin at high concentrations (200 mg/100 ml), but at usual therapeutic concentrations of 20 mg/100 ml it was without effect. Sodium benzoate at high concentrations (144 mg/100 ml) caused approximately 30% reduction of bilirubin binding capacity, but lower concentrations were without effect. This is in contras to the recently described phenomenon in which two drugs, caffeine and injectibl diazepam, commonly used in the treatment of newborn infants, were shown displace bilirubin from its binding to albumin (97). By using a combination of filtration and bilirubin spectral curves to assess the displacement, it was shown t the responsible agent was the added preservative sodium benzoate. The discrepa between investigators is probably due to methodologies.

These data regarding drug-protein interaction in newborn serum clearly de strate that plasma protein binding differs in the newborn infant from that s

the adult. Should these observations be operative in vivo, they would explain in part why drugs administered to the newborn often are associated with side effects, even though the dose used takes into consideration the smaller size of the patient. The changes in binding may be due to lower concentrations of plasma proteins (particularly albumin) seen in the newborn infant. However, the methods employed in the formulation of the Scatchard plot normalize for the amount of protein present. It is more likely that the binding characteristics reported by these several investigators are due to variations in the neonatal plasma proteins themselves. In addition, endogenous substances during the first few days of life, especially hormones transferred across the placenta and/or fatty acids, may occupy binding sites and thus reduce binding capacity.

Metabolism

In general, studies of drug metabolism in perinatal organisms of many species have shown low activity. During the postnatal and suckling period, drug metabolic capability increases quite rapidly and reaches maximum activity at varied ages according to the pathway being studied. Some pathways, for example glucuronidation and sulfation, reach peak values during the perinatal period which are much higher than later in life. Details regarding in vitro studies have been discussed in previous reviews (1–5). However, exceptions to the general concept that all newborn animals have low drug metabolizing capacity can be found for reduction (98) and sulfation (99). Both reactions are well developed at birth with in vitro activites in the adult range. One of the major questions which immediately arises concerns the regulation of drug metabolic activity and what initiates the increase that is noted soon after birth. This has been discussed in some detail in the previous section under drug metabolism in the fetus. Weaning appears to affect drug metabolism, which suggests that the maternal organism plays a role in limiting drug metabolic activity. Rats weaned early, and therefore removed from the influence of their mother, had a high 4-methylcoumarin hydroxylase activity than their unweaned counterparts. However, prolongation of weaning did not affect the development of aminopyrine demethylation (100). We have found similar results with hexobarbital oxidation in the mouse, namely a marked increase in in vitro activity and concomitant decrease in sleeping time associated with early weaning. In contrast to Henderson's findings (100), prolongation of weaning from 21–28 days delayed the increase in activity of hexobarbital oxidase (101). No explanation for this discrepancy other than species specificity is available. The effect of weaning may be mediated by hormones because profound hormonal changes take place at birth and at weaning. This hypothesis has received considerable attention with emphasis on progesterone and growth hormone. These aspects are discussed in detail in our previous review (5) and earlier in this article. It is also possible that separation from the mother via some neuroendocrine mechanism can trigger drug metabolic enzyme activity. Other environmental influences such as food, temperature, and space should also be considered, as these undergo marked changes at weaning.

In contrast to studies in the human fetus, only one in vitro study has been reported in newborn infants (102). NADPH cytochrome *c* reductase activity and cytochrome

b_5 content were found to be similar to those of adult rats in hepatic tissue obtained postmortum from one premature and one full-term newborn infant.

A number of in vivo studies of drug metabolism in newborn infants have recently been reported. The development of sensitive analytic techniques such as mass fragmentography (combination of mass spectroscopy with gas chromotography) has permitted identification of metabolites in small biologic samples. The urine of newborn infants whose mothers received therapeutic doses of chlorpromazine, pethidine, and promazine during labor contained conjugated metabolites other than those normally excreted in adults (103). This was considered an indication of a different metabolic pathway in the newborn infant. No kinetic studies were performed. A proglonged half-life of nortryptiline was found in a newborn infant whose mother took a suicidal overdose of the drug one day prior to delivery (104). Newborns of mothers taking diphenylhydantoin as an anticonvulsant were shown to excrete the drug in a significant amount only on the third day of life. This gave a half-life of 60 hr compared with 12 hr in the adult (105). It should be pointed out that this estimation of half-life is based upon a smaller number of samples. In contrast to this, it was surprising to find lower concentrations of diphenylhydantoin in the plasma of neonates given the drug for convulsive disorders than in adults (106). Both groups received the same relative dose per unit of body weight. It is conceivable that chronic administration has led to induction of hydroxlyase activity in the newborn infant. More specific investigations of this phenomenon, including measurement of hydroxylated derivatives in urine, are necessary before this decrepancy with the results reported following transplacental passage can be resolved. Analysis of the urinary metabolites following administration of digoxin to the newborn revealed a greater proportion of unchanged glycoside as compared with the older child. This was considered evidence for decreased metabolic activity in the newborn (88). The frequent administration of ethanol to women during labor has been used to determine its elimination in the infant who received the drug via transplacental passage (107). The half-life was twice as long in the newborn as in the mother.

Conjugating activity has been studied extensively because of the frequent occurrence of jaundice in the newborn infant and the demonstration, many years ago in the guinea pig, that the enzymes in the last two steps of glucuronide formation, glucuronyl transferase, and UDPG dehydrogenase, were deficient in activity when tested in vitro (108). Bilirubin conjugation, itself, was analyzed in great detail following its intravenous administration (109). The plasma half-life in infants up to 30 days of age was significantly longer than in children 4 months to 14 years of age. While this prolongation of half-life was ascribed to a diminished capacity to eliminate the bilirubin via glucuronidation, it should be emphasized that elimination also involves hepatic uptake and secretion into bile. Both of these processes have also been shown to be limited in capacity in the young animal. The plasma half-life of sulfobromophthalein, which is conjugated with different amino acids and glutathione, was studied in a large number of healthy full-terms and premature, newborn infants as well as in older children. Half-lives were twice as long during the neonatal period as in the older infants and children. Half-lives were longer in age, although these differences disappeared by four months of age. It was concluded from a comparison of the developmental course that exogenous factors were more impor-

tant than endogenous ones for maturation of the process (110). The reason for the delayed elimination of sulfobromophthalein in the newborn is unknown, although it has been suggested (111) that secretion of the test substance into bile is insufficient. There might be other contributing factors such as low concentration of hepatic carrier protein. *p*-Aminobenzoic acid, which is coupled to glucuronic acid, and glycine and N-acetyl-*p*-aminophenol, which is glucuronidated before excretion, were both eliminated more slowly in newborn and premature infants (112, 113). Besides the finding that the rate of disappearance of *p*-aminobenzoic acid increased with age, there was a qualitative difference in the pattern of metabolites between newborn infants and older children. The former excreted *p*-aminobenzoic acid mainly in the form of acetyl-*p*-aminobenzoic acid, while the latter formed mainly glycine conjugates (*p*-aminohippuric acid). In contrast to these findings the ability to acetylate sulfadiazine was found to be decreased in young children (114).

Oxidative capacity has been studied using several drugs and has generally been shown to be deficient in the neonate. When acetanalid was given to 10 newborn infants, the peak concentration of the oxidative product, paraaminophenol, appeared later than in older children (113). Tolbutamide administered orally or intravenously to 10 normal full-term infants had a prolonged plasma retention during the first two days of life (115). The plasma disappearance of the drug showed an inverse correlation with the appearance of the oxidized metabolite, carboxytolbutamide, in the urine. Further evidence for a decrease in oxidative ability was gathered when aminopyrine half-lives were measured on the first and eighth days of life in 15 normal full-term infants (116). There was a successive increase with age of aminopyrine elimination rate from plasma. The metabolism of diazepam was studied in premature, newborn infants and older children, following intramuscular administration of the drug for the management of convulsive disorders (117). Premature and newborn infants had higher and longer lasting concentrations of the drug in plasma as contrasted with older children. N-Demethyldiazepam was formed in the young infant and could be detected in the blood and urine 4 hr after administration of the parent drug. However, conjugated forms of the oxidized derivatives, N-methyloxazepam and oxazepam, could be found only in children, suggesting that the young infant cannot oxidize the drug.

More precise and quantitative investigations of the ability of human newborn infants to handle drugs are needed. For example, we assessed glucuronide formation in 14 newborn infants by administering salicylamide orally in a single dose of 20 mg/kg of body weight and determining the amount of salicylamide glucuronide in the urine. This showed an extemely wide variation among the 14 infants in whom this parameter was investigated on the fifth day of life (118). The variation ranged from as high as 45% of the dose excreted as the glucuronide (normal for adults) to as little as 8% of the dose. Furthermore, there was an inverse relationship between the serum indirect bilirubin concentration on the fifth day of life and the urinary percentage of the dose of salicylamide appearing in the urine as a glucuronide. This five- to sixfold variation in glucuronide forming capacity is striking and suggests that interplay of environmental and genetic factors are responsible.

A wide variety of drugs and environmental chemicals are known that can induce the synthesis or inhibit the activity of drug metabolizing enzymes. This subject has been extensively reviewed in 1967 (119) and again in 1973 (120). It seemed logical to determine whether this mechanism could be used to alter the low microsomal enzyme activity in the newborn. Inscoe & Axelrod (121) demonstrated that benzpyrene injected into newborn rats caused a significant increase in hepatic glucuronyl transferase activity (with ortho-aminophenol as aglycone acceptor) when compared with untreated litter mates. No increase occurred in the newborn following administration to the pregnant female just prior to term, although activity was enhanced in the mother. We were able to demonstrate a two- to threefold increase in bilirubin glucuronide conjugating activity in the newborn mouse after pretreatment of the pregnant female with sodium barbital for 4–6 days prior to delivery (122). Striking increases in the activity of this enzyme were produced on the fourth day following administration of the barbiturate directly to the newborn mouse for the first 3 days after birth. Both oxidative and reductive pathways were increased following pretreatment of newborn rabbits with phenobarbital for 3–4 days (123). The extent of enhancement of enzyme activity was not uniform and varied with the drug metabolic pathway being studied. Induction also occurred following phenobarbital administration to the pregnant doe at term, but of great importance was the observation that there was no increase in enzyme activity when treatment occurred earlier in gestation (1–2 weeks prior to term). This implied the absence of a responsive enzyme synthesizing system. Exposure of newborn animals to phenobarbital excreted into breast milk is sufficient to induce an increase in hepatic drug metabolism (124). The increased metabolism induced by treatment of the mother one week prenatally, can still be observed at 3–4 weeks of age in the young rat, while in adult animals induction lasted only 5–7 days following discontinuation of phenobarbital (125). The molecular events responsible for the inductive effect of phenobarbital and other drugs during the perinatal period have not been completely elucidated. In addition to its effect upon glucuronidation in the neonate, phenobarbital also results in increased hepatic uptake of bilirubin as well as an increase in biliary flow (122). It also results in an increase in the amount of Y protein, one of the hepatic cytoplasmic anion binding fractions shown to be low in the perinatal period (126).

This first clinical application of enzyme induction was in several infants with unconjugated hyperbilirubinemia (127, 128). Phenobarbital treatment caused a rapid decrease in serum bilirubin concentrations accompanied in one patient by an increased output of salicylamide glucuronide in the urine (127) and in the other by an increased plasma disappearance of labeled bilirubin (128). Following these observations, attention was focused on the use of phenobarbital to modulate neonatal hyperbilirubinemia. Retrospectively, it was demonstrated that infants born to epileptic mothers treated with phenobarbital throughout pregnancy had a low serum bilirubin during the neonatal period (129). In several studies, phenobarbital has been given to the mother several days prior to delivery or to the newborn infant directly, and as a result significant lower serum bilirubin concentrations were found when compared with untreated control groups (118, 130, 131). Enhancement of glucuronide formation was demonstrated, albeit indirectly, by recording an increase in the excretion of salicylamide glucuronide in infants treated with phenobarbital (118).

As mentioned previously, the mechanisms underlying the lower concentrations of bilirubin are probably complex. Other drugs such as phenobutazine, diethylnicotinamide, and ethanol have been used to decrease neonatal hyperbilirubinemia with somewhat lesser effect than with phenobarbital (116, 132, 133). In contrast to animal studies (123) induction seems to take place even during early pregnancy as judged by in vitro assay (134). This again emphasizes the marked species differences that exist particularly with respect to fetal drug metabolism.

Excretion

The final common pathway for the removal of xenobiotic substances whether unchanged or metabolized is via the kidney. It has long been known that renal function in the newborn infant is much less than that of the adult even when corrected for differences in body size (135). Adult values are not achieved until usually the latter half of the first year of life. There is usually no functional impairment noted in the normal neonate because of the high rates of body growth and of biosynthetic functions (particularly that of protein) that are physiologically present. Drugs, which are not significantly metabolized and depend upon renal excretion for termination of their action, will have a noticeably longer effect in the newborn infant. Antibiotics are the major prototype of this class of pharmacologic agent, and because of their frequent use during the neonatal period they have been studied extensively. The clearance of penicillin G (136), which is mainly dependent upon tubular secretion for elimination, was decreased in the neonate to 17% of that found in a group of 2 year old children (calculated on a surface area basis). Three other penicillins, ampicillin, methacillin, and oxacillin, also had a prolonged half-life following intramuscular injection in premature infants (137).

Despite marked differences in birth weight associated with differences in intrauterine gestational age, all of the serum half-lives approached adult values at 3 weeks of postnatal development. This suggests that the maturation of renal function, which is responsible for the decrease in serum half-life, is a postnatal phenomenon. Aminoglycoside antibiotics such as kanamycin, neomycin, and streptomycin, which are mainly excreted by glomerular filtration, had a developmental pattern in premature infants similar to that observed for the penicillins (138). This has also been demonstrated in the full-term neonate for gentamycin (139). An interesting finding involved the antibiotic, colistin, another member of the aminoglycoside group, which, although dependent upon glomerular filtration for excretion, showed no difference in serum half-lives between the very young infants and the adult (137). This suggests that colistin is handled differently by the neonatal kidney. Further investigations concerning the role of renal excretion in modifying pharmacologic effects during the neonatal period are urgently needed, particularly because renal function at this age may be seriously compromised during systemic disease.

CONCLUDING REMARKS

In this review we have attempted to summarize what is known concerning drug disposition in the fetus and newborn. Despite the extensive bibliography, more questions have been asked than have been answered. The marked differences that

exist between the human fetus and that of experimental animals in so far as drug metabolism is concerned have far-reaching implications concerning the teratogenicity of drugs administered to the pregnant woman and their preclinical evaluation. The formation of highly reactive epoxides during the hydroxylation of double bonds and the N-oxygenation of amines has great potential significance for the production of congenital malformations. Of greater importance is the need for knowledge regarding drug disposition in the human fetus as a requisite to fetal therapy. In contrast to the historical secondary adverse effects of drugs upon the fetus following administration to the mother, the future will witness drug administration to treat fetal disease. Drug disposition in the sick, newborn infant is a *sine qua non* for the establishment of sound guidelines for drug therapy in this important age. The aggressive intervention in the management of the sick newborn through the establishment of intensive care centers requires rational pharmacologic intervention as well. Despite the caution urged in extrapolating animal data to the immature organism, continued basic research is required if basic questions regarding the influence of environment upon drug disposition are to be answered. Finally, continued investigations into the phenomenon of enzyme induction in the perinatal period should prove most helpful, not only in elucidating the mechanism of action of inducing agents but also in affording a better understanding of the developmental process itself and its regulation.

Literature Cited

1. Sereni, F., Principi, N. 1966. *Ann. Rev. Pharmacol.* 8:453–66
2. Mirkin, B. L. 1970. *Ann. Rev. Pharmacol.* 10:255–72
3. Wilson, J. T. 1972. *Ann. Rev. Pharmacol.* 12:423–50
4. Yaffe, S. J. 1966. *Ann. Rev. Med.* 17: 213–34
5. Eriksson, M., Yaffe, S. J. 1973. *Ann. Rev. Med.* 24:29–40
6. Fuchs, F. 1973. *Fetal Pharmacology.* New York: Raven. 463 pp.
7. Moyer, F., Thorndike, V. 1962. *Am. J. Obstet. Gynecol.* 84:1778–92
8. Tjälve, H., Hannsson, E., Schmiterlöw, C. G. 1968. *Acta Pharmacol. Toxicol.* 26:539–55
9. McNay, J. L., Dayton, P. G. 1970. *Science* 167:988–90
10. Miller, R. K., Berndt, W. O. 1973. *Proc. Soc. Exp. Biol. Med.* 143:118–22
11. Miller, R. K., Berndt, W. O. 1972. *Fed. Proc.* 31:595(Abstr.)
12. Shoeman, D. W., Kauffman, R. E., Azarnoff, D., Boulos, B. M. 1972. *Biochem. Pharmacol.* 21:1237–46
13. Krasner, J., Giacoia, G. P., Yaffe, S. J. 1973. *Ann. NY Acad. Sci.* 7:317
14. Boulos, B. M., Almond, C. H., Davis, L. E., Hammer, M. 1972. *Arch. Int. Pharmacodyn. Ther.* 196:357–62
15. Ehrnebo, M., Agurell, S., Jalling, B., Boréus, L. O. 1971. *Eur. J. Clin. Pharmacol.* 3:189–98
16. Morgan, C. D., Sandler, M., Panigel, M. 1972. *Am. J. Obstet. Gynecol.* 112: 1068–75
17. Juchau, M. R. 1974. *CRC Crit. Rev. Toxicol.* In press
18. Rane, A., Sjöqvist, F., Orrenius, S. 1974. *Clin. Pharmacol. Ther.* In press
19. Shnider, S. M., Way, E. L. 1968. *Anesthesiology* 29:944–50
20. Fox, G. S., Houle, G. L., Desjardins, P. D., Mercier, G. 1971. *Am. J. Gynecol.* 110:896–99
21. Juchau, M. R., Dyer, D. C. 1972. *Pediat. Clin. No. Am.* 19:65–79
22. Mirkin, B. L. See Ref. 6, p. 1
23. Oh, Y., Mirkin, B. L. 1971. *Fed. Proc.* 30:2034
24. Speirs, I., Sim, A. W. 1972. *Brit. J. Anaesth.* 44:370–73
25. Ullberg, S. See Ref. 6, p. 55
26. Kennedy, J. S., Waddell, W. J. 1972. *Toxicol. Appl. Pharmacol.* 22:252–58
27. Lutwak-Mann, C. See Ref. 6, p. 419
28. Fabro, S. See Ref. 6, p. 443
29. Yaffe, S. J., Rane, A., Sjöqvist, F., Boreus, L. O., Orrenius, S. 1970. *Life Sci.* 9:1189–1200

30. Rane, A., Sjöqvist, F., Orrenius, S. 1971. *Chem. Biol. Interact.* 3:305–7
31. Rane, A., Ackermann, E. 1972. *Clin. Pharmacol. Ther.* 13:663–70
32. Jondorf, W. R., Maickel, R. P., Brodie, B. B. 1958. *Biochem. Pharmacol.* 1: 352–54
33. Fouts, J. R., Adamson, R. H. 1959. *Science* 129:897–98
34. Hart, L. G., Adamson, R. H., Dixon, R. L., Fouts, J. R. 1962. *J. Pharmacol. Exp. Ther.* 137:103–6
35. Kato, R., Vassanelli, P., Fontino, G., Chiesara, E. 1964. *Biochem. Pharmacol.* 13:1037–51
36. Dallner, G., Siekevitz, P., Palade, G. E. 1966. *J. Cell Biol.* 30:73–97
37. Short, C. R., Davis, L. E. 1970. *J. Pharmacol. Exp. Ther.* 174:185–96
38. Basu, T. K., Dickerson, J. W. T., Parke, D. V. 1971. *Biochem. J.* 124:19–24
39. Rane, A., Berggren, M., Yaffe, S., Ericsson, J. L. E. 1973. *Xenobiotica* 3:37–48
40. Pelkonen, O., Vorne, M., Kärki, N. T. 1969. *Acta Physiol. Scand. Suppl.* 330: 69–74
41. Pelkonen, O., Vorne, M., Jouppila, P., Kärki, N. T. 1971. *Acta Pharmacol. Toxicol.* 29:284–94
42. Ackermann, E., Rane, A., Ericsson, J. L. E. 1972. *Clin. Pharmacol. Ther.* 13: 652–62
43. Chatterjee, I. B., Price, Z. H., McKee, R. W. 1965. *Nature* 207:1168–70
44. Pelkonon, O., Kärki, N. T. 1971. *Acta Pharmacol. Toxicol.* 30:158–60
45. Pomp, H., Schnoor, M., Netter, K. J. 1969. *Deut. Med. Wochenschr.* 94: 1232–40
46. Juchau, M. R., Pedersen, M. G. 1973. *Life Sci.* 12:193–204
47. Juchau, M. R., Pedersen, M. G., Symms, K. G. 1972. *Biochem. Pharmacol.* 21:2269–72
48. Pelkonen, O. 1973. *Arch. Int. Pharmacodyn. Ther.* 200:259–65
49. Pelkonen, O., Arvela, P., Kärki, N. T. 1971. *Acta Pharmacol. Toxicol.* 30: 385–95
50. Welch, R. M., Gomni, B., Alvares, A. P., Conney, A. H. 1972. *Cancer Res.* 32:973–78
51. Nebert, D. W., Gelboin, H. V. 1969. *Arch. Biochem. Biophys.* 134:76–89
52. Pelkonen, O., Jouppila, P., Kärki, N. T. 1972. *Toxicol. Appl. Pharmacol.* 23: 399–412
53. Rane, A., Von Bahr, C., Orrenius, S., Sjöqvist, F. See Ref. 6, p. 287
54. Juchau, M. R. 1971. *Arch. Int. Pharmacodyn. Ther.* 194:346–58
55. Juchau, M. R., Pedersen, M. G., Fantel, A. G., Shepard, T. H. 1974. *Clin. Pharmacol. Ther.* In press
56. Juchau, M. R., Symms, K. G. 1972. *Biochem. Pharmacol.* 21:2053–65
57. Welch, R. M. et al 1968. *Clin. Pharmacol. Ther.* 10:100–9
58. Nebert, D. W., Winker, J., Gelboin, H. V. 1969. *Cancer Res.* 29:1763–69
59. Juchau, M. R. 1971. *Toxicol Appl. Pharmacol.* 18:665–75
60. Schlede, E., Merker, H. J. 1972. *Naunyn Schmiedebergs Arch. Pharmakol.* 272:89–100
61. Schlede, E., Kasper, C., Merker, H. J. 1972. *Int. Congr. Pharmacol. Abstr., 5th,* p. 204
62. Dutton, G. J. 1959. *Biochem. J.* 71: 141–48
63. Hirvonen, T. 1966. *Biologica-Geographica,* Turku, Sarja. Ser. A 11
64. Chakraborty, J., Hopkins, R., Parke, D. V. 1971. *Biochem. J.* 125:15P
65. Yaffe, S. J., Levy, G., Matsuzawa, T., Baliah, T. 1966. *N. Engl. J. Med.* 275: 1461–66
66. Ramboer, C., Thompson, R. P. H., Williams, R. 1969. *Lancet* 1:966–68
67. Stern, L., Khanna, N. N., Levy, G., Yaffe, S. J. 1970. *Am. J. Dis. Child.* 120:26–31
68. Nathenson, G., Cohen, M. I., Litt, I. F., McNamara, H. 1972. *J. Pediat.* 81:899–903
69. Juchau, M. R., Yaffe, S. J. 1969. *The Foeto-Placental Unit.* Amsterdam: Excerpta Med. Found. 260 pp.
70. Uher, J. See Ref. 69, p. 240
71. Fouts, J. R. See Ref. 6, p. 305
72. Leskes, A., Siekevitz, P., Palade, G. E. 1971. *J. Cell Biol.* 49:264–87
73. Leskes, A., Siekevitz, P., Palade, G. E. 1971. *J. Cell Biol.* 49:288–302
74. Koga, A. 1971. *Z. Analyt. Entwickl. Gesch.* 135:156–61
75. Feuer, G., Liscio, A. 1969. *Nature* 223: 68–70
76. Soyka, L. F., Long, R. J. 1972. *J. Pharmacol. Exp. Ther.* 182:320–31
77. Woods, J. S., Dixon, R. L. 1972. *Biochem. Pharmacol.* 21:1735–44
78. Klinger, W., Zwacka, G., Ankermann, H. 1968. *Acta Biol. Med. Ger.* 20: 137–45
79. Wilson, J. T. 1970. *Nature* 225:861–63
80. Netter, K. J. 1971. *Arch. Gynaekol.* 211:112–33
81. Fichter, E. G., Curtis, J. A. 1956. *Pediatrics* 18:50–58
82. Znamenacek, K., Pribylova, H. 1963. *Cesk. Pediat.* 18:104–14

83. Jusko, W. J., Khanna, N., Levy; G., Stern, L., Yaffe, S. J. 1970. *Pediatrics* 45:945–49
84. O'Connor, W. J., Warren, G. H., Mandala, P. S., Edrada, L. S., Rosenman, S. B. 1964. *Antimicrob. Ag. Chemother.*, 188–91
85. Silverio, J., Poole, J. W. 1973. *Pediatrics* 51:578–80
86. Jusko, W. J. 1973. *Pediat. Clin. N. Am.* 19:81–100
87. Weingärtner, L., Sitka, V., Ratsch, R., Grüindig, C. 1972. *Int. J. Clin. Pharmacol.* 6:358–63
88. Hernandez, A. 1969. *Pediatrics* 44: 418–28
89. Kupferberg, H. J., Way, E. L. 1963. *J. Pharmacol. Exp. Ther.* 141:105–12
90. Kandall, S. R., Thaler, M. M., Erickson, R. P. 1973. *J. Pediat.* 82:1013–19
91. Burmeister, W. 1970. *Int. Z. Klin. Pharmakol. Ther. Toxicol.* 4:32–36
92. Rane, A., Lunde, P. K. M., Jalling, B., Yaffe, S. J., Sjöqvist, F. 1971. *J. Pediat.* 78:877–82
93. Yaffe, S. J., Krasner, J. 1973. *Ann. NY Acad. Sci. Conf., January*
94. Kunin, C. M. 1967. *Ann. NY Acad. Sci.* 145:282–90
95. Krasner, J., Stern, L. J., Yaffe, S. J. 1972. *Pediat. Res.* 6:405
96. Krasner, J. 1973. *Biochem. Med.* 7: 135–44
97. Schiff, D. G., Chan, G., Stern, L. 1971. *Pediatrics* 48:139–41
98. Short, C. R., Davis, L. E. 1971. *J. Pharmacol. Exp. Ther.* 174:185–96
99. Percy, A. K., Yaffe, S. J. 1964. *Pediatrics* 33:965–68
100. Henderson, P. T. 1971. *Biochem. Pharmacol.* 20:1225–32
101. Yaffe, S. J. 1968. *Ross Conf. Pediat. Res.* 58–65
102. Soyka, L. F. 1970. *Biochem. Pharmacol.* 19:945–51
103. O'Donoghue, L. E. J. 1971. *Nature* 229:125–26
104. Sjöqvist, F., Bergfors, F. G., Borga, O., Lind, M., Ygge, H. 1972. *J. Pediat.* 80: 496–600
105. Mirkin, B. L. 1971. Ibid 78:329–37
106. Jalling, B., Boréus, L. O., Rane, A., Sjöqvist, F. 1970. *Pharmacol. Clin.* 2: 200–2
107. Idapa, J. 1972. *Am. J. Obstet. Gynecol.* 112:387–93
108. Brown, A. K., Zuelzer, W. W., Burnett, H. H. 1958. *J. Clin. Invest.* 37:332–40
109. Gladtke, E., Rud, H. 1967. *Monstsschr. Kinderheilk.* 115:231–33
110. Wichmann, H. M., Rind, H., Gladtke, E. 1968. *Z. Kinderheik.* 103:262–76
111. Vest, M. F. 1962. *J. Clin. Invest.* 41: 1013–20
112. Vest, M. F., Salzberg, R. 1965. *Arch. Dis. Childhood* 40:97–105
113. Vest, M. F., Streiff, R. R. 1949. *Am. J. Dis. Child.* 98:688–93
114. Fichter, G., Curtiss, J. I. 1955. *Am. J. Dis. Child.* 90:596–97
115. Nitowsky, H. M., Matz, L., Berzofsky, J. A. 1966. *J. Pediat.* 69:1139–49
116. Reinicke, C., Rogner, G., Frenzel, Y., Maak, B., Klinger, W. 1970. *Pharmacol. Clin.* 2:167–72
117. Garattini, S. *Pharmacol. Toxicol. Program Symp., Washington DC, May 17–19, 1971*
118. Stern, L., Khanna, N. N., Levy, G., Yaffe, S. J. 1970. *Am. J. Dis. Child.* 120:26–31
119. Conney, A. H., 1967. *Pharmacol. Rev.* 19:317–66
120. Conney, A. H., Levin, M. S., Jacobsen, M., Kuntzman, R. 1973. *Clin. Pharmacol. Ther.* 14:727–41
121. Inscoe, J. K., Axelrod, J. 1960. *J. Pharmacol. Exp. Ther.* 129:128–31
122. Catz, C., Yaffe, S. J. 1968. *Pediat. Res.* 2:361–70
123. Hart, L. G., Adamson, R. H., Dixon, R. L., Fouts, J. R. 1962. *J. Pharmacol. Exp. Ther.* 137:103–6
124. Fouts, J. R., Hart, L. G. 1965. *Ann. NY Acad. Sci.* 123:245–51
125. Mitoma, C., LeValley, S. E. 1970. *Arch. Int. Pharmacodyn. Ther.* 187:155–62
126. Reyes, H., Levi, A. J., Gatmaitan, Z., Arias, I. M. 1969. *Proc. Nat. Acad. Sci. USA* 64:168–70
127. Yaffe, S. J., Levy, G., Matsuzawa, T., Baliah, T. 1966. *N. Engl. J. Med.* 275: 1461–66
128. Crigler, J., Gold, N. I. 1966. *J. Clin. Invest.* 45:998–99
129. Trolle, D. 1968. *Lancet* 1:251–52
130. Maurer, H. M. et al 1968. *Lancet* 2: 122–24
131. Ramboer, C., Thompson, R. P. H., Williams, R. 1969. *Lancet* 1:966–68
132. Sereni, F., Perletti, L., Marini, A. 1967. *Pediatrics* 40:446–49
133. Waltman, R., Bonura, F., Nigrin, G., Pipat, C. 1969. *Lancet* 2:108–10
134. Pelkonen, O., Karki, N. T. 1972. *Int. Congr. Pharmacol., 5th,* p. 179
135. West, J. R., Smith, H. W., Chasis, H., 1948. *J. Pediat.* 32:10–18
136. Barnett, H. L., McNamara, H., Schultz, S., Tompsett, R. 1949. *Pediatrics* 3: 418–22
137. Axline, S. G., Yaffe, S. J., Simon, H. J. 1967. *Pediatrics* 39:97–107
138. Simon, H. J., Yaffe, S. J. 1970. *Proc. Int. Congr. Chemother., 6th,* 11:761–67
139. McCracken, G. H., West, N. R., Horton, L. J. 1971. *J. Infec. Dis.* 123: 257–62