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Healthy and Infarcted Brain Tissues Studied at Short Diffusion Times: the Origins of Apparent Restriction and the Reduction in Apparent Diffusion Coefficient†

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The significance of NMR water diffusion measurements performed at short diffusion times (<10 ms) for brain tissue is examined. An apparent restriction to diffusion for both healthy and cytotoxically edematous tissue is shown: cytotoxic edema lengthens the diffusion time at which this phenomenon is visible. The dramatic reduction in apparent diffusion coefficient (ADC) observed in the core of cytotoxic edema is explained in terms of the enclosure of extracellular water in non-contiguous pockets in conjunction with the shift of water from the extra- to the intracellular space. The model presented provides an explanation for the ADC reduction without recourse to changes in the cell membrane permeability to water, or unrealistic values for the extra- and intracellular diffusion coefficients.

INTRODUCTION

Ischemic insult to brain tissue results in an early and marked reduction in the apparent diffusion coefficient (ADC) of water as measured by proton MRI.[1–3] A number of explanations for this effect have been offered,[4,5] but to date none has gained widespread acceptance.

Restrictions to the strength of magnetic field gradient available on most imaging systems have meant that investigations have been performed at diffusion times (τ) such that the associated diffusion length is large compared to the cell dimensions. By investigating at shorter τ values it is possible to obtain a deeper insight into the mechanisms responsible for diffusion contrast.

The purpose of this paper is to cohere the experimental results obtained at short τ, and to present an interpretation of these based on the difference beween the intra- and extracellular diffusion coefficients, and the morphological relationship between the intra- and extracellular spaces.

THEORY

NMR diffusion studies are commonly performed using balanced pairs of pulsed gradients.[6] The diffusion induced signal attenuation is then given by:

$$\frac{S(b)}{S_0} = \exp(-bD) \tag{1}$$

where

$$b = (\gamma G \delta)^2[\Delta - (\delta/3)] \tag{2}$$

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Abbreviations used: ADC, apparent diffusion coefficient; CBF, cerebral blood flow; MCAO, middle cerebral artery occlusion.

Δ is the time between commencement of the two gradient pulses, δ is their duration, and the other characters have their usual meaning. The diffusion time (τ) is defined by:

$$\tau = \Delta - \delta/3 \tag{3}$$

In healthy brain tissue the fractional volumes of intra- and extra-cellular water (f_{in} and f_{ex}) are about $f_{in} = 0.8$ and $f_{ex} = 0.2$,[7] while the cell dimensions are of the order of 10 μm.[8] It has been shown by radio tracer studies that there is no effective hindrance to water exchange between the two compartments.[9] Provided that τ is long enough for free-exchange to occur then it is clear that the signal attenuation as a function of the b value will be given by:

$$\frac{S(b)}{S_0} = \exp[-b(f_{in}D_{in} + f_{ex}D_{ex})] \tag{4}$$

where the ADC is defined as:

$$\mathrm{ADC} = f_{in}D_{in} + f_{ex}D_{ex} \tag{5}$$

The half-life for clearance of water from the cell interior is believed to be of the order of milliseconds,[10] and it is clear that if the diffusion time could be reduced to values much shorter than this, then the attenuation as a function of the b value would be given by:

$$\frac{S(b)}{S_0} = f_{in}\exp(-bD_{in}) + f_{ex}\exp(-bD_{ex}) \tag{6}$$

i.e., if the diffusion time is short enough that the expectation value of the diffusion length r as defined by the Einstein relation:

$$\langle r^2 \rangle = 6D\tau \tag{7}$$

is smaller than both the intra- and extracellular spaces, then the system will behave as two non-exchanging compartments. *In vivo* the intracellular space is larger than the extracellular, while its diffusion coefficient is smaller[11] which implies that the maximum τ value at

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which Eqn (6) is valid is arrived at by inserting the appropriate values for the extracellular space in Eqn (7). A system which is governed by Eqn (4) will show a monoexponential dependence of the signal intensity $S(b)$ on the b value at a fixed τ, whereas a system governed by Eqn (6) will show a biexponential dependence. Furthermore it can be shown analytically that a system governed by Eqn (4) will always show at least the same and generally more signal attenuation than one governed by Eqn (6).

Another effect of possible significance is that of restricted diffusion. At sufficiently short τ values that the diffusion length is much smaller than the separation between the impermeable barriers, the diffusion will be largely unrestricted. As the τ value is increased such that the diffusion length is determined by the separation between the barriers, the signal attenuation will be reduced. Thus a decreasee in signal attenuation with increasing τ may be taken as indicative of restricted diffusion, whereas an increase implies that the free-exchange condition no longer holds.

EXPERIMENTAL BASIS

In this section a summary of the results obtained at short diffusion times is presented in sufficient detail as to provide a foundation for the arguments presented in the final two sections. Detailed experimental descriptions are presented elsewhere.[12–15] Three experimental protocols based on pulsed-gradient spin-echo sequences were developed and applied:

(i) Constant time, (*ct*), which corresponds to the conventional intra-voxel incoherent motion experiment[16] whereby the b value is varied solely by varying the gradient strength while the diffusion time is held constant. Restricted diffusion would cause the ADC measured in this experiment to increase at shorter τ values, while contravention of the free-exchange condition would result in a biexponential signal decay.

(ii) Constant gradient (*cg*), in which the gradient strength is held constant and the b value modified by varying the gradient duration, which also leads to a modification in the diffusion time. The presence of restricted diffusion would cause greater signal attenuation in this experiment than in the corresponding *ct* experiment performed over the same range of b values: the opposite would be true if the free-exchange condition were contravened.

(iii) Constant b value (*cb*), where the diffusion time is varied while the b value is held constant. A non-restricted system in free-exchange will yield a constant signal attenuation independent of τ, deviations from this can be interpreted in the same way as for the *cg* experiment.

All experiments were performed on Bruker Biospec systems operating at 4.7 T. The *ct*, *cg* and *cb* experiments were programmed as preparation experiments for the U-FLARE imaging sequence.[17] All three preparation experiments were performed at constant T_2 weighting: in the *cg* and *cb* experiments compensating delays are introduced to keep the *TE* constant. The *ct* and *cg* experiments were performed at 16 different b values equally spaced between 10 s/mm^2 and 1510 s/mm^2, the *cb* experiments were performed at a b value of 210 s/mm^2 for 16 diffusion times lying between 3.4 ms and 5.9 ms. Signal intensities were obtained by integrating over the appropriate regions of interest. The following studies were performed:

(i) Investigations of water, ethanol and acetone phantoms. These provided verification that Eqns (1) and (2) were valid, by yielding diffusion coefficients for both *ct* and *cg* experiments that were in good agreement with the literature values. Both experiments showed a monoexponential dependence of the signal attenuation on the b value. The *cb* experiment showed a constant signal attenuation independent of the diffusion time.

(ii) A study employing the middle cerebral artery occlusion (MCAO) model of cerebral infarction. Seven male Wistar rats were investigated using *ct* and *cg* experiments in the first 2 h post occlusion. Ischemia was induced *in situ* in the magnet as described elsewhere.[18] The diffusion times used for the *cg* experiments were in the range 4.5–11.8 ms; the *ct* experiments were all performed at 11.8 ms.

(iii) A study of focal ischemia in the gerbil induced by the permanent occlusion of the right carotid artery. Four animals were investigated at times from 45 min to 23 h post occlusion. The NMR protocol was as for the MCAO model.

(iv) A study of photochemically induced lesions in the rat using the Rose Bengal model. Ten male Wistar rats were investigated during the period from 24 to 168 h post insult using the same NMR protocol as above.

(v) Nine healthy male Wistar rats were studied using *cg*, *ct* and *cb* protocols. The *ct* and *cg* experiments were performed both at the diffusion times of 4.5–11.8 ms as for the infarct models, and over the considerably shorter range of 1.8–6.0 ms. Two rats were also studied directly *postmortem*. The shorter range of diffusion times was achieved by using bipolar gradient sensitization.

All experiments showed a high level of reproducibility, and the results obtained may be summarized as follows:

(i) At short diffusion times (1.8–6.0 ms) healthy and post-mortem brain tissue shows evidence of restriction.[14] This manifests itself both in the results of the *cb* experiment as shown in Fig. 1(a) and in the results of the *cg* experiment, shown alongside those of the *ct* experiment in Fig. 1(b). The signal curve for the *cg* experiment shows distinct non-monoexponentiality, and the attenuation is greater than for the corresponding *ct* experiment. The *ct* experiment shows a purely monoexponential signal decay.

(ii) At longer diffusion times (4.5–11.8 ms) cytotoxic edema shows such evidence of restriction, and this is visible over larger brain areas than that of reduced ADC, including in most instances the contralateral hemisphere.[12, 13] This is equally true for the MCAO and the gerbil models.

Figure 2 shows *ct* and *cg* attenutation curves for the latter model. At these diffusion times healthy tissue shows no evidence of restriction.

(iii) The situation in the Rose Bengal model at 24 h post insult is that cell lysis is already well advanced in the lesion centre.[19] Tissue outside the lesion shows an

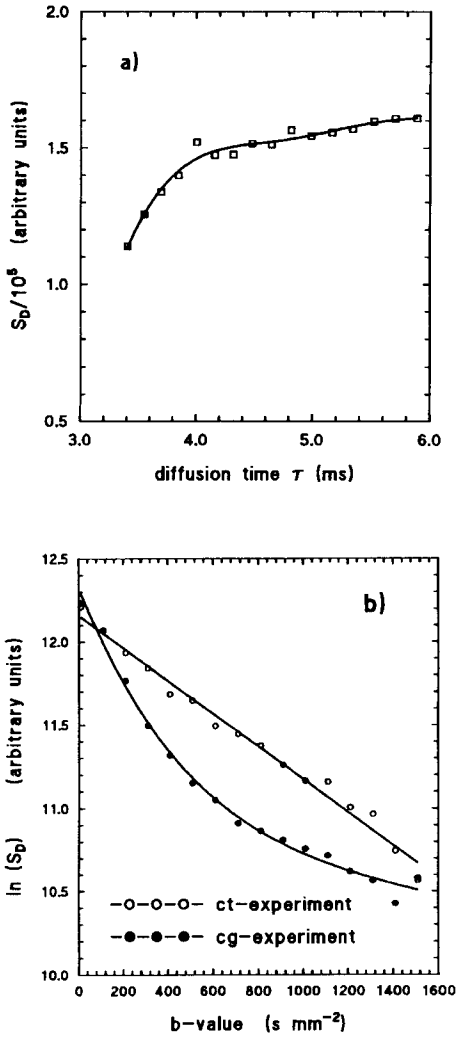

Figure 1. Signal intensities obtained from a region of interest situated in the striatum of a healthy male Wistar rat: (a) for a *cb* experiment in which the signal intensity is plotted against the diffusion time for a constant *b* value of 210 s/mm^2; and (b) for *ct* and *cg* experiments, where the diffusion time in the *cg* experiment varies between 1.8 and 6.0 ms. The signal-to-noise ratio was about 20:1 in the *cb* experiment; in the *ct* and *cg* experiments this varies as a function of *b* value, at a *b* value of 710 s/mm^2 it was 20:1 for the *ct* experiment and 12:1 in the *cg* experiment. The results are consistent with an interpretation as restricted diffusion. For further experimental details see Ref. 14.

increased ADC,[19] and it has been histologically shown that the extracellular space is enlarged in these regions. No significant difference between the *cg* and *ct* attenuation curves was found for this model.

(iv) In healthy tissue the ADC of rat brain cortex is larger at shorter τ, being 1.1×10^{-3} mm^2/s at $\tau = 6$ ms, and 0.89×10^{-3} mm^2/s at $\tau = 11.8$ ms.[14]

Taken together these results are consistent with an interpretation as restricted diffusion. There is however consistent evidence from the literature[20,21] that the ADC is independent of τ at longer τ values than those used here. If the diffusion were restricted in the classical sense then the diffusion coefficient should tend to zero as τ tended to infinity for experiments performed over the same range of *b* values. This is clearly not the case, and we have thus decided to term this phenomenon 'apparent restricted diffusion'.

EXISTING INTERPRETATIONS

This section summarizes the morphological changes that occur in the early stages of severe ischemia, and the theories that have been presented to explain the dramatic and early reduction in ADC.

It has long been known that one of the consequences of ischemic insult is a cell swelling caused by the breakdown of osmoregulation. This occurs some minutes post insult and results in a change in the intracellular fraction (f_{in}) from ≈0.8 to ≈0.9, while the extracellular fraction (f_{ex}) is halved from ≈0.2 to

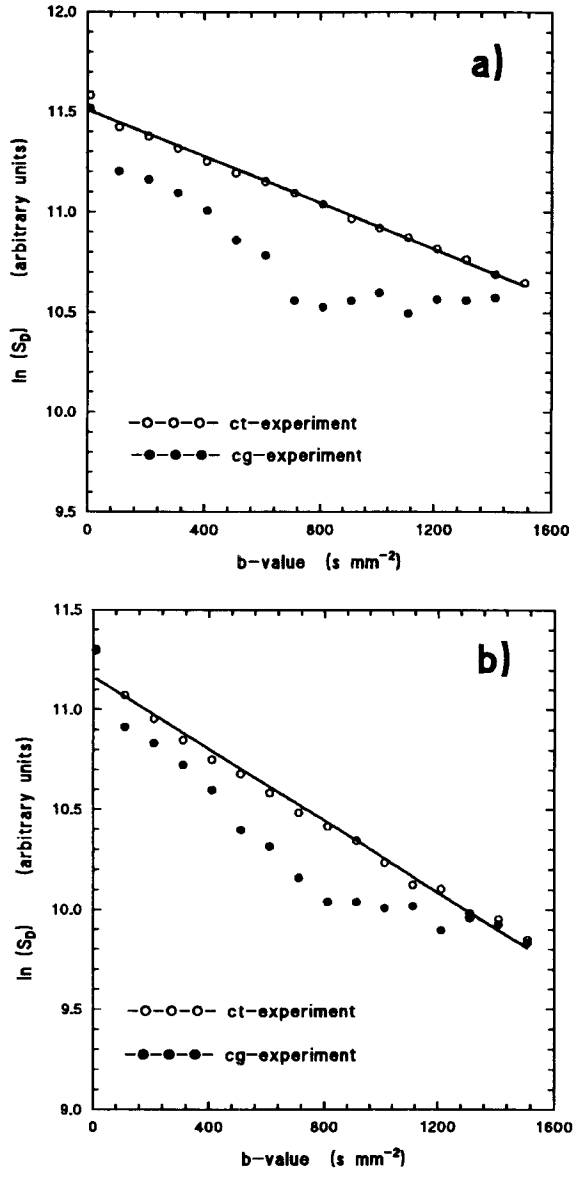

Figure 2. Signal intensity as a function of *b* value for *ct* and *cg* experiments performed on a gerbil where cerebral ischemia had been induced 23 h previously by occlusion of the anterior common carotid artery: (a) attenuation in the ipsilateral hemisphere; and (b) attenuation in the contralateral hemisphere. The change in ADC caused by the infarction manifests itself in the difference between the slopes of the *ct* experiment. In these experiments the signal-to-noise ratio at a *b* value of 710 s/mm^2 was about 40:1 for the region of interest in the ipsilateral hemisphere, and 25:1 for that in the contralateral hemisphere. It should be noted that in healthy animals examined over this range of diffusion times no appreciable difference between the curves obtained from the *ct* and *cg* experiments was measured.

≈0.1.[22] This water shift occurs at the same time post insult as the observation of the reduction in ADC. Experiments performed *post mortem*,[23] and on rats suffering from *status epilepticus*,[24] would also appear to support the assumption that the reduction in ADC is associated with cell swelling. Further support for the hypothesis that the relative sizes of the intra- and extracellular spaces are crucial in determining the ADC comes from the observation that the ADC increases in vasogenic edema, where it is also known that the extracellular volume increases,[25] and from measurements performed on maturing rat pups, in which a decrease in ADC has been correlated with a reduction in the extracellular space.[26] Cell swelling caused by extracellular NMDA[4,27] and oubain[4] have also been linked to a decrease in ADC.

It has been argued that the shift of water into the cells alone is sufficient to account for the observed reduction in ADC,[4] but this is only possible if a very low value for D_{in} ($0.145 \times 10^{-3} \times 10^{-3}$ mm^2/s) is assumed. Measurements performed on cell suspensions[14] and cell spheroids[28] have indicated that D_{in} is about 0.25×10^{-3} mm^2/s, and we have measured the ADC for cerebrospiral fluid to be 3.2×10^{-3} mm^2/s, which can be taken as an upper value for D_{ex}. By using these values a reduction in ADC from 0.84×10^{-3} mm^2/s in healthy tissue to 0.545×10^{-3} mm^2/s in infarcted tissue is predicted by Eqn (4). This does not agree with the value of 0.4×10^{-3} mm^2/s and less observed in practice.

It is also unlikely that the shift of water into the cells can in itself significantly modify D_{in} and D_{ex}. D_{in} should not be significantly modified by a mere 10% increase in water content, which would only dilute the diffusion hindering macromolecules by the same amount. The high value for D_{ex}, which is not greatly different from that of free water, implies that the extracellular concentration of diffusion hindering macromolecules is very low: doubling this cannot be expected to modify D_{ex}.

It has also been proposed that the ADC reduction can be accounted for by changes in the permeability of the cell membrane causing restricted diffusion.[5] This appears to contradict the free-exchange condition, i.e., the *ct* experiments performed at short τ values would be expected to show a non-monoexponential signal decay if this were true. There is also limited physiological evidence to support this hypothesis.

APPARENT RESTRICTION MODEL

In this section a model will be presented that is capable of explaining both the apparent restriction to diffusion observed at short τ values, and the reduction in ADC observed in cytotoxically edematous tissue. The situation will first be considered by examining the situation as a function of τ.

At very short diffusion times, shorter than those considered here, the free-exchange condition will be contravened and both intra- and extracellular diffusion will be isotropic as illustrated in Fig. 3(a). At the diffusion times used by us a local anisotropy can occur because the extracellular water will diffuse preferentially around the cells, as shown in Fig. 3(b). At somewhat longer diffusion times the diffusion length

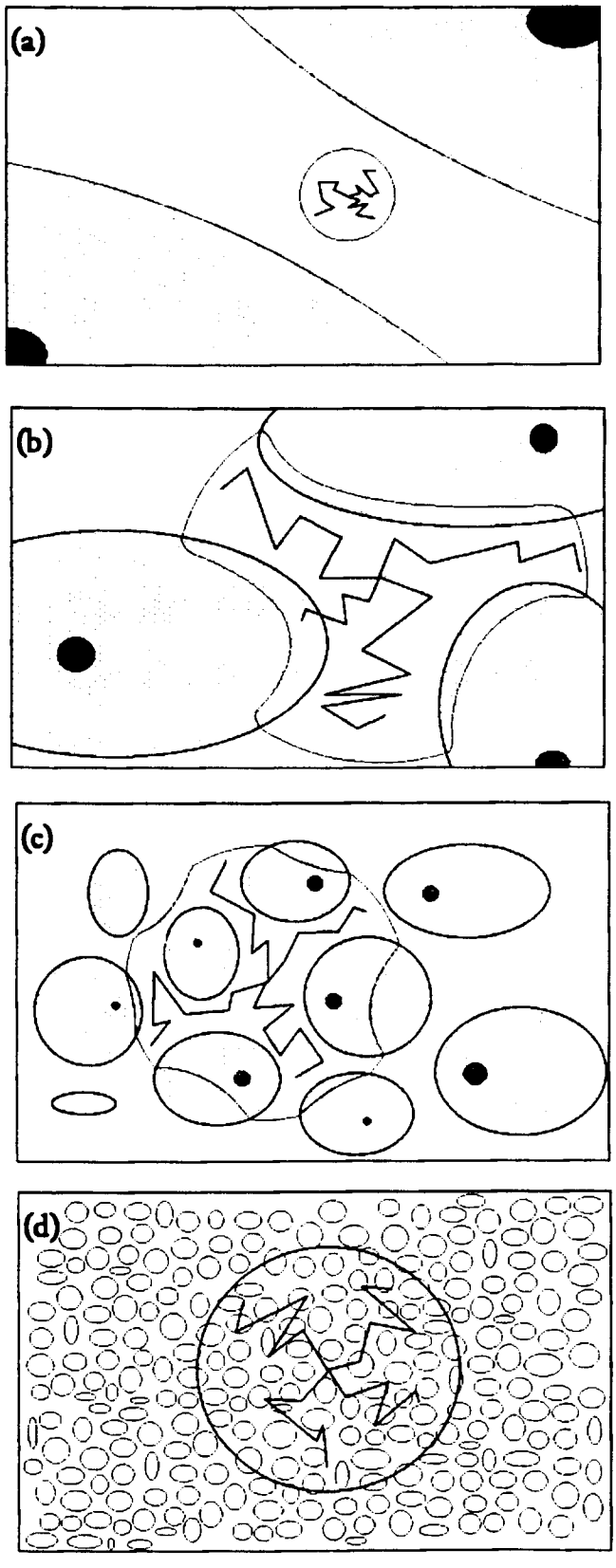

Figure 3. Schematic diagram of the form of the expectation value of the diffusion length for extracellular water as a function of diffusion time. (a) The diffusion is isotropic for extremely short diffusion times such that the free-exchange condition does not pertain, and the extracellular water does not have time to interact with the cells. (b) For longer diffusion times the extracellular water diffuses preferentially around the cells giving rise to the phenomenon of apparent extracellular restriction. (c) As the diffusion time is lengthened the water diffuses through and round the cells reducing the level of anisotropy. (d) At sufficiently long times isotropic diffusion is found.

will be greater than the cell dimensions and the diffusion will be approximately isotropic as shown in Fig. 3(c). At long diffusion times the diffusion length will far exceed the cell dimensions and isotropic diffusion will exist as shown in Fig. 3(d).

The situation shown in Fig. 3(b) is that in which apparent restriction is present. Water that is in the extracellular space at the start of the NMR diffusion sensitizing experiment will have a tensor of the expectation value of the diffusion length that is locally anisotropic, with the anisotropy being determined by the local morphology. As the extracellular space is smaller than the intracellular, and D_{ex} is greater than D_{in} this anisotropy is relevant only for the extracellular water: the water initially in the cells will diffuse isotropically. Thus only the extracellular water experiences this apparent restriction. The extracellular water can diffuse freely around the cells. It will not diffuse so far into the cells for two reasons: first because in the cell the diffusion coefficient is lower; and second because no net flow into the cells occurs and hence the extracellular water can only diffuse into the cells at the same rate as the intracellular water can diffuse out.

At longer diffusion times this local anisotropy will disappear because for diffusion lengths greater than the microscopic cell structure no preferred orientation will exist: the molecules can diffuse around or through the cells with the same probability in all directions independent of their initial positions [cf. Figs 3(c) and (d)]. This is in accordance with the observations of Moonen *et al.*[20] that at diffusion times greater than 50 ms no evidence of restriction is found in infarcted or normal tissue in the cat, and of Le Bihan *et al.* who found no evidence of restriction in human white matter at diffusion times of 17 ms and more.[21]

It is a characteristic of restricted diffusion that the diffusion coefficient measured decreases with increasing diffusion time. In apparent restriction the ADC will only depend on τ at short diffusion times. Once the τ value is so long that the situation shown in Fig. 3(c) or (d) pertains then no further dependence of the ADC on τ is predicted, as is found in practice.

Cytotoxic edema would appear to lengthen the τ value at which apparent restriction is observable. In ischemic tissue the extracellular water can diffuse less freely due to the cell swelling, and a longer diffusion time is required for the extracellular diffusion to become isotropic. In vasogenic edema the extracellular space is enlarged,[25] and it is to be expected that the τ value at which apparent restriction is observed will be shortened relative to that of healthy tissue, because extracellular diffusion will be less inhibited by the cells, and the transition between the situation shown in Fig. 3(b) to that in (c) will occur at a shorter τ value.

In a cytotoxic edema cell swelling halves the extracellular space. This will modify the cellular morphology, making it more difficult for extracellular water to diffuse around the cells, and in the extreme case some of it will be trapped in small pockets between the swollen cells. This situation is shown schematically in Fig. 4. As no net flow into the cell is expected during the diffusion sensitizing part of an NMR experiment, extracellular water can only diffuse into the cells at the same rate that intracellular water diffuses out. For example, water trapped in a pocket will have a diffusion length determined solely by D_{in} if the diffusion length is larger than the pocket size. It is hence no longer to be expected that the ADC will be the weighted sum of the diffusion coefficients of the intra- and extracellular compartments. In the ultimate infarct where, by definition, the cell swelling is so severe that all the extracellular water is trapped in such pockets, then the ADC will tend to D_{in}. This appears to offer a natural explanation for the reduction in ADC without the need for any additional effects such as changes in membrane permeability. Both compartments remain in free exchange, but the extracellular water is apparently restricted in its diffusion. No infarct will succeed in trapping all the extracellular water in this way and hence the ADC will exceed that of the intracellular water to some extent, as is found experimentally.

The theory can also be used to explain paradoxical results in anisotropic diffusion. The established view that myelinated sheaves are responsible for this effect has been called into question by the results of Beaulieu and Allen[29] who showed that the anisotropy remains after demyelination. This effect can be explained simply in terms of D_{ex} being anisotropic on a macroscopic scale, i.e., the situation shown in Fig. 3(b) pertains even at longer diffusion times. The anisotropy is then the result of the 'no net flow' condition, and is not caused by the impermeability of the myelin sheaves.

It is not clear how significant coherent interstitial fluid motion is: experimentally it is indistinguishable from diffusion. A reduction in this would reduce D_{ex} without necessarily leading to the major reduction in ADC observed in the infarct core. It would however lengthen the τ value at which apparent restriction is observed. There is a body of experimental evidence showing that a significant reduction in cerebral blood

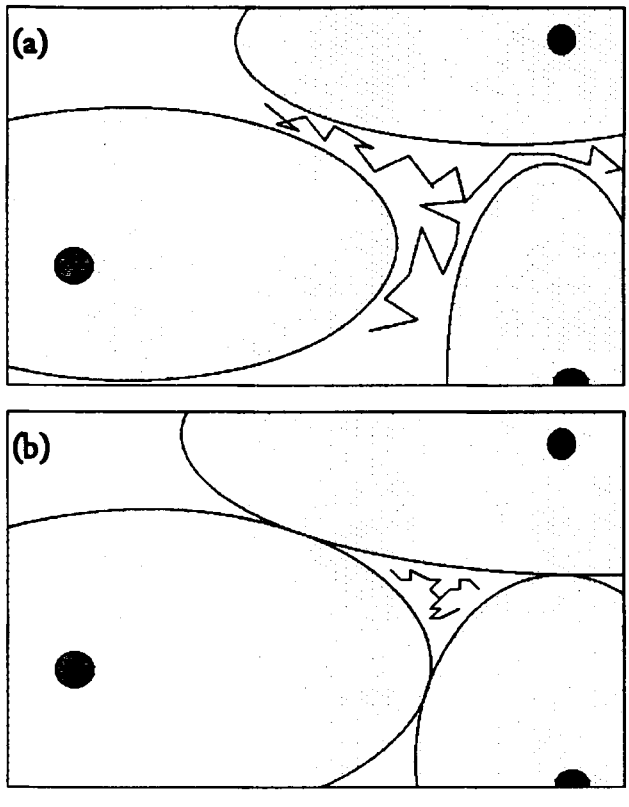

Figure 4. Schematic diagram of how extracellular water may be trapped in isolated pockets caused by cell swelling: (a) The normal situation; and (b) the situation after swelling.

flow (CBF) occurs outside the centre of ischemic damage, and in the contralateral hemisphere.[30,31] If a connection could be demonstrated between the level of CBF and coherent fluid motion in the interstitial (i.e., extracellular) space, then this could explain the detection of apparent restriction at longer diffusion times in the contralateral hemisphere.

A further unknown factor is the effect of finite permeability of the cell membrane. The theory presented above assumes that the membrane is completely permeable, the cells are considered to be like icebergs of slowly diffusing water sitting in a sea of extracellular water. It is clear that this is an oversimplification, but all the experimental evidence points to a high water flux through brain cells probably due to their high surface to volume ratios. Completely impermeable membranes would lead to restricted diffusion, a result that contradicts the experimental evidence as discussed. The presence of membranes of high but not infinite permeability would tend to increase the anisotropy of the extracellular diffusion at the short diffusion times corresponding to the situation shown in Fig. 3(b). Increasing impermeability would lengthen the τ-value at which the transition from the situation shown in Fig. 3(b) to that shown in (c) occurs. A decrease in membrane permeability within the ischemic lesion could thus explain the detection of apparent restriction at longer diffusion times in the infarct than in healthy tissue, it offers no explanation for the detection of apparent restriction in regions remote from the lesion centre.

In conclusion the theory presented here has proved itself capable of qualitatively explaining a wide range of experimental results, and has the virtue of originating simply in the difference between D_{in} and D_{ex}. A quantitative evaluation is hindered by the lack of information as to the contribution of coherent interstitial fluid motion, membrane permeability and surface to volume ratios. Other workers have recently presented a mathematical treatment which could provide a basis for performing such calculations[32] if the values of these parameters could be determined.

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