DEVELOPMENTAL PHARMACOLOGY[1]

BERNARD L. MIRKIN

Departments of Pharmacology and Pediatrics, Clinical Pharmacology Section, University of Minnesota, Minneapolis, Minnesota

Ontogenesis in the mammalian cell reflects a continuum of integrated biochemical events initated by fertilization, sustained by implantation and placentation, and exquisitely susceptible to environmental influences. Numerous investigations in this area have created a massive, primarily descriptive literature, tending to emphasize correlations between the morphologic, rather than biochemical effects of teratogenic and embryopathic drugs (1, 2). It seems desirable that the actions of pharmacologic agents on developing biologic systems be viewed from a perspective which permits not only the analysis of drug effects upon embryogenesis but also allows for an evaluation of how physiologic maturation basically influences the disposition of and response to pharmacologically active molecules. Several reviews which approach developmental pharmacology from a more fundamental viewpoint are available for the interested reader (3-5).

INFLUENCE OF MATURATION ON THE DISPOSITION AND REACTIVITY OF PHARMACOLOGIC AGENTS

Analgesics.—The molar potency and toxic effects of narcotic analgesics are generally considered to be significantly greater in the mammalian neonate than in the adult. Morphine is about 4 times more potent on a mg/kg basis when 14 day old rats are compared with 28 day old animals (6). In contrast, the LD_{50} of heroin and methadone is not significantly altered by neonatal age (6, 7). The enhanced effectiveness of analgesics during the neonatal period has been attributed to more rapid penetration and greater accumulation within the central nervous system. This generalization may not be applicable to synthetic analgesics possessing the 4-phenylpiperidine configuration. They seem to differ from morphine, in that their toxicity (LD_{50}) does not apparently correlate well with the central nervous system concentration of plasma half-life of the drug (8). Meperidine is equi-toxic to neonates and adults yet achieves plasma and brain concentrations at 30 minutes which are several times greater in the immature rat than in the adult. No

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clear explanation for this discrepancy appears to be available at the present time. Indeed, some of the 4-phenylpiperidine derivatives such as anileridine, alpha- and beta-prodine are more toxic to the adult than the neonate (see Table I).

The transplacental passage of morphine has been clinically recognized for many years in human subjects (9). Administration of H^3-dihydromorphine (subcutaneously) to pregnant rats produced maternal plasma levels of free H^3-dihydromorphine which were 2½ times greater than fetal levels ½, 1 and 2 hours after administration (10). Despite the lower fetal plasma levels,

TABLE I

EFFECT OF BIOLOGIC AGE ON LD_{50}, TISSUE DISTRIBUTION AND HALF-LIFE OF NARCOTIC ANALGESICS[a]

Drug	LD_{50} (mg/kg i.p.)		Dose Administered (mg/kg i.p.)	Brain Concentration at 30 min. (μg/g)		Plasma Concentration at 30 min. (μg/ml)		Plasma Half-life (min.)	
	Neo[b]	Adult[c]		Neo	Adult	Neo	Adult	Neo	Adult
Morphine	60	220	50	10	4	23	15.5	~40	~40
Meperidine	115	128	20	15	7	2.6	0.7	42	18
Anileridine	60	30	12	10.3	5.4	1.2	0.9	24	22
Alpha-Prodine	120	33	10	11.9	23.1	1.4	2.6	>120	~33
Beta-Prodine	95	5.5	---	---	---	---	---	---	---

[a] Data compiled primarily from references 6 and 8.
[b] Neonatal rat 14 days old.
[c] Adult rats 28 days old.

H^3-dihydromorphine concentration in fetal brain was about 3 times greater than maternal at the aforementioned time intervals. Conjugated H^3-dihydromorphine was not detectable in maternal brain until 16 hours after its administration; however, small quantities were recovered from fetal brain tissues after 2 hours. The central nervous system exhibits a selective permeability to the conjugated form of morphine in that it does not appear to penetrate either the fetal or adult brain nearly as well as the free drug. In general, higher levels of conjugated drug were found in the livers and kidneys than in the plasma of maternal rats; fetal liver-plasma and kidney-plasma relationships of conjugated H^3-dihydromorphine were extremely variable. These data demonstrate that distribution of H^3-dihydromorphine differs grossly in fetal and maternal tissues but does not differentiate the sites (maternal or fetal) at which conjugation of dihydromorphine may occur.

In the human, meperidine has been detected in fetal umbilical cord blood 2 minutes after intravenous maternal administration (11). Approximately 4 per cent of the total intravenous dose reached the fetus within ½ hour as compared to 1 per cent following a maternal intramuscular injection. Neonates who have received meperidine by transplacental passage or by the direct intramuscular route excrete 25 to 40 per cent of the dose after 48

hours, almost exclusively in the nonmethylated form. Anileridine, the N-p-aminophenethyl derivative of meperidine, also crosses the placenta rapidly and achieves significant levels in fetal plasma (12).

Local Anesthetics.—Transplacental passage of local anesthetics employed for conduction blockade during obstetrical procedures may cause fetal bradycardia (13, 14), depression (15), or stimulation (16, 17) of neuronal activity during the neonatal period.

Local anesthetics are rapidly absorbed from caudal, epidural, and paracervical sites of administration; significant concentrations are discernible in the systemic maternal circulation 3 to 5 minutes after injection with peak concentrations occurring 15 to 30 minutes thereafter. Umbilical vein levels of local anesthetics depend on a variety of factors such as the type of local anesthetic utilized, total dose administered, interval between injection and delivery, site of conduction blockade, and presence or absence of vasoconstrictor in the dose administered. Plasma levels of mepivacaine determined 30 minutes after maternal administration of 5.2 mg/kg for conduction blockade were as follows: maternal antecubital vein (2.9 μg/ml), umbilical vein (1.9 μg/ml), and umbilical artery (1.4 μg/ml). Comparable maternal levels were obtained with lidocaine; however, at equivalent maternal plasma levels the mean fetal blood level (umbilical vein) was higher after mepivacaine (2.7 μg/ml) than following lidocaine (1.3 μg/ml). Similar differences have also been noted in the partition ratios (umbilical vein/maternal vein) of mepivacaine and lidocaine which are 0.7 and 0.5 respectively (15, 18). Retrograde passage of mepivacaine from fetus to mother has been demonstrated in the guinea pig. Intracerebral injections of local anesthetic in concentrations which were lethal to the neonate proved without effect when administered to the fetus, attesting to the efficacy of this transport mechanism (19).

The human neonate appears to be considerably more susceptible to cardiovascular and respiratory depression induced by local anesthetics than are physiologically mature individuals. Plasma levels producing these toxic effects in the adult are in the range of 5.3 μg/ml for lidocaine and 6.3 μg/ml for mepivacaine as compared to 2.5 μg/ml for lidocaine in the neonate (15, 18). The shift of the dose response curve to the right with advancing age is not to be considered as conclusive at this time but does suggest an enhanced sensitivity of the newborn to compounds possessing local anesthetic activity.

Anticonvulsants.—Localization of diphenylhydantoin in fetal tissues after transplacental passage has been demonstrated in pregnant rats administered diphenylhydantoin on day 21 of gestation (20). Fetal concentrations in liver and brain were respectively 2 and 4 times greater than maternal levels one hour after intravenous administration of the drug to the mother. Thereafter, diphenylhydantoin disappeared from fetal tissues at a rate which paralleled its removal from maternal tissues. In contrast, maternal liver concentrations were approximately 15 times higher than fetal levels 4 hours after intraperitoneal administration of diphenylhydantoin to the mother, and no diphenylhydantoin or metabolite was noted in fetal brain tissue at this

time (see Table II). The metabolic degradation and elimination of diphenylhydantoin is diminished in neonatal rats until the fourth week of life when plasma and tissue decay rates approach adult values (20). Administration of diphenylhydantoin over a period of 3 days, significantly increases the drug metabolizing activity of 5- and 10-day old neonatal rats. This treatment produced an increase in hepatic microsomal cytochrome P-450 and total heme content but did not affect cytochrome b_5 (21).

Recently, the presence of diphenylhydantoin has been demonstrated in umbilical cord plasma obtained from two epileptic mothers who received diphenylhydantoin throughout their entire pregnancy. The concentration of diphenylhydantoin in both umbilical artery and vein was approximately 90 per cent that assayed in the maternal plasma. Neonatal blood levels determined 24 and 48 hours after delivery were slightly lower than those present in cord plasma at birth, with complete clearance noted on the third day of neonatal life (20).

The transplacental passage of diphenylhydantoin and its presence in developing fetal tissue has caused some concern, particularly in view of the suggested association between anticonvulsant drugs and specific teratologic effects. The chronic administration of diphenylhydantoin to primagravida rats is associated with a significant reduction in fetal weight and an increased percentage of nonviable fetuses (22). Cleft palate and lip can be produced in fetal and neonatal A/J mice by administration of diphenylhydantoin to the mother during gestation (23). Harelip and cleft palate have also been reported in the children of mothers receiving anticonvulsant therapy during their pregnancies (20, 24). Retrospective analysis of a larger epileptic population has now identified 32 additional children with cleft lip; however, a definitive cause and effect relationship remains to be established (25).

Barbiturates.—Ontogenetic factors signifiantly influence the duration of barbiturate induced sleeping times. Dose-response curves (sleeping time versus drug concentration) obtained with hexobarbital have demonstrated a potency which is 5 times greater in the 5- as contrasted to the 44-day old rat (26) and a fortyfold increase in effectiveness when mice (Bar Harbor strain #129) are compared at birth and 21 days thereafter (27). The plasma levels of hexobarbital obtained upon awakening were similar in 14, 21, and 28 day old mice despite marked differences in the duration of sleeping time (See Table II). In sharp contrast, the brain concentrations of hexobarbital were lower in neonatal than adult rats when they awoke from hypnosis (neonates—20 μg/gm; adult—30 μg/gm) and also after death due to respiratory depression (neonate: 78 μg/g; adult 257 μg/g). A similar pattern has been reported for pentobarbital wherein adult rat brains contained approximately three times more barbiturate than the neonate at death (28). These observations suggest an intrinsic sensitivity of the immature nervous system to barbiturate induced depression which cannot be attributed solely to an impaired drug metabolizing capability.

It is possible of course, to enhance the effects of some barbiturates by

retarding their biotransformation. Hexobarbital sleeping time can be significantly prolonged by pretreating rats with morphine which inhibits side chain oxidation of the barbiturate (29). This response appears to be sex and age dependent since it has only been observed in mature male rats or immature female rats treated with testosterone proprionate.

Psychopharmacologic agents.—Considerable difficulty has been encountered in developing analytical techniques which can quantitatively validate the transplacental passage and neonatal disposition of phenothiazine derivatives (31, 32). Lacomme & LeLorier (33) found minute quantities of chlorpromazine in the urine of infants whose mothers had received the drug at term. Paper chromatographic analyses have been utilized to identify chlorpromazine and promethazine in neonatal urinary samples (34). This study demonstrated placental transfer of ½ to 1 per cent of the maternally administered dose and complete clearance of the drug from the infant 48 to 60 hours after its transplacental passage.

Promazine has been identified in umbilical cord blood (35, 36). Peak levels occurred about 4 minutes after maternal administration with an estimated ½ per cent of the intravenous dose reaching the fetus. Within the 24 hours following delivery, the neonate excreted approximately 3½ per cent of the placentally transferred drug into the urine as promazine and its sulfoxide, monomethyl, and monomethylsulfoxide metabolites. The ratio of sulfoxide metabolites to promazine in urine was 4.4 (0 to 24 hours) and 2.1 (24 to 48 hours). An interesting aside to Crawford & Rudofsky's (35, 36) study was that 24 hour urine collections obtained from pregnant females given promazine (50 mg i.v). contained a significantly greater percentage of the drug and its metabolites than did similar samples from nonpregnant women. (pregnant 4.7 per cent; non-pregnant 1.8 per cent).

Drug Toxicity.—An intriguing ontogenetic relationship has recently been described for the cataractogenic activity of 2–4 dinitrophenol (37). Initial studies have demonstrated that immature rabbits and ducklings are unusually more susceptible to experimental cataract formation by dinitrophenol than are adult rabbits. Sub-lethal concentrations of 2–4 dinitrophenol administered between the tenth and sixty-second postnatal day had fivefold less cataractogenic activity than in the neonate; a similar dose given on the ninetieth postnatal day was without any effect on cataract formation. The distribution of 2–4 dinitrophenol in immature animals was characterized by higher concentrations and a longer duration of peak levels in the aqueous humor, vitreous humor, and lenses. The clearance of 2–4 dinitrophenol from the circulation of neonatal animals is also much slower and concentrations within the aqueous humor are directly proportional to the level of free, nonprotein bound drug in plasma (38). These observations suggest that some manifestations of selective toxicity in developing organisms may result from their limited transport capability leading to differential sequestration of exogenous substrates, elevations in local drug concentrations, and ultimately, noxious effects upon susceptible tissues.

The impaired metabolism of drugs in the newborn period generally tends

TABLE II

EFFECT OF BIOLOGIC AGE ON TISSUE DISTRIBUTION OF BARBITURATES AND ANTICONVULSANTS

Drug	Dose (mg/kg)	Species	Age (days)	Tissue	Tissue concentration (μg/ml or gm) at specific time (hrs) after injection of drug										Reference
					1/6	1/4	1/2	1	2	3	4	8	24	death	
Hexobarbital	100 i.p.	mice	14	plasma			36								(27)
	100 i.p.	mice	21	plasma	39										Conc. on
	100 i.p.	mice	28	plasma		34									awakening
Hexobarbital	24 i.p.	rat	5	brain		15									(26)
	30 i.p.	rat	5	brain			20								Conc. on
	38 i.p.	rat	5	brain				21							awakening
	95 i.p.	rat	44	brain		31									
	120 i.p.	rat	44	brain			29								
	156 i.p.	rat	44	brain				31							
Barbital	150 i.p.	rat	22	plasma	134		130	128		105					(30)
	150 i.p.	rat	38	plasma	174		155	165		134					Conc. at
	150 i.p.	rat	50	plasma	185		190	169		144					time indicated
Pentobarbital	23 i.p.	rat	1	brain										27	(28)
	38 i.p.	rat	5	brain										47	Conc. at
	55 i.p.	rat	10	brain										71	death
	80 i.p.	rat	20	brain										88	
	87 i.p.	rat	30	brain										95	
Diphenyl-hydantoin	100 i.p.	rat	2	brain				147			171	143	107		(20)
	100 i.p.	rat	7	brain				139			151	120			Conc. at
	100 i.p.	rat	14	brain				42			54	53	14		time
	100 i.p.	rat	28	brain				34			65	31	0		indicated

Drug	Dose (mg/kg)	Species	Age (days)	Tissue	Tissue concentration (μg/ml or gm) at specific time (hrs) after injection of drug										Reference
					1/6	1/4	1/2	1	2	3	4	8	24	death	
Diphenyl-hydantoin	100 i.p.	rat	2	liver				397			455	411	287		(20) Conc. at time indicated
	100 i.p.	rat	7	liver				360			429	336			
	100 i.p.	rat	14	liver				153			146	132	28		
	100 i.p.	rat	28	liver				76			153	69	7		
	100 i.p.	rat	Adult	liver				14	16		22	30	21		
	100 i.p.	rat	14	plasma				20			22	10	8		
	100 i.p.	rat	28	plasma				13			22	14	1		
	100 i.p.	rat	Adult	plasma				9	12		12	20	6		
	25 i.v.	rat	Adult	brain	34		30	24	23		20	2			
	25 i.v.	rat	Adult	liver	101		74	57	61		36	4			
	25 i.v.	rat	Adult	plasma	33		28	19	9		2				
	100 i.p.	rat	Maternal	brain				0	9		15	35			
	100 i.p.	rat	Fetal	brain				0	0		0	0			
	100 i.p.	rat	Maternal	liver				0	11		214	28			
	100 i.p.	rat	Fetal	liver				0	11		15	0			
	100 i.p.	rat	Maternal	plasma				0	10		16	19			
	100 i.p.	rat	Fetal	plasma				NS[a]	NS		NS	NS			
	100 i.p.	rat		placenta				0	9		48	24			
	25 i.v.	rat	Maternal	brain	8			24	7		5	8			
	25 i.v.	rat	Fetal	brain	32			48	22		14	6			
	25 i.v.	rat	Maternal	liver	0			11	25		9	3			
	25 i.v.	rat	Fetal	liver	43			58	24		10	6			
	25 i.v.	rat		placenta	49			55	35		16	5			

[a] NS=no sample.

to enhance their toxicity but does not always constitute a handicap for the neonate. This is best demonstrated by analyzing the response of newborn rats to the phallotoxins derived from mushrooms of the genus Amanita and to the teratogenic effects of cyclophosphamide and chlorcyclizine.

Phalloidin acts only if it has been administered *in vivo* and is not toxic in itself but must be converted to a toxic compound by hepatic drug metabolizing enzymes. Neonatal rats can endure extremely high doses of this substance without being killed. The basis for this resistance appears to correlate best with the absence of an active drug metabolizing system (39).

The cytotoxic action of cyclophosphamide is contingent upon biotransformation to an active alkylating agent (40, 41). Teratogenic effects resulting from the administration of this compound have been well documented and the active cyclophosphamide teratogen is probably a nonalkylating compound (42). Induction of hepatic microsomal activity by phenobarbital significantly increased the rate of conversion of cyclophosphamide to its active alkylating form and decreased the teratogenic effects of a given dose. SKF-525A decreased the concentration of cyclophosphamide alkylating product and enhanced the teratogenic action of this compound (42). The teratogenic and cytotoxic properties of cyclophosphamide may be separable with an increased teratogenic potentiality present when this compound is administered to immature organisms with limited hepatic drug metabolizing capability.

In contrast, the incidence of cleft palate in fetal rats delivered from mothers receiving chlorcyclizine is decreased when pretreated with SKF-525A (43). Chlorcyclizine is demethylated to form nor-chlorcyclizine and SKF-525A has no effect upon the teratogenic effects of the latter compound. It is likely therefore, that mutagenic activity resides primarily in the demethylated metabolite whose formation is inhibited by SKF-525A.

Metabolism of Drugs by the Feto-placental Unit

In conjunction with its role as a transport organ, the placenta contains numerous active enzyme systems which function in the biosynthesis and degradation of steroids during embryogenesis (44). Conjugation of steroids markedly diminishes their placental transport and by analogy maternally administered drugs are generally considered to cross the placenta primarily, if not exclusively, in a nonmetabolized state (45). Since pharmacologically active molecules of exogenous origin may undergo placental biotransformation in a manner described for endogenous substrates, it is essential to understand how this process may affect the transplacental passage of drugs.

The principal carbon monoxide binding pigment of placental mitochondria and microsomes has been identified as a cytochrome, P-450, which resembles that associated with hepatic, microsomally localized, drug metabolizing enzymes (46). Electron microscopy of placental tissue has demonstrated significant quantities of smooth endoplasmic reticulum in trophoblastic cells (47). The appearance of cytochrome P-450 in placental tissue may follow

an ontogenetic pattern paralleling that of the developing liver, since P-450 is not detectable in human placenta obtained between the ninth and twelfth weeks of gestation (48). The presence of P-450 and smooth endoplasmic reticulum in the human term placenta does not categorically establish that drug metabolism occurs under physiologic conditions but merely indicates a potential capability for such activity.

Several *in vitro* studies have indicated that homogenates and microsomal fractions (9,000 x g supernatant and 100,000 x g pellet) prepared from human term placenta can effectively metabolize certain drugs. Hydroxylation and demethylation of polycyclic hydrocarbons by benzpyrene hydroxylase and aminoazo dye N-demethylase has been demonstrated in both human and rat placenta (49). The metabolism of a variety of substrates has been compared in microsomal fractions obtained from human term placenta and rat liver in order to determine the relative activity and identity of degradative pathways in each tissue (50). Pentobarbital and amphetamine were metabolized in placental extracts at rates which were respectively 60 and 99 per cent that of rat liver when the comparison was based upon μM of drug metabolized/g total protein/hour. If placental drug metabolism was expressed in terms of μM of drug metabolized/g wet weight of tissue/hour the rates of metabolism for both pentobarbital and amphetamine relative to the liver were significantly lower. In contrast to these data, Welch et al. (49) obtained little or no metabolism of pentobarbital-2-C^{14} after incubation for 30 minutes with an NADPH-generating system and homogenates containing up to 400 mg of human term placenta. Meperidine is metabolized at a slower rate by placental than by hepatic homogenates and probably is degraded via a different metabolic pathway. In the liver, meperidine is primarily converted to normeperidine by N-demethylation; the placenta appears virtually devoid of N-demethylase activity for this particular substrate (50). Homogenates of rabbit placenta and liver at 14, 21, and 28 days of gestation are unable to metabolize hexobarbital whereas zoxazolamine is readily hydroxylated (51). Placental homogenates prepared from rodent tissue can catalyze the following reactions: reduction of azo-linkage of neoprontosil, reduction of the aromatic nitro group of p-nitrobenzoic acid, and conjugation of the amino group of p-aminobenzoic acid (52). These reactions do not appear to involve cytochrome P-450 of NADPH cytochrome C reductase since they are not activated by flavins nor inhibited by carbon monoxide.

A selective increase in the activity of specific hydroxylase reactions has been demonstrated to occur in the livers of developing rats (53). Hydroxylation of testosterone at the 16-α position is diminished at birth and remains low for the initial four weeks of life whereas 6-β and 7-α hydroxylation increase significantly during the first postnatal week. The inductive effects of phenobarbital and 3-methylcholanthrene on hydroxylase activity also appear to be selective (see Table III). Phenobarbital administration to immature male rats stimulates 16-α hydroxylation of testosterone several fold and 6-β and 7-α hydroxylation to a much smaller degree. In contrast 3-

methylcholanthrene has no effect on 6-β and 16-α hydroxylation but enhances 7-α hydroxylation significantly.

The human placenta at 9 to 12 weeks of gestation appears to have limited drug metabolizing capability. Homogenates prepared from such placentae can reduce the azo group of neoprontosil but are unable to reduce the nitro group of p-nitrobenzoic acid (48). In addition this tissue cannot carry out oxidative procedures such as the hydroxylation of 3, 4-benzpyrene, deamination of 1-amphetamine, sulfoxidation of chlorpromazine, side-chain oxidation of hexobarbital, N-demethylation of aminopyrine, O-demethylation of codeine, or p-hydroxylation of aniline. Dvorák (54) has demonstrated the metabolism of two anti-inflammatory drugs (ketophenylbutazone and benzopyrazone) in the human feto-placental unit during the first trimester of pregnancy. These drugs and their metabolites have been identified in chorionic tissue and in fetal organs. Unfortunately, the metabolites are identical to those obtained from maternal serum and urine, so that it is not clear whether they represent placental transport of a maternal metabolite or fetal and placental degradation of the parent compound.

Specific placental enzyme systems can be induced under appropriate conditions. Benzypyrene hydroxylase and aminoazo dye N-demethylase activity is absent or extremely low in the placentae of nonsmoking mothers whereas a high level of activity is present in the placentae of smoking mothers (49). Benzpyrene hydroxylase can be induced in rodent placenta by the injection of polycyclic hydrocarbons but not by phenobarbital administration (55). This enzyme is not detectable in the livers of 15 or 20 day old rat fetuses. However, 24 hours after the administration of 3-methylcholanthrene to rats pregnant for 14 or 19 days, benzypyrene hydroxylase activity in the fetal liver is approximately the same as that obtained in the untreated maternal liver. In contrast, the oral administration of phenobarbital failed to induce similar activity in the fetal liver (56). A similar pattern for the induction of zoxazolamine hydroxylase and hexobarbital oxidase has been noted in the pregnant doe (51). Chlordane pretreatment stimulated the metabolism of these substrates by both placental and fetal liver homogenates while phenobarbital was without effect in this regard. Pecile et al. (57), utilizing strychnine as a substrate, studied phenobarbital induction of drug metabolizing enzymes in the rodent placenta and liver. Metabolic activity was greatest in homogenates of maternal liver and virtually absent in both fetal liver and placenta. Administration of phenobarbital to pregnant animals significantly increased the drug metabolizing activity of maternal liver in both rats and rabbits with a parallel effect noted in fetal rabbit liver and a lesser one in fetal rat liver. The response of the placenta to the inducing action of phenobarbital was consistently negative in rabbits and rats. These authors concluded that participation of the fetal-placental unit in the detoxifying mechanisms of the body is possible but that fetal liver and not placental tissue is primarily involved.

An interesting yet poorly documentated relationship may exist between the urinary excretion of D-glucaric acid and hepatic microsomal conjuga-

TABLE III

EFFECT OF INDUCING AGENTS ON DRUG METABOLIZING ACTIVITY OF DEVELOPING TISSUES

Enzyme or Substrate Metab.	Fetal Liver				Neonatal Liver			Placenta				Ref.
	Untreated	After Treatment[a]			Untreated	After Treatment		Untreated	After Treatment			
		P	3-MC	CHL		P	3-MC		P	BP	CHL	
Benzpyrene Hydroxylase	Absent	0	++	—	—	—	—	Absent	0	++	—	49, 55, 56
Azo Dye N-Demethylase	Trace	0	++	—	—	—	—	Absent	—	—	—	49, 55, 56
Zoxazolamine Hydroxylase	Absent	0	—	++	—	—	—	Present	0	—	++	51
Hexobarbital Oxidase	Absent	0	—	+[b]	—	—	—	Absent	0	—	++	51
Strychnine	not metab.	++						not metab.				57
Testosterone 6-β Hydroxylase					Low	+	0					53
Testosterone 7α Hydroxylase					Low	+	++					53
Testosterone 16α Hydroxylase					Low	++	0					53

Key:

magnitude of response: ++ = several fold increase in enzyme activity or drug metabolism
+ = small increase in enzyme activity or drug metabolism
0 = no effect on enzyme activity or drug metabolism
P = phenobarbital
BP = 3-4 benzpyrene
3-MC = 3 methylcholanthrene
CHL = chlordane

[a] All inducing agents were administered into the maternal circulation.

[b] Response noted but not statistically significant.

tion reactions involving D-glucuronic acid (58). The livers of developing organisms (human and rodent) contain much lower levels of D-glucuronalactone dehydrogenase activity than those of adult specimens (59). The low D-glucaric acid excretion in newborn infants correlates well with the diminished D-glucuronalactone dehydrogenase activity noted above, and the gradual increase in D-glucaric acid excretion with age and weight reflects an increasing activity of this enzyme in liver. In premature infants, there appears to be a direct relationship between the serum bilirubin level and urinary D-glucaric acid excretion (60). D-glucaric acid excretion was significantly higher in patients taking oral contraceptives than in woman receiving no drugs (60). The primary implication of these studies is that excretion patterns of D-glucaric acid may ultimately serve as a valuable clinical index for assessing drug metabolizing capability and the influence of pharmacologic agents on this process.

Placental drug metabolism has also been studied in dogs (8 to 9 weeks gestational age) utilizing an extracorporeally perfused *in situ* uterine-placental-fetal preparation which allows parallel analyses of drug metabolism by the placenta and drug distribution in fetal tissues (61). Reactions involving the conversion of aminopyrine to 4-aminoantipyrine and 4-aminoantipyrine to N-acetyl-4-aminoantipyrine were evaluated. This preliminary study demonstrated a 29 per cent decrease in plasma aminopyrine levels after perfusion for 60 minutes, which was associated with the appearance of 4-aminoantipyrine and N-acetyl-4-aminoantipyrine in the fetal tissues.

While the diverse drug metabolizing activities of placental tissue suggest a potential regulatory role for this organ insofar as it may influence the quantity and form in which certain drugs ultimately reach the fetus, the effects of pharmacologic agents upon essential physiologic functions of the placenta must also be considered. Glucose catabolism in the human placenta occurs via glycolytic and direct oxidative pathways. The relative utilization of the pentose phosphate shunt for glucose metabolism in human placentae varies with the stage of pregnancy. Between the sixth and twentieth weeks of gestation, carbohydrate metabolism procedes along the pentose phosphate pathway to a much greater extent than it does at term (62). Protein synthesis, nucleic acid, and ribose turnover rates are increased in early placental tissue and tend to parallel elevations in pentose shunt activity (63). Carbohydrate metabolism in the placenta is affected by the following agents: liver alcohol dehydrogenase depresses glucose metabolism by the pentose route (63); oxytocin increases shunt utilization in early placental tissue (64); epinephrine increases placental glycolytic activity, lactate production, glycogen breakdown, and glucose uptake (65); monoamineoxidase inhibitors do not affect the placental response to epinephrine (64). Modification in fundamental metabolic functions of the placenta can occur as the result of specific drug action and these effects may profoundly influence fetal physiology.

Effects of Drugs on Monoamines in Developing Tissue

5-Hydroxytryptamine.—Significant phylogenetic differences have been demonstrated in the levels of 5-hydroxytryptamine, monoamine oxidase, and 5-hydroxytryptophan decarboxylase present at birth. Species which are developmentally immature at delivery, such as the rat and rabbit, contain low levels of 5-hydroxytryptamine in their cerebral tissues (66). At term, concentrations of 5-hydroxytryptamine in the neonatal goat (67) and guinea pig brain (68) are equivalent to those of the adult. The latter species by contrast are born with relatively well developed physiologic and biochemical mechanisms. The low tissue content of 5-hydroxytryptamine observed in neonatal rats and rabbits is probably not attributable to impairment of 5-hydroxytryptamine storage or binding. Fetal enterochromaffin cells of the rat contain 5-hydroxytryptamine at the eighteenth day of gestation and can also take up *l*-dihydroxyphenylalanine upon incubation with this amino acid (69). Cerebral mechanisms for the binding of 5-hydroxytryptophan seem to be fully developed at birth in the rat (70).

The activity of monoamine oxidase is respectively 38 and 43 per cent of adult levels in the brains of newborn rats (70) and livers of neonatal rabbits (71). Monoamine oxidase activity is present only in the microsomal fraction of one day old rabbit livers, appearing in the mitochondrial fraction on the fourth day of neonatal life and increasing in both fractions thereafter (71). No developmental pattern has been noted for 5-hydroxytryptophan decarboxylase which appears to be present in the brains of all newborn species thus far assayed and in concentrations equivalent to those in the adult (72). The rabbit liver, however, has low activity at birth and shows little change until the fourteenth postnatal day when a significant elevation occurs which parallels the increase in liver mass (71). Tryptophan hydroxylase seems to be the rate limiting process in the formation of 5-hydroxytryptamine (73) and the diminished levels of 5-hydroxytryptamine in cerebral tissues of the immature rat correlate well with the reduced hydroxylating capacity of neonatal brain (70).

The chronic administration of either iproniazid, reserpine, tranylcypromine, and alpha-methyldopa during pregnancy do not alter the ontogenetic sequence in which 5-hydroxytryptamine, monoamine oxidase, and 5-hydroxytryptophan decarboxylase appear. These agents produce minimal, short lived effects on 5-hydroxytryptamine tissue levels, monoamine oxidase and 5-hydroxytryptophan decarboxylase activity (70) which contrast sharply with the significant depletion of fetal and neonatal catecholamines induced by reserpine (74, 98, see catecholamine section). The placental passage of C^{14}-5-hydroxytryptamine is almost totally blocked whereas the transfer of precursors such as C^{14}-5-hydroxytryptophan is not impeded (75). Peak tissue concentrations of C^{14}-5-hydroxytryptophan occurred 20 minutes after maternal administration and were localized primarily in the adrenal medulla, intestine, and skin of the fetus.

Histamine.—While the overall rate of histamine synthesis is enhanced in pregnant animals, specific organs such as the renal cortices of pregnant mice and the hamster placenta possess unusually high histamine forming capacities. An extraordinary increase in histidine decarboxylase activity has been noted in fetal rat liver, exceeding the adult by some thousandfold (76). This enzyme appears in the kidneys of pregnant mice shortly after mating and reaches maximal levels several days prior to parturition. The fetal mouse develops a high activity of histidine decarboxylase a few days before term with rapid disappearance occurring after delivery (77).

Histamine concentrations in fetal rat brain peak at 17 days gestation, decrease sharply at 21 days and achieve maximal neonatal concentrations 5 to 10 days after birth. Significantly, no histidine decarboxylase activity has been noted in either fetal or neonatal brain tissue (78). The developmental pattern of histamine, in the central nervous system, differs uniquely from that occurring with other monoamines such as dopamine, norepinephrine, and 5-hydroxytryptamine which progressively increase in brain tissues from late gestation until maturity (66, 70, 79).

The precise role of histamine in the developing mammal is not clearly defined. It would appear that the increase in histamine content of many different tissues during embryogenesis is a reflection of the high level of protein synthesis occurring at this time. However, inhibition of protein synthesis with puromycin does not reduce the accelerated rate of histamine formation which occurs in neonatal or fetal rat liver (80). In view of these observations it has been suggested that histamine may function as a neurohormone, and in this capacity not merely reflect the active turnover of aminoacids (81).

Catecholamines.—Physiologic competency of the adrenergic nervous system during ontogenesis is contingent upon existing capabilities for synthesis, storage, inactivation, release, and response to adrenergic neurotransmitter.

The catecholamine content of most adrenergically innervated tissues is low during fetal and early neonatal life, generally approaching adult levels about 1 to 2 months after birth. This pattern is clearly demonstrable in hearts obtained from the developing rat (79), rabbit (82), dog (83), lamb (84), chicken (85), and frog (86); whereas, norepinephrine concentrations in neonatal bovine myocardium appear not to differ from those of the adult (87). The catecholamine levels of other tissues such as the spleen (rat), kidney (rat and dog), gastrointestinal tract (rat), salivary gland (rat), adrenal gland (sheep, human, rabbit), and brain (rat, rabbit, dog) also appear to follow a similar ontogenetic sequence.

The activity of catecholamine synthesizing enzymes is diminished in some fetal and neonatal tissues, resulting in lowered rates of norepinephrine formation. Tyrosine hydroxylase, the rate limiting step in catecholamine biosynthesis, exhibits very low activity in fetal sheep hearts (1/7 of adult levels) in contrast to the fetal sheep adrenal gland whose activity is equivalent to that of the adult (84). The sequential appearance of catecholamine synthesizing

enzymes in chick embryos has been described and occurs in the following pattern: tyrosine hydroxylase, day 1; dopa decarboxylase, day 2; dopamine beta-oxidase, day 4; and phenylethanolamine N-methyl transferase, day 6 (85). The activity of phenylethanolamine-N-methyl transferase is allegedly influenced by adrenal cortical steroids which exert an inducing effect upon this enzyme when administered in high concentrations (88). However, recent investigations in the neonatal rat which confirm the biphasic pattern of adrenal activity with respect to age, do not demonstrate any apparent inductive or regulatory influence of adrenal cortical hormones on phenylethanolamine-N-methyl transferase activity (89). This enzyme is of prime importance in the transmethylation of norepinephrine to epinephrine and its absence may explain the dominance of norepinephrine as the primary catecholamine in chromaffin tissues during embryologic development (90–92).

The mechanisms required for storing and binding endogenously synthesized or exogenously administered catecholamines are poorly developed in fetal life. The embryonic chick heart initially takes up H^3-norepinephrine on the fifth day of development and demonstrates an increasing capacity for uptake through the tenth day. Adrenergic innervation of the chick heart occurs on the fifth day and at this time H^3-norepinephrine can be localized in the microsomal component of subcellular fractions prepared from chick myocardium (93). Endogenous catecholamines may be identified in the total rat fetus at the thirteenth gestational day and in the fetal rat heart on the twenty-first gestational day (94). The binding of exogenous H^3-norepinephrine to microsomal fractions of fetal rat heart is not demonstrable until the twenty-first gestational day with maximal binding capacity developing between 14 and 21 days of neonatal life (94). A similar time course for the establishment of binding sites has been observed in the rat spleen. However, the gastrointestinal tract and salivary glands of neonatal rats are able to bind H^3-norepinephrine to the same degree as those of the adult (79).

The activity of catecholamine degrading enzymes appears to increase in a distinct ontogenetic sequence. Catechol-O-methyltransferase and monoamine oxidase were initially detected in the embryonic chick on the fourth day, reaching maximal levels by the nineteenth day and sharply declining on the second and third day after hatching (95). The concentration of monoamine oxidase in fetal lamb heart is about one-fourth that of the adult whereas catechol-O-methyltransferase is slightly higher in fetal myocardium than adult (84). The rat fetus and neonate are deficient in both enzymes so that catechol-O-methyltransferase and monoamine oxidase activity is present at levels about 20 per cent of that observed in the adult (96). The human neonate also appears to be deficient in its capacity for oxidative deamination of catecholamines with the result that the majority of excretory products are O-methylated (97).

The response of the neonate to the catecholamine depleting actions of

reserpine and tetrabenazine appears to be greater than that of the adult (98). Dose-response curves relating catecholamine depletion to the concentration of reserpine administered indicate that the sensitivity of the neonatal brain to the action of reserpine gradually decreases as age increases. Assays of H^3-reserpine after administering equivalent mg/kg doses reveal plasma concentrations in the neonate which are 6 to 7 times greater and plasma/brain concentration ratios twofold greater than corresponding adult values (99). The susceptibility of the newborn to the catecholamine depleting effects of reserpine appears to be caused by a diminished neonatal capacity for metabolizing reserpine and may also reflect an intrinsically greater sensitivity of immature norepinephrine storage sites to the action of this drug.

LITERATURE CITED

1. Karnofsky, D., *Ann. Rev. Pharmacol.*, **5**, 447–72 (1965)
2. Murphy, M. L. Factors influencing teratogenic response to drugs. In *Teratology, Principles and Techniques*, 145–61 (Wilson, J. G., Warkany, J., Eds., University Chicago Press, Chicago, 1965)
3. Yaffe, S. J., *Ann. Rev. Med.*, **17**, 213–34 (1966)
4. Sereni, F., Principi, N., *Ann. Rev. Pharmacol.*, **8**, 453–66 (1968)
5. Fouts, J. R. Hepatic microsomal drug metabolism in the perinatal period. In *Diagnosis and Treatment of Fetal Disorders*, 291–304 (Adamsons, K., Ed., Springer-Verlag, New York, 1968)
6. Kupferberg, H. J., Way, E. L., *J. Pharmacol. Exptl. Therap.*, **141**, 105–12 (1963)
7. Henderson, F. G., Chen, K. K., *Proc. Soc. Exptl. Biol. Med.*, **68**, 350–51 (1948)
8. Bopp, B. A., Kupferberg, H. J., *Federation Proc.*, **28**, 609 (1969)
9. Apgar, V., Burns, J. J., Brodie, B. B., Papper, E. M., *Am. J. Obstet. Gynecol.*, **64**, 1368–70 (1952)
10. Sanner, J. H., Woods, L. A., *J. Pharmacol. Exptl. Therap.*, **148**, 176–84 (1965)
11. Crawford, J. S., Rudofsky, S., *Brit. J. Anesthethia*, **37**, 927–33 (1965)
12. Hanekamp, E. H., Magaletta, G. E., Lankford, H. G., Hartnett, J., *Southern Med. J.*, **54**, 274–77 (1961)
13. Sjövall, A., Trolle, D., Boe, F., Vara, P., *Acta Obstet. Gynecol. Scand.*, **46**, (Supp. 2), (1967)
14. Gordon, H. R., *New Engl. J. Med.*, **279**, 910–14 (1968)
15. Shnider, S. M., Way, E. L., *Anesthesiology*, **29**, 951–57 (1968)
16. Finster, M., Poppers, P. J., Sinclair, J. C., Morishima, H. O., Daniel, S. S., *Am. J. Obstet. Gynecol.*, **92**, 922–24 (1965)
17. Sinclair, J. C., Fox, H. A., Lentz, J. F., Fuld, G. L., Murphy, J., *New Engl. J. Med.*, **273**, 1173–77 (1965)
18. Morishima, H. O., Daniel, S. S., Finster, M., Poppers, P. J., James, L. S., *Anesthesiology*, **27**, 147–54 (1966)
19. Morishima, H. O., Adamsons, K., *Anesthesiology*, **28**, 343–48 (1967)
20. Mirkin, B. L., *J. Am. Med. Assoc.*, in press (1970)
21. Harbison, R. D., Eling, T. E., Becker, B., *Federation Proc.*, **28**, 676 (1969)
22. Gabler, W. L., *Arch. Intern. Pharmacodyn.*, **175**, 141–52 (1968)
23. Gibson, J. E., Becker, B. A., *Proc. Soc. Exptl. Biol. Med.*, **128**, 905–09 (1968)
24. Meadow, S. R., *Lancet*, **2**, 1296 (1968)
25. Meadow, S. R., Personal Communication (1969)
26. Kalser, S. C., Forbes, E., Junig, R., *J. Pharm. Pharmacol.*, **21**, 109–13 (1969)
27. Catz, C., Yaffe, S. J., *J. Pharmacol. Exptl. Therap.*, **155**, 152–56 (1967)
28. Bianchine, J. R., Ferguson, F. C., *Proc. Soc. Exptl. Biol. Med.*, **124**, 1077–79 (1967)
29. Chaplin, M. D., Sladek, N. E., Mannering, G. J., *Federation Proc.*, **27**, 349 (1968)
30. Ebert, A. G., Yim, G. K. W., *Toxicol. Appl. Pharmacol.*, **3**, 182–87 (1961)

31. Adelman, M. H., Fisch, J., Jacobson, E., Katz, J., *Anesthesiology,* **19,** 93–94 (1958)
32. Wiser, R., Knebel, C., Seifter, J., *Pharmacologist,* **2,** 83 (1960)
33. Lacomme, M., LeLorier, G., *Bull. Federation Soc. Gynecol. Obstet, Langue Franc,* **7,** 119–27 (1955)
34. Behn, W., Frahm, M., Fretwurst, E., *Klin. Wochschr.,* **34,** 872 (1956)
35. Crawford, J. S., Rudofsky, S., *Brit. J. Anesthesia,* **37,** 303–09 (1965)
36. Crawford, J. S., Rudofsky, S., *Brit. J. Anesthesia,* **37,** 310–13 (1965)
37. Gehring, P. J., Buerge, J. F., *Toxicol. Appl. Pharmacol.,* in press (1969)
38. Gehring, P. J., Buerge, J. F., *Toxicol. Appl. Pharmacol.,* in press (1969)
39. Wieland, T., *Science,* **159,** 946–52 (1968)
40. Brock, N., Hohorst, H. J., *Cancer,* **20,** 900–04 (1967)
41. Brock, N., *Cancer Chemotherapy Rept.,* **51,** 315–25 (1967)
42. Gibson, J. E., Becker, B. A., *Teratology,* **1,** 393–98 (1968)
43. Posner, H. S., Graves, A., King, G. T. G., Wilk, A., *J. Pharmacol. Exptl. Therap.,* **155,** 494–505 (1967)
44. Diczfalusy, E., *Federation Proc.,* **23,** 791–98 (1964)
45. Levitz, M., Dancis, J., *J. Clin. Obstet. Gynecol.,* **6,** 62–75 (1963)
46. Meigs, R. A., Ryan, K. J., *Biochim. Biophys. Acta,* **165,** 476–82 (1968)
47. Staues, L., Goldenburg, N., Hirota, K., Okudaira, Y., Symposium on the placenta. In *Birth Defects, Original Article Series I* (National Foundation Pub., New York, 1965)
48. Juchau, M. R., Niswander, K. R., Yaffe, S. J., *Am. J. Obstet. Gynecol.,* **100,** 348–56 (1968)
49. Welch, R. M., Harrison, Y. E., Gommi, B. W., Poppers, P. J., Finster, M., Conney, A. H., *Clin. Pharmacol. Therap.,* **10,** 100–09 (1968)
50. Van Petten, G. R., Hirsch, G. H., Cherrington, A. D., *Can. J. Biochem.,* **46,** 1057–61 (1968)
51. Dixon, R. L., Willson, V. J., *Arch. Intern. Pharmacodyn.,* **172,** 453–66 (1968)
52. Juchau, M. R., Yaffe, S. J., *Intern. Symp. Foeto-Placental Unit,* Milan, Italy, Excerpta Medica, Intern. Congr., Ser. 170, 12 (1968)
53. Conney, A. H., Levin, W., Jacobson, M., Kuntzman, R., Cooper, D. Y., Rosenthal, O., *Proc. Intern. Symp. Microsomes Drug Oxidations,* Bethesda, Maryland, in press (1969)
54. Dvorák, K., *Intern. Sym. Foeto-Placental Unit,* Milan, Italy, Excerpta Medica, Intern. Congr., Ser. 170, 12 (1968)
55. Welch, R. M., Harrison, Y., *Pharmacologist,* **9,** 202 (1967)
56. Welch, R. M., Gommi, B. W., Conney, A. H., *Federation Proc.,* **28,** 676 (1969)
57. Pecile, A., Chiesara, E., Finzi, C., Conti, F., *Intern. Symp. Foeto-Placental Unit,* Milan, Italy, Excerpta Medica, Intern. Congr., Ser. 170, 12 (1968)
58. Marsh, C. A., Reid, L. M., *Biochim. Biophys. Acta,* **78,** 726–28, (1963)
59. Marsh, C. A., Carr, A. J., *Clin. Sci.,* **28,** 209–17 (1965)
60. Mowat, A. P., *J. Endocrinol.,* **42,** 585–90 (1968)
61. Benzi, G., Berté, F., Crema, A., Arrigoni, E., *J. Pharm. Sci.,* **57,** 1031–32 (1968)
62. Ginsburg, J., Jeacock, M. K., *Biochim. Biophys. Acta,* **90,** 166–68 (1964)
63. Beaconsfield, P., Ginsburg, J., Kosinski, Z., *Nature* **205,** 50–51 (1965)
64. Ginsburg, J., *Proc. Roy. Soc. Med.,* (Symposium on life before birth), **61,** 1244–47 (1968)
65. Ginsburg, J., Jeacock, M. K., *Am. J. Obstet. Gynecol.,* **100,** 357–65 (1968)
66. Karki, N., Kuntzman, R., Brodie, B. B., *J. Neurochem.,* **9,** 53–58 (1962)
67. Pepeu, G., Giarman, N., *J. Gen. Physiol.,* **45,** 575–83 (1962)
68. Smith, S. E., Stacey, R. S., Young, I. M., In *Proc. Intern. Pharmacol.* **8,** 101 (Uvnäs, B., Ed., Macmillan, New York, 1962)
69. Håkanson, R., Owman, C., Sjöberg, N. O., *Acta Physiol. Scand.,* **75,** 213–20 (1969)
70. Bennett, D. S., Giarman, N. J., *J. Neurochem.,* **12,** 911–18 (1965)
71. Bojanek, J., Bozkowa, K., Kurzepa, S., *Biol. Neonatorum,* **9,** 203–14 (1965/66)
72. Tissari, A., *Biochem. Pharmacol.,* **12,** Suppl. 39 (1963)
73. Cooper, J. R., Melcer, I., *J. Pharmacol. Exptl. Therap.,* **132,** 265, (1961)
74. Towell, M. E., *J. Obstet. Gynaecol. Brit. Commonwealth,* **73,** 431–38 (1966)
75. Ritzén, M., Hammarström, L., Ullberg,

S., *Biochem. Pharmacol.*, **14**, 313–21 (1965)
76. Kahlson, G., Rosengren, E., *Physiol. Rev.*, **48**, 155–96 (1968)
77. Håkanson, R., *European J. Pharmacol.*, **1**, 34–41 (1967)
78. Pearce, L., Schanberg, S., *Federation Proc.*, **28**, 353 (1969)
79. Iversen, L. L., De Champlain, J., Glowinski, J., Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **157**, 509–16 (1967)
80. Grahn, B., Hughes, R., Kahlson, G., Rosengren, E., *J. Physiol.*, **200**, 677–85 (1969)
81. Ryan, M. J., Brody, M., *Federation Proc.*, **28**, 353 (1969)
82. Friedman, W. F., Pool, P. E., Jacobowitz, D., Seagren, S. C., Braunwald, E., *Circulation Res.*, **23**, 25–32 (1968)
83. Mirkin, B., personal communications, (1969)
84. Friedman, W. F., *Proc. Soc. Pediat. Res.*, in press (1969)
85. Ignarro, L. J., Shideman, F., *J. Pharmacol. Exptl. Therap.*, **159**, 38–48 (1968)
86. Caston, J. D., *Develop. Biol.*, **5**, 468–82 (1962)
87. Vogel, J. H. K., Jacobowitz, D., Chidsey, C. A., *Circulation Res.*, **24**, 71–84 (1969)
88. Wurtman, R. J., Axelrod, J., *J. Biol. Chem.*, **241**, 2301–05, (1966)
89. Philpott, J. E., Zarrow, M. X., Denenberg, V. H., Lu, K.-H., Fuller, R., Hunt, J., *Life Sci.*, **8**, 367–71 (1969)
90. West, G. G., Shepherd, D. M., Hunter, R. B., *Lancet*, **1**, 966–69, (1951)
91. Brundin, T., *Acta Physiol. Scand.*, **66**, 406–09 (1966)
92. Comline, R. S., Silver, M., *J. Physiol.*, **156**, 424–44 (1961)
93. Ignarro, L. J., Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **159**, 49–58 (1968)
94. Mirkin, B. L., *Proc. 2nd Intern. Neurochemistry Meeting*, Milan, Italy, Sept. (1969)
95. Ignarro, L. J., Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **159**, 29–37 (1968)
96. Glowinski, J., Axelrod, J., Kopin, I. J., Wurtman, R. J., *J. Pharmacol. Exptl. Therapy.*, **146**, 48–53 (1964)
97. Brunjes, S., Castner, E., Hodgman, J., *Clin. Res.*, **12**, 88 (1964)
98. Kulkarni, A. S., Shideman, F. E., *J. Pharmacol. Exptl. Therapy.*, **153**, 428–33 (1966)
99. Mueller, R. A., Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **163**, 91–97 (1968)