# Perinatal Pharmacology and Therapeutics

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Bernard L. Mirkin

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# Preface

Perinatal pharmacology is devoted to the study of pharmacologically active molecules and the effects they produce on developing organisms. The subject matter presented in this book has been arbitrarily divided into sections dealing with the prenatal (fetal pharmacology) and the postnatal (pediatric pharmacology) consequences of drugs administered during different phases of mammalian development. This organizational format was selected to emphasize the fact that xenobiotic chemical substances may influence development in myriad ways over a broad time span, covering the periods from conception to parturition, through neonatal and childhood existence, and even into early adult life. The essential processes influencing drug disposition and pharmacodynamic action during different stages of biological maturation have been considered in a detailed and critical manner.

The broad perspective of perinatal pharmacology has necessitated that the scope of this book be restricted to allow in-depth discussions of areas currently under active investigation. Omission of apparently significant areas have occurred, not because they were deemed unimportant, but primarily because the data available was considered insufficient (at this point in time) to allow substantive conclusions to be presented. This probably is more a reflection of one's inability to assimilate the vast amount of data which has been recently generated in this area, and for this I must ask the indulgence of my many colleagues in the field.

I wish to acknowledge an indebtedness to my primary collaborators who suffered through several revisions and unforseen setbacks during which it appeared that I had placed unnecessarily stringent demands upon them. It is indeed a commentary on their overall excellence, commitment, and insight that we have come forth with a relatively integrated viewpoint which hopefully will stimulate and enlighten all readers regardless of their specific disciplinary concerns.

I am also grateful to the many individuals who knowingly and unknowingly participated as sounding boards for a variety of the concepts presented in the different chapters. In particular, I am deeply appreciative of the unseen contribution of Drs. John A. Anderson and Frederick E. Shideman who collectively shared my vision of establishing develop-

mental and pediatric clinical pharmacology as a viable discipline at the University of Minnesota. I also extend my thanks to Dr. Geoffrey S. Dawes and Paul S. Johnson for their hospitality and intellectual stimulation during my sabbatical leave at the University of Oxford. Finally, the patience and goodwill displayed by the staff of Academic Press must not go unrecognized since, together with the forebearance of my family, it is probably the single factor which "carried the day."

*Bernard L. Mirkin*

# Contents

# 1 Placental Transfer of Pharmacologically Active Molecules

Bernard L. Mirkin
Sharanjeet Singh



## I. Introduction

Chemical substances entering the body in the form of food additives, environmental pollutants, or therapeutic agents are commonly disseminated via the systemic circulation to undergo widespread tissue distribution. These compounds may also be transferred into the luminal secretions of the fallopian tube and uterine cavity through which the ovum and blastocyst must pass during the early stages of embryogenesis.

Numerous investigations have demonstrated the transplacental passage of many different types of pharmacologically active compounds as well as the untoward and capricious effects such agents may exert upon mammalian development (Wilson, 1973).

While it has proved extremely difficult to identify categorically which characteristic(s) of the host or drug molecule is (are) most influential in the causation of adverse effects upon the fetus, the following factors appear to be of great importance: stage of fetal development at the time of drug exposure; duration of exposure; amount of drug administered; quantitative rates of drug transfer to and from the fetus; distribution of drug in the fetus; and the physicochemical properties and pharmacodynamic actions of the drug molecule.

This chapter presents a comprehensive discussion of the basic mechanisms regulating placental drug transfer, fetal drug distribution, and the pharmacokinetic patterns of different classes of drugs in the maternal–placental–fetal unit.

## II. Morphological and Comparative Physiology of the Placenta

Shortly after fertilization, probably within the initial 24 hours, the ovum undergoes cleavage to produce blastomeres which are approximately equal in size. This process is initiated in the segment of the fallopian tube most proximal to the ovary and continues as the fertilized ovum proceeds toward the uterine cavity.

Blastocyst formation, characterized by the development of a cavity within the morula,* appears to be associated with the transfer of substances from the luminal fluid of the uterus into the blastocyst as well as from endogenous secretions of cells comprising the morula (Martin, 1968). At the time of implantation which generally occurs about 6 to 7 days after fertilization, formation of the placenta begins and the different histological components of this organ can be distinguished. The placenta undergoes maturational changes which may significantly influence the transfer of xenobiotic and endogenously formed molecules across the complex biological membranes contained within this organ.

The early studies of Flexner and Gellhorn (1942) suggested that the ease with which substances passed across the placental membranes was directly proportional to the number of membrane layers separating the fetal and maternal bloodstreams, i.e., the fewer the membrane layers the more rapid the transfer. The statement was based on the apparent corre-

* The mass of blastomeres resulting from the early cleavage divisions of the fertilized ovum (zygote).

lation between morphological changes in the villi of late third trimester placentas and the increased placental transfer rate of sodium observed at this gestational stage.

However, even for molecules which cross the placenta by simple diffusion, the anatomic thickness of this organ cannot be consistently related to the number of membrane layers which are either morphologically discernible or functionally operational. Placentas of all types have regions in which the membranes overlying the fetal and maternal capillaries are virtually absent or markedly attenuated. Consequently, the distance separating the maternal and fetal circulations in such areas may be no greater in a six-layered epitheliochorial placenta than in a hemochorial placenta consisting of three membrane layers (Wimsatt, 1962). The depth of the tissue layers interposed between the fetal capillaries and the maternal blood supply have been estimated to vary from 1 to 100 $\mu$m in different animal species (Metcalfe *et al.*, 1967). At term, the mean thickness of the trophoblastic membranes in the human placenta has been reported to be 3.5 $\mu$m (Aherne and Dunhill, 1966). As the placenta matures, a marked change in these structures occurs and they decrease from a thickness of 25 $\mu$m early in gestation to 2 $\mu$m at parturition (Strauss *et al.*, 1965). Recent data suggest that the relative permeability of the rat placenta to diphenylhydantoin is biphasic in nature; transfer appears to be at a maximum in the early and late stages of gestation, decreasing significantly during midgestation (Stevens and Harbison, 1974).

Histological analyses of the major types of placentas have demonstrated that the number and thickness of tissue layers interposed between the fetal and maternal vascular systems are species dependent (see Table I; Amoroso, 1952; Dawes, 1968). Anatomic classifications explicitly define the morphological distinctions existing between most mammalian placentas but do not provide additional insight regarding the functional significance of these differences. Comparative studies on the placental transfer of drugs in different species are meager and at present it can only be assumed that placentas of the hemochorial and nonhemochorial type respond similarly with respect to drug transfer. The lack of detailed information makes it virtually impossible to assess how variations in the number, composition, and characteristics of placental membranes may influence the placental passage of different drugs.

Some indication of the complexity of this problem can be obtained from studies in which the trophoblastic ultrastructure of different types of hemochorial placentas has been histologically defined. These data demonstrate that the labyrinthine hemomonochorial placentas contain spaces in which the maternal plasma is relatively stagnant due to the presence of numerous and extensive microvilli (Enders, 1967). Stasis of maternal

**TABLE I Anatomic Classification of Placentas**[a]

| | Tissues separating fetal and maternal circulatory systems | | | | | | |
|---|---|---|---|---|---|---|---|
| | Maternal (Uterine mucous membrane) | | | Fetal (Allantochorion) | | | |
| Histological type | Endothelium | Connective | Epithelium | Trophoblast | Connective | Endothelium | Typical species |
| Epitheliochorial | + | + | + | + | + | + | Pig, horse, donkey |
| Syndesmochorial | + | + | − | + | + | + | Sheep, goat, cow |
| Endotheliochorial | + | − | − | + | + | + | Cat, dog, ferret |
| Hemochorial | − | − | − | + | + | + | Man, rhesus monkey |
| Hemoendothelial | − | − | − | − | ± | + | Rabbit, guinea pig, rat, mouse |

[a] Adapted from Amoroso (1952) and Dawes (1968).

blood within the intervillous space may cause delayed and nonhomogeneous mixing of drug in the maternal placental circulation. Consequently, the diffusion of drugs into the fetal circulation of species possessing a hemomonochorial placenta (guinea pig, chipmunk) may be retarded even though it contains fewer tissue layers than the hemotrichorial placenta (rat, mouse), in which physical impediments to maternal blood flow are minimal.

Differences in transplacental electrical potentials have been observed among closely related rodent species. Potentials of 15 mV (fetus positive) were recorded in the rat, 0 mV in the rabbit, and 18 mV (fetus negative) in the guinea pig at equivalent stages in gestation (Mellor, 1969). These biogenic potentials in some manner provide an index of fetal maturity in each species, since at birth the rat is developmentally immature, the rabbit intermediate, and the guinea pig most advanced. The trophoblastic layering of the hemochorial placenta in these rodents differs* (Enders, 1967) so that a causal relationship between transplacental electrical potential, placental transfer rate, and anatomic constitution may exist.

While the nature of the relationship between structure and function in biological membranes has not been clearly elucidated, all functional membranes appear to be composed primarily of lipids and proteins. The proportion of lipid (and its constituent fatty acids) to protein differs

* The rat has a three-layered trophoblast, the rabbit two layers, and the guinea pig only one layer.

significantly in each type of membrane. Data derived from experiments using myelin sheaths as models have generally been extrapolated to, and considered relevant for, the plasma membranes of different tissues with little consideration given to the significance of differences in their respective biochemical characteristics or molecular organization.

Myelin is low in protein with a protein/lipid ratio of approximately 0.5, and contrasts markedly with other membranes in which the ratio is 2.0 or greater. Additionally, myelin's phospholipid composition differs from that of most basement membranes (Dowben, 1969; Van Bruggen, 1971). Myelin appears to be relatively inactive metabolically, in contrast to other membranes which are capable of synthesizing and degrading numerous types of cellular substrates. The placental membranes are probably best identified with the latter group because of their capacity for carrying out numerous enzymatic reactions which may be related to normal fetal development. Over 85 enzymes involving the metabolism of steroids, carbohydrates, proteins, and lipids have been identified in placental extracts (Hagerman, 1970). The biotransformation of drugs has been demonstrated in homogenates prepared from placental tissue (Juchau, 1972), however, the *in vivo* significance of this process remains unclear at present (see Chapter 2).

The structural organization of most membranes and their constituents is generally considered to be in a dynamic rather than static state of existence (Sjöstrand, 1963; Dowben, 1969). The membranes are conceived to be planar aggregates of micellar subunits (either spherical or lamellar, with an internal liquid crystalline phase) which are neither constant in their physical state nor collectively arranged in a fixed pattern (Tien and James, 1971). These subunits undergo reversible structural changes probably corresponding to phase transitions and functional needs. The rapid structural and functional modification of the placenta throughout gestation suggests that it may possess characteristics which are unique among the biomembranes. Consequently, it appears that generalizations regarding drug transfer across the placenta which are based upon data derived from investigations carried out in other nonplacental membrane systems may not be valid (Oh and Mirkin, 1971; Oh, 1973; Mirkin and Oh, 1974).

## III. Transfer of Drugs into the Preimplantation Blastocyst and Luminal Secretions of the Oviduct and Uterus

Therapeutic agents and other chemical substances may interact with the developing ovum at many different sites during its passage through the oviduct and fallopian tubes. The penetration of drugs into most por-

tions of the mammalian reproductive system has been shown to occur prior to the development of a functional placenta (see Table II).

Studies performed in a variety of species have demonstrated that the composition of fluids in the oviduct varies in accordance with the stage of the menstrual cycle, the nature of the steroid hormone present in the circulation, and the presence or absence of pregnancy (Hamner and Fox, 1969; Mastroiani *et al.*, 1961). Amino acids (Jaszczak *et al.*, 1970) and chloride ions (Brunton and Brinster, 1971) are actively secreted into the luminal fluids of the fallopian tube so that the blastocyst is exposed to high concentrations of these substances during the interval between fertilization and implantation. Since active secretory mechanisms appear to exert an important regulatory influence on the composition of fluids in the uterine and fallopian lumen, drugs affecting these processes may alter the chemical nature of such secretions and significantly influence drug distribution patterns as well as their rates of penetration into the blastocyst.

The oviductal and uterine secretions of rabbits possess a higher pH than that of plasma (McLachlan *et al.*, 1970; Vishwakrama, 1962). Thus, basic drugs would generally be anticipated to achieve lower concentrations and acidic drugs higher concentrations in these fluids if their respective pH values exceeded that of plasma. Deviations from this distribution pattern might occur via the active transport of drug or active reabsorption of water from the oviduct. Currently, little is known about either of these processes which potentially can alter drug distribution in the

**TABLE II Potential Sites at Which Drugs May Affect Ontogenesis**

| Developmental stage | Anatomic location | Primary source of drug |
|---|---|---|
| Ovum | Ovary | Maternal circulation |
| Preimplantation blastocyst | Oviduct | Luminal secretions |
| | Fallopian tube | Luminal secretions |
| Postimplantation blastocyst | Uterus | Luminal secretions |
| | | Maternal circulation (at nidation) |
| Embryo | Uterus | Maternal circulation via placental transfer |
| Fetus | Uterus | Administration directly to the fetus or indirectly via instillation into the amniotic fluid |

luminal fluids of the uterus or fallopian tubes (see Chapter 3 for a detailed discussion of drug distribution in the mammalian reproductive system).

The extent to which exogenously administered drugs can or will accumulate in the luminal secretions of the uterus appears to be primarily determined by specific physicochemical properties of each drug and possibly by the active secretory mechanisms mentioned previously. Some compounds achieve uterine fluid concentrations which are about 50% greater than those of plasma if measurements are made 6 hours after drug administration. Xenobiotic agents which exhibit this distribution pattern are nicotine, thiopental, isoniazid, DDT, and caffeine (Sieber and Fabro, 1971). It is of considerable interest to note that these compounds can be detected in the uterine secretions of pregnant animals but not in the secretions of nonpregnant animals evaluated under similar experimental conditions.

In contrast to the data of Sieber and Fabro (1971), it has been quite convincingly demonstrated that inulin, oubain, tetraethylammonium (TEA), and $\alpha$-aminoisobutyric acid (AIB), if administered systemically to the nonpregnant ovariectomized rat, will appear slowly, and in low concentrations, in the uterine luminal fluid; whereas, barbital, dimethyloxazolidinedione (DMO), antipyrine, and tritiated water are distributed into these secretions much more rapidly (Conner and Miller, 1973). The compounds investigated can be grouped into the following categories based on equilibration half-times calculated from their respective rates of penetration into luminal fluid: inulin, TEA, and AIB do not establish equilibrium with uterine luminal fluids during an experimental period of 90 minutes; barbital and DMO have equilibration half-times of 90 minutes; antipyrine and tritiated water have equilibration half-times of less than 10 minutes. These data suggest that the rate of transfer of chemical compounds into the luminal secretions of reproductive organs is closely correlated with their lipid solubility at physiological pH (7.4) and that no specific active transport system appears to be essential for this process (Table III).

The blastocyst migrating toward its eventual site of implantation in the uterus is exposed to the effects of chemical compounds which are present in secretions of the fallopian and uterine lumen. The rabbit blastocyst which has been frequently utilized as an experimental model can regulate its internal concentrations of lactic acid, bicarbonate, and glucose to a remarkable degree. Pretreatment of impregnated rabbits with a variety of drugs does not appear to alter the ability of the blastocyst to modulate these processes (Lutwak-Mann and Hay, 1962).