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VIEW AND REVIEW

Blood-cerebrospinal fluid (CSF) barrier dysfunction means reduced CSF flow not barrier leakage — conclusions from CSF protein data

Disfunção da barreira hemato-liquórica significa redução do fluxo de líquido cefalorraquidiano, e não quebra de barreira — conclusões a partir de estudos sobre as proteínas do LCR

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ABSTRACT

Background: Increased concentrations of serum proteins in cerebrospinal fluid (CSF) are interpreted as blood-CSF barrier dysfunction. Frequently used interpretations such as barrier leakage, disruption or breakdown contradict CSF protein data, which suggest a reduced CSF flow rate as the cause. **Results:** Even the severest barrier dysfunctions do not change the molecular size-dependent selectivity or the interindividual variation of the protein transfer across barriers. Serum protein concentrations in lumbar CSF increase with hyperbolic functions, but the levels of proteins that do not pass the barrier remain constant (brain proteins) or increase linearly (leptomeningal proteins). All CSF protein dynamics above and below a lumbar blockade can also be explained, independent of their barrier passage, by a reduced caudally directed flow. Local accumulation of gadolinium in multiple sclerosis (MS) is now understood as due to reduced bulk flow elimination by interstitial fluid (ISF). Nonlinear change of the steady state in barrier dysfunction and along normal rostro-caudal gradients supports the diffusion/flow model and contradicts obstructions of diffusion pathways. Regardless of the cause of the disease, pathophysiological flow blockages are found in bacterial meningitis, leukemia, meningeal carcinomatosis, Guillain-Barré syndrome, MS and experimental allergic encephalomyelitis. In humans, the fortyfold higher albumin concentrations in early fetal development decrease later with maturation of the arachnoid villi, i.e., with beginning CSF outflow, which contradicts a relevant outflow to the lymphatic system. Respiration- and heartbeat-dependent oscillations do not disturb net direction of CSF flow. **Conclusion:** Blood-CSF and blood-brain barrier dysfunctions are an expression of reduced CSF or ISF flow rate.

Keywords: Blood-brain Barrier; Barrier Dysfunction; Cerebrospinal Fluid Flow; Cerebrospinal Fluid Proteins; Neurological Disorder.

RESUMO

Introdução: Concentrações aumentadas de proteínas séricas no líquido cefalorraquidiano são interpretadas como disfunção da barreira (hemato-liquórica) sanguínea do LCR. Interpretações frequentemente usadas, como vazamento de barreira (quebra ou rompimento de barreira), rompimento ou quebra, contradiz os dados de proteína do LCR, que sugerem uma taxa de fluxo reduzida do LCR como a causa. **Resultados:** Mesmo as disfunções de barreira mais graves não alteram a seletividade dependente do tamanho molecular nem a variação interindividual da transferência de proteína através de barreiras. As concentrações de proteínas séricas no LCR lombar aumentam com as funções hiperbólicas, mas as proteínas que não passam a barreira permanecem constantes (proteínas do cérebro) ou aumentam linearmente (proteínas leptomeningeais). Toda a dinâmica das proteínas do LCR acima e abaixo de um bloqueio lombar também pode ser explicada, independente de sua passagem pela barreira, por um fluxo caudal reduzido. O acúmulo local de gadolínio na esclerose múltipla (EM) é agora entendido como decorrente da redução da eliminação do *bulk flow* pelo fluido intersticial (FIS). A mudança não linear do estado estacionário na disfunção da barreira e ao longo dos gradientes rostro-caudais normais apoia o modelo de difusão/fluxo e contradiz as obstruções das vias de difusão. Independentemente da causa da doença, os bloqueios fisiopatológicos do fluxo são encontrados na meningite bacteriana, leucemia, carcinomatose meníngea, síndrome de Guillain-Barré, EM e encefalomielite alérgica experimental. Em humanos, as concentrações de albumina quarenta vezes mais altas no desenvolvimento fetal inicial diminuem tarde com a maturação das vilosidades aracnoides, isto é, com o início do fluxo de LCR, o que contradiz um fluxo relevante para o sistema linfático. As oscilações dependentes da respiração e do batimento cardíaco não perturbam a direção do fluxo do LCR. **Conclusão:** As disfunções das barreiras hemato-liquórica e hemato-encefálica são uma expressão da redução da taxa de fluxo do LCR ou FIS.

Palavras-chave: Barreira Hematoencefálica; Disfunção da Barreira; Fluxo de LCR; Proteínas do Líquido Cefalorraquidiano; Doenças do Sistema Nervoso.

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PROTEIN DYNAMICS IN CEREBROSPINAL FLUID

Many neurological diseases[1,2] are associated with an increased concentration of blood-derived proteins in cerebrospinal fluid (CSF), interpreted as a dysfunction of the blood-CSF or blood-brain barrier[3,4,5]. However, what is often not considered are the simultaneously changing concentrations of proteins from the brain and leptomeningeal proteins in the CSF[6,7]. Since their dynamics are not influenced by changes in the molecular transfer pathways (barriers) between blood and CSF, they make a decisive contribution to the understanding of blood-CSF barrier function and dysfunction. The only consistent common explanation for these observations is a reduced CSF flow[7,8].

Many of the former mathematical barrier models[9,10,11,12,13,14] used the overall blood-CSF concentration gradients (Table 1) instead of the diffusion-related correct local concentration gradient (Figure 6 in Ref[7]). In spite of the theoretical foundation[8], this concept still has not found general acceptance. It is the aim of this review, by summarizing the known empirical neurochemical[15], neuropathological and clinical data, to substantiate this fundamental change in the concept of the biological barrier for proteins. Any barrier theory, however plausible, must be able to be measured against these data on protein dynamics in the CSF.

BARRIER CONCEPTS — AND THE STEADY STATE

Generations of neurophysiologists and neuropathologists[3,4,5,16,17,18,19,20] have contributed sophisticated knowledge about the specific structures and functions of the biological barriers in the brain, with the intercellular tight junctions in the capillary endothelium, meningeal epithelium, choroidal plexus ependyma and arachnoid epithelium being most prominent[4,18,19,20].

However, this illustrative work on the morphology of the normal barriers has completely obscured the view of CSF flow as the main modulating parameter for the barrier function for proteins in pathological processes[3,7,8,15].

H. Davson's early observation, that blood-derived molecules in CSF never reach the corresponding serum concentrations[16], was the start for the understanding of the steady state between the molecular diffusion through the barrier into CSF, J, and the elimination of the molecules by CSF bulk flow, F, as shown in the model in Figure 1. This steady state idea can be regarded as a consensus in the scientific community for the normal CSF. The dissent came, however, with the question about the cause of a pathological shift of the steady state in the case of blood CSF barrier dysfunctions[8,21].

The descriptive terms like blood-brain barrier leakage, impairment, disruption or breakdown dominate the scientific literature with together about 200.000 hits in the last ten years (https://scholar.google.de, access on 1.9.2019) including highest ranked journals. Since in most studies the cause of the increased blood-derived protein concentrations in CSF was not explicitly investigated, the term leakage in these publications is more a metaphor than a scientific finding.

It is the aim of this review to show the data that will help to resolve this hindrance in the development of new diagnostic approaches and therapies in neurology.

CEREBROSPINAL FLUID FLOW RESEARCH

Interest in the flow of CSF is very old[22]. The former experiments and observations need to be reviewed on the basis of current knowledge. With the two components, diffusion and fluid flow, all data on protein measured in CSF could be interpreted with a common biophysical model[7,8]. Under these two critical aspects the former data may have to be reinterpreted:

- Proteins diffuse from the blood through the tissue into the brain (extracellular fluid, ISF and CSF) depending on their molecular size (Table 1). Diffusion is an undirected process with non-linear concentration gradients through the tissue[7,8,21]. For proteins there is no active or facilitated transfer mechanism as is the case for glucose and vitamin C[23] or amino acids[24] and lactate;
- The transport of molecules in a fluid (solvent) takes place in the dissolved state (solute) and the transport speed of this bulk flow is not dependent on the size of the molecule[25]. The change in the albumin quotient, QAlb, is the reciprocal measure for the change in flow rate. For example, a model that associates QAlb with a molecular size-dependent exponent[13] misses the biological context.

The use of insoluble crystals (cinnabar, mercurysulfide) in the oldest CSF flow experiment of Quincke[22], 1872, is an example of a fundamental bias because insoluble crystals are not subjected to a bulk flow and thus cannot disappear from the subarachnoid space. The recent use of these experiments as an argument for a reverse direction of the cerebrospinal fluid flow[22] is therefore simply misleading.

ACTUAL CSF FLOW-RELATED QUERIES

1) CSF flow research has received a new upswing with imaging techniques. But images of CSF pulsations[26,27,28,29,30] mislead researchers to question the CSF net flow direction[14,22,26,30].

2) The use of experimental barrier models such as cell cultures and mechanical chips[4,31], missing the influences of CSF flow, brought back the focus on the morphological definitions of the blood-CSF barrier eventually restricted to the choroid plexus[4]. These models focus on the lipophilicity of molecules for the transcellular passage, different from the intercellular passage of proteins.

3) Analogies to animal models[32,33] reopen the discussion about lymphatics in the human brain and the CSF outflow ways[34].

4) Mathematical models, which ignore biological contexts[14] or do not apply Fick's 2nd law of diffusion[13,35] lead to contradictions with the empirical data[25] or miss explaining the dynamics of brain and leptomeningeal proteins presented in this review.

5) Recent findings on the bulk flow of the interstitial (extracellular) fluid[36,37,38,39] open new perspectives for the generalization of diffusion/flow relationships in the brain.

SURVEY OF DATA COMPILATION

The empirical cerebrospinal fluid protein data are structured as follows in this review

- The blood to CSF concentration gradients;
- The rostro-caudal (ventricular to lumbar) gradients;
- The interindividual, biological variations of proteins in blood, in CSF and the corresponding blood/CSF quotients;
- Developmental neurophysiology of barriers and CSF flow;
- Comparison of inflammation-dependent and mechanically blocked CSF flow or restricted CSF outflow in different neurological diseases.

The pathophysiology of representative examples of neurological diseases which show directly causes of reduced cerebrospinal fluid flow

- Purulent bacterial meningitis;
- Leukemia of the CNS;
- Meningeal carcinomatosis;
- Spinal block by spinal stenosis and spinal tumor;
- Guillain-Barré syndrome;
- Multiple sclerosis;
- Experimental allergic encephalomyelitis.

RESULTS

Source of cerebrospinal fluid proteins

The CSF proteins that enter the CSF in the ventricles and along the CSF flow way (Figure 1) derive from different sources:

- Blood;
- Brain cells (neurons and glial cells);
- Choroid plexus epithelium;
- Leptomeningeal cells;
- The largest fraction of the proteins (80%) in normal CSF originates from blood[40]. Only a small fraction of brain-derived proteins in CSF is exclusively brain-cell derived[40]. Most of the brain-derived proteins in CSF are only predominantly brain-derived, i.e., with an eventually negligible contribution from blood[6,7].

Albumin as reference molecule in cerebrospinal fluid

QAlb, the CSF/serum concentration quotient of albumin is the best reference for the evaluation of blood-derived proteins in CSF (Figure 2)[15] as it resembles all the individual biological variations such as the multitude of diffusion pathways at the blood-brain and brain-CSF interfaces as well as the individual CSF flow rate and lengths of flow ways.

CSF/serum concentration quotients, Q, are *normalized, dimensionless CSF concentrations* with values between 0 and 1.

BLOOD-DERIVED PROTEINS

Normal barrier function

The blood to CSF concentration gradients of blood-derived proteins in CSF are molecular size dependent (Table 1). The larger the molecule, the slower the diffusion is to reach the steady state concentration in CSF. The molecular flux J= -D dc/dx (Figure 1) is determined by the local concentration gradient at the diffusion/flow interface (dc/dx in Figure 1 and also Figure 2 in Ref[15]). A change of albumin concentration in blood is equilibrated with its CSF concentration in about 1 day, IgG in two and IgM much later[41].

Fact 1. Barrier passage is a molecular size-dependent random diffusion process with nonlinear concentration distribution. The net flux direction depends on the local concentration gradient at the diffusion/flow interface.

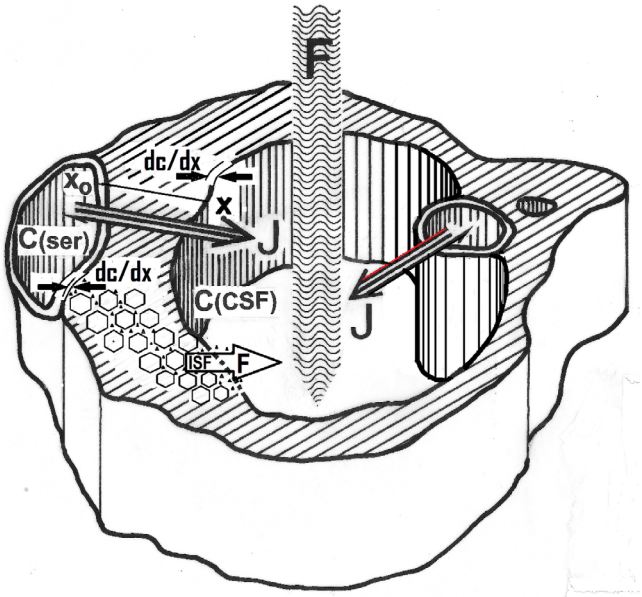


Figure 1. Idealized cross-section through the subarachnoid space with adjacent blood vessels and interstitial fluid between individual cells (hexagon). Open arrows are for bulk volume flow, F, and double arrows refer to the (bidirectional) molecular diffusion. The molecular flux, J=-D dc/dx, is defined by the diffusion coefficient, D, and the local gradient dc/dx at meningeal/cerebrospinal fluid, endothelial/cerebrospinal fluid or endothelial/interstitial fluid interface. Interstitial fluid flow transports molecules from tissue by bulk flow into cerebrospinal fluid, contributing to cerebrospinal fluid volume by about 10%.

Ventricular to lumbar (rostro-caudal) concentration gradient

In normal CSF, the concentrations of blood-derived proteins increase along the CSF flow way through the subarachnoid space (SAS)[41,42,43] because of the steady diffusion of proteins from capillaries accompanying the SAS. The albumin concentration increases 2.5-fold between the ventricular CSF and lumbar CSF[1,2,42]. From sequential extraction of lumbar CSF shown in Figure 3, we learn that the rostro-caudal gradient of blood-derived proteins increases *nonlinearly* along the CSF flow way[43]. If we invert the x-axis in Figure 3, we can fit the increasing rostro-caudal gradient with a Gaussian error function[21], the differential of the nonlinear concentration distribution function between blood and CSF[7]. The nonlinear gradient, which is molecular size-dependent (IgG *versus* albumin[43]) and steeper in barrier dysfunctions (Figure 3), can be explained with a nonlinear change of the steady state for the molecular flux dJ/dc only by Fick's 2nd law of diffusion[7,8,21].

Fact 2. The nonlinear rostro-caudal gradient for serum proteins is a basic argument for the molecular flux/CSF flow model with CSF concentration-dependent molecular flux.

Table 1. Molecular size-related overall concentration gradients of serum proteins between normal serum and normal cerebrospinal fluid.

	MW (kDa)	R (nm)	Ser:CSF mean	Serum (g/l)
Alb	69	3.58	200:1	35-55
IgG	150	5.34	429:1	7-16
IgA	160	5.68	775:1	0.7-4.5
IgM	971	12.1	3300:1	0.4-2.6
KFLC	22.5	n.d.	10:1	< 0.03

R: mean molecular radius; MW: molecular weight; means of normal cerebrospinal fluid (CSF): serum concentration quotients for IgG, IgA, IgM and kappa free light chains (KFLC)[8,25] correspond to a normal mean QAlb=5×10^{-3}.

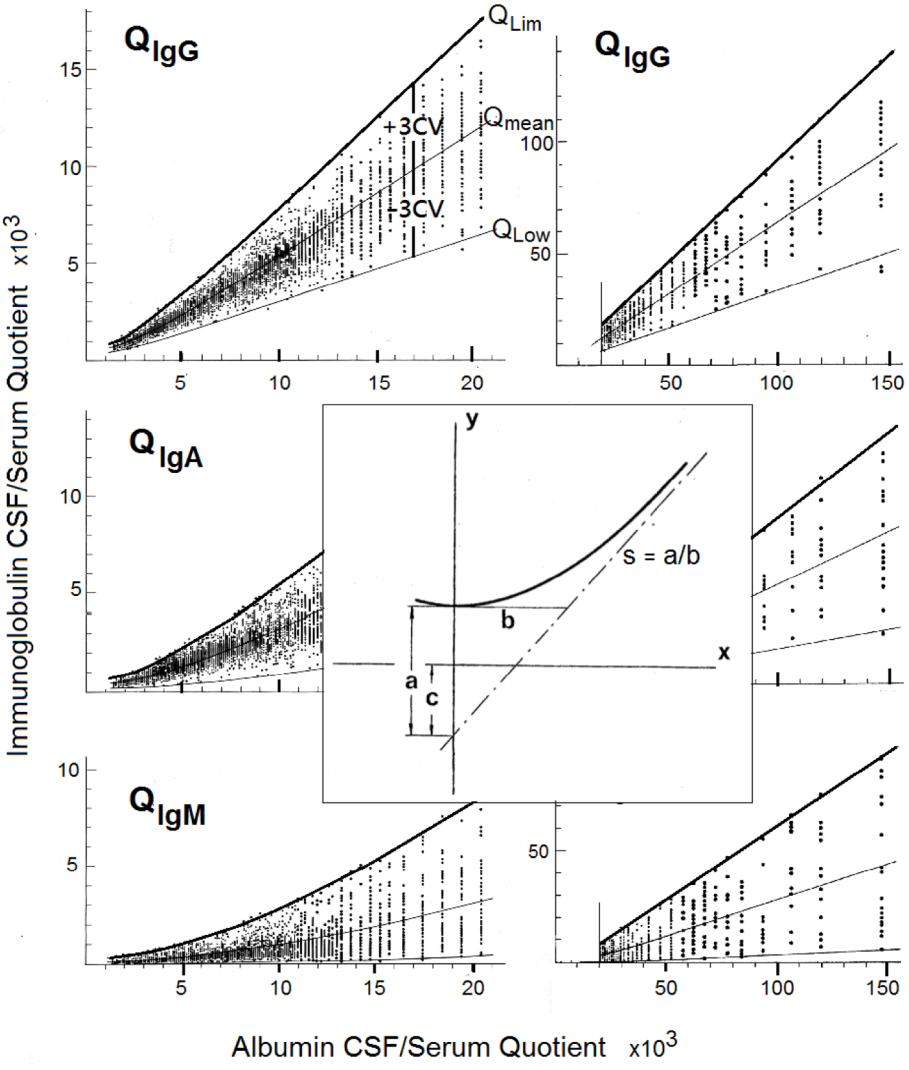


Figure 2. Reference ranges for CSF/serum concentration quotients of IgG, IgA and IgM (Q_{IgG},Q_{IgA},Q_{IgM}) relative to the albumin quotient, fitted with hyperbolic functions in lin/lin CSF/serum quotient diagrams[8]. Ranges of QAlb=1-20×10^{-3} (left side diagrams) and QAlb=20-150×10^{-3} (right side diagrams). Data base from 4154 control patients without an intrathecal immunoglobulin synthesis[8]. The reference ranges with upper and lower border include 99% of the data, corresponding to Qmean±3 standard deviations or Coefficient of Variation, CV. The inserted diagram defines the hyperbolic functions (Equation 1 in text) with c, the theoretical intercept of y at x=0 and the molecular size-dependent slope (s=a/b) of the asymptote.

Blood proteins and barrier dysfunction

Size-dependent increase of protein concentrations

The increased concentrations of blood-derived proteins in CSF are the measurable fact of a barrier dysfunction (Figure 2). The hyperbolic reference range is the base for later developments of a suitable barrier model (paragraph 3.3.4). As a second important result, we find that still the severest barrier dysfunctions do not diminish the molecular size-related selectivity of the barrier passage (QAlb/QIgG/QIgA/QIgM do not approach each other in Figure 2).

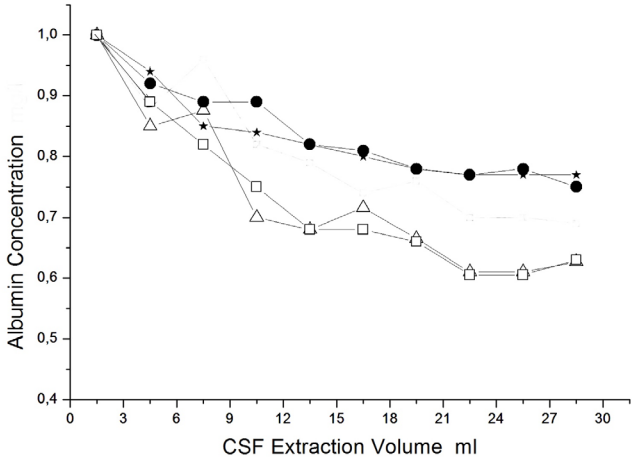
This maintenance of selectivity in barrier dysfunctions is also shown for individual patients in Table 2. Still, in diseases with CSF albumin concentrations that account for >70% of the blood concentration (so-called total barrier breakdown), we find a molecular size-related increase of CSF concentrations.

Protein kinetics in the individual patient

For a patient with bacterial meningitis (Table 3), the protein data could be compared on the first and second day after the onset of the disease. The smallest molecule, albumin, reaches its new steady state earlier than the larger molecules IgG, IgA and IgM. On the first day, albumin already reaches about 50% of the concentration reached on the second day, but the larger molecules at day one only reached 20% (IgG), 10% (IgA) and 5% (IgM) of the values measured on the second day because of their molecular size-dependent barrier passage without a compromise of the diffusion path.

This observation of the dynamics also explains the variable relations of the IgG, IgA and IgM quotients of the different patients in Table 2 as a consequence of the different time of puncture in the course of the diseases.

Fact 3. The molecular size-related increase in the approach to the new steady state confirms the unchanged barrier selectivity in acute disease of the individual patient.



Seyfert and Faulstich[43] have reported the sequential extraction of CSF (10×3 mL) from patients with a normotensive hydrocephalus with analysis of albumin and IgG in CSF and serum (presented as CSF/serum quotients, QAlb and QIgG). For comparison, their individual patient data were reevaluated and normalized (QAlb=1 for the first extracted samples). The filled circles are the compiled data from n=13 patients with a normal mean of QAlb=6×10^{-3} (1st extracted samples). The stars are the mean from two very similar data sets with a slightly increased mean QAlb=11.3×10^{-3}. The open triangles are from an individual patient with QAlb=18.5×10^{-3} and the open squares from a patient with QAlb=19.5×10^{-3}. The curves fit a theoretically expected Gaussian error function[21]. With larger lumbar QAlb; i.e., reduced CSF flow rate the rostro-caudal gradients are steeper.

Figure 3. Rostro-caudal concentration gradient of albumin in CSF.

Constant coefficient of variation in barrier dysfunctions

The statistical data interpretation in quotient diagrams (Figure 2) takes advantage of analysis of grouped QAlb intervals (explicit in Ref[25]). With this method, we calculate Qmean values and fit the Qmean curves of the reference ranges in Figure 2. This provides the interindividual coefficients of variation, CV, for subsequent QAlb concentration intervals[8,25]. As third important result from Figure 2, we find that the molecular size-dependent CVs are constant for all QAlb values, i.e., independent of the extent of the barrier dysfunction, including the severest barrier "breakdowns". This constant CV for interindividual variations means that the diffusion path is not affected.

Fact 4. The invariant transfer mechanisms (constant CV) are the strongest biophysical arguments that the increasing CSF protein concentration is not related to any change in the diffusion path (leakage) but to reduced CSF flow rate.

Table 2. Neurological diseases with extreme barrier dysfunctions, without intrathecal synthesis of immunoglobulins. Data are from first diagnostic puncture which may be at different times in the course of the disease.

Disease	QAlb (×10^3)	QIgG (×10^3)	QIgA (×10^3)	QIgM (×10^3)
Spinal tumor	172	93	67	15
Meningitis	214	155	110	84
Ependymoma	295	238	191	127
Meningitis	329	227	145	12
Spinal cyst	344	182	96	26
Men. carcinomatosis	411	256	196	16
Spinal tumor	510	333	240	154
Medulloblastoma	627	425	347	161
Bact. meningitis	731	646	466	352

QAlb; albumin quotient; QIgG: IgG quotient; QIgA; IgA quotient; QIgM; IgM quotient.

Table 3. Kinetics of molecular size-dependent protein transfer in case of a fast-developing bacterial meningitis. Comparison of data from cerebrospinal fluid punctures at day one and two after admission of the individual patient to hospital with statistically normal mean values[8].

	QAlb (×10^3)	QIgG (×10^3)	QIgA (×10^3)	QIgM (×10^3)	Cell count (cells/µL)
Normal	5	2,3	1,3	0,3	2
1. day	146	42	22	5	872
2. day	311	203	184	105	154,000

QAlb; albumin quotient; QIgG: IgG quotient; QIgA; IgA quotient; QIgM; IgM quotient.

Hyperbolic function

The fit over two orders of magnitude of the QAlb values ($1.5\text{-}150\times10^{-3}$) in Figure 2 gives the hyperbolic function its reliability. The hyperbolic function (Equation 1 and Figure 2) has a molecular size-dependent slope (s=a/b) of the asymptote, so the smaller the molecule the steeper the asymptote[7,8,25].

$$QIg=a/b\sqrt{QAlb^2+b^2} - c. \quad (1)$$

This empirically discovered fit of the hyperbolic function (Figure 2) could also be derived theoretically from the biophysics of diffusion and flow[8,21], valid as reference range up to $QIg=0.5=500\times10^{-3}$. This restriction is relevant only for molecules smaller than albumin as shown for the kappa free light chains[25] with a corresponding theoretical derivation[8,21].

The increase in the CSF concentration of a serum protein either time-dependent (barrier dysfunction with reduced flow rate) (Figure 2 and Table 4) or concentration-dependent (rostro-caudal gradient) (Figure 3) leads to a nonlinear increase (dJ/dt) in the local molecular flux into CSF (J= -D dc/dx in Figure 1 and Figure 2 in Ref[15]).

Fact 5. The hyperbolic increase of serum protein concentrations in CSF is the unambiguous indication for the rostro-caudal direction of CSF flow and the necessary application of Fick's 2nd law of diffusion (flux-flow model of barrier function).

BRAIN-DERIVED AND LEPTOMENINGEAL PROTEINS

Brain-derived proteins originate from glial, neuronal or choroid plexus cells[6,7,40]. They are released into the interstitial fluid and corresponding ventricular, cisternal and cortical CSF, but not into lumbar SAS. Their concentrations are typically higher in CSF than in blood (e.g., tau protein and S100B in Table 4).

Table 4. Concentration gradients between cerebrospinal fluid and blood and between ventricular and lumbar cerebrospinal fluid for cerebrospinal fluid proteins of different sources.

Protein	MW (kDa)	CSF:serum	IF (%)	V_{CSF}:L_{CSF}
B-T	25	34:1	>99	1:11
Cystatin C	13.3	5:1	>99	1:3.5
TP	55-74	10:1	>99	1.5:1
S-100 B	21	18:1	>99	3.5:1
NSE	78	1:1	>99	2:1
TT	54 (+21)	1:18	~90	1.1:1
sICAM	90	1:190	~30	n.d.
Albumin	64	1:200	0	1:2.5

MW: molecular weight; CSF: cerebrospinal fluid; IF: intrathecal fractions; V_{CSF}: ventricular cerebrospinal fluid; L_{CSF}: lumbar cerebrospinal fluid. Protein sources: S-100 B (glia); NSE: neuron-specific enolase (neurons); TP: tau protein (microtubules of neurons and glia); B-T: beta-trace protein and cystatin C (leptomeninges); TT: transthyretin (choroid plexus+blood, associated with retinol-binding protein); sICAM: soluble intercellular adhesion molecule-1 (leptomeninges and blood). IF (intrathecal fraction in %) is obtained by subtraction of calculated theoretical blood-derived fraction[6,7].

The arachnoid and pia mater, connected by trabeculae, between which the CSF circulates, together are called the *leptomeninges*. Leptomeningeal cells release several of the brain-derived proteins into the CSF[6,7]: beta-trace protein (prostaglandin-D-synthase) (Table 4), insulin-like growth factor (IGF)-II, IGF-binding protein-2, apolipoprotein E, beta-2-microglobulin, cystatin C (Table 4), transferrin, mannan-binding lectin[44], etc. Leptomeningeal protein concentrations are higher in lumbar CSF than in ventricular CSF (e.g., V_{CSF}:L_{CSF} for beta-trace protein and cystatin C in Table 4)

Rostro-caudal gradients

The normal rostro-caudal concentration gradient of brain proteins decreases linearly, as an expression of net diffusion outward from the CSF. The gradient of leptomeningeal proteins increases linearly due to the continuous release of proteins along the flow path (Table 4).

These processes are linear because there is no concentration-dependent feedback, as is the case with blood-derived proteins. With a wrongly postulated cisternal directed flow[14,22], there would be no brain proteins in the lumbar CSF at least not at the observed concentrations.

Fact 6. The combination of rostro-caudal gradients of brain and leptomeningeal proteins indicates a caudal direction of CSF flow.

Barrier dysfunctions and brain proteins

Brain proteins and leptomeningeal proteins, which do not have to pass the barriers, also change in the lumbar cerebrospinal fluid with a blood CSF barrier dysfunction (Figures 4A and 4B): Different from serum protein concentrations (Figure 2), the concentration of brain proteins in the lumbar cerebrospinal fluid remains constant and the leptomeningeal protein levels increase linearly (Figure 4B)[6,7].

The constant lumbar CSF concentration of the brain proteins does not contradict a reduced CSF flow rate. The slower the CSF flow, the more protein can diffuse into the ventricular and cisternal CSF, and the more will diffuse out of the SAS. Both linear processes compensate each other.

Differently, the concentrations of the leptomeningeal proteins beta-trace protein and cystatin C (Figure 4B) increase linearly with increasing barrier dysfunction[6,7]. This empirically found linearity of the increase with increasing QAlb[6,7] has to be expected because of the decreasing flow-dependent elimination and the absent feedback on the protein release from the leptomeningeal cells.

Fact 7. The reduced CSF flow rate is the common cause of the barrier-related and the non-barrier-related protein dynamics in barrier dysfunctions. Any leakage model must fail in the explanation of this coincidence.

Lumbar cerebrospinal fluid blockage: combined dynamics of blood, brain and leptomeningeal proteins

From an individual patient with spinal blockage by a leptomeningeal tumor metastasis[45], we get the data in Table 5 from two concurrent punctures at L2 and L5.

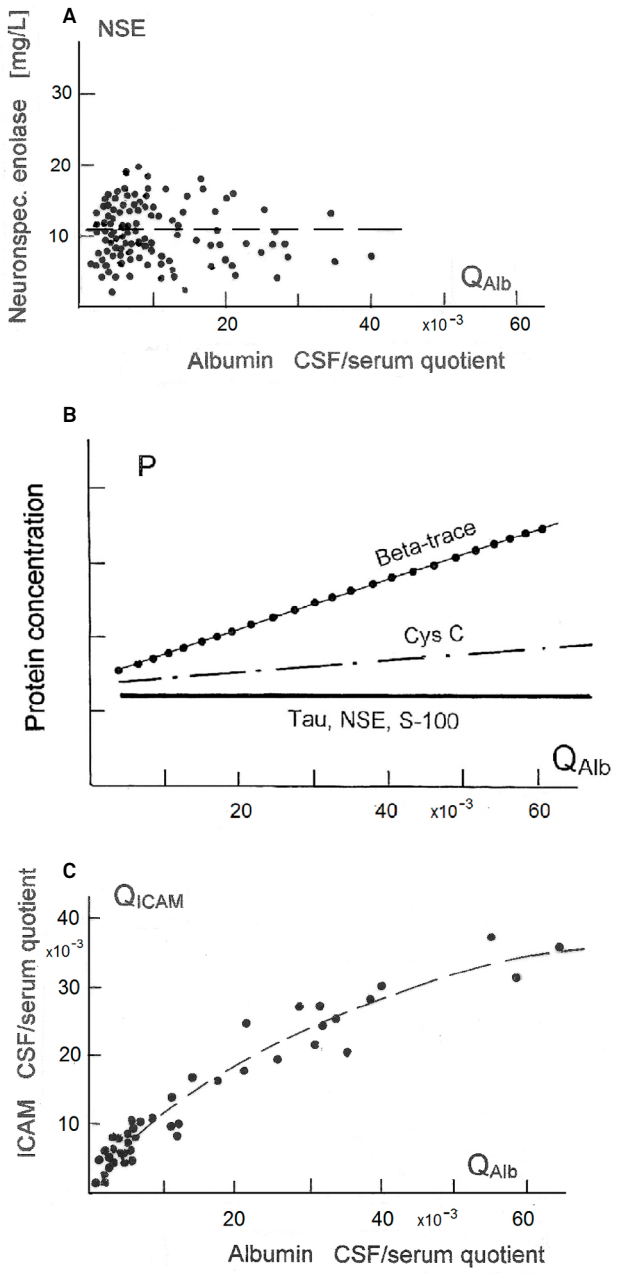


Figure 4. Influence of blood-cerebrospinal fluid barrier dysfunctions, i.e., reduced cerebrospinal fluid flow rate on cerebrospinal fluid proteins of different origins. Data of normal controls and patients with blood-cerebrospinal fluid barrier dysfunctions of noninflammatory diseases. Patients with degenerative diseases, hypoxia or stroke have been excluded from these groups. (A) Data of brain cell-derived neuron-specific enolase. (B) Regression lines of proteins released either from brain cells: glial S-100 protein, tau protein and neuron-specific enolase, or from leptomeningeal proteins beta-trace protein and cystatin C. Beta-trace protein concentrations were analyzed in lumbar cerebrospinal fluid from normal controls and patients with spinal canal stenosis. (C) Soluble intercellular adhesion molecule, sICAM. The dashed curve represents the mean of the reference range[6,7].

The albumin concentration from lumbar puncture at L2 was increased (QAlb=19.7×10^{-3} compared to normal QAlb=5×10^{-3}), but below the blockage, punctured at L5, the albumin concentration was 17-fold higher (QAlb=338×10^{-3}). Concomitantly, we have a 3-fold lower brain-derived NSE concentration and a 4-fold increase in leptomeningeal beta-trace protein (Table 5). For transthyretin (TT in Table 5) with a normally dominant brain-derived fraction above the blockage (IF in Table 4), below the blockage, the blood-derived fraction gets dominant with a threefold increase in total TT concentration.

Fact 8. The molecular flux/CSF flow model is the only model so far to give a consistent explanation of all the different particular dynamics of proteins of different sources in CSF.

Barrier dysfunction plus chemical equilibrium

Compared to the dynamics of the other proteins (Figures 4A and 4B) the soluble intercellular adhesion molecule (sICAM) in CSF[6,7] shows very different dynamics (Figure 4C). With flow-related increasing CSF concentration of blood-derived sICAM (Table 4), the equilibrium is shifted to the complex of sICAM with the membrane-bound ICAM, indicated by a classical Michaelis-Menton curve (Figure 4C).

This explanatory function of the diffusion/flow theory for sICAM dynamics shows its general validity in explanations of the barrier dysfunctions.

PATHOPHYSIOLOGICAL CAUSES OF REDUCED CSF FLOW RATE

Representative examples of neurological diseases provide the evidence from the pathological processes that a reduced CSF flow rate is sufficient to explain all kinds of blood-CSF barrier dysfunctions.

Table 5. Protein concentrations in lumbar cerebrospinal fluid above and below a spinal block. Data from a patient with a leptomeningeal infiltration (meningeosis from systemic adenocarcinoma), punctured in the same session at L2 and at L5 for diagnostic reasons[45]. The cerebrospinal fluid values were at L2: QAlb 19.7/QIgG 12.8/QIgA 8.8/QIgM 3.2×10^{-3}, CC normal, no OCB, lactate 1.6 mmol/L, TP 1.5 g/L (Hb++) QCEA<38×10^{-3}. L5: Q's 338/218/150/43.6×10^{-3}, correspondingly; CC 4/µL, lactate 2.6 mmol/L, TP 23.9 g/L (Hb 0, xanthochr.) QCEA=306×10^{-3} or 16% intrathecal synthesis. For comparison, the normal values L5 (n) were calculated from mean concentration gradients[6,7]. For protein details, see legend of Table 4.

	QAlb	CEA	NSE	B-T	TT	Ferritin
	(x 10^3)	(ng/mL)	(µg/L)	(mg/L)	(g/L)	(µg/L)
L2	19.7	<0.07	10.8	25.9	0.020	7.5
L5	338	0.52	3.0	102	0.067	32
L5(n)	<25	<0.07	>8	<40	<0.02	<15

QAlb: albumin quotient; CEA: carcinoembryonic antigen.

- *Purulent bacterial meningitis:* A very large CSF cell count and high total protein concentration in CSF (Tables 2 and 4 of Ref[2]) is associated with increased CSF viscosity and meningeal adhesions. Post-mortem studies reveal protein complexes and cellular deposits in the arachnoid granulations;
- *Leukemia of the CNS:* This disease is primarily associated with changes in the trabeculae and the arachnoid mater. Histopathological studies suggest a reduced CSF flow rate[46];
- *Meningeal carcinomatosis*[45] and spinal tumor[1,2]. A meningeal carcinomatosis can involve the cranial and spinal leptomeninges and contributes along the whole or parts of the CSF flow pathway to an obstruction of CSF flow (Table 5);
- *Spinal blockage:* This Froin's syndrome can have different causes, such as a lumbar stenosis, a spinal tumor (Table 5), disc prolaps or a flow blockage by cysticerci in an extraparenchymal neurocysticercosis[1];
- *Guillain-Barré syndrome and spinal cord schistosomiasis:* Both diseases lead to an outflow obstruction. The large protein concentrations in Guillain-Barré syndrome with up to 20-fold increased QAlb values[1], are due to the swelling in the area around the spinal nerve roots (radiculitis). In the case of spinal cord schistosomiasis, it is the direct parasite block[1] which hinders the CSF bulk outflow.
- *Multiple sclerosis*: Typical for this chronic inflammatory disease are the only slightly increased albumin quotients, observed in about 20% of MS patients. Consistant with the flow hypothesis, Liebsch et al.[47] found that the MS cases with increased QAlb values had spinal lesions. The frequently increased gadolinium extravasation (shown in MRI) in the brains of MS patients do not correlate with the increased QAlb. But they can be explained consistently by a local restriction of ISF flow as a consequence of the inflammatory local demyelination process.
- *Experimental allergic encephalomyelitis*: The chronic remitting, relapsing form of experimental allergic encephalomyelitis (crEAE) of the guinea pig, is primarily a meningeal affection[48]. The increased cisternal QAlb values can be explained as a restriction of CSF flow by the meningeal inflammation. The tight junctions of the capillaries are described as remaining intact[49].

DISCUSSION

Leak or no leak. That is not the question

Any hydrophilic molecule, no matter how large, and even entire cells can pass through the normal intercellular structures of cell layers between blood and the brain; it is only a matter of time (Table 3). The multitude of different such "barriers" from the fenestrated capillaries of the circumventricular organs to the intercellular tight junctions of different cell layers allow together a functional compartmentalization in the brain but they are never impermeable, i.e., always leaky.

But what happens if there is a pathological increase in serum protein concentrations in the CSF? What about those cases where so many authors speak of "barrier leakage"?

No change in structures

The facts 1–8 and paragraph 5 disprove a morphological barrier disorder (leakage). It is therefore not necessary to discuss the details of the different errors in those barrier models which are based on a leakage concept in one way or another[3,4,5,9,10,11,12,13,14,16,35].

The concept of leakage is based on a biologically false expectation that holes in membranes or intercellular junctions appear spontaneously in pathological conditions; it is just the other way around. To keep the intercellular fenestration open, enzymatic activity may be necessary[19]. Quite general, channels such as the sodium/potassium channels in membranes require a stabilizing protein scaffold. A membrane or intercellular leak would be like expecting a stone thrown into a lake to leave a hole.

The term barrier dysfunction still makes sense, but not the misleading terms leakage, impairment, disruption or breakdown.

Cerebrospinal fluid flow — interindividual variabilities

With the importance of CSF flow, the interindividual variations of serum protein concentrations in CSF (Figure 2 and Ref[25]) must also be assigned to the biological variations of the flow rate. These are:

- Varying ventricular CSF volumes between 7 and 60 mL correspond to the variation of the size of the choroid plexus and associated CSF production volume;
- Individual variation from the enzymes (amount and activity) involved in the water flux in choroid plexus[5,19,20];
- general variations with age[50];
- circadian (daytime) rhythms[30].

Variation of cerebrospinal fluid production in choroid plexus

The choroid plexus produces the main part of CSF volume (60–70%). The ISF, which has about the same volume as CSF in the brain, contributes also to CSF (ISF-F in Figure 1). But the ISF contribution should not be overestimated as the slow ISF flow rate is only about 10% of the CSF flow rate, and only a small fraction of the total ISF volume has access to the CSF space[36,50,51]. Phase contrast MRI at the cerebral aqueduct between the third and fourth ventricle gives a mean CSF production volume of 650 mL/day with a minimal circadian variation of 12 mL/h (at 6 pm) and 42 mL/h at night (at 2 am)[30].

Variables of individual choroid plexus function

As a basic source of biological interindividual variation of flow rate, we have to take into account a) the variable size of the ventricle volume and corresponding variability of choroid plexus and b) the active water secretion by the choroid plexus epithelium[19,20] with the many enzymes involved.

The secretion of single water molecules into the ventricles (molecular water flux) needs the water channel protein aquaporin-1[49] to facilitate this water flux 10-fold compared to the diffusion of water molecules through an elementary bilayer membrane. The direction of the passage of water is created by the high concentration of the apical Na-K-ATPase, which increases the sodium and decreases the potassium concentration in CSF. Together with the luminal sodium/bicarbonate co-transporter[52], this creates a steep osmotic gradient because of the directed molecular water flux. This secretory function of the choroid plexus is also facilitated by the fenestration of its capillaries[19], which are kept actively open by vascular endothelial growth factor (VEGF).

Any variation in the function of these four proteins (Na-K-ATPase, carboanhydrase, VEGF and aquaporin) leads to a variation in CSF production and CSF flow rate in addition to the individual size of the choroid plexus.

Development-, age- and sex-related variations in cerebrospinal fluid flow

The highest CSF protein concentrations during the human life span are found in the fetal brain. In the 15th week of gestation[53], CSF albumin concentration is 40-fold that of a 4-month-old child (Figure 5)[15,54,55].

This was previously interpreted as an expression of a missing barrier function. But this too must be revised according to the facts from developmental biology. Already at the earliest fetal stages of human development, at the 7th week of gestation, the tight junctions in the cerebral endothelial cells as well as in the choroid plexus epithelial cells appear completely functional[17]. Also, CSF production seems to function very early, concluded from the presence of carboanhydrase (water production) in the 9th week of gestation[52].

There was no indication of a functional opening of the arachnoid villi to the venous sinus (Ref.[5]) at time of first observations after the 12th week[17] in fetal development. The only slowly decreasing albumin concentration between the 12th and 41st week of gestation correlates with the slow maturation of the arachnoid villi. CSF data from human newborns[55] with a maximal CSF flow rate at about 4 months after birth (mean QAlb=2×10^{-3})[54,55] demonstrate this maturation. From Figure 5 (right diagram) we learn also that already at the time of birth, before the maximal flow rate, the relation between molecules of different size (QIgG/QAlb) fits a hyperbolic function characteristic of the mature morphological barrier[8].

Later, after the first years of life, with increasing age, QAlb increases again as the mean CSF production rate decreases, e.g., from 0.41 mL/min (mean age 29 years) to 0.19 mL/h (mean age 77 years)[50]. The reasons are hormonal changes as well as natural calcification in the choroid plexus with increasing age[56].

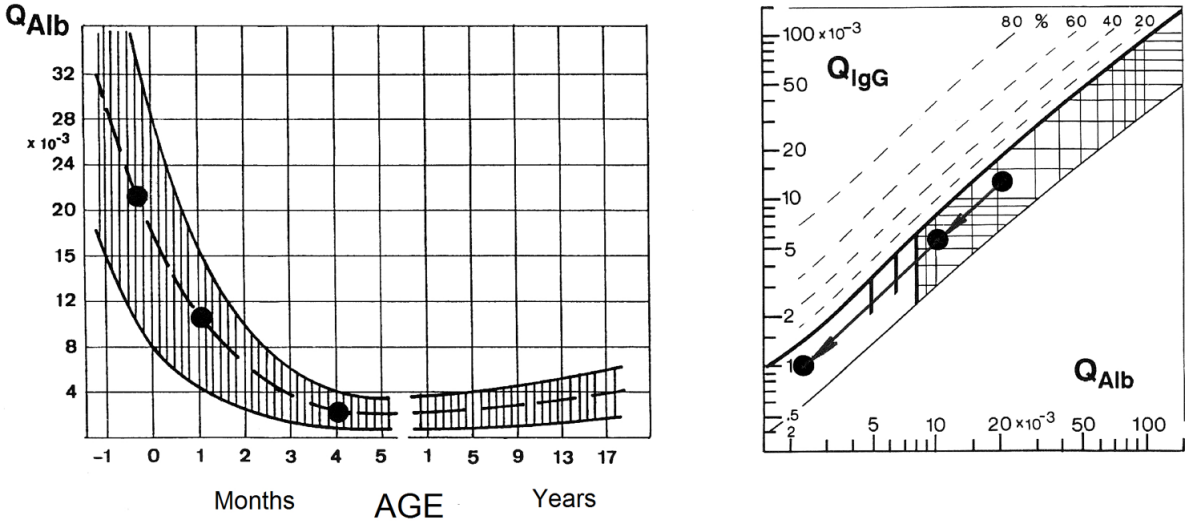
The early function of barriers together with early CSF production lead to high protein concentrations in prenatal CSF as there is no outflow, which appears only with the maturation of the arachnoid villi, to be considered as the main outflow way for CSF.

The sex-related difference between mean CSF concentrations of serum proteins with about 20% higher QAlb for men compared to females[54] may depend mainly on different body size and corresponding longer flow ways with increasing rostro-caudal gradient (Figure 3) and barely on hormonal differences in CSF production.

Related queries in cerebrospinal fluid research

Lymphatics

In rats and sheeps, CSF drains via the cribriform plate and nasal mucosa to cervical lymph nodes[32,57] which is



Left diagram: the initially high albumin quotients at time of birth with a steep decrease in the first 4 months to the lowest albumin quotients and later slowly increasing with increasing age. Right diagram: In the quotient diagram, the changing relation of QIgG to QAlb follows the hyperbolic mean reference curve for a blood-derived IgG fraction in cerebrospinal fluid already at time of birth. Age-related points (mean values) in left and right diagram are corresponding.

Figure 5. Age-dependent protein concentrations in cerebrospinal fluid of the newborn and at older age.

considered to account for the elimination of approximately 50% of CSF outflow in these animals already in the earliest developmental stage.

From the missing CSF outflow before maturation of the arachnoid villi, we learn that there is no comparably relevant lymphatic outflow in humans. Various experiments to show the importance of lymphatics in humans are seriously misinterpreted. Post-mortem tracer studies are not reliable as the energy-dependent CSF production stops with death[58], and the experimentally induced tracer gradient (applied post-mortem[32]) contributes only by diffusion. These experiments cannot give any information about flow.

Albeit a minor, pathophysiologically relevant connection along the olfactory nerve to the lymph system cannot be excluded in humans, there is no relevant volume fraction of CSF passing to the lymph system.

Pulsations and oscillations of cerebrospinal fluid

Brain pulsation and heartbeat- or respiration-related CSF dynamics have been investigated by a variety of imaging techniques[26,27,28,29]. With flow-sensitive magnetic resonance imaging[59], these pressure pulses are visible as to-and-fro motions in the SAS with impressive jet-like streams through the foramina backwards into the 4th ventricle. But this local pulse is about 100-fold faster than the mean CSF flow rate (3.7–7.6 mm/s compared to mean CSF net flow <0.03 mm/s at foramen Magendie). Additionally, the overall pressure gradient, which drives CSF flow, is not changed.

The *net flow* of CSF in the SAS was found by MRI at all times a caudally directed flow[28]. This is consistent with the protein data in CSF: CSF pulsation[26] does not eliminate or disturb the nonlinear concentration gradients for proteins between ventricles and lumbar CSF (Figure 3); i.e., the jet-like backflow into the ventricles should not lead to the misinterpretation as a reversed net flow. The wrong reinterpretation of Quincke's experiments[22] has been mentioned in the introduction.

The pulsation of heartbeat and respiration are of negligible relevance for net flow of CSF and overall protein dynamics in CSF.

PERSPECTIVES

Biophysical communalities of the blood-brain and blood-cerebrospinal fluid barriers

With the improved knowledge about the bulk flow of the extracellular fluid or ISF[36,37,38,39,51], we gained a new view on the intercellular barrier functions and dysfunctions. The most exciting aspect of bulk flow of ISF comes with the possibility to apply the biophysical principle of the molecular diffusion/flow model[7,8] not only at the meninges/CSF interface but also at the lowest structural level at the tight junction/ISF interfaces all over the brain. Regarding the barrier dysfunctions as a reduced bulk flow, in biophysical terms, we do not need to discriminate the blood brain- and blood-CSF barrier dysfunction due to their common biophysical principle at the capillary/ISF, the capillary/CSF or meningeal/CSF interface (Figure 1).

With these considerations, we face indeed a culture change[60], at least a change of an old paradigm, the opening for new diagnostic and therapeutic concepts in neurology.

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