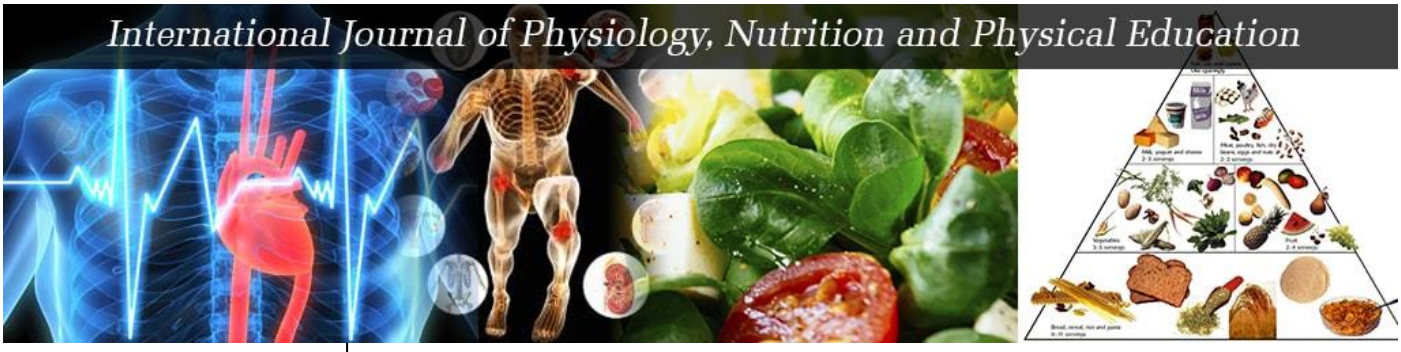

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A comprehensive study regarding a better insight into physiology of fluids and barriers of the brain

Dr. Amitabha Kar and Dr. Partha Majumder

Abstract
This review discusses Fenstermacher's contribution to the field of fluids and barriers of the CNS. Specifically, his fundamental work on diffusion of molecules within the brain extracellular space and the research on properties of the blood–brain barrier in health and disease are described. Fenstermacher's early research on cerebrospinal fluid dynamics and the regulation of cerebral blood flow is also reviewed, followed by the discussion of his more recent work involving the use of magnetic resonance imaging (MRI).

Keywords: Cerebrospinal fluid, brain extracellular space, radiolabelled tracers, MRI contrast agent

Introduction
In this article, we will discuss Fenstermacher's contribution to the field, and also reflect on how his early work and the experimental tools at his disposal allowed him to describe the physiological phenomena. We also show that with the help of modern research technologies, these can now be confirmed with an improved understanding. This review will start with a section written by Charles Nicholson introducing the reader to Fenstermacher's early work on diffusion of radiotracers within the brain extracellular space (ECS) and the assessment of its volume fraction. The next section, written by Adam Chodobski and Jean-François Ghersi-Egea, will focus on early investigations of cerebrospinal fluid (CSF) formation and space, followed by early studies of the regulation of cerebral blood flow (CBF). The final part, written by Tavarekere Nagaraja, will be devoted to the last seventeen years of Fenstermacher's work during which he and his colleagues validated modern technologies such as magnetic resonance imaging (MRI) by concurrently employing previously established radioisotopic techniques.

Review
Ventriculocisternal perfusion applied to the study of diffusion in ECS
The ECS is the totality of the narrow gaps that separate adjacent cells of the brain. In the 1950's and 1960's there was much controversy about this domain. The width of the ECS was thought to be much less than a micrometer; nonetheless, because a tiny atmosphere of ECS surrounds each cell membrane and there are a huge number of cells, the fraction of brain tissue occupied by ECS (volume fraction) could be appreciable. The problem in the early days was to assign a numerical value to the volume fraction and the numbers obtained ranged widely. On one side were the electron microscopists who obtained different, and usually small, values depending on how they euthanized the animals and fixed the tissue, because the width of the ECS is exceedingly sensitive to ischemia and the water content of the cells [1]. On the other side were the physiologists who bathed a volume of tissue in a small radiotracer that was supposed to permeate only the ECS, and then measured how much had equilibrated with the total tissue volume [2]; unfortunately, many tracers used are far from impermeable.
As is often the case when the topic engenders heated discussion, the resolution required a better technique and this duly appeared in the form of ventriculocisternal perfusion with radiolabeled inulin. Rall, Oppelt and Patlak [3] used this method to establish the volume fraction of the ECS in the caudate nucleus of mongrel dogs. They based their results on tissue samples taken at different time points from the first few millimeters from the ventricle.

Because they took samples all the way through the thickness of the caudate and cortex, the in their paper clearly shows that they had established a gradient of concentration. Such a gradient is a characteristic of diffusion and a measurement of the gradient has the potential to yield the effective diffusion coefficient for the substance of choice in the tissue.

After the ventriculocisternal perfusion method became established there was a need to refine it and expand the work. This is where Fenstermacher appears in the publications with this method. In fact, he had made an earlier attempt to measure ECS volume fraction using subarachnoid perfusion but the results gave a low value, which even the authors themselves questioned [5]. The paper by Fenstermacher, Rall, Patlak and Levin [6] is primarily about the application of the ventriculocisternal perfusion method and it further established that sucrose and inulin were good ECS molecules for studying diffusion in the ECS. Levin, Fenstermacher and Patlak [7] used the technique to make extensive measurements of ECS volume fraction in rabbits, cats, dogs and monkeys. They concluded that the volume fraction (α) in the cerebral cortex, averaged across all four species, was 19.7% for sucrose and 18.3% for inulin. It may be noted that the volume fraction values are close to those reported with more recent ion-selective microelectrode measurements that will be described below. These investigators were also able to make a rough estimate of the effective diffusion coefficients for these two substances based on the depth profile of concentration.

By 1975, it was also becoming clear that radiolabeled sucrose was the probe of choice for a molecule that remains predominantly in the ECS (inulin is less reliable). In many practical situations, however, one wants to study molecules that penetrate cells or move across the BBB. One example is molecules that are used for chemotherapy in brain tissue. In one of several studies, Blasberg, Patlak and Fenstermacher [15] applied ventriculocisternal perfusion to the study of the diffusion properties of five different chemotherapeutic drugs in monkey brain and concluded that they differ in their distribution space and capillary permeability thus showing that different application regimens will be required for effective drug application. The distribution space is defined as the space that the molecules actually occupy; depending on the technique used to make the measurement, the volume fraction obtained may exceed unity if molecules are actively accumulated in cells. In 1976, a series of studies with John Kessler expanded the ventriculocisternal perfusion methodology to subarachnoid perfusion in monkey spinal cord, e.g. [16], paving the way for better drug application to this important part of the CNS. The studies on the spinal cord lacked some of the precision seen in the cortex, doubtless because the small cross-section of the spinal cord does not lend itself well to perfusion studies.

The point-source paradigm for ECS diffusion measurements

One of the advantages of ventriculocisternal perfusion with radiotracers is that it may be used with a wide variety of substances subject only to the condition that they can be formulated as a radiotracer. There are significant disadvantages to the method, however. Only one time-point can be obtained per animal and the blocks of tissue harvested to determine concentration must be sufficiently large to achieve an accurate reading, which limits spatial resolution and promotes the use of large brains and consequently, large animals, such as dogs. Only tissue fairly close to the perfusion surface may be analyzed and must broadly conform to a one-dimensional diffusion problem. This also implies that the tissue under analysis must be homogeneous in structure. Finally, radioactive substances must be used with their attendant practical issues.

Some of the disadvantages of the radiotracer method were overcome through the introduction of the 'point-source paradigm' by Charles Nicholson and co-workers. The idea is simply to release a substance from a micropipette and then measure the concentration as a function of time and distance, fit the appropriate solution of the diffusion equation and extract α and λ. The first detailed implementation of this concept was by Nicholson and Phillips [18] where both small cations and anions were employed. The ion of choice was released by iontophoresis from a micropipette located in the brain and the concentration measured as a function of time about 100 μm away using an ion-selective microelectrode. Subsequently, tetramethylammonium (TMA^+) was used almost exclusively as the probe ion and the method became known as the Real Time Iontophoretic (RTI) method or TMA method. The technique was refined to allow for a small amount of loss of TMA^+ [19] and also to permit ions to be delivered by pressure injection from a micropipette (the Real Time Pressure or RTP method).

The RTI method has been used extensively by the Nicholson laboratory in New York with emphasis on the normal brain and by Eva Syková and colleagues in Prague, where many pathophysiological states have been investigated (for a comprehensive review see Syková and Nicholson [20]). The RTI-TMA method has amply confirmed the earlier studies by Fenstermacher and colleagues using sucrose or inulin; however, the RTI method has permitted studies with much higher spatial and temporal resolution so that, for example, anisotropic [22] and inhomogeneous properties [23] could be determined and the behavior of the ECS quantified in ischemic tissue [24, 25].

The main limitation of the RTI and RTP methods is that the sensor is an ion-selective microelectrode so the method is constrained to a few select substances of low molecular weight (MW). It is thus best thought of as a probe of the structure of the ECS, as encapsulated in α and λ, although the method has been used to explore the interaction of Ca^{2+} with the extracellular matrix [26]. To extend the point-source paradigm to a wider variety of molecules, Nicholson and Tao introduced the Integrative Optical Imaging (IOI) method [27]. In this method, a fluorescent macromolecule was released from a micropipette by a brief pulse of pressure and the resulting cloud of diffusing molecules was imaged with a standard epifluorescence microscope. At a sequence of times, the solution to the diffusion equation as a function of distance (a Gaussian curve, see Eq. (3) as described for the RTP method) was fitted to fluorescence intensity and the tortuosity measured. The method is not able to measure volume fraction because, unlike the iontophoretic method, the number of ejected molecules cannot be precisely controlled. The measurement of λ for a variety of molecules, including dextrans and albumins, has proved to be interesting and shown that λ increases with molecular weight rising from a typical value of 1.6 for TMA^+ (measured with RTI) to greater than 2 for 70,000 MW dextran or 66,000 MW bovine serum albumin [20]. The method has even permitted the width of the ECS to be estimated as between 40–60 nm, using quantum dots [28]. The IOI approach has also revealed how proteins like lactoferrin interact with the heparan sulfate component of the extracellular matrix [29].

Diffusion studies are now a valuable adjunct in multimodal studies of function. For example, the RTI method has revealed that the ECS expands during sleep to facilitate the removal of waste products [14] and most recently both the RTI and IOI methods have helped elucidate the role of the extracellular matrix component hyaluronan and the ECS in epileptic seizures [30].

Quantitation and modeling of diffusion in the ECS

All the diffusion studies of Fenstermacher and colleagues have relied on implicit models of diffusion in ECS. As noted above, the way the experiments were conducted led to diffusion in a single axis normal to the brain surface that was perfused with radiotracer. This mandated a one-dimensional solution to the diffusion equation for a slab of tissue subject to a constant concentration at one surface. This solution took the form of a complementary error function (Eq. (1)) and Fenstermacher's longtime colleague, the late Clifford Patlak, a noted mathematician, devised special graph paper, which was based on the inverse of this function, to facilitate the analysis of early experiments. Deviations from the expected straight-line solution indicated that significant loss of the diffusing molecule from the ECS was taking place, through intracellular accumulation for example [15]. Another source of loss might occur across the BBB and this was modeled in the paper by Patlak and Fenstermacher [10]. It is worth noting the many important contributions of Patlak to the quantitative interpretation of diffusion in the brain; a study of Ca^{2+} diffusion in normal and thick slices [30] probably represents the most sophisticated analysis of radiotracers to date and may be compared with the later study using the RTP method by Hrabětová *et al*. [26].

The point-source paradigm also centers on a one-dimensional solution to the diffusion equation, but in this case the spherical symmetry of the problem dictates that the solution is carried out in a spherical coordinate system. In this approach, the diffusion is not driven by a constant concentration boundary condition, but either by a constant flux point source (RTI) or a small bolus (less than 1 nL) of injected substance (RTP and IOI). The very small source enables diffusion characteristics to be determined in a volume of tissue with a characteristic length of ~100 μm and in periods of 30–90 s.

The pathophysiology of the CSF

The diffusion of molecules within the ECS was not the only aspect of brain fluid physiology on which Fenstermacher focused his research. Indeed, he also had a keen interest in the CSF. It is quite likely that some of Fenstermacher's early work related to CSF function was inspired by his interactions with neurosurgeons Thomas Milhorat and Mary Hammock, whose primary interest was in the pathophysiology of hydrocephalus. These were the late 1960's and early 1970's, and at that time ventriculocisternal perfusion was the technique of choice to measure the rate of CSF formation in experimental animals. Ventriculolumbar perfusion, a modification of the ventriculocisternal perfusion method, was sometimes used to measure the rate of CSF production in humans. Using this technique, Fenstermacher, Milhorat, Hammock, and others have shown that in a two-year-old child diagnosed with choroid plexus papilloma in one of the lateral cerebral ventricles, the removal of the tumor resulted in a considerable reduction in the rate of CSF formation. Initially, inulin was employed as a non-diffusible marker for ventriculocisternal perfusion; however, concerns have arisen that the CSF formation rate could have been overestimated because of some diffusional loss of inulin from the perfusate into the brain tissue. Fenstermacher together with Robert Curran and other colleagues approached this problem by simultaneously using ^{3}H- or ^{14}C-labeled inulin and ^{131}I-albumin as non-diffusible markers. These studies clearly demonstrated an uptake of inulin by brain tissue surrounding the cerebral ventricles and emphasized the importance of using the high molecular weight markers, such as albumin, in the ventriculocisternal perfusion experiments. The group then went on to use Blue Dextran 2000, with a MW of 2×10^6 Da, as a non-diffusible marker. Since then Blue Dextran 2000 has been predominantly chosen as a marker to measure the rate of CSF formation with the ventriculocisternal perfusion technique.

The anatomy and physiology of CSF compartments in the brain

His long-lasting interest in CSF physiology prompted Fenstermacher to investigate the peculiar tracer diffusion phenomena that were often observed in autoradiographic studies in the vicinity of fluid-filled spaces in the brain, the areas generally discarded from the analysis in most studies. He undertook an effort to track the flow of CSF and the fate of CSF-borne substances. His group combined cerebroventricular infusion of polar tracers that did not cause any disturbance in CSF flow with quantitative autoradiography (QAR) that involved brain tissue sampling from the frozen head to keep all fluids in place. This allowed the group to analyze the complex CSF circulatory system of cisternal and subarachnoid compartments, including the routes of CSF flow through the cisterns of the velum interpositum and anterior medullary velum. Together with his colleagues, Fenstermacher generated the tracer concentration-time profiles for multiple compartments and described a dual physiological role of glia limitans both in restricting the rate of tracer diffusion into neuropil and in acting as a reservoir for solutes carried by the CSF. He also delineated the movement of CSF-borne tracers within the arterial/arteriolar perivascular spaces, supporting the pioneering work of Helen Cserr on the drainage of brain interstitial fluid along the Virchow-Robin perivascular spaces. In particular, Fenstermacher showed that the movement of solutes occurs from ventricle-to-interstitium-to-perivascular space. He also provided the tracer-based functional evidence for differences in perivascular anatomical organization and local perivascular flow along the cerebral arterioles and venules that had previously been discovered by Roy Weller's group. This paved the way to further studies of the role of brain fluids in the elimination of potentially deleterious endogenous metabolites. Applying the previously used techniques to study the clearance of CSF-borne substances from the brain, Fenstermacher together with the Blas Frangione group from New York University showed a biphasic CSF clearance rate for amyloid-β $peptide_{1–40}$. While moving along the ventricular system, this peptide was initially rapidly cleared, which was proposed to occur across either the BBB or the BCSFB in the choroid plexus. This hypothesis has later been tested by his and other laboratories. After an initial rapid phase of clearance from the CSF, the elimination of amyloid-β $peptide_{1–40}$ was slower when this peptide was moving through the subarachnoid/cisternal spaces to be eventually retained around pial arteries and arterioles. More recently, the role of the peculiar anatomical organization of CSF compartments and interconnected perivascular spaces in the clearance of amyloid-β peptide has been revisited. In these studies, a new

brain imaging methodology was used and new hypotheses concerning the pathophysiology of Alzheimer's disease were proposed [13, 14]. The detailed description of CSF flow pathways also had an impact on the research on neuroinflammation. In fact, the choroid plexus together with the subarachnoid and cisternal fluid spaces described by Fenstermacher and his colleagues are now considered to be key players in both normal neuroimmune surveillance and pathological neuroinflammation.

Studies on the clearance of CSF-borne substances from the brain were continued in Fenstermacher's laboratory at the Henry Ford Hospital in Detroit, Michigan. Using ^{125}I-insulin-like growth factor-1 (IGF1) as a tracer for this growth factor, the group demonstrated in a rat model that the entry of IGF1 into normal brain parenchyma after its intracerebroventricular administration was limited by a rapid clearance from CSF and brain, and by slow diffusion into the periventricular brain tissue. Apart from these observations, two more novel facts were reported in this study. The first one was the deceptively similar-shaped temporal plasma profiles of radioactivity following both properly performed intracerebroventricular and erroneous intraparenchymal injections of the tracer. The second was the construction by Patlak of an 'emergence function' that could be used to estimate the rate of appearance of the tracer in the venous system.

The physiology of the BBB

From early on in his scientific career, Joseph Fenstermacher had a significant interest in physiology of the BBB. In his first study published together with John Johnson, he assessed basic properties of the BBB, such as the filtration and reflection coefficients, for water and several solutes, including glucose, sucrose, and urea. Later on Fenstermacher teamed up with Patlak to measure the influx rates across the BBB for water and various solutes using the ventriculocisternal perfusion technique in dogs [10]. It seems that for Fenstermacher it was critically important to know all the advantages and disadvantages of the techniques he was using. This is reflected by his interest in theoretical analyses, like for example the analysis of the experimental conditions for the measurement of the blood-to-brain transfer constants that was done together with Patlak and Ronald Blasberg.

The opening of the BBB in acute ischemic stroke

Previous studies had indicated that the BBB damage after stroke not only varies in regard to its spatial distribution, but also in its magnitude within a given brain region. Such information was indirectly inferred from the differential distribution of radiolabeled tracers with two different molecular sizes, such as sucrose and inulin. Our previous data suggested that the gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) enhancing regions observed immediately after reperfusion developed hemorrhagic transformation (HT) at 24 h post-stroke. This prompted Fenstermacher and his colleagues to postulate that an acute extravasation of blood-borne substances must be limited by the size of the opening of the BBB. This hypothesis was investigated in a series of innovative experiments. In the first set of experiments, the transvascular movement of plasma and red blood cells (RBC) in acute stroke and 24 h after reperfusion was studied. Classical QAR tracers available for such studies were radioiodinated serum albumin (RISA) and ^{55}Fe-labeled RBC. Instead, the Fenstermacher group used a technique previously employed in tumor blood flow experiments with Evans blue, a plasma marker (MW of ~66 kDa after binding to plasma albumin), administered together with fluorescein isothiocyanate (FITC)-labeled autologous RBCs. The data showed that at 3 h after reperfusion, Evans blue leaked, but the RBCs remained intravascular, whereas at 24 h post-reperfusion both Evans blue and RBCs were found in the extravascular space, confirming the previous observations by Knight *et al*. The next question to address was the maximal size of blood-borne molecules that can extravasate acutely after ischemia. For these investigations, once again Evans blue bound to plasma albumin was chosen as the reference tracer, which was used together with a series of FITC-labeled dextrans of varied sizes. These studies demonstrated that FITC-dextran of 2×10^6 Da remained intravascular acutely after reperfusion, suggesting a possible size limit for blood-borne substances penetrating the BBB after cerebral ischemia.

The demonstration of variable magnitude of BBB damage within the ischemic lesion was done later using the MRI technique and employing for the first time Gd-DTPA and Gd-DTPA-albumin, two contrast agents with a significant molecular size difference. Gd-DTPA-albumin was also linked to Evans blue, enabling the fluorescent microscopic confirmation of the patterns of magnetic resonance (MR) distribution of Gd-DTPA-albumin. These data conclusively proved for the first time that the BBB damage varies within the ischemic lesion and that it could be imaged *in vivo*.

Concurrent application of QAR and terminal tracer techniques with MRI and other imaging modalities

The last seventeen years of his career Joseph Fenstermacher spent working at the Henry Ford Hospital in Detroit, Michigan. A significant part of his work there involved employing MRI to investigate cerebrovascular pathology in animal models of ischemic stroke and brain tumors. Several other studies investigating the drug distribution in the prostate gland and the theoretical aspects of water proton tracking, as well as the analysis of histopathological changes in ischemic brain underlying MRI signal changes were also done there. In all these studies, Fenstermacher and his team used QAR and histopathological techniques as a gold standard to validate MR imaging signals for assessing CBF, BBB permeability, ECS, etc. This approach aided in precise localization and quantification of cerebrovascular pathology by MRI. Several 'Aha' moments were a part of the scientific discussions. Over the decades Fenstermacher and his colleagues painstakingly perfected techniques for measuring CBF, blood-to-brain transport kinetics, ECS, fluid bulk flow, diffusion, convection, etc., forming the basis for imaging applications in clinical practice.

Stroke studies: CBF measurements using MRI

One of the first major undertakings was to compare two MRI arterial spin tagging (AST) methods for estimating CBF with the classic IAP-based QAR technique. A rat ischemic stroke model with suture occlusion of the middle cerebral artery for 2 h was employed in these studies. This model had the advantage of producing an extended range of CBF rates for testing the sensitivity of the measurements and a normal contralateral side for comparison in the same rat. Two MRI approaches were used: spin echo (SE) and variable tip angle gradient echo (VTA-GE) readouts. Despite the widespread use of AST-MRI techniques to estimate CBF, very few studies were done till then to compare the MRI data with established terminal tracer studies. For instance, single-coil AST has been used in the laboratory as an imaging technique for assessing the state of cerebral perfusion in a variety of rat

models of ischemia. Questions have been raised regarding whether it is advisable to eliminate signal from vascular spins, whether the technique produces signal linear to flow, and under what conditions the technique might produce an unbiased estimate of flow. It was in this framework that the group examined the operating characteristics of the AST-MRI measurement across the wide range of flows. For the AST studies, both SE and VTA-GE readings were used to assay tissue magnetization. The aim of this study was to determine whether there is a correlation between CBF estimates produced by the two AST-MRI methods; whether the CBF rates measured by either or both of the AST-MRI methods are concordant with those determined by IAP-QAR; and finally whether the AST-MRI methods yield robust estimates of CBF.

The blood-to-brain influx of an MRI contrast agent in ischemic stroke and the Patlak plot

The MRI-QAR correlation of CBF in stroke demonstrated that the approach was feasible and valuable in confirming proton MRI data. This led to the next step of evaluation of BBB opening in stroke, its localization by contrast-enhanced (CE)-MRI, confirmation and quantification by an appropriate QAR technique. Such studies were essential since CE-MRI was employed to image BBB injury in stroke, brain tumors, and other CNS diseases; however, the quantification of BBB damage by MRI needed some more refinement. To this end, a rat model of transient cerebral ischemia resulting in hemorrhagic transformation at 24 h post-stroke was chosen. A 3-step series of experiments was planned to accomplish the objectives. Methods were very similar to those adopted for CBF studies, with each MRI session immediately followed by a QAR experiment in the same rat and the data from QAR being a gold standard to confirm the MRI results. The MRI contrast agent used in all these studies was Gd-DTPA. Gadolinium analogs are the most commonly used clinical paramagnetic agents. The first QAR tracer chosen for comparison was ^{14}C-sucrose. The distribution of ^{14}C-sucrose in the brain had been previously studied by Fenstermacher and colleagues [7, 8, 9, 10]. The MW for ^{14}C-sucrose (302 Da) is close to that of Gd-DTPA (565 Da), and most importantly, both Gd-DTPA and sucrose do not have any known uptake mechanisms in the brain. Therefore, their diffusion within the ECS or their back efflux to the vasculature should result in identical distribution patterns enabling one-to-one comparisons.

The superior contrast enhancement after SDI administration of Gd-DTPA suggested that this might be useful in a better demarcation of the BBB opening. This idea was tested by CE-MRI studies using again the ischemia-reperfusion rat model with SDI administration of Gd-DTPA in the magnet and Gd-^{14}C-DTPA on the for QAR. Identical patterns of CA blood levels were recorded in both procedures. The normalized plasma concentration-time integrals were identical for Gd-DTPA and Gd-^{14}C-DTPA, indicating that the MRI protocol yielded reliable estimates of plasma Gd-DTPA levels. In rats with BBB opening, 14 spatially similar regions of extravascular Gd-DTPA enhancement and Gd-^{14}C-DTPA leakage, including one very small area, were observed. The terminal tissue-plasma ratios from QAR tended to be slightly higher than those from MRI in these regions, but the differences were not statistically significant. The MRI-derived K_i values for Gd-DTPA closely agreed and correlated well with those obtained for Gd-^{14}C-DTPA. Apart from these confirmations, a salient feature of these studies was that compared to bolus injections, spatial resolving power of the SDI input was greater with BBB openings as small as 0.5 mm^3 detected by CE-MRI. Subsequent analysis also showed that unlike the bolus injection, the SDI protocol allows for the magnetic resonance contrast agent (MRCA) to spread beyond the ischemic core. The distribution of water in brain always occupied Fenstermacher's thoughts due to its crucial role in health and disease and its significance as the basis for proton MRI.

Discussions and conclusion

The various scientific accomplishments has been discussed in this review were not necessarily done in chronological order, but, rather, were often performed simultaneously, frequently influencing each other. The early works of Fenstermacher and colleagues on CBF, ECS, and the distributions of water and radiotracers across the BBB evolved seamlessly into the use of modern technologies, such as MRI and CE-MRI. Techniques were borrowed from one application to develop the tools for another project. Examples include, but are not limited to, FITC-labeled RBC used both for tumor blood flow studies and to estimate temporal evolution of BBB damage in acute stroke, the MRI-CAs utilized both in stroke and tumor studies, and the list goes on.

In the modern and fast developing world of science, when researchers frequently specialize in a unique and narrow discipline, scientists like Joseph Fenstermacher are unusual. He represents a rapidly diminishing generation with a deep understanding of the principles of physiology. Nowadays, many consider this discipline of biomedical science as rather archaic and unattractive. However, when a holistic approach to the problem, such as systems biology, is needed and the understanding of the basics of physiology is lacking, the use of even the most advanced and sophisticated technologies to solve the problem may not provide the correct answers. The quest for a better insight into physiology of fluids and barriers of the brain championed by Joseph Fenstermacher laid the groundwork for many of us to build upon. Although he retired in 2012 from the Henry Ford Hospital, he continues to write manuscripts and consult on grant applications, and stays in close touch with his colleagues. Jean-François Ghersi-Egea and Tavarekere Nagaraja, the contributors to this review, had the privilege to train with Fenstermacher at different stages of his career.

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