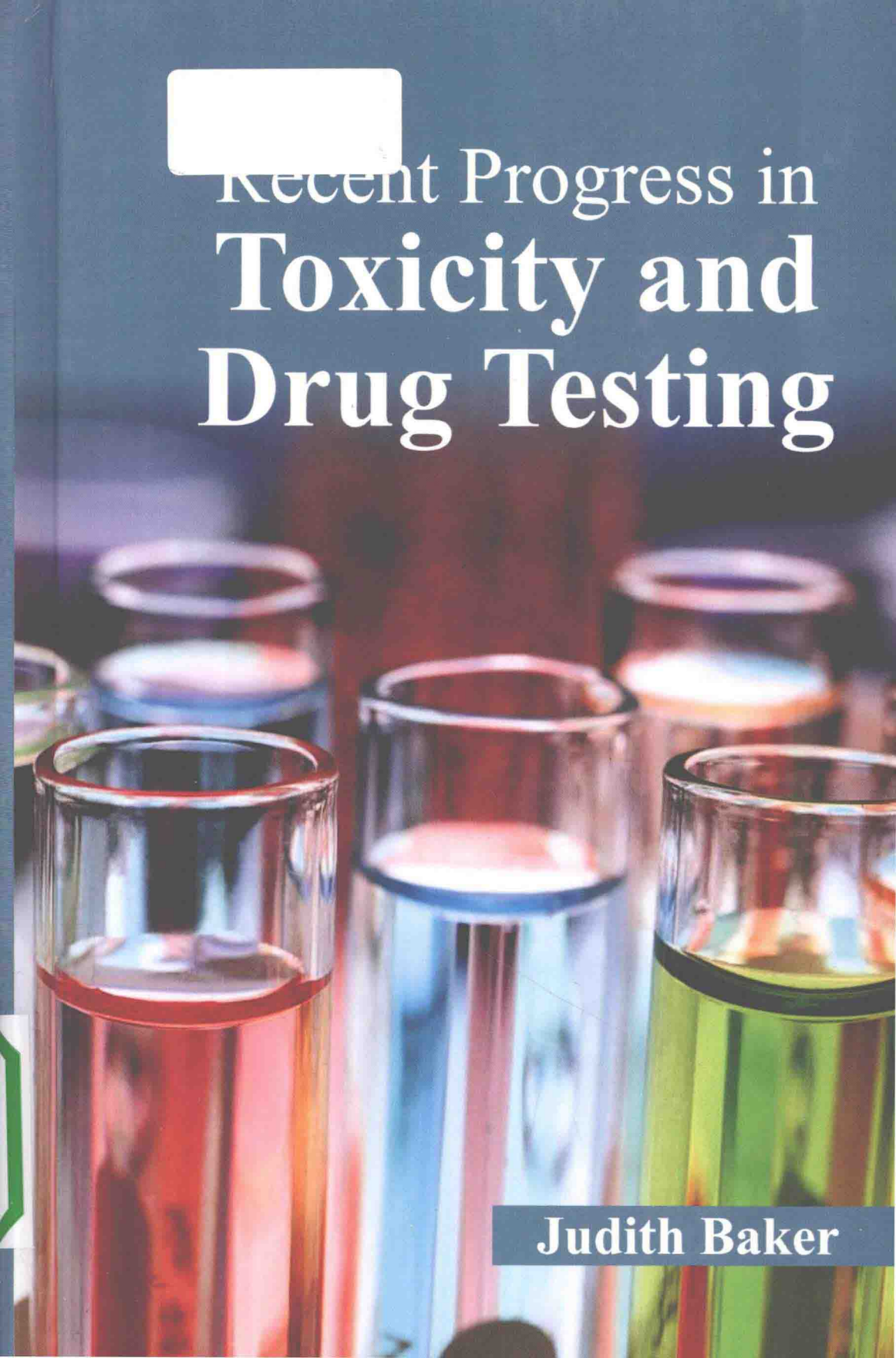
Recent Progress in
Toxicity and
Drug Testing
Judith Baker

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Edited by **Judith Baker**

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Preface

This book was inspired by the evolution of our times; to answer the curiosity of inquisitive minds. Many developments have occurred across the globe in the recent past which has transformed the progress in the field.

Present-day drug design and testing includes experimental in-vitro and in-vivo measurements of the drug candidate's adsorption, distribution, metabolism, elimination and toxicity (ADMET) properties in the initial stages of drug discovery. Just a poor percentage of the recommended drug candidates get the authorization of government and reach the market place. Disadvantageous pharmacokinetic properties, bad bioavailability and efficiency, negative side effects, poor solubility and toxicity matters are responsible for most of the drug failures confronted in the pharmaceutical industry. Authors from across the globe have provided information elucidating pharmaceutical concerns, regulatory policies and clinical properties in their respective countries hoping that the open trade of scientific ideas and outcomes compiled in this book will result in enhanced pharmaceutical products. This book has a section on drug design covering several research chapters.

This book was developed from a mere concept to drafts to chapters and finally compiled together as a complete text to benefit the readers across all nations. To ensure the quality of the content we instilled two significant steps in our procedure. The first was to appoint an editorial team that would verify the data and statistics provided in the book and also select the most appropriate and valuable contributions from the plentiful contributions we received from authors worldwide. The next step was to appoint an expert of the topic as the Editor-in-Chief, who would head the project and finally make the necessary amendments and modifications to make the text reader-friendly. I was then commissioned to examine all the material to present the topics in the most comprehensible and productive format.

I would like to take this opportunity to thank all the contributing authors who were supportive enough to contribute their time and knowledge to this project. I also wish to convey my regards to my family who have been extremely supportive during the entire project.

Editor

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Drug Design

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Blood Brain Barrier Permeation

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1. Introduction

The large surface area and the short diffusion distance from capillaries of the blood brain barrier (BBB) to the neurons facilitate the drugs and nutrients access to the brain. Penetration of chemicals to the BBB occurs using a combination of intra and intercellular passages. Tight junctions regulate the intracellular passage of molecules according to their physico- chemical properties (e.g. lipophilicity, ionisation and polarity), where inter cellular penetration is regulated by influx and efflux transporters, endocytosis and passive diffusion. Poor pharmacokinetic properties (absorption, distribution, metabolism and excretion) and toxicity are responsible for most of the failures in drug discovery projects. This problem is more evident for CNS drugs because of the restrict barrier function of blood brain barrier. The CNS drug discovery attracted more attentions since the diseases pattern has been changed during recent decades and aging disorders are one of the major health problems. Drug exposure is controlled by plasma pharmacokinetic properties of drug which are different from brain pharmacokinetic and can be studied using common pharmacokinetic studies, where BBB permeability depends on physicochemical properties of drug compound and physiologic function of the BBB (physical barrier, transport, metabolic, ...) and need special study techniques. In this chapter, fundamentals of BBB, permeation mechanisms, penetration measurement methods and penetration prediction methods are discussed.

2. Fundamentals of BBB

2.1 Cellular properties of Blood Brain Barrier

BBB consisted of a monolayer of brain micro vascular endothelial cells (BMVEC) joined together by much tighter junctions than peripheral vessels and formed a cellular membrane which known as the main physical barrier of BBB (Abbott, 2005; Cardoso et.al., 2010). The main characteristics of this cellular membrane are, uniform thickness, no fenestrae, low pinocytotic activity, continues basement membrane and negative surface charge. In addition to the BMVECs, the neurovascular unit consisted of the capillary basement membrane, pericytes, astrocytes and microglia. The BMVECs are surrounded by a basement membrane which composed of structural proteins (collagen and elastin), specialized proteins (fibronectin and laminin) and proteoglycans. This structural specificity gives the basement membrane a cell establishment role. Pericytes are cellular constituents of microvessels

including capillaries and post capillary venules that covered about 22-32% of the capillaries and shared the same basement membrane. Pericytes are responsible for a wide variety of structural and non-structural tasks in BBB. In summary they synthesis some of structural and signalling proteins and they are involved in the BMVECs proliferation, migration and differentiation. More details and references about pericytes role in BBB can be found in the literature (Cardoso et al., 2010). Fine lamellae closely opposed to the outer surface of the capillary endothelium and respective basement membrane formed by astrocytes end feet. Like pericytes, astrocytes involve in various functional and structural properties of neurovascular unit.

Microglia is immunocompetent cells of the brain that continuously survey local micro environment with highly motile extensions and change the phenotype in response to the homeostatic disturbance of the CNS (Prinz & Mildner, 2011). The interactions of brain micro vascular endothelial cells with basement membrane, neighbouring glial cells (microglia and astrocytes), neurons and perivascular pericytes leads to specific brain micro vascular biology. Presence of matrix adhesion receptors and signalling proteins form an extensive and complex matrix which is essential for maintenance of the BBB (Cardoso et al., 2010). Figure 1 shows a schematic illustration of neurovascular unit and BBB cellular components.

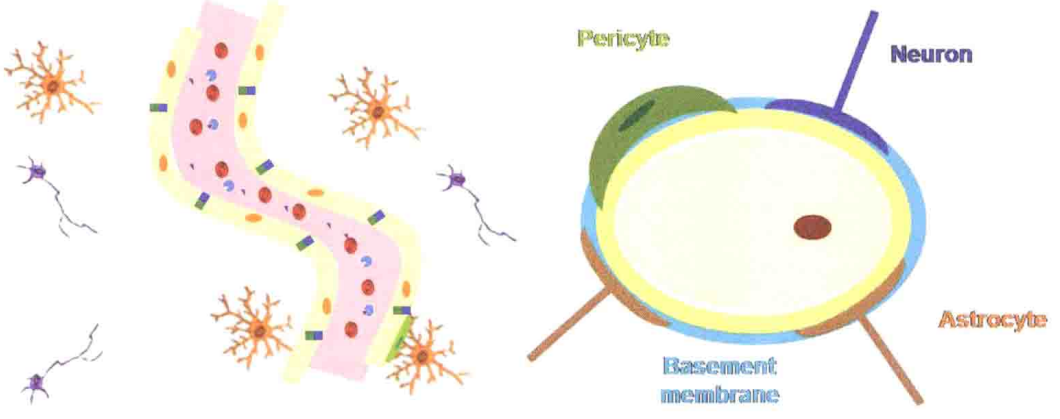

Fig. 1. Schematic illustration of the neurovascular unit and BBB cellular components adopted from (Cardoso et al., 2010).

2.2 Molecular properties of BBB

The BMVECs assembly are regulated by molecular constituents of tight junctions, adherence junctions and signalling pathways. Tight junctions are highly dynamic structures which are responsible for the barrier properties of BBB. Apical region of the endothelial cells sealed together by tight junctions and paracellular permeability of BMVECs are limited by them. Structurally tight junctions formed by interaction of integral transmembrane proteins with neighbouring plasma membrane. Among these proteins junction adhesion molecules, claudins and occludins (inter membrane) which bind to the cytoplasmic proteins (e.g. zonula occludens, cinguline, ...) are well studied and their role in tight junctions and BBB have been evaluated (Figure 2). Beyond the main role in physical restriction of BBB, other functions such as control of gene expression, cell proliferation and differentiation have been

suggested for tight junctions. Below the tight junctions, actin filaments (including cadherins and catenins) linked together and form a belt of adherence junctions. In addition to the contribution in the barrier function some other events such as adhesion of BMVECs to each other, the contact inhibition during vascular growth, the initiation of cell polarity and the regulation of paracellular permeability have been suggested for adherence junctions. A dynamic interaction between tight junctions and adherence junctions through signalling pathways regulate the permeability of BBB. These signalling routes mainly involve protein kinases, members of mitogen - activated protein kinases, endothelial nitric oxide synthase and G-proteins. Dynamic interactions between these pathways control the opening and closing of the paracellular route for fluids, proteins and cells to move across the endothelial cells through two main types of signal transduction procedures (e.g. signals from cell interior to tight junctions to guide their assembly and regulate their permeability, signals transmitted from tight junctions to cell interior to modulate gene expression, proliferation and differentiation). The molecular mechanisms of these interactions can be found in the literature (Ballab et al., 2004; Abbott et al., 2006). In addition to the proteins with enzymatic activities, there are other specific proteins (drug efflux transporters, multi drug resistance proteins, organic anion transporting polypeptides) work as BBB transporters which are responsible for rapid efflux of xenobiotics from the CNS (Losscher & Potschka, 2005) and delivery of the essential nutrients and transmitters to the brain.

The combined effect of the special cellular and molecular properties of central nervous system result in the specific barrier functions of BBB which is important for preventing CNS from harmful xenobiotics. Because of these properties drug delivery to the CNS is among the most challenging drug development areas. In order to develop successful drug candidates for CNS disorders drug uptake mechanisms should be studied. In the next section, these mechanisms are briefly reviewed.

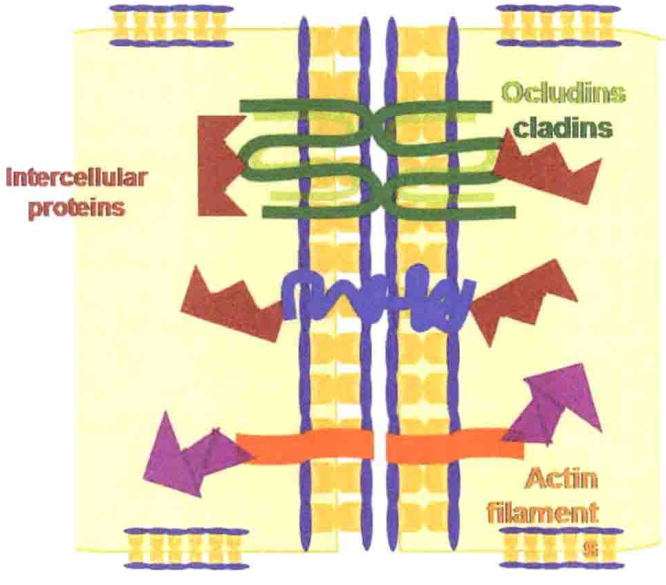

Fig. 2. Tight junctions and adherent junctions.

3. BBB permeation mechanisms

Like other cellular membranes in the body, permeation through BBB can occur by passive diffusion, endocytosis and active transport (Diagram 1). Combined effects of the mentioned mechanisms modulate the compound (e.g. Drugs) penetration to the brain.

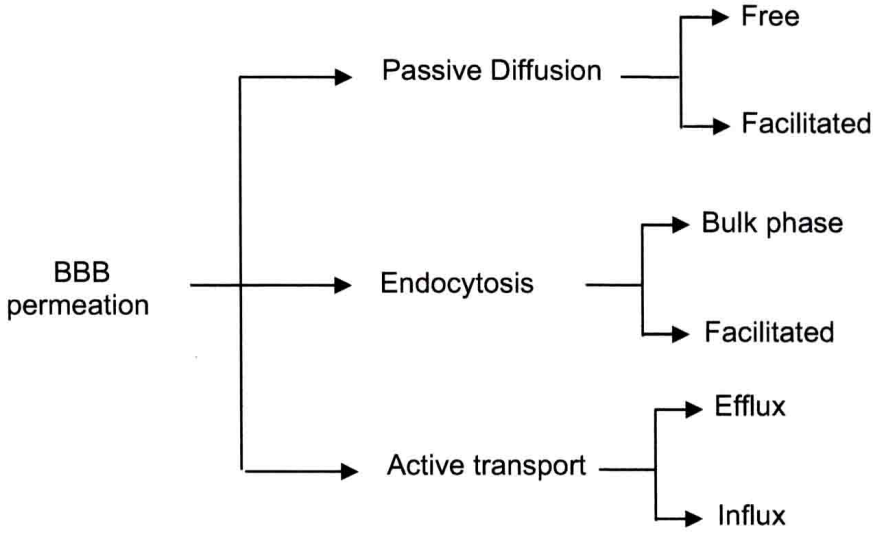

Diagram 1. Main permeation mechanisms in the brain.

3.1 Passive diffusion

A limited number of drugs and drug like compounds with high lipophilicity and low molecular size can penetrate to the brain mainly by passive diffusion. In order to overcome the surface tension difference between a compound and cellular membrane, physical work is needed and the smaller molecules will need less work. The uncharged forms of the weak acidic and basic compounds have higher permeability rate in comparison with charged molecules in physiologic pH of brain. The charged forms possess hydrophilic characteristics and hydrophilic drugs distribute within blood and cannot cross the endothelial cells and excreted from brain parenchyma. Therefore, the molecules with higher fraction of uncharged form in physiologic pH have higher permeability rate (Fischer et al., 1998).

Passive diffusion occurs via two mechanisms (Figure 3):

- Free diffusion in which some compounds move freely paracellularly (e.g. sucrose) between cells to a limited extent due to tight junctions or transcellularly (transcytosis) across the cells for lipophilic substances (e.g. ethanol) (Alam et al., 2010). These mechanisms are non-competitive, nonsaturable and occur in downhill concentration direction.
- Facilitated diffusion in which target compounds bind to a specific membrane protein and carry to the other side of the membrane through conformation change of the protein. This mechanism is a form of carrier mediated endocytosis which occurs from high to low concentration like free diffusion and contributes for transport of some amino acids, nucleosides, small peptides, mono-carboxylates and glutathione (Alam et al., 2010).

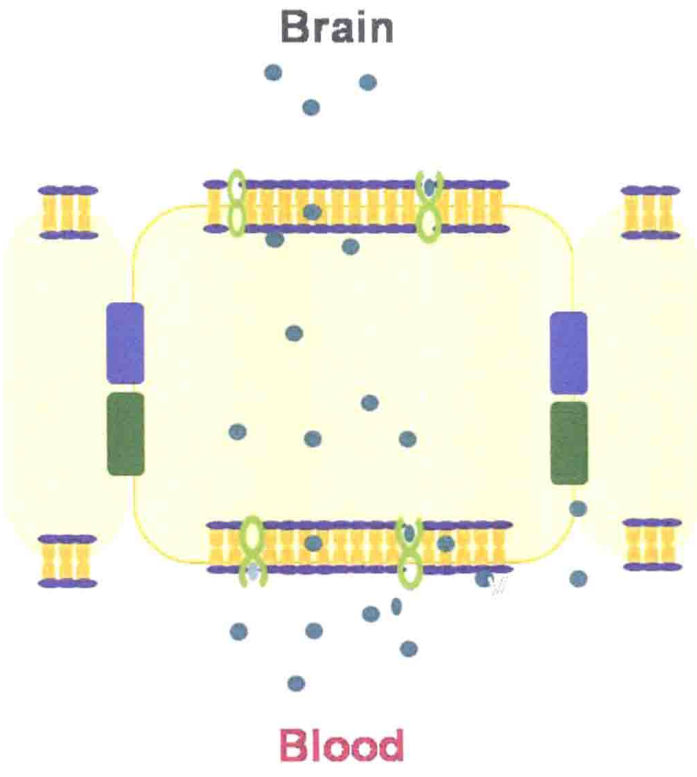

Fig. 3. Free and facilitated passive diffusion.

3.2 Endocytosis

In this method, substances (e.g. macromolecules) are engulfed by membrane and pass through the cell by vesicles and release in the other side (Kerns & Di, 2008). Endocytosis occurs via two main methods: bulk phase endocytosis (fluid phase or pinocytosis) and mediated or facilitated endocytosis (receptor and absorptive mediated). Fluid phase endocytosis is a nonsaturable, non-competitive and non-specific method for uptake of extra cellular fluids which is temperature and energy dependent.

Receptor mediated endocytosis facilitates the larger essential molecules uptake selectively using specific receptors present in luminal membrane. Hormones, growth factors, enzymes and plasma proteins are targets for specific receptors (Pardridge, 2007).

Absorptive mediated endocytosis is based on an electrostatic interaction between negatively charged plasma membrane luminal surfaces (glycocalyx which is a negatively charged proteoglycan or glycosaminoglycan) with cationic substances (e.g. peptides) and uptake it in a vesicle into the endothelial cell and release it on the other side (Figure 4) (Ueno, 2009).

This has lower affinity and higher capacity than receptor mediated endocytosis (Alam et al., 2010). Mechanism of vesicle formation (caveolin dependent, dynamin dependent and caveolin- dynamin independent) is not discussed in this chapter and more details could be found in the literature (Lajoie et al., 2010).

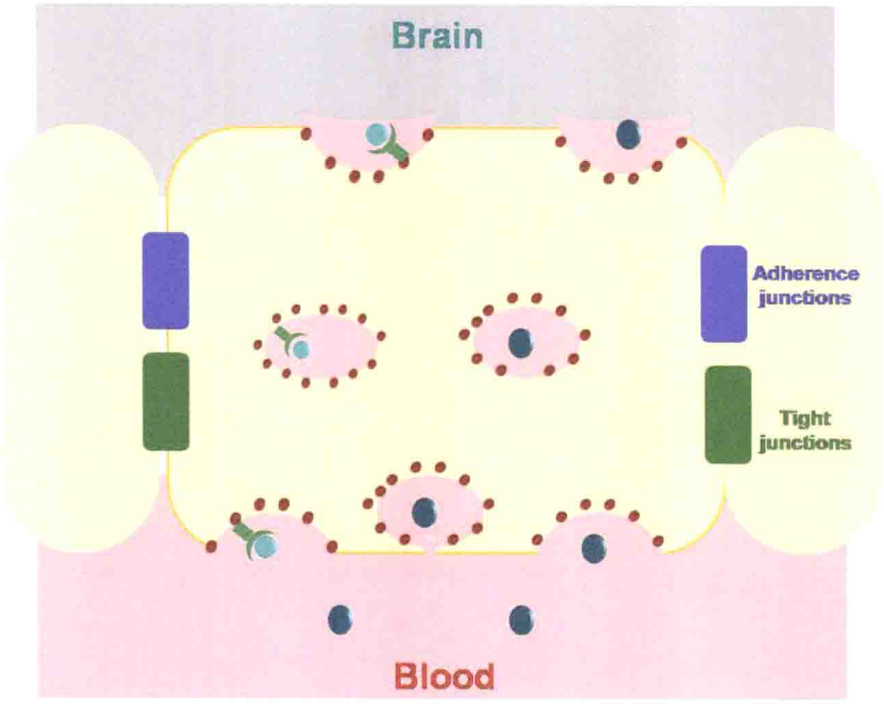

Fig. 4. Bulk phase and facilitated endocytosis.

3.3 Active transport

Hydrophilic drugs which cannot penetrate the brain through passive diffusion and lipophilic drugs which cannot penetrate the brain, in contrast of their suitable characteristics for BBB permeation are substrate for drug transporters of the BBB. Also some compounds are substrates for transporters and at the same time they are delivered by passive diffusion or endocytosis. Drug transporters are integral membrane proteins which is able to carry the drug usually against the concentration gradient into and out of the cell.

The overall exposure of xenobiotics to brain through these transporters depends on their location and expression level according to the normal and pathophysiologic conditions. Two types of drug transporters according to their driving forces (ATP dependent and ATP independent) are known. Active transporters broadly categorized as primary (ATP dependent), secondary or tertiary (ATP independent) (Murk et al., 2010).

There are two types of transporters:

1. Carrier mediated transporters which express on both the luminal and abluminal membranes and operates in both blood to brain and brain to blood directions.
2. Active efflux transporters which mediate extruding drugs and other compounds from brain (Alam et al., 2010). Although the main role of the drug transporters is carrying the drugs and other xenobiotics into and out of the brain but they are responsible for other cell processes such as inflammation, differentiation of immune cells, cell detoxification, lipid trafficking, hormone secretion and development of stem cells (Murk et al., 2010).