- i. **Title: Medications for pregnant women: A balancing act between the interests of the mother and of the fetus.**
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- vii. The authors state they have no conflicts of interest.
- viii. Part of the work reviewed is from a study funded by the CASS Foundation, Victoria.
- ix. What's already known about this topic:
- Many medications are taken by pregnant and breast-feeding mothers.
 - Unlike non-pregnant adults there is only limited evidence from epidemiological studies and little or non from clinical trials on which medical advice may be given to these patients.
 - Very few studies based on animal experimental work investigated entry of drugs into developing brain across blood/brain barrier interfaces.
 - Almost all studies reported suffer from lack of sampling of fetal blood and cerebrospinal fluid, making interpretation difficult.
- What does this study add
- Animal studies in appropriate species, with proper sampling would improve evidence for clinical advice and a basis for future studies for minimising potential harms.
- X. Data availability. All data cited has been derived from the literature.

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XI. ABSTRACT

Drug entry into the adult brain is controlled by efflux mechanisms situated in various brain barrier interfaces. The effectiveness of these protective mechanisms in the embryo, fetus and newborn brain is less clear. The longstanding belief that “the” blood brain barrier is absent or immature in the fetus and newborn has led to many misleading statements with potential clinical implications. Here we review the properties of brain barrier mechanisms in the context of drug entry into the developing brain and discuss the limited number of studies published on the subject. We noticed that most of available literature suffers from some experimental limitations, notably that drug levels in fetal blood and cerebrospinal fluid have not been measured. This means that the relative contribution to the overall brain protection provided by individual barriers such as the placenta (which contains similar efflux mechanisms) and the brain barriers cannot be separately ascertained. Finally, we propose that systematic studies in appropriate animal models of drug entry into the brain at different stages of development would provide a rational basis for use of medications in pregnancy and in newborns, especially prematurely born, where protection usually provided by the placenta is no longer present.

Keywords : Fetal and placental pathology

xii. Acknowledgements. We should like to thank our many colleagues and collaborators who have contributed to much of this work that had led us to start studying mechanisms of drug entry into the developing brain.

xiii. Main text

INTRODUCTION

It is generally believed that the fetal brain is vulnerable to damage from maternally administered drugs; pregnant women are therefore often given advice to not take any medication if possible. Accordingly, some women risk their health by stopping treatments for fear of harming their baby or are otherwise anxious throughout pregnancy. In some instances withholding medication from a woman, when she becomes pregnant, could be harmful, both to her and her baby. In many cases this group of vulnerable patients is disadvantaged because, unlike non-pregnant adults, there is no evidence base from clinical trials and only limited evidence from clinical reports and epidemiological studies that can be used by medical practitioners when deciding on best therapies for pre-existing medical conditions or conditions that arise after the onset of pregnancy.

The Federal Drug Administration have abandoned their advisory letter code of A to E and X, because too few drugs fell into the A category (thought to be safe) or X (high risk and therefore to be avoided). The FDA (Pregnancy and Lactation Labelling Rule) has replaced this with a requirement that all packs of drugs to be prescribed to pregnant women should include an insert which details all of the information known about risks and side effects of the drug in pregnancy (1).

Currently the main sources of information for clinicians and their pregnant patients are the Australian Medicines Handbook (2) hospital databases and fact sheets such as that at the Royal Women's Hospital, Melbourne (3). There is also the comprehensive US volume that used to be known as "Yaffe's Index" and is now authored by Briggs et al (4). This assembles the published human clinical and animal experimental data on each of the more than 1200 drugs that have been taken by pregnant and breastfeeding women. On the basis of these data Briggs et al (4) offer a risk assessment which ranges from "Compatible (Maternal benefit>> Embryo-Fetal Risk) to "Contraindicated". There are many categories in between, sometimes assessing risks differentially, depending on the stage of pregnancy. However, we have noted that some key publications are not cited, which is hardly surprising given the massive number of papers in the literature.

There has been a gradual shift from a preoccupation with the possibility of drugs taken in the 1st trimester causing congenital malformations, to concern about possible effects of drugs taken later or throughout pregnancy having effects on the offspring in the years after birth. This was well summarised by Yaffe (5): “The physician is confronted with two imperatives in treating the pregnant woman: alleviate maternal suffering and do no harm to the fetus. Until now, the emphasis has been on the amelioration of suffering, but the time has come to concentrate on not harming the fetus”. There are now moves to involve pregnant women in clinical trials (6, 7).

The FDA has published draft guidance for industry (1). It does seem unlikely that clinical trials will be undertaken for many drugs currently in use and clinical trials in pregnant women are only a tiny fraction of trials carried out in the adult population (8). We are therefore proposing an alternative approach for which we have instituted some initial studies (30 and in preparation). We suggest that well-defined and relevant animal models are required to conduct controlled experimentation under physiological conditions that can then be applied to human pregnancy.

A specific major concern is whether or not a drug taken during pregnancy will cross the placenta and enter the brain of the embryo/fetus across the various brain barrier interfaces and if it does enter, whether it will have deleterious effects on brain development that may manifest as abnormal in the offspring after birth and perhaps even many years later.

There is almost no published experimental evidence addressing this problem (9). There are less than a dozen papers published that we could identify in PubMed in which a drug (usually radiolabeled) has been injected into a pregnant animal (usually a rodent) and the brain examined to determine if the drug had reached the brain (Table 1). The results of these papers are difficult to interpret because in no case was the radiolabeled drug estimated in the fetal blood or cerebrospinal fluid (CSF). In the absence of data on fetal blood levels the contribution to drug protection provided by the placenta cannot be separated from that provided by the brain barriers that in the adult exclude many drugs from entering the brain (see below). Data on CSF are important because this differentiates entry into the brain via the choroid plexuses and CSF and the direct entry across cerebral blood vessels. The

former is thought to be more important in the fetus because choroid plexus development occurs well in advance of much vascularisation of the brain (21).

One reason for the neglect of this field is perhaps the longstanding belief that the blood-brain barrier is absent or poorly functioning in the fetus and newborn. This appears to stem from a combination of poor research and misunderstanding or mis-citation of published work (for review see 22). More recently and supported by substantial experimental evidence, it has been accepted by most that the brain grows within a well-controlled environment that changes as the brain develops. The tightly controlled environment is provided by cellular and morphological mechanisms that comprise the brain barrier interfaces (23).

In the adult most drugs do not enter the brain to any significant level. Even in the case of drugs developed specifically to treat neurological and psychiatric conditions it has been estimated that 98% do not enter the brain (24). This is largely because the drugs were developed using *in vitro* systems that do not take account of the properties of brain barrier mechanisms. The mechanisms responsible for limiting or excluding drug entry into the brain are efflux transporters generally belonging to the family of ATP-binding cassette (ABC) transporters. Very little is known about the presence, let alone functional status, of these transporters in the developing brain. The limited amount known about these transporters in human and animal fetal brains will be summarized in this review, but first we outline in general the current understanding of barrier mechanisms in the developing brain.

BARRIER MECHANISMS IN THE DEVELOPING BRAIN

The term “blood-brain barrier” is of limited value and is confusing because it encompasses not only a physical restraint on passage of molecules across the cerebral vessels, whereas it also involves a range of physiological mechanisms that control entry into and exit from the brain of a wide range of molecules including drugs and toxins (9, 23).

In addition, these exchange mechanisms occur across five barrier (six in the developing brain) interfaces in the brain, not just the blood-brain barrier proper, which is present in cerebral blood vessels (BBB in Figure 1). The other interfaces are: the blood-cerebrospinal fluid (CSF) barrier in the choroid plexus within each brain ventricle (BCSFB, in Figure 1);

circumventricular organs; ependyma in the adult brain; the meningeal barrier, which consists of three separate interfaces and in the embryo the CSF-brain barrier (CSF-BB in Figure 1). A key morphological specialization in most of these interfaces is the presence of tight junctions between the adjacent cells of the barrier. These limit the movement of all but the smallest water-soluble molecules between the cells of the interface. This has the functional effect of conferring the properties of individual cells in the interface across the full extent of the interface (for example endothelial cells of cerebral blood vessels and epithelial cells of the choroid plexuses). Inserted into these morphological interfaces are various cellular mechanisms.

The cellular mechanisms can be divided into those which have a net inward transport function (e.g. for glucose, amino acids and monocarboxylates, Figure 2) and those which efflux potentially deleterious compounds such as drugs and toxins (Figure 3). The sites of these barrier mechanisms at the three best-studied interfaces (blood-brain barrier, BBB; blood-CSF barrier, BCSFB and CSF-brain barrier, CSFB) are illustrated in Figure 4.

The situation during development is even more complex. The neuroepithelium in the fetus generates many of the neurons and later glial cells of the developing brain (25). In the early fetus, when neurogenesis predominates, the CSF-brain interface limits intercellular diffusion of water-soluble molecules by the presence of strap junctions between adjacent neuroepithelial cells; these disappear at around the time of the switch from neurogenesis to gliogenesis in the neuroepithelium (26, 27). The cells of the neuroepithelium then become ependymal cells of the adult ependyma that lines the cerebral ventricles. They are linked by gap junctions which do not impede the passage of molecules even as large as proteins into the brain interstitial fluid (28). In the adult the concentration of protein in CSF is very low, which given the free exchange of proteins across the ependyma explains the low concentration of protein in the interstitial fluid of the brain.

DRUG ENTRY IN THE DEVELOPING BRAIN

In spite of the large number of drugs prescribed to pregnant and breast-feeding mothers there is very little experimental or clinical evidence on whether such drugs enter the brain of a fetus or neonate. At present this can only be studied in experimental animals, usually

rodents. There are, however, very sensitive radiolabeled drug imaging methods being developed which may eventually allow human studies to be undertaken (29).

Published studies, that we identified in PubMed, in rodents are summarized in Table 1, including information on the dose, route of administration, detection method and the ABC transporter for which the drugs are thought to be a substrate (usually ABCB1, P-glycoprotein). The experiments were mainly conducted in the last third of gestation (E14 to E21). Most of the drugs were detected in the developing brain, but the results are difficult to interpret. No measurements in fetal blood or cerebrospinal fluid are reported. The results are generally expressed as a ratio of fetal brain/rest of fetus or fetal brain/maternal blood. This means that the relative contributions of the placenta, blood-brain and blood-CSF barriers to restricting entry of drug into the developing brain cannot be determined.

This limitation has been overcome in a recent study of three drugs (digoxin, cimetidine and paracetamol) in E19 pregnant and P4 postnatal rats compared to pregnant and non-pregnant adults (30). In these experiments, entry of drug into fetal blood, brain and CSF at all three ages was estimated using radiolabeled drugs. Two treatment regimens were used: single intraperitoneal doses or twice daily treatment over 5 days. Radiolabeled drug was included in the injectate in the single dose experiments and with the last dose of the 5-day treatment. Blood, brain and CSF samples were taken at 30 min after injection. An example of results from this approach in a study of paracetamol (acetaminophen), which illustrates the importance of sampling fetal and neonatal CSF, is shown in Figure 5. For both acute and chronic treatment groups the ratios (brain/plasma and CSF/plasma) were higher in brain and CSF in younger animals. In the adults the ratios for both brain and CSF were lower, probably because of upregulation of ABC transporters (30) in response to repeated drug doses. At E19 following chronic doses ratios for both brain and CSF were higher, probably indicating that any limitation by ABC transporters at this age was overwhelmed by the dosage and at this age upregulation did not occur (30).

Mechanisms protecting the brain from drug entry:

ABC EFFLUX TRANSPORTERS IN DEVELOPING BRAIN

Around 50 members of the ABC protein superfamily have been identified in the human; they are present in many different tissues (31). In human adult brain at the blood-brain barrier efflux transporters that appear to be functional are: ABCB1 (also known as P-glycoprotein, PGP or Multidrug Resistance Protein 1, MDR1), ABCG2 (Breast Cancer Resistance Protein, BCRP), ABCC1 (Multidrug Resistance Protein 1, MRP1), ABCC2 (MRP2), ABCC4 (MRP4), ABCC5 (MRP5)- see (31, 32, 33). Suhy et al (34) identified transcripts of many more ABC transporters in an RNA-Seq study of human cerebral cortex from two brains enriched with isolated human brain microvessel endothelial cells (BMECs). However, given that whole cortex was used the localization of the transporters is unclear as is whether there is translation to functional efflux transporter protein. ABCC1 (Multidrug Resistance Protein 1, MRP1) appears to be the main efflux transporter at the blood-CSF barrier (choroid plexuses); but ABCC4 (MRP4) and ABCG2 (BCRP) are also present at this interface (35, 36).

In human developing brain there is only one comprehensive immunohistochemical study of the distribution of three of the main brain ABC transporters and this was restricted to 5 to 21-weeks post conception, wpc (37). BCRP and ABCC1 were present at the earliest age examined (5wpc). This is a stage when the human embryo has a closed neural tube comprising forebrain, midbrain, hindbrain, spinal cord, but no choroid plexuses and the only brain barrier is an inner eCSF-brain barrier. PGP did not appear until 7wpc and then only in 4th ventricular plexus cells. All 3 were present in lateral ventricular choroid plexus epithelial cells at 8wpc.

Of key importance is when these transporters appear in developing brains of experimental animals as it is these that will be required for studies of drug entry in pregnancy and lactation. ABC transporters have mainly been studied in rodents, with a few studies in the developing brain. These have been reviewed in (9). It appears that at least two of the key transporters have similar temporal profiles in rats and humans during development (Table 2).

EFFECTS OF MATERNAL INGESTION OF DRUGS DURING PREGNANCY ON BRAIN & BEHAVIOUR IN THE OFFSPRING.

This has mainly been studied epidemiologically rather than in prospective randomised blinded clinical trials, although there have been a few prospective animal studies. There are several sources that attempt to bring together the published data on drugs that have been used in pregnancy and breast feeding; most of these studies are case reports or reports on small numbers of patients. There is usually no attempt to assess the value of these studies so they are little more than summaries of published reports (3, 4, 38).

Because of space constraints we can only give a few examples and point to their limitations. In our view it would be preferable to have information about the extent of drug entry into the developing brain and its distribution in the brain as a prelude to undertaking studies of potential deleterious effects on brain structure and consequent behavioural problems. Leclercq et al (39) studied, what they describe as “low dose”, effects of penicillin on gut microbiota, brain cytokines and behaviour in the offspring of pregnant mice. Pregnant and postnatal mice were treated with either penicillin B or penicillin B and *Lactobacillus rhamnosus* JB-1 compared to no drug controls. There are several problems with this study. In addition to a notable lack of randomisation and blinding, the penicillin dose was at the upper limit of that used clinically and treatment was for a clinically improbable length of gestation and into the postnatal period. These problems render the conclusions to be of doubtful value and certainly do not provide the evidence that appropriately prescribed penicillin during pregnancy and breast feeding is hazardous for the babies. In a better designed study (including randomisation and blinding) Viberg et al (40) examined the effects on locomotor behaviour of two doses of paracetamol in 10-day old mice. Only the larger dose of two treatments had a significant effect compared to control or the lower dose level. The authors acknowledge that the larger dose was above any that would be used clinically. Nevertheless, the finding of a substantial entry of paracetamol into the brains and CSF of fetal and newborn rats (30) suggests that caution is warranted when considering taking paracetamol during pregnancy and breast-feeding and advice that paracetamol is safe (2) may not be entirely warranted.

Another epidemiological study is that of Stergiakouli et al (41) who claimed an association between paracetamol (acetaminophen) use in pregnancy and behavioural problems in childhood. This work has been criticised on a number of grounds. Their data show an

association between the partner's use of paracetamol and behavioural problems in the children at 5 years of age. But this was dismissed in favour of the hypothesis that the behavioural problems correlated with an intrauterine mechanism (42). Damkier et al (43) amongst a series of critical comments questioned the adequacy of the data used for the study and point to the uncritical use of parent-assessed questionnaires. Much of the key data were hidden away in supplementary tables which obscured some relevant findings that were ignored by the authors. For example, nearly as many of the children of mothers who had never taken paracetamol also showed behavioural problems (44).

All of the correspondents commenting on the limitations of this study expressed concern about such weak data alarming pregnant women past and present. Little et al (45) made the point that the criticisms of the study are not just a matter of methods or reporting but the studies raise ethical issues given the potential of the claims to evoke guilt or anxiety in the people for whom the research is most relevant.

ANIMAL MODELS FOR THERAPUTIC STUDIES OF HUMAN DEVELOPMENTAL PROBLEMS

The most important decision about which species to study in relation to a particular problem is its relevance to the problem, rather than convenience or availability. Nevertheless compromises often have to be made, particularly in developmental studies, so it is important to acknowledge the limitations of the species and approach used.

It is generally desirable for the animals' physiological state to be monitored as this may affect the entry of molecules into the brain and CSF as happens in hypercapnia (46). However, this may not be possible for very early stages of brain development. Because of being born at such an early stage of development, marsupial species have the advantage that for simple drug studies they are already exteriorised so that injections of test compounds result in much less of an interference than would be the case for example with a pregnant rat or mouse. Marsupials also provide an opportunity to carry out drug studies at very early stages of brain development in the absence of a placenta, because the animals have been born and they survive much better than rodents at equivalent (*in utero*) stages of development. Nevertheless some have questioned the value of marsupial species for developmental

studies on the grounds of lack of research tools compared to rodents, particularly mice. However, the genomes of two experimental species have now been sequenced (47, 48). Table 3 provides information about developmental milestones and ages at which experiments have been performed in the key mammalian species that have been used for developmental brain barrier studies. For more details on brain development see (49) and <http://www.translatingtime.org/translate>.

In Table 3, we have divided the species into four groups based on stages of brain development at birth.

In Group IV, the sheep fetus is particularly important and has been the traditional species for a whole range of developmental physiological studies. The sheep placenta is made up of around 100 cotyledons, each with its own circulation (50). Access to the fetal circulation can be obtained with minimal disruption of the placental circulation, which is a significant problem in non-ungulate species. Permeability studies in fetal sheep using monitored and reasonably well-controlled physiological conditions have been possible as early as E50 (51) although most fetal sheep studies have been carried out later in gestation e.g. E87 to term (52). The monitored studies in fetal sheep are relatively expensive and require more space and instrumentation, but they provide important background validation to studies in species where monitoring has so far not been technically possible. However, they are unsuitable for some human related problems. For example they have been suggested as a model for disorders in prematurely born infants. This has been very successful for studies of lung immaturity and developing effective ventilator and other treatments (53, 54, 55). But the sheep fetus is less suitable for studies of brain development compared to humans as at the earliest stages of viability (E120) the brain is already as mature as in postnatal infants (<http://www.translatingtime.org/translate>). The rat is a more appropriate species for brain drug entry studies during development. The rat brain develops at a much faster rate than human but the stage of its development in first few days of life is in important respects similar to that of the human brain at 22-26 weeks' gestation (49) and (<http://www.translatingtime.org/translate>). This provides an opportunity to study a stage of development when the brain may be more vulnerable because of the loss of the placenta.

A specific reason why the rat is a suitable species is that the temporal development of some of the key efflux transporters is similar in the human and rat (36).

Because each species comes with inherent limitations, it is preferable to study problems in more than one animal species. This provides complementary evidence on a particular question, an approach that has been described by (56) as “triangulation”.

CONCLUSION

There is only very limited experimental information about drug entry into the developing brain and most of the few published studies are difficult to interpret because the drug levels in fetal blood and CSF were not measured. An approach which provides this information for 3 drugs (digoxin, cimetidine and paracetamol) has been published recently for fetal and neonatal rat brain (29). Such studies need to be extended to other clinically important drugs, particularly those that are likely to be required throughout pregnancy and during breast-feeding (e.g. anti-epileptics, antidepressants and anti-psychotic drugs). Such drugs also should be tested for long term effects on brain development and behaviour in offspring of mothers taking them. Even if deleterious effects are identified, caution needs to be exercised in advising pregnant and breast-feeding mothers as such information is likely to be mainly derived from animal studies. Nonetheless, such information would provide clinicians with better quality advice for their patients than is currently available.

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xv. Tables

Drug/toxin	Method	Species	Route	Dose	ABC substrate	Age	Drug distribution	Reference
Digoxin	³ H	Mouse	i.v.	0.05mg/Kg	ABCB1	E15	Fetus/maternal plasma 25%	(10)
Saquinavir	¹⁴ C				ABCB1		Fetus/maternal plasma 2%	
Paclitaxel	HPLC				ABCB1		Fetus/maternal plasma 2%	
Fluoroquinolone DW-116	¹⁴ C	Rat	oral	501mg/Kg		E18	Fetal brain/maternal plasma 140%	
Cimetidine	³ H	Rat	i.v. infusion	14 nmol in 1ml /100g for 60min	ABCG2	E18 E21	Fetal brain/maternal plasma 6% Fetal brain/maternal plasma 3%	(11)
Digoxin	³ H	Mouse	i.v.	50µg/Kg	ABCB1a	E15.5 E18.5	Fetal brain/body 55% Fetal brain/body 45% (ns)	(12)
Apixaban	¹⁴ C	Rat	gavage	5mg/Kg	ABCB1* ABCG2	Not given* *	Fetal brain less than other organs. Fetal brain/maternal blood=11% 12% in adult; cleared more rapidly	(13)
Mitoxantrone	³ H	Mouse	i.v.	5mg/Kg	ABCG2	E15.5 E18.5	Fetal brain/body 60% Fetal brain/body 60%	(14)
Talinolol	LC–MS/MS	Rat	oral	100 mg/kg/day	ABCB1	E15–E21	Fetal Brain/maternal serum 7.4% Adult Brain/mat serum 4.1% ^^	(15)
Carbamazepine	³ H	Mouse	Oral (in drinking water)	100µg/L	ABCB1	E0–	Detected in embryonic brain	(16)
Fluoxetine				10µg/L	ABCB1	E10	None in embryonic brain	
Venlafaxine				50µg/L	?		None in embryonic brain	
Genisten	LC/MS	Mouse	Oral	30 µmol/kg	BCRP	E14	Fetus/maternal plasma 25% Fetal brain/fetal body 60% Higher ratios in Bcrp-/- mice	(17)

Digoxin	3H	Mouse	i.v.	50 µg/kg and 1 µCi/animal	ABCB1	E15.5	Fetal brain/body 50% at 4h 75% at 24h. Incr by TLR-3 lig PolyI:C	(18)
Saquinavir	14C	Mouse	oral	5.0mg/Kg	ABCB1	E15	Fetal brain/maternal blood 8h, 5.7% calc from Fig 3. Incrin Mdr1a KO	(19)
Olanzapine	14C	Rat	oral	18mg/Kg		E12, E18	Autoradiography. E12 Emb/plasma 63% (est). E18 no values given	(20)

Table 1. Published studies identified in PubMed of drug entry into fetal brain. Note lack of estimations of drug in fetal blood or CSF.

	Cerebral vessels		Choroid plexus	
AGE ^a	ABCB1 (PGP)	ABCG2 (BCRP)	ABCB1 (PGP)	ABCG2 (BCRP)
Human				
5	NP (d)	++ (d)	Plexus not present ^b	
8	++ (c)	+++ (c)	++ (c)	++ (c)
13	+++	+++	+++	+++
20 ^e - 23 ^d	+++ + NP (c d e)	+++ (c, e)	+++ + (c e)	+++ NP (c e)
Adult	++++ (d, e)	++++ (e, f, g)	+++ ++ (e h)	NP (e)
Rat				
E13	+ / NP (i)	++ (i)	Plexus not present ³	
E15	+	++ (i)	+	++ (i)
P1	++ (i)	++ (g)	+	++ (i)
P21	+++ (i)	NP (i)	+	NP (i)
Adult	++++ (i, j)	NP (i)	+	NP (i)

Table 2. Developmental presence of PGP (ABCB1, red) and BCRP (ABCG2, green) in human and rat brain. Intensity of immunostaining scored from +/- (very few positive stained vessels) to ++++ (dense and consistent staining). Immunostaining not present indicated by NP. (a) Developmental ages in weeks post conception (wpc) for humans, embryonic (E) and postnatal (P) days for rats. (b) Choroid plexus not yet present at earliest stages investigated in both species. (c) Data from (36); (d) data from (58); (e) data from (59); (f) data from (60); (g) data from (61); (h) Data from (62); (i) data from (34);(j) data from (63). Modified from (36).

	Group I short gestation, at birth cortex undifferentiated		Group II short gestation, at birth neurogenesis near complete		Group III intermediate gestation, brain more mature than Group II		Group IV long gestation, larger brain at birth neurogenesis & gliogenesis mostly complete		
	Opossum	Wallaby	Mouse	Rat	Rabbit	Guinea Pig	Sheep	Pig	Human
Days post conception	14	28	20-21	21-22	30-32	67-70	147	115	266
Neural tube closure	E10.5 [^]	~E18 ⁺	E9- E9.5 [#]	E11 [#]	E9 [#]	14 [#]	E21 [#]	E17-E18 [#]	E26
¹ Published permeability studies	P0* P5	P0-3	E11* E12	E13	E28-30	E60-65	E21* E50	E20*	E35*

Table 3. Developmental milestones of mammalian species commonly used for blood-bran and blood-CSF barrier studies

[#](64) [^](65). ⁺(66). ¹Permeability studies include blood-brain and blood-CSF barriers.

*BBB impermeability to plasma proteins demonstrated by immunohistochemistry only. Data sources for permeability studies: Opossum (*Monodelphis domestica*) (67); tammar wallaby (*Macropus eugenii*) (68); mouse (69); rat (70); sheep, (51, 71, 72, 73); pig (74); human (36); guinea pig (75); rabbit (76). From (22).

xvi. Figure legends

Figure 1. Schematic diagram (center left) of the five main barrier interfaces (a-e) in the brain and an additional one in the embryo (f). The barrier-forming cellular layers at each interface are colored green.

(a) The meningeal barrier is structurally the most complex of all the brain barriers. (the Arachnoid barrier cells [ABC]) have tight junctions (arrowheads) between adjacent cells forming a barrier between the outer cerebrospinal fluid (o-CSF) and more superficial dural layers (dural border cells [DBC] and the dura mater. Blood vessels (BV) in the subarachnoid space (SAS) have tight junctions but no surrounding pericytes and astrocytic end-feet. Blood vessels within the dura mater are fenestrated (f-BV); bm = basement membrane, gl = glia limitans.

(b) The blood-brain barrier: cerebral blood vessels (BV) with tight junctions (tj, arrowhead) between the endothelial cells (EC); bm = basement membrane, PC = pericytes, AE = end feet from astroglial cells.

(c) The blood-CSF barrier: choroid plexuses within each brain ventricle. The epithelial cells (CPE) have tight junctions (27) at their apical side (CSF facing, arrowheads). Blood vessels (BV) are fenestrated.

(d) Circumventricular organs (including median eminence, pineal gland, area postrema, subfornical organ). Blood vessels have permeability characteristics similar to elsewhere in the body. Allow feedback penetration of peptide hormones controlled by the hypothalamic-pituitary axis. Peptides and other molecules are prevented from entering CSF by tanycytes (TC), connected by tight junctions between their apices (arrowhead); entry into rest of the brain prevented by tight junctions between astroglial cells (GC). Ependymal cells lining rest of ventricular system are linked by gap junctions that allow free exchange between the CSF and brain interstitial fluid (broken arrow).

(e) Ependyma in adult brain. Apart from areas with tanycytes, ependymal cells are linked by gap junctions that do not restrict exchange of even large molecules between CSF and interstitial space of brain (solid arrows).

(f) The embryonic CSF-brain barrier. In early brain development, strap junctions (open arrowheads) are present between adjacent neuroepithelial cells (NE); these form a barrier restricting the movement of larger molecules, such as proteins, but not smaller molecules. From (57).

Figure 2. Influx transporters at the blood–brain barrier

These are mainly SLC (solute carrier) transporters. See (22) for comprehensive review. Many of these transporter genes are found in both endothelial cells of the blood–brain barrier and epithelial cells of the choroid plexuses. Others are unique to each interface. Note, many metal ions that are potentially toxic can be carried in via some of these transporters. From (22).

Figure 3. Efflux transporters at the blood–brain barrier

These are mainly ATP-binding cassette (ABC) transporters. Some, e.g. P-glycoprotein (PGP; ABCB1), reduce entry into cells. Others, e.g. multidrug resistance protein (MRPs; ABCCs), ligand (drug or toxin) combines with glutathione, glucuronic acid or sulphate in cells before efflux. BCRP, breast cancer resistance protein (ABCG2). ‘Others’ include SLC efflux (SLCO) transporters. From (22).

Figure 4. Sites of main brain barrier interfaces

BBB, blood-brain barrier; BCSFB, blood-SCF barrier; CSF-BB, CSF brain barrier.

Secreted CSF moves through ventricular system by bulk flow and is important for reducing the level of compounds entering the CSF (“sink effect”).

From (22).

Figure 5. Brain/plasma and CSF/plasma concentration ratios for [³H]-paracetamol

Acute (white bars) and chronic (grey bars) experiments. Means, * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$. At E19 paracetamol was administered by i.p. injection to the mother. Individual fetuses were serially sampled starting at 30min following maternal injection up to approximately 2.5h. Adult and P4 animals were injected i.p. and samples taken at 30min. Note that for both acute and chronic treatment groups the ratios were higher in brain and CSF in younger animals. At E19 following

chronic doses ratios for both brain and CSF were higher; in the adults they were lower. In these experiments the fetal/maternal plasma ratio was 42% in acute dose experiments and 43% in chronic dose experiments. From (29).

Figure 1.

Figure 2.

Figure 3

Figure 4

PARACETAMOL

Figure 5

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