Characteristics of mammalian development

III. CHARACTERISTICS OF MAMMALIAN DEVELOPMENT

The development of the conceptus consists of two fundamental processes: cell multiplication and differentiation. In the embryonic stages, the cells multiply actively and produce inductors which mediate the morphogenesis of the organs. Hence, in the first stages of development, the physiology of the embryo is, in fact, cellular. The true physiology can be defined in the foetal stage when the organs are sufficiently differentiated to perform a number of vital functions (Table 4).

This development depends on the continuous supply of nutrients which are transferred from the mother across the placenta. In mammals, digestion, respiration and thermogenesis are almost entirely controlled by the maternal organism, and the prenatal life is thus dominated by the placental function.

A. Prenatal physiology

During the prenatal development, two main stages can be distinguished: the embryonic phase, which occupies the first two months, and the foetal period from the third month to the end of pregnancy.

1. *The embryonic period:* this stage is characterized by cellular events, mainly proliferation and migration.

- From fecundation to the end of gastrulation, the embryo consists essentially of a population of dividing cells of similar appearance. Disturbances at this stage will lead to death while teratogenic effects are rare.
- The period of the great cellular movements occurs at the stage of implantation and placentation of the egg. These movements are determined by selective mitotic stimulations in cell groups having already a prospective tissular differentiation. They result in differential growth and modelling.

The determinants of this stimulation are protein inducers, so that all the factors interfering with protein synthesis have a marked teratogenic effect at this stage through an impairment of the sequence of the normal induction mechanism.

The general metabolic functions are characterized by a predominance of anaerobic glycolysis of carbohydrate metabolism, the pentose pathway being very active. Specific metabolic functions commence activity within the cells.

2. *The foetal period* is mainly characterized by the differentiation of the anlagen into definite organs, the general growth, and the storage of energy substrates. At this stage the great morphogenetic events are over and the susceptibility of the conceptus to teratogenic agents greatly decreases. The important processes are the induction of new specific enzymes in the developing organs. If disturbances occur at this stage of maturation, they are unlikely to have any morphological effects and may be harmless to the foetus

Table 4

Period of cleavage	All cells of similar appearance and mitotic activity.	One to n cell stages in oviduct. Morula, early blastocyst in uterus.	
Period of determination	Prospective significance of cell groups, "selective" differences in mitotic activity. Polarization of blastocyst. Beginning of "chemo-differentiation"	Blastocyst	Implantation Placentation
Period of differentiation	Histo- and organogenesis. Further "chemo-differentiation"	Embryo	Junction to maternal blood circulation
	Auxano-differentiation. Organs assume "normal" shape and function.	Foetus	

since most organs are not functional and do not use their specific enzyme assortment.

The foetal physiology is more simple than that of the neonate. The central nervous system does not play an important role; normal development can even be observed in decapitated foetuses. For the most part, excretion is performed by the placenta; nevertheless, the kidneys function very early and it is possible to find urine in the bladder of human foetuses from the third month of gestation. The circulation is regulated at an early stage by adaptative processes: hypoxia produces acceleration of heart rate in the lamb foetus at mid-gestation; vagal bradycardia reflexes function about the 60th day.

Since the important physiological functions are performed by the maternal organism, the energy requirements of the foetus are low. It seems that all the metabolic reactions proceed under low oxygen tension. The oxygen uptake of the foetal lamb is 4 to 5 ml/kg/min while it is 12 ml/kg/min in the newborn.

3. *The newborn period* is characterized by the adaptation to extra-uterine life. It is important as a test period in which metabolic anomalies acquired in the course of gestation may cause a dysfunction of the enzymatic systems required for the autonomic life of the newly born.

The general metabolism performs a switch-over *from an anabolic situation to a catabolic one.* Before birth, the metabolic flow was oriented from the mother to the foetus, which stored glycogen and lipids. After birth new needs of energy arise for thermogenesis, respiration and motility. This energy is supplied by utilization of stored glycogen and lipids.

Interference with storage or utilization processes may have a deleterious effect on the survival of the newborn.

To sum up, the foetal physiology is dominated by growth and storage processes; the most important functions are performed by the placenta.

B. The placenta and the nutrition of the embryo

1. *The placenta* mediates the attachment of the embryo to the uterine wall and displays a more or less intimate intricacy of maternal and foetal tissues in different mammalian species. In man, and Insectivora, the placenta is haemochorial; the chorionic villi are directly in contact with the maternal blood. In Chiroptera and Carnivora the placenta is endotheliochorial: the chorion is separated from the maternal blood by the endothelium of the uterine vessels. In cattle it is syndesmochorial: the chorion is not directly in contact with the maternal vessels; there is an intermediate layer of connective tissues. In some species (pig, horse, monkey) the epithelium of the uterine wall is intact and the chorion is adjacent to it: the placenta is epitheliochorial.

Whatever its structure the placenta has the same essential role in the transfer of molecules in both directions. An impairment of the placental function can be a limiting factor for foetal nutrition.

Moreover, the placenta synthesizes hormones. It performs a great number of enzymatic activities directed towards detoxifying processes: oxidation, reduction, conjugation and hydrolysis.

The early stages of the formation of the placenta, its permeability and its detoxification activity need further study. Most of the investigations performed so far relate to later stages which are less important in teratology. The nutritional role is first performed by the yolk-sac membrane: its modification by trypan blue or kidney antisera has been suspected to be the cause of teratogenic effects.

The transfer of drugs across the placenta may be considered a particular case of transfer across a biological membrane analogous to the intestinal wall. If a drug is adequately absorbed after oral administration and distributed in the tissues it may be capable of crossing the placenta.

The different processes involved are the following (Fig. 3):

– *Active transport* requires a particular structure of the molecule to fit with the specific membrane carrier (Scheme 1). Very few drugs possess this specificity. In this case a competitive effect between molecules of related structures is observed.

– *Passage through membrane pores* cannot be achieved by molecules with a molecular weight higher than 100, which is the case for the great majority of drugs.

– *Pinocytosis* may account for the passage of very small quantities of macromolecules, especially viruses and immunologically active substances.

– The most important process for the transfer of most compounds through the placenta is *the simple diffusion process*.

In fact, the chief characteristics of the passage through the placenta for most drugs are in keeping with the general laws of diffusion through a lipoprotein membrane. The rate of diffusion is proportional to the concentration of the drug; there is no saturation effect, no energy requirement, and no competition between related molecules.

Considering the fundamental properties of a lipid membrane it can be predicted that the rate of diffusion of a compound through the placenta will depend mostly on its lipid/water partition and also on its degree of ionization.

Concerning the *lipid/water ratio*, it has been observed, in fact, that lipophilic drugs pass readily into the foetal circulation. It has been shown experimentally that the lipophilic oestrogens cross the placenta in contrast to their hydrophilic glucuronides, which are hardly able to pass the membranes.

On the other hand, *the degree* of ionization of the drug at a physiological pH must be considered. The non-ionized form of the molecule diffuses but completely ionized compounds penetrate very slowly through the placenta. For instance, strongly quaternary bases used as polarizing muscle relaxants can be detected in the foetus only if several hundred times the pharmacological doses are administered.

The majority of drugs are, in fact, moderately lipophilic and only weakly basic or acidic so that they have little difficulty in crossing the placenta.

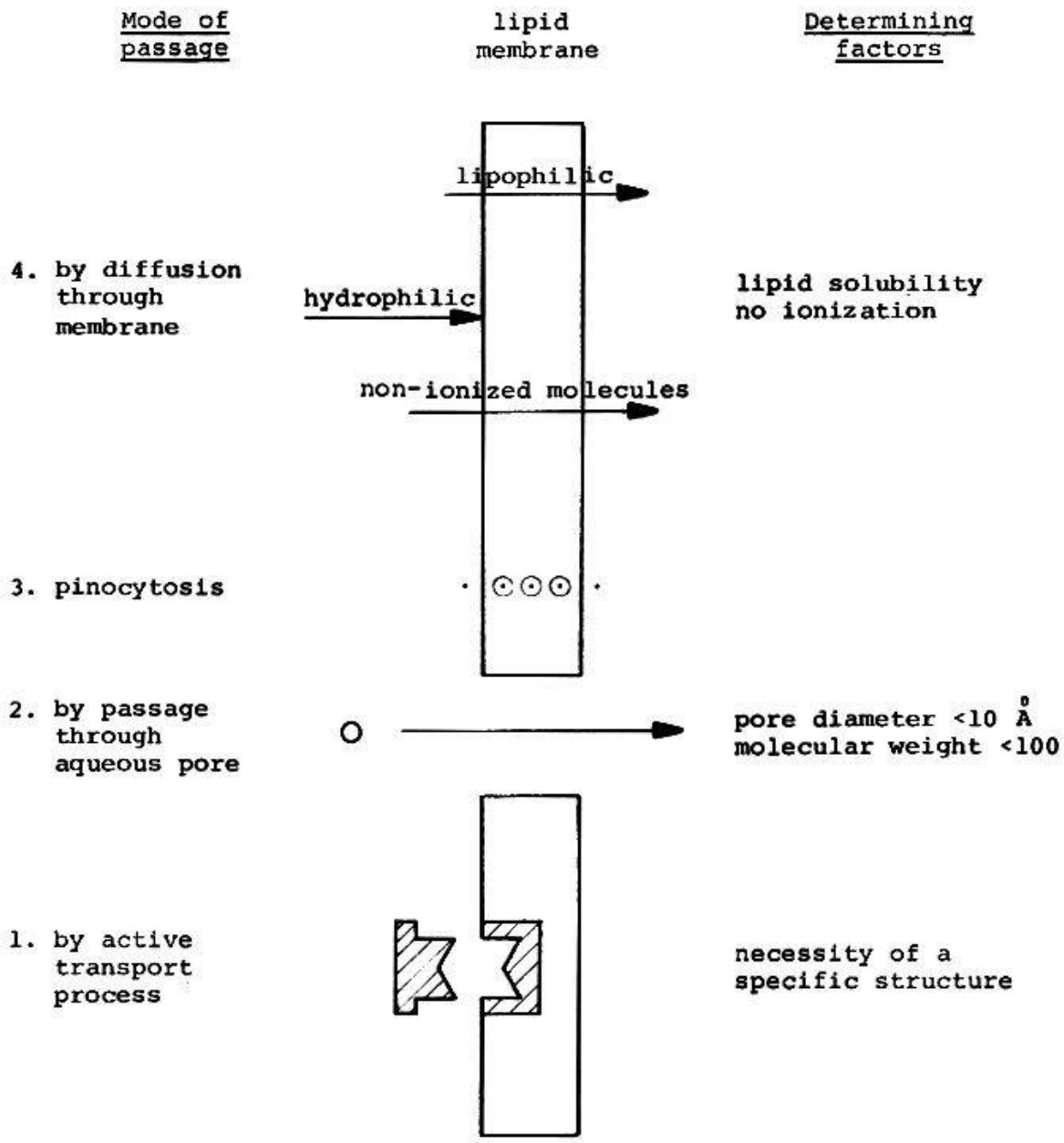

Fig. 3. Placental transfer of various compounds.

In addition to the rate of passage through the placenta several factors may affect the concentration of the drug in the foetus.

The degree of binding to plasma proteins: salicylate was found to bind *in vitro* to a greater extent to maternal than to foetal plasma. *In vivo*, foetal blood levels of sulphonamide have been found to be 10% to 30% below the maternal level, perhaps owing to differences in protein-binding capacity.

The pH of the blood is different in mother and foetus and this could give rise to an unequal concentration of ionizable drug. The fact that the level of pethidine in newborn infants is higher than in the mother has been attributed to the lower pH of the neonatal blood.

The destruction or binding of a compound in the placenta may also limit its concentration in the foetus. This limiting factor is important for endogenous

catecholamines, hydroxytryptamine and tyramine, but it does not apply to drugs in general.

The distribution of the drug throughout the body. Some drugs are known to show specific affinity for certain organs or tissues. Whole-body autoradiography of the foetus in experimental assays serves to compare the disposition of the drug in the foetus with that in the parent animal.

The metabolism and excretion of a compound are the factors which limit the duration of its action. The fact that many enzymes of drug metabolism are absent from the foetal liver may constitute a potential mechanism of protection. In the case of an even tissue distribution, and as soon as the concentration of the compound decreases in the maternal blood, there is a reversal of the diffusion through the placenta and the compound contained in the foetus passes back into the mother's blood. However, if a lipophilic compound were metabolized into its hydrophilic metabolites in the foetus, these metabolites would not cross back through the placenta and could remain in the foetus.

This situation does not hold good after birth because of the absence or low activity of oxidative and glucuronide-forming enzyme in the newly born. Compounds retained during pregnancy may remain for a long time in the newborn and may produce toxic effects. An example is provided by chloramphenicol, which, in addition to being retained, enters into competition with the excretion of bilirubin.

Generally speaking, the fact that a compound crosses the placenta does not mean that it will have an embryotoxic or teratogenic potential. The nature of the compound or of its metabolites and the possibility of their accumulation in the foetus are very important.

Although the study of placental transfer mechanisms is of great interest, it seems that the nature of these phenomena is only of limited relevance to the manifestation of embryotoxicity or teratogenicity of absorbed compounds.

2. The nutrition of the embryo

The mammalian embryo, being practically devoid of nutritional stores, is highly dependent on the maternal organism supplying nutrients via the placenta. The nutrition of the mother is thus particularly important since nutritional deficiencies may cause embryonic death, malformations, and stunted foetuses.

The nutritional requirements of the mother are increased during pregnancy: some parts of the nutrients are utilized for the growth of the foetus and others for energy storage. Placental hormones with a growth-hormone- and prolactin-like activity produce a shift to a state of anabolism in the maternal organism characterized by the storage of free amino acids and peptides, minerals, and other substances. This pool of nutrients is reutilized during the suckling period.

Both the quantity and the balance of the different components of the mother's diet may affect the embryo.

Concerning the quantitative requirements, severe overall restrictions in the diet, occurring during the embryonic period, may result in the death of the conceptus. When they occur during the foetal period, its growth is impeded. Sensitivity to hypocaloric diets varies greatly from species to species: whilst cows and rabbits are not very sensitive, rats show a marked sensitivity; a 50% decrease in the diet causes sterility and a hypocaloric diet may increase the teratogenic action, e.g. of cortisone.

Low-Protein diets alter the growth during the foetal period. They are more noxious in the course of the embryonic period, causing disturbances in pituitary and ovarian function so that the uterine mucosa is no longer adapted to the implantation and nutrition of the young embryo, which cannot be maintained. Moreover, deficiencies in one of the essential amino acids may result in abnormalities, resorptions, and stillbirth: the essential processes of morphogenesis are in fact very sensitive to the supply of amino acids. The optimal balance in amino acids is found in protein of animal origin, not in vegetal protein.

In the rat, deficiencies in *essential fatty acids* such as linoleic acid result in sterility and abortion.

Carbohydrates represent the first source of energy for the embryo and foetus: glucose and fructose are the sugars most readily used. Some organ systems, such as the nervous system, are more sensitive than others – the heart for instance – to carbohydrate deficiencies.

Inorganic substances are also important for the development of the embryo. Some are essential as enzymatic constituents. Zinc deficiency is associated with important malformations in the rat. Other elements are important components of intercellular substances, such as sodium in connective tissues and calcium, phosphorus and manganese in bone. In these cases deficiencies do not cause true malformations: bone curvatures due to defective calcification are observed.

Vitamins readily cross the placenta and are largely utilized by the foetus. If the mother's supply is not increased during pregnancy, the normal level of vitamin in the mother's blood decreases progressively and is often very low at the end of the gestation. Malformations through vitamin deficiencies have not been reported in the human, except for folic acid. A lack of vitamin B_2, vitamin A, pantothenic acid, folic acid, or vitamin B_{12}, is markedly teratogenic in the rat and in other animal species. Deficiencies of other vitamins, such as ascorbic acid, nicotinic acid, thiamine, biotine, and vitamin K have not proved teratogenic. An excess of some vitamins, such as vitamin A, may also disturb the prenatal development in rats, mice, rabbits and guinea-pigs.