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Proteins in cerebrospinal fluid and blood: Barriers, CSF flow rate and source-related dynamics

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Abstract. Cerebrospinal fluid (CSF) routine analysis for diagnosis of neurological diseases is based on the concepts for discrimination of blood-derived and brain-derived immunoglobulin fractions in CSF. The actual molecular flux/CSF flow theory of the blood/CSF barrier function, which founded the hyperbolic discrimination lines in quotient diagrams, is derived from the laws of molecular diffusion combined with CSF flow rate. It emerged from this theory that the decrease of CSF flow rate is sufficient to explain quantitatively the increase of CSF protein concentrations as observed in many neurological diseases. With this concept of CSF flow rate as the modulator of the normal and pathological blood-CSF barrier function, we got for the first time a theoretical frame work to explain also quantitatively the dynamics of brain-derived proteins and their source related (neurons and glial cells or leptomeningal cells) differences. The review of the anatomical, physiological and biophysical knowledge points to the new interpretations: The changing albumin quotient is an indicator of changing CSF flow rate and not for a morphological "leakage" of the blood-brain barrier.
As an application of these concepts the dynamics of brain-derived molecules in blood are discussed with two examples: beta trace protein, flowing with CSF into venous blood, and neuron-specific enolase, passing from tissue into blood the opposite direction of serum proteins, again a gradient-dependent protein diffusion across the intact blood vessel wall.

Keywords: Blood-CSF barrier function, blood-brain barrier, Cerebrospinal fluid, CSF flow, brain-derived proteins, blood-derived proteins, protein dynamics, theory

1. Introduction

For about hundred years protein analysis in CSF dealt with the blood-derived proteins, in particular with the immunoglobulins and their pathological intrathecal synthesis. Correspondingly, interpretation concepts referred to various models of the blood-CSF barrier function to discriminate an intrathecally synthesized fraction from a blood-derived fraction in CSF. The most prominent procedures used to identify an intrathecal IgG synthesis were linear functions [1–3]. The early observation of a different relation of the immunoglobulin CSF/serum quotient, QIgG, to the albumin quotient, QAlb, in the range of a blood CSF barrier dysfunction [4] found a first, still linear response [3] soon followed by several nonlinear approaches using sigmoidal [5], exponential [6,7] or unspecified arbitrary curves [8]. The discovery of the hyperbolic function as a best empirical fit [9] was finally confirmed as the correct description by its biophysical derivation from laws of diffusion combined with CSF flow rate (molecular flux/CSF flow theory [10]). With the application in quotient diagrams for IgG, IgA and IgM and the description of disease-related immunoglobulin patterns [11,12] CSF analysis for diagnosis of neurological diseases gained sensitivity and specificity. The theoretical foundation of the hyperbolic discrimination functions between blood- and brain-derived protein fractions in CSF [10] contributed to a new view of the blood CSF barrier function: the pathological change of

CSF flow rate is sufficient to explain quantitatively the dynamics of blood-derived proteins in CSF.

Brain-derived proteins in CSF and blood, which are of increasing diagnostic relevance [13–19], present an important extension of the basic CSF analysis program, in particular the observation that the dynamics of brain-derived proteins are, source-related, modified by CSF flow rate [19]. Neither a systematic investigation nor a general theoretical approach to describe the dynamics of brain-derived proteins in CSF has been available so far.With this recent decription of the dynamics of brain-derived proteins [19] the molecular flux/CSF flow theory [10] gained evidence as a valid concept to describe the normal and pathological protein dynamics in CSF. Subsequently this theory led to a new interpretation of the physiological observations regarding blood-brain and blood – CSF barrier functions.

Earlier ideas, still frequently referred to, explained an increased protein concentration in CSF as a morphological "leakage" or "breakdown" of the barrier. A part of this view is the misleading idea that the intact blood-brain barrier is impermeable for large proteins or for proteins at all. This is definitively wrong (see below).But the most crucial, wrong asumption is that the local pathological molecular changes [20,21] at the wall of some brain capillaries (blood-brain barrier) have a particular relevance for the pathological concentration change in CSF (blood-CSF barrier dysfunction). The empirical physiological observations justifying this statement which might irritate some readers, are summarized below and in [10]. In contrast to the asumption that such a local molecular approach counts for the observed changes in CSF the molecular flux/CSF flow theory [10] gives a much wider view of the blood-CSF barrier as a functional entity of diffusion and CSF flow rate. This "barrier-function" has to be understood as a process, which by restricted molecular diffusion (including capillary walls among other structures) and associated biochemical and physiological processes maintain a dynamical blood/CSF concentration gradient. With this concept all different causes of increased CSF protein concentrations like inflammation or mechanical blockade in the lumbar spinal canal got a common interpretation.

In this contribution I summarize the anatomical, physiological and biophysical knowledge base for the molecular flux/CSF flow model, its relevance for the barrier concepts regarding blood-derived proteins and the relevance for the dynamics of brain-derived proteins in CSF.

Based on this concept of diffusion and CSF flow, I report a first draft for the interpretation of the observed dynamics of brain-derived proteins in blood passing the "barriers" in the opposite direction.

2. Physiology

2.1. Origins of CSF proteins

The main fraction of proteins in the normal cerebrospinal fluid originates from blood, e.g. albumin which constitutes 35–80% of total protein in CSF [11].

About 20% of the protein in CSF are predominantly brain-derived, but rarely brain-specific [22]. For example,90% of transthyretin in CSF originate from choroid plexus (Table 1) but is also synthesized in the liver or, another example, the gamma monomer of neuron-specific enolase is synthesized in erythrocytes and thrombocytes as well.

The basic feature of predominantly brain-derived proteins is their higher concentration in CSF compared to serum, inducing a net flux out of CSF compared with blood-derived proteins with a net flux into CSF (Table 1). The particular dynamics of the brain-derived proteins depend on their sources which are either the brain cells (neurons, glial cells) or the leptomeningeal cells [19]. For some of the predominantly brain-derived proteins their high blood concentration contributes a not negligible fraction to the CSF concentration (Table 1).

2.2. Physiology of CSF space

The CSF space (Fig. 1) involves the ventricles and the subarachnoid space with its enlargements, the cisterns. The subarachnoid space between the arachnoidea and the pia mater has a particular structure with trabeculae connecting the two meninges. CSF, flowing from the ventricles into the cisterns, divides then into a cortical and lumbar subarachnoid space (Fig. 1). The CSF, including cells and proteins, leaves the subarachnoid space through the arachnoid granulations or villi, which function like a valve, letting pass the whole unfiltered CSF("bulk flow") into the dural venous sinuses. As shown by several investigators [23], the main, if not the only, exit routes of bulk CSF are in the cortical and spinal subarachnoid spaces (arachnoid villi and spinal nerve roots).

Table 1
Concentration gradients for blood- and brain-derived proteins between CSF and blood and between ventricular and lumbar CSF, with calculated intrathecal fractions (IF)

Proteins[1)]	MW (kDa)	CSF : serum Ratio	IF[2)] (%)	V-CSF:L-CSF Gradient[6)]
β-trace-Prot.	25	34 : 1	> 99	1 : 11
Cystatin C	13.3	5 : 1	> 99	1 : 3.5
Tau-Protein	55–74	10 : 1[3)]	> 99	1.5 : 1
S-100 B	21	18 : 1	> 99	3.5 : 1
NSE	78	1 : 1	> 99	2 : 1
Transthyretin	55 (+21)[4)]	1 : 18	$\sim$90	1.1 : 1
s-ICAM	90	1 : 190	$\sim$30	–
Albumin	67	1 : 205[5)]	0	1 : 2.5
IgG	150	1 : 440[5)]	0	–
IgA	170	1 : 800[5)]	0	–
IgM	900	1 : 3400[5)]	0	–

1. Source of data as indicated in *Material and Methods of ref.* [19].
S-100 protein is an acidic, calcium-binding protein found in the brain as homodimer or heterodimer. S-100 B, the beta, beta dimer, is present in high concentrations in glial cells and Schwann cells.
Tau proteins modulate assembly and stability in axonal and dendritic microtubules of neurons, but are also found in astrocytes and oligodendrocytes.
β-trace protein (prostaglandin D synthase) is a small secretory protein and originates exclusively from the brain, mainly from the leptomeninges.
Cystatin C, the former gamma-trace protein, a cysteine proteinase inhibitor is produced by all nucleated cells, including neuroendocrine cells and cortical neurons Besides a dominant release from the choroid plexus compared to the residual brain a release of cystatin C from leptomeningeal cells in vitro has been reported.
Transthyretin, the former prealbumin, functions as a carrier protein for thyroxine and for vitamin A by one to one association with retinol binding protein and originates in CSF predominantly from the choroid plexus.
2. IF = intrathecal fractions, are calculated as shown in [19].
3. Calculated with reference to median of serum concentration 20 ng/L ($n = 60$ normal blood donors). Tau protein concentrations in blood vary extremely between < 10 and > 100 ng/L.
4. Transthyretin (54 kDa) passes from blood into CSF associated with the retinol-binding protein (21 kDa). Data represent the mean from $n = 27$ control patients.
5. The ratios refer to mean quotients ($Q_{\mathrm{IgG}} = 2.28 \times 10^{-3}$, $Q_{\mathrm{IgA}} = 1.26$ and $Q_{\mathrm{IgM}} = 0.295 \times 10^{-3}$) calculated with the hyperbolic functions for mean $Q_{\mathrm{Alb}} = 4.9 \times 10^{-3}$ [10].
6. The mean values of data from ventricular CSF refer to groups of 7 to 13 patients [19].

2.3. CSF flow – source, evidence and relevance

Awareness of the essentially uni-directional CSF flow has very old roots and achieved scientific relevance by the experimentally founded physiology of CSF [24]. Direct evidence for steady CSF flow is given by magnetic resonance imaging [25–27], which can visualize flow of CSF through the aqueduct. When referring to variable CSF flow rate we mean average flow rates. The short-, intermediate- and long-term variations or local fluctuations in the subarachnoid space [27] can be neglected for diagnostic or theoretical purposes [10].

CSF is continuously formed in the choroid plexus of the ventricles. Both, active secretion by the choroid epithelium and filtration of plasma across the endothelial wall of the choroid capillary constitute the ventricular CSF.

The daily CSF production is age-dependent and varies between about 500 mL/day in the young human (0.4 mL/min) to 250 mL/day (0.19 mL/min) for the elderly humans [28].

The driving force for CSF flow is the arterio-venous pressure gradient.

The CSF pressure is influenced by wide variations in individual anatomical and physiological parameters which in turn vary with age and, in particular in pathological processes, with increasing resistance to flow.

If we regard CSF as a sink [24] to drain the brain extracellular fluid with its brain-derived metabolites, we have to consider two particular ratios: The daily produced CSF volume (500 mL) represents about 10% of total blood volume in the adult human and with a relationship of 165 mL CSF space to a mean of 1.4 kg brain weight, we have again about 10% CSF volume/total brain volume. We have also to take into account the alternative, direct diffusion of brain metabolites into blood. This can be a very fast process as seen for

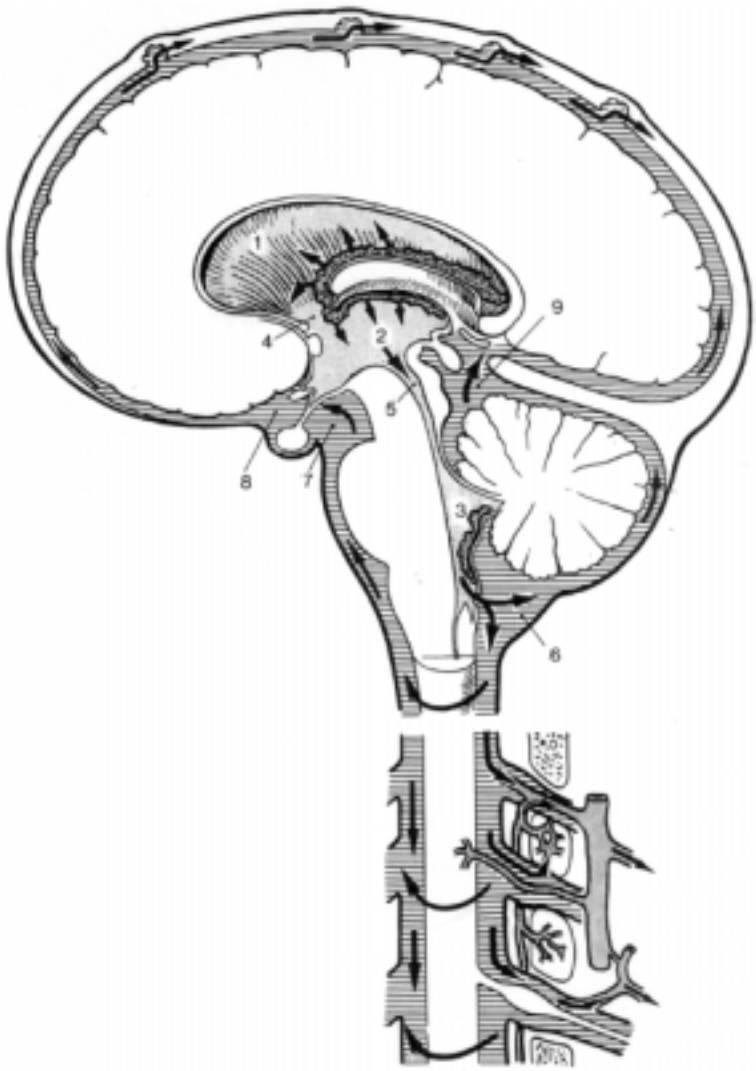

Fig. 1. Anatomy and physiology of cerebrospinal fluid (CSF) flow [10].
CSF is produced in choroid plexus of the four ventricles: 1, I. + II. lateral ventricles; 2, III. ventricle; 3, IV. ventricle. CSF passes through the foramen of Monroe, 4, into third ventricle and through aqueduct of Sylvius, 5, into fourth ventricle and leaves into the cisternae, 6–9, via foramen of Mangendie (into cisterna magna) and via both lateral foramina of Luschka (into cisterna pontis). From cisterns CSF divides into a cortical and a lumbar branch of subarachnoid space. Finally, CSF drains through the arachnoid villi into venous blood.
Mean CSF volumes (on the basis of NMR imaging): Total CSF 165 mL; ventricles 32 mL (third ventricle 1 mL); extra-ventricular CSF 133 mL which recruits 30 mL from spinal subarachnoid space and, in particular, from a large CSF reservoir in the cortical sulci. The age related, mean daily turnover is about 500 mL CSF for the adults.
CSF bulk flow is driven by the arterio-ventricular pressure gradient, modulated by short term variations from autonomic nervous system functions.

neuron-specific enolase [15] in case of cerebral hypoxia or head brain trauma (Fig. 9).

2.4. *Normal and pathological variations of CSF flow rate*

The albumin CSF/serum concentration quotient is generally accepted as the best measure to characterize the individual blood-CSF barrier function (Fig. 2) for blood-derived proteins in CSF. This is in particular due to the fact that albumin in CSF exclusively originates from blood and therefore its lumbar CSF/serum concentration quotient is representative for a manifold of different sources and diffusion ways (see next paragraph), by which serum-derived proteins reach CSF. But, as shown [10], the CSF flow rate is the main mod-

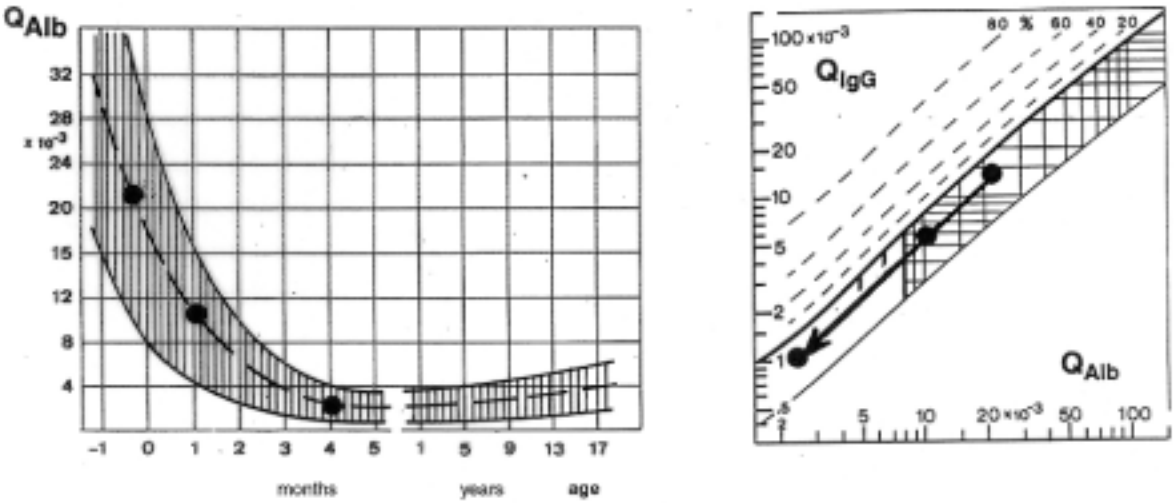

Fig. 2. Albumin-CSF/serum concentration quotient – age-dependency of the reference range in childhood.The left diagram demonstrates the initially high albumin quotients at time of birth with a steep decrease in the first 4 months to the lowest albumin quotients and later slowly increasing with increasing age. The right diagram, the quotient diagram of QIgG versus Q_{Alb}, shows that the selectivity of the barrier function is already the same as for the adults. The ratio of the IgG quotient to the albumin quotient with age related change of CSF flow rate follows the hyperbolic reference range for a blood-derived fraction in CSF.
The high albumin quotients at time of birth are a consequence of the low CSF flow rate due to the immature arachnoid villi. After maturation (4th month) the CSF flow rate is maximal, subsequent we get the lowest protein concentration in CSF. Later, in life of the mature adult, an age-related decrease of CSF production rate in the choroid plexus reduces CSF flow rate, and subsequently the value of Q_{Alb} becomes higher.

ulator of blood-derived protein concentrations in CSF in normal and in particular for pathological conditions of neurological diseases. So the change of the albumin concentration (used in its normalized form, the albumin CSF/serum quotient, Q_{Alb}) in particular represents a nonlinear reciprocal measure of changing CSF flow rate, Δr (def. in Fig. 4) with $\Delta r \sim \Delta\,(Q_{\mathrm{Alb}})^{-0.5}$. An application of this particular function is shown for the relation of ascorbic acid in CSF and blood [29].

For normal CSF flow rate we have to consider the age-related changes visualized by changes of the albumin CSF/serum quotient in Fig. 2. Around birth, before maturation of arachnoid villi, the subsequently slow CSF turnover allows high concentrations of the blood-derived proteins in CSF, i.e. a large albumin quotient. About four months after birth, with maturation of arachnoid villi, a maximal CSF flow rate with a minimum of CSF albumin concentration is observed (Fig. 2). After this time, again a slow increase in the concentration of blood-derived proteins in CSF takes place, now due to an increasing length of the spinal canal with the juvenile development and much later by a decreasing CSF production rate in the elderly people [28]. As shown in the quotient diagram in Fig. 2 (right side) the IgG concentration in CSF changes together with the albumin concentration in a hyperbolic relation.

The pathological decrease of CSF flow rate [10], i.e. the blood-CSF barrier dysfunction as reported in many neurological diseases [11] with an up to 100-fold increase of protein concentrations in CSF, can have different causes: like a reduced passage of CSF through the arachnoid villi in inflammatory diseases or a blockade of the subarachnoid space by a tumor or a disc prolaps etc. [10].

2.5. Barriers between blood, brain and CSF

The restricted exchange of material between blood and the perivascular, extracellular fluid (ECF) is manifested in terms of "barriers" – blood-brain barrier, blood-testicular barrier, blood-retinal barrier, etc. The site of exchange between blood and adjacent tissue fluids (Fig. 4) is undoubtedly the capillary, the small non-muscular channel, interposed between arteriole and venule, i.e. blood/tissue barriers represent the general aspects of vascular permeability.

Brain capillaries show a wide range of morphological variations (see below), but differ from capillaries in other organs. In addition to the very different structures of the capillary cell wall in CNS, several particular structural elements have to be considered in brain, like basal membrane with pericytes, connective tissue space and perivascular glial sheath [23].

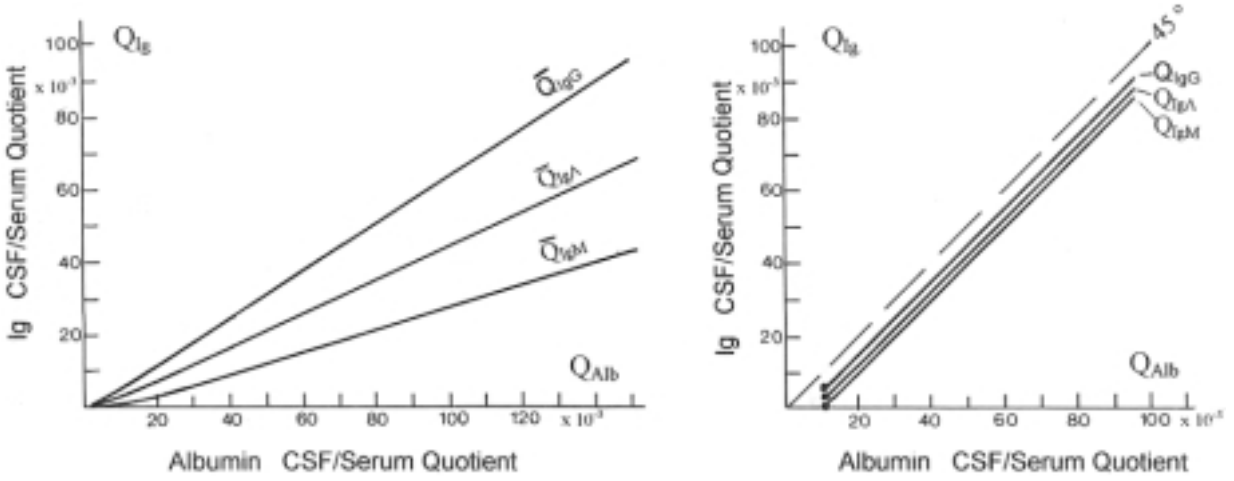

Fig. 3. Change of immunoglobulin concentration in CSF as a function of increasing albumin quotient (blood-CSF barrier dysfunction)- a comparison.
The left diagram shows the means of the empirical data of immunoglobulin CSF/serum quotients [10] with increasing albumin quotient characterized by a hyperbolic function. For extreme values see also Table 2. The diagram on the right side represents an experimental approach, by which the serum of a patient is successively titrated into the CSF sample. The empirically measured data follow a 45° line. There is no molecular size dependent discrimination as seen from the parallel lines of QIgG, QIgA and QIgM. This discrepancy to the empirical data (left side) presents an argument against a "leakage" phenomenon, in which the molecular size-dependent discrimination (selectivity) would be lost.

The passage of dye-molecules from blood into brain extracellular fluid is timely delayed, i.e. restricted, compared to the fast stain of extravascular, i.e.intercellular fluid of other tissues. In the original experiments by Ehrlich (Ref. in [24]), when the animal was injected intravenously with Evans blue dye (which is bound principally to albumin), the entire animal turned blue with the exception of the brain which remained white. This led to the idea that certain molecules do not pass the blood-brain barrier. It is meanwhile clear that all molecules as well as whole cells [30] can pass from blood into brain. The extent of protein transfer depends on the molecular size-dependent diffusion [31]. Larger molecules have steeper overall concentration gradients between blood and CSF and a larger time delay for arrival in CSF (Fig. 5). In particular, for the relevant interpretation of pathological processes which involve "barrier dysfunctions", it is important to understand this dynamical nature of the barriers, not merely based on morphological changes.

In general terms, a barrier function allows to sustain a biological systems individuality, i.e. it permits single compartments (cell, organ, circulation system) to maintain an inner state different from its environment (e.g. extracellular fluid or blood). The barrier function maintains concentration gradients against the dissipative forces which would otherwise lead the system into a chemical equilibrium (and death). But the steady state of a gradient is not only the consequence of the thickness or tightness of structures which restrict the molecular diffusion, the actual gradient depends crucially on the connected consumption- or elimination-reactions, e.g., without the CSF flow (Fig. 4) the concentrations of the serum proteins would equilibrate on both sides of the barrier after some time and the gradient would disappear (as observed shortly after death in the corpse).

The non-linear nature of these relations in such dynamic systems were first described as reaction-diffusion equations by Turing [32]. The molecular flux/CSF flow theory [10] represents a particular application of this concept to describe the non-linear dynamics of the blood-CSF barrier function (Fig. 5).

The particular blood-CSF barrier function for proteins includes a manifold of diffusion pathways. Only a fraction of them is directly connected with the capillary wall and associated structures, called *blood-brain barrier*. A protein, like albumin, originating from blood can enter CSF in the ventricles, cisterns or lumbar and cortical subarachnoid space. Blood-derived proteins can have passed very different structures at different locations [23] when they finally appear in lumbar CSF:

1. the choroid plexus with capillaries of the fenestrated type and tight epithelial lining
2. the ventricular surface with a less tight ependymal layer (gap junctions) and correspondingly less re-

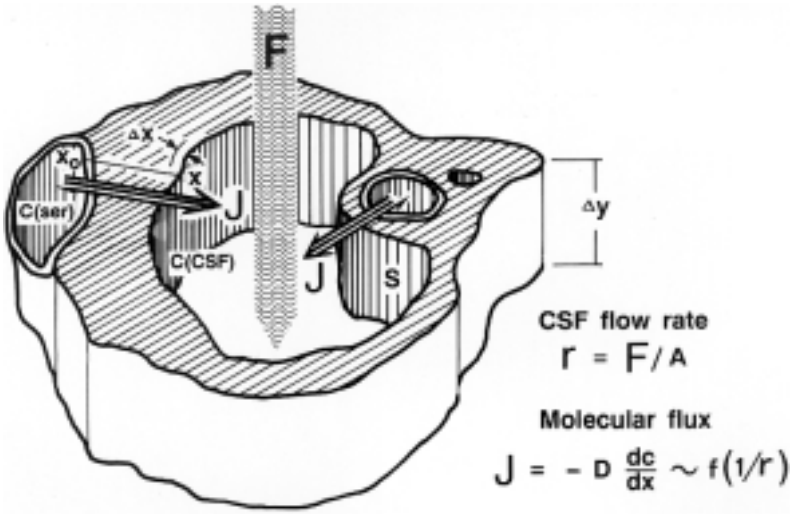

Fig. 4. Scheme of a cross section through subarachnoid space with two blood vessels [10]. Molecules diffuse from serum with a concentration c(ser), through tissue along the diffusion path, x, into subarachnoid space with a concentration c(CSF). The rate of transfer (n moles/s) of diffusing substance through unit area (1 cm^2) of a section is proportional to the concentration gradient measured normal to the section. This molecular flux, J, at the border to the subarachnoid space, is (Fick's 1st law): J = -D dc/dx [nmoles/s · cm^2]. D is the diffusion coefficient. The concentration gradient dc/dx is negative as its slope is negative in the direction of molecular flux.
The age related CSF volume turnover rate is indicated by F(F = 500 mL/day for the adult human). The CSF concentration of a single blood-derived molecule increases with decreasing volume exchange, i.e. decreasing F. The flow rate of a molecule in CSF is $r = F/A$ with A, the unknown, varying cross section of subarachnoid space.

strictions to extracellular fluid (ECF) to communicate with CSF.
3. the circumventricular organs with fenestrated capillaries but a tight ependymal cell lineage
4. the caudal subarachnoid space with tight capillaries in the pia mater and arachnoid mater (leptomeninges).
5. Less tight cell lineage at the inner border to subarachnoid space allows a less restricted exchange between extracellular fluid (ECF) from brain parenchyma into CSF.
6. Along the whole subarachnoid space ECF easily can communicate with the CSF space through the glia/pia epithelial lining with gap junctions.
7. The whole subarachnoid space represents a net of capillaries, most of which have tight junctions.

With the albumin CSF/serum quotient, Q_{Alb}, in a single person, we get a measure integrating all influences on its way between blood and lumbar CSF representative for all blood-derived proteins. In particular, for pathological changes, the albumin quotient, Q_{Alb}, became the most relevant parameter to indicate a change in blood-CSF barrier function (i.e. now a reduction of CSF flow rate). This quality of Q_{Alb} led very early (see introduction) to the development of quotient diagrams (like in Fig. 2, right diagram), in which the immunoglobulin quotients are referred to the albumin quotient.

For most sensitive detection of a brain-derived fraction besides the blood-derived fraction of immunoglobulins [11], it was necessary to characterize a discrimination line in the quotient diagrams between these both fractions [10]. Different discrimination functions caused discussions over decades (Ref. in [10]). The derivation of the biophysically derived, physiologically confirmed hyperbolic discrimination function [10] is described below. The clinical relevance of a linear vs the hyperbolic function for CSF data evaluation is compared in [11].

2.6. The "leakage" metaphor

The increase of protein concentration in CSF was and still is frequently interpreted as a "leakage" or "barrier breakdown". There is a tremendous number of references which report on the properties of the blood-brain barrier [21] frequently referring to the pathological change at the intercellular connections, e.g. tight junctions [20]. The most frequent flaw regarding the blood-CSF barrier function for proteins is the asumption that the pathomechanisms at the capillary wall are relevant to explain the observed protein dynamics in CSF. The following list summarizes the arguments

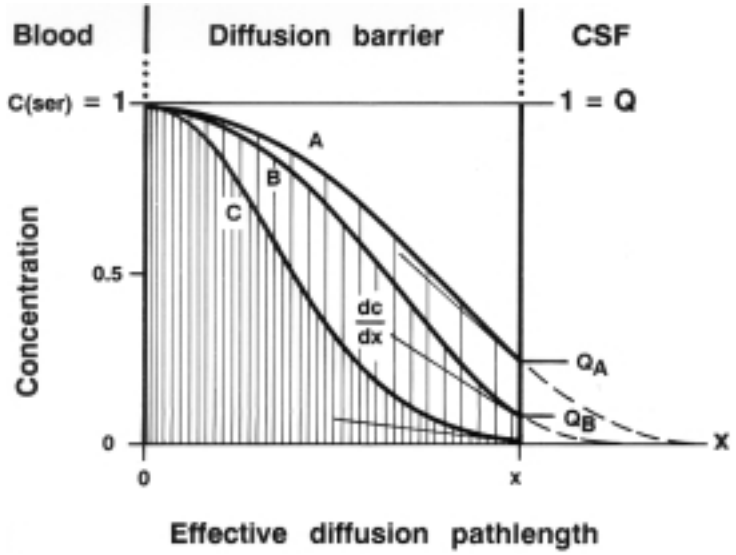

Fig. 5. Idealized protein concentration gradient between blood and CSF [10].
The diffusion controlled protein transfer between blood and CSF is described by an idealized diffusion barrier (homogeneous and unique instead of multistructural, inhomogeneous diffusionpath). The change of concentration c_i (in blood as c(ser), normalized for c(ser) = 1, and in CSF as Q = c(CSF)/c(ser)), along the effective diffusion pathlength, x (x_p, the value at the border to subarachnoid space for the single person), shows the sigmoidal curve, derived from Fick's second law of diffusion. These curves represent steady states for three protein species of different molecular size, like albumin (A), IgG (B) and IgM (C). The smaller molecules A has a larger "penetration depth", i.e. higher mean concentration, in the tissue than the larger molecules B, and a steeper local gradient corresponding to a higher CSF concentration, Q_A.

which give evidence for the molecular flux/CSF flow theory of the blood-CSF barrier function:

1. Figure 3 shows the most striking experiment indicating the discrepancy between the empirical blood-CSF barrier dysfunction and the idea of a "leakage" due to a "hole" or increasing pores, i.e. a massive extravasation of blood proteins in the absence of a molecular size-dependent transfer: On the left side of Fig. 3 the empirical mean relation between two molecules of different size, e.g. IgG and albumin, follows a hyperbolic function over a wide range of increasing albumin concentrations (decreasing flow rate) and the empirical relation between the mean concentration of IgG, IgA and IgM shows that the molecular size-related selectivity of the barrier function is maintained in spite of serious blood-CSF barrier dysfunction. The Table 2 gives a collection of single cases with CSF albumin quotients up to 73% of the serum concentration, but still with a molecular size-related selectivity. The alternative, hypothetical model of a "leakage" is shown on the right side of Fig. 3. If small volumes of the blood of a patient are successively titrated into its CSF sample, we get a linear increase (45°) of all proteins with a loss of molecular size-related selectivity.

Table 2
Data sets of albumin- and immunoglobulin CSF/serum concentration quotients from patients with an extreme blood-CSF barrier dysfunction without an intrathecal humoral immune response

Disease	Q_{Alb} ($\cdot 10^3$)	Q_{IgG} ($\cdot 10^3$)	Q_{IgA} ($\cdot 10^3$)	Q_{IgM} ($\cdot 10^3$)
Spinal tumor	**172**	93	67	15
Spinal cyst	**344**	182	96	26
Medullablastoma	**627**	425	347	161
Bacterial meningitis*	**731**	646	466	352

*Early phase before onset of humoral immune response.

2. The dynamics of the initial increase of protein concentrations in CSF of patients with a bacterial meningitis (Table 3) clearly indicate the unchanged molecular size-dependent selectivity in spite of the barrier dysfunction. The smaller molecules reach faster the steady state than the larger molecules [10]: Albumin reaches at the first day after start of clinical symptoms 50%, IgG 20%, IgA 10% and IgM 5%, respectively, of the concentrations at the second day. According to the leakage model (Fig. 3, right side) the IgM concentration should increase relatively faster then albumin between normal and the first day which is in contrast to the empirical data in Table 3.

Table 3
Dynamics of the blood-CSF barrier dysfunction in case of a bacterial meningitis at time after onset of symptoms, before onset of an intrathecal humoral immune response. The change of the CSF/serum concentration quotients, $Q_{\mathrm{Alb}}, Q_{\mathrm{IgG}}, Q_{\mathrm{IgA}}, Q_{\mathrm{IgM}}$ represent molecular size-dependent selectivity

	Q_{Alb} $(\cdot 10^3)$	Q_{IgG} $(\cdot 10^3)$	Q_{IgA} $(\cdot 10^3)$	Q_{IgM} $(\cdot 10^3)$	Cell count
Normal*	5	2.3	1.3	0.3	2/μL
1st day	146	42	22	5	872/μL
2nd day	311	203	184	105	154000/μL

*Mean values of normal controls.

3. The biological coefficient of variation of a blood-derived protein in CSF in a group with severly increased albumin quotient, Q_{Alb}, is the same as in a group with a normal albumin quotient [10].
4. The biological coefficient of variation of a blood-derived protein in CSF does not approach the value of the biological CV in the blood of the same group of patients ($n = 4300$) in spite of the most severe barrier dysfunction with CSF protein concentrations approaching the concentration in serum [10].
5. Increased albumin quotients in multiple sclerosis patients correlate with a spinal involvement, but most of the patients which have an increased gadolinium extravasation (shown in MRT) have a normal blood-CSF barrier function.
6. In addition to the manifold of brain capillary structures(above) one has to keep in mind that only a very restricted volume of the brain tissue, next to the CSF space like the circumventricular organs, drains into CSF.
7. The relation between the dynamics of the IgG to albumin quotients in the newborn (Fig. 2 right side) indicates that the barrier selectivity is already at time of birth the same as in the mature adult- but due to the slow CSF flow rate the total protein concentration is high. So the earlier asumption that the high total protein in CSF of the newborn is due to an immature "barrier" is definitively wrong as we also know from investigations of animals (Ref in [10]).
8. The age-related dynamics of the leptomeningeal protein cystatin C in the normal CSF of the newborn [33] corresponds to the dynamics of the albumin concentration (Fig. 2, left side). This can be explained by the change of the CSF flow rate – a common determinant for both molecules- but one from brain cells entering via extracellular fluid and the other passing the normal blood-brain barrier.
9. Along the spinal canal with the dorsal root ganglion an increase in the flux of protein molecules has been reported as a significant contribution of these 60 dorsal roots to the elevation of total protein concentration. But this has not a morphological cause, it comes from the steeper local concentration gradient (see biophysics).
10. In the CSF of patients with a blood-CSF barrier dysfunction the dynamics of the brain-derived like the blood-derived proteins can be quantitatively explained by changing CSF flow rate.

These facts give strong evidence for CSF flow rate as the main modulator of the protein dynamics in the normal and pathological blood-CSF barrier function. In the research for the pathomechanisms connected with a changing molecular transfer through barriers, we have to consider associated biochemical, physiological or metabolic causes for a change in the gradient, but not necessarily a change in morphological structures (for detailled derivation see biophysics paragraph).

2.7. Transfer mechanisms at barriers and selectivity

The time required for a molecule to pass through certain structures (cell membrane or tissue with cell layers) varies between seconds to hours and does not only follow the rules of free diffusion (down-hill) but is also observed "up-hill", i.e. against a concentration gradient leading to accumulation of products by energy-dependent reactions (active transport) or also by transporter facilitated mechanisms. In this sense the blood-CSF barrier function for proteins (passive diffusion, i.e. molecular flux) is different from that for aminoacids (carrier facilitated), glucose or vitamin C (active transport). So, for different molecules we have to discriminate different types of "barriers", regarding their different modes of transfer.

Selectivity for transport across a barrier comes for proteins from their molecular size-dependent delay of diffusion but for other molecules selectivity can come from the specific recognition by their particular transporters and different strength of binding. Only transport by pinocytosis is lacking selectivity for different molecules.

3. Biophysics of diffusion and flow- the molecular flux/ CSF flow theory

Blood-derived proteins enter CSF space passive by diffusion along the concentration gradients. Diffusion

processes are described by Fick's first and second law of diffusion. The second law with a differential equation of second order has no explicit, general solution. This is the reason why the history of physics of diffusion is characterized by the invention of implicit solutions for particular physical conditions, i.e. for particular models.

The model of Reiber (1994) [10] for the blood-CSF barrier function (Figs 4–6) describes a diffusion system where on one side of a restricted range (tissue) we have a quasi infinite source of solutes (blood) and at the other side a solvent (CSF), which, most relevant, is the source of a steady elimination process by the flowing CSF.

Figure 4 shows these both determinants in a schematic cross section of the subarachnoid space with blood vessels.

The particular target for a mathematical treatment of this model was to describe the changing relation of CSF concentrations of two blood derived molecule species with different molecular size, in the case of a changing elimination rate (CSF flow rate). For this purpose we have to regard: 1) the non-linear concentration gradient (Fig. 5); 2) the relevance of the local concentration gradient for the diffusion rate (molecular flux into CSF (Fick's 1st law, $J = -D_i\ dc_i/dx$); and 3) the change of CSF concentration as source of the time dependent change in the local gradient (Fick's 2nd law, $dc/dt = D(d^2c/dx^2)$).

The basic laws of diffusion, their application to the model of blood CSF barrier function and the derivation of the hyperbolic function with the molecular flux/CSF flow theory are described in detail [10]. For the extension of the application of the theory to integrate the brain-derived proteins, I will outline the base of the theory.

The concentration gradient of a protein between blood vessel and CSF (model in Fig. 4) is non-linear as shown in Fig. 5 for an idealized homogeneous medium. According to Fick's 1st law the molecular flux into CSF is determined by the local concentration gradient, $\Delta c/\Delta x$, as shown in the cross section through subarachnoid space (Fig. 4), or more correct as the differential, dc/dx, the slope of the tangent of the non-linear concentration gradient at the border to subarachnoid space (Fig. 5).

As molecules in a diffusion process are not coupled to each other, it is always the local gradient which is relevant for the local molecular flux, not the fictive, calculated overall gradient (Fig. 6, right diagram). But the local gradient depends on the overall gradient in a non-linear way (Fig. 5). This non-linear gradient is a part of the experience each biochemist can have by watching column chromatography: a sharp dye band becomes broader and broader with increasing time of diffusion and by densitometry one can show a concentration distribution like a Gaussian error curve. The same physical base leads to the curves in Fig. 5. These curves represent steady states for three protein species of different molecular size,idealized as albumin (A), IgG (B) and IgM (C). The smaller molecules A have a larger "penetration depth", i.e. higher mean concentration, in the tissue than the larger molecules B (Fig. 5), a steeper local gradient at the border to CSF space and a higher CSF concentration, QA than QB or QC. The differences are physically characterized by the diffusion coefficient, D, used in the equation of Fick's 1st law (legend of Fig. 4). The diffusion coefficient D_i of a molecule i, represents a value which depends on its molecular size and on the properties of the solvent (e.g. viscosity) in which the molecule (solute) is solved. The variation of the value of D_i along a diffusion path through tissue with different viscosities can be neglected in our task as this change is relevant for all molecules (A, B, C) in a corresponding way.

Fick's 1st law for molecular flux is a linear expression of a local concentration gradient. Now under pathological conditions of a neurological disease, where the CSF protein concentration is increased, subsequently the local gradient changes as a function of concentration or time. Exactly this is described by Fick's second law, a non-linear function, shown in the equation: $dc/dt = D(d^2c/dx^2)$.

This means that in case of reduced CSF flow rate a molecule C in Fig. 6 (e.g. IgM), has a primarily increasing concentration in CSF from Q_C to Q_D to Q_E with a subsequent increase in penetration depth, i.e. increasing mean protein concentration in the tissue (Fig. 6). With this increase of CSF concentration, also the local molecular flux $J \approx dc_i/dx$ at the border to CSF space changes: the local gradient dc/dx is increasing!, i.e. with primarily increasing CSF protein concentration there is also secondarily a faster molecular flux, additionally increasing the CSF protein concentration. Correspondingly, also the concentration ratio between two molecules of different size, like Q_A to Q_B in Fig. 5, would change. This change of the gradient has now to be described by Fick's second law of diffusion.

By a mathematical procedure, based on introduction of a geometrical series, called error function complement, erfc [10], I could derive a function which contained only the ratios of diffusion coefficients. This function in Eq. (1) describes the changing concentra-

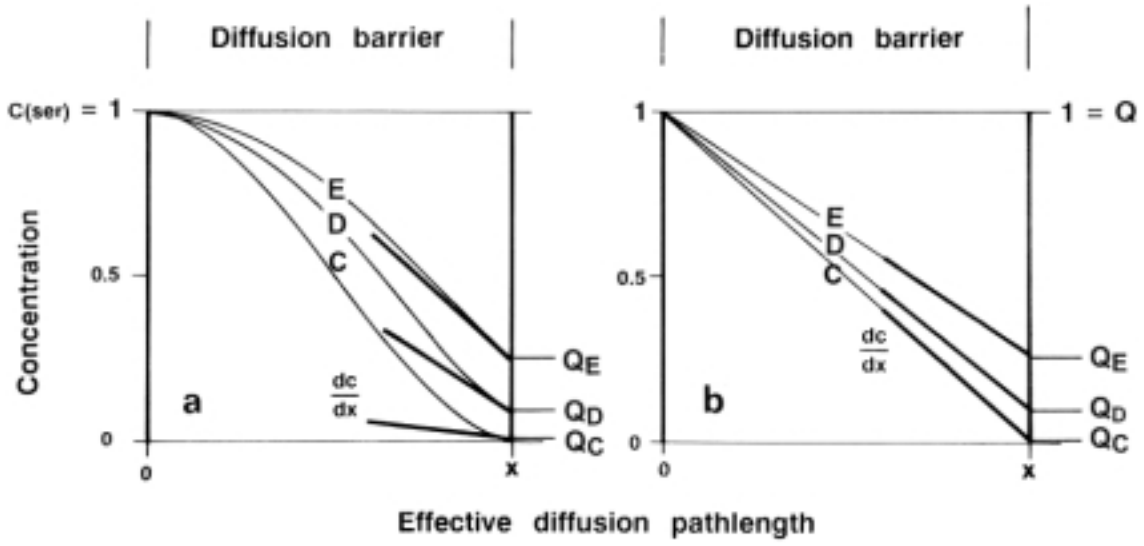

Fig. 6. Dynamics of blood-derived proteins between blood and CSF after pathological reduction of CSF flow rate.
Comparison of non-linear (left side) and linear models of blood CSF protein concentration gradients. The blood protein concentration of a single protein is normalized with maximal values C(ser) = 1. Corresponding CSF protein concentrations are given as dimensionless CSF/serum quotients (Q_C, Q_D, Q_E) with values between 0 and 1. x, represents the effective diffusion pathlength of the idealized barrier according to the boundary conditions described in [10]. C, D, E represent the curves for the concentration distribution of the same protein (e.g. IgM) at different times: before (to, curve C) and after (t_1, t_2, curve D and E) the onset of a disease with decreasing CSF flow rate and, subsequently, increasing concentration of IgM in CSF. The local concentration gradient, dc/dx, at the border to subarachnoid space (at x) and correspondingly the molecular flux, J, increase in the non-linear model (left side) from C to E, but decrease in the linear model (right side) from C to E. In this case the decreasing fictive, calculated overall concentration gradient would be indentical with the local gradient dc/dx, but decreasing from C to E in contrast to the case in the left diagramm.

tion ratio of the two molecules, e.g. A and B in CSF in Fig. 5, if the CSF flow rate changes (i.e. with increasing Q_A):

$$Q_B = (erfc\, z\, (D_B/D_A)^{0.5}/erfc\, z) \cdot Q_A \quad (1)$$

I could also show that this function in Eq. (1) is a hyperbolic function [10], i.e. the corresponding concentration values of Q_B and Q_A fit the well known, explicit form of the hyperbolic function [9]:

$$Q_B = a/b \cdot (Q_A^2 + b^2)^{0.5} - c \quad (2)$$

This means that the concentration ratio of molecules of different size diffusing through the same matter (e.g. plane sheet, slab) is generally characterized by a hyperbolic function. The shape of this hyperbolic curve is determined only by the ratio of the square roots of both diffusion coefficients, in Eq. (1).

It is important to understand that this hyperbolic function is valid for the normal as well as for the pathological range of albumin quotients, i.e. still under pathological conditions the change of protein concentrations in CSF follows the rules of diffusion with a molecular size-dependent discrimination (Fig. 3, left diagram).

The values of the actual parameters a, b, c of this function in Eq. (2) for IgG, IgA or IgM are given in [10] with calculation examples in [11,12].

3.1. Physical, selfreferential feed back

Figure 6 shows the time dependent change with decreasing CSF flow rate, the subsequent increase of protein concentration in CSF due to reduced volume turnover and a subsequent increase in the steady state concentration in the tissue: the sigmoidal curve for the concentration of molecule C at time t_c, changes into a curve D at time t_d and to E at time t_e. The particular consequence of this increase of the concentration in the tissue between blood and CSF in our model is the change at the border to the CSF space: the local gradient is increasing (increasing slope of tangents in Fig. 6, left side), in spite of the decreasing overall concentration ratio between blood and CSF (Fig. 6, right side). The slope of the tangents is non-linearly increasing as long as $C_{CSF} < C_{ser}/2$ or $Q < 0.5$ but decreases for the rare case of quotients $Q > 0.5$ ($> 500 \cdot 10^{-3}$).

As shown in Fig. 6, right side, for a wrong, linear model the local concentration gradient would de-

crease with increasing CSF concentration and correspondingly the local molecular flux into CSF. In this sense of a negative feed back the barrier dysfunction would be a selflimiting event. This is in contradiction to observations, like the faster appearance of a blood-derived molecule in lumbar, before ventricular CSF (the higher lumbar CSF concentration corresponds to a steeper local gradient).

Figure 6 describes a difference between former linear barrier models [35] and the actual molecular flux/CSF flow theory, which is aware of the physical, positive feed back of increasing CSF concentration on the local concentration gradient, inducing a faster molecular flux with increasing CSF concentration. This selfreferential system (analog to autocatalytic processes in chemistry) is the cause of the observed non-linear relation, the hyperbolic function (e.g. in Fig. 3, left side). This is the main cause of increased molecular passage from blood into tissue and CSF- with or without any change at the local "microscopic" conditions of capillary walls: The transfer of molecules from blood into CSF can become faster just by reduced CSF flow rate without any change of morphological structures!

Figure 7 shows that this theory fits for all blood-derived proteins, like prothrombin or carcinoembryonic antigen in addition to the well known immunoglobulins. The only difference between the curves comes from their different molecular size as to be expected from Eq. (1).

3.2. CSF flow and the rostro-caudal gradient

The concentrations of blood-derived proteins in CSF increase between ventricles and lumbar subarachnoid space (normal CSF flow rate) e.g. 2.5-fold for albumin (Table 1), due to the steady molecular flux of the proteins into CSF along its flow way As a consequence of the concentration-dependent positive feed back (Fick's 2nd law), the local concentration gradients must increase along the rostro caudal concentration gradient, too, i.e. the molecular flux into CSF increases non-linearly with increasing distance from the ventricles. This is consistent with the report that labeled proteins from blood appear first in lumbar CSF and later in cisternal or ventricular CSF (Ref. in [10]). A corresponding set of albumin concentration values from a series of CSF samples extracted by lumbar puncture in serial fractions should show this non-linear increase to some extent [34].

4. Dynamics of brain-derived proteins in CSF

Table 1 and Figs 7 and 8 describe the main differences between brain-derived proteins and blood-derived proteins. The differences are best characterised by the two concentration gradients: the CSF/blood concentration ratio (known as CSF/serum quotients) and the ventricular/lumbar CSF concentration gradient (rostro-caudal gradient).

We find large *CSF/serum concentration ratios* between 1:1 (NSE) and 34:1 (β-trace protein) compared to 1:205 (albumin) or 1:3400 (IgM) for blood-derived proteins in CSF. The calculated brain-derived intrathecal fraction (IF) is $>$ 99% for tau protein, NSE, S-100B, Cystatin C or β-trace protein, but 0% for the proteins which, under normal conditions, are exclusively blood-derived. Brain-derived proteins with a non-negligible blood-derived fraction in CSF, like transthyretin, angiotensin converting enzyme [19] or soluble intracellular cell adhesion molecule (s-ICAM) have intermediate CSF: serum ratios (1:18 to 1:190) with intrathecal fractions between about 90% to 30%, correspondingly (Table 1).

The *ventricular to lumbar CSF concentration gradients* of brain-derived proteins show source-related differences.Proteins which are released from brain cells (NSE, S-100B and tau protein) have the highest concentration in ventricular CSF steadily decreasing along the CSF flow path through the subarachnoid space (Table 1). Vice versa, proteins released from leptomeninges into CSF are increasing in concentration along the subarachnoid space up to 11-fold (β-trace protein). This is higher than the rostro-caudal 2.5-fold increase of the albumin concentration. The gradient for transthyretin in Table 1 refers to the choroid plexus-derived fraction in CSF calculated from total transthyretin in CSF by subtraction of the blood-derived fraction [19]. Already the concentration of total transthyretin is decreasing slightly between ventricular and lumbar CSF, in spite of the small but steady flux of blood-derived molecules into CSF, as consequence of the overall blood/CSF gradient shown in Table 1.

With these data in Table 1 we are able to discriminate three different groups according to their source, with different consequences for the dynamics (Fig. 8):

1) proteins originating from neurons and glial cells, like tau protein, S-100 and neuronspecific enolase, which are primarily released into ventricular and cisternal CSF,

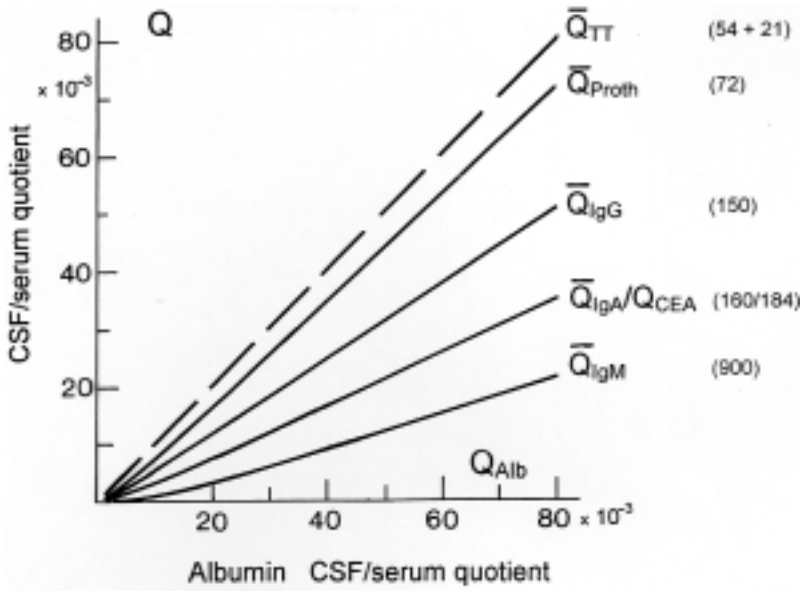

Fig. 7. Blood-derived proteins and blood-CSF barrier dysfunction [12].
The hyperbolic curves show the mean CSF/serum quotients, $\overline{Q}$, of blood-derived proteins with different molecular size as a function of decreasing CSF flow rate. The decreasing flow rate or increasing blood-CSF barrier dysfunction is indicated by the increasing albumin CSF/serum quotient, Q_{Alb}. The theoretical, calculated blood-derived fraction of transthyretin (TT) is shown by the dashed line with 45° (TT passes the barrier associated with the retinol binding protein [19] and has therefore a size similar to albumin). Empirical mean quotients for prothrombin (Proth.), carcinoembryonic antigen (CEA) (Reiber, unpublished) and immunoglobulins IgG, IgA, IgM [10] are shown together with their molecular weights (kDa).
Hyperbolic functions are a consequence of non-linear interaction of molecular flux with CSF flow rate as derived from the laws of diffusion [10] with the general equation: $Q_{\mathrm{IgG}} = (erfc\ z\ (D_{\mathrm{IgG}} : D_{\mathrm{Alb}})^{0.5}/erfc\ z)\ \cdot Q_{\mathrm{Alb}}$. This function shows that the relation depends only on the ratio of diffusion coefficients ($D_{\mathrm{IgG}} : D_{\mathrm{Alb}}$), i.e. on the different size of the molecules. The hyperbolic functions for the single proteins are characterized empirically by three variables (a, b, c) in a more usual form of the hyperbolic function, $Q_{\mathrm{IgG}} = a/b((Q_{\mathrm{Alb}})^2 + b^2)^{0.5} - c$.

2) proteins which are primarily released from leptomeninges into CSF, like β-trace protein and cystatin C and,
3) brain-derived proteins with a non-negligible blood-derived fraction in CSF, like transthyretin, angiotensin converting enzyme [19] or the soluble intercellular adhesion molecule (s-ICAM).

For these three groups of brain-derived proteins the theoretical concept, regarding diffusion and CSF flow, offers a quantitative description of the empirically observed dynamics.

The following summary articulates the differences in the three different groups of brain-derived proteins.

1) *CSF proteins from brain cells* which enter CSF primarily in the ventricular and cisternal spaces:

a) their concentration is decreasing between normal ventricular and lumbar CSF (Table 1) in contrast to the (non-linearly) increasing concentrations of blood-derived proteins along the rostro-caudal flow way.
b) their concentration does not vary with pathologically decreasing CSF flow rate, i.e. in cases of a so-called blood CSF barrier dysfunction for blood-derived proteins (Fig. 8).

The first observation, the decreasing rostro-caudal gradient of neuronal and glial proteins, is easily explained by the inversion of the tissue to CSF concentration gradient dc/dx between the ventricles and the cisternal to lumbar CSF spaces. Tau protein, NSE or S-100B, due to their neuronal or glial source, must have higher concentrations in the tissue around the ventricular and cisternal CSF space than around the lumbar space in the leptomeninges. With only a small local release of neuronal or glial proteins, the outside/in gradient in the range of ventricles is inverted to an inside/out gradient in lumbar CSF where the concentration in CSF is larger than in the surrounding tissue (leptomeninges).

The second observation, that protein concentrations in lumbar CSF do not change with decreasing CSF flow rate, i.e. increasing Q_{Alb} (Fig. 8), again can be explained by the laws of diffusion and by changing CSF flow rate. With decreasing CSF flow rate, i.e. reduced CSF volume turnover, the ventricular CSF concentration of the brain cell-derived proteins is increasing but

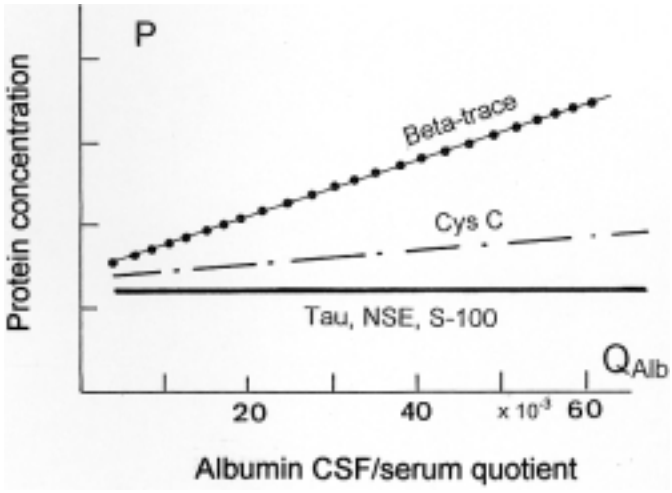

Fig. 8. Dynamics of brain-derived proteins in lumbar CSF.
The relative protein concentrations are shown as a function of the albumin CSF/serum concentration quotient, Q_{Alb}, increasing with blood CSF barrier dysfunction, i.e. decreased CSF flow rate. The mean slopes for the predominantly leptomeningeal proteins β-trace and cystatin C are derived from the data in [19]. The lumbar concentration of glial and neuronal proteins which enter CSF in the ventricular and cisternal space, e.g. tau protein, neurospecific enolase and S-100 protein, are invariantly constant in cases of blood-CSF barrier dysfunctions, i.e reduced CSF flow rate [19].These dynamics of the brain derived proteins are derived from the laws of diffusion and CSF flow as shown in [19].

with increased ventricular CSF concentration there is downstream also an increased concentration gradient for a larger molecular flux inside/out. Both effects which can be described by the linear Fick's 1st law compensate each other quantitatively, what keeps the lumbar CSF concentration constant.

At this point we hit the crucial difference between blood-derived (Fig. 7) and brain-derived (Fig. 8) protein dynamics in CSF in case of pathologically decreasing flow rate: the increasing CSF concentration in case of brain-derived proteins does not have a positive feed back on the local gradient as blood derived proteins do. In the first case we have a linear change (explained more detailled below for β-trace protein in Fig. 8), in the other case we have the hyperbolic relation (Fig. 7). Only in case of a linear relation, described by Fick's 1st law, we have this possibility for compensation described above for the concentration changes along the rostro caudal flow way.

The observed empirical data [19] for NSE, S-100, tau protein or choroid plexus-derived transthyretin fraction (summary in Fig. 8) are quantitatively explained by the theoretical model in which CSF flow rate represents the main modulator of CSF protein concentrations in cases of neurological diseases with a blood-CSF barrier dysfunction.

2) *CSF proteins from leptomeningeal cells*, like β-trace protein or cystatin C, show:

a) An increasing concentration between normal ventricular and lumbar CSF (similar to blood-derived proteins).
b) A linearly increasing concentration in CSF in case of pathologically decreasing CSF flow rate (Fig. 8) in contrast to a hyperbolic increase in blood derived proteins.
c) A particular modification is observed in inflammatory meningeal processes [19].

a) The normal mean β-trace protein concentration is increasing from 1.5 mg/L in the ventricles to 16.6 mg/L in the lumbar subarachnoid space (Table 1 and [19]). Obviously the β-trace protein concentration is much higher in the leptomeninges than in the periventricular tissue, i.e., there is no inversion of the gradient dc/dx downstream, which is different from the first group of brain-derived proteins.

This 11-fold rostro-caudal increase of β-trace protein and a 3.5-fold increase of cystatin C concentrations (Table 1) under normal conditions are easily understood: the steady release of proteins into CSF along its flow path, is due to a local outside/in concentration gradient at the border of the subarachnoid space.

b) The second observation, the linear increase of β-trace protein concentration in lumbar CSF in case of pathologically decreasing CSF flow rate (Fig. 8), is the simple consequence of a steady release of the protein into CSF which, due to a slower volume turnover rate, gains a higher protein concentration per volume.

This is similar to the primary concentration change of blood-derived proteins. But in contrast to the blood-derived proteins with a secondary positive feed back mechanism increasing additionally the molecular flux, this can not happen for the leptomeningeal cell-derived proteins,because it would mean that an increasing CSF concentration of leptomeningeal proteins in ECF would induce an increased release of these proteins from brain cells. Such a positive feed back (like an autocatalytic process) would be an unphysiological destabilization of the steady state of the cell.

c) A negative feed back loop, by which with increasing concentration the expression and release or the kinetic activity of enzymes is inhibited, represents a common mechanism of metabolic control. Such a case seems to be realized by s-ICAM [19], a molecule species of the third group. This absence of a feed back in the cases of brain derived proteins explains why the dynamic of these proteins can be described by Fick's 1st law with a linear relation between molecular flux and concentration gradient.

3) *The third group of proteins with non-negligible blood-derived fractions* in addition to brain-derived fractions like transthyretin, soluble intercellular adhesion molecule (s-ICAM) as well as ACE represent a mixture of different influences. In particular, in this group it is important to decide whether a quotient or absolute concentration of the brain-derived protein is preferred for most sensitive evaluation with reference to the albumin quotient [19]. The saturation like curve observed for s-ICAM was a surprize. As an explanation, with increasing s-ICAM concentration in CSF the stripping of the soluble part from the cell surface could be reduced by a negative feed back control (see above), approaching an equilibrium (saturation function in [19]). The detection of a metabolic control of CSF protein concentration as shown for the first time for s-ICAM [19] would not have been possible without the molecular flux/CSF flow model.

These findings from pathology give further evidence that the molecular flux/CSF flow theory represents a complete conceptual framework for explaining the dynamics of both, the blood- and brain-derived proteins in CSF.

5. Brain-derived proteins in blood

The so called marker proteins which allow to investigate pathological brain processes by blood analysis, like S100 [18] or NSE [15] represent a relevant diagnostic approach. Yet there are no clear demonstrations how and where particular brain- derived proteins, e.g. after a brain hypoxia [15] enter the blood. In principle, the laws of diffusion described above are relevant also for the way out of the brain and the arguments against a leakage model must be relevant, too :All proteins pass a biological barrier, like blood vessel walls, according to the local concentration gradient and the size of the molecule (Table 1).

Quite frequent we are confronted with the question whether a certain brain-derived substance can become a relevant marker molecule to be detected in blood. The answer has to consider the intrathecal concentration (e.g. a main difference between S100 and NSE), the relative concentration change by the pathological process (release from cells, compensated by metabolic consumption or re-uptake into other brain cells), the molecular size and the half life time in blood. Again, proteins or larger peptides are the best candidates for a successful application.

With three examples I want to shed some light on the complexity of this topic:

β-trace protein in blood (mean 0.5 mg/L) originates primarily from CSF flow into venous blood. A 34-fold higher concentration (17 mg/L) in lumbar CSF will be diluted about 1:10 by flow of 500 mL CSF/day into 5 L blood, i.e. a contribution of about 0.07 mg/L/hour. A half life time of guessed 4 hours for β-trace in blood would reduce the concentration in blood by 0.065 mg/L/hour. Under these conditions of such a low half life time, the concentration of this leptomeningeal protein in blood could be maintained just by the CSF flow into blood. For any other protein in the Table1 with much less steep gradients between CSF and blood, the CSF flow could not count solely for the blood concentration. But, what happens with the blood concentration of β trace protein in blood in case of barrier dysfunction with a reduced flow rate but increased CSF concentrations? The next example can shed some light on this topic.

The *Vitamin C concentration in CSF and blood* presents an interesting example to answer the above question: Ascorbic acid (vitamin C) is transferred in the choroid plexus from blood into CSF against a concentration gradient, inducing a 6–12 fold higher ascorbate concentration in CSF. With increasing barrier dysfunction, i.e decreasing CSF flow rate, but unchanged vitamin C concentration in CSF, we observed a decreasing vitamin C concentration in blood [29]. The reported correlation of the blood concentration of ascorbic acid with the CSF flow rate, r, with $\Delta r \sim \Delta l\ (Q_{\mathrm{Alb}})^{-0.5}$

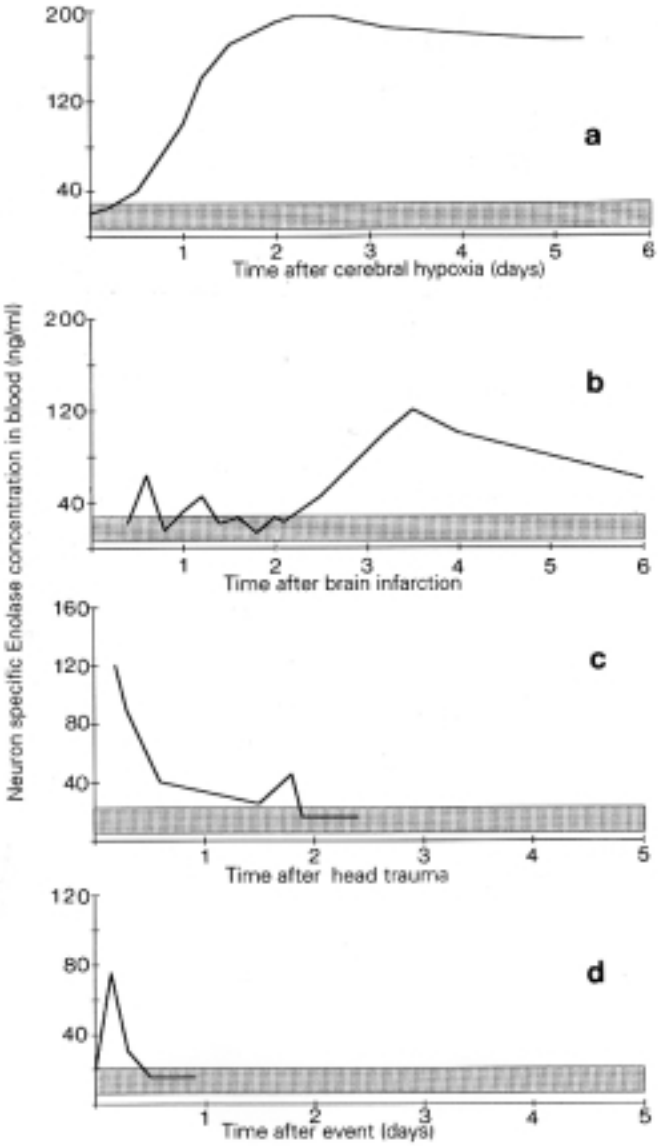

Fig. 9. Neuronspecific enolase (NSE) in blood: Dynamics of release from brain in different neurological diseases.
a) Data from a patient after cardiac arrest hypoxia with a Glasgow-outcome scale score of 1 [15].
b) Time course of NSE concentration in plasma in case of brain infarction and Glasgow-outcome scale score 2 [15]. The late increase of NSE in blood between day 2 and day 3 corresponds with a secondary edema preceeding the clinical symptoms by about a day.
c) Time course of NSE concentration in blood after head trauma. The fast decrease in between 1 day corresponds to a benign clinical outcome.
d) Data from a patient after electroconvulsive therapy. This occasional event is characterized by a fast decrease (in between 2 hours NSE values in blood are normal). Peak values have been observed up to 120 ng/mL. These reactions are seen only in about 10% of the treatment cycles (R. Thomas and H Reiber, unpublished results).

could be a helpful model for evaluation of the corresponding dynamic in blood.

Neuron-specific Enolase (*NSE*), the gamma, gamma dimer of the ubiquiteous distributed glycolytic enzyme enolase, with a molecular weight of 78 kDa originates predominantly from neurons and neuroendocrine cells. The large amount found in the brain, means that NSE can be a very sensitive marker for fast neuronal degradation. Increased concentrations have been detected in CSF for different neurological diseases [36], in particular in Creutzfeldt-Jakob disease [14]. But due to its high brain concentrations it is also easily detected in blood [15] with diagnostic relevance for the prognosis of clinical outcomes after cerebral hypoxia, brain

infarction or head/brain trauma (Fig. 9 and [15]).

With a CSF: serum concentration ratio of $1:1$ (Table 1) the intrathecal (brain-derived) fraction of NSE in CSF can be calculated to be still $> 99\%$ (the blood-derived fraction is smaller than 1% due to an interpolated molecular size-dependent CSF: blood gradient of about $1:200$ for a theoretical, solely blood-derived fraction). The concentration decreases between ventricular and lumbar CSF ($2:1$, Table 1). The mean NSE concentration in lumbar CSF does not change with increasing Q_{Alb} in case of a blood-CSF barrier dysfunction as shown [19], i.e. is invariant to reduced CSF flow rate. Anyway the dynamics in blood (Fig. 9) can not be explained by a CSF related change of blood concentrations by CSF flow into venous blood as the pathological concentrations in CSF [14,36] are not high enough to count for the blood concentration after a $1:10$ dilution (see above). The increased NSE release from brain into blood vessels can be interpreteed on the base of molecular diffusion due to a locally increased brain-blood gradient via an intact barrier. There is no necessity to postulate a barrier dysfunction for the sudden increase of NSE in blood. A certain evidence for this statement is also given from the observations in Fig. 9, that very different causes like hypoxia, hemorrhage, secondary brain edema and electroconvulsive treatment can cause an increased NSE concentration in blood.

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