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DETERMINANTS OF DRUG DISPOSITION AND EFFECT IN THE FETUS

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INTRODUCTION

The disposition of pharmacologically active molecules in the maternal-placental-fetal unit has been discussed in several recent books and reviews (1–3). Considerable data have accumulated to indicate that nearly all drugs enter the fetal circulation following maternal administration and may exert different effects upon the fetus. Hence, it is of considerable significance to determine the influence of biological maturation on placental transfer, the effects of drugs on placental function, fetal drug disposition, and the reactivity of the fetus to pharmacologic agents.

This review considers aspects of developmental pharmacology that have not been extensively considered in previous publications, examining in particular the following subject areas:

1. The determinants of placental transfer with emphasis on how biological maturation modifies these factors and influences fetal drug disposition.
2. The fetal hepatic circulation and its effect on drug metabolism.
3. The role of the adenyl cyclase–cyclic nucleotide system in regulating receptor reactivity and the fetal response to drugs.

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PLACENTAL TRANSFER DURING BIOLOGICAL MATURATION

The maternal-placental-fetal unit is a unique pharmacological system, consisting of two independent circulations, each autonomously regulated with differing chemical composition and physical properties. While serving to isolate mother from fetus in some respects, the intervening placenta must allow bidirectional transfer of water, electrolytes, nutrients, and organic wastes. As the requirements of the fetus change quantitatively and qualitatively during gestation, so must the factors that limit placental transfer, if fetal growth and homeostasis are to be maintained. The details of this process have been difficult to unravel experimentally because in vivo models such as the chronically catheterized sheep or primate fetus can only be established near term. Additionally, it has become apparent that many of the factors governing placental transfer differ fundamentally between species. Histological differences noted in the placentas of these species often correlate with differences in the mechanisms controlling the transplacental passage. Hence, nonprimate placentas do not approximate the properties of the human placenta. Because these data are very difficult to obtain from humans, we must generally rely on primate models and selected experiments from other species that must be cautiously interpreted in light of the effects species-dependent factors can exert on placental transfer.

This section considers the influence of biological maturation on three factors governing drug transfer in the placenta, namely placental circulation, placental permeability, and drug-protein interactions.

Placental Circulation

Blood flow constitutes a major rate-limiting factor determining placental transfer of the more lipid-soluble drugs. At least two properties of the placental circulation must be considered, vessel geometry and blood flow.

The importance of vessel geometry has been elegantly explained by Faber (4, 5). He performed an analysis of the limitations on exchange imposed by geometry of the placental vessels drawn from the theory of heat exchangers and examined simple models for exchange including countercurrent, cross-current, and pool flow types. A few mathematical expressions with a minimum of terms describe this interesting system:

$$T^M = C_A^M - C_V^M / C_A^M - C_A^F \text{ and } T^F = C_A^F - C_V^F / C_A^M - C_A^F,$$

T is the normalized arteriovenous difference of a tracer compound on one side of the membrane and denotes to what degree the concentration of the tracer compound approaches the arterial concentration on the opposite side of the placental membrane; C denotes the concentration of

a tracer; superscripts M and F denote maternal and fetal locations while subscripts A and V indicate arterial and venous concentrations; d is a permeability variable which includes the membrane permeability P and the flows through maternal ($\mathring{Q}^M$) and fetal ($\mathring{Q}^F$) sides of the placenta: $d = P/(\mathring{Q}^M \cdot \mathring{Q}^F)^{1/2}$. Only unbound flow-limited tracers are considered here to illustrate the influence of vessel geometry on exchange. This model is used below as a framework for understanding the contributions of other determinants.

Each of the model systems can be described by a T diagram (Figure 1). Abcissa and ordinate are T_F and T_M. Since $T_M/T_F = \mathring{Q}^F/\mathring{Q}^M$ follows from Fick's principle, a family of straight lines, passing through the origin and radiating out with a slope $\mathring{Q}^F/\mathring{Q}^M$, can be superimposed demonstrating the influence of relative changes in the flow of the fetal and/or maternal circulations. Isotherms, with constant d, shown in the diagrams, illustrate that at any given flow rate, transfer will be more efficient for a more permeable marker.

Visual inspection yields important information about the comparative characteristics of different vessel geometries. At any given $\mathring{Q}^F/\mathring{Q}^M$ and d, the T values will be greater for the countercurrent exchanger than for other geometries, whereas an increase of d above a value of 1.0 in the concurrent or pool flow models increases transfer very little. Comparison between models also confirms that for less permeable molecules (e.g. $d = 0.1$) there is no limitation by vascular geometry on transfer, because the d-constant isotherm is essentially unchanged from one model to another. When the blood flows measured are uterine and umbilical flows, the analysis of trans-

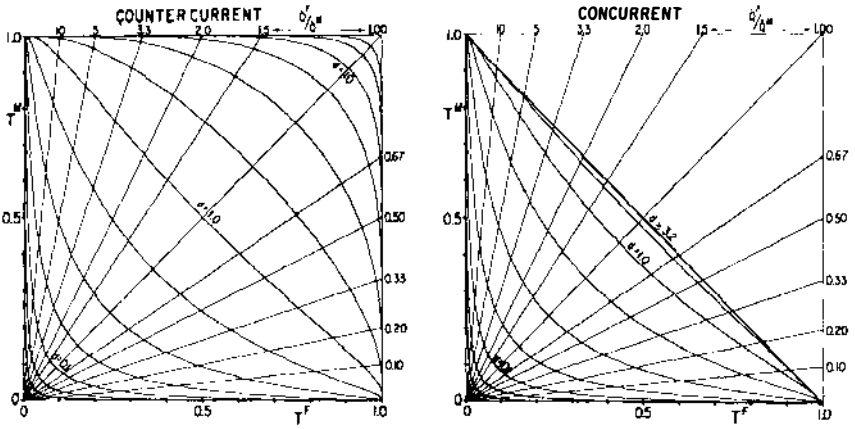

Figure 1 T diagrams computed for the countercurrent and concurrent exchangers, showing the relationship between T^M and T^F as a function of d [reproduced from (5) with permission].

fer is distorted, because an unknown fraction of each circulation will not in fact perfuse the exchanging surface but will be directed to other sites (6). This type of analysis allows evaluation of nonexchanging shunts in either circulation. A given d-constant isotherm will intercept the maternal or fetal axis at a fraction below 1.0 equal to the fraction of blood shunted away from the exchanging membrane.

Data from physiologic studies have been used to assign tentative vessel geometries following analysis by this method. Schröder & Leichtweiss (7) studied the transfer of tritiated water across the isolated guinea pig placenta by varying $\mathring{Q}^F/\mathring{Q}^M$. Since the isotherm obtained on a T diagram yielded $T^M + T^F > 1.0$ for most values, it was concluded that the placenta mimicked a more efficient exchanger, perhaps the countercurrent or cross-flow type. Further, the isotherm intersected the T^M axis at 0.8, implying a nonexchanging flow of about 20% (this agrees with shunt determination by microsphere method). Retrograde perfusion decreases the relative rate of transfer. These data are consistent with Schröder & Leichtweiss's hypothesis that orthograde flow is countercurrent with retrograde perfusion, which produces, therefore, a concurrent flow. Bailey's data (8) confirm the conclusion that placental flow in the guinea pig is countercurrent but suggest the absence of placental shunts.

Using the same method of analysis, Rankin & Peterson (9) carried out similar investigations in the goat placenta. Their results suggested a concurrent exchange system with sizable maternal and fetal shunts. The magnitude of the shunts was subsequently confirmed by another technique. Faber's analysis of previously published data (5) concerning transfer of oxygen and other flow-limited tracers shows blood flow in the sheep and goat placentas to conform to the concurrent model while that in the guinea pig and rabbit placentas mimics the countercurrent exchanger.

Anatomical and histologic studies have complimented and confirmed results obtained by physiologic methods. For example, Lee & Dempsey (10), using scanning and transmission electron microscopy on casts of rat placental vessels, confirmed a countercurrent vessel pattern. These techniques may be particularly valuable in understanding the limitations imposed by flow geometry in primate species, including man. Such methods suggest the presence of countercurrent flow in limited areas and concurrent flow in other areas (11). Anatomic studies may allow mathematical modeling that is more directed than the simple models mentioned above. Development of new physiologic models and anatomic techniques has allowed appreciation of species differences in vessel geometry and an understanding of the limits geometric configuration imposes upon the exchange of certain molecules.

While vessel geometry constrains the maximum rate of transfer of flow-limited molecules, the vessels themselves are subject to a number of influences during gestation that alter flow rates. Recent reviews (6, 12) on the control of placental blood flows have dealt at length with experimental methodologies. Technique is critical, since many studies have quantitatively determined uterine and umbilical flows, whereas the respective placental circulations may behave quite differently. On the other hand, the placenta is not innervated and neurogenic influences are mediated through control of preplacental and postplacental nerves as well as via neurohumoral mechanisms. There have been many conflicting observations and marked interspecies differences in response to specific agonists and antagonists.

Placental blood flow increases throughout pregnancy. Late in pregnancy this tendency continues at a rate that is proportional to fetal size, although placental mass, relative to the fetus, is reduced (6). Hormonal mediators effect profound circulatory changes during pregnancy. Since hormonal balance appears to be critical in the timing of many events during pregnancy, it is not hard to also imagine a role for hormones in blood flow regulation. Reactivity of the uterine vasculature to estrogens in fact may be confined to pregnancy. Data from sheep (13) show a locally mediated uterine vessel dilatation in response to estrogens which is prominent early in pregnancy but diminishes toward term. In the rabbit, estrogens have been shown to increase uterine blood flow, apparently, however, at the expense of placental flow (14). This response is apparently mediated by cholinergic vasodilator nerves which are present only in preplacental vessels (15) and activated primarily during pregnancy or following estrogen treatment (16).

Although α- and β-adrenergic receptors have been located in the uteri of experimental animals, their relative contributions to the maintenance of vasomotor tone may vary between species (12). Elnäs and associates (17) concluded from their studies in sheep that salbutamol, a β-agonist, caused a decrease in utero-placental blood flow in the absence of contractions. α-Agonists have also been shown, in several species, to decrease uterine blood flow. Experiments with adrenergic blockers have given varying results (12, 18).

Because the placenta is not innervated and yet is able to maintain flow to the fetus under a variety of perfusion pressures (19), some autoregulatory system is probably operational. Speroff (20) has speculated on the possible contribution of prostaglandins in this role. He notes that prostaglandin synthesis occurs in the pregnant uterus and is increased by angiotensin II. Further, inhibition of synthesis lowers basal blood flow and prevents the response to angiotensin II. Renin, or a renin-like substance, has been found to be elaborated by the gravid uterus in several species (21). This substance

may mediate vasodilatation locally via the uteroplacental angiotensin-prostaglandin pathway and through angiotensin promote additional effects systemically. Another study demonstrating autoregulation of blood flow in the placenta at varying perfusion pressures found no adverse influence of inhibiting prostaglandin synthetase (19). A number of other vasoactive hormones (e.g. serotonin, histamine, bradykinin) have been shown to affect the maternoplacental circulation; however, the nature of their physiologic role, if any, has not been defined (6, 22).

Nonpharmacological events may also influence uteroplacental blood flow and, therefore, nutrient and drug transfer. Butler et al (23) developed a mathematical model for assessing changes in blood flow during uterine contractions and predicted that oxygen transfer would be most sensitive to intensity of contraction and maternal blood pressure. Past difficulties in obtaining flow data during uterine contractions make this sort of theoretical analysis very helpful. Karlsson & Kjellmer (24) have quantitatively estimated placental blood flow using radioactive microspheres in pregnant rabbits subjected to hyperoxia. They found a decrease in blood flow accompanying a rise in resistance, suggestive of true placental vasoconstriction.

It is clear that regardless of the model used, alterations in the ratio of flow rates ($\mathring{Q}_F/\mathring{Q}_M$), such as might be produced by neurochemical mediators whose access was limited to one side of the placenta, would alter the T^F and T^M of most substances (see Figure 1). Walker (25) showed decreases in the placental clearance of antipyrine, a flow-limited tracer, during the maternal administration of epinephrine. At the same time passage of a diffusion-limited agent, urea, was not affected. Heymann (26) has discussed the probability that maternally administered local anesthetics, by decreasing fetal flow selectively, may have inhibited oxygen exchange. It should also be noted that changing flow proportionately on both sides of the placenta would also influence transfer rates as the permeability variable, *d,* will depend in some measure on the magnitude of flows.

Placental Permeability

The movement of drugs from the maternal to fetal circulation seems to occur primarily by diffusion. Active transport and facilitated diffusion, although important for endogenous molecules, must play a much more limited role for drugs. The sum of all factors limiting diffusion will be reflected in *P,* the permeability constant. The contribution made by chemical properties of the drug such as lipid solubility, pK_a, and molecular weight are well understood and have been elaborated upon by Mirkin (1). Placental metabolism of drugs might also modify the quantitative aspects of their transplacental passage. While the metabolism of hormones by this system has been shown to be developmentally significant (27), the importance of

xenobiotic metabolism by the placenta remains unclear and interested readers are referred to excellent reviews on this subject by Juchau (22, 28). In this section the physical properties of placental membranes, the water content of the placenta, and hypothesized mediator-controlled permeability of the placenta, all of which may be important in limiting transfer of some molecules, are considered with emphasis on changes occurring during biological maturation.

Although early work suggested that placental membrane thickness or number of placental layers limited diffusion, it is now clear that the situation is more complex. For example, the observed decrease in number of tissue layers and decrease in thickness of the trophoblast does not predict the decrease in permeability found between the first and second trimester in pregnant rodents (1). Thornburg & Faber (29) studied the resistance to diffusion among the three layers of the rabbit placenta. By comparing the resistance to diffusion with small molecular weight compounds, they concluded that the fetal endothelium, which is not markedly altered during pregnancy, is the layer responsible for diffusional resistance to larger molecular weight substances. For smaller polar molecules, diffusion through the sheep and rabbit placenta is consistent with presence of interstitial water-filled pores of fixed diameter. However, once again species differences are predominant; the calculated pore radii are 0.4 nm for sheep (30) and approximately 30 nm for the rabbit (29).

Ion metabolism and water content of the placenta can be expected to directly affect the permeability of hydrophilic molecules. Cittadini and co-workers (31), examining the distribution of water and ions in the rabbit placenta, found a decrease of 18% in tissue water between the ninth gestational day and the end of gestation. This was accompanied by a shift of fluid from intracellular to extracellular compartments, a corresponding increase in placental sodium, and a decrease in placental potassium. Concurrently, the total water content of the fetus also decreased consonant with a relative decrease in extracellular water and ions and increase in intracellular water and ions (32, 33). These changes in water content may affect not only the rate of diffusion of hydrophilic substances from the maternal circulation to the placenta and from the placenta to the fetal circulation, but the total quantity of drug moving from mother to fetus. Alterations in the nature of these relationships during gestation imply that the kinetics of drug transfer will also change correspondingly.

Water movement in the maternal-placental-fetal unit changes as pregnancy progresses and can influence drug transfer via solute drag as water is transferred from mother to fetus. Since maternal plasma is hyperosmotic with respect to fetal plasma (34), simple osmotic forces, which would draw water from the fetus to the mother, do not account for water transfer.

Conrad & Faber (35) were able to account for the movement of water across sheep placentae by a mechanism dependent upon hydrostatic pressure and the active transport of some solutes. Apparently, controlling the increased rate of water acquisition noted during gestation was the developmentally dependent alteration in partial placental permeability to major electrolytes. These investigators were able to show that variation of this parameter alone, and not alteration of other possible variables, accounted for the accelerated water transfer.

It has been hypothesized that permeability of the placenta to a variety of hydrophilic molecules may be modified by the placental cholinergic system (36, 37). Acetylcholine is without effect on placental vasculature, yet abundant acetylcholine, probably bound to membranes of the syncytiotrophoblast, is found in the human placenta (36). The concentration increases until 21–24 weeks of gestation and then gradually declines. Choline acetyl transferase also is present but acetylcholinesterase is conspicuously absent (38). Release of acetylcholine into the maternal circulation occurs spontaneously, but can be increased by either increasing the calcium concentration or adding nicotine to the perfusing medium (39). The nicotine effect did not occur in the absence of calcium, whereas cocaine decreased the spontaneous and nicotine-induced release of acetylcholine. The placenta was able to take up acetylcholine by an active, saturable process against a concentration gradient (40). Judging from the effects of drugs on this process, the uptake differs from that which occurs in nervous tissue (41). The lack of any acetylcholine-induced effects upon ^{32}Pi incorporation into phospholipids of the placenta implies that its mechanism of action may be different as well (37). In nervous tissue preparations, incorporation of Pi into polar phospholipids has been thought to be linked with the metabolic turnover of these compounds as well as control of permeability in excitable membranes. This effect apparently cannot be demonstrated in the human term placenta.

Harbison et al (42) have noted a correlation between uptake of phenytoin and α-aminoisobutyric acid during gestation with activity of the placental cholinergic system. While acetylcholine can affect membrane permeability in other tissues and is present in abundance in the placenta, the direct demonstration of such a role in this organ is lacking. If a link between the cholinergic system and placental permeability to hydrophilic molecules is established, however, one might be able to pharmacologically modify permeability with anticholinergic agents. Honey et al (43) have proposed, in a similar vein, that 5-hydroxytryptamine may reduce placental membrane permeability; however, further work is needed in this area.

Drug-Protein Interactions

The binding of drugs to plasma or tissue proteins has been thought to be a major determinant of placental transfer, but direct experimental data on

this subject are difficult to obtain. Goldstein, Aronow & Kalman (44) reasoned that protein binding might decrease the rate of equilibration across the placenta. In their example, one drug, *Y,* not protein bound, would take time, *t,* to reach equilibrium. Another drug, *X,* 90% protein bound on both sides of the placenta but transferred at the same rate, would require much longer to saturate its protein binding sites and reach the eventual same free drug equilibrium concentration as *Y.* Recent placental transfer data and extrapolations from protein binding studies in other systems indicates that this is only one possible effect of protein binding. It is a valid analysis only for drugs whose permeability across the placenta is rate limiting and whose protein binding characteristics are similar on both sides of the placenta.

Dancis, Jansen & Levitz (45) have used an in vitro human placental perfusion system to determine the effect of protein binding on the placental transfer of palmitic (46), hexanoic, and decanoic acids (45). The extent of transfer was ascertained with no protein present in the perfusate solutions, with protein on each side alone and finally with equal concentrations in both perfusates. The transfer of decanoic acid in the presence of equivalent concentrations of protein on both sides of the placenta was significantly slower than transfer determined when both sides were perfused with buffer. Significantly, this effect was less marked with hexanoic acid, which is protein bound less avidly. These observations are consistent with the predictions of Goldstein et al that protein binding would decrease transfer rates. However, the experiments demonstrated that transfer was more rapid with protein on both sides of the placenta than would be predicted by diffusion of the unbound compound alone. This outcome indicates that either permeability of the free drug is not limited and that protein binding is rapidly reversible or that the bound form is available for trans-placental passage. The authors speculate that for highly lipophilic compounds whose water solubility is limited, protein binding may allow an increased delivery to the placenta and subsequently result in greater transfer. An analogous situation would be the transfer of oxygen, whose unbound fraction constitutes a very small percentage of the total blood content, yet rapid reversibility of binding essentially allows a flow limited transfer of total blood oxygen.

Whether protein binding will hinder or promote placental transfer depends on a variety of factors. Even when bound drug is not itself allowed transfer through the placenta, a possibility considered later, if diffusion through membranes is rapid and the dissociation constant (k_1) is large relative to rate of blood flow through the placenta, protein binding will effectively increase the amount transferred by allowing more drug to be presented to the placenta. It is possible that a drug that is tightly bound (high association constant, *Ka*) might have a rapid rate of dissociation, as seen from the equation $Ka = k_1/k_{-1}$, provided that an avid association process accounts for the high equilibrium constant. The interplay of these

factors has varying effects in other physiologic systems. A high degree of protein binding inhibits removal of drug from the plasma when the elimination processes are relatively inefficient, e.g. glomerular filtration, but increases elimination when the active systems are very efficient, e.g. hepatic enzymes or the active transport systems of the renal tubule (47).

The possibility that the protein-bound drug itself may undergo transport remains a distinct possibility and would alter the kinetics of placental transfer (48). The placenta is not impermeable to proteins and a wide variety of proteins have been shown to cross the placenta (49, 50). While gamma globulin seems actively transported, the smaller proteins including albumin appear to be dependent upon diffusion alone. Whether such a pathway exists for drug molecules is unknown, although it conceivably could represent an important mode of transfer for highly protein-bound, poorly soluble substances. For example, vitamin A is transported through the rat placenta by the retinol binding protein to which it binds in maternal serum (51).

The actual and hypothesized effects of protein binding on placental drug transfer that have been discussed so far are based on the assumption that the degree of protein binding on each side of the placenta is similar. In fact, the protein binding of specific drug molecules differs significantly in the fetal and maternal circulations (52, 56). The apparent reasons for this phenomenon and its consequences are discussed below.

Factors that determine the extent of drug binding to proteins include temperature, pH, drug concentrations, presence of substances (e.g. bilirubin, fatty acids, hormones, other drugs) competing for same binding sites or allosterically altering the albumin molecule and, finally, the concentration and association constants of the binding sites. These parameters differ in varying degrees when maternal and fetal serum proteins are compared. The difference in temperature and pH is too small to affect binding, but each of the other factors bears examination.

Miyoshi et al (57) demonstrated that albumin isolated from umbilical cord and adult sera by ammonium sulfate precipitation differed in their electrophoretic and chromatographic patterns as well as in their resistance to proteolytic hydrolysis and alkali denaturation. They suggested that at birth 50% of the total plasma albumin pool consists of the fetal form with the remainder present in the adult form. However, Ganapathy & Cohen (58) found albumin from these sources to have identical molecular weights by ultracentrifugation, very similar spectroscopic properties, and indistinguishable association constants for the binding of sulfadiazine. They concluded that no qualitative differences existed. This discrepancy was resolved by three groups. Chignell and colleagues (55) observed a decreased binding affinity for sulfenazole in albumin obtained from neonatal serum when

compared with adult albumin. This difference was eliminated by pretreatment of the neonatal protein with charcoal, a process that removes endogenous ligands such as fatty acids and bilirubin. Charcoal-treated fetal and adult bovine serum albumins were studied by Huntley, Neitzel & Elson (59) who found the two preparations to be similar in their electrophoretic properties and in the binding of diphenylhydantoin, salicylate, and six fatty acids. Tuilié & Lardinois (60) determined the bilirubin binding capacity of albumin, serum, and lipid-free albumin of adult and cord origin and concluded that endogenous protein-bound ligands accounted for the previously demonstrated differences in albumin properties.

Other factors also contribute to the decreased drug binding capacity of fetal sera when compared with maternal sera. Analysis of rat sera obtained at different stages of gestation has demonstrated that the albumin concentration in the fetus is very low early in pregnancy (61), a finding that would support the argument that binding is decreased in the fetal circulation (54). The albumin concentration then increases markedly near term (61). In addition, several unique fetal globulins are present and it has been shown that binding to nonalbumin proteins can be important. Bilirubin, although bound with greater avidity to albumin, binds to other serum proteins when bilirubin/albumin molar ratio is above two (60). Bupivacaine, a local anesthetic, is 80–92% bound to maternal plasma, but only 30–35% to purified albumin in the same concentration (53). There are also significant quantitative differences in the globulin proteins between mother and fetus. The differences in globulin concentrations are more marked than those noted for albumin, especially early in pregnancy (53, 54). The contributions of individual nonalbumin proteins to drug protein binding and the variation with gestation would require an extremely tedious study. Nonetheless, differences in the concentrations of albumin and globulins account for an important part of the decreased protein binding of many drugs found in fetal sera. In passing, it is interesting to note that plasma protein concentrations may vary considerably from patient to patient and are subject to variation during pathophysiologic states. Studies from India (62) and Iran (63) showed significantly lower serum protein concentrations in the less affluent socioeconomic classes, perhaps reflecting an unfavorable nutritional state. Plasma proteins may also be selectively affected in the fetus by other conditions such as maternal infections (64).

The consequence of unequal protein binding on opposite sides of the placenta can best be understood by returning to Faber's model for placental exchange. Faber used an additional factor, f, for tracers that bind rapidly and reversibly to blood components. It is defined as the ratio of tracer present per volume of blood divided by the concentration present in physical solution. All expressions where flow, $\mathring{Q}$, appears are replaced with $f\mathring{Q}$;

for example, with oxygen, f would be about 100 at 50% saturation. The factor f can be shown to be related to the association constant, Ka, by the equation $f = Ka \cdot [A] + 1$, where $[A]$ is the concentration of drug binding sites; the expression is strictly true only if $[A]$ is large relative to the number of occupied sites. As drug concentration increases, f will not be a constant, but will fall toward a limit of one as free drug concentration increases. (Note also that this use of the term Ka, when we are considering drug binding to whole serum rather than a purified protein fraction, represents a composite of the Ka's of each binding site.) It follows that the influence of f on placental transfer will be significant when $Ka[A]$ is greater than one. This will be true for most protein-bound drugs, and f will be greater at higher values of Ka. This phenomenon is best illustrated by the data of Dancis el al (45) obtained during studies with hexanoic and decanoic acid which have Ka's of 1.5×10^4 M^{-1} and 1.0×10^5 M^{-1}, respectively. When both sides of the placenta were perfused with solutions containing similar concentrations of albumin and the results compared with the experiment in which no albumin was present, the transfer of hexanoic acid was marginally affected, whereas in a parallel experiment the transfer of decanoic acid was markedly affected. Since $\mathring{Q}^F/\mathring{Q}^M$ was constant between experiments, the most likely responsible factor was a critical difference in the permeability variable d, due to the difference in f.

Some of the consequences that may result from having different degrees of protein binding on opposite sides of the placenta are discussed below. The smaller Ka and lower concentrations of binding protein on the fetal side will lead to lower f values than on the maternal side. This discrepancy will affect the flow ratio $\mathring{Q}^F/\mathring{Q}^M$, since it will now be $f^F\mathring{Q}^F/f^M\mathring{Q}^M$, decreased by a factor f^F/f^M. The permeability variable d will also be changed; it will become $d = P/(f^F\mathring{Q}^F \cdot f^M\mathring{Q}^M)½$. The effects of these changes on the transfer of four theoretical drugs with Ka's contrived to yield a convenient percentage of drug bound at some fixed, arbitrary drug concentration are described in Table 1. Values of f were calculated and a value of d in the absence of protein binding was selected. The value of T^F was then derived from Figure 1 in the absence of protein and then derived again considering the given values for binding. Dissociation was assumed to be rapid compared to the placental circulation time.

Example A in Table 1, a loosely bound drug with a small maternal-fetal difference, indicates that protein binding would lead to a slightly higher T_F for a more lipid-soluble molecule ($d = 10$) but to a small decrease for a less permeable one ($d = 1$). Example B, a more tightly bound drug with slightly greater differential in binding, shows that the effect of protein binding can be dependent on vessel geometry. With the more permeable molecule, protein binding results in a slight decrease, 0.91 to 0.88, when

Table 1 The influence of differential protein binding on drug transfer

	Percentage of drug bound			Countercurrent model		Concurrent model	
Example	Maternal	Fetal	d[a]	T^F[b]	T^F + protein	T^F	T^F + protein
A	50.0	36.1	10	0.91	0.93	0.50	0.57
			1	0.50	0.40	0.43	0.38
B	89.1	71.4	10	0.91	0.88	0.50	0.72
			1	0.50	0.23	0.43	0.23
C	98.4	98.0	10	0.91	0.17	0.50	0.17
D	99.2	96.1	10	0.91	0.32	0.50	0.32

[a] d = Permeability variable in the absence of protein binding.
[b] T^F = Normalized fetal arteriovenous drug concentration difference determined with and without protein in perfusate (see text for further explanation).

placental flows are countercurrent, but a rather large increase, 0.50 to 0.72, when the flows are concurrent. The T_F decreases sharply in both models when $d = 1$, since protein binding decreases the permeability variable further and makes it essentially flow-independent. Examples C and D illustrate situations where protein binding is considerable; in C a small difference and in D a large difference exist between the extent of protein binding in each of the circulations. Although d without binding is large, d with this high degree of binding makes permeability independent of flow. Comparison of the two examples, however, shows that the greater discrepancy in protein binding in example D is more important than the more avid protein binding on the maternal side and allows considerably greater transfer: T_F's equal 0.32 and 0.17 in D and C, respectively.

Protein binding is also one factor that may affect the rate of nonplacental elimination of the drug from the maternal circulation (65). An understanding of how maternal drug kinetics can influence placental transfer is found in Goldstein, Aronow & Kalman (44). They describe the kinetics of a flow-limited drug using flow rates approximated from humans and a pool-flow vessel geometry. Drugs that achieve steady state levels in the mother will take about 40 min to reach equilibrium in the fetus. Drugs whose half-life is short in relation to this equilibrium time will have significant elimination from the mother during this period and hence may reach equilbrium much sooner. One must conclude, however, that levels approaching those seen in the mother will be seen only in situations where a steady state or relatively slow elimination exists. Other drugs may cross the placenta, but will reach equilibrium at levels considerably below the peak levels achieved in the mother. The rapid redistribution and elimination of thiopental, when considered in this analysis, explain the lack of effect seen in babies born to anesthetized mothers. One must also remember that this

is a flow-limited analysis. Drugs that do not readily cross the placental barrier for a variety of reasons will reach equilibrium much more slowly. Further, any interference with blood flow via drugs that modify the placental flow characteristics (see above) or contractions (66) will further impede the process. These factors will be significant in preventing fetal exposures to anesthetic agents at term. Drug-protein binding as it affects maternal drug kinetics will alter the amount of drug transferred to the fetus. The avid protein binding of the cholecystographic dye iophenoxic acid prevents elimination from the mother, maintains a steady state concentration in maternal serum, and allows equilibrium of maternal and fetal serum to be reached (67).

The conclusion must be that the extent of protein binding as determined by maternal and fetal protein concentrations, intrinsic association constants, and competing drugs and endogenous ligands is an important determinant of the rate of placental transfer. This will be more significant when protein binding is strong and when maternal-fetal differences are present. The transfer of more lipid-soluble drugs might be enhanced, especially if their aqueous solubility is limited; the transfer of more permeability limited ones may be retarded. Because our present knowledge of permeability constants, association constants, and flow rates is limited and because vessel geometrics rarely relate perfectly to the models, empirical determinations of the effect of protein binding of a given drug in a given species is not possible. More carefully controlled in vitro models, perhaps patterned after that of Dancis et al (45), may allow more accurate assessment of the contribution of this determinant.

FETAL HEPATIC CIRCULATION AND DRUG METABOLISM

The fetal liver occupies a unique, anatomical position, standing interposed between the umbilical vein and the inferior vena cava, so that all pharmacologically active molecules passing the placenta must traverse this organ prior to entering the fetal systemic circulation (Figure 2). Consequently, factors that modify total blood flow and its intrahepatic distribution within the fetal liver may significantly alter the metabolic degradation of drugs by the fetal liver.

Aside from its primary role in providing essential substrates and oxygen for tissue growth, the fetal circulation can also be viewed as a drug delivery system. It is important, therefore, to understand which characteristics of the developing cardiovascular system undergo change during maturation, and, in particular, whether these significantly affect the delivery of drugs to their

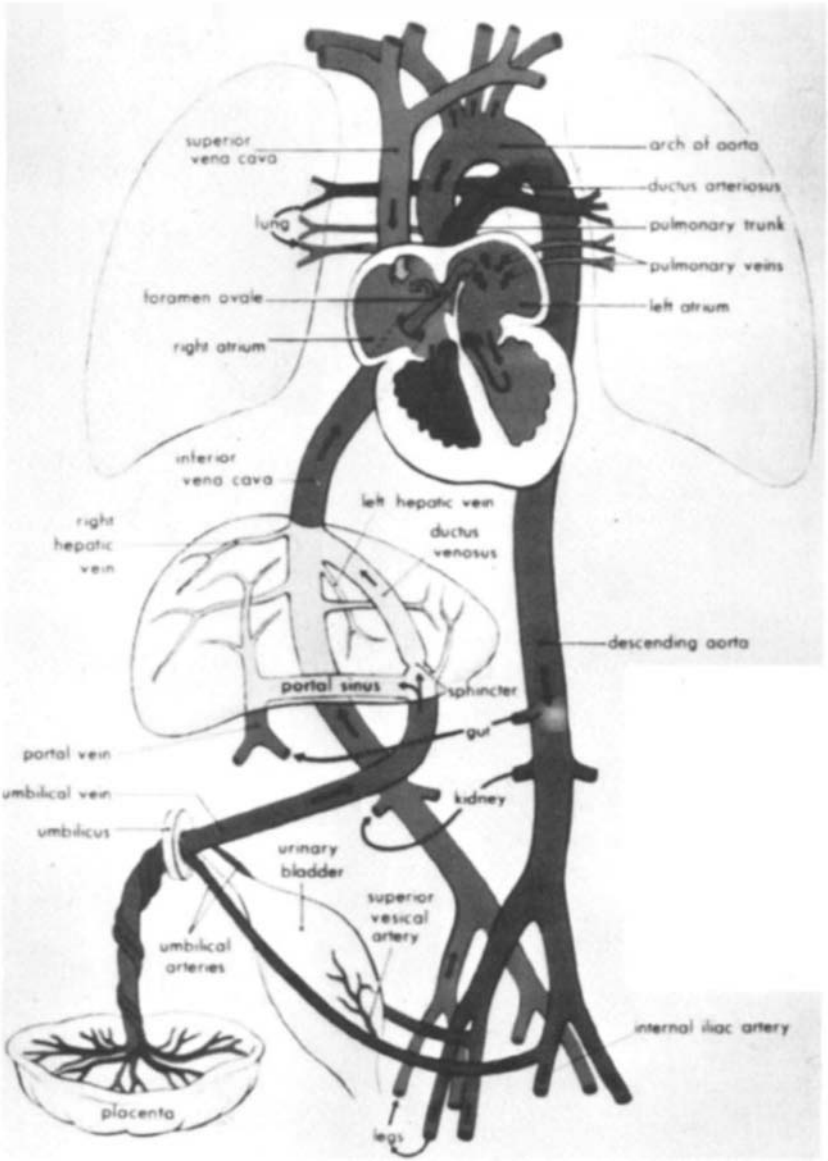

Figure 2 Diagram of the ovine placental and fetal circulations.

ultimate sites of biotransformation. The analysis of drug disposition in the maternal-placental-fetal unit must consider not only whether drugs cross the placenta or what the fetus's capacity to respond is, but also what factors other than maturation can alter the fetus's ability to modify drug molecules to inactive, active, or potentially toxic compounds. It is from this perspective that the fetal hepatic circulation and, in particular, its low pressure–high flow vascular shunts are reviewed.

All drugs pass through the liver before they are carried by the blood into the inferior vena cava to the fetal right heart. The umbilical venous flow is diverted in the fetal liver, so that substantial portions may enter the ductus venosus and effectively bypass the liver, while the remainder flows into the portal vein and perfuses the hepatic parenchymal cells. The proportion of umbilical blood flow which is distributed between these circuits may profoundly affect the quantity of metabolized and nonmetabolized drug circulating in the fetus at any particular point in time.

If a major portion of the total umbilical venous flow were shunted through the low pressure–high flow conduit provided by the ductus venosus, relatively high concentrations of pharmacologically active drugs would perfuse the fetal right heart and also be distributed to the central nervous system. Drugs that exhibit a high hepatic extraction ratio following a single pass would be greatly affected by the intrahepatic diversion of blood flow into the ductus venosus, since these highly metabolized molecules would be relieved of this constraint and potentially achieve hazardous drug concentrations. Since the concentration of drug achieved in peripheral tissue is flow and diffusion limited, increasing the initial plasma concentration of drug by selective intrahepatic partitioning would lead to a higher plasma-to-tissue drug concentration gradient and, ultimately, elevated tissue levels.

It has been well established that the human fetal liver can oxidize many xenobiotic substrates as early as the sixteenth week of gestation (68). Drugs that undergo extensive biotransformation by this organ could be significantly affected by any shift in the proportion of umbilical venous blood flow that normally passes through the ductus venosus or portal vein of the fetal liver.

Development and Anatomy of the Fetal Hepatic Circulation

The intrahepatic circulation, in a manner paralleling that of the cardiopulmonary circuit, contains numerous vascular shunts. The degree of potency and vascular resistance of these alternative flow routes regulates what percentage of total umbilical venous flow enters directly into the inferior vena cava without perfusing the hepatic parenchyma (69). The ductus arteriosus and the foramen ovale of the cardiopulmonary circuit might be considered somewhat analogous to the ductus venosus of the hepatic circulation, even

though the factors that regulate flow and vascular tone in the latter are not nearly as well understood (70).

The major vessel through which placental venous return bypasses the hepatic portal circuit is the ductus venosus and much of the ensuing discussion deals with this major vessel. Vascular communications in the fetal liver, other than the ductus venosus, have been observed between the umbilical vein and the inferior vena cava but their physiologic significance remains unclear because they have been demonstrated following high pressure injections of dye into the umbilical vein.[4]

The development of the ductus venosus begins quite early in mammalian ontogenesis. It appears to take form at approximately seven weeks of gestation in the human fetus, following degeneration of the vitelline veins that ultimately form the portal vein and hepatic sinusoids. Development of the fetal hepatic vasculature proceeds rapidly, so that the ductus venosus is well established within the fetal liver by approximately 10–11 weeks of age (71). It continues to grow until parturition, at which point the cross-sectional area is approximately one third that of the umbilical vein and between 2–3 cm in length.

Regional Distribution of Blood Flow in Fetal Hepatic Circulation

One of the main problems confronting studies in this area has been related to the development of appropriate means for determining blood flow within this highly inaccessible component of the circulatory system. The quantitative estimation of blood flow in the fetal liver, under near physiologic conditions, has proven to be a vexing experimental problem.

The initial studies in this area employed angiographic techniques to demonstrate that radioopaque media injected into the umbilical vein also flowed into the inferior vena cava and ductus venosus of the fetal lamb (72, 73) and human infant (74). This method possessed the obvious deficiency of not providing a quantitative measure of flow. An additional shortcoming of these procedures was that they required removal of the fetus from the uterus leading to cardiovascular stress and changes in fetal blood flow patterns (75, 76). Despite these restrictions, Barclay et al (73) concluded from their morphologic data that the internal caliber of the ductus venosus in the fetal lamb was approximately one third that of the umbilical vein. Since both vessels were presumed to be circular in nature, the ductus venosus was estimated to have a cross-sectional area equal to one ninth that

[4]Vascular communications in the fetal liver, other than the ductus venosus, have been observed between the umbilical vein and the inferior vena cava (P. Johnson, unpublished observations, 1973).

of the umbilical vein. This deduction led to the highly dubious assertion that only *11%* of the umbilical blood flow reached the inferior vena cava via the ductus venosus with the remaining 89% entering directly into the hepatic sinusoids and veins. It is of interest that in experiments where contrast media were infused into fetuses at near-physiologic pressures, visualization of the fetal ductus venosus was readily observed following umbilical venous injection but less frequently after intraportal injections (75, 77). These data suggested that blood flow through the ductus venosus was probably not derived from the portal venous contribution but more likely from the umbilical venous circuit.

A significant technological breakthrough occurred with the development of alternative methods for measuring blood flow, in particular those that utilized radioactive microspheres and blood flow probes. These procedures enabled the quantitative determination of blood flow in selected regions under near physiologic conditions, so that more precise and realistic estimates could be obtained. Studies carried out in the fetal lamb (78) demonstrated that 34 to 91% of umbilical venous flow passed through the ductus venosus. The proportion of total umbilical venous flow entering this shunt was directly proportional to its magnitude; that is, the greater the umbilical venous flow the higher the percentage shunted. Similar observations have been made in the fetuses of other mammalian species, such as the baboon [55% of umbilical flow passes through the ductus venosus (79)] and the rhesus monkey [31 to 70% of umbilical flow passes through the ductus venosus (80)]. These rather high degrees of shunting can have considerable significance, when one considers that between 40 to 50% of the fetal cardiac output is distributed to the placenta and that venous return to the fetal right heart at any given moment is affected by the quantity of blood diverted through the ductus venosus. The rhesus monkey, which has a high umbilical blood flow, shunts approximately 40% of fetal cardiac output via the ductus venosus (80), whereas the proportion is 21.5% in sheep (81) and 23% in the baboon (79). Another important consequence of this phenomenon is its effect upon drug distribution in the fetal liver as well as other fetal organs.

The partitioning of umbilical venous flow between the portal and ductus venosus circuits in the human has been investigated within the last decade. These data are quite striking since they indicate a wide intrasubject range in the percentage of total umbilical flow bypassing the liver via this route. Studies using previable human fetuses that were injected with radioactive microspheres have shown the percentage of placental blood flow passing through the ductus venosus to range from 8 to 92% (76). A frequency distribution plot of these data revealed that 30 of the 58 observations (52%) had ductus venosus flows exceeding 50% of the umbilical venous flow and

in 43 of the 58 (76%) it exceeded 40% of this value (1). Similar findings were reported by Gurpide et al (82) who used a pharmacologic tracer to study this phenomenon and found that 70% of a progesterone dose infused into the umbilical vein reached the fetal inferior vena cava unmetabolized. Based on these observations, this group estimated that 60% of the fetal placental flow was shunted through the ductus venosus. Collectively, all these data appear to suggest that a variable, but highly significant proportion, of umbilical blood flow is diverted via the ductus venosus during in utero fetal existence.

Neurohumoral and Pharmacologic Regulation of Fetal Hepatic Blood Flow

Vascular tone within the hepatic circulatory system is probably under the control of neurohumoral processes similar to those acting upon vascular smooth muscle in other parts of the body. The ductus venosus is histologically unique in that a sphincter formed from smooth muscle tissue surrounds the opening of this vessel at its confluence with the umbilical vein (83, 84) and in that it probably has some functional importance in diverting blood at this site (see Figure 1).

Neurohumoral modulation of muscle tone in the ductus and associated vessels has been suggested by the demonstration of synaptic aborizations of adrenergic nerve fibers at these sites. The identification of these neural elements as postganglionic adrenergic nerve fibers was shown by fluorescence histochemistry (85, 86). However, it is quite clear that cholinergic fibers also run in close association with adrenergic neurons, and the presence of both adrenergic and cholinergic fibers in the vicinity of the ductus venosus sphincter has been reported in human fetuses, at approximately 28 weeks of age (84).

While evidence has accumulated to demonstrate that the ductus venosus may be innervated by adrenergic and cholinergic neurons, there are only scanty data describing the effects of autonomic agents upon the vascular tone of this structure. Studies by in vitro tissue bath techniques of sphincters isolated from the ductus venosus have shown that this anatomical component of the hepatic circulatory system is not very responsive to either tyramine or noradrenaline, but contracts dramatically to acetylcholine and 5-hydroxytryptamine (85, 86). In contrast to the rather desultory in vitro response to catecholamines and indirect acting sympathicomimetics, in vivo studies suggest that the hepatic vasculature and the sphincter of the ductus venosus contract when norepinephrine is administered via the umbilical vein (77, 87). These investigations revealed a marked elevation in umbilical venous pressure following epinephrine or norepinephrine injected by this

route. There are also presumptive data suggesting that adrenergic mechanisms may be activated during fetal development since ductus venosus flow has been shown to increase sharply with fetal maturation (81). This corresponds with a period of rapid development of the adrenergic system and any increases in ductus venosus flow during acute hypoxia might be mediated via an increase in sympathetic activity known to occur during this type of stress (88).

Generally, most data would support the view that the ductus venosus, as well as other components of the hepatic vascular tree, are responsive to autonomic drugs and neurohumoral stimuli during fetal life. There is no indication how agents that modify adrenergic and/or cholinergic neuronal activity affect hepatic blood flow or shunting of umbilical venous flow through the liver.

From a phylogenetic viewpoint, comparable studies have not been carried out in lower mammalian species with the exception of the fetal lamb. It is of interest that the ductus venosus in this species develops at a gestational stage which is comparable to that of the human. The importance of this observation is that physiologic studies have shown diversion of umbilical venous blood flow through the ductus venosus to be comparable in both the lamb and human so that it may be the best model for studying the fetal hepatic circulation.

Hepatic Blood Flow and Fetal Drug Metabolism

Studies carried out over the past decade have clearly demonstrated that the fetus is able to actively metabolize a wide variety of xenobiotic compounds via the hepatic microsomal enzyme system (89). The fetal liver plays an integral role in converting lipophilic molecules with high affinity for hepatic parenchymal cells to compounds that are more hydrophilic and generally less pharmacologically active. This in turn may also significantly alter the distribution pattern of many drugs.

In this context, therefore, it is interesting to speculate upon the effects that could result from diverting umbilical venous blood flow into the ductus venosus and thereby diminishing "first pass" hepatic metabolism by this route. Drugs with a very high affinity for the liver whose intensity of pharmacologic response is modulated by the so-called first pass effect might be expected to be greatly influenced (90). The importance of this process has been shown for a variety of drugs such as propranolol, oxyphenbutazone, and lidocaine. These agents, if allowed to bypass the liver, would produce very large plasma to tissue gradients resulting in altered patterns of distribution and unanticipated magnitude of pharmacologic response.

While this is merely a hypothesis at present, it is of considerable therapeutic significance to determine how drug disposition may be modified by

changes in fetal hepatic blood flow and how different pathophysiologic states or pharmacologic agents affect hepatic perfusion. The importance of this parameter to drug metabolism is further emphasized by recent observations indicating that the metabolic clearance of drugs with a high "first pass" effect are influenced more by hepatic blood flow than by the intrinsic enzymatic activity of the liver (91).

CYCLIC NUCLEOTIDES, DRUG RECEPTORS, AND PHARMACOLOGIC RESPONSE IN THE FETUS

The role of the adenosine 3',5'-monophosphate (cyclic AMP) system in modulating receptor reactivity and the response of the fetus to drugs has not been precisely defined. Weiss & Strada (92) commented on the paucity of data linking cyclic AMP to developmental processes, yet they suggested that such a relationship was probable in view of the central position that cyclic AMP, and perhaps cyclic GMP, occupies in regulatory processes. This hypothesis remains tenable, especially insofar as a number of laboratories have demonstrated the importance of cyclic AMP as a regulator of growth, proliferation, and differentiation in a number of cell systems (93). In extension of this theme, data linking the development of drug-receptor systems in the fetus to a specific cyclic nucleotide–modulated effector apparatus are discussed in this section. The relationship between development or activity of this system in the fetal myocardium, central nervous system (CNS), and pulmonary circuit and exposure of the maternal-placental-fetal unit to drugs is also examined.

The Developing Myocardium

During fetal and postnatal life, the immature heart gradually acquires the ability to respond to cardioactive agents. The developmental stage at which pharmacological responsiveness appears varies widely for different agents and, in the case of the physiological neurohumoral transmitters, norepinephrine and acetylcholine, the developmental stage actually precedes innervation of this organ (94). If the adenylate cyclase system is an integral part of the receptor-effector complex in developing tissues, it should be sensitive to stimulation by catecholamines, and changes in tissue cyclic AMP levels, associated with neurohormonal modulation of cardiac function, should be detectable at the very earliest stages of fetal development. Furthermore, developmental changes in myocardial metabolism brought about by neurotransmitters should also be promoted by cyclic AMP and those agents that alter cyclic AMP metabolism. These questions are addressed below and, in essence, underlie the discussion of fetal receptor-effector systems in the CNS and lung as well.

MYOCARDIAL INNERVATION AND CATECHOLAMINES The concentration of endogenous norepinephrine in a peripheral tissue reflects the density of the mature postganglionic sympathetic nerves (95). The catecholamine concentration of most adrenergically innervated mammalian tissues is low during fetal and early neonatal life, generally approaching adult concentrations about four weeks after birth (96, 97). Glowinski et al (98) and Iversen et al (95) showed that cardiac muscle of the neonatal rat manifests a progressive age-dependent increase in endogenous stores of norepinephrine, and in the ability to take up and retain exogenous norepinephrine. Using fetal rat hearts, Mirkin (96, 97) reported that endogenous stores of norepinephrine were not detectable at prenatal day 15, whereas measurable levels were seen on day 21 (parturition). These studies complement the finding (99) that mature adrenergic nerve fibers were identified in the 19-day-old fetal rat heart, and the observation of Friedman et al (100) that sympathetic innervation in the fetal rabbit heart was less dense, consisting of large, intensely fluorescent preterminal trunks.

Functionally, adrenergic innervation of the fetal myocardium is also immature. Following intravenous administration of ^{3}H-norepinephrine, the uptake and binding of catecholamine to rat heart microsomes was not observed in the 16-day-old fetal heart and minimally detected in hearts studied on the eighteenth or twenty-first gestational day (96, 97). In contrast, one-day-old neonatal and adult hearts accumulated significant quantities of the exogenous radioligand. Similar results were obtained when cardiac tissue slices from fetal, neonatal, and adult rats were incubated with radioligand in vitro, data suggestive of a maturational sequence of neuronal proliferation (96, 97).

CYCLIC AMP AND MYOCARDIAL DIFFERENTIATION The presence of adenylate cyclase in fetal and neonatal tissues from humans (101, 102), rats (103), mice (104), and chicks (105) has been reported. Menon et al (101) measured adenylate cyclase activity in a number of tissues taken from human fetuses at 12–13 weeks gestation. The crude membrane fraction prepared from cardiac tissue (ventricle) had the highest basal and fluoride-stimulated adenylate cyclase activity of all the tissues studied. Human ventricular adenylate cyclase was stimulated in all of the fetuses by both epinephrine and glucagon in a concentration-dependent manner. In an elegant study using human myocardial tissue obtained from eighteen fetuses of 6–17 weeks gestation, Dail & Palmer (102) observed prominent basal adenylate cyclase activity throughout the 6–17 week developmental period, with the onset of fluoride stimulation at 8–9 weeks gestation. In contrast to the report of Menon et al (101), glucagon-stimulated cyclase was not observed until 17 weeks gestation. In addition, varying concentrations

(10^{-6}–10^{-3} M) of catecholamines either slightly inhibited or elevated (6%) enzymatic activity at all ages. These same workers also observed a progressive increase of cyclic AMP phosphodiesterase (PDE) activity from 8 to 9 weeks gestation onward, with little inhibition by the PDE inhibitor aminophylline until 10–11 weeks gestation.

Although some discrepancies do exist among these reports, they are quite interesting in that adrenergic nerve fibers are not present in the human fetal heart at this early stage of gestation. Dail & Palmer (102), employing the fluorescent histochemical technique of Falck (106), observed small, intensely fluorescent cells oriented along blood vessels, axons, and large nerve trunks which resemble the catecholamine-containing chromaffin cells described in the adult heart of several species (107). However, this technique did not demonstrate preterminal adrenergic fibers in the 6–17 week human fetal myocardium. The authors suggested that this failure to demonstrate fluorescent adrenergic fibers might be attributed to the activity of monoamine oxidase prior to freezing of the hearts. However, Partanen & Korkala (108) also were unable to observe fluorescent adrenergic terminal nerve fibers in the atrial or ventricular wall or in the nodal tissues of 10–16 week human fetuses, although the small intensely fluorescent (SIF) cells described above were noted. It has been suggested (102) that the lack of terminal adrenergic nerve fibers in the cardiac wall of human fetuses may account for the humoral, rather than neural, adrenergic control of the human fetal heart during the first half of pregnancy.

Correlative data implying an association between human fetal myocardial contractility and development of adenylate cyclase have been presented (109, 110). A progressive development of fetal contractile response to catecholamines from 12 to 22 weeks gestation was demonstrated, which roughly paralleled a spontaneous increase in both basal adenylate cyclase and norepinephrine-stimulated Ca^{2+} uptake. No data on catecholamine-stimulated adenylate cyclase were provided; thus it is not possible to say with assurance that all these components are linked to excitation-contraction coupling.

Investigations using hearts from chicken embryos have demonstrated the usefulness of this species for the ontogenetic study of β-adrenoreceptors. Specific receptors responsive to epinephrine and norepinephrine are present in embryonic chick hearts prior to establishment of sympathetic innervation (day 5 of embryogenesis) (111). Polson et al (105) monitored the changes in contractility and cyclic AMP levels in response to β-adrenergic agonists in isolated 4-day-old (noninnervated) and 7-day-old (innervated) embryonic hearts. Isoproterenol (10^{-7}M) and norepinephrine (5×10^{-8} M) evoked an increase in contractile amplitude and cyclic AMP in the noninnervated hearts. The magnitude of the cyclic AMP increase in the noninnervated

heart was greater than that occurring in the innervated (7-day-old) chick embryo. In all cases, the responses were blocked by propranolol, a β-adrenoreceptor antagonist. Although an exhaustive developmental survey of adenylate cyclase-hormonal responsiveness was not carried out in these studies, they clearly establish a relationship between β-adrenoreceptor stimulation and cyclic AMP accumulation at a stage of myocardial development preceding sympathetic innervation. Another interesting finding of this study was that the 4-day-old embryonic heart displayed a greater cyclic AMP increase in response to catecholamines than the innervated 7-day-old heart. Although this may reflect a more efficient coupling of the β-adrenoreceptor adenylate cyclase complex to the contractile mechanism in the older hearts, it is possible that the sensitivity of the β-adrenoreceptor to further catecholamine stimulation diminishes following exposure of the receptor complex to an ontogenically regulated increase in endogenous neurotransmitter. As has been recently demonstrated for a number of physiologic systems (112), repetitive exposure of β-adrenergic receptors to catecholamines evokes a diminished (subsensitive) response to subsequent stimulation; conversely, the absence of antecedent adrenergic stimulation was associated with an enhanced (supersensitive) response. Although in vitro studies with myocardial tissue obtained from mature sheep fetuses (gestational age 140 days) have not revealed a developmental difference in the reactivity or affinity of fetal β-adrenoreceptors for isoproterenol or propranolol (113), detailed radioligand-binding studies on immature, non-sympathetically innervated fetal preparations have not been performed.

Mitochondria from fetal and newborn lambs had a significant increase in oxygen consumption in the presence of ADP, when compared to the adult (113). The uncoupling agent, dinitrophenol, also stimulated mitochondrial oxygen consumption in fetal and newborn lamb heart, a finding compatible with the presence of increased cytochrome *c* oxidase activity in immature myocardium. In conjunction with the known increase in anaerobic capacity of hearts from young animals (114), this increase in the aerobic capacity of immature cardiac mitochondria may enable the fetus to better tolerate periods of hypoxic stress. In addition it may facilitate mobilization of glycogen stores in the fetal heart to provide substrate for glycolysis (94, 103, 104).

It has been known for some time that stimulation of adult rat hearts by epinephrine and glucagon can evoke positive chronotropic and inotropic responses and stimulate glycogenolysis, apparently by producing an increase in cyclic AMP (115). Clark et al (103) observed that epinephrine, not glucagon, stimulated myocardial adenylate cyclase and cardiac glycogenolysis in fetal rats of 16–20 days gestational age. Responsiveness to glucagon, for both of these parameters, did not develop in myocardial tissue until 4

weeks after birth. In contrast to these findings, fetal hepatic cyclase activity and glycogenolysis were stimulated by both hormones. Similar observations have been made with epinephrine which activated fetal mouse myocardial adenylate cyclase at 18–22 days gestation, whereas glucagon had no effect on cyclase at any fetal stage (104). A point of some interest was that glucagon did enhance cardiac contractility and glycogenolysis in the fetal mouse, thus dissociating, in part, its metabolic effects from activation of an adenylate cyclase.

Although phylogenetic differences may underlie some of the discrepancies observed, these findings and those presented earlier clearly demonstrate (*a*) that the myocardial receptor–adenylate cyclase complex may develop a functional capacity in the absence of a defined neurotransmitter input and (*b*) its separate components may express an individualized development sequence. As shown by Dail & Palmer (102), fluoride-stimulated and basal adenylate cyclase activity was present, at 6 to 8 weeks gestation in the human myocardium, whereas glucagon stimulation was not discernible until the seventeenth week when catecholamine stimulation was completely absent. These findings are consistent with the hypothesis that the catalytic portion of the adenylate cyclase–receptor complex develops at an earlier stage in the fetal heart than does the receptor component, and that receptor binding by hormones and adenylate cyclase activation are separate processes.

BIOCHEMICAL ASPECTS OF MYOCARDIAL DIFFERENTIATION The rates of DNA synthesis and cellular proliferation in a variety of normal, transformed, and tumor cells are inversely related or directly related, respectively, to the intracellular concentration of cyclic AMP or cyclic GMP (93). During the early neonatal period, in the rat (1–22 days), there is a progressive, striking decline in DNA synthesis and cell proliferation of terminally differentiating cardiac muscle (116). Throughout this period, cardiac tissue concentrations of cyclic AMP are increasing and cyclic GMP levels are declining (117, 118). Employing neonatal rats of 3–5 days of age, Claycomb (119) observed that injections of either isoproterenol or N^6, O^2-dibutyryl adenosine 3':5'-monophosphate (dibutyryl cyclic AMP) produced a significant inhibition of [^{3}H]-thymidine incorporation into the DNA of differentiating cardiac muscle. This effect was not evoked by cyclic GMP, dibutyryl cyclic GMP, or the α-adrenoreceptor agonist, phenylephrine. In addition, β-adrenoreceptor agonists, such as isoproterenol, enhanced the uptake and incorporation of [^{3}H]-phenylalanine into total cardiac muscle and the contractile protein, myosin. This finding is consistent with the observation that cell proliferation ceases with initiation of cell enlargement and contractile protein synthesis on the twenty-second postna-

tal day in the rat (116, 120). Although these studies used relatively large concentrations of β-adrenoreceptor agonists (10^{-6}–10^{-3} M) and lacked data clearly showing β-antagonist blockade of the inhibition of DNA synthesis, it is interesting that the sequence of metabolic events coincide well with the maturation of cardiac adrenergic nerve fibers (95–98, 100).

Thus, it appears likely that (*a*) the functional integrity of the autonomic nervous system may be instrumental in influencing cellular proliferation and functional differentiation in the developing myocardium and (*b*) perturbation of this relationship in the fetus by drugs may impede this closely regulated process.

The Developing Nervous System

ONTOGENESIS OF CENTRAL ADRENERGIC NEURONS The maturation of neuronal structures in the mammalian brain progresses in defined patterns, unique to the species investigated. During the late fetal and early neonatal periods, the rat brain is functionally quite immature. Early reports (121) demonstrated that the endogenous concentration of norephinephrine in the newborn rat brain was very low, but achieves adult levels by 7 weeks of age. Examining fetal rat brains, Mirkin (96, 97) reported extremely low levels of norepinephrine ($<$ 5 ng/gm) at gestational day 15 with a progressive increase in concentration occurring during the first 21 days of life. During the initial 30 postnatal days, the rat brain approaches functional maturity in that both behavior and electrophysiologic activity are changing from fetal to adult patterns (122). Brain protein increases 50–60% in the first 8 weeks of life (123) and the microscopic appearance of the cerebral cortex achieves nearly its adult form between the twelfth and fifteenth postnatal day (124). The total DNA content of the cerebrum rises rapidly between the seventh and fifteenth days peaking at about 21 days of age (125) in parallel with enhanced rates of ganglioside production (124), sulfatide synthesis (126), and development of complex synaptic structures (127). In the developing human, the process of brain maturation is more prolonged. Increases in cellular protein, RNA, and lipid and water content of the cerebral cortex extend through adolescence. In contrast to these growth parameters, neuroblastic proliferation is completed by 20 weeks gestational age in man and glial proliferation by the end of the first postnatal year (128–129).

The central nervous system thus represents a dynamic, yet complex, system for the study of the role of cyclic nucleotides in the expression of receptor-linked developmental events. The relative lack of data on mammalian fetuses is offset by fascinating studies in neonatal animals, such as the rat, where various components of the receptor-cyclic nucleotide system can be ontogenically dissociated.

CYCLIC NUCLEOTIDE LEVELS IN THE DEVELOPING BRAIN Defining the role of the cyclic nucleotides in the central nervous system has been difficult because of the neuronal complexity of the tissue and the still incomplete identification of natural hormones that might act as first messengers on neurons and glia. In spite of these obstacles, it has been demonstrated (130) that cyclic AMP may modulate the postsynaptic effects of endogenous neurotransmitters on central neurons. In these studies, iontophoretically applied cyclic AMP mimicked the ability of norepinephrine to produce a specific patterned depression of spontaneous firing in rat cerebellar Purkinje and hippocampal pyramidal neurons. Using the superior cervical ganglion, Greengard & McAfee (131) and Greengard & Kebabian (132) demonstrated that a dopaminergic, cyclic AMP–linked system hyperpolarizes the postganglionic neuron causing a slow inhibitory postsynaptic potential. This response is then modulated by a cholinergic, cyclic GMP–linked depolarization of the cell, resulting in a slow excitatory postsynaptic potential. These associations between neuronal function and cyclic nucleotides have not been approached developmentally. However, the available data (see below) suggest a significant correlation between brain maturation and cyclic nucleotide metabolism.

The concentration of cyclic AMP in whole rat brain tissue increases progressively from birth (133, 134). Over the first 40 days of life, this increase (threefold) was suppressed by administration of I^{131} to newborn rats, inducing a complete destruction of thyroid tissue. Inhibition of the increase in brain cAMP by thyroidectomy was not accompanied by an attenuation of the spontaneous increases in adenylate cyclase or phosphodiesterase seen with maturation. Thus, it probably reflects the striking inhibition of brain and body growth seen in classical cretinism, yet may represent a sensitive biochemical indicator of CNS damage seen in the offspring of mothers treated prenatally with antithyroid agents (135).

Radioimmunoassay and rapid freezing procedures, to minimize anoxia-induced increases in cyclic nucleotides (136), were used by Steiner et al (137) to examine the sequential changes in mouse brain cyclic AMP and cyclic GMP during early postnatal life. The levels of cyclic nucleotide were not significantly altered during the initial week of life (postnatal days 1–7). Beginning on day 7, cyclic AMP levels in the forebrain, brain stem, and cerebellum doubled; forebrain levels of cyclic GMP were stable, whereas brain stem levels increased 2-fold and cerebellar cyclic GMP rose 13-fold during postnatal days 14 to 21. In contrast to these findings, basal levels of cyclic AMP in brain slices from neonatal rats remained constant throughout the first 18 days of life (138). Although variations in tissue preparation may partially explain discrepancies in these data, maturational alterations in cyclic nucleotide levels coincide with the striking changes occurring in

other aspects of brain development, and suggest a causal relationship between these phenomena.

ADENYLATE CYCLASE AND CATECHOLAMINE RESPONSIVENESS

Homogenates of nervous tissue, particularly those prepared from the cerebellum and cerebral cortex, are rich sources of adenylate cyclase (139). A membrane-bound enzyme in brain as well as in other tissues, adenylate cyclase is particularly concentrated in synaptosomal fractions of brain tissue (140). If such preparations can be shown to respond to added neurotransmitters, they represent the simplest in vitro systems for demonstrating the coupling of neurotransmitter receptors to cyclic AMP formation, and for studying the pharmacological specificity of such receptors (141). In addition to cell-free systems, numerous studies have shown that putative neurotransmitters such as norepinephrine, serotonin, and histamine could elicit accumulation of cyclic AMP in brain slices (142).

The initial report linking catecholamine responsiveness to cyclic AMP generation in the developing CNS was provided by Schmidt et al (133). Utilizing brain slices obtained from newborn and immature rats, these workers reported that norepinephrine failed to increase the concentration of cyclic AMP in brain slices of newborn animals, but raised the level of the cyclic nucleotide severalfold in rats 6 days of age and older. Furthermore, this response was not altered by neonatal thyroidectomy (134). These studies suggested that although the catalytic activity of adenylate cyclase was present in newborn brain, the norepinephrine insensitivity might be due to the lack of specific adrenergic receptors during the early stages of development. More recently, Schmidt & Robison (143) demonstrated a progressive increase in norepinephrine-sensitive cyclic AMP generation in tissue slices from various regions of the rabbit brain during the first 2 weeks of life followed by a decline thereafter. Although these fluctuations may reflect relative ontogenetic alterations in biosynthetic and degradative capacity of the specific tissue, the enhanced reactivity noted at a stage in development when synaptic innervation is incomplete may reflect a supersensitive response similar to denervation supersensitivity. Other laboratories (138) did not observe a cyclic AMP response to norepinephrine in cerebral cortical rat brain slices until day 11, although adenosine and the combination of norepinephrine with adenosine stimulated an increase in cyclic AMP prior to the development of sensitivity to catecholamines alone. Perkins (138) has suggested that either the immature receptor does not bind norepinephrine in the absence of adenosine or that the coupling function is not fully operative in the absence of adenosine.

The ontogenetic development of brain adenylate cyclase activity per se was first described by Weiss (123). Employing homogenates prepared from

rat cerebrum, cerebellum, and brain stem, this worker observed a rapid (three to six fold) increase in cerebral and brain stem enzyme in the first 14 postnatal days, whether measurements were made in the absence or presence of the activator anion, sodium fluoride. Adenylate cyclase activity of cerebellum increased more slowly and reached a maximum at about 30 days of age.

Although a component of the peripheral autonomic nervous system, the rat pineal gland has proven to be a very useful model system for the study of end-organ regulation by nerves (144). Weiss (123) observed that basal adenylate cyclase activity in the neonatal rat pineal did not increase with age; however, both sodium fluoride and norepinephrine-stimulated activity increased over the first 4 weeks of life. Catecholamine stimulation was not observed on day 1, a time which precedes innervation of the parenchymal cells by nerve fibers arising in the superior cervical ganglion (145). The ontogenetic development of catecholamine-stimulated adenylate cyclase activity was not prevented by removing the superior cervical ganglion; thus, receptor-mediated adenylate cyclase activity in the pineal appears to be independent of a presynaptic input, data qualitatively consonant with observations made in the embryonic chick heart. This concept was further supported by the demonstration that norepinephrine and dopamine-stimulated adenylate cyclase were present in rat cortical and subcortical particulate fractions at all stages of development (146). Thus, regulation of biochemical modulators such as cyclic AMP develops prior to maturation of the synaptic structures they may modulate.

Ontogenetic expression of adenylate cyclase activity in the human CNS has been minimally examined. Weiss & Strada (92) reported that brain cyclase from 10- to 14-week-old fetuses was 10% as active as the adult brain enzyme, an interesting and complementary finding insofar as neuroblastic differentiation in the human is not complete until 20 weeks gestation (129).

PHOSPHODIESTERASE AND PROTEIN KINASE IN NEURAL DEVELOPMENT The steady state concentration of cyclic AMP in any tissue reflects the combined activities of adenylate cyclase and phosphodiesterase (PDE). Like adenylate cyclase, PDE activity displays a defined ontogenetic pattern in activity. Schmidt et al (133) observed a severalfold increase in both soluble and particulate PDE in neonatal rats between ages 1 and 23 days, an increase that paralleled changes in Na+-K+-ATPase in mitochondria, nerve endings, and nerve ending membranes (147).

The postnatal development of PDE activity varies with the brain area analyzed (123). In the neonatal rat, brain stem and cerebral PDE increased severalfold between 2 and 16 days of age, whereas cerebellar PDE increased very little. Later work by this group (92) revealed that the activity of the

neonatal high K_M PDE (148, 149) increased 5-fold whereas the low K_M enzyme increased only 2.5 fold. These workers also measured both high affinity and low affinity PDE in human fetal brain at 10–14 weeks gestation. Total PDE activity, like adenylate cyclase, was substantially lower in human fetal than adult brain, although its specific activity was greater than enzyme from fetal heart, adrenal, and liver. More recently, Kang (150) examined both cyclic AMP and cyclic GMP PDE in brain tissues from human fetuses of 14–20 weeks gestation and in brains from young adults. The mature cortex had 10 times more activity than the fetal brain for cyclic AMP hydrolysis and 15–20 times more activity for cyclic GMP hydrolysis with a relative shift in localization of high K_M activities to the particulate fraction with age. At all substrate concentrations studied, the fetal cortical PDE was more sensitive to the inhibitory effects of theophylline than the mature cortical enzyme. Although millimolar concentrations of theophylline are rarely, if ever, achieved in vivo, these data suggest that a differential response to methylxanthines may be exerted in fetal and maternal tissues.

Phosphorylation of endogenous neural proteins by cyclic nucleotide–dependent protein kinases has been postulated to be the intracellular effector mechanism involved in cyclic nucleotide action (151). Gaballah et al (152) measured high levels of enzyme in brains from 18-day fetal and 3-day-old rats and reported a sharp increase in activity at 6 days of age. Of interest was the finding that the relative degree of activation by cyclic AMP was actually greater in the fetal and neonatal brain than in tissues from older animals. Although these data suggest that protein kinase from immature brains may be "supersensitive," relative to mature tissues, Schmidt & Robison (134) observed a significant, age-dependent phosphorylation of endogenous components of rat brain during ontogenetic development. Thus, the activity of protein kinase in the fetus and neonate may be critically limited in vivo, by low levels of the activator, cyclic AMP, or by the availability of natural substrates. Agents or conditions that may alter one or both of these components may ultimately influence the characteristics of the receptor-cyclic AMP effector system in the developing organism.

The Developing Lung

Adaptation of the fetal lung to air breathing is critically dependent upon maturation of the alveolar surfactant-producing system. Deficiencies of this surface-active lipoprotein at birth results in the respiratory distress syndrome (RDS) of the newborn, characterized by atelectasis and inadequate ventilation (153). Saturated phosphatidyl choline, dipalmitoyl lecithin, is the major lipid component of the surfactant material (154). Its synthesis occurs primarily through a de novo pathway where the rate-limiting step appears to be the conversion of CDP-choline to choline and also through a secondary route whereby phosphatidyl ethanolamine methylation occurs

in the presence of S-adenosylmethionine (155). More recently, a number of reports have appeared demonstrating an accelerated rate of phosphatidyl choline synthesis in fetal lung after maternal administration of glucocorticoids (156). This therapeutic intervention has been reported to exert a beneficial effect in premature human neonates at risk for developing RDS (157).

The role of cyclic AMP in pulmonary maturation has not been elucidated. A number of biogenic amines, such as norepinephrine, epinephrine, and histamine have been shown to promote a dose-dependent increase in cyclic AMP in adult rabbit lung slices (158). In fetal rabbit lung, cyclic AMP is responsive to catecholamines and histamine stimulation by 21 days of gestation (158) with near-adult responsiveness manifest at day 25 of gestation. Adenylate cyclase activity itself is present in fetal rabbit lung, by day 21, and can be stimulated by epinephrine in vitro (159). Several reports have stated that glucocorticoids can enhance cyclic AMP concentrations in various tissues, presumably via inhibition of PDE (160, 161). Barrett et al (156) examined changes in phosphatidyl choline synthesis and cyclic AMP metabolism after administering glucocorticoids and aminophylline to pregnant rabbits. Both agents inhibited lung PDE activity, augmented tissue cyclic AMP concentrations, and stimulated incorporation of labeled choline and methionine precursors into phosphatidyl choline, suggestive of a common, cyclic AMP-linked pathway leading to lung maturation. These studies and others (155) have shown that methylation of phosphatidyl ethanolamine to phosphatidyl choline contributes only a small fraction to the total lipid synthesized. It is of great interest, however, that the specific activity of methionine adenosyltransferase, the enzyme catalyzing synthesis of the methyl donor S-adenosylmethionine, increases dramatically in the lungs of fetal humans, monkeys, and rabbits shortly before the time when extrauterine survival is possible (162).

The presence of two methyltransferases in adrenal microsomal and erythrocyte membranes that catalyze the stepwise methylation of phosphatidyl ethanolamine to phosphatidyl choline, resulting in a rapid transmembrane transfer of the final product, phosphatidyl choline, to the external surface of the membrane has recently been demonstrated (163, 164). Furthermore, these workers stated that the stimulation of β-adrenergic receptors, and presumably cyclic AMP synthesis, caused a marked increase in membrane phospholipid synthesis, membrane fluidity, and "flip-flop" across membranes. This response was coupled to an actual increase in β-adrenergic receptors as phosphatidyl choline synthesis progressed. While these data have obvious implications regarding comprehension of the processes that modulate fetal lung maturation, they also may provide a valuable new tool for the study of regulatory membrane phenomena in developing organ systems.

CONCLUSION

This review has explored several unique aspects of the maternal-fetal-placental unit. It has considered selected determinants of placental drug transfer, fetal drug distribution, and biochemical regulators of end organ response to pharmacologically active molecules. It is clear that more carefully designed in vitro models and more precisely defined in vivo observations are needed to further understanding in the discipline of developmental pharmacology.

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