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Transport Processes in the Formation of the Cerebrospinal Fluid

ERNEST M. WRIGHT *

Contents

I. Introduction

The cerebrospinal fluid (CSF) occupies the ventricles, canals, and spaces surrounding the central nervous system (CNS). In man the total volume of the CSF is 140 ml, and about 25 ml of this is contained within the ventricles of the brain (see Fig. 1). CSF production amounts to 600 ml/day, and it is agreed that about two-thirds of this originates in the ventricles from the choroid plexuses. These plexuses are richly vascularized epithelial tissues and in man weigh approximately 2 g. There are two plexuses in each of the two lateral ventricles, and one in both the IIIrd and IVth ventricle. In experimental animals 55–65% of the CSF formed within the

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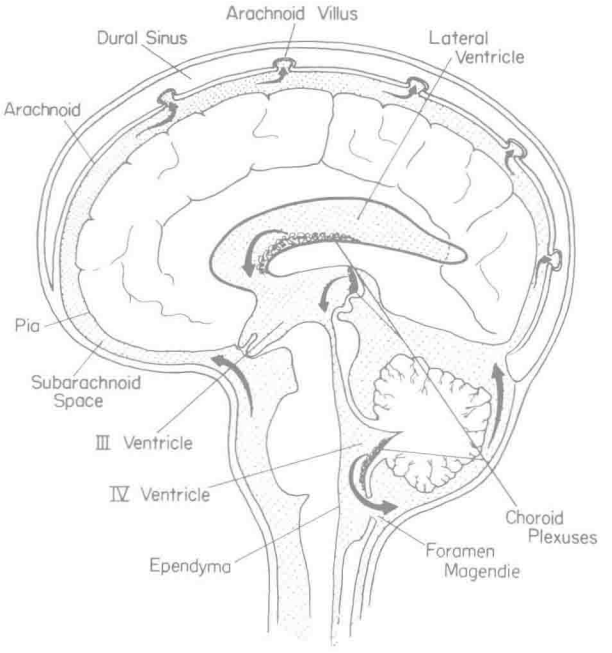

Fig. 1. A diagrammatic illustration of the human brain showing the relationship between the CSF and brain. Fluid secreted by the choroid plexuses in the lateral, IIIrd and IVth ventricles flows through the foramina into the cisterns and then over the surface of the brain. The CSF enventually reaches the blood in the dural sinus via the arachnoid villi. The CNS is enclosed by the arachnoid membrane and the dura (not shown in this diagram). A major function of the CSF is to provide physical support for the brain. (After *Milhorat*, 1972)

ventricles is produced in the IVth ventricle. CSF is secreted against a hydrostatic pressure of 15 cm H_2O, and once formed, the direction of bulk flow is from the lateral ventricles to the IVth ventricle where it passes via three exits (the foramina of Luschka and Magnedie) into the cisterns on the external surface of the brain. Fluid in the cisterns passes over the surface of the brain into the subarachnoid spaces, until it reaches the arachnoid villi, where it returns to the vascular system by way of the dural sinuses. The mechanism of CSF flow is unknown, but it is thought that pressure gradients brought about by fluid secretion, postural changes, and vascular pulsations are all involved. Ciliary activity contributes to the flow of CSF over the walls of the ventricles and canals, but the role of cilia in bulk CSF flow is probably insignificant.

CSF is separated from the neurones and glia of the CNS by the ependyma, which lines the ventricles and canals, and the pia, which covers the external surface of the brain and spinal cord. The choroid plexuses and

the arachnoid membrane separate the CSF from blood, and these two membranes form the so-called "blood-CSF barrier".

The composition of the bulk CSF closely resembles an ultrafiltrate of plasma (see Table 1). The protein concentration of CSF is less than 0.5%

Table 1. Composition of human CSF and plasma (in mM/kg H_2O)

	Plasma	CSF
Sodium	150	147
Potassium	4.6	2.9
Magnesium	3.2	4.5
Calcium	2.4	1.2
Chloride	99	113
Bicarbonate	27	23
Glucose	5.2	3.4
Glycine	0.22	0.02
pH	7.40	7.31
Osmolarity	289	289
Protein (mg/100 ml)	6800	28

These average estimates of CSF composition are taken from *Milhorat* (1972), *Dayson* (1967), and *Katzman* and *Pappius* (1973), and they represent values for lumbar CSF. There are regional differences in the composition of the CSF from the surface of the choroid plexuses to the arachnoid granulations.

of that in plasma, while the levels of the major cations and anions, the pH and osmolarity of the two fluids are quite similar. A major difference is that the concentrations of glucose and amino acids, e.g., glycine, are significantly lower in CSF than in plasma. A remarkable feature of the CSF is that its composition remains fairly constant in the face of wide fluctuations in plasma, e.g., CSF K remains between 2 and 4 mEq/liter even when plasma K is varied between 1 and 12 mEq/liter. Homeostatic mechanisms are known to maintain CSF K, pH, Mg, Ca, amino acids, catecholamines, organic acids and bases, and polyatomic anions concentrations at constant levels. The importance of these homeostatic mechanisms becomes clear in experiments where CSF composition is varied artificially (see *Winterstein,* 1961, and *Leusen,* 1972). Very small changes in CSF composition produce dramatic effects, e.g., respiratory ventilation rates increase four fold from the resting value upon lowering the CSF pH 0.05 U, and elevation of CSF glycine produces hypothermia, hypotension, and motor incoordination. These observations are not at all surprising in light of the fact that

the CSF is in free communication with the interstitial fluid of the brain parenchyma. Thus, changes in CSF composition produce changes in the fluids surrounding the neurones and synapses of the CNS, and this in turn influences the normal function of the brain. Obviously, it is important to identify the mechanisms responsible for controlling the chemical composition of the CSF in order to achieve a complete understanding of the operation of the CNS in health and disease.

II. Factors Influencing CSF Composition

The physiology of the CSF is complicated by the fact that the fluid comes into direct or indirect contact with many structures as it circulates through the ventricles and over the surface of the brain and spinal cord (see Fig. 1). Thus, the composition of the CSF in any region of the brain is directly related to the transport processes occurring at all the membranes exposed to the CSF. The relationships between the fluid compartments of the brain are represented diagrammatically in Figure 2.

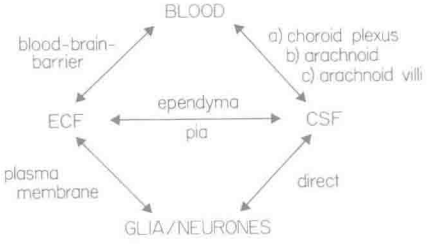

Fig. 2. A diagram summarizing the relations between the various fluid compartments in the CNS. The CSF communicates with the blood directly through the choroid plexuses, the arachnoid, and the arachnoid villi, and indirectly by way of the extracellular fluids of the brain parenchyma and the blood-brain barrier. The composition of the CSF is also influenced by the neurones and glia indirectly via the ECF and the ependyma and pia, and directly via those cellular elements that project into the CSF

CSF is in direct contact with blood across the choroid plexuses, arachnoid membrane, and the arachnoid villi. Fluid is actually secreted by each of the four choroid plexuses, and in addition, the choroidal epithelium is able to actively modify the composition of the ventricular CSF by transporting solutes back into blood. The arachnoid membrane also modifies the composition of the CSF as the fluid passes through the subarachnoid spaces and the arachnoid may actually secrete CSF. The CSF is in free communication with the extracellular fluid (ECF) of the brain parenchyma, and this in turn is in contact with neurones and glia, and with blood

through the "blood-brain barrier". There is mounting circumstantial evidence that the brain capillary endothelium is actually the "blood-brain barrier". Finally, there are some neurones and glia in direct contact with the CSF, particularly in the IIIrd ventricle, and these cells may exchange substances directly with the CSF.

This review is limited to a discussion of CSF production by the choroid plexuses and the regulation of CSF composition by the plexuses and the arachnoid membrane. Transport of substances across the "blood-brain barrier" has been reviewed recently (see *Partridge* and *Oldendorf,* 1977, and *Cserr* et al., 1975). Studies of the blood-brain barrier have been mainly limited to in vivo experiments on the extraction of solutes from blood by the brain (see *Rapoport,* 1976), but there have been preliminary reports on the use of isolated brain capillaries (*Sershen* and *Lajtha,* 1976; *Mrsulja* et al., 1976; and *Goldstein* et al., 1977). The exchange of solutes between the CSF and brain has also been reviewed (see *Cserr* et al., 1975), and it is concluded that there is free diffusion of ions and molecules between the CSF and ECF compartments. Nevertheless, specialized regions of the ependyma are involved in the secretion of hormones into the CSF, and this subject has been covered by *Rodriguez* (1976) and *Cserr* et al. (1975). Readers are referred to the following monographs and reviews for general coverage of the physiology and pathology of the CSF: *Davson* (1967), *Cserr* (1971), *Milhorat* (1972, 1976), *Katzman* and *Pappius* (1973), *Welch* (1975), *Netsky* and *Shungshotli* (1975), and *Rapoport* (1976).

III. CSF Secretion

A major proportion of the CSF secreted in the ventricles is produced by the choroid plexuses. In mammals there is one plexus in each of the four ventricles, but in amphibians they are only found in the IIIrd and IVth ventricles. Each plexus is a highly vascular tissue that projects into the ventricle, and during development they originate from the pia mater and blood vessels in subarachnoid space. Scanning electron micrographs of the ventricular surface of the choroideus rhombencephali are shown in Figure 3, and the histologic features of the same plexus are illustrated schematically in Figure 4. The surface is composed of highly complex, interlocking folds that are covered with a single layer of cuboidal epithelial cells. The epithelium rests upon a thin stroma of collagen fibers, fibroblasts, and blood vessels. The vascular elements include small arteries, arterioles, large venous sinuses, and capillaries. Blood flow to the plexuses amounts to about 3 ml/min/g, and this is about twice as high as the kidney (see *Csaky,* 1969). The capillaries are fenestrated unlike most other capillaries in the brain. It has been estimated that the epithelial cells account for 25–40% of the

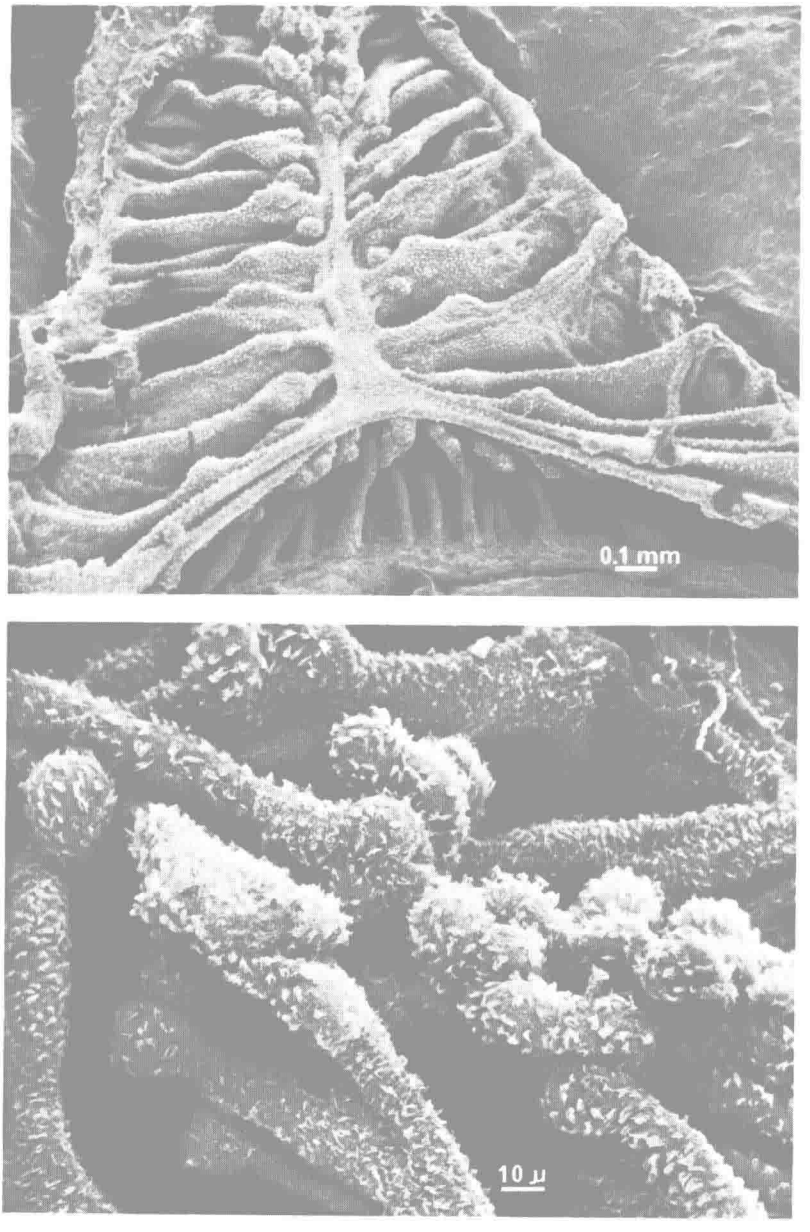

Fig. 3. Scanning electron micrographs of the ventricular surface of the posterior choroid plexus of the frog. Note that the structure becomes more complex toward the dorsal surface, and that the granular appearance of the low power micrograph is due to the clumps of cilia which project from each cell into the CSF. Taken from *Nelson* and *Wright* (1974)

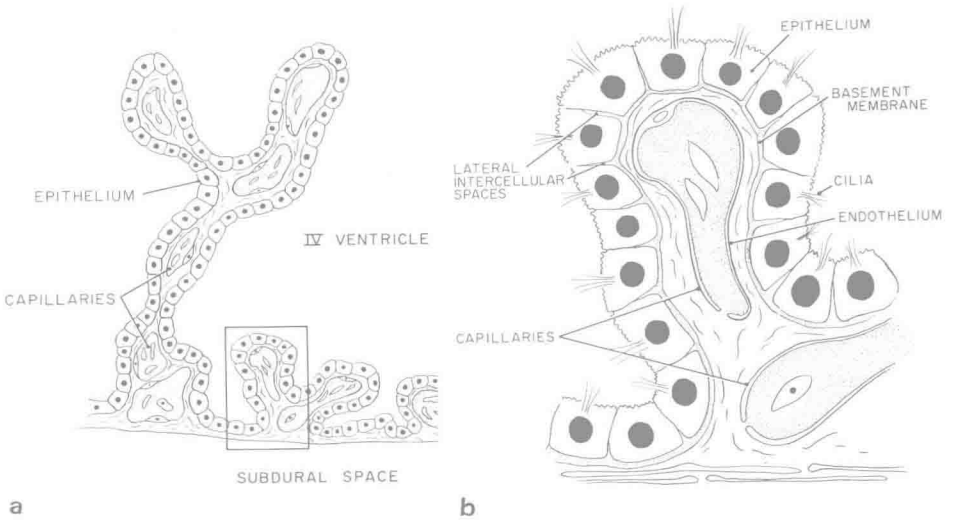

Fig. 4 a and b. Diagrams showing the major histologic features of the frog posterior choroid plexus. In the low power drawing the villi, which project into the CSF of the IVth ventricle, are covered with a single layer of cuboidal epithelial cells, and the core of these villi consists of a loose connective tissue containing numerous blood vessels. The higher power drawing shows an area of the plexus in more detail. It should be noted that the structure of the IVth ventricle choroid plexus in mammals is essentially identical to that in amphibians, but it is buried in the recesses of the traverse fissure of the cerebellum by the hind brain. (From *Wright,* 1972a)

total number of cells in the plexus, and that they occupy 65–95% of the total cell volume (*Quay,* 1966).

A transmission electron micrograph of the epithelium is shown in Figure 5. The cells are cuboidal in shape with a large, central nucleus and irregular, highly packed microvilli on the apical surface of the tissue. In many species, particularly during development, the epithelium is heavily ciliated. In the mature frog 20–40 cilia, each about 20 μ long, project from each cell into the CSF. These cilia beat at a frequency between 5 and 30 cps, and this ciliary motion promotes the flow of CSF over the surface of the epithelium (*Nelson* and *Wright,* 1974).

The cytoplasm contains numerous mitochondria and these appear to be concentrated at the apical end of the cell. Of the total number of mitochondria in the plexus, 80–95% are in the epithelium, and these account for the very high oxygen consumption of the epithelium (*Quay,* 1963; *Quay,* 1960; *Krebs* and *Rosenhagen,* 1931).

Each cell is joined to its neighbors by tight junctions, zonulae occludendes, at the apical surface. In this region the lateral faces of contigious epithelial cells fuse to form girdles around each cell. The junctions prohibit the passage of large molecules (proteins) between the lateral intercellular

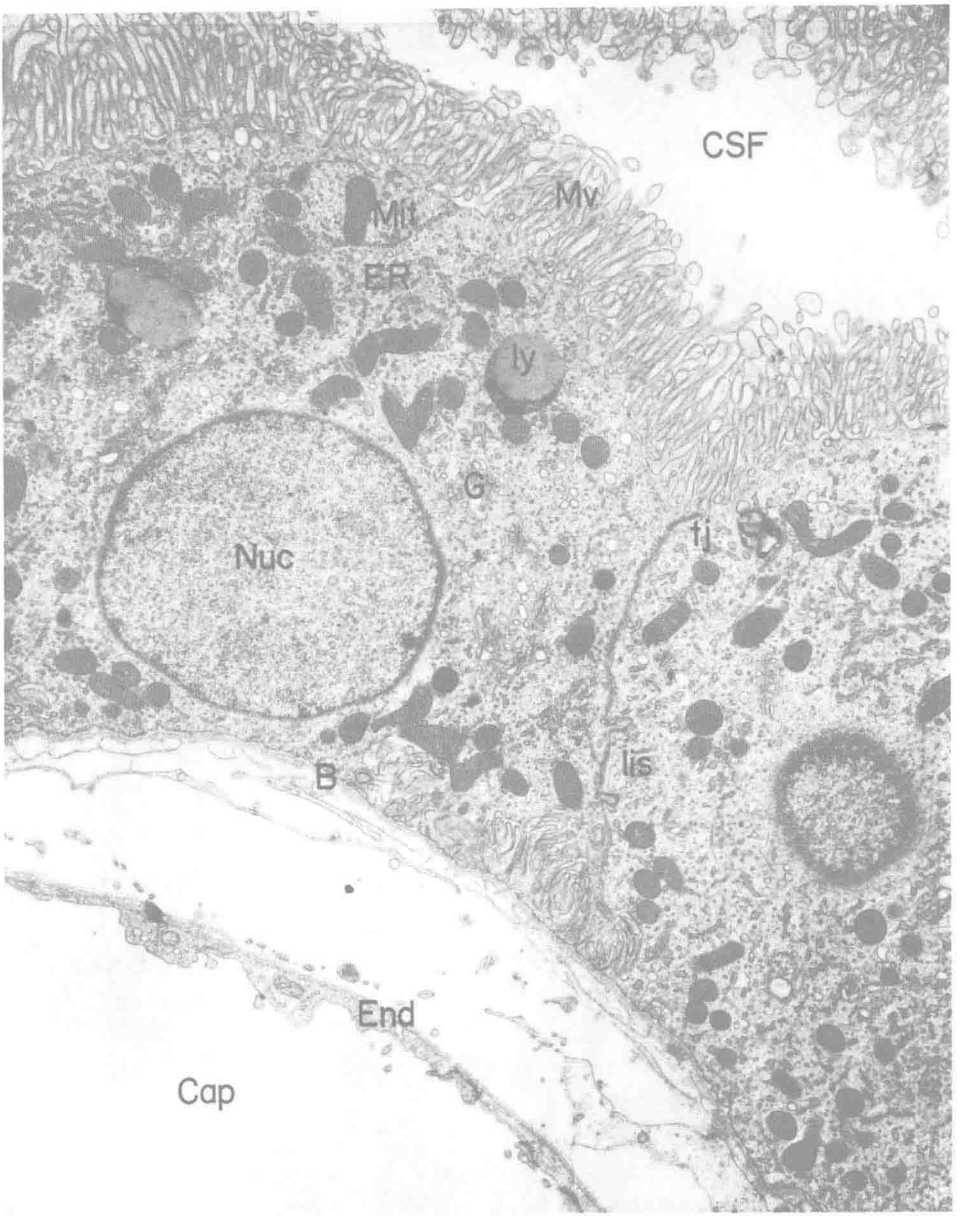

Fig. 5a. An electromicrograph of the rat choroid plexus (x 12,000). The cuboidal epithelial cells separate the CSF from the blood in the plexus capillaries (*Cap*). The cells rest upon a thin basement membrane (*B*) and are joined together at the apical surface by so-called tight junctions (*tj*) which form a girdle around the circumference of each cell. The lateral intercellular spaces (*lis*) separate each cell from the tight junctions to the basement membrane. The apical surface of the epithelium is composed of tightly packed, irregular microvilli (*Mv*), the large nucleus (*Nuc*) is centrally placed in the cytoplasm, and mitochondria (*Mit*) are more numerous near the apical membrane than other parts of the cell. The endoplasmic reticulum (*ER*) is well-developed and the Golgi apparatus (*G*) is in a supranuclear position. Lysosomes (*ly*) are also prominent in the cytoplasm. The endothelium (*End*) of the plexus capillaries is of the fenestrated type. (Taken with kind permission from *Peters* et al., 1976)

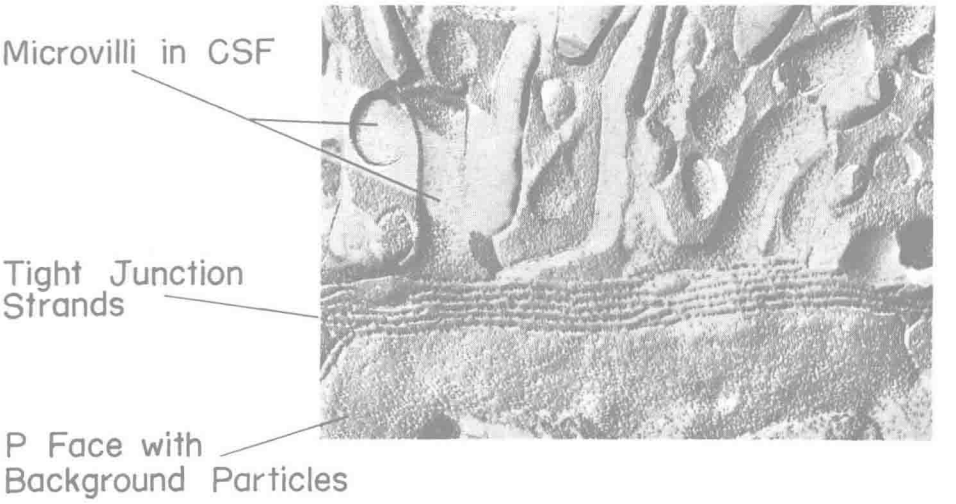

Fig. 5b. Freeze-fracture replica of a tight junction in the choroid plexus epithelium of the mouse IVth ventricle choroidal epithelium (x 47,000). The microvilli project into the CSF in the IVth ventricle, and the strands in the tight junction run parallel with the apical surface of the cell. The strands, or ridges, are composed of particles which are interrupted in places. The micrograph was kindly provided by Dr. *Milton W. Brightman*

spaces and the CSF, but there is substantial evidence to show that they are leaky to small ions (*Wright*, 1972a, 1974a; *Castel* et al., 1974; *Bouldin* and *Krigman,* 1975). Thus, the choroid plexus belongs to a group of epithelia with "leaky" so-called tight junctions; other members of the group include the gall bladder, intestine, and renal proximal tubule. In other epithelia, notably the frog skin and urinary bladder, junctions are tight even to small ions such as Na and Cl. Freeze-fracture studies of junctions in epithelia reveal a network of intramembraneous strands or fibrils (see Fig. 5b), and it has been suggested that the number of strands is correlated with the permeability of the junction. However, a survey of different epithelia under a variety of experimental conditions does not support this hypothesis (e.g., *Martinez-Palomo* and *Erlij,* 1975, and *Møllgard* et al., 1976). The physiologic implications of "leaky" tight junctions are considered below, and a more detailed account of the structure of the choroid plexus is given by *Peters* et al., 1976.

A. Methods of Study

There are four major approaches to the study of solute and water transport across the choroidal epithelium: 1) in vivo ventriculo-cisteral perfusion (see *Cserr,* 1971, and *Rapoport,* 1976, for details of this approach),

2) in vivo isolation of a choroid plexus (e.g., *Miner* and *Reed,* 1972), 3) extracorporeal perfusion of the isolated plexus (*Pollay* et al., 1972a, 1972b, 1973; *Segal* and *Pollay,* 1977), and 4) mounting the IVth ventricle plexus between Ussing type flux chambers (first introduced by *Patlak* et al., 1966). I have developed an in vitro preparation of the frog posterior choroid plexus, and this model system offers a number of advantages: 1) frogs provide convenient, simple in vitro preparations that are viable for many hours, e.g., active ion transport rates are stable for up to 8 h, and they tolerate a very wide range of experimental conditions that are not possible with mammalian in vivo or in vitro preparations, and 2) the ultrastructure of the plexus, the composition of the CSF, and the regulation of CSF composition in the frog are essentially identical to those in higher animals. When the posterior choroid plexus is mounted between flux chambers, it is possible to measure 1) unidirectional fluxes across the epithelium, 2) unidirectional fluxes across the apical surface of the epithelium, 3) the steady-state concentrations of solutes within the epithelium, 4) transcellular electric potentials and conductances, 5) intracellular electric potentials, and 6) water fluxes across the plexus (for experimental details see: *Wright,* 1972a, 1974a, and *Wright* et al., 1977). This in vitro approach has been largely responsible for the tremendous progress in our understanding of epithelial tissues such as the frog skin, urinary bladder, gall bladder, and intestine, and when applied to the choroid plexus offers unique insights into mechanisms of CSF secretion. This review provides a forum for the progress made with this model system over the last decade.

B. Passive Ion Permeation

With identical Ringer's solutions on each side of the frog choroid plexus, the transepithelial potential difference (p.d.) was less than 1 mV, and the electric resistance was 200 Ω cm^2 (*Wright,* 1972a). Similar results have been obtained for shark and mammalian plexuses (*Patlak* et al., 1966; *Wright,* 1972a; *Welch* and *Akari,* 1975; and *Eisenberg* and *Welch,* 1976). Passive ion permeability coefficients have been obtained from both diffusion potential and radioactive flux measurements. In the frog, the cation permeability sequence was P_{Ca} (1.32) > P_K (1.23) $\sim$ P_{Rb} (1.23) > P_{Cs} (1.02) $\sim$ P_{Na} (1) > P_{Li} (0.86), and the absolute sodium permeability coefficient was 5 x 10^{-6} cm s^{-1} (*Wright,* 1972a). The sodium conductance of the plexus was found to be sensitive to the drug 2,4,6-triaminopyrimidine (*Moreno,* 1975; *Eisenberg* and *Welch,* 1976; and unpublished observations). Similar results were obtained for the cat choroid plexus by *Welch* and *Akari* (1975). In the case of anions, the permeability sequence was P_I (1.39) > P_{Br} (1.29) > P_{SCN} (1.02) $\sim$ P_{Cl} (1) $\sim$ P_{HCO_3} (1) > P_{TcO_4} (0.54),

and the absolute chloride permeability coefficient was 8 x 10^{-6} cm/s^{-1} (*Wright,* 1974a). There was no evidence for chloride exchange diffusion across the plexus as judged by the fact that the Cl flux ratios were close to those predicted by the Ussing flux ratio equation (*Wright,* 1972a). The ion permeation sequences belong to those predicted by *Eisenman's* theory of ion selectivity (see *Diamond* and *Wright*, 1969; *Wright* and *Diamond,* 1977; *Wright,* 1977a).

There is considerable evidence that the major pathway for passive ion permeation across the plexus is via the extracellular shunt path, i.e., tight junctions and lateral intercellular spaces (*Wright,* 1972a, 1974a; *Castel* et al., 1974; and *Bouldin* and *Krigman,* 1975), and there is indirect evidence that diffusion along the lateral intercellular spaces may rate limit passive permeation under certain circumstances (see *Smulders* et al., 1972; *Bindslev* et al., 1974). In tight junctions, permeation was controlled by fixed charges with a pK of 4 and an isoelectric point of 3 (*Wright* and *Prather,* 1970). The mechanism of ion permeation across the tight junctions of the choroid plexus appears to be very similar to that in other leaky epithelia (see *Moreno* and *Diamond,* 1975).

C. Nonelectrolyte Permeability

The passive permeability of the choroid plexus to nonelectrolytes resembled that seen in other epithelia and in single cells (*Wright* and *Prather,* 1970; *Wright* and *Pietras,* 1974). In general, the permeability of a solute was directly proportional to the solute's oil:water partition coefficient, the higher the partition coefficient the higher the permeability coefficient (Table 2). Detailed analysis of permeability measurements on the plexus show that: 1) P's ranged from about 1– 1000 x 10^{-7} cm/s (Table 2), 2) the membrane lipids of the plexus were more "fluid" than those in other cells and tissues, and 3) small molecules, such as urea and water, permeated across the plexus more rapidly than expected from their oil: water partition coefficients. Rapid permeation of the small molecules may have been in part due to the presence of "pores", but it was in part due to high permeation of these substances through membrane lipids.

1. Water

The osmotic water permeability (L_p) of the frog choroid plexus was 2.2 x 10^{-3} cm/s (*Wright* et al., 1977), while that for the rabbit plexus was 4.0 x 10^{-3} cm/s (*Welch* et al., 1966). These values are similar to those obtained for the frog urinary bladder (see *Wright,* 1977b). The resistance to osmotic flow across the plexus increased when the lateral intercellular

Table 2. Permeability of frog choroid plexus to nonelectrolytes

Nonelectrolyte	K_{oil}	P (cm/s x 10^7)
Sucrose	1×10^{-6}	16
Mannitol	1.2×10^{-6}	21
Erythritol	3×10^{-5}	49 *
Glycerol	7×10^{-5}	69
Urea	1.5×10^{-4}	120
Water	7×10^{-4}	680
Acetamide	8.3×10^{-4}	125
1,2-propanediol	1.7×10^{-3}	98
1,4-butanediol	2.1×10^{-3}	96
Nicotinamide	5×10^{-3}	171 *
N-butyramide	1.7×10^{-2}	166
Iso-butyramide	1.4×10^{-2}	136
1,7-heptanediol	3.1×10^{-2}	283
Antipyrine	3.2×10^{-2}	242
Caffeine	3.3×10^{-2}	432

The P values are quoted as the means estimated from an average of 30 estimates. The standard errors of the means were less than 10% of the means, except for those two cases marked by asterisks where the errors were less than 20% of the means. All permeability coefficients were corrected for the presence of unstirred layers. The permeability coefficients were arranged according to the solute olive oil partion coefficients. (Taken from *Wright* and *Pietras,* 1974).

spaces collapsed, e.g., when 100 mM sucrose was added to the CSF the L_p dropped from 2.2×10^{-3} to 1.6×10^{-3} cm/s, and this was accompanied by an increase in the electric resistance of the tissue (see *Wright* et al., 1977). This showed that the lateral spaces are a common pathway for ion and water permeation across the choroid plexus, and that flow across the plexus decreased when the resistance of the spaces increased.

The diffusional water permeability coefficient for the frog choroid plexus was 0.07×10^{-3} cm/s (*Wright* and *Pietras,* 1974), and unpublished experiments showed that this was unaffected by the presence of the amphibian antidiuretic hormone arginine (vasotocin), phloretin, and PCMBS (p-chloromercuribenzenesulfonate).

2. Sugars

Sugars permeated across the frog choroid plexus by facilitated diffusion (*Prather* and *Wright,* 1970). Those sugars with a pyranose ring in the

D-glucose chair conformation were able to use the transport carrier, and the affinity of the sugar for the carrier was related to the number of hydroxyl groups on the sugar that were in the equatorial plane of the ring. The sugar with the most hydroxyl groups in this conformation, i.e., D-glucose, had the greatest affinity for the transport system. I have no evidence for active sugar transport either across the plexus or into the epithelium, even though there are reports of active sugar accumulation in mammalian plexuses (*Csaky* and *Rigor*, 1964; and *Segal* and *Pollay*, 1977). It is reasonable to conclude that the lower concentration of glucose in the CSF with respect to blood (Table 1) is due to the facilitated exchange of the sugar between blood and CSF and the high rate of glucose metabolism in the brain (see also *Rapoport*, 1976).

3. Amino Acids

The concentration of amino acids in the CSF is generally lower than in blood (Table 1), and it is generally accepted that amino acids are actively transported out of the CSF into blood (see *Rapoport*, 1976). It has long been maintained that the choroid plexuses were the sites of the active transport step, but this does not appear to be the case in the frog. Although the frog plexus, like mammalian plexuses, could accumulate neutral, basic, and acidic amino acids within the epithelium, there was no net transport of these solutes across the plexus (*Wright*, 1972b). It appears that the amino acids were unable to diffuse from the epithelium into the blood across the basolateral cell membrane. In the frog, it is the arachnoid membrane that is a more reasonable candidate for the active transport of amino acids from CSF to blood (*Wright*, 1974b).

The actual permeability of the frog choroid plexus to amino acids was quite low, i.e., 4×10^{-6} cm/s, and so it is the slow leak of amino acids into the CSF from blood combined with an active transport mechanism in the arachnoid and brain metabolism that accounts for the low concentration of amino acids in the CSF.

D. Active Ion Transport

To approach the question about the mechanism of CSF secretion across the choroid plexus, I first measured the unidirectional fluces of Na, K, Cl, and Ca in the absence of appreciable electrochemical potential gradients across the epithelium (*Wright*, 1972a). The results are summarized in Table 3, where it can be seen that there was a net transport of sodium from blood to CSF of 1 μmol/cm^2/h. Chloride was also transported in the same direction, but in this case the rate of transport was only about 60%